Chemically modified sporopollenin as a solid support for heterogeneous catalysts

being a Thesis submitted for the Degree of

Doctor of Philosophy

in the University of Hull

by

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December 2019

Dedication

This thesis is dedicated to Almighty God, the giver of life and knowledge; and also, to the duo; my maternal grandmother, Alice Adegbenjo Olabode and my late mother, Elizabeth Modupeola Abimbade who both laboured tirelessly to influence my academic and other aspect of life by instilling dedication, discipline and hardworking in me. I will live to always remember your instructions.

Acknowledgements

All glory be unto the Lord Jesus Christ and to God the Father for their mercies and protections throughout the period of my research in the UK.

I would like to offer my sincere gratitude to my supervisor, Dr A.N. Boa, who through patience, learn to understand my perspective and orientation about life despite our different cultural backgrounds. Honestly, encouragement from Dr Andrew Boa has been the pivot that motivate my passion to diligently work on the results from my research with critical analysis. I appreciate his availabilities to attend to all my questions, even when his schedule is stringently tight. I love to confess that your immeasurable friendliness and assistance during the period of my trials reshaped my focus. The thoroughness while reading my thesis is also appreciated. All your valuable instructions and guidance will forever be in the archive of remembrance. I am also very grateful to my second supervisor, Dr Grahame Mackenzie, your enthusiasm and cheerfulness; especially your morning greetings, are major catalysts that often make my days great. Your contributions to my work and painstaking reading of my thesis in a constraint time is currently occupying a special place in my heart. I say a big thank you. The academic and technical staff that contribute to my work, especially, Dr Rob Lewis – for teaching me how to operate NMR machine, Mrs Carol Kennedy – for elemental analysis, Mr Bob Knight – for carrying out ICP-MS analysis on the SpECs samples, Dr Kelvin Welham – for GC-MS and ESI-MS analysis of the samples, Dr Rob Macdonald – for his support, and Mr Timothy S. Dunstan – for SEM analysis on the SpECs samples, are gratefully acknowledged. To Arundhasa Chandrabalan, Oluremi Isola Adeniran, Salim Al-habsi, I say thank you very much. Daniel Grahame-Mackenzie, I will not forget your kindness. Guy Entract, your understanding is unique. All the MChem., MSc. And exchange students; Harry, Liban Saney, Julian Dellen, Julio, Maoulud, Elizabeth, Steve, Connor and Ezinne, are well appreciated.

I also want to thank Pastors Victor Ebenuwa, Bothwell Tsiga, John Adeyemi, Atilade Ewetola and Kayode Obajaja as well as all other brethren in the UK and Nigeria that do not only support me in prayers but also give their moral support. My colleagues in Federal University, Oye Ekiti (FUOYE), Nigeria, especially Professor Cecilia Akintayo (your motherly role and love are exemplary), Professor Olayide Samuel Lawal, Professor Patrick Okechukwu Okolo, Dr Olaiya Folorunso, Dr Oluwatimilehin Joshua Oluwadare, Dr Emmanuel Afolabi Bakare, Dr Olaide Sikiru Hammed, Dr Olushola Sunday Ayanda,

Dr Bolanle Motunrayo Babalola, Dr Habibat Omolara Adubiaro, Dr Dosu Malomo, Dr Emmanuel Gbenga Olumayede, Dr Adeniyi A. Adebayo, Dr Ayodele Odo, Dr Olusayo Olubosede, Dr Oyebamiji O. Oketayo etc who always call to know the state of my welfare, are well appreciated. My uncle, Mr Emmanuel Adelani Olabode, who shoulder the responsibilities of my tertiary educations and often intimately relate with me, after the death of my parents is highly appreciated and acknowledged. My grandmother, you a sweet mother. My Late father, Mr Gabriel Ajayi Abimbade and my siblings – Omolara Beatrice Adebisi (Nee Abimbade), Abidemi Janet Oladele (Nee Abimbade), Abiona Samson Abimbade, Tope Adebayo, Olaleye Edward Olabode, Oladele Hezekiah Olabode etc, you are the best. A million thanks to my sponsors, Federal University, Oye Ekiti, Nigeria, through the TETFund, and the department of chemistry, University of Hull, UK, through the Faculty of Science and Engineering Chemistry PGR Scholarship, for the opportunity. My former teachers; Late R.A. Ajayi (principal in my secondary school days), Professor Sunday Oladoye, Dr Ganiyat Oloyede, Professors Dayo Sonibare, Patricia Onocha and Ezekiel Temidayo Ayodele, thank you for your contributions and supports in the early days of my academics. My past students that are always in touch with me, thank you. My Friends, Pastors Kayode Oladokun, Saka Hassan, Dr Donatus Onwuli and family, Dr Sogo James Olatunde, Dr Olalekan Oluwabamise Faboya, Dr Adeolu Adediran, Dr Ebenezer Adewuyi Ademola, Dr Abimbola Adekambi, Dr Gbenga Samson Ajayi as well as Mr Ademola Michael Ojo, Mr Lanre, Ibrahim Amobire Akolga and Mrs Nkiru Ogidi are well appreciated. To my future wife, I appreciate your patience. Thank you all.

Sunday Felix Abimbade
10/12/2019

Publications and Conferences

Parts of this work have been published as:

S.F. Abimbade, A.N. Boa and G. Mackenzie (2018) Sulfonated Sporopollenin: Solid acid catalyst derived from natural organic polymer (Postal). Presented at the Post-Graduate Colloquium, Department of Chemistry, University of Hull, UK

S.F. Abimbade, A.N. Boa and G. Mackenzie (2019) Sulfonated sporopollenin (SpEC-SO_3H) as a recyclable heterogeneous acid Catalyst in the environmentally benign synthesis of di-substituted benzimidazoles (Postal). Presented at 34th Annual Postgraduate Symposium, AstraZeneca, Macclesfield, UK

Abstract

Application of the principles of 'Green Chemistry' has been a focus in recent years in the synthesis of fine chemicals, and solid-phase catalysts are useful tools in that endeavour compared to stoichiometric reagents. Another important principle of 'Green Chemistry' is in the choice of renewable feedstocks. With those two aspects in mind, the plant-derived polymer sporopollenin, isolated in the form of sporopollenin exine capsules (SpECs), has been extracted from the spores of *Lycopodium clavatum*. Modification of these polymer capsules with chlorosulfonic acids gave rise to sulfonated SpECs (SpEC-SO_3H), which have been investigated as a heterogenous acid catalyst in a range of organic reactions. Other modifications of the SpECs investigated were zinc (SpEC-Zn), brominated (SpEC-Br) and thiolated (SpEC-SH) forms of the SpECs.

In this work, sulfonated SpECs were used as a heterogeneous catalyst for the: (i) synthesis of disubstituted benzimidazole derivatives; (ii) protection of carbonyl compounds to form spiro ketals; (iii) formation of tetrahydropyranyl ethers from alcohols; (iv) formation of ketals from monosaccharides; (v) Fischer glycosylation of monosaccharides; (vi) *O*-glycosylation of phenols and alkyl alcohols; (vii) *N*-glycosylation of urea and urethane; (vii) dehydration of monosaccharides; (ix) Beckmann rearrangement of benzophenone oximes; (x) transesterification of fatty acid triglycerides.

In these reactions, the sulfonated sporopollenin exine capsules (SpEC-SO_3H) proved to be an efficient catalyst in reactions that were either carried out in water, in aqueous solvent mixtures, in various organic solvents, or in some cases in solvent-free processes. For example, the synthesis of disubstituted benzimidazoles from *o*-phenylenendiamine and substituted benzaldehydes was investigated in water at 70 °C, under solvent-free melt conditions at 70 °C, in aqueous ethanol or in ethanol at room temperature. The catalytic action of SpEC-SO_3H resulted in a good yield of the benzimidazoles (43% - 90 %) in all of these processes except in the reaction of *o*-phenylene diamine with 2-methoxybenzaldehyde in aqueous ethanol that gave 6 %. Likewise, similar yields were obtained when the reaction of *o*-phenylene diamine with benzaldehyde or 4-hydroxybenzaldehyde catalysed by SpEC-SO_3H using microwave heating was compared to the conventional heating. The reaction of aliphatic aldehydes and *o*-phenylenediamine to form alkylated benzimidazoles in the presence of SpEC-SO_3H was performed at room temperature to avoid the formation of aldol adducts. In the formation of benzimidazole

from aliphatic aldehyde, the reaction time was shorter and with minimal by-products resulting from competing aldol side reactions.

SpEC-SO_3H also proved effective in catalysing glycosylation reactions of alcohols; however, *O*-glycosylation of phenols and *N*-glycosylation of benzamide, acrylamide, purine, imidazole and derivatives were unsuccessful. However, *N*-glycosylation of urea and urethane was successful and the *O*-glycosylation and *N*-glycosylation reactions that worked, yielded high products using SpEC-SO_3H catalysis.

The protection of carbonyl compounds (as ketals) and alcohols (as THP ethers) also revealed the effectiveness of the catalytic performance of SpEC-SO_3H as a bio-based heterogeneous catalyst. Application of SpEC-SO_3H to dehydration of fructose led to the formation of a high yield of hydroxymethylfurfural (HMF). Also, SpEC-SO_3H was effective in the transesterification reaction of sunflower fatty acid triglycerides with methanol, and in the Beckmann rearrangement of benzophenone oxime.

Another aspect that portrayed potential usefulness of the SpEC-SO_3H catalyst is its recyclability. In most of the reactions studied, the SpEC-SO_3H catalysts was able to be isolated, recycled and re-used.

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List of abbreviations

AHS	–	Hydrochloric acid-hydrolysed sporopollenins
AHS-SO_3H	–	Sulfonated sporopollenin from AHS
BHS	–	Base-hydrolysed sporopollenins
BHS-SO_3H	–	Sulfonated sporopollenin from BHS
^{13}C CP/MAS NMR	–	Carbon-13 Cross-polarization and magic-angle spinning nuclear magnetic resonance
^{13}C-NMR	–	Carbon-13 nuclear magnetic resonance
DCM	–	Dichloromethane
DMSO	–	Dimethylsufoxide
DVB	–	Divinylbenzene
ESI-MS	–	Electrospray Ionization mass spectrometry
FTIR	–	Fourier transform infrared
^{1}H-NMR	–	Proton nuclear magnetic resonance
ICP-MS	–	Inductively Coupled Plasma Mass Spectrometry
MS	–	Mass Spectroscopy
PAHS	–	Orthophosphoric acid treated AHS
PAHS-SO_3H	–	Sulfonated PAHS
PBHS	–	Orthophosphoric acid treated BHS
PBH-SO_3H	–	Sulfonated PBHS
PHS	–	Orthophosphoric acid-hydrolysed sporopollenin
SEM	–	Scanning Electron Microscope
SpEC-Br	–	Brominated Sporopollenin
SpEC-SO_3H	–	Sulfonated sporopollenins from phosphoric acid
SpEC-OSO_3H	–	Sulfated Sporopollenin
SpEC	–	Sporopollenin microcapsules
SpEC-SH	–	Thiolated sporopollenin
SpEC-Zn	–	Zinc sporopollenin
TLC	–	Thin Layer Chromatography
UV	–	Ultraviolet Lamp

Chapter 1: Pollens and Spores

Pollen and spores grains, are the reproductive organs of the plant.[1] They are used as a means of dispersal of their reproductive organs by plants.

The size of their grains ranges between 7-200 μm[2] and despite the size of the grains of pollen, their morphology is often very complex. This feature has been used to trace their origins and their environmental settings.[2,3]

Pollens are composed of hard biopolymer, flavonoid and carotenoid pigments, lignin, pectin, proteins, lipids, carbohydrates, and nucleic acids in the inner cells.[4,5] The pollen wall is multi-cellular and comprises of cellulose and the outer polymer, known as sporopollenin, that is resistant to chemical and physical attack.[2,6] The outer cell wall, therefore, provides mechanical protection that prevent the male gametophytes from dehydration, microbial attacks and environmental challenges.[6] The outer walls of pollen, if kept in a dry environment, can be preserved for several years.[2]

Pollens and spores have various uses in palynology studies, investigation of geological age and geographical location of a substance,[3,7,8] forensic analysis,[9] civil and criminal investigations, biology, and chemistry.[2] Due to their size, complex morphology and resistance to decomposition, they have advantage over other materials of biological origin for palynology studies and an image of a sunflower pollen grain is shown in **Figure *1.1-1***.

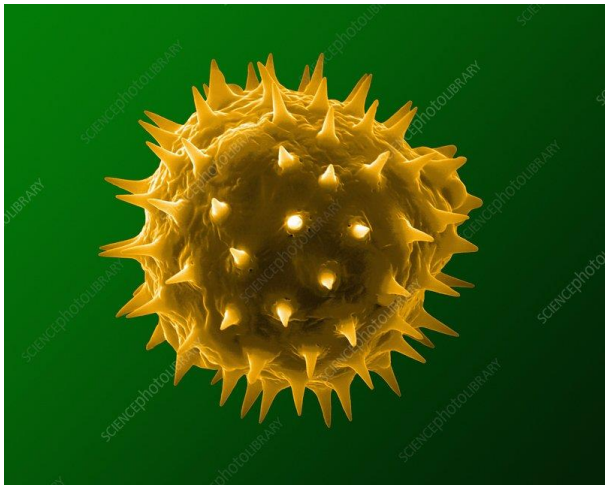


Figure 1.1-1*:* SEM Image of sunflower (*Helianthus annuus*) pollen grain[10]

1.1 Pollen or spore?

Sporangia is a receptacle that serves as the production site for the formation of the spores or pollen in lower plants.[11] Spores are the means by which the sexual organs are dispersed in the lower plant thereby becomes their reproductive organ. Examples of these plants include algae, ferns, fungi, liverworts, lycopods, hornworts, lichens and mosses, which include club moss *L. clavatum*.[2]

Pollens are produced within anthers in flowers or highly modified leaves within cones of conifers.[2] They are very important to pollination and germination.[6] They carry the male sex cells from one plant to the female part of the flower (stigma) or the female cone of a different plant (more often) in the same species for fertilisation and seed production.[2]

The germination of spores and fertilization of pollen grains depend on the human factor, animal activities, insects and environmental factors like water and wind to execute their reproduction.[2]

1.2 Lycopodium clavatum

L. clavatum, which is alternatively called club moss, clubfoot moss, foxtail, ground pine, or wolf's claw, is one of the members of family *Lycopodiaceae* in the plantae kingdom.[12] The plant is widespread and found mainly in tropical, subtropical climates and in many European countries.[12]

L. clavatum is a non-timber, evergreen fern-like plant.[11] It is terrestrially grown with rhizomatous vascular stem running above or beneath the ground surface.[11,13] The existence of *L. clavatum* is reported to be age-long plant with adventitious roots and had survived different environmental changes.[11,13-16] *L. clavatum*, therefore, is a perennial plant that has survived several climate changes and also, it has clonal nature.[11,15] The leaves of *L. clavatum* are very small with length of about 3-5 mm and breadth of around 0.7 – 1mm width.[12,17] The leaves are separated from one another by the space of about 8 mm.[17] The diagram below (**Figure *1.2-1***) explains the description of *L. clavatum* plant.

Figure 1.2-1*: Lycopodium clavatum* plant[15]

At full maturation age, the aerial branches of the stem of *L. clavtum* often develop strobili or spores cones (**Figure *1.2-2***).[2,11] Strobili is a green-yellow peduncle that produce the enclosed capsule, known as sporangia, which house the production of spores and it has the life span of 4 to 6 years.[2,11,17]

Figure 1.2-2*: Close-up of strobili for spore production*[15]

L. clavatum is a very useful plant. Lycopodium powder is very useful as a natural powder lubricant and base for cosmetics.[18] It also found its use in herbal medicine.[12,13,19,20] Traditionally, the spore is used in the treatment of stomach pain,[12,21] against rheumatic disease,[12,21,22] treatment of muscle pain or as an analgesic agent,[12,21,22] Alzheimers disease,[12,13,23] treatments of aneurisms,[12,21] constipation,[12,21] chronic lung and bronchial disorders,[12,21,23,24] and fevers.[12,15,21] It is also used as a medicine in the treatment of gastric inflammations,[22,25] aids digestion,[21] to treat chronic kidney disorders,[12,15] skin irritation and itching,[21] kidney stones,[15] hepato-protective,[15,19,21,26] as an anti-carcinogenic[19,23,24,27]

and in the treatment of urinary tract infections.[12,21,28] Scientists have extended the use of spores and pollen to medicine and health.[19,24,26,27,29,30] The presence of flavonoid (apigenin), alkaloids (huperzine A, lycopodine, lycoflexine, alphaonocerin), organic acid (vanillic, coumaric, ferulic acids and syringic acids), polyphenolic acids, including dihydrocaffeic and triterpenes in *Lycopodium clavatum*, contributes immensely to its medicinal and biological activities.[13,24,31] The chemical structure of the compounds isolated from *L. clavatum* spore are shown in **Figure *1.2-3***. Also, the extract from the spores of *L. clavatum* has been reportedly used as an antioxidant.[32]

It has been reported that the crude ethanolic extract of *L. clavatum* spores has no fewer than 201 different alkaloids.[13] The bioactivities of some of these alkaloids has been tested and known, while the bioactivities of the majority of the remaining alkaloids are not yet known.[23]

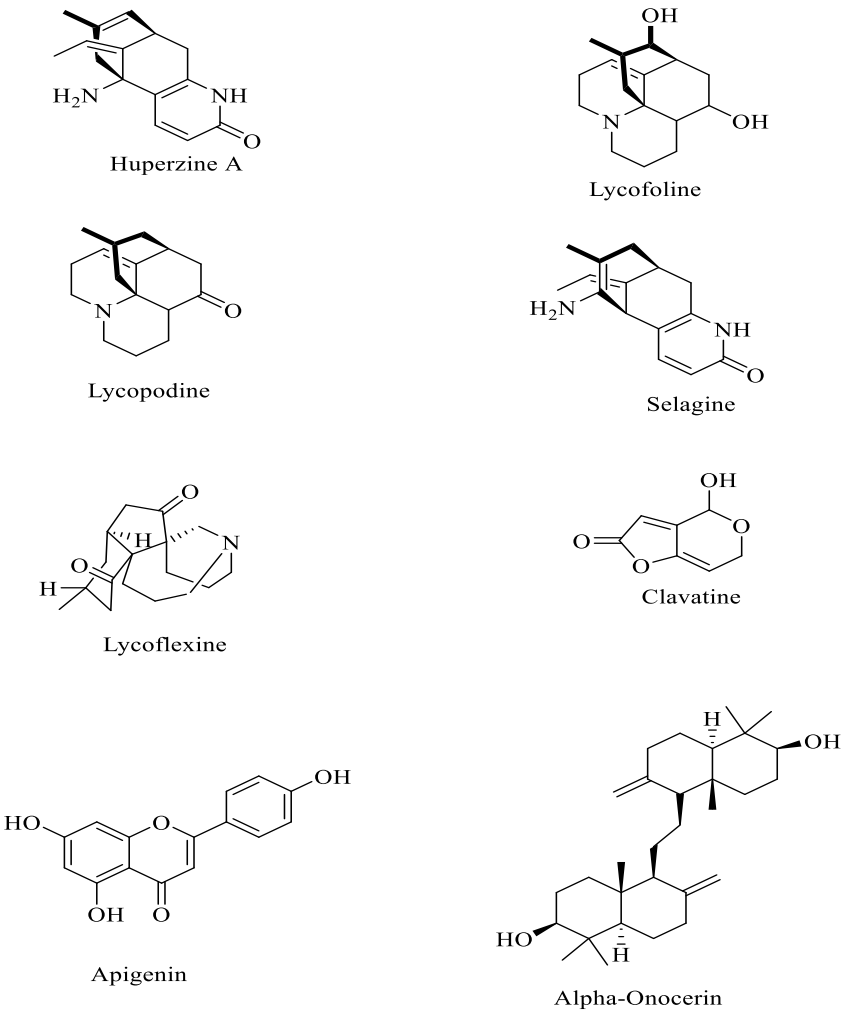


Figure 1.2-3*: Some compounds isolated from Lycopodium clavatum* [23]

There are several published articles that have reported the effectiveness of constituents from the *L. clavatum* against cancer cells. For example, lycopodine and apigenin, which are respectively alkaloids and flavonoid constituents obtained from the extract of *L. clavatum*, had been reported to have exhibit efficient inhibition of the proliferation of cancer cells and they are classified as potential anti-cancer drugs.[20,23,33,34] Pathak's work on the animal liver in 2006 indicates that the extract from the spore of *L. clavatum* protects the liver against hepatocarcinogen.[24,26] It was further revealed that apigenin is an antioxidant which protect skin keratinocytes from ultraviolet light by stimulation of nucleotides to repair the body genes.[41] Other potent antioxidant and anti-inflammatory compounds were also reported present in *L. clavatum* [35-37] for example, Graf investigated and reported that ferulic acid inhibits the propagation of free radicals chain reaction.[35] The bioactivities of club moss is largely due to the presence of flavonoids and alkaloids.[37]

The use of the crushed spores of lycopodium in homeopathic medical system is dates back to the period of the practice of traditional medicine.[29] Sundaram *et al*. (2013) explained the analgesic activity of lycopodium by testing its extract on rats for 30 days.[22] Another researcher also reported that lycopodium is used in the treatment of the reproductive anomalies in human beings like the reduction of enlarged prostrate, resuscitation of the male reproductive organ and reduction of the vagina dryness.[12,22] It was reported that petroleum ether and chloroform extracts of lycopodium plant exhibit antiprotozoal activity by inhibition of the growth of *plasmodium falciparum* and also displays potency against leishmania due to the presence terpenes.[38] Orthan in 2003 had used the triterpenoid compounds, α-onocerin, from *L. clavatum* to inhibit acetylcholinesterase in the central nervous system. This same experiment further investigated on rat in 2012 by Konrath.[39,40]It was also reported that α-onocerin is effective against oxidative and proliferative effects.[24,26,33] The methanolic extract was investigated and show that it plays some roles on thyrotropin-releasing hormone.[41]

The external layer (see section 1.3.1, **Figure *1.3-1***) of the spore of *L. clavatum* has been in use since the 19th century. The uses of sporopopollenins are enormous due to its unique resistance to chemical and physical damages.[31,42,43]

1.3 Morphology of pollen/spores

1.3.1 The ultrastructure of pollen and spores

The identification of pollen is uniquely different from other biological organism because it is prone to cause an allergic reaction. Unlike other organisms that owe their identification to molecular parameters, pollen can only be identified based on its morphological information obtained through optical or electron microscopy.[4] The method of identification of pollens, which is mainly using their morphological information from light microscopy and image analysis, increases human exposure to pollen and thereby causes an increase in the prevalence of pollen allergies.[44] The need to acquire more efficient information about the pollen identification led to the development of pollen allergy-warning systems with fast chemical information.[4] The pollen allergy-warning system makes use of Raman spectrum to reveal different classes of pollen through their biochemical fingerprint.[4] The classification and characterization of individual fresh pollens with their morphological components, using Raman spectroscopy, requires no prior preparation and Raman spectroscopy is non-destructive. The Raman spectroscopic analysis can even be carried out with single pollen grains or fragments, therefore the result or spectrum used for characterization and classification of pollen is often obtained *in situ*.[4,45] In this inelastic scattering process, scattered photons with changed energy are resulted from the interaction of the molecular vibrations of the sample and photons emanated from a laser source. The energy difference corresponds to the energy of the molecular vibration that was involved in the interaction. The superimposed vibrational information on structure, composition and interactions from all classes of molecules are generated from the resulting spectra.[4,45,46]

Figure 1.3-1*:* Morphology of *Lycopodium clavatum* spore[47]

1.3.2 Pollen and spore grain capsules are multi-layered structure

The walls of a pollen or spore grain are multi-layered. The membrane of pollens and spores are similar. They have been found to contain some components like cellulose, lipids and polysaccharides.[48] Spores are reported to contain resistant exine material, i.e. the external layer, known as sporopollenin, that envelope some contents like base-hydrolyzable fatty acids, hydroxycinnamic acids, cellulose and other carbohydrates or polysaccharides.[49]

The inner layer, or intine, is made up of a polysaccharide / cellulose and may be sub-divided into two major distinguishable layers. The outer layer of this polysaccharide is known as exintine while the inner layer that is majorly cellulose is termed enditine.[50]

The exine is the outer layer of the pollen grains or the spore, that protects the contents from external physical and chemical attack. The layer of exine has a thickness of less than 1 μm and it is composed of three sub-layers. The outer layer is the ektexine while the inner layer that is next to intine in the spore or pollen grains, is called the endexine (**Figure *1.3-2***). The layer between ektexine and endexine is known as mesexine.[50-52] Erdtman reported that ektexine is often flattened at the poles more than the endexine (**Figure *1.3-2***). The ektexine can be distinctively differentiated from endexine by hydrolysis. The outer layer can be easily hydrolysed with 2- ethanolamine or 3-ethanolamine and experiences a deep colour change when stained with fuschin, while the inner layer is resistant to hydrolysis and staining.[50,52]

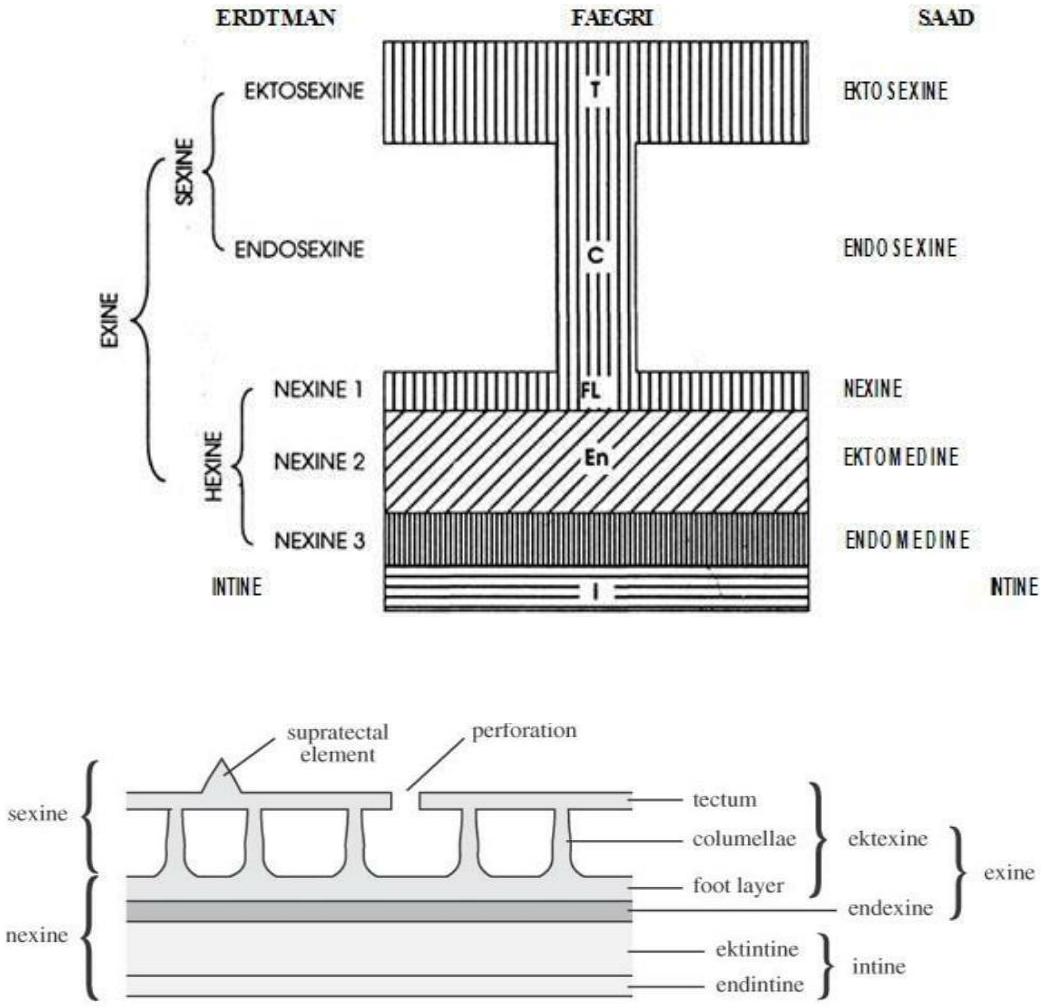


Figure 1.3-2: Layers of exine and intine[53,54]

1.3.3 Sporopollenin

Mature pollen grains or spores from most plant species are enclosed in a distinct and unique shells, of which the outer exine layer that consists mainly of a biopolymer called sporopollenin. The word "sporopollenin" was first used in 1931 by Zetzsche.[55] John had earlier named the outer wall of the pollen of tulip "pollenin" due to the unreactive nature of the compound.[56] About fifteen years later, Braconnot extracted compound with similar chemical characteristics from *Scirpoides holoschoenus* and referred to it as "sporonin".[57] Zetzsche first used the word "sporonin" in 1928,[58] but the word "sporonin" was join with the word "pollenin" when he discovered that the exine of the spore of *Lycopodium clavatum* has the same chemical properties with those discovered by John and Braconnot.[55,59] "Sporopollenin", therefore, is the term used to describe the external cell wall (resistant exine) extracted from spore and pollen grain, since they have the same or

a very similar chemical properties. Sporopollenin is a chemical term, independent of botanical taxonomy, used to describe only the constituent component of the spore exine.[57,59] It has been described as a polymer wall that protect pollen, spores, algae and some bacteria from external attack.[60] In a general description, sporopollenin is a complex polymer, with high stability to both non-oxidative chemical and physical activities, it is free of nitrogen, protein and intine.[55,59] Sporopollenin, after the removal of intine and other spore/pollen constituents, is often isolated as a capsule which retains the morphology of its original raw spores or pollens.[59] The SEM structure of *L. clavatum* sporopollenin is as shown in the diagram below (**Figure *1.3-3***).

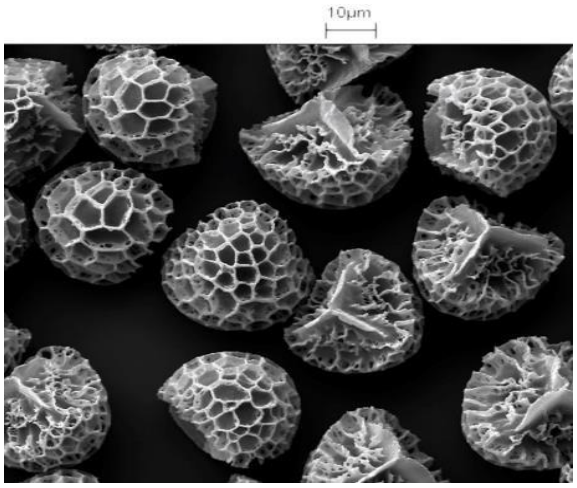


Figure 1.3-3*:* Scanning electron microscopy (SEM) picture of *L. clavatum* spores[61]

Maeda reported in 1984 that sporopollenin polymer has various structure and the location in different pollens or spores depending on each species.[42] Sporopollenins are enclosed around the spores which are mostly sited on the stalk sheath of fruiting bodies in angiosperms, gymnosperms, pteridophytes, fungi and algae.

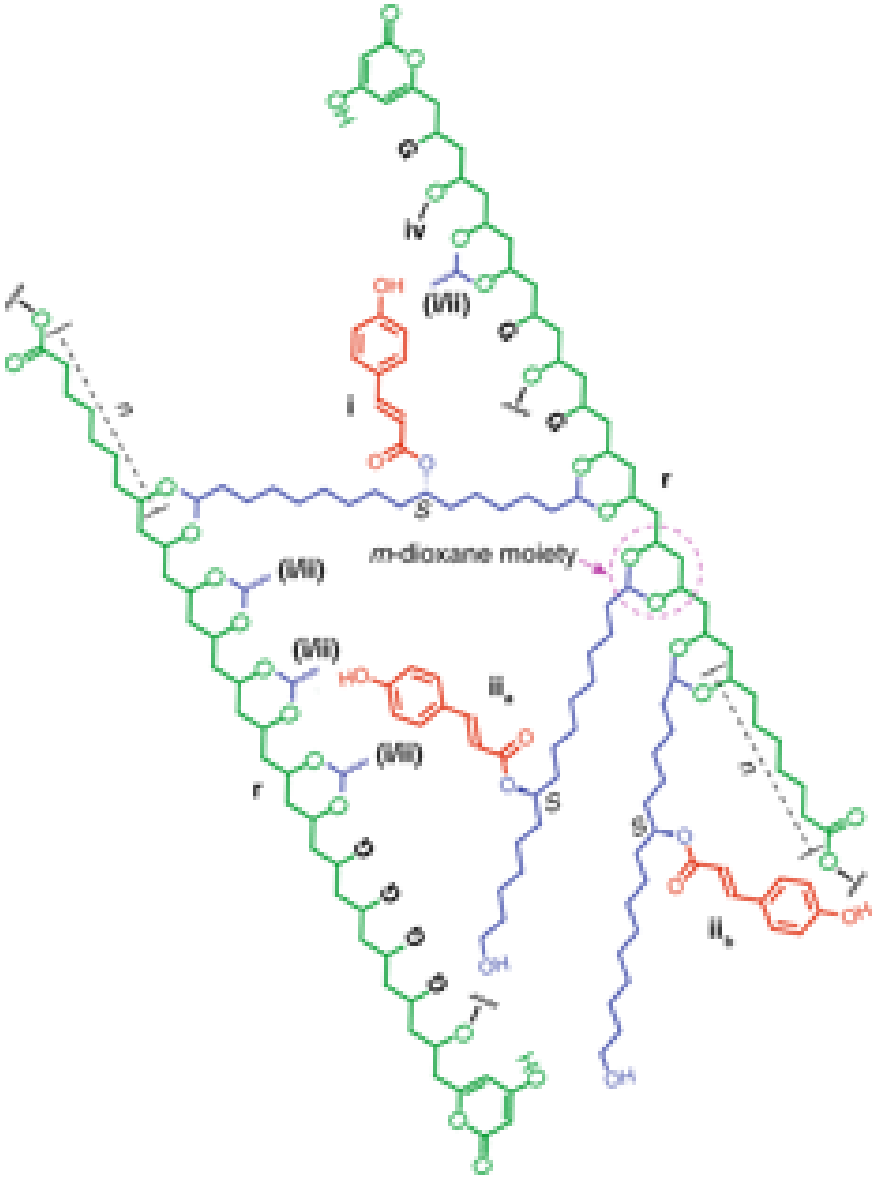
1.3.3.1 Evidence for the chemical structure of sporopollenin

Little is known about the detailed structure of sporopollenin. The structure has been very difficult to elucidate mainly because of sporopollenin's chemical resilience, but it is knownfrom combustion elemental analysis that it has carbon, hydrogen and oxygen as the components.[55,58,59,62-64] Bubert *et al* predicted from CHN analysis data that the structure of sporopollenin is corresponding to chemical formula in the range $CH_{1.49}O_{0.27}$ to $CH_{1.57}O_{0.28}$, they concluded from ^{13}CCPMAS-NMR spectra that sporopollenin comprises of CH and CH_2 carbons with a very low number of CH_3 carbons and quaternary carbons.[65] The empirical formulae for sporopollenin has since, the early investigations,

been expressed based on ninety carbon atoms,[55,58,59,62] though, it was later established that the number of carbon atoms in sporopollenin relative to H and O atoms varies depending on the method of extraction and the source of the plant.[59] Therefore, it was previously concluded that the exact structure of sporopollenins could not be predicted[66] but it was believed that sporopollenin contains polymeric unbranched aliphatics that has a variable amount of aromatics compounds with conjugated side chain as major compounds.[67-69]

Recently, the long-standing elusiveness about the molecular structure of sporopollenin was resolved. Li *et al*[70] used thioacidolysis for the degradation of the sporopollenin from pine and solid-state NMR for the identification of the constituting compounds. It was reported from their work that polyvinyl alcohol units derived from aliphatic polyketide cross-linked with 7-*O*-*p*-coumaroylated C_{16} aliphatic units through an acetal (dioxane) moiety is the major constituent of pine sporopollenin (**Figure *1.3-4***) while the minor component was identified to be naringenin.[70] Li *et al*'s discovery confirmed the reports of Osthoff *et al*[71] and Herminghaus *et al*[72] on the presence of phenolic compounds as the major components in sporopollenin. Osthoff *et al*[71] had reported in 1987, after sporopollenin was degraded by saponification, potash fusion and nitrobenzene oxidation**,** that sporopollenin from pine pollen has phenols or phenolic compounds that are non-covalently bonded to their surface and/or their inner part as their integral components. The report further stated that with the use of TLC, HPLC, UV and mass spectroscopies to analyse the degraded products of pine sporopollenin, *p*-coumaric acid, *p*-hydroxybenzoic acid, vanillic acid, vanillin, *p*-hydroxybenzaldehyde, and ferulic acid were identified as its main components.[71] A year later, Herminghaus *et al* reported the presence of stable carboxylic acids (such as *p*-coumaric acid and ferulic acid) and phenolic compounds like *p*-hydroxybenzaldehyde in sporopollenins (SpECs) obtained from *Corylus avellana* pollen.[72] However, the chemical composition and molecular weight of sporopollenin may vary from one plant species to another.[73] The variation results from the ratio of the aliphatic to aromatic compounds of a particular sporopollenin.[74] Method of isolation of sporopollenin from the pollen/ spore may also contributes to the variation in its chemical compositions.[75] For example**,** Herminghaus *et al* clearly stated that *p*-coumaric acid constituted the major components in the sporopollenins (SpECs) extracted with 6 N KOH at 100 °C while phenolic compound

such as *p*-hydroxybenzaldehyde dominated the sporopollenins (SpECs) obtained from enzymatic and some other extraction methods.[72]



G = Glycerol-like moieties, **r** = polyketide-derived PVA-like which contains 38 carbons[70]

Figure 1.3-4: Chemical structure of sporopollenin from pine pollen[70]

Generally, it can be concluded that the chemical structure of sporopollenin mainly contains phenolic compounds such as *p*-coumaric acid, *p*-hydroxybenzaldehyde, long-chain aliphatic compounds[76] and polyvinyl derivatives (**Figure *1.3-4***). It is noteworthy that the presence of phenylpropane in the structure of sporopollenin differentiate it from the lignin or lignin-like compounds.[71]

1.3.3.2 Evidence for functional groups present in sporopollenin

Sporopollenin is resistant to selected chemical degradation;[42] therefore, it is insoluble in most of the common organic and inorganic solvents.[66] As a result of its insolubilities, it was almost impossible to characterize sporopollenin with the use of chemical reagents but most reports shows that the structure of sporopollenins was mostly studied by the solid-state analyses such as nuclear magnetic resonance (NMR) and Fourier-transform infrared spectroscopy (FT-IR).[69,75-77] The FT-IR spectrum of the sporopollenin from *Pinus pinaster*, *Betula alba*, *Ambrosia elatior* and *Capsicum annuum* (**Figure *1.3-5***) reveals absorptions due to the O-H stretch of -OH groups (3450 cm^{-1}), the C-H (ν_a) stretch (2910 cm^{-1}) and C-H (ν_b) stretch (2840 cm^{-1}) of CH_2 groups, the O-H stretch (2693 cm^{-1}) of H bonded -CO_2H groups, the C=O stretch (1680 cm^{-1}) of -CO_2H groups, the C=C stretch (1650 cm^{-1}), (-CH_2-) scissoring (1474 cm^{-1}), (-C-O-C-) stretch (1130 cm^{-1}), and (-CH_2-) rocking (731 cm^{-1}).[78]

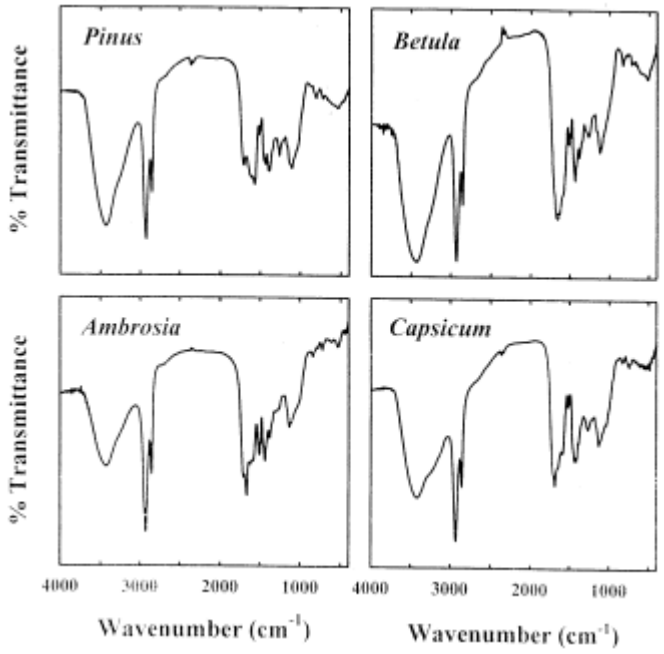


Figure 1.3-5: FT-IR spectral of sporopollenin from different pollens[78]

Also, Dyab *et al*[79] revealed that the FT-IR spectrum (**Figure *1.3-6***) of *L. clavatum* sporopollenin showed absorptions -OH groups (3416 cm^{-1}), C-H stretch (2925 cm^{-1}) of -CH_2- groups, C=O containing groups (1712 cm^{-1}), C-O stretch of ethers (1138 cm^{-1}) and medium aromatic (C-H) out of plane wagging (848 cm^{-1}). It was further stated that the

spectrum did not show any absorption in the characteristic region of ester (1750 - 1735 cm^{-1}) as shown in **Figure *1.3-6***.

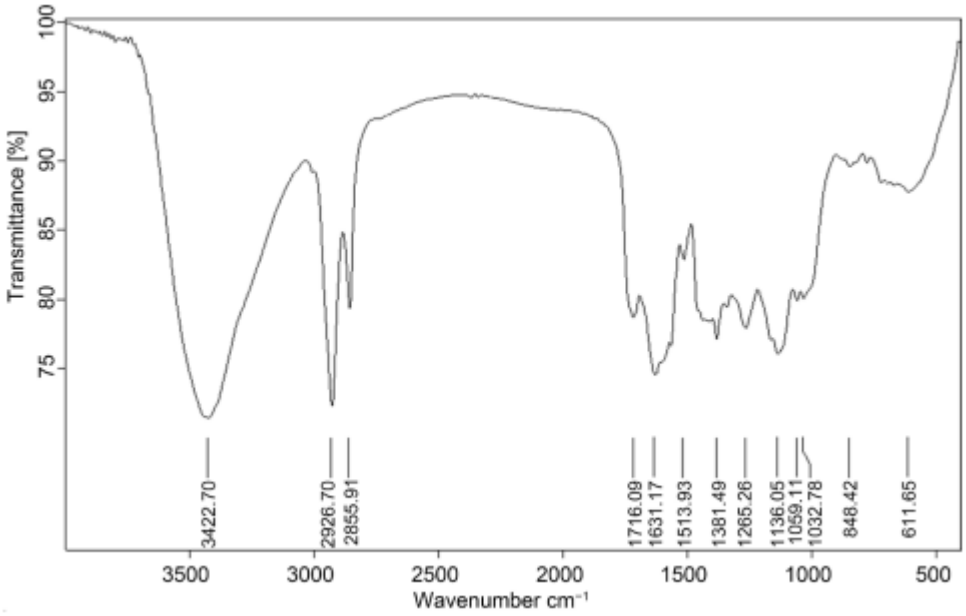


Figure 1.3-6: FT-IR spectrum showing the functional groups present in the sporopollenin of *L. clavatum*[79]

Guilford *et al* used high resolution ^{13}C-NMR to reveal that sporopollenins from six different plants, which are wheat (*Triticum aestivum*), pine (*Pinus thunbergiana*), corn (*Zea mays*), ragweed (*Ambrosia trifida*), *Lycopodium clavatum* and *Chlorella fusca*, have ether, unsaturation, and carboxylic acid functional groups.[77] Espelie *et al*[76] in 1989, through the use of ^{13}C-NMR, also reported that 47 % of the carbon on the carbon-13 spectrum of the sporopollenin from *Lilium longiflorum* are in the spectrum regions with distinct characteristics of CH_3 and CH_2, 10 % are in the regions of carboxyl carbon and 2 % in the characteristics region of ketone carbonyl. Shaw and Apperley reported that lycopodium sporopollenin has predominantly methylenic character of the biopolymer along with ether, unsaturations, and carboxylic acid functional groups.[80] In addition, it was reported that the ^{13}C-NMR spectra of *Lilium longiflorum*, cutin and *Botryococcus hraunii* sporopollenins are very similar.[76] In addition, the ^{13}C-NMR spectra of the sporopollenins obtained from the megaspores and microspores fossil cone of lycoposids plants are similar.[75] The above works, therefore, evidently revealed that sporopollenins (SpECs), irrespective of the source, have similar structures and functional groups.

The biosynthetic pathways to sporopollenin are still subject to debate.[65,73] The physicochemical characteristics of the cell wall of pollen and spores might likely be resulted from the resistance ability of sporopollenin.[42] The structure of sporopollenin was predicted to have been derived from carotenoids compounds[69,76,81] but this could not be established by radiochemical labelling.[73,77,82,83] Using radiochemical labelling techniques, precursors such as acetate, mevalonate, glucose and phenylalanine had been employed by various scientist to trace the biochemical synthetic pathway of the exine but the clarity in the pathways could not be ascertained.[43,73] However, the results obtained from tracer experiment revealed that sporopollenin contains phenolic compounds.[84,85] The tracer experiment is in line with investigation reported on the pyrolysis of lycopodium sporopollenin. Schenck stated that pyrolysis of lycopodium sporopollenin yields benzene and phenol derivatives.[86] Further works on the biosynthesis and chemical origins of sporopollenin spurred Lallemand and his team to do more investigation on the possible precursor of sporopollenins. Lallemand and his co-workers drew inference from the works of Dominguez, *et al*; Bubert, *et al* and Ahlers, *et al* and concluded that long polyhydroxylated aliphatic chains and small amounts of aromatic rings derived from the phenylpropanoid metabolism are the major constituents of sporopollenins.[65,66,87] However, Kettley[88], in her PhD research at university of Hull, conducted lots of experimental research to establish and identify the type of various functional groups that are present in SpECs. Through chemical analysis, she reported the presence of carboxylic, hydroxyl, alkene, carbonyl groups of ketone and aldehyde, and aromatic functional groups in SpECs.[88] Earlier before the work of Kettley, Ahler, in two different studies, confirmed that sporopollenins (SpECs) contain phenolic compounds, other nitrogen-free aromatic compounds and conjugated unsaturated chain.[89,90] The study of sporopollenin and their possible degradation played major role as part of the basis of the science of palynology.[57] Sporopollenins, therefore, are organic polymer of biological origin which are insoluble in organic solvent, resistant to non-oxidative chemicals and physical degradation.[42,57,91]

1.4 Sporopollenin Exine Capsules (SpECs) from *Lycopodium clavatum*

1.4.1 Extraction of SpECs

The isolation of sporopollenin exines (micro)capsules for the subsequent investigation of the chemical structure was found initially to be difficult because sporopollenin can be

resistant to both chemical and biochemical reagents.[43] But this has resulted in the development of various methods using reagents that are capable of penetrating into the inner layer of the spores or pollen to remove intine layer and sporoplasm, leaving only the intact sporopollenin exine capsules (SpECs) as a solid.[92] Recently, several new ways of isolating sporopollenin from its spores or the pollens have been developed.[88,93-96] For example, sporopollenin was extracted by hydrolyzing the spores or pollen with 9 M hydrochloric acid over a period of one hour,[94] or with the 6 % sodium hydroxide for a period of 24 hours.[88,93] Zetzsche described a method using acetone, alkaline and 80 % of ortho-phosphoric acid for the extraction of sporopollenin.[55,58,62] The phosphoric acid and potassium hydroxide extracted Sporopollenins obtained from *Lycopodium clavatum* were reported to be insoluble.[93] Barrier attributed the insolubility of the phosphoric extracted sporopollenin to cross-link formation by Michael addition or Claisen condensation or Knoevenagel reaction of active methylene during heating was claimed to have caused.[93]

Several approach had been developed and used by different researchers, over a hundred years ago, to totally get rid of the intines from the spore grains, so leaving no trace of non-sporopollenin materials in the compounds.[52,55,58,62,77,80,88,97] Zetzsche and co-workers[55,58,62]pioneered the work on the preparation of intine and cellulose-free sporopollenins that retained its original morphological shape and different reagents were used, ranging from alkaline conditions to different acids, in the different hydrolysis experiments to obtain sporopollenin.[93]

1.4.1.1 Acetic Anhydride Based Extraction (Acetolysis)

Isolation of sporopollenins from pollens was first carried out by Erdtman when he centrifuged the pollen in glacial acetic acid and later treated it under reflux, for one minute, with acetic anhydride and sulphuric acid in the ratio 9:1 v/v.[52,93,98] Barrier and other researchers claimed that the sporopollenin extracted by this method is not totally free of nitrogen, impurities from protein and cellulose materials.[93,96,99] Barrier also reported the likelihood of the introduction of sulphur contamination on the sporopollenins by sulphuric acid.[93] Acetic anhydride in the acetolysis method was reported to have acetylated the sporopollenins thereby introducing a new functional group to the compound.[88,100] Though acetolysis was reported to be better at preserving the pollen or spore capsule, compared to other extraction methods, it can cause swelling of the raw material.[99] More so, Domínguez *et al* reported that if the ratio of acetic anhydride to

sulphuric acid is not accurately measure, it could cause readjustment of the structure of the sporopollenins (SpECs).[99] In addition, sulphuric acid is very corrosive and cause hazard if not handled with precaution.[98]

1.4.1.2 Enzyme Based extraction

Herminghaus *et al* in 1988 reported the removal of intine and isolation of exine from pollens to obtain sporopollenins.[72] Also in 1999 Ahlers[89,90] *et al* degraded the intine of pollens through enzyme hydrolysis to isolate sporopollenin. Enzyme hydrolytic extracted sporopollenins was reported to have phenolic compounds as the major components both in the exines.[71,72,89,90,93] However, the sporopollenins obtained by this type of process may not be totally free from nitrogenous materials except if it is further treated with various organic solvents which usually are exhaustively executed for about 200 days.[72] Though, Barrier suggested that one of the enzymes used for the extraction may have introduced the nitrogenous material as a contaminant into the sporopollenins (SpECs) during the process of extraction.[93] It is worthy to note that the extraction involving enzyme hydrolysis method takes unnecessary lengthy extraction periods (about a year) before it reaches completion.[72,93] It is also required and involved the use of up to five different types of enzymes (such as pronase, lipase, cellulase, amylase, cellulysin); each for the degradation of different components of the spore.[71,72]

1.4.1.3 Base Hydrolysed Extraction (Alkaline lysis)

1.4.1.3.1 Hydrolysis with NaOH or KOH

Several researchers have experimented with the removal of the internal component of spores and pollens in an attempt to get a pure sporopollenin. It was reported by Southworth in 1974 that different researchers used either potassium hydroxide or sodium hydroxide to remove protein and fatty acids or to study the cytoplasmic contents in pollens and spores.[101] Pollens retains their shapes intact after the treatment with alkali but the assertiveness of this statement is not universal for all the pollens[48] as Mundargi *et al* reported that the microstructure of sporopollenins extracted from sunflower by alkaline treatment was completely damaged.[102] Potassium hydroxide has been used in the recent times to remove the cytoplasmic material before the complete removal of intines, cellulose and other non-sporopollenin material.[59,102-104]

1.4.1.3.2 Extraction of sporopollenins with cuprammonium hydroxide

The use of cuprammonium hydroxide for the removal of non-sporopollenins material from spores/pollen was reported to not have been totally successful despite the long period of hydrolytic process that the method involved.[88,93,96] Consequently, the method was abandoned by subsequent researchers and therefore there is limited information about this process.[93]

1.4.1.3.3 Extraction of sporopollenin by *N*-methylmorpholine

One of the methods reported for the extraction of sporopollenins involved the use of *N*-methylmorpholine as a solvent to dissolve the polysaccharides and release the sporoplast or protoplast.[105] *N*-Methylmorpholine causes the swelling of the inner components, which is called protoplast or sporoplast, and thereby limits the complete removal of intine from the sporopollenins and other cellulosic contents. [80,99,106,107] The release of the inner components of pollen or spores was reported to have been prevented by the constrained size of the exine capsule.[106] It was reported that the remaining nitrogenous material left in the *N*-methylmorpholine-extracted sporopollenins and makes it to look rough.[93] According to Kettley, the expected sporopollenins were recovered at the end of the treatment.[88]

1.4.1.4 Extraction of sporopollenin by acid hydrolysis (acidolysis)

In recent time, acid-based extraction is the most widely used method. Though, the use of acid for the extraction of sporopollenins dates back to the time of the discovery of sporopollenins[58,97] but the method has undergone modification as more researchers have worked on sporopollenins recently.[108] The use of only one acid e.g. 85% phosphoric acid in the extraction process was reported not to be effective enough to produce sporopollenins that are completely free of cellulose and other non-sporopollenins components.[80,96] To obtain a complete sporopollenins that is free of "contaminants", either the acid is used as a complement to another method e.g. complement to acetolysis method or two acids are used to complement one another.

1.4.1.4.1 Extraction of sporopollenin using anhydrous hydrofluoric acid

In an attempt to totally rid the acetolysis derived sporopollenins of cellulose and other non-sporopollenins material, Domínguez *et al*[99] introduced the use of anhydrous hydrogen fluoride to complement the acetolysis method.[88,96,99] The researcher proved,

with FT-IR result as the evidence, that the method efficiently removed the intine and cellulose from the pollens but concluded that anhydrous hydrogen fluoride effected a slight alteration in the structure of the sporopollenins especially its carbon skeleton.[99] However, FT-IR provided not enough evidence to prove that the method was totally effective because both the sporopollenin and cellulose have the hydroxyl group as one of their functional groups.[77,96] Anhydrous hydrogen fluoride is also highly corrosive and can be very hazardous to use in the laboratory.[96]

1.4.1.4.2 Extraction of sporopollenin with ionic liquids

Removal of inner components from pollen or spores by ionic liquids is a recent method and was reported for the first time by Chiappe *et al*.[109] It was reported in the work that morphological shape and particle size of the ionic liquid extracted sporopollenins were intact and similar to that of the untreated pollens. The idea of using ionic liquids to isolate sporopollenin was hinged on the ability of ionic liquids, with anions that has strong affinity for hydrogen bonds, to effect the removal of intine by dissolving the cellulose contents of the pollens. On the other hand, Brønsted acidic ionic liquids, such as 1-(4-sulfonic acid) butyl-3-methyl-imidazolium hydrogen sulphate $[MIMC_4SO_3H][HSO_4]$, can also affect or change the functional groups on the surface of the exine.

Chiappe *et al*[109] carried out the experiment by dispersing the pollen in ionic liquids and heated the mixture at 160 °C for 90 minutes. Seven different ionic liquids were investigated: tetra-n-butylphosphonium hydroxide, $[Bu_4P]OH$; 1-butyl-3-methylimidazolium chloride, [BMIM]Cl; 1,3-dimethylimidazolium methylphosphosphonate, $[DMIM][(MeO)(H)PO_2]$; 1,3-dimethylimidazolium methyl methylphosphonate, $[DMIM][(MeO)(Me)PO_2]$; and 1,3-dimethylimidazolium dimethylphosphate $[DMIM][(MeO)_2PO_2]$. These are all ionic liquid with anions that have strong affinity for hydrogen bonding. Other ionic liquids used in the experiment had reactive anions: 1-(4-sulfonic acid)butyl-3-methyl-imidazolium hydrogen sulfate $[MIMC_4SO_3H][HSO_4]$ and 1,8-diazabicycleundec-7-eneninium hydrogen sulfate, [DBUH] $[HSO_4]$. The ionic liquids with reactive anions have catalytic activities capable of modifying the functional groups of exines and are termed brønsted acidic ionic liquids. Chiappe *et al*[109] further reported that are tetra-n-butylphosphonium hydroxide, $[Bu_4P]OH$ and 1-butyl-3-methylimidazolium chloride, [BMIM]Cl dissolved the entire pollen- both intine and sporopollenin biopolymer. 1,3-dimethylimidazolium methylphosphosphonate, $[DMIM][(MeO)(H)PO_2]$; 1,3-dimethylimidazolium methyl methylphosphonate,

[DMIM][(MeO)(Me)PO_2]; and 1,3-dimethylimidazolium dimethylphosphate [DMIM][$(MeO)_2PO_2$], which are alkyl phosphates, removed lipid components but they could not completely remove cellulose and other intine materials from the pollen. The study further revealed that the sporopollenins obtained from the treatment of pollen with 1,8-diazabicycleundec-7-eneninium hydrogen sulphate, [DBUH] [HSO_4] was not too different from those recovered from alkyl phosphate treated pollen. This was attributed to the acidic character of 1,8-diazabicycleundec-7-eneninium hydrogen sulphate, [DBUH] [HSO_4] which was termed to be less acidic than 1-(4-sulfonic acid)butyl-3-methyl-imidazolium hydrogen sulphate [$MIMC_4SO_3H$][HSO_4].[109]

The report stated that the sporopollenin obtained, at 160 °C for 90 minutes, from the treatment of pollen with 1-(4-sulfonic acid)butyl-3-methyl-imidazolium hydrogen sulfate, [$MIMC_4SO_3H$][HSO_4] was completely free from protein, polysaccharides, cellulose and other intine materials. This was confirmed with the FTIR spectrum of the sporopollenin, however, an intense band -SO_3 asymmetric stretching at 1176 cm^{-1} and a small band attributed to lipid at 1708 cm^{-1} were observed. It was therefore deduced that 1-(4-sulfonic acid)butyl-3-methyl-imidazolium hydrogen sulfate, [$MIMC_4SO_3H$][HSO_4] had modified the hydroxyl functional group on the sporopolenin with sulfur-related functional groups. Chiappe *et al*[109] trusted the FTIR spectrum result of ionic liquid treated sporopollenin on the integrity of Zimmermann *et al*'s[110] report on FTIR result sporopollenin. Chiappe *et al*[109] also established more facts about the purity of the ionic liquid treated sporopollenin by with the use of elemental CHNS analysis, fluorescence microscopy and scanning electron microscopy (SEM).

The elemental analysis confirmed the % sulfur presence in the sporopollenin to be 2.83 %. The presence of sulfur-related functional groups on the sporopollenins was initially attributed to lack of thorough washing but -SO_3 band was still reported to be present after washing the sporopollenin with different type of solvents, therefore the presence of sulfur was attributed to sulfination of the sporopollenins rather than inefficient washing.[109,111] It is noteworthy that the proteinaceous materials were not totally removed when the extraction of sporopollenins with 1-(4-sulfonic acid)butyl-3-methyl-imidazolium hydrogen sulphate, [$MIMC_4SO_3H$][HSO_4] was carried out at 100 °C.[109] Chiappe *et al*[109] concluded that 1-(4-sulfonic acid)butyl-3-methyl-imidazolium hydrogen sulfate,

[MIMC4SO3H][HSO4] extraction method is recyclable and the sporopollenins obtained can directly be used as a solid acid catalyst.

1.4.1.4.3 Extraction of sporopollenin using hydrochloric acid

Three different research groups reported the use of hydrochloric acid for the hydrolysis of sporopollenins (SpECs).[93] It was reported that the method involved stirring the pollen or spores in 6 M hydrochloric acid for 24 hours at a regulated temperature of 110 °C.[93] However, with the increase in the concentration of the acid from 6 M to 9 M and the temperature reduced to 90 °C, the extraction period reduced to one hour.[95] The sporopollenins obtained through this process was claimed to be protein-free and intine-free but contaminated by the presence of chlorine. [93,96] However, the impurity-free claim for the sporopollenin isolated by HCl was contested by Mundargi *et al* when the sporopollenin obtained from sunflower was reported to have pollen debris such as cellulosic and protein materials.[102] Chlorination may have occur as a result of addition reaction of HCl across an unsaturated bond formed from the dehydration of hydroxyl group. Though, hydrolysis of pollens or spores with HCl is one of the shortest extraction method, the chlorination resulting during the extraction process and the presence of protein material as claimed by Mundargi *et al*[102] makes the HCl extracted sporopollenin to be considered impure.

1.4.1.4.4 Extraction of sporopollenins using sulfuric acid

In an attempt to get pure sporopollenin, it was reported that 72 % sulphuric was used, at room temperature, and was able to remove hemicellulose polysaccharide and other intine components from the spore/pollen by the earlier researchers in an attempt to get pure sporopollenins.[96,101] Sulfuric acid was also used to complement other extraction processes; such as in combination with acetic anhydride[52,88,98,101] or as a successive extraction process after acidolysis[80] or as one of the reagents used in the oxidative degradation of the intines.[101] However, the sporopollenins recovered from all the methods, involving the use of sulfuric acids, was reported to have sulfur contamination or distortion of the particles.[52,88,93,96,97] The presence in the sporopollenins renders it unsuitable for the application in the food and pharmaceutical companies.[93] Kettley suggested that the hydroxyl group in sporopollenins may have reacted with sulfuric acids to form a sulfate.[88] She further reported that subsequent treatment of such sporopollenin with sodium hydroxide (NaOH) or potassium hydroxide (KOH) drastically reduced the percentage of

sulfur in the sporopollenins. However, other observations, like degradation of SpECs into smaller particles during extraction with sulfuric acids, made her to conclude that methods of extraction involving the use of sulfuric acids is not suitable for the preparation of SpECs.[93]

1.4.1.4.5 Extraction of sporopollenin using phosphoric acid (H_3PO_4)

It has been reported that cellulose decomposes when spores are treated with phosphoric acid at a temperature of 80 °C or above.[80,93] However, cellulose was not completely removed from the spores at 30 °C when spores were treated with 85 % phosphoric acid for six days.[77,80] Shaw *et al* reported further that cellulose was not also remove completely at an increased temperature ranging from 80 to 90 °C when the spores were suspended for two weeks in 80 % phosphoric acid.[80] Complete removal of cellulose from spores could be achieved by subjecting sporopollenin extracted with phosphoric acid to further acidolysis using H_2SO_4.[80,88] Barrier reported[93] that the extraction carried out using orthophosphoric acid (H_3PO_4) might possibly have resulted in the sporopollenin containing more unsaturated double bonds because of its ability to dehydrate the hydroxyl groups forming alkenes.[112] In view of the Barrier report, sporopollenin obtained from orthophosphoric acid (H_3PO_4) showed more unsaturated carbon-carbon double bonds than those obtained from enzyme based extracted sporopollenin,[93] possibly because of the ability of H_3PO_4 to dehydrate hydroxyl-containing organic compounds. It was also revealed that sporopollenin extracted by phosphoric acid effectively remove cellulose better than any other methods of extractions and the surface of the capsules was unchanged.[93,102,113,114]

1.4.1.4.6 Extraction of sporopollenin using oxidizing agents

Attempts were made by the earlier researchers to use oxidizing reagents to degrade the intine. Different oxidizing solutions were used for the treatment of pollens[101] to remove lipids and fatty acid related components.[115]

1.4.1.4.7 Combination of acetolysis, alkaline lysis and acidolysis

In recent times, morphologically intact SpECs are extracted by the use of the combination of reagents and solvents. Sporopollenins devoid of contamination was achieved by defatting the spores with acetone, and subjecting the defatted spores to alkali treatment. The alkali-treated spores were then treated with 80 % orthophosphoric acid at 80 °C.[59,77,102,103] The extracted SpECs was also treated with NaOH and HCl to adjust the

pH/ionisation of its functional groups. It was observed by Shaw *et al* that the use of 85 % orthophosphoric acid at 30 °C in the combination treatment described above did not yield a cellulose-free sporopollenin, therefore, the temperature was increased to between 80 °C and 90 °C.[80] Other combinations of reagents were also investigated by different researchers, but the extraction method involving orthophosphoric acid was adjudged the best because the SpECs obtained after the extraction processes was reported to have maintained the same microstructure as the raw materials – i.e. untreated pollens or spores.[93,102,103]

1.4.2 Degradation of sporopollenins

Sporopollenins, depending on the extraction methods, can be dissolved in strong oxidizing solution.[101] The preparation of sporopollenin for elemental analysis was carried out by its dissolution in the mixture of sulfuric acid and hydrogen peroxide.[101] The sporopollenins or wall of some pollens and spores were dissolved / treated with 40 % chromic acid, mixtures of sulfuric acid and dichromate (*Ambrosia trifida*),[101] hydrogen peroxide (*Ambrosia trifida*),[101] ozone (*Pinus silvestris* and *L. clavatum*)[48,116] or sodium hypochlorite; acidified with HCl (*Artemisia pycnocepha* and *Lilium humboldtii*).[101,117] Other reagents used in dissolution and degradation of sporopollenins include 2-aminoethanol (*Typha angustifolia* and many other sporopollenins from spores and pollens)[101,118] and potassium hydroxide (*Ambrosia trifida, Pinus silvestris* and *L. clavatum*)[48,101] The fusion of the membrane of *L. clavatum* with potassium hydroxide led to the degradation and production of alkanoic acids of five to eleven carbons as revealed by gas chromatography. On extraction of the organic acids fraction with ether, a mixture of the following phenolic acids were obtained: *p*-hydroxybenzoic acid and *m*-hydroxybenzoic acid, *m,p*-dihydroxybenzoic acid (protocatechuic acid), traces of *p*-hydroxy-*m,m*-dimethoxybenzoic acid (syringic acid) and *p*-hydroxy-*m*-methoxybenzoic acid (vanillic acid).[48,88] It is worth noting that Barrier observed that phosphoric acid hydrolysed sporopollenins have high number of cross-link carbon-carbon bonds and therefore, He explained that it is an insoluble organic polymer and it's difficult to degrade.[93]

1.4.3 Derivatisation of Sporopollenin

The surface of sporopollenin exine capsules (SpECs) have been derivatised for use in various kinds of way. Derivatisation is possible because of the presence of hydroxyl groups and carboxyl groups on its surface, as well as the presence of unsaturated long

aliphatic chain. The derivatisation of sporopollenin can occur through substitution reactions, which involve the replacement of the hydroxyl group by another functional group e.g. halogen, electrophilic substitution reaction of its aromatic rings, or amide or ester forming reactions of the carboxylic acid functions.

Barrier[93] reported that amidation of sporopollenin could be achieved by reacting either ammonia, or primary aliphatic amine or aniline with sporopollenin. The carboxylic group on the surface of sporopollenin reacted with the amino group to form the amidated sporopollenin. He also halogenated sporopollenins *via* their unsaturated double bonds and by the substitution of hydroxyl groups. Chloromethylation has also been carried out on the aromatic rings of sporopollenin.[88,93] Barrier also then converted the halogenated sporopollenin to azide and thiol derivatives by substitution reactions.[93] The thiol derivative was later converted to disulfide derivatives of sporopollenin. Acetylation, amination, methylation and silylation of sporopollenins were also reported in the work of previous researchers.[87,88]

1.5 Uses of sporopollenin exine capsules

SpECs are empty hollow shells[114] with hydroxyl, carboxyl benzyl, aryl, and olefin functional groups on the inner and outer surface.[48,69,71,72] Sporopollenin exine capsules (SpECs) and its derivatives have been found useful in various kinds of way. The use of SpECs and their derivatives range from catalytic activities in organic synthesis, encapsulation of substances,[119-121] protection against oxidation and photo-oxidation,[93,120] and drug delivery.[122] Sporopollenin has also been found useful in the analysis of coals; which often contain a large proportion of fossil spores and pollen which are sporopollenin.[49,123,124]

1.5.1 Using encapsulation

Sporopollenin is a polymer that is found in hollow shell-like, forms in plant pollens and spores, with function some functional groups.[114] SpECs have been filled with different types of organic or inorganic substances. Organic or inorganic compounds can be protected from chemical, physical and external attack by encapsulating them in SpECs.[120,121] SpECs have the ability to encapsulate either hydrophilic or hydrophobic substances or materials that contain both features.[119] In 2007, Diego-Taboada *et al* reported that SpECs have the ability encapsulate two or more drugs at the same time.[119]

Encapsulation of two different molecules was also demonstrated by Hamad *et al* when SpECs was used to simultaneously encapsulate living cells and magnetic nanoparticles.[120] The sporopollenin itself, has the ability to shield the encapsulated substances or compounds from external attacks. For example, fish oil was protected from oxidation or photo-oxidation when it was encapsulated into SpECs.[93,120] The antioxidant activity of sporopollenin was reported to be due to the presence of the conjugated phenol constituents in its structure.[61] Both the SpECs with encapsulated substances and/or their derivatives have found potential applications in drug delivery,[61,108,113,114,120,125-130] uses in medicine,[22] vaccines,[113] adsorption of proteins,[114,127,131] encapsulation of living cells,[120] fish and plant oils,[108,120,127-129] food supplements,[120,128] vitamins,[120] in taste-masking,[125,127] as an antioxidant protection,[121] and as an emulsifier of oil and water mixtures.[63] The encapsulation of substances into sporopollenin may involve pressing the sporopollenins into pellets[120] for solid drugs or mixing with the liquid active components to form a solution.[61]

1.5.1.1 Medicine, vaccine and drug delivery

After the removal of allergen containing cytoplasm or sporoplasm, sporopollenins is most likely to be free from causing harms to human body and health.[132] Allergen-free SpECs has been used by different researchers to encapsulate vaccines and drugs which are proposed of having capacity of being administered orally, inhaled, rubbed on the skin or delivered into the human body through injection no irritation, side or harmful side effect.[93,132,133] It is possible to chemically attach a drug to the functional groups on the surface of sporopollenin but such a method would mean the product would have to be registered with the regulatory bodies as a new medicine.[119] Diego-Taboada *et al* and Shakya *et al* reported an increase in the efficiency of delivery of actives drugs and nutraceuticals encapsulated with SpECs due or their mucoadhesion to intestinal tissues.[61,134] Previous studies show that the drug incorporated into the SpECs remained intact after its exposure to intestinal gastric acids/fluids,[113,119,131,135] but they were also reported to be susceptible to degradation by enzyme in human blood plasma during the circulatory system and thereby releasing the incorporated drugs into the bloodstream.[93,119,126,132]

The small particle size, porous shell and the antioxidant property, are some of the characteristics that help in proposing SpECs for the formulation of drug and vaccine.[113,119]

In 2007, drug formulations for asthma and other lung-related diseases were reported to be inefficient in clearing the lung from infections. The report further stated that 20 % or less of the formulation reached the lung alveoli through inhalation.[119] The diameter of the grain particle size of *Aspergillus niger* spore is 4 µm while the diameters of most others pollens/spores are of bigger such range as 200 µm.[61,136,137] The important characteristics for the carrier of asthma drug formulation are the falling speed of the carrier to reach the lung alveoli and its particle size; which should be between 3 µm to 5 µm.[119] The SpECs from *Aspergillus niger* spores were, therefore, proposed to have the potential to deliver a drug into the lung for curative purposes. *Aspergillus niger* SpECs were proposed as the spore itself is able to travel to the same remote part of the lungs as the causative agent of most lung infections.[119]

Other research on drug deliveries and vaccinations include encapsulation of ibuprofen with *Lycopodium clavatum* SpECs,[125] encapsulation of antibiotics (erythromycin and bacitracin) into SpECs from *L. clavatum* by Dyab *et al*,[135] and Uddin *et al* demonstrated that SpECs from *L. clavatum* and ragweed pollen have the potential to deliver oral vaccines. Also, studies revealed the ability of SpECs from *L. clavatum* to deliver mucosal vaccines.[113,134] 5-Fluorouracil was loaded into *L. clavatum* in three ways: passive loading, (passive loading is prepared by thermo-shaking the vortexed mixture of the suspended of *L. clavatum* spore and 5-fluorouracil solution), compression loading (compression loading is prepared by centrifugation of the compressed pellet/tablet-like *L. clavatum* spore soaked in 5-Fluorouracil solution) and vacuum loading (vacuum loading is prepared by freeze-dry the vortexed mixture of the suspended of *L. clavatum* spore and 5-fluorouracil solution).[138] Lorch *et al* incorporated gadolinium (III) into sporopollenins of *L. clavatum* for radiopharmaceutical treatment and revealed that magnetic resonance imaging was enhanced in the detection of MR contrast agent in the encapsulated gadolinium (III) when released into the human blood.[126]

1.5.1.2 Encapsulated oil, antioxidants and food supplements

Thomasson[94] and Barrier,[93] at different times, used sporopollenin for the encapsulation of sunflower and cod liver oils. Their works revealed that sporopollenin protected the oils against photo-oxidation from UV lamp, aerial oxidation and therefore, from the resulting rancidity.[61,93,108] Also the bioavailability of eicosapentaenoic acid (EPA) in fish oil was found to be enhanced when it was encapsulated with SpECs.[128] All the various types of

methods employed in the encapsulation of oils were reported to be efficient in protecting oils from oxidation.[127] The *L. clavatum* SpECs-encapsulated oils (SpEC and oils in the weight of 1:2) were dry fine powders with the SpECs mask flavour of the fish oil.[108]

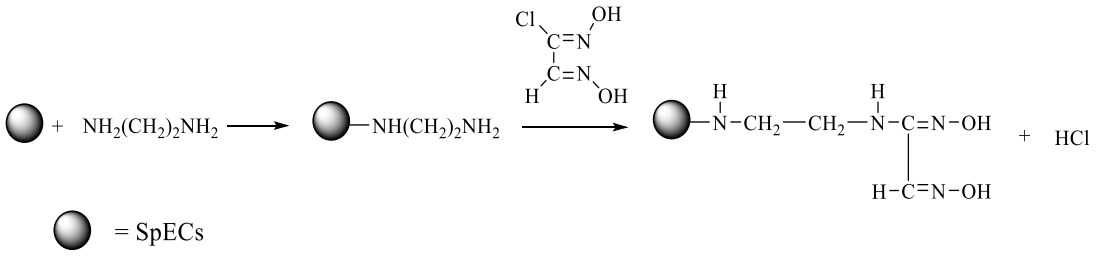
1.5.1.3 Living cell, enzymes and proteins

It is claimed by Hamad *et al* that living cells have been encapsulated into SpECs.[120] It was revealed that the cells encapsulated in the SpECs were strongly viable and biologically active when placed into the culture medium.[120] Also, the report stated that SpECs controlled the *in situ* bio-reaction and fermentation during the delivery process. Research revealed that none of the enzymes, proteins and other sensitive molecules experienced denaturation or spoilage during the reaction and delivery process,[127,133] therefore, living cells encapsulated in SpECs can be used for live vaccines delivery in pharmaceutical industries and protection of food probiotics in the food industries.[120]

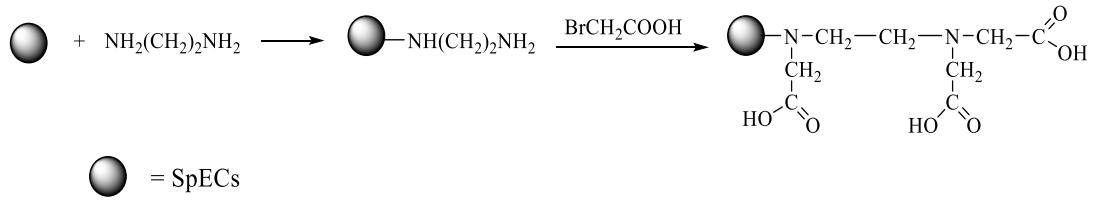
In addition, Wang *et al* developed surface modified SpECs with a micromotor that is capable of transferring bovine serum albumin into the internal micro-cavity.[122] Bovine serum albumin was reported to have been encapsulated into the SpECs through vacuum loading technique and delivered internal micro-cavity with a speed of about 26.40 μms−1.[122]

1.5.1.4 Purification

SpECs were reported to have the ability to remove heavy metals and humic acid from aqueous solutions,[79,122] and this can result in purification of water and environmental protection.[139] Bis-diaminoethyl glyoximated sporopollenin and carboxylated diaminoethyl sporopollenins made from diaminosporopollenin were prepared by Ersoz *et al* and Pehlivan *et al* to adsorb metal ions from aqueous solution (**Scheme 1-1** and **Scheme 1-2**).[130,139,140]

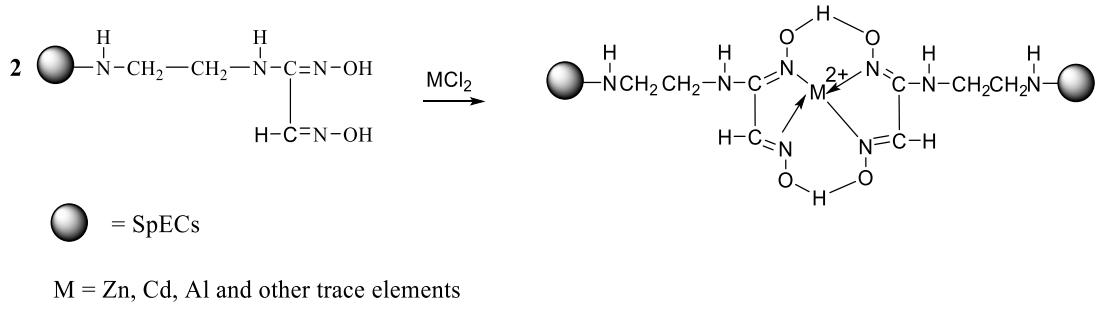


Scheme 1-1: **Preparation of bis-diaminoethyl glyoximated sporopollenin from diaminoethyl sporopollenin**[130]

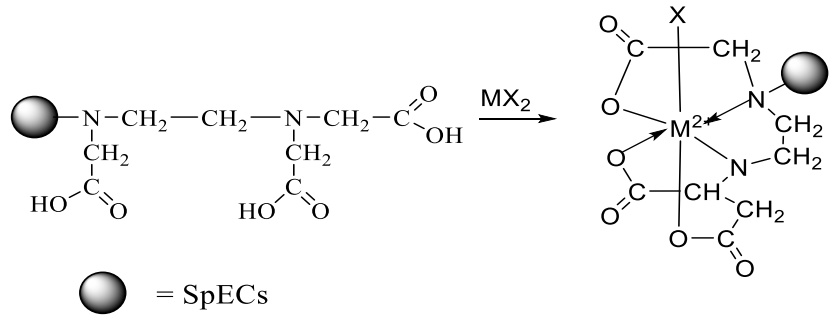


Scheme 1-2: **Preparation of carboxylated diaminoethyl sporopollenin from diaminoethyl sporopollenin**[130]

The strong chelating properties of the sporopollenin derivative were reported to be responsible for the selective removal of the heavy metals (like Zn^{2+}, Cd^{2+}, Pb^{2+} and Al^{3+}) and other harmful trace elements from the aqueous solution (**Scheme 1-3 and Scheme 1-4**).[140]



Scheme 1-3: **Chelation of metals by bis-diaminoethyl glyoximated sporopollenin**[130]



Scheme 1-4: **Chelation ability of carboxylated diaminoethyl sporopollenin**[130]

Also, the surface of humic acid was modified with nanoparticles of zinc and successfully incorporated into sporopollenins to enhance the magnetic properties of iron.[79] Base hydrolysed sporopollenin was reported to be more efficient than acid hydrolysed sporopollenin.[93]

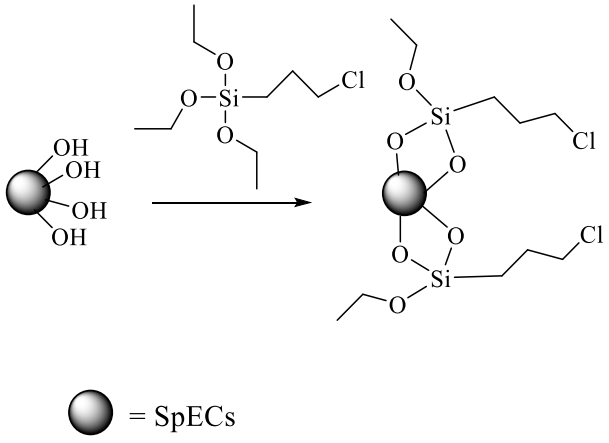
The ability of sporopollenin exine capsules to remove heavy metals from the waste water is due to the presence of some functional groups, such as carboxyl groups,[65] on its surface which is liable to form coordination with heavy metals and thereby remove them

from effluents or waste water.[67,122,130,141-143] The presence of hydroxyl[65,87] and the carboxyl groups on the surface of sporopollenin exine capsules (SpECs) is a possible factor that contributes to its ability to form carboxylate salts with metal ions.[122] This leads to the prediction of the ability of sporopollenin to also act as a ligand with electropositive metals.[139,140]

1.5.2 Using derivatisation

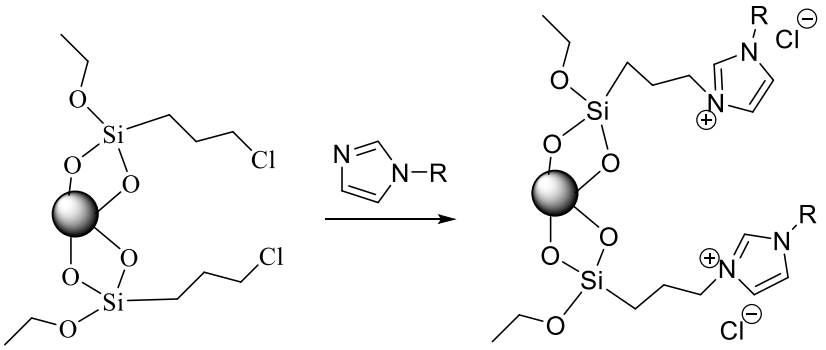
It was reported that SpECs, their acetylated, metallic and other derivatives were used to sequestrate oil from water and surfactant-stabilised emulsions.[129] Yaacob and co-workers reported that magnetic hybrid β-cyclodextrin functionalized sporopollenin was used as an adsorbent for the extraction of nonsteroidal anti-inflammatory drugs from water.[144]

Recently, Palazzo *et al*[111] prepared different types of sporopollenin-supported ionic liquid-type compounds for use in the synthesis of organic compounds. They first reacted 1-(4-sulfonic acid)butyl-3-methyl-imidazolium hydrogen sulphate ($[MIMC_4SO_3H][HSO_4]$) extracted SpECs with (3-chloropropyl) triethoxysilane to obtain (3-chloropropyl) triethoxysilyl sporopollenins (**Scheme 1-5**).



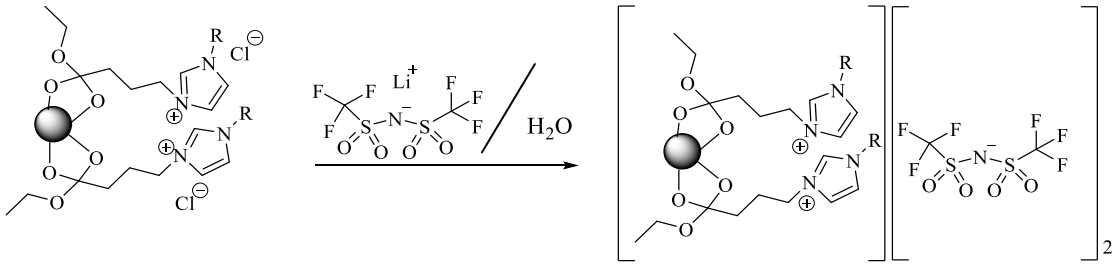
Scheme 1-5: **Reaction of SpECs with (3-chloropropyl) triethoxysilane**[111]

Then, (3-chloropropyl) triethoxysilyl sporopollenins were transformed by substituting the chloro group with different 1-alkylimidazole nucleophiles with a chiral centre to form sporopollenin-supported ionic liquid-like compounds[111] (**Scheme 1-6**).



Scheme 1-6: **Transformation of (3-chloropropyl) triethoxysilyl sporopollenin to sporopollenin-supported ionic liquid**[111]

Hydrophobic ionic liquids were prepared by modification of the properties of sporopollenin alkylimidazoles. The property modifications were reported to involve the treatment of sporopollenin alkylimidazoles chloride with bis(trifuoromethylsulfonyl)imide anion. The chloride anion of the supported ionic liquid was replaced by bis(trifuoromethylsulfonyl)imide anion to reduce or totally remove the hydrophilicity of the surface of sporopollenin[111] (**Scheme 1-7**)



Scheme 1-7: **Displacement of chloride ion from sporopollenin alkylimidazoles chloride by bis(trifuoromethylsulfonyl)imide anion**[111]

Another useful SpEC derivative is glyoximated diaminosporopollenin. Glyoximated diaminosporopollenin derivatives were used as ion exchangers in the separation of nucleosides and nucleic acid bases from aqueous solution.[130,141,145-147] The separation was carried out by running the experiment using a column chromatography set up.[145-147] It was reported that the glyoximated diaminosporopollenin ion exchanger adsorbed nucleic acid bases better than the nucleosides.[146]

Yusuf *et al* incorporated sporopollenin microparticles into the hexyl methacrylate-based monolithic columns as a stationary phase for capillary liquid chromatography.[148] The efficiency activity of hexyl methacrylate was enhanced and the retention time of capillary chromatography was reduced with better resolution of each corresponding peaks when sporopollenin was incorporated into the methacrylate mixture. It was reported that sporopollenin efficiently improved the separation of mixture of substances during a column chromatography.[148] This showed that sporopollenin is an efficient stationary phase for column chromatography.[148]

Archibald, Young and co-worker's work on the interaction of sporopollenin with iron[149] predicts the possibility of derivatising the sporopollenin with metal which can be used as Lewis catalyst in organic synthesis. However, the use of sporopollenin exine capsules, such as sulfonated derivative, as an acid catalyst has not been found prominent in the chemistry of organic synthesis.

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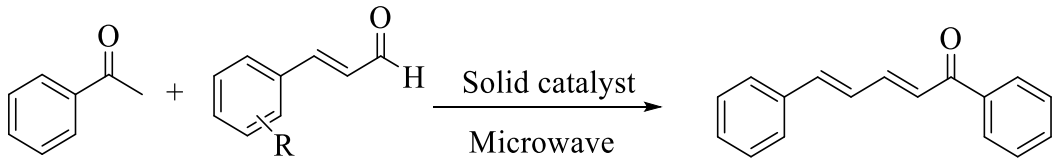
Chapter 2: Solid-phase (heterogeneous) acid catalysts

2.1 Heterogeneous acid catalysis

Catalysis has become very important in organic synthesis in recent times.[1,2] Catalysts play major roles in reducing the costs of production and establishment of economic strength in the chemical industries.[3] There are two general types of catalysts used in organic synthesis: homogeneous and heterogeneous catalysts. A catalyst is termed to be homogeneous if such a catalyst is in the same phase with the reactants during the reaction. Heterogeneous catalysts are in different physical phase from the reactants during the reaction and they are usually present in solid form.[4]

Some homogeneous catalysts are hazardous to health, therefore, handling is one of the limitations of homogeneous this type of catalyst.[1,5] Also, the necessity of isolating the products from the reaction mixture,[6] quenching reaction processes through neutralisation and other purification techniques often results in generation of large amount of different potentially environmental unfriendly wastes.[1,2,6,7] In addition, disposal of the generated waste may make a process less economically viable.[1,2,8] Therefore there has been emphasis on the use of solid-phase or heterogeneous catalysts in industrial organic chemistry.[5,8]

Heterogeneous catalysts are gaining wide acceptability in organic synthesis because they are recoverable with simple separation techniques from the products[3,9-16] thereby reducing the post-treatment of the product.[17,18] The use of heterogeneous catalysts in organic synthesis and chemical industries has overcome most of the challenges that homogeneous catalysts pose to product and production processes.[11,15,16,19,20] The selectivity abilities, i.e. regioselectivity, stereoselectivity and chemoselectivity,[21] (**Scheme 2-1**) of solid catalysts is another advantage that has attracted the attention of scientists.[22,23]



Solid catalyst = Amberlyst-15

***Scheme 2-1*: Selectivity of heterogeneous catalyst in aldol condensation reaction**[24]

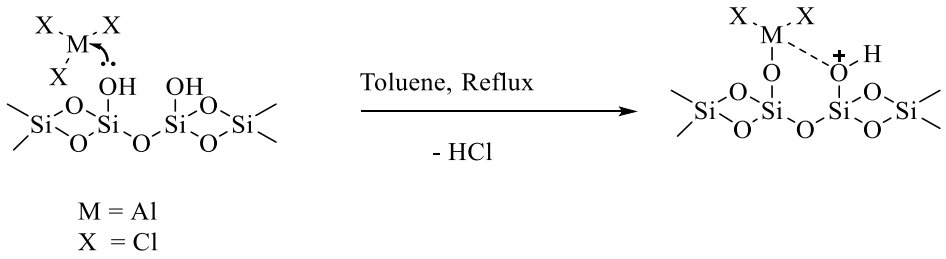
2.2 Solid acid catalysts in organic synthesis

The role played by catalysts in organic synthesis is very important especially in the stabilization of economic strength of chemical industries.[3,25] Solid acid catalysts are also important in controlling the environmental pollution and reducing the rate of corrosion of the reaction equipment or reactors.[2,5,7,25,26] They are recyclable and the mechanism of their recovery, at the completion of reaction, is friendly and easy.[3,9,27] The recovered solid acid catalyst often retains its acid strength or any other catalytic activities after use.[1,26]

The performance and the activities of solid acid catalysts depend on the type of the solid support used in the preparation of the catalysts.[1] Differences may be in acid strength, thermal, or mechanical sensitivities and have different production costs.[26]

The description of a solid acid catalyst is based on various features of the support material, especially the porosity of the materials, the surface area, nature of acidity (e.g. Brønsted or Lewis), acid strength of the catalysts, the distribution and the number acid sites.[1,2,28] The descriptors mentioned above are often the key properties responsible for the selectivity of the product formation by the catalyst.[1-3] Some reactions require a Brønsted acid catalyst, whilst others require a Lewis acid catalyst, some require a catalyst with strong acid sites, whilst others require a catalyst with medium or weak acid sites.[1,2,28] For example, catalysts with medium acidic strength or sites are required in the ketalization / acetalization reactions or their hydrolysis, while catalysts with strong acidic strength or sites are needed in skeletal rearrangements, electrophilic additions and esterifications.[1,2] Also, the Friedel-Crafts alkylation of toluene with benzyl chloride requires a catalyst with a Lewis acid site, while the alkylation using benzyl alcohol requires a catalyst with Brønsted acid sites.[1,2,28]

Brønsted and Lewis solid-acid catalysts have dominated the field of organic synthesis in recent times, and continue to attract the interest of researchers. For example, Lewis acid solid-catalysts is increasingly employed to ease the increasing commercial processes that involve hydrogenation reactions.[29] However Lewis solid acid catalysts can sometimes have Brønsted acid site through complexation and polarization of hydroxyl moieties with metal ions in a Lewis catalyst (**Scheme 2-2**).[1,2]



***Scheme 2-2*: Conversion of a Lewis solid acid catalyst to Brønsted solid acid catalyst**[1-3]

This type of polarization interaction often occurs between the hydroxyl groups adjacent to the aluminium atom on silica supported aluminium chloride (**Scheme 2-2**).[2,3]

The solid support material used for solid acid catalysts are usually either derived from inorganic materials, synthetic polymeric materials or natural organic polymers.[1] The material that may be used must be chemically and thermally stable during the reaction and separation process stages. In addition, they must possess good accessibility and dispersal of the active acid sites.[1,25]

2.2.1 Inorganic-based solid catalyst

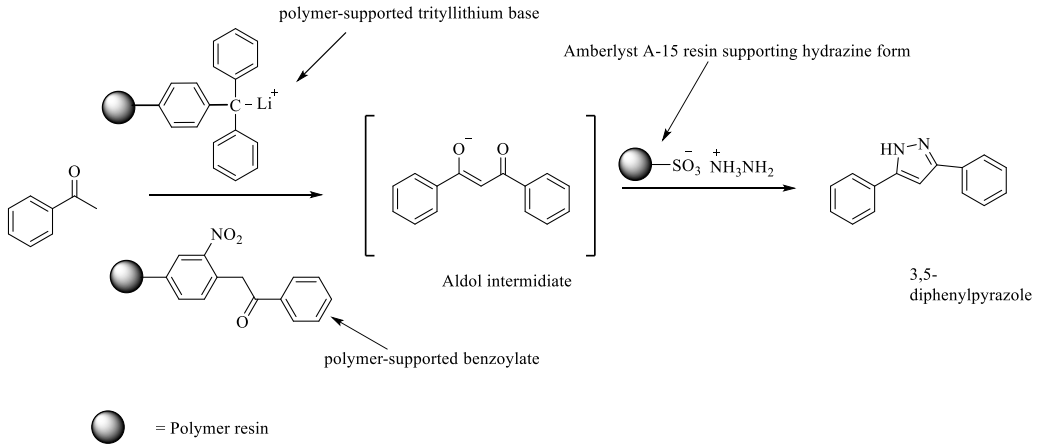
Inorganic support materials that have been used in heterogeneous catalysis are silicon oxide, also known as silica, clay,[6] inorganic carbon or graphite,[30,31] zeolites (composes of silica−aluminium),[6,32] hydroxyapatite, as well as oxide of titanium,[33] zirconium oxide solid support, lanthanium, tungsten, iron, molybdenum, vanadium and even the rarer metals, tantalium pentoxide solid support, etc.[1,34,35]

Examples of solid acid catalysts based on inorganic substances include Filtrol-24 clay,[6] K10 clay, tungstophophoric, silica, sulfated zirconia (SO_4^{2-}/ZrO_2), sulfated tin oxide (SO_4^{2-}/SnO_2) and sulfated titanium oxide (SO_4^{2-}/TiO_2), sulphated lanthanum oxide (SO_4^{2-}/La_2O_3), sulphated mesoporous tantalum pentoxide,[36] etc.

The inorganic-based solid catalysts are not found useful in the industry because of cost of their production.[30]

2.2.2 Organic polymer-based solid acid catalysts

Solid acid can also be supported on insoluble polymeric materials.[37] The use of polymer-based materials in the preparation of solid acid catalysts dates back to 1940s[38-40] and their usage has been progressively growing in the chemical synthesis industries e.g. pharmaceutical industry.[38] Polymer-based solid support materials are either made from synthetic or natural organic polymeric materials. Synthetic polymer solid-support catalysts are widely used in organic synthesis[3,41] as the polymers have the ability to be tailored to covalently or ionically bound with the supported reagents and thereby effect the selective changes or transformation of specific functional group and new bonds' formation.[38] They have served as a solid support for both Brønsted and Lewis solid acids. Reactions involving a polymer-based heterogeneous catalyst often lead to a targeted product of better purity compared to those involving homogeneous catalysts.[38-40] The use of polymer-supported scavenger (catch-and-release techniques) and/or polymer-supported reagent(s)[37-40] in addition with the polymer-based heterogeneous catalysts in a multistep organic synthesis, aid the by-product, which are most times difficult to separate from the product,[37,38,40] to selectively attached or trapped to the solid surface and as a result, lead to ease separation of the product(s) from the reaction mixtures, mostly by filtration.[2,3,37-40] For example, in the synthesis of 3,5-diphenylpyrazole from acetophenone in which the reaction was initiated by the abstraction of hydrogen atom from the acyl group of acetophenone with the polymer-supported trityllithium and *C*-benzoylation of the abstracted acetophenone using polymer-supported benzoylate. The reaction proceeded, without isolation, to form 3,5- diphenylpyrazole when catalysed by Amberlyst A-15 resin-supported hydrazine form (**Scheme 2-3**)[40]



Scheme 2-3: **Isolation of the target product using polymer support reagent, scavenger and catalyst**[40]

The by-products and other impurities can be removed from the surface of the catalyst by washing them off with the appropriate solvents.[42] For instance, polystyrene-supported aluminium chloride was filtered and only washed with chloroform ($CHCl_3$) after it was used to catalyse the reaction that led to the formation of pyrrole and xanthenes.[42,43]

The polymeric material can change or alter the specificity, selectivity and activity of an acid site that they support.[39] For example, the nature of the polymeric materials used for solid support can add to the enhancement of the yield of a product because the polymeric materials with large pore and good swelling ability will allow the fast influx of many / large amount of the reactants into the catalyst to have a contact with the acid sites and thereby influence the yield of the products.[26]

Also, the polymeric solid support can form intermolecular bond with the starting materials and thereby lead to the reduction of the product's yield.[37,39]

Polymer-based solid supports must also possess the ability to swell in the reaction solvent, and create a large interior area for the pore of the polymer to expose the active acid site of the catalyst, in order to afford the reactants, through the diffusion of soluble reagents in the reaction solvent or solution, the accessibility to the acid sites for easy reactivity.[26,44] The poor swelling ability of a polymer used as a solid support[45] will result in the failure of reagents or the reactants to penetrate the network of the polymer to the acid site or reaction site and therefore will limit the activity of the catalyst.[46] The swelling depends on the ratio of the network crosslinking of a polymer used in the solid support. [45] The high swelling-ability, or degree of swelling, of the polymer indicates its low level

cross-linking and vice versa.[26] However, the compatibility of the support material with solvents and the reactants is very important for easy diffusion of the reagents to the acid site of the catalyst.[45] The swelling behaviour of a polymer or resins depends on the nature and compatibility of the solvent.[47] Selection of a good solvent results in the quick swelling and fast diffusion of the reactants through the crosslinked polymeric networks to the catalyst acid sites.[45]

The polymer material used as solid support for the acid catalyst can be categorized into two groups:

(1) Biopolymer based solid support
(2) Polystyrene based solid support for soluble acid catalysts and Ion-exchange resin based solid support [1]

2.2.2.1 Biopolymer based solid support

Heterogeneous catalysts derived from bio-polymers - such as cellulose-supported solid catalysts, alginate-supported solid catalysts (alginate is an anionic polysaccharide in the cell walls of brown algae, Laminaria and Ascophyllum species) and chitosan-supported solid catalyst (chitosan is a linear polysaccharide built by randomly distributed β-(1→4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit)) are reported to have shown better selectivity and reaction yields over the synthetic polymer-supported catalysts.[48]

Some researchers, in the recent past, have used cellulose as a solid support for sulfuric acid.[1] These catalysts have been used successfully in the synthesis of oxazolines,[49,50] α-amino nitriles, production of 2,4,5-triarylimidazoles,[51] imidazoazines,[49] imidazolines, thiazolines,[49] reaction leading to the formation of xanthenes,[52] synthesis of quinolines and quinazolin-4(1H)-ones,[53] 3,3'-indolyloxindole derivatives,[54] substituted-9H-purines,[55] β-acetamido carbonyl derivatives,[56] 5-unsubstituted-1,4-dihydropyridines and substituted pyrroles,[57,58] Knoevenagel condensation[59] and also in the protection of hydroxyl groups.[1,50]

Recently, a Brønsted acid catalyst supported on peanut shell, which consists of cellulose, hemicelluloses, and lignin microfibrils,[60] was reported by Zeng and co-workers to have an acidity stronger than the acidity of HZSM-5(Si/Al=75) but weaker than 100%

H_2SO_4.[12] Pua *et al* also reported the sulfonation of aromatic rich Kraft lignin obtained from the industrial pulping of softwood,[61] and its uses in catalysing the production of biodiesel from triglycerides.[10] Also, lignin from pine wood was sulfonated by Liang *et al.*[62] Though, it was reported that the concentration of the sulfonic acid groups on pine wood lignin-supported sulfonated acid catalyst is lower than that of the Amberlyst 15 but catalytically performed better in esterification of acetic acid with ethanol than Amberlyst 15 and sulfonated carbon.[1,62] Its high catalytic activities performance was attributed to the contribution from many macro-pores on the lignin which afford the reactants in solution to gain good access to the sulfonic acid sites / groups of the catalyst.[1,62] However, the catalytic performance of the pine wood lignin-supported sulfonated catalyst in the hydration of 2, 3-dimethyl-2-butene was very low when compared to the catalytic activities of Amberlyst 15 and sulfonated carbon, this was due to the less hydrophobic nature of the pine wood lignin.[1,62]

2.2.2.2 Polystyrene based solid support for acid catalysts

Polystyrene-based solid supports have been reported for a variety of different acid catalysts and subsequently used in organic synthesis.[1] For example, a polystyrene support has been reported for aluminium chloride ($AlCl_3$),[1,43,63-68] ferric chloride ($FeCl_3$),[1,64] 1-vinylimidazolium based ionic liquids supported on polystyrene,[1,69] sulfonic acids (-SO_3H),[1,70]

The above-mentioned catalysts have been used in one or more types of reaction and exemplified by, for example: Friedel–Crafts alkylation and acylation,[1,63,65,66,68,71] tetrahydropyranylation reactions,[1,65] synthesis of bis-indolylmethanes,[1,67] synthesis of N-substituted pyrroles,[1,42] xanthenes,[1,43] dithioacetalization of carbonyl compounds,[1,64] acetal formation,[1,72] synthesis of acylals from aldehydes,[1,73] dehydration of xylose to from furfural,[1,70] esterification reactions, transesterification reactions,[1,70] amidation of benzhydrol and tertiary alcohols with nitriles,[1,72] synthesis of β-amino ketones,[1,74] acetylation reactions,[1,73,75] conversion of biomass and carbohydrates into 5-hydroxymethylfurfural (HMF).[1,76]

2.2.2.2.1 Ion-exchange resin based solid support (Styrene cross-linked with Divinylbenzene polymer based solid support) for acid catalysts

Ion-exchange resins are insoluble polymers (resins) used to support Brønsted acid catalyst or base functional groups. The polymers are macroporous with ionogenic groups that have strong capabilities of exchanging specific ions with another certain ion in the same polymers.[1] The ion-exchange resin are most often composed of styrene and divinylbenzene which co-polymerised together form a cross-linked resin.[77,78] The Brønsted ion-exchange resins typically have sulfonic acids groups, grafted onto the phenyl ring, as the acid sites on the resins.[1,77,79] The rigidity and the pore size (macro-porosity) of the catalyst depends mainly on the proportional amount of divinylbenzene (DVB) in the catalyst.[1,78] However, the swelling ability of the resins in the reaction solvent determines the catalytic activities of the catalyst.[1,78,79] The lower the DVB amount in the resin, the higher the swelling ability of the catalysts and their catalytic activities.[234,235] The implication of the low amount of DVB in the resin of the catalysts is that it reduces the rigidity and macro-porosity of the resins.[78] It is worth noting that the greater the swelling of the catalyst, the higher the rate of penetration of the reactants into the resin particles and their rate of accessing the acid sites of the catalysts thereby influences the catalysts' activities.[1,79] Due to the uniqueness of the acidity of ion exchange resins in the presence of water, they have been applied in dehydration and hydration reactions, esterification, hydrolysis, condensation, and alkylation reactions.[1]

Ion exchange resins are known commonly *via* their commercial tradenames: Dowex, Amberlyst, Amberlite, Nafion, Indion and Lewatit.[1,6,80]For each brand, there are often many different subtypes, which reflects differences such as Dowex 50W, Dowex G-26, Dowex 66, MBIR Dowex-11 etc. for Dowex brand; Amberlyst 15, Amberlyst 16, Amberlyst 18, Amberlyst 36, Amberlyst-35, Amberlyst 39, Amberlyst 46, Amberlyst 70 Amberlyst NX 1010, etc. for Amberlyst brand; Amberlite IR120, Amberlite IRA900N_3, etc. for Amberlite; Nafion 811, Nafion SAC-13 etc. for Nafion brand; Indion 140, Indion-130, Indion-190 etc. for Indion brand; and Lewatit SPC 112 etc. for Lewatit brand.[1,6,80]

2.2.3 Common commercially available sulfonated polymer-based catalysts

Sulfonated polymer-based catalysts are ion exchange resins with strong acid catalysts or its corresponding salt.[47] They have cation exchange nature and are recyclable when used as catalysts. Sulfonated polymer-based catalysts have many advantages over their corresponding homogeneous counterparts in organic chemistry. Different brand names

are used to describe sulfonated polymer-based catalysts but the three most prominent commercially available brand names are Amberlyst, Amberlite and Dowex. These three brand types of acid catalyst are the most widely used in organic synthesis and, due to their strong concentration of acid active sites, have shown very good effectiveness in the world of catalysis.[81] They are recyclable, with little acidity depreciation and can be used several times over. A few examples were given above on some of the commercially available ion exchange resin supported acid catalysts. Various types of sulfonated polymer-based acid catalysts are commercially available. The commercially available sulfonated polymer-based acid catalysts can be categorized as macroporous resin-supported sulfonated acid catalysts or microporous resin-supported sulfonated acid catalysts.[82] The macroporous resins can either have low or medium or high degrees of crosslinking.[82] The macroporous-supported sulfonated catalysts with a low degree of crosslinking includes Amberlyst 39 and Amberlyst 70 etc. while Amberlyst 16, Amberlyst 36 etc. are macroporous-supported sulfonated catalysts with a medium degree of crosslinking while Amberlyst 15, Amberlyst 35 etc are of high degree. [82] The microporous resins are also known as gels. Examples of microporous resin-supported sulfonated catalysts include Amberlyst 31, Amberlyst 121, Dowex 50 X4 and Dowex 50 X2.[82]

Polymer-based sulfonated catalysts can also be categorized according to the numbers of their sulfonic acid sites. Some are mono-sulfonated while others are over or multi-sulfonated.[82] Examples of the polymer-based sulfonated catalysts in the category of mono-sulfonated polymer-based are Amberlyst 15, Amberlyst 16, Amberlyst 39, Dowex 50Wx8, Dowex 50Wx4, Amberlyst 31, Dowex 50Wx2 and Amberlyst 121 while that of the over or multi-sulfonated includes Amberlyst 35 and Amberlyst 36.[82] It is worth noting that Amberlyst 35 and Amberlyst 36 are respectively the over-sulfonated types of Amberlyst 15 and Amberlyst 16.[82] However, for Amberlyst 46, the sulfonation is at the surface of the macro-porous resin.[82]

Amberlysts, especially Amberlyst 15, have found their applications in the major organic reactions such as esterification and transesterification reactions, Michael and Aza-Michael addition reactions, Friedel-Crafts reactions, ring opening of epoxides, condensation of carbonyl compounds, halogenation, hydrolysis of acetates, formation of heterocyclic and homocyclic compounds, synthesis of phosphonates, enaminones and derivatives etc.[83] Amberlyst 15, Amberlyst- 36, Dowex 50W, Amberlite IR-120 and

Lewatit S1467 catalysts were also reported to be effective for the esterification of acetic acid with ethanol.[84] Amberlite IRA900N_3 was used in the azidation of α,β-unsaturated Ketones.[85]

2.2.4 Summary and limitation of solid acid catalyst

Some of the solid acid catalysts described above were produced from inorganic materials while the others were based on polystyrene-sulfonic acids.[3,6,37,80] The inorganic-based solid-phase catalysts, depends on the catalyst/metal, are very expensive and not often found useful in industry because of their cost of production.[30] Polystyrene-based catalysts are produced from styrene, as by-products obtained in the pyrolysis of petroleum crude.[86,87] However it has been reported that both styrene[87-91] and perfluoroalkyls[92-95] can cause cancer and other risks associated with human health.

Petroleum / crude oil, the main source of styrene, will eventually run out[96,97] because of over dependence of some countries on its products, and also the campaign against environmental pollution. Resulting in the current development/orientation for the utilisation of renewable materials. Therefore, there is a need for a better alternative to serve the purpose of the current solid acid catalysts. Since petroleum is generated from the fossilisation of animal and plant materials,[98-100] this suggests that the components from animal or plants that are cross-linked are capable of being sulfonated and can serve as an alternative or replacement to the current sulfuric / sulfonic polystyrene catalysts.

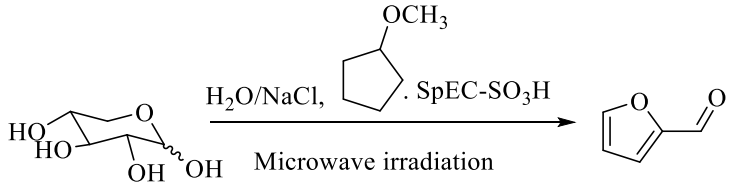
The limitation of the polystyrene and the campaign for the protection of environment are the major calls for the utilisation of sustainable polymers to support the acid catalysts. Cellulose and lignin are renewable materials and naturally abundant biopolymers[58,62] with several applications in organic chemistry.[1,50] Many researchers reported the application and effectiveness of their sulfonated derivatives, as sustainable heterogeneous catalysts, in the synthesis of different organic compounds.[1,50,62] Both cellulose and lignin supported sulfonated acid catalyst were reported to possess strong acid sites of sulfonic acid groups, high hydrothermal and thermal stability at 100 °C.[50,62] The report further confirmed that the amount of the sulfonic acid groups in lignin supported sulfonated acid catalyst remained intact even after boiling the catalyst in water for 2 hours.[62] However, lignin-supported lost mechanical stability at the temperature above 700 °C as there decrease in the mass of the support materials due the modification of its chemical composition and distruption of the bonds between the strands of the polymer.[48,62]

Sporopollenin, a polymer with high physical and thermal stabilities, is another example of polymeric material that is naturally produced by plants and requires no industrial polymerization process,[101] and so is a prime candidate for derivatisation and use as a solid support for catalysts.

2.3 Sporopollenin exine capsules as a solid-phase support for catalysts

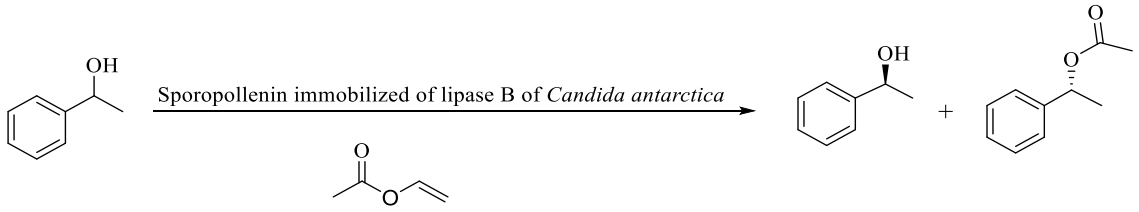
Sporopollenin, a complex cross-linked polymer,[18] is biosynthetically produced in the plant and requires no industrial polymerization process.[101] Sporopollenin and derivatives have previously been used as a solid support catalyst in the chemical[101-103] and enzyme[104] catalysed synthesis of some organic compounds.

An example in which sporopollenin has been used as a support for / as a heterogeneous sulfonic acid catalyst is found in the dehydration of D-xylose and xylan into the formation of furfural.[105] (**Scheme 2-4*)***



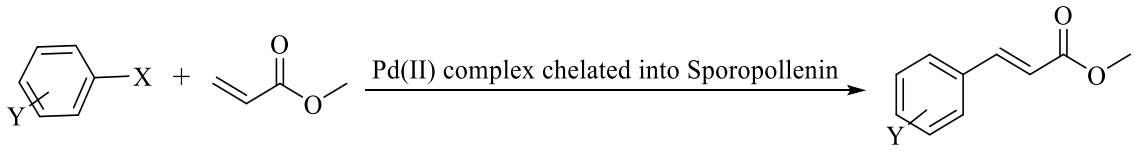
Scheme 2-4: **Dehydration of xylose by sulfonated sporopollenin *(*SpEC-SO_3H*)*** [105]

An example of an enzyme mediate process was reported by Souza *et al*, who used aminoalkyl derivatised sporopollenin exine capsules (from *L. clavatum* spores) to immobilize enzyme *Candida antarctica* lipase B to efficiently catalyse the reaction of vinyl acetate and 1-phenylethanol[104] **(Scheme 2-5*)***

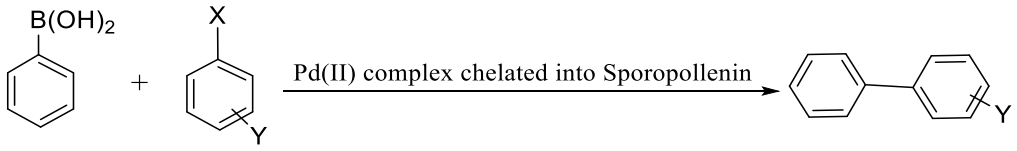


Scheme 2-5: **Sporopollenin immobilized enzyme catalyzed reaction** [104]

Keleş and Baran separately applied the complex of palladium (II) chelated into the sporopollenin, through the sporopollenin immobilised Schiff bases, as catalysts to catalyse Heck and Suzuki coupling reactions to form their respective products.[48,103] **(Scheme 2-6 and Scheme 2-7*)***



***Scheme 2-6*: Palladium (II) sporopollenin catalysed Heck coupling reaction**[103]



***Scheme 2-7*: Synthesis of biphenyl compounds (Suzuki reaction) in the presence of palladium derivatised sporopllenin**[48]

2.3.1 Possible advantages of sporopollenin as heterogeneous catalysts

Catalysts are used to speed up the rate of reaction by lowering the activation energy of a process. In industrial processes and synthesis, it is not only desirable that the catalyst should be efficient but must also be highly recoverable without degeneration,[106] be recyclable, be inexpensively available and be thermally, chemically and physically stable. Their precursors, and other materials used in their productions, are required to be environmentally friendly and safe to human health.[107] Sporopollenin is a renewable compound being produced by plants.[48,108]

Sporopollenin, as a cross-linked natural polymer, has numerous potential advantages such as its resilience to physical attack. Their previous usage showed that they are consistent and recyclable. Sporopollenin (SpECs) have lost its inner protein and other allergic materials to the extraction process and therefore, considered and reported not hazardous to the health of humans.[109,110]

There are various articles that have reported the stability of sporopollenin. SpECs are thermally stable beyond 200 °C,[48,111,112] possess resistance to non-oxidative degradation by chemical, [113,114] biological[113] and / or physical substances.[114] Baran *et al* reported the thermal stability of sporopollenin up to 470 °C.[48] The above mentioned paper described several properties serving as major advantages over the previously available polystyrene resins which are thermally unstable[5,115,116]and liable to suffer setback from blockage during chemical reactions.[117,118]

Stability is one of the major properties required for a solid-phase catalyst. Sporopollenin often maintains the precise morphology of their original pollen/spore shell

and this aids its resistance to attack from reagents during chemical reactions.[48]Sporopollenin, unlike polymer-based catalysts, have not been reported to exhibit or undergo molecular / structure deformation.[6,47,101,119,120] Also, sporopollenins have monodisperse grains, so are easy to selectively filter without the filter getting blocked and are easy to process.

Sporopollenin is a multifunctional compound, its structural morphology helps it to have a constant particle size which makes it a useful free-flowing column material. The constant particle size of sporopollenin was reported to aid the constant flow rate, for a long period of time, of the mobile phase in column chromatography,[101] therefore, sporopollenin can be used a catalyst in the continuous reaction process.

Several researchers have changed the surface of SpECs by functional groups interconversion depending on the intended use. For example, Souza *et al*[104] methylated the surface of the SpECs, then aminated using a diamine in order to use the free amine to immobilise a *C. Antarctica* B lipase. Sahin *et al* immobilised Schiff base derivatives on the SpECs' surface to form complex with ruthenium (III),[102] through the use of (3-chloropropyl) triethoxysilyl sporopollenins.[121] Similarly, different derivatives of sporopollenin were used by Keleş and Baran *et al* for the formation of palladium (II) complexes, which were used as solid supported catalysts in organic reactions.[48,103]

2.4 Aim of the research

This project aims to develop acid catalysts from *L. clavatum*. To achieve this, the surface of sporopollenin, being a thermally stable organic compound from *L. clavatum*, will be modified with suitable reagents to form solid-phase (heterogeneous) catalysts. In this research, SpEC-SO_3H and other SpEC derivatives will be applied as heterogeneous catalysts in organic synthesis. Specifically, SpECs will be extracted from raw spores of *L. clavatum*, the surface or the functionally groups of sporopollenin particles will be chemically modified to provide new surface functionality (by sulfonation, sulfation, thiolation and metalation) and the chemically modified sporopollenin exine capsules will be used as a solid-phase reagent or heterogeneous acid catalyst in various acid catalysed organic syntheses. The results obtained will be compare with those using the commercially available solid acid catalysts, Amberlyst 15, amberlite IR-120(H) and Dowex 55W.

In order to build on the ability of sporopollenin and derivatives as a solid catalyst and intensify specifically the investigation on the use of sulfonated SpECs (SpEC-SO_3H) as an acid catalyst in organic synthesis, the catalytic activities of SpEC-SO_3H and sulfated SpEC (SpEC-OSO_3H) will be tested in a variety of reactions such as glycosylation, dehydration, protection of organic compounds, transesterification, rearrangements and heterocyclic syntheses.

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Chapter 3: Extraction and derivatisation of SpECs

3.1 Extraction of SpECs from raw spores of *Lycopodium clavatum*

Various methods of extracting sporopollenin exine capsules have been reported. Many of the first methods reported centred their focus on the removal of the inner layer (intine) of the pollen or spores, and the intactness of exine capsule. Chemicals and reagents were rigorously investigated, so as to avoid the degradation of the exine polymer. Some have reported enzymatic extraction,[1] whilst others utilised chemical reagents[2] to get rid of the nitrogenous and polysaccharide content of the spores, including the intine layer.[3]

In this work, three different methods were employed to extract SpECs from *Lycopodium clavatum*, viz:

1) The long method (by time) involving 85% *ortho* phosphoric acid as the final reagent to hydrolyse the polysaccharide-containing intine layer of *L. clavatum* (producing PHS-SpECs);
2) A 'quick' acid hydrolysis, involving the use of 9 M hydrochloric acid (producing AHS-SpECs);
3) A base hydrolysis method, which involved the use of 6 % aqueous sodium hydroxide only (producing BHS-SpECs).

The above-mentioned methods have all previously been reported widely in the literature,[4,5] and so were therefore adopted for this work.

Orthophosphoric acid extracted SpECs (PHS-SpECs) are sporopollenin capsules prepared by the orthophosphoric acid method. Free fatty acids and other lipids are removed from the spores of *L. clavatum* by stirring them with acetone at reflux for 4 hours. The lipid-free spores are then filtered, air dried and treated with 6 % aqueous potassium hydroxide at 83 °C for 6 hours. This hydrolysed the nitrogenous compounds, including proteins and nucleic acids, which are components of sporoplasm. The alkali treated spores are filtered and re-suspended in another portion of potassium hydroxide, of equal volume, at the same temperature and number of hours as the first treatment. This is done to ensure complete hydrolysis of the nitrogenous compounds. The alkali-treated

spores are thoroughly washed with deionised water until the filtrate becomes neutral, to avoid contamination of the SpECs through with inorganic salts when the acid in the final step was added. The washed, alkali-treated spores are finally treated with 85 % orthophosphoric acid for 7 days at 83 ˚C to remove the polysaccharide intine.[6] At the same time several other reactions are possible. For examples phenolic / hydroxyl groups on the surface of the capsules[7] could react with orthophosphoric acid and form a phosphorylated surface,[8] as well as dehydration and cyclo-etherification processes.

After this process was conducted the final SpECs were washed with water to remove excess acid, treated with ethanol to remove excess water, then vacuum dried to constant weight. The CHN combustion elemental analysis of the prepared SpECs indicated that this first batch, referred hereafter as SpEC1, had 66.54 % C, 8.61 % H, and 0.00 % N by weight. Assuming the balance is made up of oxygen, this gives the approximate formula of $C_{90}H_{139}O_{25.}$ Since the SpEC polymer is not regular, Zetzsche *et al* presumed that the formula of the polymer can be represented with 90 carbon atoms.[9] Therefore, the combustion elemental analysis results from this and subsequent batches are in good agreement with the observation reported in **Table *3.1-1***. The formula of orthophosphoric acid extracted SpECs was almost the same with the finding of Berkaloff *et al*[10] in which it was claimed that *L. clavatum* has the formula of $C_{90}H_{138}O_{28.}$ Over the course of the project, five further batches of SpECs extracted with orthophosphoric acid were produced. Each batch of the extracted SpEC, after drying, produced a similar quantity. The six batches had the percentage weight of carbon content in the range of 63.74% to 70.51 % and percentage weight of hydrogen in the range of 7.03 to 9.19 % (**Table *3.1-1***). The result obtained from various batches of extraction showed the repeatability and the effectiveness of the method.

Table 3.1-1: Summary of combustion elemental analysis results of extracted SpECs

Entry	Sample	C Content %	H Content %	N Content %	Approximate empirical formula	Remark
1	SpEC1	66.54	8.61	0.00	$C_{90}H_{139}O_{25}$	Orthophosphoric acid extracted SpECs
2	SpEC2	63.74	7.03	0.00	$C_{90}H_{118}O_{31}$	Orthophosphoric acid extracted SpECs
3	SpEC3	67.97	9.19	0.00	$C_{90}H_{145}O_{23}$	Orthophosphoric acid extracted SpECs
4	SpEC4	68.70	7.76	0.00	$C_{90}H_{121}O_{23}$	Orthophosphoric acid extracted SpECs
5	SpEC5	65.23	8.40	0.00	$C_{90}H_{138}O_{27}$	Orthophosphoric acid extracted SpECs
6	SpEC6	70.51	8.44	0.00	$C_{90}H_{128}O_{20}$	Orthophosphoric acid extracted SpECs
7	AHS	65.01	7.96	0.00	$C_{90}H_{131}O_{28}$	Hydrochloric acid extracted SpECs
8	BHS	58.90	8.65	0.50	$C_{90}H_{158}O_{37}$	Sodium hydroxide extracted SpECs
9	PAHS	64.15	8.03	0.00	$C_{90}H_{134}O_{29}$	AHS further treated with ortho phosphoric acid
10	PBHS	64.73	8.61	0.00	$C_{90}H_{143}O_{28}$	BHS further treated with ortho phosphoric acid

NOTE: CHN combustion elemental analysis only determines the % of carbon, hydrogen, and nitrogen by weight. The weight % of oxygen was obtained by subtracting the sum of % carbon, % hydrogen and % nitrogen content from 100 and assumes that no other elements or contaminants are present.

Hydrochloric acid-hydrolysed SpECs (AHS) and based-hydrolysed (BHS) SpECs were prepared by the abbreviated methods described in the experimental section. The results of combustion elemental analysis showed that acid hydrolysed SpECs have the

formula $C_{90}H_{131}O_{28}$ and base hydrolysed SpECs (BHS) have $C_{90}H_{158}O_{37}$. It was observed that base hydrolysed SpECs has the lowest % carbon content, when compared to SpECs (PHS) and AHS, this might be as a result of formation of the sodium salt and therefore, the % weight of the inferred oxygen content obtained BHS was higher than those obtained from acidolysis. Obviously, the reagent used in the extraction may have contributed to the lower % of carbon content obtained. Sodium hydroxide is a basic reagent and therefore, the greater percentage weight of inferred oxygen observed in BHS may likely be attributed to inability of sodium hydroxide to remove cellulose / polysaccharide[4] and possibly incomplete hydrolysis of sporoplasm and intine content.[4,6,11] Due to the incomplete removal of intine and other cellulose contents by sodium hydroxide as reported by previous researchers, BHS was therefore treated with orthophosphoric acid for the total removal of intine and other polysaccharide materials as it was reported by Shaw *et al* that orthophosphoric acid possess a better ability to remove the polysaccharide.[12] The treatment of BHS with orthophosphoric acid, whose product was recorded as PBHS **Table *3.1-1***, led to an increase in the % weight content of carbon and a decrease in the % weight of other elemental contents; including oxygen and possibly sodium, that are most likely to be present in the SpECs after the treatment. Also, the % weight content of carbon and oxygen from the CHNS elemental combustion analysis of BHS, after its treatment with orthophosphoric acid, have great similarity with the SpEC originally extracted by orthophosphoric acid. This observation confirmed the report of Shaw *et al*[12] that the complete removal of cellulose from spore can only be attained by treatment of the base hydrolysed sporopollenin with 80 % phosphoric acid. It can also be concluded that hydrolysis of spores with base prior to the treatment with orthophosphoric acid is more economical and saves time. The procedure has proved that alkalis are capable of removing both the fatty acid and compounds containing nitrogen (including protein and nucleic acid) without prior treatment with acetone.

The treatment of BHS with orthophosphoric has a great effect on the colour of the SpECs. The colour of the SpECs recovered changed from beige or light brown to a dark brown. The colour of PBHS was identical to orthophosphoric extracted SpECs. The combustion elemental analysis result showed that the PBHS was nitrogen free and the difference between the percentage weight of carbon contents of PBHS and BHS is around 4 % with PBHS having a higher percentage carbon content (**Table *3.1-1***).

The time of the hydrochloric acid hydrolysis method, which was used in the extraction that led to the production of AHS, was very short. The SpECs were produced in a greater yield than methods involving the other two reagents. The brown colour of AHS was darker than the PHS and BHS SpECs. The possibility of chlorination of double bonds in the sporopollenin, or chloride contamination cannot be over emphasized as reported by the previous researchers.[4,11]

Modification of the surface of acid-hydrolysed SpECs involved treatment of AHS with orthophosphoric acid to obtain PAHS (Table 3.1-1). The difference between AHS and PAHS, as measured by CHN elemental combustion analysis, was not too significant. The percentage carbon content showed less that a 1 % decrease. This may be as a result of the strength of both acids. Hydrochloric acid is a stronger acid than orthophosphoric acid. Hydrochloric acid may have probably removed nearly all the intine content[4,11] leaving little or none in the AHS before its treatment with orthophosphoric acid.

The table below shows the summary of the combustion elemental analysis and the empirical formula of all the extracted sporopollenins.

3.2 Sulfonation and sulfation of SpECs

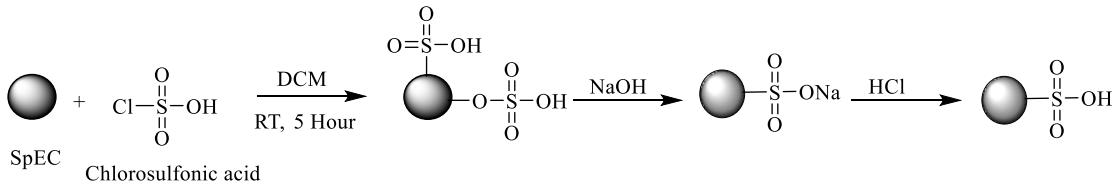
3.2.1 Sulfonation of SpECs

There are factors that are important for a material or polymer to be considered useful as support for a catalyst. Some of these factors include its mechanical stability, stability to heat and different chemical reagents during the reactions, accessibility of its micro / meso / macro pores for derivatization and good distribution of the functional groups that can be derivatised to form active sites.[13,14] The dispersibility of such material in the solvent(s) is another major factor to be considered while selecting a solid support material. SpECs possess all the aforementioned attributes with good dispersibility[7] in organic solvents and therefore could be used to support a catalyst. The aim of this work is to transform SpECs to a solid catalyst for organic synthesis. SpECs is a micro / macroporous polymer which, depending on the method of extraction, mainly possesses carboxyl, phenolic, polyvinyl alcohols and aliphatic polyketide functional groups.[15] The presence of aromatic carboxyl and the phenolic groups[4,16-18] as part of the chemical constituents of SpECs are the main factors that are responsible for and aid the derivatization. Moreover, Binks *et al* had earlier reported that an increase in the pH increases the negative charge of the ionisable groups on the SpECs extracted from the *L.*

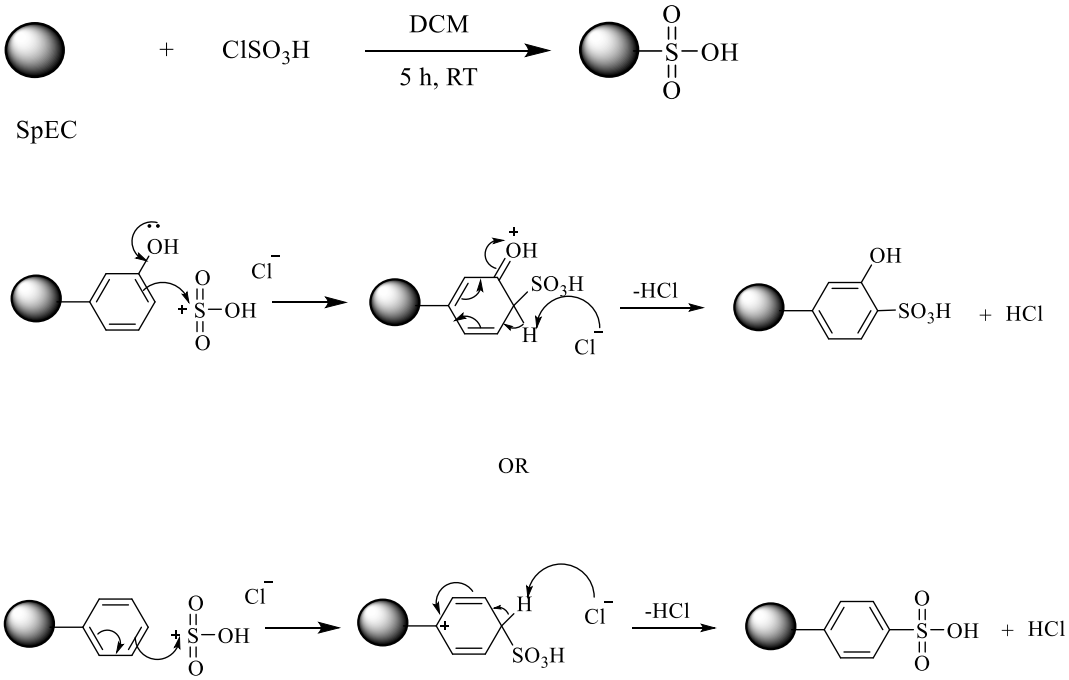
clavatum.[7] Therefore both the carboxyl and phenolic groups possess the ionizable ion, which are capable of becoming negatively charged as the pH increases (**Scheme 3-2**).[7]

3.2.1.1 Sulfonation of classic extracted SpECs

The classic method of extracting SpECs from the spores or pollen is a long extraction process that involved combination of defatting of spores or pollens with acetone and alkaline lysis with KOH then acidolysis using orthophosphoric acid. The orthophosphoric acid hydrolysed SpECs were sulfonated using chlorosulfonic acid in dichloromethane.[5] The chlorosulfonic acid treated SpECs was washed thoroughly with deionised water until the filtrate became colourless and neutral. The black filtrate that came out during the initial washing stages is an indication that part of the polymer degraded in the chlorosulfonic acid or the functional groups of some of the constituents were oxidised to afford the sulfonation of the polymer. Cremlyn[19] reported that the reaction of equimolar quantity of aromatic substrate and chlorosulfonic acid leads to the formation of a sulfated product at the first step but the product can slowly transform into its sulfonyl chloride derivative by replacing the sulfonic acid proton with a chlorine atom and liberate sulfuric acid, if the chlorosulfonic acid is in excess. More so, one of the components of SpEC, polyvinyl alcohol,[15] is capable of forming an addition reaction with chlorine atom of chlorosulfonic acid. With the possibility of formation of a chloride derivative and having remnant chlorosulfonic acid adhering to the SpECs, the treated SpECs were therefore later subjected to further treatment with potassium hydroxide to neutralise the remaining trace of chlorosulfonic acid, to remove the chloride ions and also to remove the unstable sulfated groups attached to the surface of the sporopollenin (**Scheme 3-1**). The alkali treated sulfonated SpECs were then treated with hydrochloric acid to neutralise the salts formed from the potassium hydroxide step, and to re-protonate the sulfonate groups. At each stage of treatment, the derivatised sporopollenin was thoroughly washed with water to avoid contamination, through the introduction of unwanted elements into the SpECs, from the reagents used in the sulfonation process (**Scheme 3-1**). The sulfonated SpECs (SpEC-SO_3H) were vacuum dried over phosphorus pentoxide to a constant weight in the desiccator. The percentage weight and the sulfur loading were investigated through ICPMS and combustion elemental analysis. The results are shown in the **Table *3.2-1***.



Scheme 3-1: **Reaction of sporopollenin with chlorosulfonic acid**



Scheme 3-2: ***Mechanistic steps of the reaction of sporopollenin with chlorosulfonic acid***

Consideration of the elemental and ICP-MS analyses on the samples reveals that the percentage weight of sulfur is within the range of 9.48 - 12.87 % for all the sulfonated SpECs, as shown in **Table *3.2-1*** below. The percentage by weight of the sulfur present in each of the sample is affected by the extraction process employed. Sulfonated base hydrolysed SpECs (BHS-SO_3H) have the highest % weight of sulfur content possibly because of the incomplete removal of sporoplasm and intine[4,6,11] and also the possible reaction of the sulfonic group with nitrogen compounds in the sporoplasm.[20]

Table 3.2-1: Summary of combustion elemental analysis and ICP-MS analysis results of sulfonated sporopollenin

Sample	Elemental Analysis					ICP-MS	
	C content (wt. %)	H content (wt. %)	N content (wt. %)	S content (wt. %)	S in mmol/g	S content (wt.%)	S in mmol/g
SpEC-SO_3H1	39.42	4.81	0.03	10.62	3.31	10.47	3.265
SpEC-SO_3H2	40.11	5.27	0.00	10.31	3.22	-	
SpEC-SO_3H3	37.66	5.04	0.09	10.36	3.23		
SpEC-SO_3H4	34.71	5.04	0.10	9.78	3.05		
SpEC-SO_3H5	36.52	4.79	0.00	7.95	2.48		
SpEC-SO_3H6	39.17	4.85	0.00	8.35	2.60		
SpEC-SO_3H7	38.89	5.01	0.00	7.88	2.46		
SpEC-SO_3H8	29.61	5.87	0.00	11.19	3.49		
SpEC-SO_3H9	27.96	3.71	0.00	9.80	3.06		
SpEC-SO_3H10	44.08	5.26	0.00	7.99	2.49	8.30	2.66
SpEC-SO_3H11	38.95	4.50	0.00	8.72	2.72	7.32	2.35

NOTE:(1) **SpEC-SO_3H** = Sulfonated sporopollenin from orthophosphoric acid extracted SpECs

It worth noting that SpEC-SO_3H, except otherwise stated, is generally referred to as the sulfonated derivative polymer extracted with a long reaction process involving defatting with organic solvent, treatment with KOH and orthophosphoric acid.

3.2.1.2 Comparison of sulfonation of other types of extracted SpECs

In order to compare the sulfonated products of various extraction methods, hydrochloric acid hydrolysed SpECs (AHS), base hydrolysed SpECs (BHS) as well as both orthophosphoric acid treated AHS and BHS were also sulfonated using chlorosulfonic acid in dichloromethane.[5] The method used in the sulfonation of the classic extracted SpECs was strictly followed for the sulfonation of these other SpECs.

Sulfonated SpECs, referred to as AHS-SO_3H, BHS-SO_3H, PAHS-SO_3H and PBH-SO_3H are respectively sulfonated derivatives obtained from the sporopollenins polymers SpEC, AHS, BHS, PAHS and PBHS respectively (see **Table *3.2-2***). The results of ICP-MS analysis, combustion elemental analysis of each was recorded in **Table *3.2-2***.

Table 3.2-2: Combustion analysis of other types of extracted SpECs

Sample	Elemental Analysis					ICP-MS	
	C content (wt. %)	H content (wt. %)	N content (wt. %)	S content (wt. %)	S in mmol/g	S content (wt. %)	S in mmol/g
AHS	65.01	7.96	0.00				
BHS	58.90	8.65	0.50				
PAHS	64.15	8.03	0.00				
PBHS	64.73	8.61	0.00				
AHS-SO_3H	38.16	5.30	0.00	11.75	3.664	10.97	3.42
BHS-SO_3H	36.55	5.21	0.07	12.87	4.014	11.99	3.74
PAHS-SO_3H	34.75	4.46	0.00	9.67	3.016	-	
PBHS-SO_3H	36.56	4.57	0.00	9.48	2.956	-	

AHS-SO_3H = Sulfonated sporopollenin from hydrochloric acid hydrolysed sporopollenin

BHS-SO_3H = Sulfonated sporopollenin from sodium hydroxide hydrolysed sporopollenin

PAHS-SO_3H = Sulfonated sporopollenin from AHS treated with orthophosphoric acid

PBHS-SO_3H = Sulfonated sporopollenin from BHS treated with orthophosphoric acid

The sulfonation of AHS, BHS, PAHS and PBHS have a reduced percentage of carbon content to the amount relatively close to the carbon content of the classic extracted SpECs (**Table *3.2-1***). The acidic group loadings of AHS-SO_3H, PAHS-SO_3H and PBHS-SO_3H were almost the same as SpEC-SO_3H but BHS-SO_3H has a bit higher acidic group loading than the others (**Table *3.2-2***). This may due to the fact that BHS has more hydroxyl groups, which may be available for sulfonation, than other extracted SpECs. Acidolysis extracted SpECs, especially those extracted with orthophosphoric acid, were reported to have hydrophobic parts containing of aliphatic polyvinyl compounds which may be liable to limiting or shielding the functional groups from easily interacting with chlorosulfonic acid.

3.2.2 Sulfation of SpECs

Sulfation of organic molecules, containing phenolic group, was reported to be more easy to produce than the sulfonation.[19] It requires the reaction between the hydroxyl group of an organic compound and the sulfation agent (in a suitable solvent, if the organic compound is a solid). Due to the presence of the hydroxyl group on the aromatic and aliphatic components of SpECs,[15] formation of sulfated SpECs can easily be carried out through esterification of alcoholic groups with a sulfating agent. Cremlyn[19] reported that the most favourable reagent for the production of quality sulfated product is chlorosulfonic acid. Also, the sulfated products are postulated to have the capability of rearranging to form the sulfonated derivative.[19] The rearrangement of sulfated products will lead to the production of polysulfonated products in a reactive phenolic compound[19] and polysulfonation will add to the catalytic performance of SpECs by making available more acid proton to be released in a catalytic organic synthesis. Therefore, sulfation of SpECs was carried out using the same method for the preparation of sulfonated derivative except that the derivatized sporopollenins were not subsequently treated with NaOH. It was reported that treatment of phenolic compounds with chlorosulfonic acid often leads to the formation of a mixture of *O*-sulfonation (sulfate derivative) at the hydroxyl functional group and *C*-sulfonation on the aromatic ring.[19] Treatment with NaOH was therefore omitted to avoid cleavage of the sulfate functional group. Therefore sporopollenins, suspended in dichloromethane, were treated with chlorosulfonic acid under the condition described in the sulfonation reaction. Chlorosulfonic acid was added while the mixture was in the ice bath. The mixture was removed from the ice bath after the addition of chlorosulfonic acid and was stirred at room temperature for 5 hours. After 5 hours, the sulfated sporopollenin (SpEC-OSO_3H) was recovered through filtration and thorough washing with water until the pH of the washings was neutral. The recovered sulfated SpECs were vacuum dried over phosphorus pentoxide to a constant weight. The percentage of sulfur loading on the SpECs was determined by combustion elemental analysis and ICP-MS as before, and the results are shown in **Table *3.2-3***.

Table 3.2-3*:* The result of elemental and ICP-MS analysis

Sample	Carbon Content (weight %)	Hydrogen Content (weight %)	Nitrogen Content (weight %)	Inferred Oxygen Content (weight %)	Sulfur Content (weight %)	Sulfur in mmol/g	ICP-MS (weight %)	Sulfur in mmol/g
SpEC-OSO_3H 1	23.11	2.49	0.00	69.98	4.42	1.38	8.07	2.58
SpEC-OSO_3H 2	42.72	5.85	0.12	42.27	9.04	2.82	10.14	3.24
SpEC-OSO_3H 3	40.94	5.87	0.14	42.17	10.88	3.39	9.96	3.19
SpEC-OSO_3H 4	39.89	5.29	0.12	42.19	12.51	3.90	11.56	3.70

3.3 Microstructure determination of SpECs and SpEC-SO_3H by Scanning Electron Microscope (SEM)

During the isolation of SpECs, the mass loss was significant. For example, from 200 g of the raw spores about 50 g of SpECs were isolated, in general. This mass loss was due to hydrolysis of the sporoplasmic components that were soluble in the various reagents used for the extraction. The derivatisation of SpECs with chlorosulfonic acid also caused damage to the capsules, as shown by scanning electron microscope (SEM) (**Figure *3.3-1*** and **Figure *3.3-2***). The SEM image of SpEC-SO_3H (**Figure *3.3-2***), as well as whole capsules (**Figure *3.3-1***), shows that there are fragmented SpECs, which must have been damaged during contact with the chlorosulfonic acid. It is known that sporopollenin can be broken down by strong oxidising agents,[21-23] and also that chlorosulfonic acid is an oxidizing acid. This observation is therefore not unexpected, and indicates that there is likely to be an optimum time and temperature for sulfonation, which if exceeded will lead to extensive degradation of the capsules.

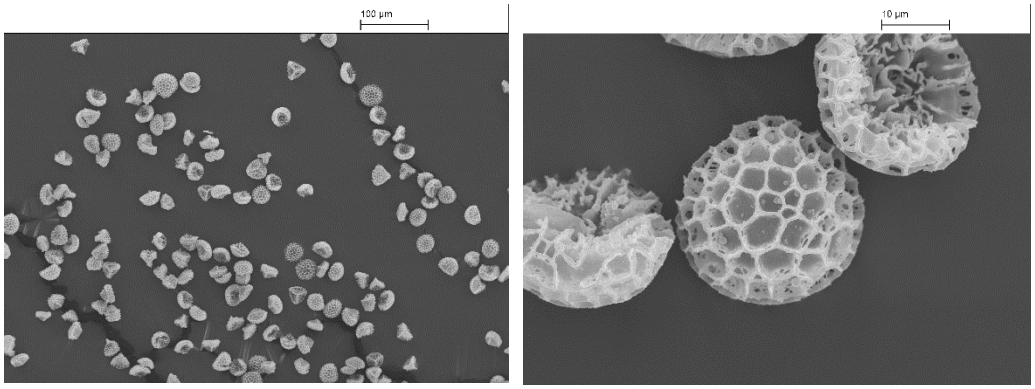


Figure 3.3-1: SEM of SpECs extracted with classical method (see section 3.6.5)

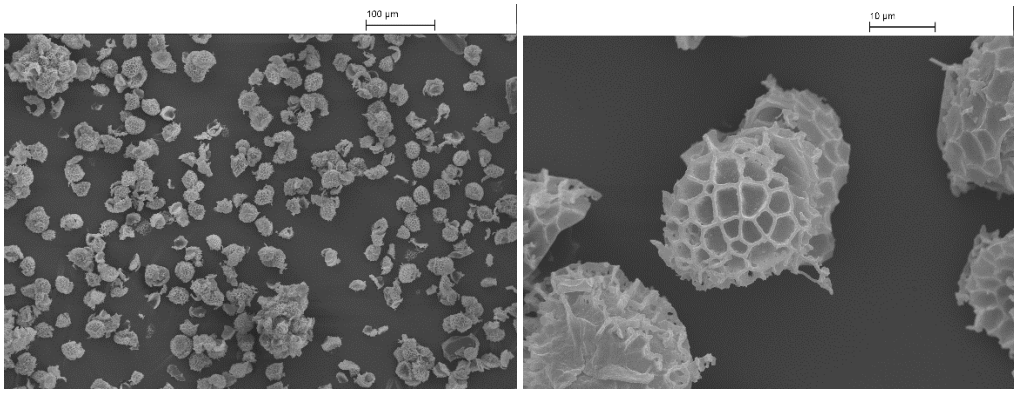


Figure 3.3-2 : SEM of SpECs-SO_3H (sulfonated SpECs from the classical method) as shown in section 3.6.5

3.3.1 Leaching

In using sulfonated SpECs (SpECs-SO_3H) during its application in chapters four to seven, it was noted that the reaction mixtures often became darker, as if some coloured species were leaching from the SpECs-SO_3H despite the extensive washing used in their preparation. The effect of different organic solvents on SpEC-SO_3H was therefore investigated, with the aim of optimising the procedure used in their preparation. SpEC-SO_3H (0.1 g) was soaked in each of seven different organic solvents for a specified period. The solvents ranged from non-polar through moderately polar to polar solvents which are n-hexane, toluene, diethyl ether, ethyl acetate, dichloromethane, ethanol and methanol. To investigate how important a factor contact time was, a portion of SpEC-SO_3H was soaked in each organic solvent for thirty weeks in a tightly sealed container, and another portion for a week in another tightly sealed container. After the appropriate time, the SpEC-SO_3H was filtered from the solvents and the filtrate was examined with UV-vis spectroscopy (**Table *3.3-1***). All of the filtrates, except those from ethanol and methanol, retained the colourless appearance of the original solvents, but most of them absorbed in the UV region (**Table *3.3-1***). At thirty weeks, the filtrates had more intense absorptions than those soaked for one week (**Table *3.3-1***). As SpEC-SO_3H is insoluble in organic solvents, the UV absorption is an indication of leaching of some of the materials from the SpEC-SO_3H (**Table *3.3-1***).

Table 3.3-1: Measurement of leaching on SpECs in various organic solvents

Entry	Solvent	Solution at one week			Solution at thirty weeks		
		Colour	Absorption maxima (nm)	Absorbance values	**Colour**	Absorption maxima (nm)	Absorbance values
1	n-Hexane	Colourless	210	1.300	Colourless	210	1.400
2	Toluene	Colourless	220, 290	-0.600, 0.150	Colourless	220, 290	-0.60, 0.150
3	Diethyl ether	Colourless	210	2.500	Colourless	210	2.600
4	Ethyl acetate	Colourless	230	0.580	Colourless	230	3.000
5	DCM	Colourless	270	1.500	Colourless	280	1.750
6	EtOH	Dark Brown	220	1.600	Dark Brown	220	2.400
7	MeOH	Dark Brown	220	0.400	Dark Brown	220	1.300

It was therefore concluded that it may be appropriate to wash the SpEC-SO_3H first with the solvent intended to be used in the reaction, so as to avoid leachate contaminating the product(s) and thus increasing the product purity. SpEC-SO_3H could be washed with all above solvents during the extraction process, but that was considered to be uneconomic.

3.4 Determination of sulfur loading

Table *3.2-1* shows the sulfur loading of the sulfonated SpECs. The sulfur loadings, in mmol per gram, of all the sulfonated SpECs are comparable for each sample, whether calculated from combustion elemental analysis or ICP-MS analysis. The calculated results for eleven different batches of SpEC-SO_3H gives the sulfonic acid loading in the range of 2.46 to 3.31 mmol per gram. This an indication that the process for the production of sulfonated SpECs is reproducible.

The determination of the loading of sulfonic acid group on SpEC-SO_3H was later evaluated by titration. A carefully weighed sample of sulfonated sporopollenin (0.05 g) and their corresponding underivatised SpECs (0.1 g) were stirred respectively in 0.500 M

sodium hydroxide at room temperature for up to 16 hours (**Table *3.4-1***, **Table *3.4-2***, **Table *3.5-1*** and **Table *3.5-2***). Each sample was filtered and the filtrate was titrated against 0.5 M hydrochloric acid, with methyl orange as an indicator. The titration of each sample was performed in triplicate. The loading of the acidic characteristics of both underivatised SpECs and that of the sulfonated SpECs were determined, in order to calculate the loading of sulfonic acid groups of the latter. The loading of sulfonic acid groups was derived by deduction of the calculated acidic groups (in mmol/g) of each extracted SpECs from that of the acidic groups (in mmol/g) of its corresponding sulfonated derivative.

The equations below were used to calculate sulfur and sulfonic acid (-SO_3H) loading on SpECs in dm^3/g (**Equation *1***) and mol/g (**Equation *2***) respectively. The results are reported in **Table *3.4-1***.

$$\text{SpECs or SpEC-SO}_3\text{H in dm}^3\text{/g} = \frac{(20 - \sum \left(\frac{Titre\ Values}{3}\right))ml\ /g}{1000} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Equation 1: Calculation of the concentration of SpECs or SpEC-SO_3H in dm^3/g

$$\text{SpECs or SpEC-SO}_3\text{H in mol/g} = Molarity\ (0.5\ mol/vol)x\frac{Vol}{g}(or\ equation\ 1) \ldots\ldots 2$$

Equation 2: Derivation of the acid loading on SpEC-SO_3H

The result of **Equation *2*** is reported in **Table *3.4-1***.

The sulfonic acid group (-SO_3H) loading on the SpEC-SO_3H (mol/g) was measured by subtracting acid loading of SpECs in mol/g from the acid loading of SpEC-SO_3H in mol/g as shown in **Table *3.4-2***.

Table 3.4-1: Total loading of acidic functional groups on SpECs and SpEC-SO_3H

SpECs (mmol in 1g)	**Time stirred with 0.5 M NaOH (h)**		
	2	4	16
SpECs	5.50	7.00	6.83
SpEC-SO_3H	15.33	19.00	15.33
AHS	1.50	1.83	7.00
AHS-SO_3H	6.67	8.33	15.3
BHS	-	-	2.17
BHS-SO_3H	4.667	6.33	10.33

From the calculated loading per gram of SpEC-SO_3H, it was observed that, in general, the acidic group loading on the SpECs increases with increase in time. The apparent increase in loading of acidic groups could be caused by many factors; there could be degradation of the SpECs leading to increase in acidic functional groups, or it could also due to the fact that a long period of time is needed to fully wet the surface of the SpECs and for the base to reach all of the acidic groups inside and outside of the capsule.

The result recorded in **Table *3.4-2*** suggest that the catalytic functionality SpEC-SO_3H may gradually decline after a long time and if used repeatedly. For BHS-SO_3H, the acid loading could be not be measured until the sixteenth hour.

Table 3.4-2: The loading of sulfur functional group on sulfonated derivatives

SpECs (mmol in 1g)	**Time (h)**		
	2	4	16
SpEC-SO_3H	9.83	12.00	8.50
AHS-SO_3H	5.17	6.50	8.16
BHS-SO_3H	-	-	8.16
PAHS-SO_3H	5.40	6.50	
PBHS-SO_3H	6.17	8.17	

The loading of acidic groups in the SpEC-SO_3H could contain the weak acid protons and other acidic functional groups. Therefore, investigation of the acid strength of the acidic groups in SpEC-SO_3H led to the use of a pH meter. SpEC-SO_3H and Amberlyst 15 were separately stirred in an equal volume of deionized water at room temperature for 16 hours and filtered. The pH of each of the filtrates was measured using a pH meter and the results are recorded in **Table *3.4-3***.

Table 3.4-3: Results of the acidity comparison of SpECs from various methods, their sulfonated derivatives and Amberlyst 15

Entry	Sample	pH Meter	Difference between the pH of each SpECs and their corresponding sulfonated derivatives
1	**Amberlyst 15**	4.60	
2	**SpECs**	7.32	5.13
3	**SpEC-SO_3H**	2.19	
4	**AHS**	5.36	4.35
5	**AHS-SO_3H**	1.01	
6	**BHS**	8.76	8.01
7	**BHS-SO_3H**	0.75	
8	**PAHS**	8.76	7.66
9	**PAHS-SO_3H**	1.10	
10	**PBHS**	4.35	3.24
11	**PBHS-SO_3H**	1.11	

Though the acid concentration per gram of Amberlyst 15 (around 4.6 mmol / g) is higher than the calculated concentration per gram of SpEC-SO_3H, it appeared from pH meter values that the SpEC-SO_3H is a stronger acid than Amberlyst 15. This might have resulted from the ionization of acidic protons from sulfonic acid group and the support from other ionizable groups on the surface of sporopollenin. It is worth noting that SpEC-SO_3H is presumed to have several ionisable sites, [15,16,24] which could be responsible for the generation of more acidic proton in water than Amberlyst 15.[25] This observation is in line with the report of Binks *et al* about how the increase in pH often influences or leads to an increase in the ionization of the functional groups on the surface of SpECs.[7] This claim leads to the prediction of the possibility of catalysing a reaction with SpEC-SO_3H, at room temperature, for an extended period without it losing its catalytic activities.

The difference between the acidity of the protons of sulfonic acid group of Amberlyst 15 and the protons on the sulfonic acid group of SpEC-SO_3H is between 2– 3 pH units.

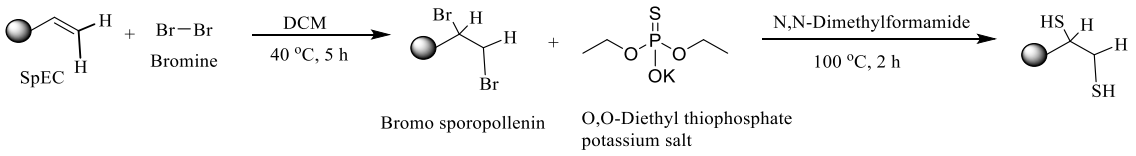
The pH measurement of the sulfonated product of SpECs extracted from other methods also corroborate the acid concentration of the sulfonated SpECs obtained from the classic method (**Table *3.4-3***). The acid concentration of other sulfonated products was found to be higher than that of SpEC-SO_3H, especially BHS-SO_3H, this may largely be due to the

contribution from the proton of carboxyl groups. Base hydrolysed SpECs are believed to have more carboxylic functional and acidic hydroxyl groups than those extracted through other methods because NaOH is not a dehydrating agent. More so, the pH measurements of other extracted products were taken from the batches produced 3 years ago from the SpECs extracted by the classical methods, there could have been variance in the buffer of the pH meter which probably influenced the measurement. However, it is clear, as revealed by the difference in pH of each SpECs and its corresponding sulfonated derivative (**Table *3.4-3***) that the sulfonic acid group is mainly responsible for the acidity of the sulfonated derivatives of SpECs.

The investigations of the sulfonated sporopollenin discussed above, indicate that they can be a replacement for the sulfonated polystyrene based Brønsted acid catalysts.

3.5 Brominated and thiolated SpECs

The importance of thiol-functionalized SpECs (SpEC-SH) as an alternative to direct sulfonation made us consider converting brominated SpECs (SpEC-Br) into a thiol SpECs through nucleophilic substitution with a thiophosphate (***Scheme 3-3***). The thiol could be oxidized to the sulfonic group

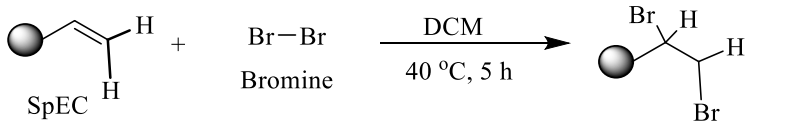


Scheme 3-3: **Thiolation of sporopollenin exine capsule**

3.5.1 Bromination of SpECs

Brominated SpECs were required as the starting material for the production of thiolated SpECs. Brominated SpECs were made by suspending SpECs in a solution of bromine in dry dichloromethane and stirring overnight at 40 °C.[4,23] After filtration and subsequent washing with methanol, water, 2 M HCl, water, ethanol and diethyl ether, the brominated SpECs were dried under vacuum over phosphorus pentoxide to a constant weight (**Scheme 3-4**). The experiment was repeated twice to confirm the reproducibility of the results. The samples were subjected to combustion elemental analysis to determine the % carbon, hydrogen and nitrogen contents. The amount recovered (in mass) at the end

of first experiment and the results obtained from combustion elemental analysis were consistent with the second experiment as shown **Table *3.5-1***.



Bromo sporopollenin

Scheme 3-4: **Bromination of sporopollenin exine capsule**

Table 3.5-1: Summary of combustion elemental analysis results of brominated SpECs

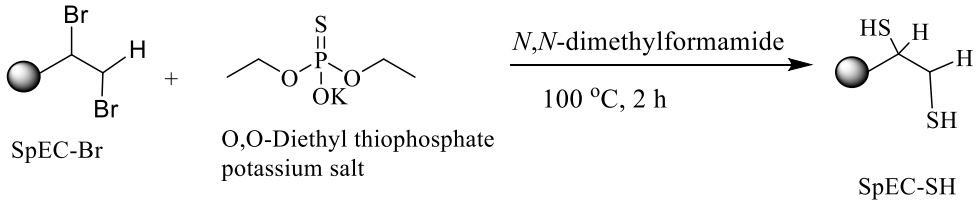
Sample	Amount of SpECs used	Amount recovered	Elemental Analysis		
			C weight %	H weight %	N weight %
SpEC1	-	-	66.54	8.61	0.00
SpEC-Br 1	3.00 g	2.78 g	30.97	3.15	0.10
SpEC-Br 2	3.00 g	2.71 g	30.88	3.09	0.09

Since the reaction was carried out, at low temperature, in a moderately polar solvent and the fact that Liu *et al*[15] reported the presence of aliphatic unsaturated bond in the organic structure of SpECs, the bromination of SpECs will mainly occur by electrophilic addition reaction of the olefinic double bonds of in the SpECs with halogen[4,23,26] as shown in **Scheme 3-4**. Also, the presence of phenolic compounds in SpECs might likely lead to the substitution of hydrogen at the *ortho*- or *para*-position of the phenol in the SpECs to form a mixture of monobrominated products[27] but this type of bromination of phenolic components in SpECs was reported to be a minor product.[4]

3.5.2 Thiolation of brominated SpECs

Thiolated SpECs were obtained when potassium *O,O*-diethyl thiophosphate was added to brominated sporopollenin suspended in dry *N,N*-dimethylformamide at 100 °C under reflux for 2 hours. The resulting mixture was filtered, and the SpECs washed with water, methanol and diethyl ether to give thiolated SpECs which were dried under vacuum to a constant weight (**Scheme 3-5** and **Scheme 3-6**). The experiment was repeated four times using 1.6:1 ratio (w/w) of the potassium-*O,O*-diethyl thiophosphate to brominated sporopollenin. The results of element analysis of the experiments were similar and are

reported in **Table *3.5-2***. The presence of nitrogen in the brominated sporopollenin, as revealed in the combustion elemental analysis, was presumed to come from *N*,*N*-dimethylformamide that was used in the reaction process, because the starting SpECs were nitrogen free. However, with the use of an equal mass (w/w) of potassium *O*,*O*-diethyl thiophosphate and brominated SpECs, the combustion elemental analysis result showed that there was no nitrogen atom in the product, and the % weight of carbon, hydrogen and sulfur were not significantly different from the first four experiments. The lack of nitrogen atom is likely to have been due to better washing of the thiolated SpECs with water.

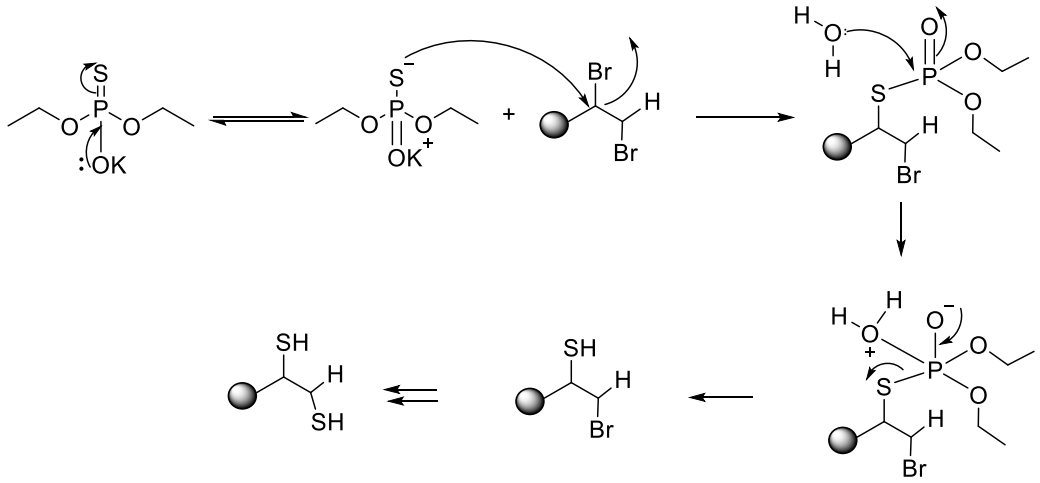


Scheme 3-5: **Production of thiolated SpECs from brominated SpECs**

Table 3.5-2: Summary of combustion elemental analysis results of thiolated SpECs

Sample	**Amount of SpECs used (g)**	**Amount Recovered (g)**	**Elemental Analysis**			
			% Carbon weight	**% Hydrogen weight**	**% Nitrogen weight**	**% Sulphur weight**
SpEC-SH1	0.500	0.406	39.21	4.10	0.21	3.65
SpEC-SH2	0.500	0.324	39.36	3.99	0.44	4.14
SpEC-SH3	0.500	0.380	39.43	4.13	0.28	4.11
SpEC-SH4	0.500	0.408	33.30	3.46	0.28	3.24
SpEC-SH5	2.100	1.647	38.74	4.10	0.00	3.47

NOTE: CHN combustion elemental analysis indicates only the % weight of carbon, hydrogen, nitrogen and sulfur in the SpEC derivatives.

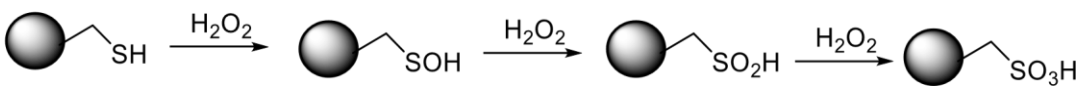


Scheme 3-6: **Mechanism leading to the formation of thiolated SpECs**

3.5.3 Conversion of thiolated sporopollenins (SpEC-SH) into sulfonated derivative

An attempt was made to oxidize the thiolated SpECs to sulfonated SpECs with hydrogen peroxide. In this attempt, thiolated SpECs (406 mg) were suspended in H_2O_2 – acetic acid (20 mL; 1:1) and the mixture was stirred for 12 hours at room temperature. The product was filtered and vacuumed dried over phosphorus pentoxide to a constant weight (**Scheme 3-7**). Combustion elemental analysis was carried out to ascertain the percentage sulfur content in the product.

Oxidation of SH would have been almost instantaneous if the surface wetted easily but since SpECs has aliphatic components, which are hydrophobic in nature, the polymer was on the surface of the solvent until serious agitation in order to overcome the immiscibility. The yield by weight was poor (0.308 g obtained from 0.406 g). The expected SpEC-SO_3H has additional oxygen atoms therefore it was expected that mass of the latter should have been higher than the starting material. The decline in weight may probably indicated either loss of some SpECs during transfer, or the oxidation caused some degradation of the SpECs. Even if the oxidation of the thiol had been successful, the % weight of sulfur by elemental analysis was low compared to the direct sulfonation route.



Scheme 3-7: **Oxidation of thiolated sporopollenin (SpECs)**

3.6 Experimental

3.6.1 General

Spores (*L. clavatum*) used for sulfonated sporopollenin were from Sporomex, UK. The results of elemental analyses were obtained from Fisons Carlo Erba EA 100 C H N S machine by Mrs Carol Keneddy. The ICP-MS experiment was carried out by Mr Bob Knight by digesting the sample in super-purity spectroscopy grade nitric acid using a CEM MARS 5 microwave assisted reaction system and the sample was diluted with the use of Ultra High Quality (UHQ) water. Finally, the quantity of the sulfur content was measured on a Perkin Elmer Optima 5300DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and calibrated with Romil PrimAg*-plus certified element reference solutions. All the above-mentioned analyses were carried out in the Department of Chemistry at the University of Hull. SEM was carried out by faculty of science and engineering resource person; Mr Timothy Dunstan, on Carl Zeiss EVO-60. The UV-visible and pH were recorded on 6705 UV/Vis. Spectrophotometer and Mettler Toledo FE20/EL20 respectively.

SpEC samples were dried over calcium chloride under vacuum, except where it is otherwise stated in the experimental procedure. All the solvents and chemicals in this work were used as purchased. Their purities were trusted on the integrity of the companies where they were purchased. When required solvents were dried over either 4 Å molecular sieves or sodium sulfate.

3.6.1 Analytical Quantification of Sulfur Content

Quantification of the sulfur content in the sulfonated and sulfated sporopollenin exine capsules was carried out either by combustion elemental analysis and / or inductively coupled plasma mass spectroscopy (ICP-MS) to know the percentage sulfur content in the sulfonated and sulfated sporopollenin exine capsules.

3.6.2 Extraction of SpECs

3.6.2.1 Preparation of phosphoric acid hydrolysed SpEC (PHS)

Raw spores of *Lycopodium clavatum* (200 g) were stirred with acetone (900 mL) at 60 °C under reflux for 4 hours. The mixture was filtered through a sinter funnel (porosity grade 2) and vacuum dried overnight to a constant weight. The recovered spores were treated with 6 % aqueous potassium hydroxide (900 mL) and heated with continuous

stirring at 83 °C for 6 hours. The SpECs were filtered, the aqueous solution of 6 % potassium hydroxide (900 mL) and the solid SpECs heated again 6 % potassium hydroxide (900 mL) at 83 °C was continued for a further 6 hours. After this time the cooled suspension was filtered and the recovered SpECs were washed with deionized water (18 x 600 mL) until the filtrate was neutral, and then dried under vacuum overnight (17 hours). The dried solid residue was stirred with orthophosphoric acid (1000 mL) at 83 °C for 7 days. The mixture was filtered and washed with boiling (100 °C) distilled water (22 x 300 mL) until a clear neutral filtrate was obtained. The resulting SpECs were dried under vacuum overnight. The part dried SpECs was suspended in ethanol (800 mL) and stirred for 2 hours at 80 °C, filtered, washed with absolute ethanol (3 x 200 mL) and vacuum dried to a constant weight (22 hours). The extraction experiment was repeated five times (**Table *3.6-1***).

Table 3.6-1: Amount recovered and Results of Combustion Elemental Analysis of classical extracted SpECs

Sample	Amount of raw spores used (g)	Amount SECs recovered (g)	**Combustion Elemental Analysis**		
			% Carbon Content by weight	% Hydrogen Content by weight	% Nitrogen Content by weight
SpECs 1	200	50.72	66.54	8.61	0.00
SpECs 2	250	51.28	63.74	7.03	0.00
SpECs 3	250	58.72	67.97	9.19	0.00
SpECs 4	250	56.83	68.70	7.76	0.00
SpECs 5	150	46.65	65.23	8.40	0.00
SpECs 6	150	47.28	70.51	8.44	0.00

3.6.2.2 Preparation of acid hydrolysed sporopollenin (AHS)

Raw *L. clavatum* spores (50 g) were stirred with freshly prepared 9 M aqueous hydrochloric acid solution (300 mL) at 90 °C for 1 hour. The mixture was allowed to cool and filtered under vacuum through a sinter funnel (porosity grade 3). The residual sporopollenin exines were washed with deionized water (12 x 500 mL) until the filtrate was clear and neutral. The exines were then washed with absolute ethanol (100 mL) to remove the excess water. The residual exines were stirred and heated at reflux in absolute ethanol (400 mL) for 20 minutes. The mixture was filtered and this process was repeated

four times to completely remove water from the SpECs. The recovered SpECs were left under vacuum over night to obtain a constant weight. After drying, the AHS recovered was 18.06 g which showed combustion analysis result of C, 65.01 %; H, 7.96 %; and N, 0.00 %

3.6.2.3 Preparation of base hydrolysed sporopollenin (BHS)

Raw *L. clavatum* spores (50 g) were stirred with freshly prepared 6 % w/v aqueous sodium hydroxide (300 mL) at 85 °C for 24 hours. The mixture was filtered through a sintered funnel (porosity grade 3) and the SpECs were washed with deionized water (24 x 400 mL) until the filtrate was clear and neutral. The SpECs were then washed with absolute ethanol (400 mL) to remove excess water. The residual sample was stirred for four hours in absolute ethanol (400 mL) under reflux, filtered and washed twice with absolute ethanol (2 x 400 mL). The recovered SpECs were left in a desiccator under vacuum over night to obtain a constant weight. After drying, the 14.04 g BHS which showed combustion analysis result of C, 58.90 %; H, 8.65 %; and N, 0.50 % was recovered.

3.6.2.4 Treatment of AHS & BHS with orthophosphoric

The dried AHS (3 g) was stirred with orthophosphoric acid (21 mL) at room temperature for 5 days. The mixture was filtered and washed several times with excess hot distilled water until a colourless filtrate was obtained that was neutral to pH paper. The resulting SpECs were dried under vacuum overnight. The dried SpECs were suspended in ethanol (50 mL) and stirred for 2 hours at 80 °C under reflux. The SpECs were recovered by filtration, washed with absolute ethanol (3 x 10 mL) and vacuum dried to a constant weight (22 hours) to provide PAHS.

PBHS was obtained from BHS using the above procedure except that BHS (3 g) was stirred and heated in orthophosphoric acid (21 mL) at 80 °C for 5 days (**Table *3.6-2***).

Table 3.6-2: Amount recovered and results of elemental and ICP-MS Analysis for PAHS and PBHS

Sample	Amount of AHS and BHS (g)	Amount recovered (g)	% weight C	% weight H	% Weight N
PAHS	3.00	2.99	64.15	8.03	0.00
PBHS	3.00	2.25	64.73	8.61	0.00

3.6.3 Sulfonation of sporopollenin exine capsules

Dry dichloromethane (200 ml) was added to SpECs (10 g) and cooled in ice-water bath. Chlorosulfonic acid (30 ml) was added drop-wise to the stirred mixture. The flask was removed from the ice-water bath and the mixture was stirred for 5 hours at room temperature. The mixture was filtered through a sintered funnel (porosity grade 2). The recovered chlorosulfonic acid treated sporopollenin exine capsules were washed with dichloromethane (200 mL) and then with water (4 x 2L) until the filtrate was colourless and neutral. The sulfonated SpECs were mixed with 2 M aqueous sodium hydroxide (500 mL), checking that the pH was still basic, and stirred at room temperature overnight. The sulfonated particles were filtered, and the residue was washed with water until the filtrate was clear and neutral (4 x 200 mL). The base treated sulfonated SpECs were suspended in 2 M aqueous hydrochloric acid (300 mL) and stirred for 3 hours at room temperature. The SpEC derivatives were recovered by filtration and the particles washed thoroughly with deionized water (5 x 500 mL) until a clear filtrate that was neutral was obtained. The particles were left in the vacuum funnel overnight. The air-dried sulfonated SpECs were dried over phosphorus pentoxide under vacuum for 24 h, or until a constant weight was obtained.

The above procedure was repeated for the sulfonation of AHS, BHS, PAHS and PBHS to obtain the sulfonated sporopollenin exine particles tagged AHS-SO_3H, BHS-SO_3H, PAHS-SO_3H and PBHS-SO_3H respectively (**Table *3.6-3***). The volume of the reagent used was determined by the amount of the starting material.

Table 3.6-3: Amount recovered from sulfonation experiment and results elemental and ICP-MS Analysis

Sample	Starting SpECs (g)	Sulfonated SpECs (g)	% weight C	% weight H	% weight N	% weight S	ICP-MS (%) S
SpEC-SO_3H1	10.00	11.16	39.42	4.81	0.03	10.62	10.47
SpEC-SO_3H2	10.00	12.01	40.11	5.27	0.00	10.31	-
AHS-SO_3H	5.00	4.11	38.16	5.30	0.00	11.75	10.97
BHS-SO_3H	5.00	5.09	36.55	5.21	0.07	12.87	11.99
PAHS-SO_3H	1.00	1.13	34.75	4.46	0.00	9.67	-
PBHS-SO_3H	1.00	1.40	36.56	4.57	0.00	9.48	-

3.6.3.1 Determination of loading of acidic functional groups on SpECs and Sulfonated SpECs using volumetric analysis

Sporopollenin exine capsules (0.400 g), accurately weighed to 3 decimal places, were mixed with 80 mL of 0.50 M aqueous sodium hydroxide solution and stirred 4 hours. The mixture was filtered through a small pad of Celite® (0.5 g) to remove the exines and the filtrate was collected. A sample of the filtrate was accurately measured with pipette (20.0 mL) and titrated against 0.50 M hydrochloric acid aqueous solution with methyl orange as an indicator. The titration was carried out three times.

The acid test was also carried out by stirring of SpECs in the base for 2 hours and 16 hours to compare the result of titrations.

The above procedure was used for SpECs, AHS, BHS, PAHS and PBHS. The same procedure was also used to test the acidity of the sulfonated sporopollenin exine capsule (i.e. SpEC-SO_3H, AHS-SO_3H, BHS-SO_3H, PAHS-SO_3H, PBHS-SO_3H) but the quantities were reduced to 0.200 g (**Table *3.6-4***).

Table 3.6-4: Acidity strength of the proton of SpECs and its sulfonated derivative (SpEC-SO_3H)

Total loading of acidic functional groups on SpECs	Time (Hours)		
	2	4	16
SpEC-SO_3H (mmol / g)	15.33	19.00	15.33
SpEC (mmol / g)	5.50	7.00	6.83
Sulfonic acid groups loading (mmol / g)	9.83	12.00	8.50
AHS-SO_3H (mmol / g)	6.67	8.33	15.00
AHS (mmol / g)	1.50	1.83	7.00
Sulfonic acid groups loading (mmol / g)	5.10	7.50	8.00
BHS-SO_3H (mmol / g)	4.67	7.0	10.33
BHS (mmol / g)	-	-	2.17
Sulfonic acid groups loading (mmol / g)	-	-	8.16
PAHS-SO_3H (mmol / g)	11.00	15.00	
PAHS (mmol / g)	5.60	8.50	
Sulfonic acid groups loading (mmol / g)	5.40	6.50	
PBHS-SO_3H (mmol / g)	12.67	15.67	
PBHS (mmol / g)	6.50	7.50	
Sulfonic acid groups loading (mmol / g)	6.17	8.17	

Note: (1) Time is the number of hours used to stir SpECs and their sulfonated derivatives in deionised water at room temperature (2) mmol / g is the concentration is the total loading of acidic functional groups or sulfonic acid groups in mmol per 1 gram of SpECs.

3.6.3.2 Determination of the pH of suspensions of sulfonated SpECs and Amberlyst 15 in water

The catalyst (0.2 g) (sulfonated sporopollenin or Amberlyst 15) was stirred in deionized water (40 mL) at room temperature for 16 hours. The mixture was centrifuged and the acidity of the filtrate was tested using both indicator paper and pH meter (**Table *3.4-3***).

3.6.4 Sulfation of sporopollenin (SpEC-OSO_3H)

Dry dichloromethane (previously dried over 4 Å molecular sieves) was added to SpEC (10 g) in a reaction flask cooled in an ice-water bath. Chlorosulfonic acid (30 mL) was added drop-wise to the stirred mixture. The flask was removed from the ice-water bath and the mixture was stirred for 5 hours at room temperature. The mixture was filtered through a sintered funnel (porosity grade 2). The recovered chlorosulfonic acid-treated SpECs were washed with dichloromethane (200 mL) and then with water (4 x 2 L) until the filtrate was colourless and neutral. The particles were left to air dry in the funnel connected to the vacuum overnight. The dried sulfated SpECs were dried over phosphorus pentoxide under vacuum until a constant weight was obtained (**Table *3.6-5***).

Table 3.6-5: The elemental and ICP-MS analysis result for sulfated sporopollenin

SpECs	**Combustion Elemental Analysis**					**ICP-MS**	
	% C Content	**% H Content**	**% N Content**	**%S Content**	**S in mmol/ g**	**% S Content**	**S in mmol/ g**
SpEC- OSO_3H 1	23.11	2.49	0.00	4.42	1.38	8.07	2.58
SpEC- OSO_3H 2	42.72	5.85	0.12	9.04	2.82	10.14	3.24
SpEC- OSO_3H 3	40.94	5.87	0.14	10.88	3.89		
SpEC- OSO_3H 4	39.89	5.29	0.12	12.51	3.90		

3.6.5 Determination of microstructure of SpECs and its sulfonated derivative using SEM

The SpECs extracted by classical method and its sulfonated derivative were subjected to SEM (Carl Zeiss EVO-60) to determine and identify the difference in their microstructure.

3.6.6 Production of brominated and thiolated of SpECs

3.6.6.1 Preparation of brominated SpECs:

SpECs (3 g) were suspended in a solution of bromine (12 mL) in dry dichloromethane (120 mL) and stirred overnight at 40 °C. Bromosporopollenin was recovered by filtration through a sinter funnel (porosity 3) and washed extensively with DCM until the filtrate was colourless (10 x 20 mL), then washed with methanol (10 x 10 mL), water (10 x 10 mL), 2 M HCl (5 x 10 mL), water (5 x 10 mL), ethanol (3 x 10 mL) and diethyl ether (3

x 10 mL) in that order, and finally dried under vacuum over phosphorus pentoxide to a constant weight (**Table *3.6-6***). The experiment was carried out twice.

Table 3.6-6: Mass recovered and results of Elemental Analysis of brominated sporopollenin

SpECs	Amount of SpECs used (g)	Vol. of Br_2 used	Amount recovered	Elemental Analysis		
				% weight C	**% weight H**	**% weight N**
SpEC-Br 1	3	12 mL	2.78 g	30.97	3.15	0.10
SpEC-Br 2	3	12 mL	2.71 g	30.88	3.09	0.09

3.6.6.2 Preparation of thiolated sporopollenin

Potassium *O*,*O*-diethyl thiophosphate (800 mg) was added to brominated sporopollenin (500 mg) suspended in dry *N*,*N*-dimethylformamide (25 mL) (previously dried over 4 Å molecular sieves) at 100 °C, and left for 2 hours. After cooling, the SpECs were filtered and washed with water (5 x 10 mL), methanol (3 x 10 mL) and diethyl ether (3 x 10 mL), and dried under vacuum over phosphorus pentoxide to a constant weight. The experiment was repeated five times (**Table *3.6-7***) in which one experiment was on an increased scale, with the quantities and volumes above also increased correspondingly.

Table 3.6-7: Production of thiolated SpECs

SpECs	Amount of SpECs used (g)	Amount Recovered (g)	Elemental Analysis			
			% weight C	**% weight H**	**% weight N**	**% weight S**
SpEC-SH 1	0.50	0.406	39.21	4.10	0.21	3.65
SpEC-SH 2	0.50	0.324	39.36	3.99	0.44	4.14
SpEC-SH 3	0.50	0.380	39.43	4.13	0.28	4.11
SpEC-SH 4	0.50	0.408	33.30	3.46	0.28	3.24
SpEC-SH 5	0.21	1.647	38.74	4.10	0.00	3.47

3.6.6.3 Conversion of thiolated SpECs (SpEC-SH) into a sulfonated derivative (SpEC-SO_3H)

Thiolated SpECs (406 mg) were suspended in H_2O_2 (35 %) –acetic acid (20 mL; 1:1) and stirred for 12 hours at room temperature. The mixture was filtered and dried under vacuum over phosphorus pentoxide to a constant weight (**Table *3.6-8***).

Table 3.6-8*:* Oxidation of thiolated SpECs

SpECs	Amount of Thiolated SpECs (g)	Amount Recovered (g)
SpEC-SO_3H	0.406 g	0.308

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Chapter 4: Application of sulfonated SpECs to heterocyclic chemistry

Heterogeneous catalysts using as bio-based solid supports have proven to be more effective in organic synthesis in terms of product yield and selectivity than their homogeneous counterpart.[1] Polymer-supported oxidant performed well in coupling the aromatic amine with aldehyde to yield benzimidazole product.[2,3] Therefore bio-based solid catalyst seems promising in the synthesis of benzimidazole.

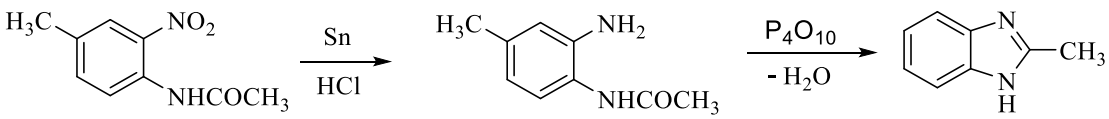
4.1 Benzimidazole and derivatives

Benzo[d]imidazole, or more commonly benzimidazole, is an aromatic organic compound composed of a benzene ring fused to the heterocycle imidazole. Benzimidazole is an important intermediate in the production of some other organic compounds, especially, those of biological[2,4] and pharmacological importance.[5-7] Benzimidazole is found to be an important nucleus in the production of antiviral[5,8,9] and other anti-infectious disease,[10-12] anticancer,[5,13,14] antiulcer,[6,15] antitumour,[11,16] anti-inflammatory[5] and anthelminthic[6] agents as well as the agents used in the treatment of physiological disorders and hypoglycemia.[5] It was also reported that some of the derivatives exhibit antihistamatic,[10,17] antibacterial,[11,18] analgesic,[14] anti-arrhythmic,[10] antihypertensive[4] properties etc.

4.2 Previous syntheses of benzimidazole

4.2.1 Synthesis of benzimidazole

Benzimidazole was first synthesised in 1872 by Hoebrecker. Hoebrecker selectively reduced the nitro group of 2-nitro-4-methylacetanilide to get 2-amino-4-methylacetanilide, and he subsequently dehydrated the latter to obtain 2,5-dimethylbenzimidazole (**Scheme 4-1**).[14,19,20]

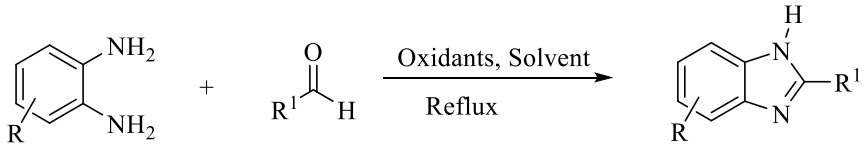


Scheme 4-1: **The Hoebrecker reaction for the synthesis of mono-substituted benzimidazoles**[14,20]

4.2.2 Further research on benzimidazole

Several researchers, after Hoebrecker, have devised, developed, and modified many different methods to synthesize benzimidazoles, with the aim of getting product of better purity, quality and high yield.[21] Therefore, there are various ways by which benzimidazole can generally be synthesized depending on the procedural methods and other reaction factors.[22] But most of these procedures are specifically based on the reaction of *o*-phenylenediamine, or its substituted derivative(s), and compound(s) with the carbonyl functional group. The reaction between *o*-phenylenediamine and carboxylic acids (or other carbonyl and related derivatives, such as nitrile, imidates or orthoesters)[5,22] is considered to be the standard and general method for the synthesis of benzimidazoles, but this reaction methodology often requires harsh condition for dehydration of an intermediate to form the target product.[5] However, the harsh condition does not reduce the tendency of requiring long time of reaction, generation of environmentally and unfriendly harmful by-products, low yield and formation of lumps during the reaction.[5,23]

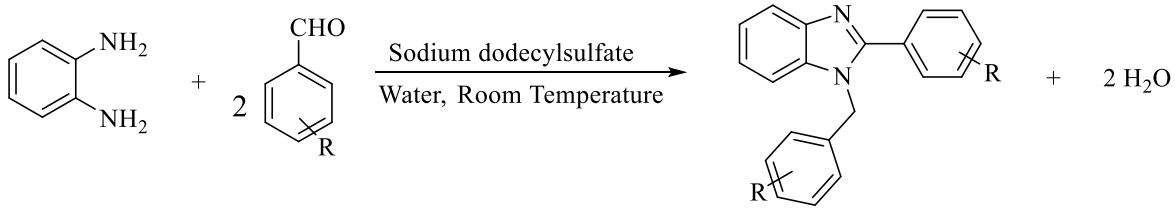
Oxidants[24-26] including $I_2/KI/K_2CO_3/H_2O$, metabisulfite and $Na_2S_2O_5$[24] have also been used as reagents in the synthesis of benzimidazole (***Scheme 4-2***). Most of the oxidants used as reagents in the synthesis of benzimidazole are toxic or can lead to the generation of toxic derivatives of by-products, which in turn can complicate the separation or purification process of the target product.[4,5]



R = Organic group or any substituet(s)
R^1 = Aromatic or aliphatic alkyl groups

***Scheme 4-2*: Synthesis of benzimidazole in the presence of oxidant catalysts**

Other researchers employed an organic salt, e.g. sodium dodecylsulfate (**Scheme 4-3**), to initiate the reaction process through the formation of Schiff base as an intermediate.[4]

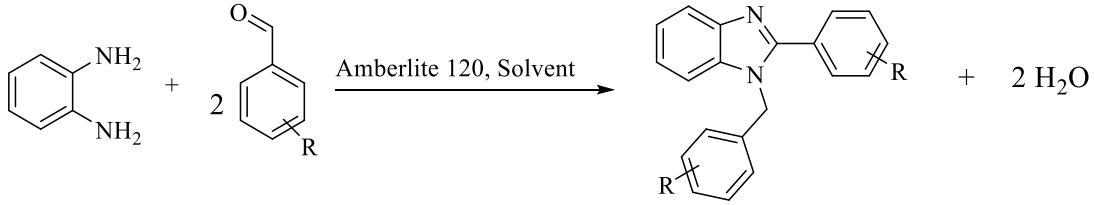


R = Aromatic or Aliphatic group

Scheme 4-3: **Synthesis of benzimidazole *via* SDS**

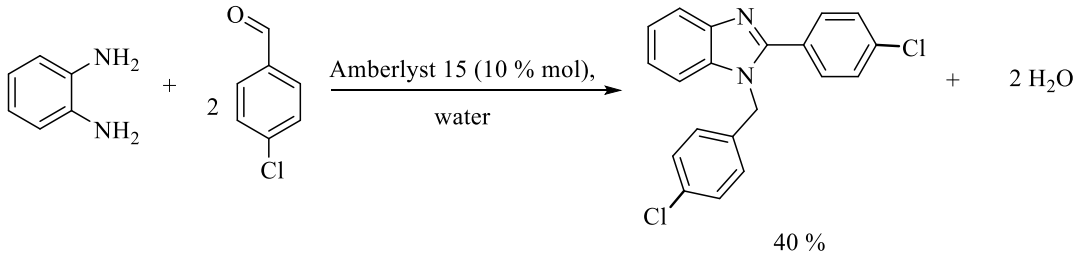
4.2.3 Synthesis of benzimidazole in the presence of heterogeneous catalysts

Reactions involving carboxylic acids are mostly useful for the production of 2-substituted derivatives of benzimidazole.[27] However, with the introduction of ion-exchange polymer or silica supported solid acid catalysts, disubstituted benzimidazoles can be produced by reacting *o*-phenylenediamine and an aliphatic or aromatic aldehyde. Some of the researchers applied homogeneous catalysts, whilst others applied heterogeneous catalysts such as sulphuric acid on silica, Dowex 50W, Amberlyst 15, or Amberlite IR-120 (**Scheme 4-4**).[28,29]



Scheme 4-4: **Solvent-mediated synthesis of benzimidazole in the presence of Amberlite 120**[28]

The syntheses of benzimidazole in the presence of Amberlite 120[28] (**Scheme 4-4**) and Dowex 50W[29] were successful with high yields. By contrast the product recovered from the water mediated synthesis of benzimidazole in the presence of Amberlyst 15 was as low as 40 % (**Scheme 4-5**)



Scheme 4-5: **Amberlyst 15 catalysed synthesis of 1,2-disubstituted benzimidazole**[29]

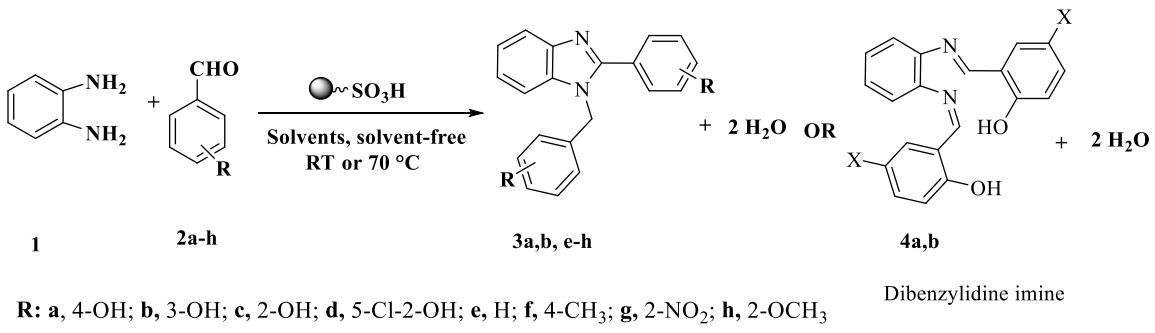
Solid acid catalysts have been reportedly applied to catalyse the synthesis of several derivatives of benzimidazoles from different aldehyde including 4-chlorobenzaldehyde. For example the synthesis of 1,2-di(4'-chlorophenyl)-1H-benzimidazole, using water as

the solvent medium, in the presence of Amberlyst-15 (10 % mol)[29] at 70℃ gave a 40 % yield, Silica-H_2SO_4 (30 %)[6] at 35 ℃ yielded a 50 % product, Dowex 50 (10 % mol)[29] at 70 ℃ gave 88 % yield, polystyrene sulfonic acid (10 % mol)[29] at 80 ℃ yielded 36 % benzimidazole product, and Silica-$HClO_4$ (10 % mol)[29] at 80 ℃ yielded 45 % product. L-proline (10 % mol)[12] was also used at 35 ℃ in chloroform to form 1,2-di(4'-chlorophenyl)-1H-benzimidazole.

4.3 Synthesis of 1,2-disubstituted benzimidazoles in the presence of sulfonated sporopollenin exine capsules (SpEC-SO_3H)

The application of an inexpensive sulfonated solid based on SpECs offers an attractive way of building a more environmentally friendly route for the synthesis of benzimidazoles. Mukhopadhyay *et al* were able to employ an aqueous medium while synthesizing 1,2-disubstituted benzimidazole using a Dowex 50W,[29] as a solid sulfonated polystyrene catalyst. It was therefore reasoned that sulfonated sporopollenin exine capsules (SpEC-SO_3H) could also be applied as a catalyst in the synthesis of di-substituted derivatives of benzimidazole.

The synthesis of benzimidazoles was investigated in the presence of (SpEC-SO_3H) in solvent-free conditions and using water, aqueous ethanol or ethanol mediated as solvent (**Scheme 4-6**).



R: a, 4-OH; **b,** 3-OH; **c,** 2-OH; **d,** 5-Cl-2-OH; **e,** H; **f,** 4-CH_3; **g,** 2-NO_2; **h,** 2-OCH_3

Scheme 4-6: **Reactions of ortho-phenylenediamine and benzaldehyde derivatives in the presence of sulfonated SpECs (SpEC-SO_3H) at various methods**

4.3.1 With solvent

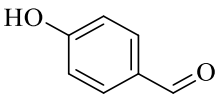
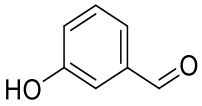
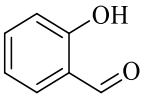
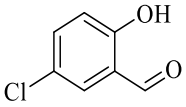
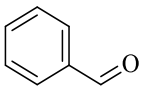
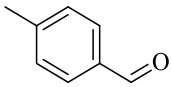
The use of less harmful solvents in organic synthesis is gaining major interest in sustainable chemistry.[29-33] Several researchers reported, with high yield, the synthesis of different organic compounds, including benzimidazole, in water and different aqueous media.[29-35] The waste generated from the experiment using water, as solvent medium, is

not as harmful to the environment.[31] In addition, the separation of the organic compounds synthesized in water medium can be simple.[31]

4.3.1.1 Synthesis in water medium

In this work, the oil bath was first/pre-heated to 70 °C. The sulfonated SpECs were suspended in water (15 mL) in a reaction flask and the benzimidazole precursors, *o*-phenylenediamine (4 mmol) and 4-hydroxybenzaldehyde (8 mmol), were added successively. The mixture was stirred for 8 hours to allow the reaction to reach completion (**Scheme 4-6**). At the end of the reaction, ethanol was added to the mixture to dissolve the product, much of which had absorbed to the surface of the catalyst. Ethanol was chosen because it is fully miscible in water and can also dissolve the final product. The SpEC-SO_3H was filtered from the mixture and the solvent from the filtrate was evaporated to obtain a crude product. Pure products obtained by recrystallisation of the crude using aqueous ethanol, were subjected to different analyses such as NMR, CHNS elemental analysis, and mass spectrometry. The data collected were compared with those obtained from the literature, and the results, as shown in **Table *4.3-1***, confirmed that SpEC-SO_3H was effective in catalysing the formation of 1,2-di-substituted products. SpEC-SO_3H proved to have acid catalytic ability in the production of this type of heterocyclic compound because the yields obtained at the end of each reaction are comparable to those in the literature who used solid supported sulfonated catalysts.

Table 4.3-1: Reaction of *o*-phenylenediamine and substituted benzaldehyde in water at 70 °C for 8 hours

Entry	Products	Aldehyde	SpEC-SO_3H (mmol)	Yield (%)
1	3a	 4-hydroxybenzaldehyde	0.26	79
2	3b	 3-hydroxybenzaldehyde	0.52	66
3	3c	 salicyaldehyde	0.52	78
4	3d	 5-chloro salicyaldehyde	0.52	75
5	3e	 benzaldehyde	0.52	43
6	3f	 Tolualdehyde	0.13	90

With the aim to increase the mole equivalent of the sulfonic acid group in the SpECs for the reaction process, double the amount of the SpEC-SO_3H in the initial reaction was used to catalyse the reaction process but it was observed that the product got stuck to the surface of the catalyst. Removing the product from the SpEC-SO_3H required a large volume of ethanol. The attachment of the product to the surface of excess SpEC-SO_3H may have possibly been caused by the formation of partial hydrogen bond between the nitrogen atom at the position 3 of the product (disubstituted benzimidazole) and the hydrogen atom of the sulfonic group or the oxygen of the sulfonic group and the hydrogen of the hydroxyl group at the para position of the substituent hydroxyphenyl groups or both the nitrogen atom and the hydroxyl group proton of the benzimidazole. (**Figure *4.3-1***)

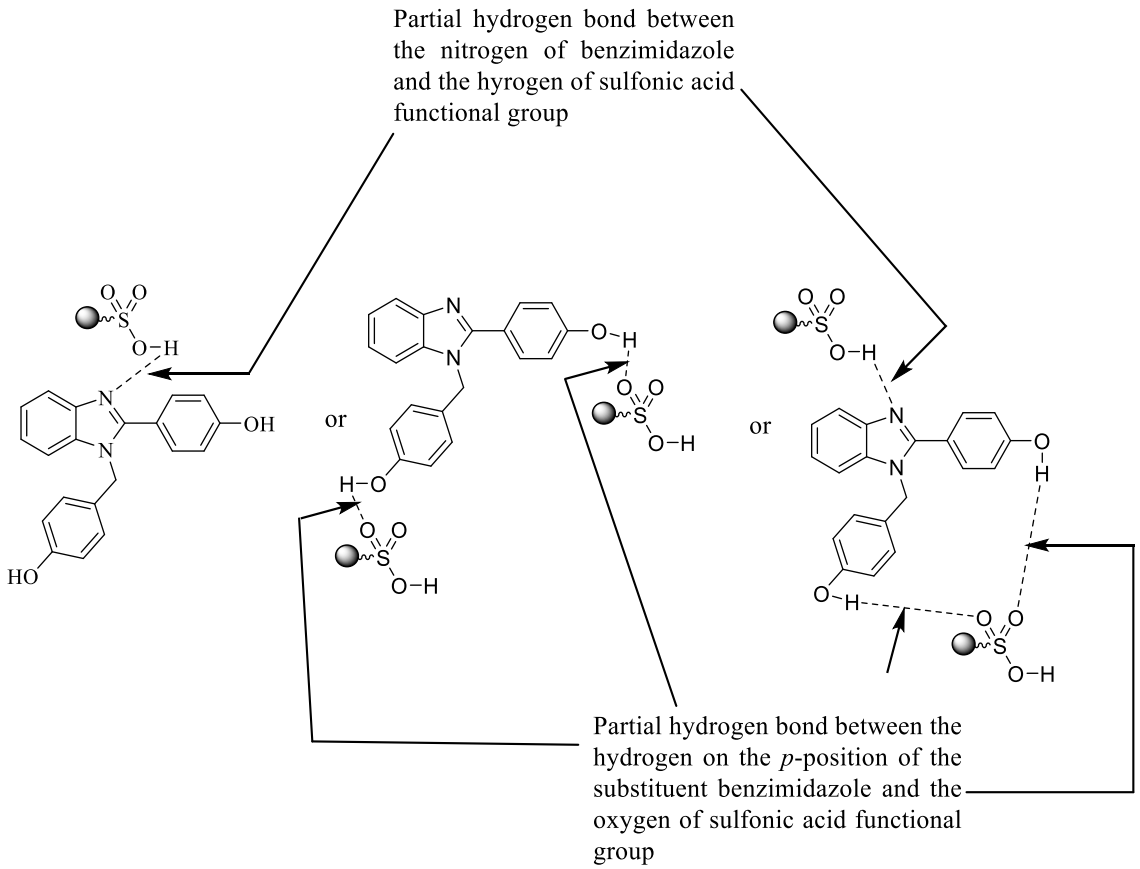


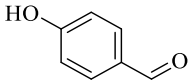
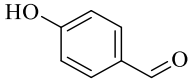
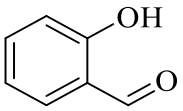
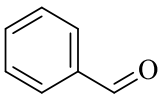
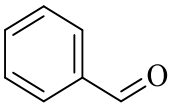
Figure 4.3-1: Possibility of hydrogen bond formation between benzimidazole and the sulfonic group of the excess SpEC-SO_3H

The reactions involving aldehydes with low solubility in water was a bit complex as they adhered to SpEC-SO_3H and a small amount of ethanol was added to mediate the reaction. For example, the reaction involving 2-methoxybenzaldehyde and 2-nitrobenzaldehyde, could not successfully be carried out or completed in water alone. The mixture of *o*-phenylenediamine in water rapidly adhered to the SpEC-SO_3H and became a deposit on the around the stirrer bar almost immediately after the addition of the aldehyde. In the reaction that led to the formation of compounds **3b**, **3e** and **3f** (**Table *4.3-1*, entries 3, 5 and 6**), the addition of aldehyde also caused the formation of lumps around the stirrer in less than an hour but with introduction of 0.5 mL of ethanol to the reaction mixture, the lump dissolved and the reaction continued. It is worth noting that 2-nitrobenzaldehyde is not soluble in water while the solubility of 2-methoxybenzaldehyde, benzaldehyde and 4-paratolualdehyde in water are relatively low.

4.3.1.2 Using ethanol or aqueous ethanol as solvent

The need to extract the product from the SpEC-SO_3H in water with ethanol necessitated the experiment to be carried out in ethanol. Especially the aldehyde which stuck to the SpEC-SO_3H. Ethanol, therefore, has the ability to dissolve both reactants, i.e. aldehyde and *o*-phenylenediamine and turn the entire mixture into solution in order to ease the reaction process. The procedure was slightly modified, and the reaction temperature was changed because the whole of the reactants had totally gone into solution in the ethanol. The reaction was carried out at room temperature throughout the period of the reaction process. Here, SpEC-SO_3H (0.130 mmol equivalent) was suspended in ethanol (6 mL) at room temperature. *o*-Phenylenediamine (4 mmol) and benzaldehyde (8 mmol) were added successively to the suspension at that same temperature and the whole mixture was stirred for 7 hours and the reaction was monitored by thin layer chromatography. At the end of the reaction, the SpEC-SO_3H were filtered and the solvent was evaporated to obtain a crude product (**Scheme 4-6**). The crude product was purified by recrystallization in ethanol-water in a 1:1 ratio. The pure product was filtered and dried under vacuum. Benzaldehyde was also used and the results are reported in **Table *4.3-2***. Surprisingly, the reaction involving 4-hydroxybenzaldehyde in ethanol was complete in 2 hours. This shows that the hydroxyl substituent at the *para* position on its phenyl ring contributed to its solubility in the ethanol. The hydroxy group also contributed to the reactivity of the aldehyde because of the lone pair electrons donated by the oxygen of the hydroxy group to the aromatic ring resulting in super conjugation and increases the reactivity of aldehyde. Therefore, the high solubility and the reactivity of this type of aldehyde in ethanol enhanced the reaction process.

Table 4.3-2: SpEC-SO_3H catalysed formation of disubstituted benzimidazole in ethanol at room temperature

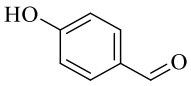
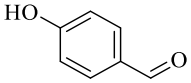
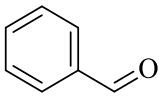
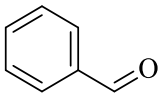
Entry	Products	Aldehyde	Catalyst (mmol)	Yield (%)
1	**3a**	 4-hydroxybenzaldehyde	0.13	71
2	3a	 4-hydroxybenzaldehyde	0.26	73
3	3c	 salicaldehyde	0.26	79
4	**3e**	 benzaldehyde	0.13	87
5	3e	 benzaldehyde	0.26	69

A smaller volume of ethanol, compared to the reaction that was carried out in water, was used to wash the SpEC-SO_3H at the end of the reaction. Despite the use of ethanol as the solvent for the synthesis of benzimidazole, the product yield obtained from the reaction involving SpECs with 0.26 mmol equivalent of sulfonic acid group as catalyst (table 4.3-2, entry 5) was lower than that recovered from the reaction catalyzed by SpEC with 0.13 mmol sulfonic acid group equivalent (**Table *4.3-2*, entry 4**).

To determine the effect of the temperature on the rate of reaction, the reaction mixture in ethanol was repeated and monitored at 70 ℃. For entry 3 in **Table *4.3-3***, the reaction reached completion at two hours earlier than the one that was carried out at room temperature and with better product yield (**Table *4.3-3***), the reaction ended at 5 hours. This shows that temperature had an influence on the speed of reaction and the catalytic activity of SpEC-SO_3H. It was observed that there is not much difference between the yields of the reaction at room temperature (**Table *4.3-3***) and those obtained after carrying out the reaction at 70 ℃. However, the synthesis of 3a (**Table *4.3-3*, entries 1 and 2**) at

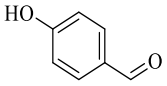
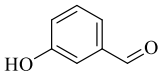
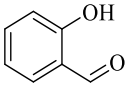
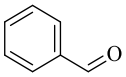
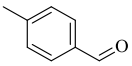
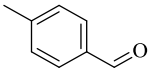
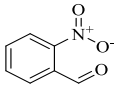
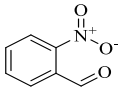
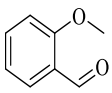
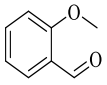
70 ℃ in ethanol was monitored with TLC and the reaction completed at 90 minutes. The reason may be that 4-hydroxybenzaldehyde is highly soluble in ethanol and the ability of heat to speed up the rate of reaction.

Table 4.3-3: Result of SpEC-SO_3H catalysed reaction of *o*-phenylenediamine and benzaldehyde in ethanol at 70 ℃

Entry	Products	Aldehyde	Catalyst (mmol)	Yield (%)
1	3a	 4-hydroxybenzaldehyde	0.13	64
2	3a	 4-hydroxybenzaldehyde	0. 26	67
3	3e	 benzaldehyde	0.13	89
4	3e	 benzaldehyde	0.26	74

To know the effect of solvent on the catalytic performance of SpEC-SO_3H and the entire reaction, the experiment was also carried out in an aqueous solution of ethanol. Ethanol was mixed with water in a 2:1 ratio and the mixed solvent was used to mediate the reaction. Here, SpEC-SO_3H (0.130 mmol equivalent) was suspended in the mixture of 1:2 water-ethanol (6 mL) at room temperature.

Table 4.3-4: Reaction of *o*-phenylenediamine and substituted benzaldehyde in aqueous ethanol

Entry	Products	Aldehyde	Catalyst (mmol)	Solvent ratio (H_2O: EtOH v/v)	Temp. (°C)	Time (hours)	Yield (%)
1	3a	 4-hydroxybenzaldehyde	0.26	1:2	R T	7	83
2	3b	 3-hydroxybenzaldehyde	0.52	1:2	R T	7	61
3	3c	 salicaldehyde	0.26	1:2	R T	7	90
4	3e	 benzaldehyde	0.26	1:2	R T	7	43
5	3f	 Tolualdehyde	0.26	1:5	R T	7	81
6	3f	 Tolualdehyde	0.52	1:2	70	8	70
7	3g	 2-nitrobenzaldehyde	0.26	1:7	R T	26	61
8	3g	 2-nitrobenzaldehyde	0.52	1:3	70	24	56
9	3h	 2-methoxybenzaldehyde	0.26	1:2	R T	7	47
10	3h	 2-methoxybenzaldehyde	0.52	3:5	70	24	6

This procedure was repeated with only ethanol as the reaction mediated solvent at room temperature. In each case, the crude products obtained were recrystallized with aqueous ethanol at 1:1 ratio.

At room temperature, the water solubility of the aldehyde used in the production of compound 3f (**Table *4.3-4***, **entry 5**) is very low, therefore more ethanol was added, until the ratio of water-ethanol was 1:5 to enhance its solubility and to make all the reactants to go in solution for ease accessibility of the acid site of the SpEC-SO_3H. However, for the heat-treated (70 ℃) production of **3f** (**Table *4.3-4***, **entry 6**), the solubility of the reactants was increased and thereby did not require beyond 1:2 water-ethanol ratio to proceed the reaction. The lack of solubility of 2-nitrobenzaldehyde in water made the production of compound 3g difficult. It required an increase in the ratio of ethanol to water and a longer reaction time before the reaction could be executed. The reaction at room temperature was carried out in 1:7 water-ethanol ratio for 26 hours (**Table *4.3-4***, **entry 7**) while the reaction at 70 ℃ was executed in the solvent medium of 1:3 water-ethanol ratio for the reaction period of 24 hours (**Table *4.3-4*entry 8**). Also, product 3h (**Table *4.3-4***, **entries 9** and **10**) was produced from 2-methoxybenzaldehyde, which is insoluble in water. The aldehyde adhered to SpEC-SO_3H and formed a lump in the reaction medium. The original intention of the reaction carried out at 70 ℃ (**Table *4.3-4***, **entry 10**) was to use only water as the solvent medium but due to the lack of solubility of the aldehyde, ethanol was added to make the solvent medium up to 3:5 water-ethanol ratio. Despite the addition of ethanol, the compound (**3h**) yield was as low as 6 %, even when the reaction mixture was heated for 24 hours. The yield of **3h** (**Table *4.3-4***, **entry 9**) produced at room temperature was relatively low compared to those obtained from other aromatic aldehydes. Generally, the low yield of **3h** may be attributed to the steric hindrance produced by the methoxyl group at the ortho position on the phenyl group of the aldehyde which may hinder the aldehyde from reacting with the amine.

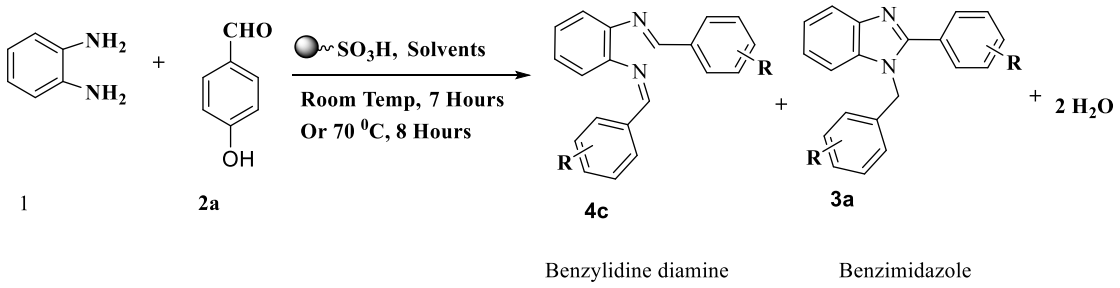
The results obtained from the synthesis of **3a** was compared to that of 2-(4-chlorophenyl)-1-(4-chlorophenylmethyl)-1*H*-benzimidazole[29] because both of them are attached to the para position on the aromatic aldehyde.

The percentage yield of **3a** was approximately 80 %, was obtained with the SpEC-SO_3H as the catalyst **(Table 4.3-5)**. This is comparable to the 84 % obtained using Dowex 50 as catalyst.[29]

Table 4.3-5: Results for the production of 3a using SpEC-SO_3H as a catalyst

Entry	SpEC-SO_3H mmol/g	Solvent ratio (H_2O: EtOH v/v)	Temp. ℃	Time (hours)	Yield (%)
1	0.78	1:0	70	8	43
2	0.52	1:0	70	8	80
3	0.26	1:0	70	8	79
4	0.13	1:0	70	8	70
5	0.52	1:2	R. T	7	79
6	0.26	1:2	R. T	7	83
7	0.13	1:2	R. T	7	82

Attempts to modify the procedure to get the mono 2-substituted benzimidazoles, using *ortho*-phenylenediamine and 4-hydroxybenzaldehyde in 1:1 ratio, led to the formation of the mixture of benzylidene intermediate and di-substituted products (**Scheme 4-7**).



***Scheme 4-7*: Synthesis of different derivatives of benzimidazole**

This result is in line with the result obtained by Sharma *et al*[28] when Amberlite was used to syntheses benzimidazole. It appeared that sulfonated catalysts were useful only for the synthesis of the di-substituted products.[28,29,36] It was reported that oxidation of dihydrobenzimidazole derivatives, formed from the reaction of aromatic aldehyde / aldehyde and *o*-phenylenediamine, is required before its transformation to 2-substituted bezimidazoles derivatives.[6] One of the chemical constituent of SpECs is known to be phenolic compounds,[37] which is an antioxidant. Therefore, SpECs have antioxidant properties[38] which may have been responsible for inhibiting the oxidative formation of 2-substituted benzimidazoles derivatives.

As above, replacement of water with either aqueous ethanol or ethanol, as a solvent medium, resulted in the isolation of higher yields with lower amounts of catalyst (**Table**

4.3-5). This may be caused by the relative solubility of the reactants, especially the benzaldehyde derivatives, and the expected product in ethanol. Sharma *et al* reported a similar result when benzimidazoles are synthesised in aqueous ethanol in the presence of amberlite 120 which resulted in a higher yield of product than those that were synthesised in water.[28] This may be as a result of the much higher solubility of the aldehyde in ethanol compared to water.

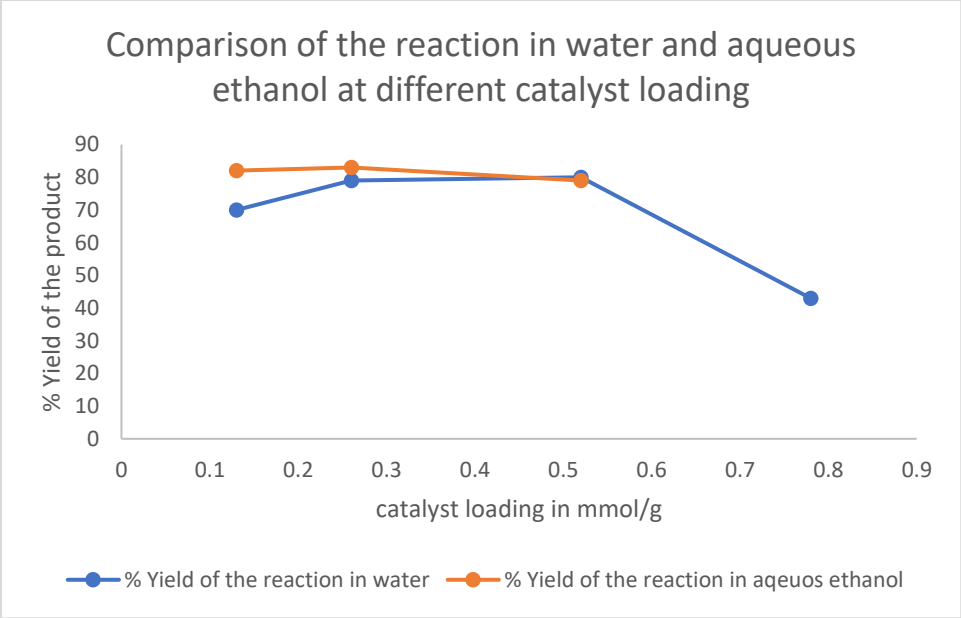


Figure 4.3-2: Comparison of the yields of the benzimidazole 3a in water or ethanol with catalyst loading of SpEC-SO_3H

Also, the yield increased by 4 % even if the amount of catalyst was reduced from 0.52 to 0.26 mmol equivalent (**Figure *4.3-2***). The yield was almost the same with the latter when 0.13 mmol equivalent of catalyst loading was used. For example, the yield of the product obtained in the presence of 0.13 mmol equivalent of SpEC-SO_3H was higher than the yield obtained when an increased amount of the catalyst of 0.52 mmol equivalent was used to catalyse the reaction. This result was similar to the observation of Sharma *et al*[28] that an increase in the amount of Amberlite 120 in the synthesis of disubstituted benzimidazole did not increase the yield of the product. However, it should be noted that the catalytic activity of Amberlite 120 is different from that of the SpEC-SO_3H because with Amberlite, the maximum amount of the yield was relative to certain amount of the catalyst and anything lesser will cause a decline in the amount of the product yield. Qian *et al*[39] that the same observation, as reflected by catalytic activity of SpEC-SO_3H, was recorded, but the catalyst involved in their work was nano ZnO. It was also observed that,

when a large amount of the SpEC-SO_3H catalyst was introduced into the reaction, the products got stuck to the catalyst. With the use of large amount of solvent, it might be possible to remove the product from the catalyst, but this would be expected to lead to a slower reaction due to decreased concentration, as well as the generation of more waste than necessary.

It is noteworthy that procedures involving the use of aqueous ethanol or ethanol as solvent, carried out at room temperature, gave yields that were comparable to those produced by heat and in water alone. (**Figure *4.3-2***)

It is thought that the high hydrophobicity[40] of the sporopollenin extracted by the *ortho* phosphoric acid method can limit the interaction between the SpEC-SO_3H catalyst and the reactant dissolved in water, and this contributes to the slower reaction rate. However, application of heat and serious agitation of the mixture increased the interaction between the catalyst, solvent and the reactants.

Table 4.3-6: Reaction of ortho-phenylenediamine and benzaldehyde in ethanol

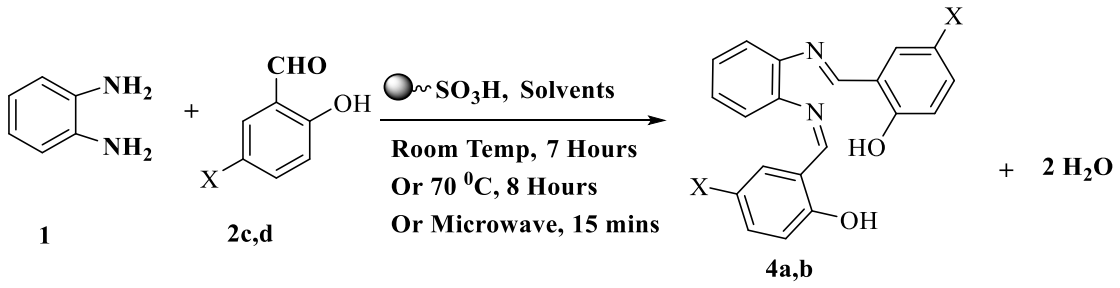
Entry	Products	Catalysts (mmol)	Temperature ℃	Time (hours)	Yield (%)
1	3e	0.13	R T	7	87
2	3e	0.13	70	5	89
3	3e	0.26	R T	7	69
4	3e	0.26	70	5	74

Catalysing the reaction involving benzaldehyde and *ortho*-phenylenediamine by SpEC-SO_3H in depends on the reaction solvent medium because benzaldehyde is more soluble in ethanol than in water. The reaction in high polarity solvent resulted in lower yield, as shown in the **Figure *4.3-2*** above, even when the reaction was carried out at higher temperature.

It was observed from the synthesis of **3e** in ethanolic medium using different mmol equivalent of SpEC-SO_3H, as shown in the **Table *4.3-6*** above, that an increase in temperature has a significant effect on driving the reaction to completion. The reaction carried out at 70 ℃ in the presence of SpEC-SO_3H catalyst went to completion 2 hours

earlier than the one carried out at room temperature as shown in **Table *4.3-6***. The effect of the amount of the catalyst on the reaction process cannot be over emphasized, the intermediate product got stuck on the catalyst and thereby inhibits the forward moving of the reaction when the 0.26 mmol equivalent of the catalyst was used for the reaction. It can be concluded that the higher the amount of the catalyst, the lower the yield of the product that was obtained.

4.3.1.3 Reaction leading to formation of benzylidine diamine

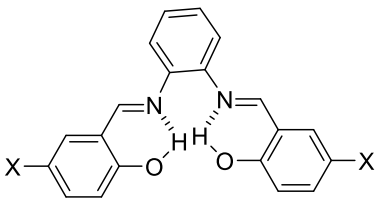


Dibenzylidine imine

Where X = H for Salicyaldehyde
or X = Cl for 5-Chlorosalicyaldehyde

Scheme 4-8: **Reaction of salicylaldehyde or derivative with *ortho*-phenylenediamine**

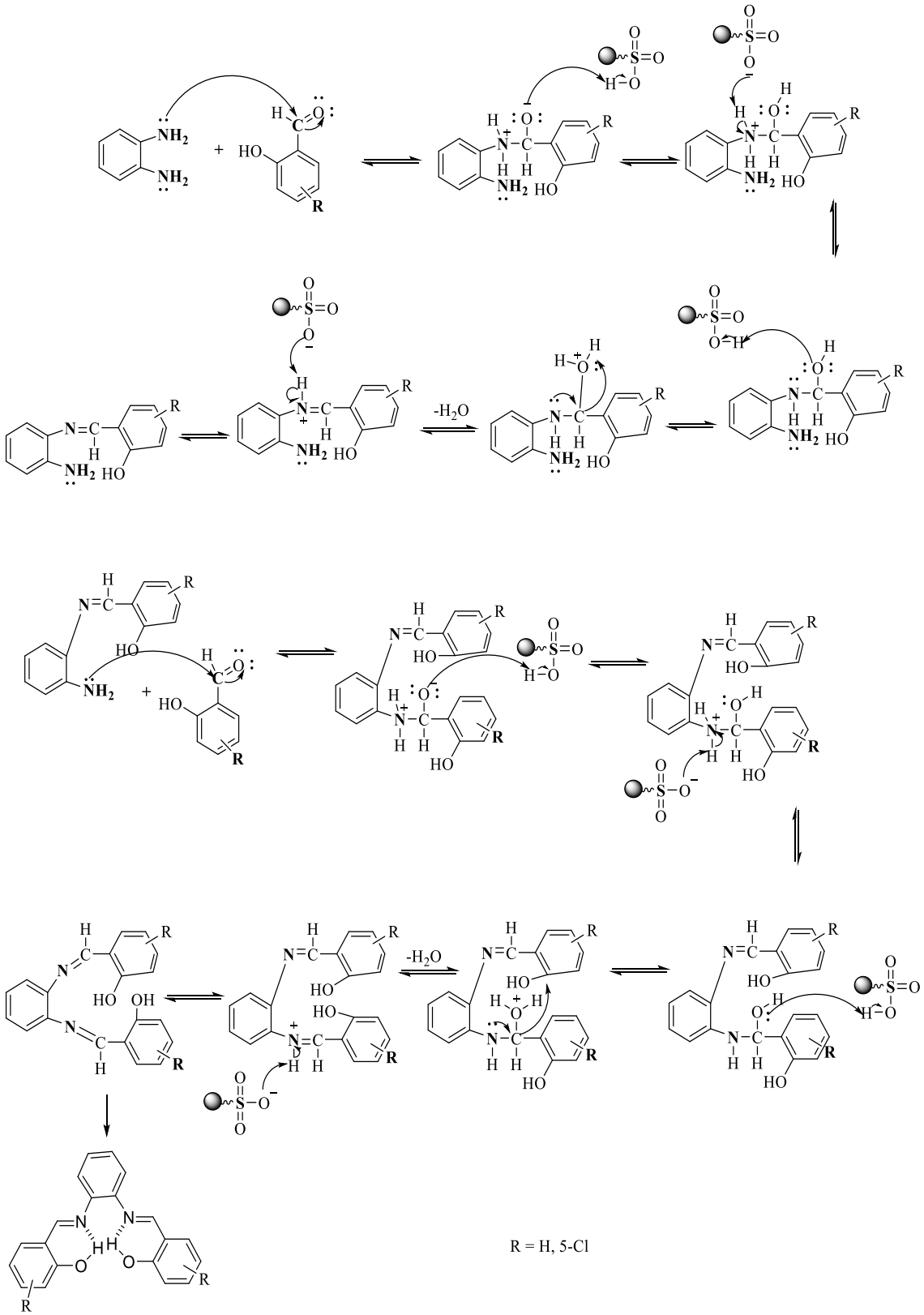
The reactions of *o*-phenylenediamine and either salicylaldehyde or 5-chlorosalicylaldehyde resulted in the formation of the dibenzylidene imines (**Scheme 4-8**). Even when the reaction was heated at 70 ℃ for 8 hours, only diimine was obtained (**Scheme 4-8**). The reaction was also carried out in a microwave reactor (water, 70 ℃, 200 W and 200 PSI) but the ^{1}H NMR spectrum of the resultant product indicated that the reaction of salicylaldehyde and *ortho*-phenylenediamine led to the formation of diimine. The difficulty in getting the reaction to completion was presumed to be due to the formation of hydrogen bond between the hydrogen of the hydroxyl atom at the *ortho* position on the benzene ring and the nitrogen atom of the benzylidene bond. (**Figure *4.3-3***).



X = H or Cl

Figure 4.3-3: Hydrogen bond formation between heteroatom and hydrogen atom

The reaction mechanism that led to the formation of dibenzylidene amine is shown in **Scheme 4-9**.

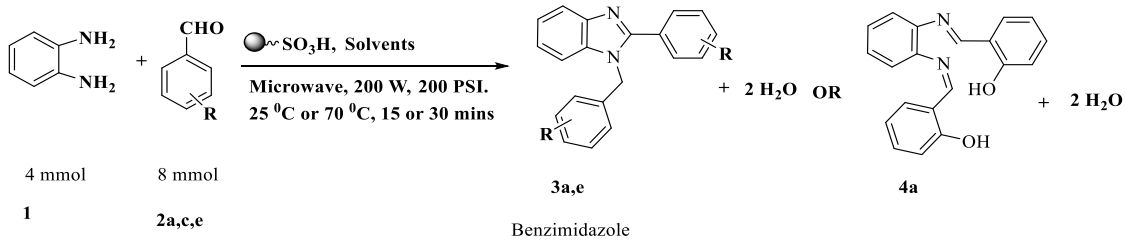


Scheme 4-9: **Mechanism of the dibenzylidene imine**

Table 4.3-7: % Yields of dibenzylidene imines

Entry	Product	Catalyst (mmol)	Solvent ratio (H_2O: EtOH v/v)	Temp. (°C)	Time (hours)	Yield (%)
1	3c	0.52	1:0	70	8	78
2	3c	0.26	1:2	R T	7	90
3	3c	0.26	0:1	R T	7	79
4	3d	0.52	1:0	70	8	75

4.3.1.4 Microwave experiment



Scheme 4-10: **Microwave reactor in the synthesis of benzimidazole from benzaldehyde and derivatives of benzaldehyde**

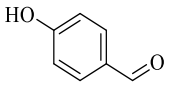
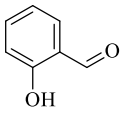
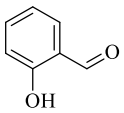
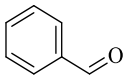
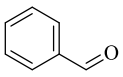
Organic reactions carried out by conventional heating methods are most often tedious, require long reaction time and the use of excess solvents.[41] In the past few decades, microwave heating device was introduced to induce organic synthesis and it has been demonstrated to be effective in the heating of chemical reactions through microwave irradiation without the need for heat conduction.[42,43] Under the microwave heating system, the catalyst and the substrates are directly heated and induced,[44] without the transfer of heat through any medium, by the irradiation of the microwave.[43] Microwave irradiation is a green heating method that enhance the selectivity and activity of the catalyst by increasing the rate of reaction[41,42] and also by reduction of the reaction time.[41,43] Introduction of microwave reactor enables the researchers to strictly control the temperature of the reaction and perform organic reactions at a precise temperature.[43] It was therefore reported that the heat produced by microwave irradiation are always uniform throughout the reaction mixtures and period.[41] Also, the microwave-assisted reaction often lead to the product of high yield and of better purity than those obtained from conventional heating.[41,44]

Use of a microwave reactor was applied to the synthesis of benzimidazoles **3a**, **3c** and **3e** in water and in ethanol. This investigation involved the reaction of *ortho* phenylenediamine with three different aromatic aldehydes in water or in ethanol viz; 4-

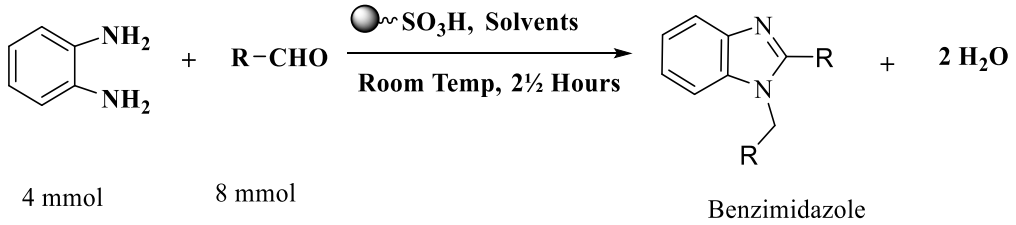
hydroxybenzaldehyde (in water); salicaldehyde (in water or ethanol); benzaldehyde (in water and ethanol). The yields obtained from the microwave heated reactions were comparable to the conventionally heated route (**Table *4.3-8***).

Sporopollenin is thermally stable and can withstand heat up to 400 ℃ without undergoing degradation.[1,45-47] Therefore, its sulfonated derivative can be subjected to high temperature and will function properly without degradation.

Table 4.3-8: Microwave reactor in the reaction of Ortho-phenylenediamine and benzaldehyde and derivatives of benzaldehyde

Entry	Product	Aldehyde	Catalyst mmol	Solvent	Temp ℃	Time	Yield %
1	3a	 4-hydroxybenzaldehyde	0.26	Water	70	15 mins	77
2	3c	 Salicylaldehyde	0.26	Water	70	15 mins	81
3	3c	 Salicylaldehyde	0.26	EtOH	25	15 mins	90
4	3e	 benzaldehyde	0.26	Water	70	30 mins	83
5	3e	 benzaldehyde	0.26	EtOH	25	15 mins	89

4.3.1.5 Reaction of *ortho*-phenylenediamine with aliphatic aldehyde in the presence of SpEC-SO_3H



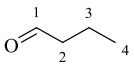
4 mmol 8 mmol Benzimidazole

Scheme 4-11: **Reaction aliphatic aldehyde with *ortho*-phenylenediamine**

The reactions involving aliphatic aldehydes and *o*-phenylenediamine were used to test the activity of SpEC-SO_3H in the synthesis of benzimidazole. In this work, the synthesis of benzimidazole involved the use of both primary and secondary aliphatic aldehydes. The primary aldehyde was butyraldehyde and isobutyraldehyde was the secondary aldehyde used in the experiment. Benzimidazole was produced by mixing 4 mmol *o*-phenylenediamine and 8 mmol aliphatic aldehyde in ethanol in the presence of SpEC-SO_3H. The reaction time was shorter than the reaction involving aromatic aldehydes. The products were purified by column chromatography and the yields are recorded in **Table *4.3-9***.

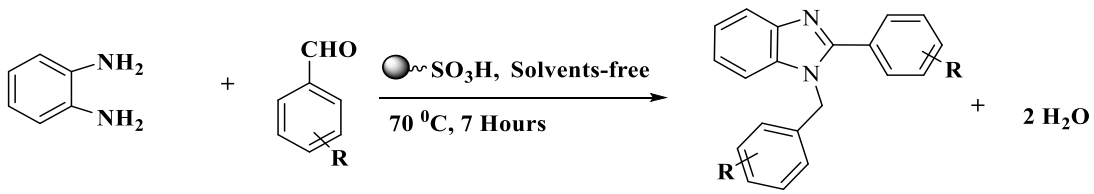
It was observed that the secondary aldehyde gave a product of higher yield than the primary aldehyde. The primary aldehyde is more reactive and highly prone to acid catalysed aldol self-condensation. The low yield obtained from this reaction may be due to the possibility of the aliphatic aldehyde to react with itself in the presence of an acid catalyst to form aldol products as a by-product and thereby reduced the amount of the aldehyde available for the reaction.

Table 4.3-9: Synthesis of benzimidazole from aliphatic aldehyde in the presence of SpEC-SO_3H

Entry	Product	Aldehyde	Catalyst (mmol)	Solvent	Temp (°C)	Time (hours)	Yield (%)
1	3i	 Isobutyraldehyde	0.26	Ethanol	R T	2½	68
2	3j	 Butyraldehyde	0.26	Ethanol	R T	2½ Hours	58

4.3.2 Solvent free reaction of *o*-phenylenediamine and benzaldehyde

The desire to simplify the 'green' production of benzimidazoles via this method, led towards investigation of a solvent free synthesis. A 1:2 mmol ratio of *o*-phenylenediamine and benzaldehyde were mixed together in a reaction pot. SpEC-SO_3H (0.13 mmol/0.26 mmol) was added to the mixture. The mixture was heated at 70 °C for 7 hours and the progress of the reaction was monitored periodically by taking a small sample and using thin layer chromatography (**Scheme 4-12**).



4 mmol 8 mmol Benzimidazole

Scheme 4-12: **Solvent-free synthesis of benzimidazole from benzaldehyde**

The old bottle of liquid benzaldehyde used could have contained impurities such as benzoic acid, which may be formed through oxidation. The contaminant might negatively affect the rate of reaction, resulting in lower yield or hindering the reaction from taking place. Trace benzoic acid can be removed with aqueous 10 % sodium bicarbonate solution. Thus, a solution of benzaldehyde in dichloromethane was shaken with a cold saturated solution of sodium bicarbonate solution. The organic layer was then washed with water and dried with sodium sulfate. The separation of benzaldehyde from the dichloromethane was by distillation because of the difference in their boiling points. The % yield of the benzimidazole obtained from purified and 'impure' aldehyde was used to compare their ability in the reaction process. The results of the solvent-free synthesis were recorded in **Table *4.3-10***.

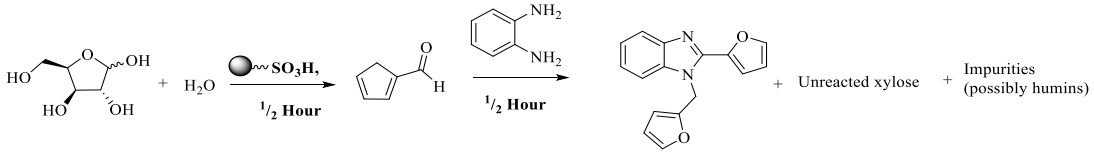
Table 4.3-10: Solvent free reaction of Ortho-phenylenediamine and benzaldehyde

Entry	Products	Catalyst (mmol)	Method	Time (hours)	Yield (%)	comment
1	3e	0.13	Melt (with stirring)	7	39	Aldehyde from the bottle
2	3e	0.26	Melt (with stirring)	7	58	Aldehyde from the stock
3	3e	0.13	Melt (with stirring)	7	37	Purified aldehyde
4	3e	0.13	Melt (without stirring)	7	68	Aldehyde from the stock
5	3e	0.13	Melt (without stirring)	7	52	Purified aldehyde

4.3.3 Attempted production of benzimidazoles using D-xylose as a precursor for furfural

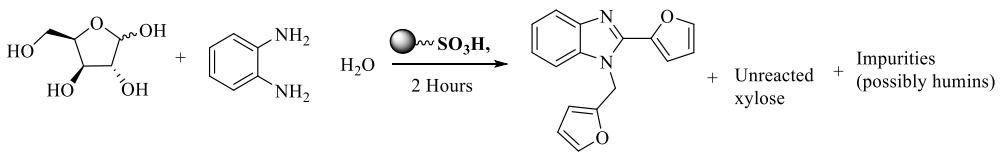
Verma *et al*[247] showed that reaction of *o*-phenylenediamine and D-xylose in presence of sulfonated graphitic carbon nitride (Sg-CN) as catalyst produced 2-(furan-2-yl)-1-(furan-2-ylmethyl)-1H-benzimidazole as Wang *et al*[48] showed that furfural could be produced from the SpEC-SO_3H acid catalyzed dehydration of xylose, a one-pot reaction similar to Verma *et al* was envisioned, using xylose and *o*-phenylenediamine in the presence of SpEC-SO_3H to produce 1,2-di (furan-2'-yl)-1H-benzimidazole. Thus D-xylose (1 g, 6.66 mmol) and SpEC-SO_3H (0.2 mmol equivalent) were suspended at 100 °C in water for 30 minutes, by which time furfural was produced in the reaction pot whereupon the 1,2-phenylenediamine (18.51 mmol equivalent) was added and heating continued with stirring for another 30 minutes. The reaction was monitored by TLC, in which the spots on the TLC plate were visualised by UV and potassium permanganate stain. At the end of the set time, when the *o*-phenylenediamine had been consumed, the mixture was cooled to room temperature and filtered in the sinter funnel through a small pad of Celite® to remove the catalyst. The mixture was concentrated by rotary evaporation to provide a crude product. The crude product was then dissolved in water. Normally, benzimidazoles products are not soluble in water, so the aim of using water

was to dissolve unreacted *o*-phenylenediamine and xylose. Therefore, the target product was extracted from the solution by ethyl acetate. The organic layer was dried over sodium sulfate, then concentrated under reduced pressure, and the product obtained was subjected to NMR analysis. The NMR analyses seemed to indicate the formation of di-substituted benzimidazole derivative of furfural, but many peaks of other organic materials were present in the spectrum. From the explanation of Wang *et al*,[48] the extra peaks in the NMR spectra of our product(s) seems to be an indication that humins had formed in the reaction. Humin is a polymeric compound composed of many furan rings bonded together, and is one of the compounds that usually results from the acid catalysed reactions of xylose (**Scheme 4-13**). Formation of humin when monosaccharide sugars are in contact with acid catalyst and in the presence of heat is not strange in sugar chemistry. Therefore, humin can possibly poison the activity of the catalyst by blocking the active site on the catalyst[49] and inhibit the reaction leading to the formation of benzimdazole. This undesired side product can also cause other unreacted sugar to degrade and thereby decrease the amount of substrate available to react with *o*-phenylenediamine in the reaction vessel.



Scheme 4-13: **Attempted one-pot synthesis of benzimidazoles from xylose**

A second attempt (**Scheme 4-14**) increased the reaction time to two hours, and the starting materials were dissolved first in water before the addition of SpEC-SO_3H. Observations from TLC and NMR were an improvement over the previous attempt, but the presence of sugar speaks was still prominent. The crude yield was also very low. Performing the reaction for five hours did not help as nothing significant was extracted from the reaction. Application of the microwave method for 15 minutes did not make any difference to the reaction, therefore the work was abandoned.



Scheme 4-14: **Optimised one-pot reaction of xylose and ortho-phenylenediamine**

4.3.4 Recyclability of SpEC-SO_3H

The recyclability of SpEC-SO_3H (0.26 mmol equivalent) was investigated in the synthesis of benzimidazole 3a from the reaction of *o*-phenylenediamine (4 mmol) and *p*-hydroxybenzaldehyde (8 mmol) in water at 70 ℃. The catalyst was reused for four subsequent times to catalyse reactions. At each time, the catalyst was recovered through vacuum filtration and washed thoroughly with ethanol until no product was remained on its surface. The catalyst was properly vacuum dried and desiccated in order to eliminate the remaining solvent. SpEC-SO_3H, when removed from the desiccator, was weighed and reuse/recycle in successive reactions. There was a decline in the mass of the SpEC-SO_3H, due to loss in handling during washing and weighing in the subsequent reactions. Despite the decline in the weight of SpEC-SO_3H, the amount of the reactants was not scaled down in the subsequent reactions. The mass amounts of the reactants i.e. *o*-phenylene diamine and *p*-hydroxybenzaldehyde, used in the first reaction were maintained in each of the later reactions. The result of the recyclability of SpEC-SO_3H was recorded in **Table *4.3-11***.

Table 4.3-11: Recyclability of SpEC-SO_3H in the synthesis of benzimidazole 3a from 4-hydroxybenzaldehyde

Entry	Product	Time (Hours)	Yield (%)
1	3a	4½	73
2	3a	4½	75
3	3a	4½	82
4	3a	4½	52

It was observed that there was an insignificant increase in the amount of the product obtained from the recycled catalyst in the third reaction. This might likely due to the decrease in the mass amount of the catalyst (SpEC-SO_3H) during the catalyst recovery, preparation of the catalyst for the subsequent reactions, handling and transfer of the catalyst. Also, the remnant product that stuck in the pore of the catalyst may not be properly or thoroughly washed off and as a result of that, it may have contributed to the amount of the subsequent products. However, at the fourth reuse of SpEC-SO_3H, the yield significantly declined to 52 %.

4.4 Experimental

4.4.1 General.

All the chemicals were purchased in the United Kingdom and their purities were trusted on the integrity of the companies where they were purchased. Precoated aluminum TLC silica gel 60 F254 plate was used for thin layer chromatography. ^{1}H and ^{13}C NMR spectra were obtained on a Jeol JEOL ECZ 400S spectrometer instrument at 400 and 100 MHz respectively. Melting points were determined on Fischer-Johns melting point apparatus with an open capillary. ESI-Mass spectra of the compounds were recorded on a Mass Spectrometer instrument.

4.4.2 Synthesis of benzimidazole from benzaldehyde or substituted benzaldehyde in water at room temperature

To a suspension of sulfonated sporopollenin SpEC-SO_3H (0.1 g, 0.26 mmol) in water (25 mL) were added successively *ortho*-phenylenediamine (4 mmol) and aromatic aldehyde (8 mmol). The mixture was stirred at 70 °C for 8 hours. At the end of the reaction, the mixture was allowed to cool down to room temperature. Ethanol (25 mL) was added to the cooled reaction mixture and the total mixture was filtered through a small pad of Celite® in the sinter funnel (porous grade 3) to remove the catalyst. The filtrate was kept at 4 °C overnight to obtain almost pure crystals of 1,2-disubstituted benzimidazole. The product was recrystallized again from aqueous ethanol (1:1) to obtain pure crystals.

4.4.3 Synthesis of benzimidazole from benzaldehyde or substituted benzaldehyde in aqueous ethanol at room temperature

To a suspension of sulfonated sporopollenin (SpEC-SO_3H) (0.1 g, 0.26 mmol) in aqueous ethanol (20 mL, 3:1 v/v) were added successively *ortho*-phenylenediamine (4 mmol) and the aromatic aldehyde (8 mmol). The total mixture was stirred for 7 hours at room temperature. The mixture, at the end of the reaction, was filtered through a pad of Celite® in the sintered funnel and the catalyst was wash thoroughly with ethanol. The filtrate was concentrated to obtain a crude product and recrystallized with ethanol and water in 1:1 ratio v/v. The product was then kept in the refrigerator till the following day

Note: Dichloromethane was use to wash the catalyst at the end of the reaction involving *o*-phenylene diamine with salicylaldehyde or 5-chlorosalicylaldehyde.

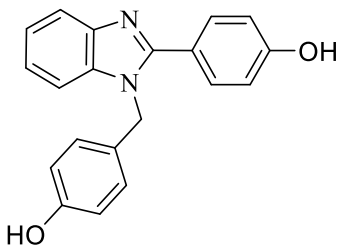
4.4.4 Solvents-free synthesis of benzimidazole from benzaldehyde

Sulfonated sporopollenin (SpEC-SO_3H) (0.1 g, 0.26 mmol) was added to the mixture of *ortho*-phenylenediamine (4 mmol) and the benzaldehyde (8 mmol). The total mixture was stirred for 7 hours at 70 ℃. The mixture, at the end of the reaction, was dissolved in ethanol and filtered through a sintered funnel to remove the catalyst. The catalyst was thoroughly washed with ethanol until no product remained on its surface. The filtrate was concentrated to obtain a crude product and recrystallized with ethanol and water in 1:1 ratio v/v. The product was later kept in the refrigerator for 3 days.

4.4.5 Microwave reactor in the Synthesis of benzimidazole from benzaldehyde or substituted benzaldehyde

A sample mixture of phenylenediamine (4 mmol), benzaldehyde or substituted benzaldehyde (8 mmol) and SpEC-SO_3H catalyst (0.13 mmol) in water (4 mL) was added to in a thick-walled microwave vessel fitted with a Teflon cap. The solution was heated in a CEM SP Benchmate microwave for 15 or 30 mins at 70 ^oC, at a maximum power of 200 W and maximum pressure of 200 PSI. The reaction was allowed to cool *via* a venting program and removed for purification immediately. Ethanol (5 mL) was added to reaction mixture and the total liquid was filtered through a pad of Celite® using sinter funnel (porous grade 3) to remove the catalyst (sulfonated sporopollenin). The filtrate was kept in the refrigerator (4 ℃) overnight to obtain almost pure crystals of 2-aryl-1-arylmethyl-1*H*-benzimidazoles or other substituted benzimidazole. The product was recrystallized again from aqueous ethanol to obtain a pure crystal.

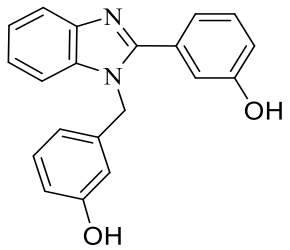
1-(4-Hydroxybenzyl)-2-(4-hydroxy-1-phenyl)-1-H-benzimidazole (3a) (as the NMR data compared with Sharma *et al*[28] and Fekri *et al*[50])



White solid, 79-83 %; **^{1}H-NMR (400 MHz, DMSO-D6)** δ 5.37 (s, 2H), 6.63 (d, J = 7.8 Hz, 2H), 6.83 (dd, J = 28.0, 7.8 Hz, 4H), 7.16 (s, 2H), 7.37 (d, J = 6.4 Hz, 1H), 7.57 (dd, J = 35.8, 7.3 Hz, 3H), 9.39 (s, 1H), 9.96 (s, 1H) (m, 2H), **^{13}C-NMR (101 MHz, DMSO-D6)** δ 47.6, 111.5, 116.0, 116.1, 119.4, 121.3, 122.4, 122.7, 127.6, 128.0, 131.1, 136.4,

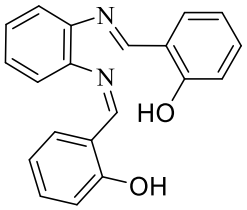
143.2, 154.1, 157.2, 159.4**,** *m/z* (**ESI-MS): [**M + 1] $^{+}$ 317; **elemental Analysis** $C_{20}H_{16}N_2O_2$ requires: C, 75.93 %; H, 5.10 %; O, 10.11 %; N, 8.86 %; found: C, 69.52 %; H, 5.37 %; N, 8.09 %.

1-(3-Hydroxybenzyl)-2-(3-hydroxy-1-phenyl)-1-H-benzimidazole (3b) (as the NMR data compared with Wan *et al*[22])



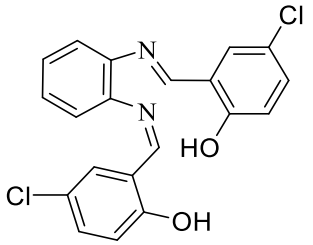
White solid, 61-66 % yield; **^{1}H-NMR (400 MHz, DMSO-D6)** δ 5.46 (d, J = 14.2 Hz, 2H), 6.35-7.81 (m, 12H), 9.70 (s, 2H), **^{13}C-NMR (101 MHz, DMSO-D6)** δ 48.0, 111.7, 113.2, 115.0, 116.6, 117.1, 117.5, 119.7, 122.7, 123.2, 130.4, 131.7, 136.4, 138.9, 143.1, 153.9, 158.2, *m/z* (**ESI-MS): [**M + 1] $^{+}$ 317 e**lemental Analysis** $C_{20}H_{16}N_2O_2$ requires: C, 75.93 %; H, 5.10 %; O, 10.11 %; N, 8.86 %; found: C, 74.93 %; H, 5.03 %; N, 8.63 %.

N,N'-bis(salicylidene)-o-phenylenediamine (3c) (as the NMR data compared with Deng *et al*[51])



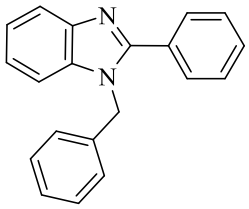
Yellow solid powder, 98 %; **^{1}H-NMR (400 MHz, $CDCl_3$)** δ 6.91 (dd, J = 7.5 Hz, 2H), 7.04 (d, J = 8.3 Hz, 2H), 7.23 (dd, J = 9.3, 4.2 Hz, 2H), 7.32-7.38 (m, 6H), 8.62 (s, 2H), **^{13}C-NMR NMR (101 MHz, $CDCl_3$)** δ 117.6, 119.1, 119.3, 119.8, 127.8, 132.4, 133.5, 142.6, 161.4, 163.8, *m/z* (**ESI-MS): [**M + 1] $^{+}$ 317, **elemental Analysis** $C_{20}H_{16}N_2O_2$ requires**:** C, 75.95 %; H, 5.10 %; O, 10.11 %; N, 8.86 %; found **:** C, 75.55 %; H, 5.16 %; N, 8.73 %.

N,N'-bis(5-chlorosalicylidene)-1,2-phenylenediamine (3d)



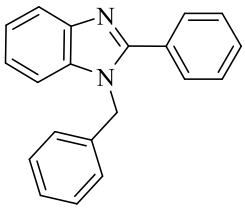
Orange colour, solid 75 %; **^{1}H-NMR (400 MHz, DMSO-D6)** δ 6.96 (d, J = 9.2 Hz, 2H), 7.38-7.47 (m, 6H), 7.73 (d, J = 2.8 Hz, 2H), 8.89 (s, 2H), **^{13}C-NMR (101 MHz, DMSO-D6)** δ 119.3, 120.2, 121.3, 123.1, 128.7, 131.3, 133.5, 142.6, 159.6, 162.8, *m/z* (**ESI-MS): [**M + 1] $^{+}$ 385.24; e**lemental Analysis** $C_{20}H_{14}Cl_2N_2O_2$ requires: C, 62.36 %; H, 3.66 %; O, 8.31 %; N, 7.27 %; Cl, 18.40 %; found: C, 61.82 %; H 3.39 %; N = 7.09 %.

1-Benzyl-2-phenyl-1-H-benzimidazole (3e) (as the NMR data compared with Ghosh *et al*,[4] Shitole *et al*[13] and Fekri *et al*[50])



Milk colour powder, 87-89 %; **^{1}H-NMR (400 MHz, DMSO-D6**) δ 1H-NMR (400 MHz, DMSO-D6) δ 5.55 (s, 2H), 6.96 (d, J = 7.1 Hz, 2H), 7.18-7.24 (m, 6H), 7.42-7.50 (m, 6H), 7.62-7.70 (m, 3H), 8.15 (d, J = 7.3 Hz, 1H), **^{13}C-NMR (101 MHz, DMSO-D6)** δ 47.60, 111.93, 120.17, 122.48, 122.90, 126.95, 128.16, 129.54, 130.85, 136.39, 137.85, 143.18, 151.28, 153.59, *m/z* **(ESI-MS): [**M^{+} + 1] 285; **elemental Analysis** $C_{20}H_{16}N_2$ requires**:** C, 84.48 %; H, 5.67 %; N, 9.85% found: C, 82.11 %; H, 5.46 %; N, 10.98 %.

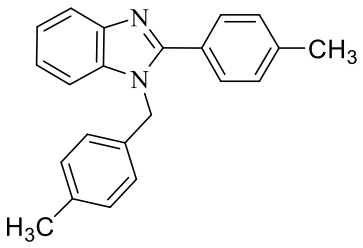
1-Benzyl-2-phenyl-1-H-benzomidazole from melt reaction (3e) (as the NMR data compared with Ghosh *et al*,[4] Shitole *et al*[13] and Fekri *et al*[50])



Light yellow powder, 68 %**, ^{1}H-NMR (400 MHz, DMSO-D6)** δ 8.15 (d, J = 7.3 Hz, 1H), 7.84-7.06 (m, 16H), 6.96 (d, J = 7.3 Hz, 2H), 5.54 (s, 2H); **^{13}C -NMR (101 MHz, DMSO-**

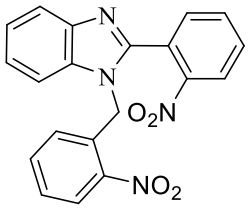
D6) δ 47.98, 111.76, 119.70, 122.75, 126.61, 128.01, 129.31, 130.37, 130.98, 136.20, 137.46, 143.36, 151.57, 153.79,

1-(4-Methylbenzyl)-2-(4-methyl-1-phenyl)-1-H-benzomidazole (3f) (as the NMR data compared with Wan *et al*[22])



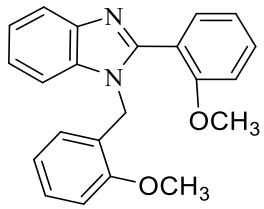
White solid, 81 %; **^{1}H-NMR (400 MHz, DMSO-D6)** δ 2.18 (s, 3H), 2.33 (s, 3H), 5.47 (s, 2H), 6.85 (d, J = 7.8 Hz, 2H), 7.05 (d, J = 7.5 Hz, 2H), 7.28-7.33 (m, 4H), 7.58 (d, J = 8.0 Hz, 3H), 8.02 (s, 1H), **^{13}C-NMR (101 MHz, DMSO-D6) δ** 21.1, 21.5, 47.7, 111.6, 119.7, 122.6, 123.0, 126.5, 126.9, 127.8, 129.4, 129.9, 134.5, 136.4, 137.2, 140.1, 143.2, 151.9, 153.9, **ES-MS:** *m/z* 312.42 (%): **[**M^+ + 1] 313**, elemental Analysis** $C_{22}H_{20}N_2$ requires**:** C, 84.58 %; H, 6.45 %; N, 8.97 %; found: C, 82.17 %; H, 6.25 %; N, 10.20 %.

1-(2-Nitrobenzyl)-2-(2-nitro-1-phenyl)-1-H-benzimidazole (3g)



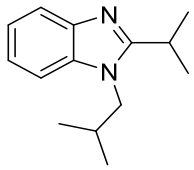
Yellow solid, 61 % yield; **^{1}H-NMR (400 MHz, DMSO-D6)** δ 7.25-8.36 (m, 29H), 6.78 (s, 1H), 5.63-5.95 (m, 2H), 1.02-1.18 (m, 1H) **^{13}C-NMR (101 MHz, DMSO-D6)** δ 149.75, 147.63, 144.14, 143.27, 135.41, 134.31, 134.22, 133.17, 132.37, 131.68, 129.45, 128.45, 125.75, 125.06, 123.62, 122.43, 119.97, 112.21, 45.32 *m/z* (**ESI-MS): [**$M + 1]^+$ 375; **elemental Analysis** $C_{20}H_{14}N_4O_4$ requires**:** C, 64.17 %; H, 3.77 %; O, 17.09 %; N, 14.97 %; found: C, 62.56 %; H, 3.54 %; N, 15.30 %.

1-(2-Methoxylbenzyl)-2-(2-methoxyl-1-phenyl)-1-H-benzimidazole (3h) (as the NMR data compared with Wan *et al*[22])



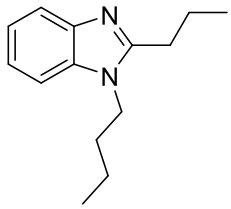
Brown solid**,** 47 % yield; **^{1}H-NMR (400 MHz, $CDCl_3$)** δ 3.57 (s, 3H), 3.77 (s, 3H), 5.22 (s, 2H), 6.67-6.83 (m, 3H), 6.94 (d, J = 8.2 Hz, 1H), 7.04 (t, J = 7.3 Hz, 1H), 7.16-7.28 (m, 4H), 7.43 (d, J = 8.9 Hz, 1H), 7.52 (d, J = 5.9 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), **^{13}C-NMR (101 MHz, $CDCl_3$)** δ 43.6, 55.2, 55.3, 110.0, 110.9, 119.9, 120.5, 120.9, 122.0, 122.5, 124.6, 127.8, 128.5, 131.5, 132.5, 135.6, 143.4, 152.6, 156.6, 157.7, *m/z* (**ESI-MS): [**M + 1] $^{+}$ 345; **elemental Analysis** $C_{22}H_{20}N_2O_2$ requires**:** C, 76.72 %; H, 5.85 %; O, 9.29 %; N, 8.13 %; found: C, 75.57 %; H, 5.96 %; N, 8.13 %.

1-Isobutyl-2-isopropyl-1H-benzimidazole (3i) (as the NMR data compared with Kumar *et al*[52])



Brown syrup-like, 68 %, **^{1}H-NMR (400 MHz, $CDCl_3$)** δ 0.93 (dd, J = 6.4, 4.8 Hz, 6H), 1.42 (dd, J = 6.8, 4.9 Hz, 6H), 2.20 (dd, J = 11.2, 6.6 Hz, 1H), 3.17 (dd, J = 11.5, 6.6 Hz, 1H), 3.90 (dd, J = 7.6, 4.6 Hz, 2H), 7.19-7.27 (m, 3H), 7.73 (d, J = 3.9 Hz, 1H), **^{13} C - NMR (101 MHz, $CDCl_3$)** δ 20.33, 21.98, 26.44, 29.36, 50.87, 109.76, 119.36, 121.68, 121.84, 135.27, 142.78, 160.28

1-Butyl-2-propyl-1H-benzimidazole (3j) (as the NMR data compared with Nguyen *et al*[53])



Brown syrup-like solid, 58 %, **^{1}H-NMR (400 MHz, $CDCl_3$)** δ 0.95 (td, J = 7.3, 3.1 Hz, 3H), 1.06 (td, J = 7.4, 3.1 Hz, 3H), 1.36-1.44 (m, 2H), 1.74-1.98 (m, 4H), 2.80-2.84 (m, 2H), 4.07-4.10 (m, 2H), 7.20-7.30 (m, 3H), 7.71 (dd, J = 5.7, 3.4 Hz, 1H), **^{13} C -NMR**

(101 MHz, $CDCl_3$) δ 13.86, 14.20, 20.31, 21.29, 29.50, 32.08, 43.50, 109.32, 119.24, 121.67, 121.87, 135.12, 142.81, 154.97

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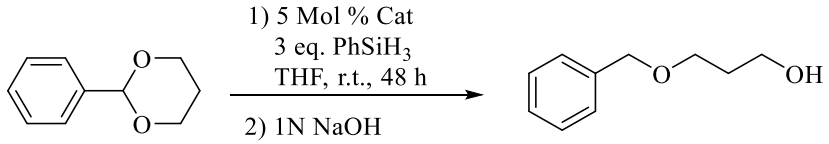
Chapter 5: Functional group protection using sulfonated sporopollenin exine

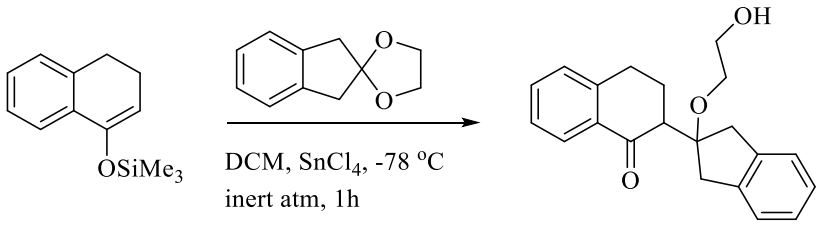
Protection of functional groups involves shielding specific and sensitive functional group(s) of an organic compound to avoid problems of chemoselectivity in a multiple step reaction.[1] Also, some functional groups are sensitive to certain reagents and so these must be chemically modified by a protecting group, which may depend on the aim of the synthesis, to avoid complexity in the reaction process and formation of unwanted products.

Protection of carbonyl functional group through ketal or acetal formation was speculated by Adam *et al* to probably be the most used method in organic synthesis.[2-4] With the formation of ketals or acetals, other functional groups in an organic compound can be manipulated and transformed to another moieties during synthetic reactions.[5] Ketals and acetals are also used in the production of ether compounds (**Scheme 5-1**).[5-7]

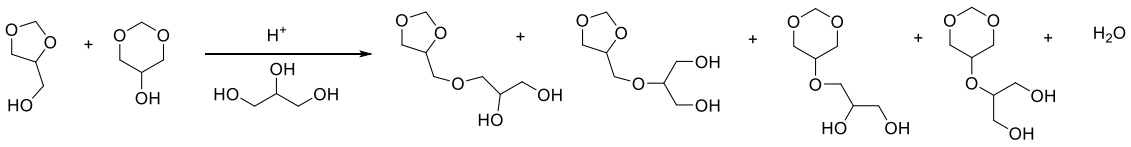


***Scheme 5-1*: Transformation of acetal to ethers**[6]

They are reportedly used in carbon to carbon (C-C) bond formation through Mukaiyama coupling reaction (**Scheme 5-2**)[5,8] and help in formation of chiral carbons as a building block in organic synthesis (**Scheme 5-3**).[9,10]



***Scheme 5-2*: Reaction of silyl enol ether and acetal**[8]



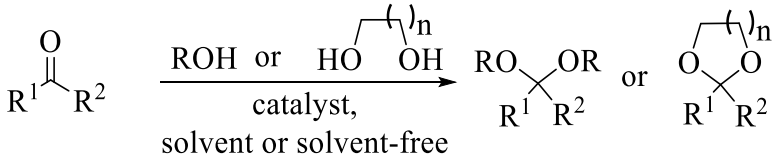
***Scheme 5-3*: Formation of Chiral centre by reacting acetal with glycerol**[10]

The application of ketals and acetals is not limited to synthesis, but they are also found in fragrances,[11-13] used as reactants in the production of steroids,[12] detergents,[13] lacquers,[13] cosmetics,[13] pharmaceutical products,[11,12] biodiesel fuels,[9,13,14] food and beverage additives,[9,13] antiseptics,[9] gasoline and diesel.[9,13,14]

5.1 Formation of ketals and acetals

Carbonyl compounds in the presence of a nucleophile can result in the formation of an addition product, and with bases to the formation of an aldol condensation product.[9] Both of these processes may constitute hindrance to formation of another desired product *via* an alternative reaction pathway. The ready ability of carbonyl group to participate in such reactions often necessitate the protection the carbonyl group before proceeding.

Ketal / acetal formation is a means of selectively protecting a carbonyl group[2,15] in the presence of many other functional groups, and is useful when a multifunctional organic compound is used in organic synthesis.[13] Neither ketals nor acetals are attacked by nucleophiles or strongly basic reagents.[4,15,16] Ketals are formed from the protection of ketones with an alcohol or diol (**Scheme 5-4**), while acetals are the compounds formed from the protection of aldehydes.[9]

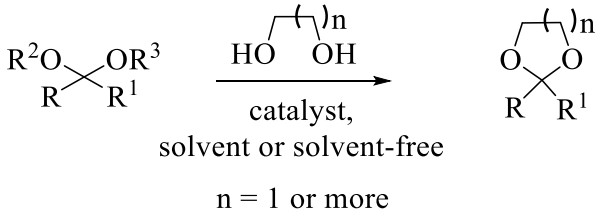


R1 = H or alkyl or aryl; R^2 = alkyl or aryl

***Scheme 5-4*: Formation of ketals or acetals from ketone or aldehyde**

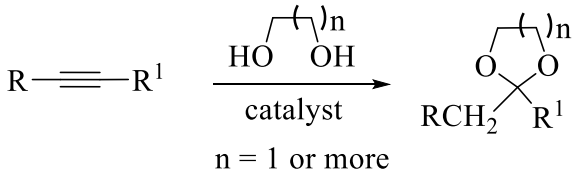
Both cyclic and acylic ketalization or acetalization reactions involve the reaction between mono or dihydric alcohols and aldehydes or ketones, with provision for the removal of water either through azeotrope or by using dehydrating reagent, in the presence of acid catalyst.[12,17-19] The formation of ketals and acetals most commonly involves the use of protic acid catalysts and high temperature.[5,20] High temperature enables the elimination of water through a Dean-Stark trap and thus prevents the reverse reaction. The reaction can only be forced to completion when there is total removal of water from the reaction mixture.

Smith *et al* reported that cyclic ketals or acetals can also be obtained by transketalisation/ transacetalisation in solvent or solvent-free reaction (**Scheme 5-5**).[18,21]



***Scheme 5-5*: Transacetalisation and transketalisation reactions**

In addition, the report by Santos *et al* reveals that ketals or acetals can also be formed by the reaction of dihydric alcohol with alkyne compounds in the presence of acid catalyst (**Scheme 5-6**).[19]



***Scheme 5-6:* Synthesis of ketals or acetals from alkynes**

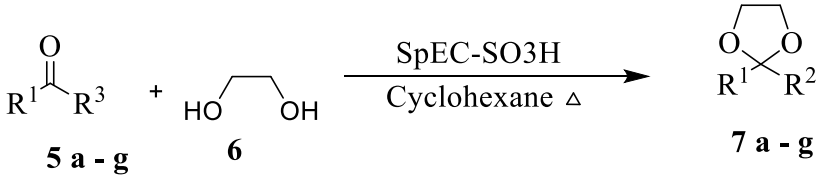
Due to the importance of ketals and acetals in organic synthesis, it is important to investigate various catalysts employed for an increase in their safer and environmental friendliness in the reaction process. Most catalysts previously employed in the protection of carbonyl compounds are strong protic acids, and these are beset with problems resulting from corrosion, catalyst recovery and product separation.[12] A good catalyst must be selective, cost effective and possess application to the reaction process or to the protection of wide range of compounds, irrespective of the class of the organic compound, with similar functional groups. However, some of the previously used homogeneous catalysts, such as *p*-TsOH, hydrochloric acids, phosphoric acids, sulphuric acids and other homogeneous catalysts,[13] were reportedly cost ineffective, difficult to recover and tedious to separate from the products and corrosion of the reactor.[9,12,22] Because of the disadvantages exhibited by homogeneous catalyst, researchers moved to the use of heterogeneous catalysts in the synthesis of acetals and ketals compounds. Though some heterogeneous catalysts have selectivity affinity towards protection of certain carbonyl compounds than the other but each have its advantages.[16,22] For example, some the heterogeneous catalysts can only promote the protection of aldehydes and cannot exhibit catalytic action in the protection of ketones.[22]

Heterogeneous catalysts, do have significant advantages over their homogeneous counterparts. These advantages range from recoverability,[11]reusability, they are normally easy and safe to handle, are separated easily from the of product, and environmentally hazard-free and have thermal stability.[23] Both Lewis[18,19,21] and Brønsted solid acid catalysts, such as Amberlyst 15[12], silica-gel supported sulfamic acid,[23] silica-gel supported sulfonated catalyst[9] etc, had been previously employed as heterogeneous catalysts in the ketalisation or acetalisation of carbonyl compounds. As part of the effort of introducing sustainable acid catalyst to the ketalisation and acetalisation reactions, sulfonated sporopollenin exine capsules (SpEC-SO_3H) were investigated.

5.1.1 Ketalization reactions in the presence of SpEC-SO_3H

Amberlyst 15 is a sulfonated resin-supported acid catalysts that has been used in several synthetic reactions. SpEC-SO_3H is an acid catalyst supported by natural polymer that is inert to attack from thermal attack at temperature below 400 °C,[24] non-oxidative chemical reagents,[25,26] non-oxidative biological reagents[25] and physical activities.[26]

In this work, SpEC-SO_3H were studied as catalyst in the formation of a ketal (**Scheme 5-7**), from a ketone and ethylene glycol. The yields are recorded in **Table *5.1-1*** (**entries 1-6**).



5 a - g **6** **7 a - g**

a: R^1 & R^2 = cyclopentyl; **b:** cyclohexyl
c: R^1 = phenyl, R^2 = methyl
d: R^1 = phenyl, R^2 = CH_2Br
e: R^1 = phenyl, R^2 = CH_2Cl
f: R^1 = R^2 = phenyl
g: R^1 = phenyl, R^2 = CH_2OH

Scheme 5-7 : **Synthesis of ketals in the presence of SpEC-SO_3H**

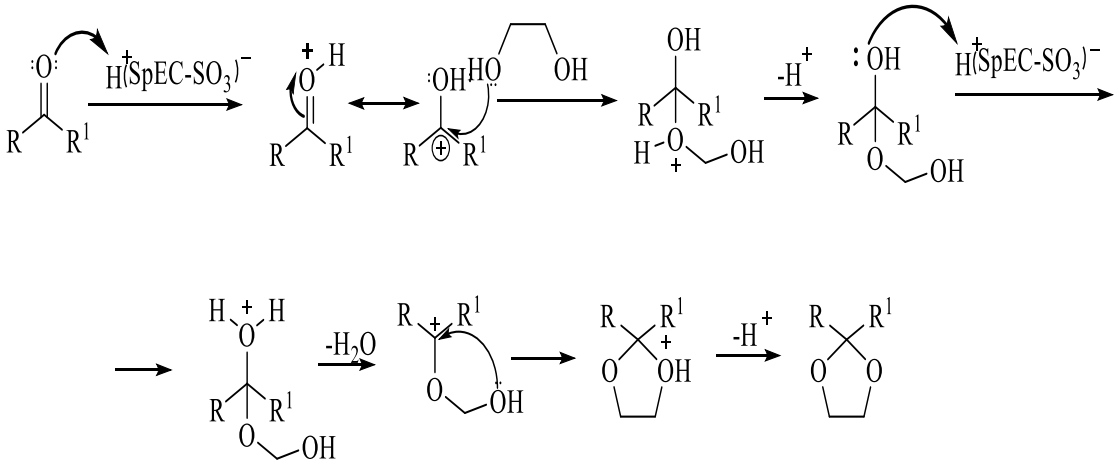
Table 5.1-1: Yields of ketals obtained in the presence of SpEC-SO_3H

Entries	Ketone	Products	Time (Hour)	Yield (%)
1	**5a**	**7a**	2	65
2	**5b**	**7b**	2	82
3	**5c**	**7c**	7.5	71
4	**5d**	**7d**	3	86
5	**5e**	**7e**	5	68
6	**5f**	**7f**	8	94
7	**5g**	**7g**	5	79

The aim was to test the catalytic activity of SpEC-SO_3H in reactions involving aliphatic and aromatic ketones.

The activity of the SpEC-SO_3H was investigated in the reaction involving dried 1,2-ethanediol and carbonyl compounds such as cyclopentanone, cyclohexanone, acetophenone, phenacyl bromide (2-bromoacetophenone), phenacyl chloride (2-chloroacetophenone), benzophenone and 2-hydroxyphenylacetone (**Scheme 5-7**). It is worth noting that the selected ketones were liquid except bromo acetophenone and benzophenone which are solids. Cyclohexane was used as the solvent medium and the by-product, water, was azeotropically removed with the aid of Dean-Stark apparatus (**Scheme 5-8**). Thorough mixing / agitation was used to increase the contact of the solution with the surface of the SpEC-SO_3H particles in the reaction vessel.

In order to stop the side reactions and low reactivity of some of the starting reactants; such as acetophenone etc, the mol ratio of SpEC-SO_3H to the reactant was increased to aid the protection of ketone. For example, SpEC-SO_3H equivalent to 6 % mole of acetophenone was used in its ketalisation reaction unlike SpEC-SO_3H of 2.5 % equivalent of the mole of each of cyclopentanone and cyclohexanone was used in their transformation to respective ketals. It was observed that the ketone with highly electronegative substituents e.g. chloroacetophenone yielded lower product than those that are free of electronegative atoms (**Table *5.1-1***). Also, the sensitivity of ketal / acetal to water in the presence of an acid catalyst may have contributed to the deprotection and reversibility of the product back to the starting ketone.



Scheme 5-8: **Reaction mechanism for ketal formation**

5.1.1.1 Application of SpEC-SO_3H to the protection of aliphatic ketones

SpEC-SO_3H was used to catalyse the transformation of two different cyclic aliphatic ketones, viz; cyclopentanone and cyclohexanone, to their respective ketals. Each of the ketones was reacted with ethylene glycol in a round bottom flask fitted with Dean-Stark trap at 80 °C. The Dean-Stark trap was used to azeotropically removed water from the reaction in order to avoid reversibility of the product back into the reactants. The experiment was carried out with ratios 1:40 and 1:16 mole equivalent of the SpEC-SO_3H to ketones. At the end of each reaction, SpEC-SO_3H was recovered by filtration and thoroughly washed with ethyl acetate. The excess or unreacted ethylene glycol was removed by washing the combined organic extracts (cyclohexane and ethyl acetate) with distilled water. The organic extracts were dried with sodium sulfate and evaporated to a constant weight. The products were further purified by column chromatography. The formation of the product was confirmed by NMR analysis and comparison to data in the literature. Ketals of cyclopentanone and cyclohexanone, like their starting materials, are liquids in nature.

When equal mole ratios of the reactants i.e. compound **5a** or **5b** to compound **6**, in the presence of SpEC-SO_3H catalyst, were used in the ketalisation reaction (**Table *5.1-2***, entries 1 & 3), the yields obtained were 37 % for compound **5a** and 44 % for compound **5b**. The yields of **7a** (1,4-dioxaspiro[4.4]nonane**)** and **7b** (1,4-dioxaspiro[4.5]decane), were lower compared to the yields obtained from the reaction involving 1:2 ratio of compound **5a** or **5b** and compound **6** which were 65 % and 82 % respectively (**Table *5.1-2***, entries 2 & 4).

Ethylene glycol (**6**) is a viscous liquid and may be capable of being stuck to SpEC-SO_3H at room temperature in cyclohexane. Using a large amount of **6**, or larger mass of SpEC-SO_3H, above 2.5 % mole% compared to **5a** or **5b**, may slow down the reaction rate due to the glycol coating the catalyst particles. For example, when 3.07 mmol of SpEC-SO_3H was used, which is around 6 mol % of compound **5a** or **5b**, the resulting product yields were low and it took up to 3 hours for the reaction to complete (**Table *5.1-2*, entries 1 & 3).** However, with the use of SpEC-SO_3H at 2.5 % mole equivalent of **5a** or **5b**, the reaction was complete within 2 hours and in a product yield of above 50 % yield (**Table *5.1-2*, entries 2 & 4**). It was observed that ketalisation of compound **5b** resulted into the product of better yield than compound **5a**.

Table 5.1-2: SpEC-O_3H catalysed reaction of cyclic aliphatic ketones and ethylene glycol

Entries	Ketone	Ethylene glycol	SpEC-SO_3H	Time (Hour)	% Yield
1	**5a**	50 mmol	3.07 mmol	3	37 %
2	**5a**	100 mmol	1.25 mmol	2	65 %
3	**5b**	50 mmol	3.07 mmol	3	44 %
4	**5b**	100 mmol	1.25 mmol	2	82 %

5.1.1.2 Formation of aromatic ketal compounds

In this work, the catalytic ability of SpEC-SO_3H was also tested in the protection of four different derivatives of acetophenone and a benzophenone derivative. Following the same general method described above, SpEC-SO_3H was applied to the synthesis of aromatic ketals by reacting ethylene glycol with each of the different aromatic ketones, stated above, in cyclohexanone at 80 °C except the reaction that involved benzophenone was carried out in an oil bath at 120 °C (**Table *5.1-1*, entries 3 to 7**). The water produced was removed through the use of a Dean-Stark trap. The period for each reaction was dictated by the amount and the ratio of SpEC-SO_3H to ketone. SpEC-SO_3H was removed with aid of filtration and washed with ethyl acetate. Then the organic layer was washed with distilled water to remove excess ethylene glycol. The filtrate was dried over sodium sulfate and solvent evaporated by rotary evaporator at room temperature. Compound **7c** sublimed when the temperature of the evaporation was increased to above 26 °C. All the

aromatic ketals obtained from the experiment were solids, therefore they were purified by two successive recrystallisation from n-hexane overnight in the refrigerator. The crystallised products were analysed by NMR spectroscopy and the spectra were compared to data from the literature. Like the cyclic aliphatic counterparts, the reactivity of carbonyl group to addition are major factors that influence the yield of each ketal. Generally, it was reported that ketalisation of acetophenone is difficult and in some cases, leads to generation of byproducts.[27] Therefore, an attempt to protect 2-hydroxyaceptophenone gave complicated result, even, after two successive attempts to purify the same compound through column chromatography. This confirmed the report of Ono *et al* that acid-catalysed ketalization of hydroxyacetophenone resulted in a poor yield.[27] The researcher further stated that obtaining a good yield in the ketalization of hydroxyacetophenone, under an acid-catalysed condition, requires prior protection of the hydroxy group before embarking on the protection of the carbonyl group with glycol.[27] However, the protection of 2-hydroxyphenylacetone with ethylene glycol, in the presence of SpEC-SO_3H, resulted in the mixture of starting ketone and 2-hydroxyphenyl-1,3-dioxolane as shown by the chemical shifts in the NMR spectrum. When the product was spotted on the TLC, after purification and before subjecting it to NMR spectroscopy, there were two spots, one of which has the same retention factor as the starting ketone. The mass recovered, after concentration, was 1.379 g. On the ketalization of other acetophenone, the protection of 2-chloroacetophenone or phenacyl chloride has the lowest yield among aromatic compounds possibly because of the influence of greater electron withdrawing ability of chlorine attached to its methylene group than substituent attached to other compounds.

The ratio of SpEC-SO_3H to compound **5c** greatly influenced the yield of its corresponding ketal. For example, the use of 6% mole equivalent of SpEC-SO_3H to catalyse a reaction that involved equimolar amount of compound **5c** and compound **6** yielded only 29 % of compound **7c (Table 5.1-3, entry 1)**. When the mole % equivalent ratio of SpEC-SO_3H to compound **5c** was increased to 7 %, and with the reduction of compound **5c** by 10 % while the amount of ethylene glycol was maintained, the reaction was complete at 4 hours and the yield of the product (compound **7c**) was increased to 45 % (**Table 5.1-3, entry 2**). Furthermore, with the ratio of SpEC-SO_3H to compound **5c** maintained at 6 % mole equivalent and the mole of ethylene glycol increased to the double of the amount of compound **5c,** the yield of the product **7c** increased to 71 % but it took 7.5 hours to complete the reaction (**Table 5.1-3, entry 3**). However, when the mole ratio

of SpEC-SO_3H was reduced to 3 % equivalent of compound **5c** and the ratio of the same ketone to ethylene glycol was maintained at 1:2 moles, the yield was of the reaction was 22 % (**Table 5.1-3, entry 4**). The ratio of SpEC-SO_3H to ketone, the amount of ethylene glycol, serious agitation and reaction time contribute immensely to the yield of ketals.

Table 5.1-3: Application of SpEC-SO_3H to the production of aromatic ketals

Entries	Compound 5c	Compound 6	SpEC-SO_3H	Time (h)	Yield (%)
1	50 mmol	50 mmol	6 mole%	9	29 %
2	45 mmol	50 mmol	7 mole%	4	45 %
3	50 mmol	100 mmol	6 mole%	7.5	71 %
4	50 mmol	100 mmol	3 mole%	7.5	22 %

Benzophenone (**5f**) is a more stable compound, because of the influence of inductive effects on its structure, than compound **5c.** Therefore, ketalization of compound **5f** in the presence of SpEC-SO_3H gave a better yield than the ketals **5c**, **5d**, **5e** and **5g**. **5f** was reacted with 5 mole% of SpEC-SO_3H over a period of 5 to 7 hours. The product was subjected to recrystallisation then further purified with column chromatography. However, a pure product was not obtained until an excess amount of **6** was used in the protection of **5f**, using 11 mole% of SpEC-SO_3H relative to **5f**, and with a reaction over 8 hours.

Other aromatic ketones were reacted with ethylene glycol and 12.5 mole% of SpECs-SO_3H to obtain their respective ketals (**Table *5.1-1***). As expected, the yield of product **7d** and **7e** were higher than compound **7c**. Product **7d** was obtained in 86 % yield after 3 hours, and product **7e** in 83 % after 5 hours. Product **7g** is a liquid and could not be purely separated from the unreacted compound **5g**.

5.1.1.3 Recyclability of SpEC-SO_3H in the protection of carbonyl compounds

The recyclability of a catalyst is very important for application to industrial processes.[28] The recyclability of SpEC-SO_3H for use in the synthesis of benzimidazole was good (Chapter 4), therefore the recyclability of SpEC-SO_3H was tested in the protection of carbonyl compounds as ketals. The recyclability of the catalyst was studied by recovering the SpEC-SO_3H by filtration after a reaction, and thorough washing with ethyl acetate to remove the product remnant that maybe stuck on the surface of the

catalyst. Then, the recovered SpEC-SO_3H was allowed to dry properly in a vacuum desiccator. It worth noting that it was impossible to avoid some mass loss during transfer of the recovered SpEC-SO_3H from the filter to the next reaction vessel. Some of the particles were stuck to the pores of the filter, others were, because of their lightness, lost in the fume hood. Therefore, in subsequent reactions the amount of SpEC-SO_3H was fractionally lower in the following reaction cycle. The dried recovered SpEC-SO_3H were in this way subjected to three successive recycled reactions involving the protection of the carbonyl functional groups of cylopentanone and cyclohexanone. In the recyclability of SpEC-SO_3H in the reaction involving **5**, the ketone was fixed at 50 mmol, whereas for **6** it was at 100 mmol. In the first cycle of SpEC-SO_3H was 1.25 mmol equivalent of acid groups. All the recycled reactions were carried out over a period of 2 hours.

SpEC-SO_3H proved to be recyclable in the ketalization of cyclopentanone as there was not much difference in the percentage yield of the products obtained from the first and second reaction cycle. However, a sharp decrease in the yield between the product of the second and third reaction cycle was observed (**Figure *5.1-1***). This reduction may be attributed to a decrease in the mass of the catalyst during the transfers stages of each reaction. The fourth reaction cycle produced a yield similar to the third cycle.

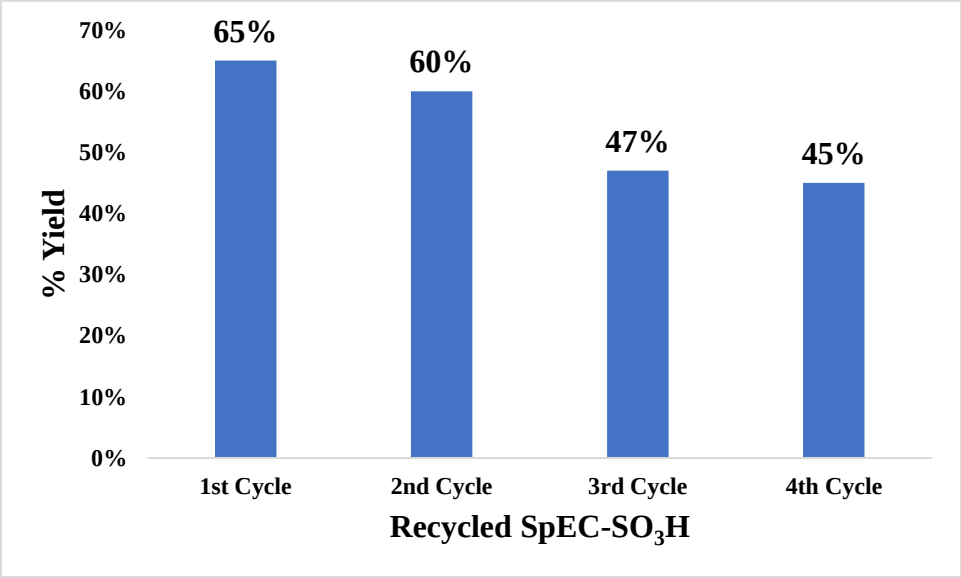


Figure 5.1-1: Recyclability of SpEC-SO_3H in the protection of cyclopentanone with ethylene glycol

Another ketone used to study the recyclability of SpEC-SO_3H was cyclohexanone (**5b**). The reaction process followed the same procedure used for **5a** and results are

graphically showed in **Figure *5.1-2***. The mole ratio of the reactants and SpEC-SO_3H together with the time of the reaction were maintained. However, the addition of 2 mmol to compound **5b** reduced the losses during the work up and also, greatly influenced the yield of product at the third and fourth reaction cycle. Of course, it is possible that some of the product are clogged into the SpEC-SO_3H from the previous reaction cycle and therefore may also contribute to the maintenance of the yield across each cycle.

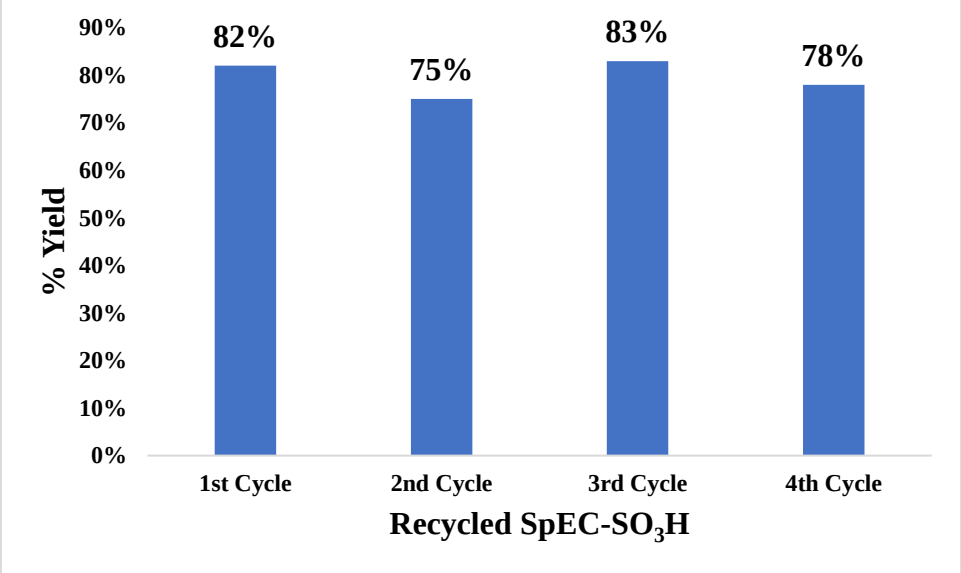


Figure 5.1-2: Recyability of SpEC-SO_3H in the protection of cyclohexanone with ethylene glycol

5.2 Synthesis of tetrahydropyranyl ethers (THPEs)

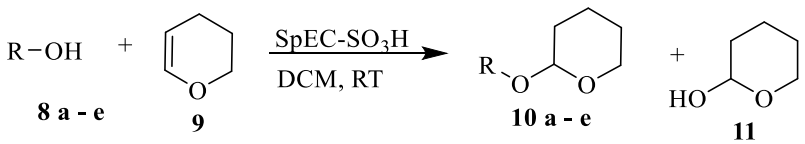
The protection of a hydroxyl group in organic compounds containing other functional groups is an important strategy in organic syntheses involving multistep reactions.[29,30] Many protecting groups have been used to protect hydroxyl groups in the past. The use of 3,4-dihydropyran, leading to the formation of a tetrahydropyranyl ether (THPE), is one of the important protection strategies in alcohol chemistry.[31] Since THP ethers are easy to de-protect,[29] they are extensively used as intermediates in organic synthesis.[31] They are resistant to strong base, Grignard reagents, acylating agents, oxidative conditions, alkylating reagents, and hydride reducing agents.[29,30,32] THPEs are de-protected by acid hydrolysis.[30,31]

THPEs are inexpensive and can easily be produced.[33,34] They are typically produced by reacting equimolar amounts of a hydroxyl containing compound with 3,4-dihydro-2H-pyran (DHP) in the presence of an acid catalyst. Several catalysts have successfully been

used in the tetrahyropyranlation of alcohols.[31,33] Some of these catalysts were reported to be very effective for the production of THPEs, but other reports state disadvantages; for example some are hazardous to the environment, require high temperatures to function, use long reaction time and harmful organic solvents, are incompatible with acid sensitive functional groups or sensitive to water.[33,35-37] Also, a good number of the these catalysts were only claimed to be effective in the protection reaction but the deprotection activities of most of them were not discussed in the literature.[38,39]

Tetrahyropyranlation of alcohols has been carried out by Poon *et al* using the polymer supported acid catalyst Dowex 50WX4–100.[32] Therefore, this work was considered as a useful comparator for the results obtained involving our SpEC-SO_3H.

In this experiment, the tetrahyropyranlation of the hydroxyl group of different classes of alcohols - including phenol and derivatives, in the presence of SpEC-SO_3H, resulted in a high yield of the expected corresponding THP ether. A mixture of each alcohol, 3,4-dihydropyran (DHP), SpEC-SO_3H and dichloromethane were stirred at room temperature for the hours specified in **Table *5.2-1***. The progress of the reaction was monitored by TLC until the alcohol had disappeared from the mixture. After completion, the reaction mixture was filtered and the SpEC-SO_3H was washed with more dichloromethane. The combined filtrates were concentrated to obtain a crude product, which was purified using column chromatography (eluent hexane–ether 9:1) to give the tetrahydropyranyl ethers of each alcohol. The products were subjected to NMR analysis to ascertain the formation of the product (**Scheme 5-9**).



R =Benzyl = **a**
Cholestanyl = **b**
beta-Sitosteryl = **c**
Phenyl = **d**
2,4-dihydroxyBenzophenone = **e**

Scheme 5-9: **Preparation of tetrahydropyran ether**

Table 5.2-1: Mole of the reactants, SpEC-SO_3H and reaction time

Entry	Alcohol	Amount (mmol)	3,4-DHP (mmol)	SpEC-SO_3H (%)	Time (mins)	Product 10a-e (%)
1	**8a**	10	15	6.14	120	82
2	**8b**	2.57	3.75	3.11	180	85
3	**8c**	2.5	5	3.20	180	88
4	**8d**	10	15	6.14	180	79
5	**8e**	11	30	5.58	180	97

Apart from the SpEC-SO_3H-catalysed formation of tetrahydropyranyl ethers (**10a-e**), the by-product tetrahydro-2H-pyran-2-ol (**11**) formed, albeit in very low yield, due to the presence of water in the dichloromethane (DCM) solvent. The formation of tetrahydro-2H-pyran-2-ol was first noticed during the TLC screening before the crude product was subjected to the NMR analysis for confirmation of the formation of product. In each of the ^{13}CNMR spectrum of the crude products, there were small peaks at 94.53, 63.91, 32.10, 25.24 and 20.37 ppm that could only be associated with chemical shift of compound **11**.[40-42] The yield of the products **10a-e** after purification, are represented in **Table *5.2-1*** above.

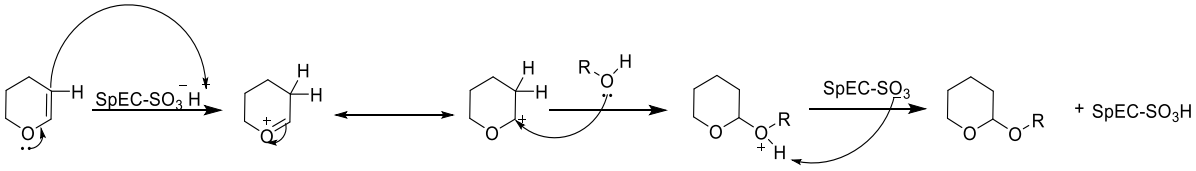
The hydroxyl group of an aromatic primary alcohol, two steroids, which are secondary alcohols, a phenol and a hydroxybenzophenone were protected by 3,4-dihydropyran (DHP) in the presence of SpEC-SO_3H. Phenol yielded lower amounts of product compared to those from aliphatic alcohols.

In phenol (**8d**) the hydroxyl group is attached to the conjugated phenyl ring, which reduces its nucleophilic reactivity. The yield of the product obtained from the tetrahydropyranylation of **8c** was a bit higher than **8b**.

Due to the presence of chiral centres in compounds **8b** and **8c**, coupled with the presence of the new stereogenic centre in the tetrahydropyran ring, tetrahydropynylation of those two steroids led to the formation of diastereoisomers. The formation of diastereoisomers was confirmed by ^{13}CNMR spectra in which doubling number of peaks close to the newly formed stereocentre was observed. These results were corroborated by comparison with data from the literature.[30,31]

5.2.1 The ability of SpEC-SO_3H to initiate chemoselective reaction in tetrahydropyranylation

With the formation of tetrahydropyran ethers **10a-d**, SpEC-SO_3H proved to be able to initiate a chemoselective reaction of the alcohols and 3,4-dihydropyran as shown in **Scheme 5-10**.



Scheme 5-10: **Mechanism leading to the formation of tetrahydropyran ethers**

5.2.2 Regioselective protection of hydroxyl group and chemoselectivity of SpEC-SO_3H in a multifunctional compound

The SpEC-SO_3H catalysed tetrahydropyranylation of **10e** was an interesting reaction. It was expected that both of the hydroxyl groups (i.e. the hydroxyl groups at *ortho* and *para* positions to the carbonyl functional group) would be protected, but in fact only the *para* hydroxyl group was protected. This could be due to intramolecular hydrogen bonding between the proton of the 2-position hydroxyl group and the carbonyl group on the *ortho* side of the hydroxyl group (**Figure *5.2-1***) thereby leaving only the hydroxyl group at the *para* position to the carbonyl group active for tetrahydropyranation,[43,44] but also the distance of the *para* hydroxyl group to the carbonyl group could be another factor that the hydroxyl group at the 4 – position is available for tetrahydropyrnation.

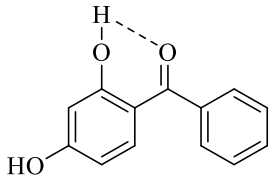


Figure 5.2-1: Intramolecular hydrogen bonding in benzophenone

Even, when the amount of **9** was doubled in a repeat reaction, only mono-tetrahydropyranlated compound **10e** was formed. The excess DHP, was hydrated forming tetrahydro-2H-pyran-2-ol (**11**) as by-product. Compound **10e** {2-benzophenonoxytetrahydro-*2H*-pyran or (2-hydroxy-4-((tetrahydro-*2H*-pyran-2-yl)oxy)phenyl)(phenyl)methanone}, was isolated as a transparent viscous orange syrup or semi solid compound with a sweet odour. The pure compound **10e** eluted out of the column chromatography at 1:9 of ethyl acetate-hexane mixture with a 97 % yield.

In the reaction above, SpEC-SO_3H showed chemoselectivity catalysing the protection of hydroxyl group in the presence of carbonyl functional group. Therefore, depending on the protecting reagent and reaction condition, SpEC-SO_3H can function in the catalysing the selective protection of either carbonyl or hydroxyl functional groups.

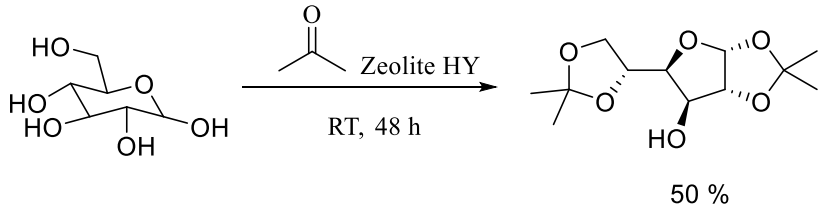
5.2.3 Application of SpEC-SO_3H in solvent-free DHP protection of alcohols

An attempt was made to use SpEC-SO_3H in a solvent-free tetrahydropyranylation reaction, mixing both reactants and catalyst with the aid of a pestle and mortar at room temperature for 2 minutes. Using this approach with compound **8a**, the yield (15 %) of the product, after purification by column chromatography, was lower than the yield obtained from the reaction carried out in solution which was 82 %. This leaves a good proportion of the alcohol substrate unreacted.

A solvent-free reaction was not effective for the solid alcohol substrates. For example, the reaction of substrate **8c** and compound **9** was not successful because compound **8c** is insoluble in the latter reactant. It was only **8a**, which is a liquid, that properly reacted with DHP but nevertheless a greater percentage of the alcohol remained unreacted**.**

5.3 Protection of monosaccharide compounds

2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose is a useful precursor in the synthesis of a range of chiral compounds, such an anti-inflammatory drugs, oligosaccharides and other important compounds that are biologically active.[45-48] Sugars have been protected as ketals in the past with various solid catalysts such as zeolite HY (**Scheme 5-11**), $FeCl_3.6H_2O$ on silica, $NaHSO_4$ on silica, $HClO_4$ on silica etc,[49-54] but the attempts have resulted in either low yields or separation problems.[49,50]

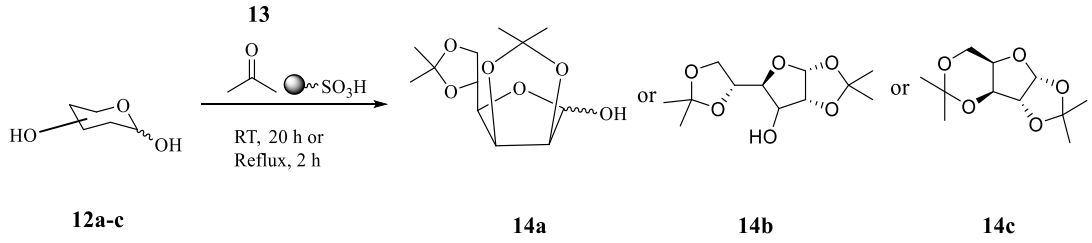


Scheme 5-11: **Isopropylidation of glucose catalysed by zeolite HY**

Rajput *et al*[50] successfully reported the application of silica-H_2SO_4 to the protection of different sugars. Inhaling silica loaded with sulfuric acid is however dangerous to health.[55,56] This calls for the use of environmental-friendly and renewable catalyst.

5.3.1 Acetonation of mannose at room temperature in the presence of SpEC-SO_3H

Sulfonated sporopollenin (SpEC-SO_3H), at a loading of 6 mol% relative to the monosaccharide,[57] was used as a heterogeneous catalyst in the protection of D-mannose with acetone. This reaction was first tried by Huang,[58] but reinvestigated here to acquire more data. The mixture of mannose (5 g, 28 mmol) and SpEC-SO_3H (0.5g, 1.66 mmol) in acetone was stirred at room temperature for 20 hours, filtered to remove the catalyst and evaporated to give the crude product as a solid. The crude product was dissolved in diethyl ether and crystallized by addition of n-hexane (3:5 ether-hexane). The crystallized product was left at room temperature for a few hours for slow crystallization, and later kept at 0 °C overnight to obtain a better yield. The pure product obtained was a crystalline white solid in a 64 % yield (**Scheme 5-12**).



12a-c **14a** **14b** **14c**

a: mannose; **b:** glucose; **c:** xylose

Scheme 5-12: **Protection of D-sugar**

In the course of recrystallization, it was observed that the longer the mixture was kept at room temperature, the slower the recrystallization but with a purer product. A longer time spent at reduced temperature, however, gave the higher yield (64 %). The product obtained from the longer recrystallization at refrigerator temperature was subjected to NMR analysis, and it was concluded that the increase in yield observed was most likely due to the crystallization of both α- and β-anomers in a 4:1 ratio. The α- and β-anomeric carbons appeared at 101.26 ppm and 97.07 ppm on ^{13}C-NMR spectrum. A trace of unreacted D-mannose was also detected.

Table 5.3-1: Summary of the effect of recrystallization solvent ratio on the product yield obtained from the protection of D-mannose at room temperature with 6 % SpEC-SO_3H

Entries	diethyl ether (ml)	*n*-hexane (ml)	Period (hours)	Yield (%)	Remark
1	3	4	18	31	a low yield, but pure as shown on NMR spectra with 4:1 α: β-anomer
2	3	4	~ 24	56	4:1 α: β - anomer
3	2	3	18	37	4:1 α: β- anomer
4	1	4	18	71	Yielded a very light brown product (indicated the presence of impurity)
5	7	10	18	56	Yielded the product and a trace of unreacted sugar
6	3	5	20	42	Pure product (2:1 α: β - anomer)
7	3	5	68	64	Pure product (4:1 α: β - anomer)

The NMR spectra of all the experiments were nearly the same. The compounds recorded in **Table *5.3-1***, **entry 1, 6 and 7** were crystalline and white by mere sight but their anomeric ratios differed. The compound recorded **Table 5.3-1**, **entries 4 and 5** contained traces of impurity and unreacted sugar. **Table 5.3-1**, **entry 5** contains peaks of unreacted mannose.

The samples were subjected to NMR analysis and the melting point confirm the formation of the expected product, by comparing the data obtained to those data reported in the literature. The NMR spectrum confirmed the same chemical shifts with those that were reported by Rajput *et al*,[49,50] Appelt *et al*[59] El Nezhawy *et al*[60] and Charonnat *et al*.[61]

However, the observed melting point of the product (**Table 5.3-1**, **entry 7**), which was 106-109 ºC, was lower than 121–122 °C reported by El-Nezhawy *et al* for the α-anomer.[60] The ^{13}CNMR chemical shifts of our product showed spectra peaks that double the number of those reported in the literature, indicating that our compound was a mixture of α- and β-anomers in a 5:1 ratio.

The method was further investigated in an attempt to discover the effect of the amount of SpEC-SO_3H on the yield of isopropylidation of D-mannose. The mole % ratio of SpEC-SO_3H to the sugar substrate was increased to 10 % (**Table 5.3-2**, **entry 1**) and the reaction

time was fixed at 20 hours. The recrystallisation reaction was carried out in diethyl ether-hexane solvents (3:5). With the increase in the amount of the catalyst, the yield of the product (isopropylidation of D-mannose) increased to 84 % at room temperature. The NMR spectra was compared to those of Rajput *et al*[50] and Maddani *et al.*[62,63] The chemical shifts from our work are in consistent with those reported by Rajput *et al*.[50]

However, when 2.5 % mole ratio of SpEC-SO_3H (**Table *5.3-2*, entry 3**) was employed to catalyse the reaction involving acetone and mannose, only 34 % product was obtained. Even, when the reaction was left for a week (168 h), the yield of the isolated product remained around 34 %. It can therefore be concluded that 10 mole % SpEC-SO_3H gave better yield in the isopropylidation of mannose at room temperature.

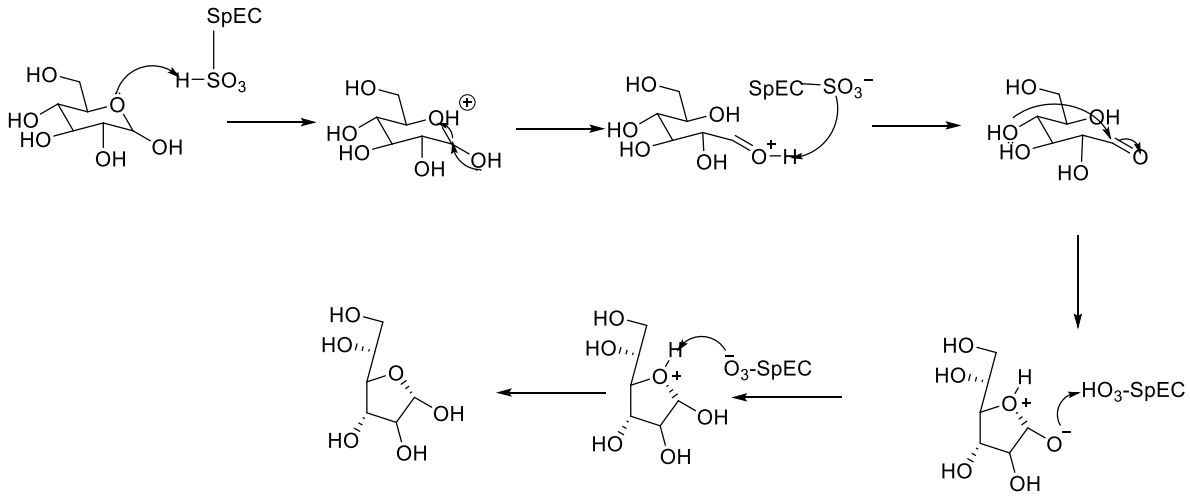
Table 5.3-2: Isopropylidation of mannose at different mole ratio of SpEC-SO_3H

5.3.2 Influence of the stereo centre on the isopropylidation of other monosaccharide sugars in the presence of SpEC-SO_3H

Entry	SpEC-SO_3H (mmol)	SpEC-SO_3H (mol%)	Time (h)	Yield (%)
1	3.07	10.0	20	84
2	1.66	6.0	20	64
3	0.75	2.5	168	42

The use of SpEC-SO_3H was also investigated for the protection of D-glucose and D-xylose in the isopropylidation chemistry described above. The relative stability of each sugar and its structure in solution is peculiar and mostly different from another. For example, D-mannose structure is predominately more stable in its α-anomer while D-glucose and D-xylose structures are predominantly β-anomers in the solvation state.[64-66] More so, D-xylose has a lower number of carbons therefore the ring size was smaller than D-mannose and D-glucose. D-glucose, in its free state, is mostly stable as the α-anomer in which the anomeric hydroxyl group is in an axial position and therefore caused an increase in steric and also the mutarotation. In solvent, D-glucose is catalysed, in the reference of an acid catalyst, to β-anomers with its anomeric hydroxyl in the equatorial position which is energetically stable and limits the 1,3-axial hydroxy interaction. At equilibrium therefore, glucose anomeric carbon will interact with axial hydroxyl on the carbon in position 4 to form an unstable furanose hemiacetal (**Scheme 5-13**). In addition,

during acetonation reaction, the anomeric hydroxyl group in equatorial position and the axial 2-hydroxyl group are protected to form the isopropylidene product. The large distance between the equatorial hydroxyl group on carbon position 1 and axial hydroxyl group on carbon 2 results in slowing down the reaction and make the formation of the isopropylidene of glucose difficult. The glucopyranose form with many equatorial hydroxy group protections is more stable than the glucofuranose (**Figure *5.3-1***).



Scheme 5-13: **Formation of glucofuranose hemiacetal**

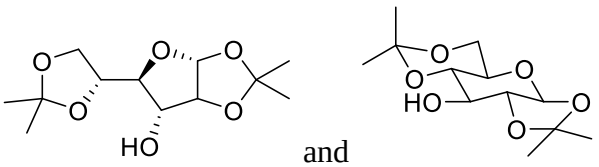


Figure 5.3-1: Di-isopropylidene glucofuranose and glucopyranose

Following the same general method as described above D-glucose and D-xylose were reacted with SpEC-SO_3H (10 mol %) in acetone for 48 h at RT. The recrystallisation of the crude products in both cases, because of their low melting points, were difficult and were not subjected to further purification. More so, the products were semi solid or syrup-like in nature due to the mixture α- and β- anomers together with by-products, as indicated in NMR spectra of the crude products.

The protection of xylose with acetone at room temperature in the presence of 5 % mole ratio (1.5 mmol) SpEC-SO_3H took four days and a crude product of 61 % was obtained. The longer reaction time may, together with the nature of the crude products, may be due to the interaction of xylose with solvent and mutarotation of its anomeric hydroxy group.

5.3.3 SpEC-SO_3H catalysed formation of di-O-isopropylidene sugars from the reaction of acetone and a monosaccharide at reflux.

The reaction time for the formation of *O*-isopropylidene compounds from anhydrous acetone and a monosaccharide at room temperature was too long, and this could lead to the degradation of sugars, which are acid sensitive substrates. Therefore, increased reaction temperatures were investigated with the aim of facilitating ring-opening that lead to equilibrium, during the transition or interconversion of α- anomer to β- anomer or *vice versa*, in a reduced time and thereby reducing the reaction time.[57,67,68] Though, an increase in temperature will increase the rate at which the substrate convert to the product but also the rate of degradation of the product and / or the substrate but a large amount of the substrate must have been converted to the product in a short reaction time. With an increase in temperature to reflux, the acetonation of mannose was complete after 2 hours (**Scheme 5-12**). Specifically, SpEC-SO_3H (10 mol%), D-mannose (30 mmol) in dry acetone (dried with potassium carbonate before use, 150 mL) were heated at reflux for 2 hours. The reaction was monitored by TLC. At the end of the reaction, the mixture was filtered through a pad of the Celite® to remove the catalyst and the SpEC-SO_3H was washed with dry acetone. The crude product, which was later purified by recrystallisation in a mixture of 1:4 diethyl ether - n-hexane, was obtained by evaporation of the combined filtrates. The recrystallized solid was dried in a vacuum desiccator to constant weight. The recrystallised solid was subjected to NMR spectrometry to confirm the formation of the product. The result showed 91 % pure product (**Figure *5.3-2***) composed of 4:1 α-anomer to β-anomer.

The effect of using SpEC-SO_3H at different ratios - ranging from 2.5 % mole to 10 % mole- was tested in the acetonation of mannose (30 mmol) at the reaction period of 2 hours. As expected, 10 mol % SpEC-SO_3H (3.07 mmol) gave the highest product yield when compared to the yields of the products obtained from the SpEC-SO_3H of lower mole% ratios (**Figure *5.3-2***). The percentage yield of the products declined with a decrease in the amount of SpEC-SO_3H used (**Figure *5.3-2***). However, with persistence and elongation of the reaction time, the yield of the product might increase even with this lower level of catalyst, but there is possibility of degradation of the sugar and formation of humins or other by-products.[57,67,68]

In view of the above explanation, 10 mole % of SpEC-SO_3H appears best for the acetonation of sugar substrates when heat is used to accelerate the reaction.

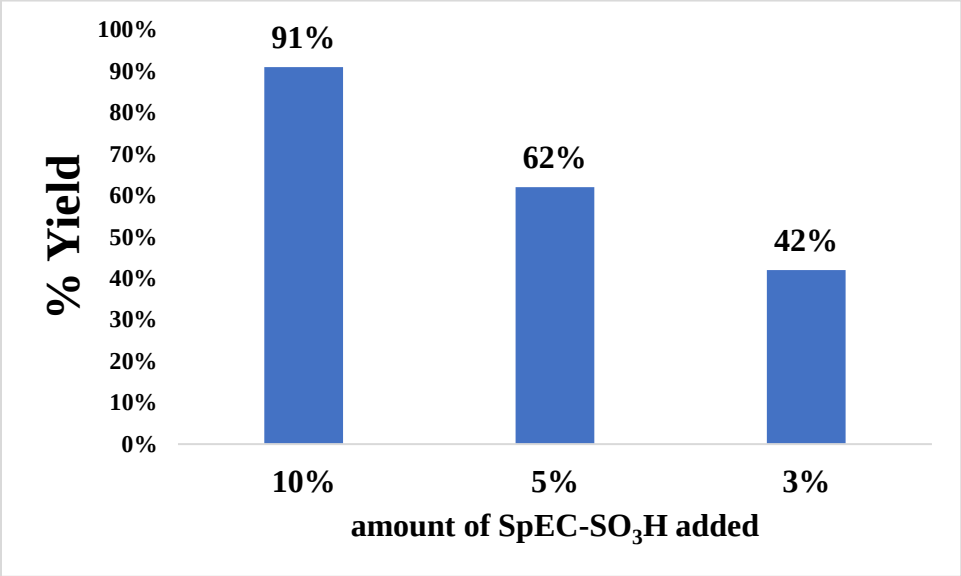
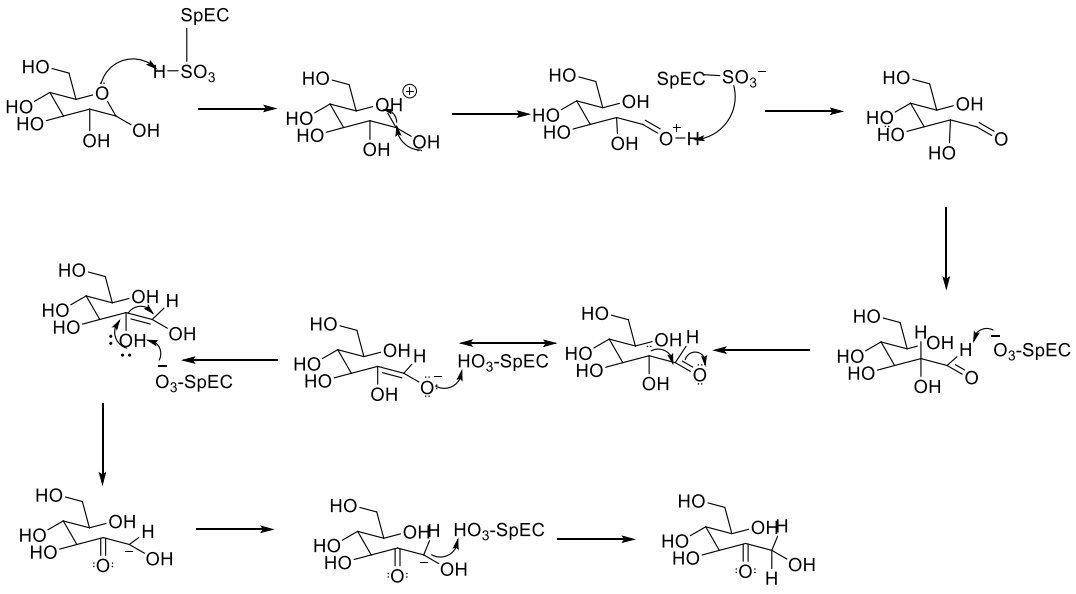


Figure 5.3-2: Yields of mannoside using different SpEC-SO_3H mole ratios (amount of mannose was 30 mmol in each case)

Since the reaction was carried out on a pre-heated oil bath, the di-O-isopropylidene furanose product of mannose (**Figure *5.3-2***) under the condition described above, according to Rajput *et al*,[50] might likely contain some product in pyranose form. The formation of pyranose product from the isopropylidation of sugar substrate is controlled kinetically. The kinetically controlled products are more stable but not easy to obtain because its reaction is slow and requires long reaction time to overcome the activation energy barrier that lead to its production. Mostly, strong acid catalysts often speed up the rate of reaction and thereby lead to the formation of thermodynamically favoured product.[69,70] SpEC-SO_3H can be reported to be a moderately strong acid catalyst because the chlorosulfonic acid used in its production has a strong acid strength with active sulfur site and also, it presumably has many active sites[71-73] which can aid in overcoming the activation energy barrier and therefore influence the reaction to form the thermodynamically favoured furanose product. Isopropylidation of mannose using SpEC-SO_3H as catalyst can be reported to be a thermodynamically controlled reaction because the reaction predominantly led to the formation of isopropylidene mannofuranose. To justify this conclusion, some researchers have previously reported that the thermodynamically controlled reaction in the protection of monosaccharide sugars often favour the formation of the products in furanose form.[70,74]

Though the protection of other sugars, such as D-glucose and D-xylose, were also investigated using the SpEC-SO_3H acid catalyst, the crude products were not subjected to further purification after a trial recrystallisation was attempted. The NMR spectrum of the crude product from the protection of glucose indicated that a mixture of α- and β-anomers (because the chemical shifts appear at 111.9 (β-anomeric carbon), 109.7, 105.3 (α-anomeric carbon), 85.3, 81.2, 75.2, 73.5, 67.7, 63.8, 54.0, 31.8, 31.8, 31.1, 29.3, 27.7, 26.9, 26.8, 26.2, 25.3, 20.7 ppm) of di-O-isopropylidene glucofuranoside was formed in the reaction in 1:3 ratio. TLC, which was visualised with potassium permanganate solution, was used to monitor the reaction at 30 minutes intervals, and the reaction was stopped at the end of six hours to avoid degradation of sugar substrate. There was an observation of five different spots on the TLC plate, in addition to the unreacted sugar at the baseline. The ^{13}C-NMR spectrum indicated ten major peaks that could be associated with methyl groups of isopropylidene glucofuranoside between the region of 20.7 ppm to 31.8 ppm (31.8, 31.8, 31.1, 29.3, 27.7, 26.9, 26.8, 26.2, 25.3, 20.7 ppm) and the peaks of acetal quaternary carbon at 111.8 ppm and 109.7 ppm. The peaks of ^{13}C-NMR spectrum of the crude product matched with those reported in the literature by Maddani *et al*[62] and Rajput *et al*[50] and this evidence indicated the formation of α- and β- anomers of 1,2:5,6-di-*O*-isopropylidene glucofuranoside. Further information from ^{13}C-NMR revealed the formation of a by-product which might likely be unsaturated in nature, because of two peaks in the region of 124.26 and 155.28 ppm. There was also evidence of a peak at 198.97 ppm, which may be due to isomerisation of the sugar into a keto form (**Scheme 5-14**).

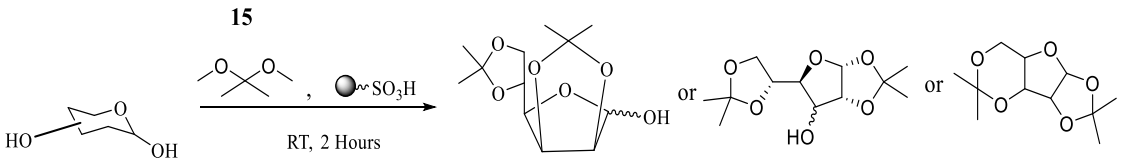


Scheme 5-14: **Isomerisation of glucose to its keto form**

5.3.4 Protection of monosaccharides with DMP at room temperature in the presence of SpEC-SO_3H

As well as employing acetone in the protection of sugars, more reactive reagents such as 2,2-dimethoxypropane (DMP)[70,75] and 2-methoxypropene[70] can be employed, in the presence of an acid catalyst, in the transformation of a monosaccharide to O-isopropylidene compounds. Employing the more reactive 2,2-dimethoxypropane for the protection of a sugar allows the reaction to be conducted under mild conditions, and helps avoid of degradation of the sugar substrate by the acid catalyst.[69]

In the protection of mannose with DMP, in the presence of SpEC-SO_3H, the procedure employed by Khan *et al*[70] was used with a slight modification. In this reaction, a mixture containing a D-sugar (5.4 g, 30 mmol), 2,2-dimethoxypropane (DMP, 67 mmol) and SpEC-SO_3H in acetone (dried with potassium carbonate before use, 60 mL) was stirred at room temperature for 2 hours. Then the mixture was filtered through a pad of Celite® and the SpEC-SO_3H washed with dry acetone. The combined filtrates were evaporated to obtain the crude product. The crude product was dissolved in diethyl ether (5 ml) and allowed to crystallize by addition of hexane (20 ml). The recrystallized solid was dried in a vacuum desiccator to constant weight (**Scheme 5-15**).



12a-c **14a** **14b** **14c**

a: mannose; **b:** glucose; **c:** xylose

Scheme 5-15: **Isopropylidation of sugar with DMP**

Different mole ratios of SpEC-SO_3H to substrate were employed in the investigation of catalytic activity of SpEC-SO_3H in the protection of sugars with DMP. 10 mol%, 5 mol % and 2.5 mol % SpEC-SO_3H were employed over various reaction times to catalyse the protection of mannose, and the results are shown in **Figure *5.3-3***. The product from the SpEC-SO_3H catalysed isopropylidenation of mannose with DMP gave the mannofuranoside like those reported in the literature by Khan *et al*.[70] The difference in yields (i.e. 95 %, 86 % and 75 %) between the different mole ratios of SpEC-SO_3H (3.07 mmol, 1.50 mmol and 0.75 mmol) employed in the reaction was around 10 % (**Figure *5.3-3***). The shortened reaction time and milder temperature conditions, which reduce the

rate of degradation of the sugar substrate are likely to be responsible for the consistency in the yields of the products in this reaction.

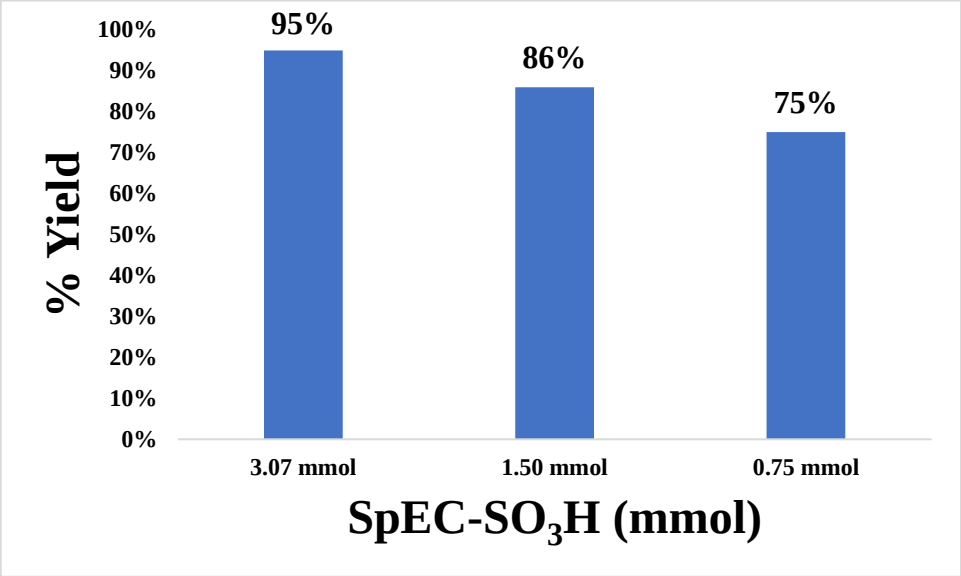


Figure 5.3-3: DMP protection of D-mannose

As a result of low concentration of H^+ in SpEC-SO_3H, the rate of reaction was also slower and thereby, the yield was affected and lower than what was obtained when the SpEC-SO_3H of higher concentration H^+ was employed. 10 % mole ratio has higher concentration of H^+ than the other mole % of SpEC-SO_3H, therefore, it led to higher yields of isopropylidene product in the reaction of mannose with DMP.

Due to the high reactivity of dimethoxypropane (DMP), short reaction times and mild temperature conditions, other sugar substrates were used to investigate the catalytic activity of SpEC-SO_3H. The period for the reaction of xylose with DMP in the presence of 5 mole % SpEC-SO_3H was 4 hours. Even so, unreacted xylose was observed (TLC) at the end of 4 hours, and since there was no indication that more xylose was been transformed to the expected product, the reaction was stopped and the crude product was obtained by filtration through a pad of Celite® and evaporation of solvents from the filtrate. The NMR of the crude product indicated formation of the product as a mixture of anomers. An attempt was made to recrystallise the crude with DCM and n-hexane but the effort was futile. Therefore, the crude product was subjected to column chromatography for purification with the hope of separating the crude product into each anomer. After several attempts of running and re-running the column chromatography, the product could not be separated into the different isomers, maybe because of the similarity of the Rf. However, the mixture of α- and β-anomers of diisopropylidene xylofuranose in 1.5:1 ratio

respectively together with 1,2-isopropylidene xylofuranose (also, in α- and β-anomers in 1:1 ratio) were isolated, which amounted to a total yield of 70 %, from the reaction of xylose with DMP. Judging from the peak height, 1,2-isopropylidene xylofuranose was only 25 % of the total yield. Also, there was an observation of a chemical shifts in the β-isomer region (109.6 ppm) that could be assumed to be a resonance signal of the β-isomer products.

The use of D-glucose as sugar substrate in the isopropylidenation with DMP required longer reaction times. The reaction of DMP with D-glucose substrate (5.41g, 30 mmol), in the presence of 5 mol% of SpEC-SO_3H, at room temperature was stopped at the end of 6 hours. Despite the longer reaction time, D-glucose had not completely reacted with DMP; there was physical observation of the unreacted sugar in the mixture. The unreacted sugar was filtered off along with the catalyst at the stoppage time of the reaction. The reaction, which was monitored with TLC, indicated the formation of isopropylidenated product. This was confirmed when the crude product was subjected to NMR spectroscopy and the selected peaks on spectra was compared to those from the literature. A complication observed when protecting the hydroxyl groups of glucose in the presence of SpEC-SO_3H, was the consistent formation of a by-product that contained unsaturated signals in the aromatic region of the ^{13}C-NMR spectrum. There was also the presence of a carbonyl peaks at 208 ppm in the ^{13}C-NMR spectrum. With the same catalyst ratio, when the reaction time was shortened to 4 hour and later 1 hour, the amount of crude product recovered was almost the same as that of the reaction that was allowed to continue for 6 hours. The crude product was obtained in the range of 49 % to 61 %. Due to observation of unreacted substrate in the mixture and also, some peaks thought to be aromatic and carbonyl in the NMR spectrum, it was concluded that the reaction was incomplete; the crude product is a mixture of intermediate and expected products together with other by-products. Therefore, the reaction was left for 19 hours in order to drive the reaction to completion. At stoppage of the reaction, the TLC showed the spot associated with the product was more prominent. Also, there was total disappearance of the peaks in the aromatic and carbonyl regions on ^{13}C- NMR spectrum of the crude product. The crude yield obtained from the latter reaction was increased to 72 %. At this juncture, and the crude product was subjected to column chromatography for further purification. The isolated product, which was 54 %, was subjected to NMR spectroscopy. The NMR

spectrum revealed that the product is a mixture of α- and β- anomers of furanoside in 57:43.

5.3.5 Comparision of activity of 10 mol% SpEC-SO3H and the yields of isopropylidene products from the three methods discussed.

It was reported that the isopropylidene products formed from isopropylidation of D-mannose was similar to those obtained from DMP.[74] Therefore, the yields resulting from each reaction method tried here was compared to one another. It was observed that ispropylidenation of D-mannose with DMP gave better yields than other methods in the reaction that involve 10 mol% of SpEC-SO_3H (**Figure *5.3-4***). The higher yield may have been as a result of the high reactivity of DMP. This reactivity advantage minimises the rate of sugar degradation, either on the acid catalyst or degradation resulting from heat. Moreso, DMP is often used to protect acid sensitive sugar substrates.[70] Protection of mannose with DMP was carried out at room temperature in a shorter reaction period (2 hours), therefore all the sugar substrate employed in the reaction may have been totally utilised and protected. On the other hand, the heat-aided O-isopropylidenation of mannose with acetone was not only faster (2 h) than the one reaction performed at room temperature (20 h), the reaction also resulted in a higher yield. It was observed that the isopropylidenation reaction performed with the same mol % ratio amount of SpEC-SO_3H, yielded a pure product of 84 % at room temperature while the product obtained from the reaction involving reflux of acetone yielded 91 % pure product (**Figure *5.3-4***).

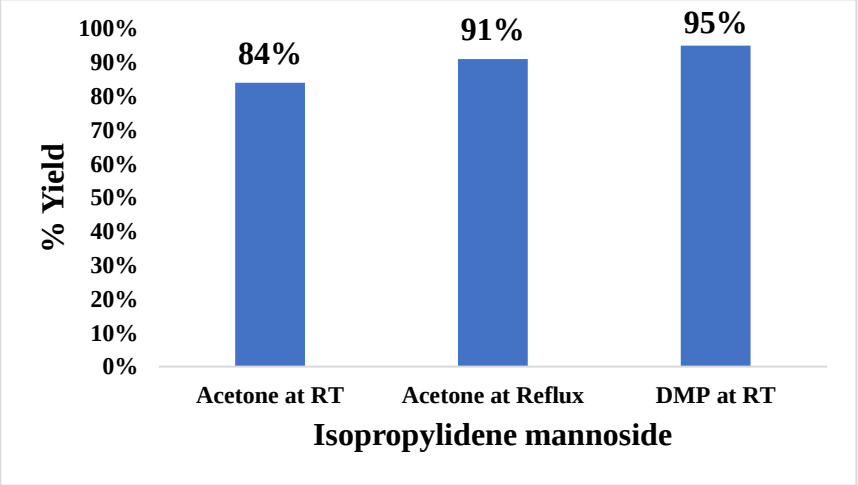


Figure 5.3-4: Comparison of the yields from the protection of D-mannose using 10 mol % SpECs-SO_3H

The difference between the yields of the two reactions performed at room temperature is about 11 %. This showed that isopropylidenation with DMP is more efficient than acetonation of sugar at room temperature.

5.3.6 Recyclability of SpEC-SO_3H in isopropylidation reactions

In order to ascertain the recyclability SpEC-SO_3H in the protection of D-mannose, the SpEC-SO_3H was recovered from O-isopropylidation reactions involving acetone at reflux, and also DMP at room temperature. After reaction, the recovered SpECs-SO_3H were washed thoroughly and dried in a desiccator to constant weight. The thoroughly dried recovered SpEC-SO_3H, from each of the methods, was then reused in three successive cycles of the same reaction. It was noted that there was steady decline in the weight of SpEC-SO_3H recovered each time of reuse.

At the second reaction cycle of the heat/acetone reaction the yield of the product dropped from 92 % to 90 % (**Figure *5.3-6***), but increased in the third and fourth reaction cycles to 99 %. The reason may be that some of the product clogged to the catalyst, during the previous use, was transferred to the successive reaction cycle. In summary, SpEC-SO_3H is highly recyclable in the heat-aided acetonation of D-mannose.

The recyclability of SpEC-SO_3H in isopropylidenation reaction using DMP was also successful. Whilst the yield of the protected mannose increased from 95 % in the first reaction cycle to 98 % in the second reaction cycle, the yield declined to 80 % in the fourth cycle (**Figure 5.3-5**). The decline in the yield was largely due to the reduction in the amount of SpEC-SO_3H resulting from the loss of some particles during washing and drying process. However, it was concluded that SpEC-SO_3H showed a good performance during these recyclability and reuse experiments.

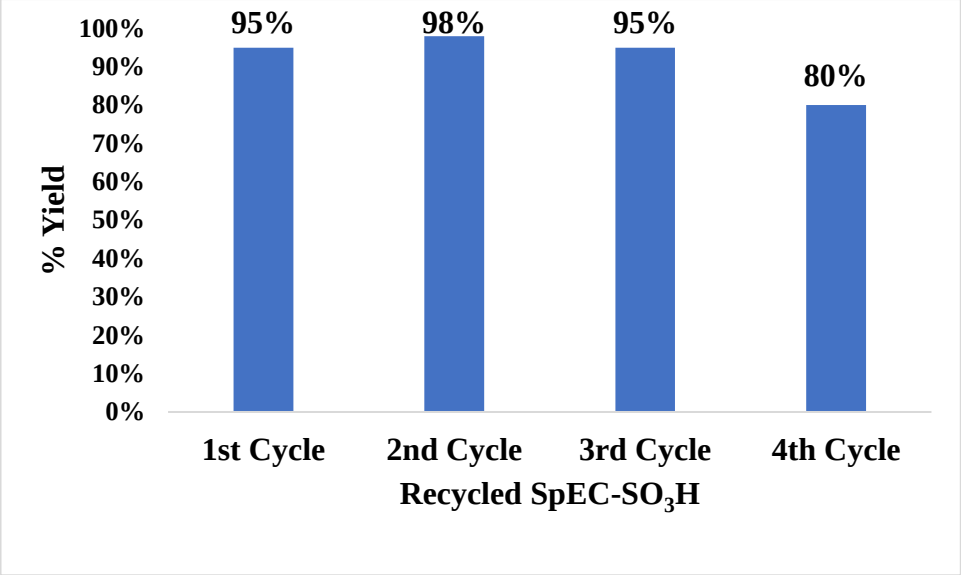


Figure 5.3-5: Recyclability of SpEC-SO_3H during the protection of *D*-mannose with DMP at room temperature

5.3.6.1 Comparison of the recycled SpEC-SO_3H catalysed isopropylidene products from reflux acetonation and DMP

The catalytic performance of SpEC-SO_3H in the isopropylidation of D-mannose was highly successful. The exploration of the catalytic activities of SpEC-SO_3H at different temperatures and as well in the reaction involving different acetal or isopropylidene reactants has shown that the catalyst performed relatively well at 10 % mole ratio in the three methods used. The recyclability of SpEC-SO_3H in the isopropylidation of mannose with DMP and its acetonation at the reflux temperature of acetone was therefore compared. The yield from the reaction involving DMP was higher than the acetonation of mannose, at reflux temperature of acetone, in the first two reaction cycles (**Figure *5.3-6***) but surprisingly, the yield of the acetonated products at the third and fourth reaction cycles jumped up and was higher than that of the DMP isopropylidenated products (**Figure *5.3-6***). The reason may not from lack of thorough washing of SpEC-SO_3H or transfer of the residue from the previous reaction cycles to the latter.

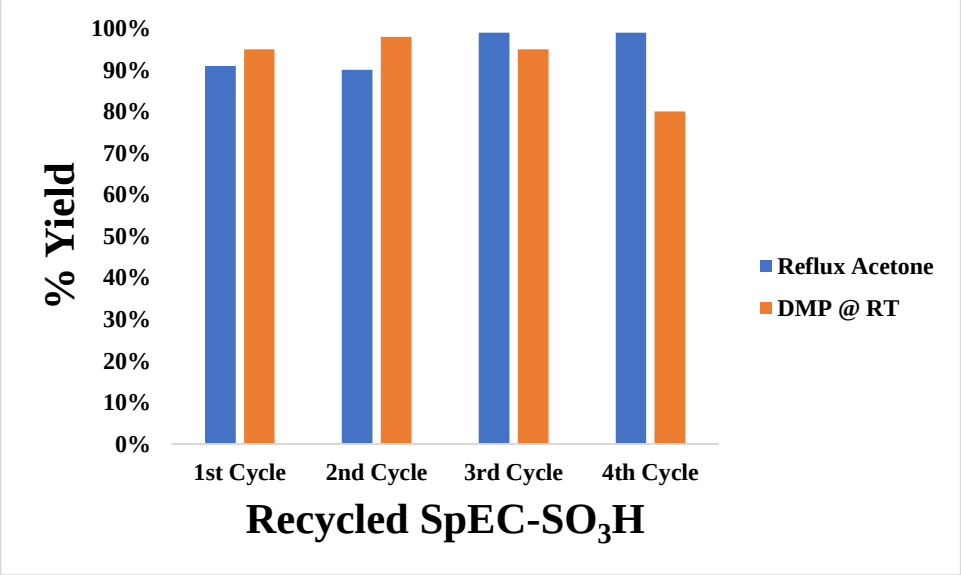


Figure 5.3-6: The yield of 2,3:5,6-Di-O-isopropylidene-α-D-mannofuranose using a recycled catalyst sample

5.4 Experimental

5.4.1 General method

SpEC-SO_3H was prepared according to the method discussed in **heading 3**. All other general methods followed those explained in **heading 4**.

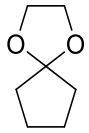
5.4.2 Preparation of ketal compounds

Ethylene glycol (molar amount specified in **Table *5.4-1***) and ketone (molar amount specified in **Table *5.4-1***) were added to a round-bottomed flask with sulfonated sporopollenin SpEC-SO_3H (2.5 – 12.5 % mole of ketone) and cyclohexane (5 ml), stirred at reflux with a Dean-Stark trap at 80 °C for the period recorded in **Table *5.4-1***. Then the mixture was filtered through a pad of Celite®, and the SpEC-SO_3H washed with ethyl acetate (30 ml). The combined filtrates were washed with water to remove the excess of unreacted alcohol. The organic layer was dried with anhydrous sodium sulfate then evaporated under vacuum until a constant weight was obtained. Further purification was through recrystallisation in n-hexane or column chromatography (ethyl acetate-n-hexane in 0.5 to 9.5 ratio).

Table 5.4-1: Reaction of ethylene glycol and ketone

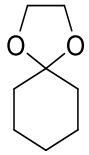
Entry	ketone	Amount of ketone (g, mmol)	Ethylene glycol (mmol)	SpEC-SO_3H (mmol, mole %)	Time (h)	Yield (%)
1	**4a**	4.21, 50	100	1.25, 2.5	2	65
2	**4b**	4.91, 50	100	1.25, 2.5	2	82
3	**4c**	6.01, 50	100	3. 07, 6.0	7.5	71
4	**4d**	1.99, 10	11	1.25, 12.5	3	86
5	**4e**	1.54, 10	11	1.25, 12.5	5	68
6	**4f**	1.00, 5.5	80	0.60, 10.9	8	94
7	**4g**	1.36, 10	11	1.25, 12.5	5	79

1,4-Dioxaspiro[4.4]nonane (7a) as compared with Torok *et al* [20]and Nikolic *et al*[76]



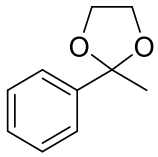
Liquid, 65 %; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 1.64-1.67 (m, 4H), 1.75 (t, J = 6.9 Hz, 4H), 3.87 (d, J = 0.9 Hz, 4H); **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 23.61, 64.27, 35.97,118.57.

1,4-Dioxaspiro[4.5]decane (7b) as compared with Nikolic *et al,*[76] Smith *et al,*[21] Ikeda *et al,*[77] and Hon *et al*[78]



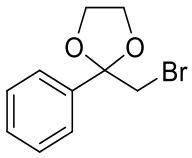
Liquid, 82 %; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 1.37 (s, 2H), 1.57 (s, 8H), 3.90 (d, *J* = 6.9 Hz, 4H), **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 24.06, 25.23, 35.24, 64.23,109.09.

2-Methyl-2-phenyl-1,3-dioxolane (7c) as compared with Smith *et al,*[21] Hong *et al,*[12] Aoyama *et al,*[23] Gregg *et al,*[17] Soderquist *et al,*[4] and Adam *et al*[2]



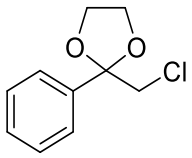
Solid, 71 %; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 1.65 (s, *J* = 7.1 Hz, 3H), 3.75-3.81 (m, 2H), 3.99-4.05 (m, 2H), 7.25-7.36 (m, 3H), 7.48 (d, J = 8.2 Hz, 2H), **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 27.72, 64.52, 108.91, 125.34, 127.91, 128.28, 143.35.

2-(Bromomethyl)-2-phenyl-1,3-dioxolane (7d) as compared with Zheng *et al,*[79] Han *et al,*[80] and Carlson *et al,*[81]



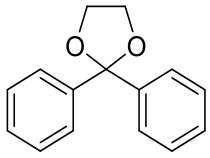
Solid, 86 %; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 3.66 (s, 2H), 3.85-3.93 (m, 2H), 4.14-4.22 (m, 2H), 7.31-7.38 (m, 3H), 7.50 (dd, J = 7.6, 1.8 Hz, 2H), **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 38.35, 65.91, 107.30, 126.06, 128.41, 128.89, 139.74.

2-(Chloromethyl)-2-phenyl-1,3-dioxolane (7e) as compared with Zheng *et al,*[79] Carlson *et al,*[81] and Ivanova *et al,*[82]



Solid, 68 %; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 3.75 (s, 2H), 3.90 (t, J = 6.9 Hz, 2H), 4.16-4.19 (m, 2H), 7.36 (d, J = 7.3 Hz, 3H), 7.50-7.52 (m, 2H), **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 49.50, 65.91, 107.94, 126.09, 128.40, 128.91, 139.76.

2,2-Diphenyl-1,3-dioxolane (7f) as compared with Akkapeddi *et al,*[79] Ueno *et al,*[83] and Fei *et al,*[84]



white crystalline solid, 94 %; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 7.20 - 7.94 (10H), 4.00 - 4.12 (4H); **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 64.98, 109.49, 126.24, 128.15, 128.23, 128.38, 130.17, 132.52, 137.69,142.19.

5.4.3 Preparation of DHP ethers

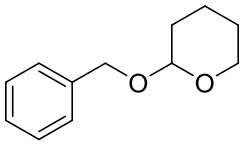
5.4.3.1 Preparation of DHP ethers in DCM

A mixture of the alcohol (10 mmol), 3,4-dihydropyran (X ml or g, 11 mmol), SpEC-SO_3H (0.2 g, 3.07 mmol.g^{-1}, 0.614 mmol), and dichloromethane (20 ml) was stirred at room temperature, and the progress of the reaction was monitored by TLC (petroleum ether-EtOAc; 8:2). After completion, the reaction mixture was filtered and the residue was washed with dichloromethane. The filtrate was concentrated to obtain a crude product. The crude product was purified over a column chromatography (eluent hexane–ether 9:1) to obtain the tetrahydropyranyl ethers.

5.4.3.2 Solvent free method for the preparation of benzyl DHP ether

Benzyl alcohol (1.08 g, 10 mmol) was added to sulfonated sporopollenin SpEC-SO_3H (0.2 g, 3.07 mmol.g^{-1}, 0.614 mmol) with 3,4-dihydropyran DHP (1.2 g, 15 mmol) in a mortar and was ground with pestle for 7 minutes. The progress of the reaction was monitored by TLC (petroleum ether- EtOAc; 8:2). After the disappearance of the starting alcohol, the mixture was treated with dichloromethane (10 mL) or ethyl acetate (10 mL) and the mixture filtered through Celite®. The filtrate was concentrated under vacuum to obtain the crude product. The product was purified with column chromatography (ethyl acetate-n-hexane 1:9) to afford pure benzyltetrahydropyranyl ether (0.39 g, 20 %).

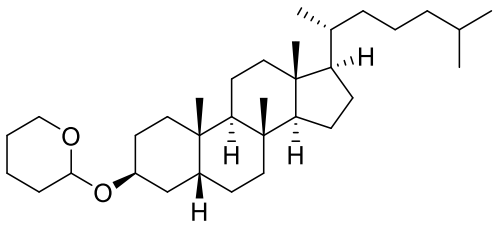
2-(Benzyloxy)tetrahydro-*2H*-pyran (10a)



2-(Benzyloxy)tetrahydro-2*H*-pyran

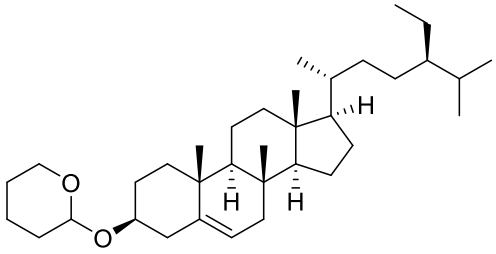
82 % yield, colourless liquid, **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 1.56-1.87 (m, 6H, 3 x C**H_2**), 3.56 (t, J = 5.3 Hz, 1H, -C**H_2**-O), 3.93 (s, 1H, O-C**H_2**-O), 4.51 (q, J = 5.8 Hz, 1H, -C**H**-O), 4.72-4.82 (m, 2H, Ar-C**H_2**-O), 7.28-7.37 (m, 5H, Ar-**H**), **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 19.45, 25.59, 30.67, 62.20, 68.91, 97.80, 127.61, 127.91, 128.47, 138.39, *m/z* (**ESI-MS): [**$M]^+$ 193.2

2-Cholestanyltetrahydro-*2H*-pyran (10b)



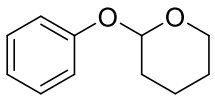
85 % yield, white solid, **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 0.67-2.00 (m, 54H), 3.45-3.60 (m, 2H), 3.84-4.04 (m, 1H), 4.69 (s, 1H), **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 12.15, 12.39, 18.75, 19.86, 20.12, 20.21, 21.29, 22.66, 22.92, 23.92, 24.30, 25.53, 25.60, 27.89, 28.10, 28.34, 28.82, 28.99, 29.54, 30.78, 31.39, 32.20, 34.39, 35.58, 35.72, 35.77, 35.88, 36.25, 37.07, 37.28, 39.59, 40.12, 42.67, 44.83, 45.20, 54.47, 56.35, 56.56, 62.88, 62.99, 75.35, 75.69, 94.75, 96.66, 96.93.

2-Sitosteryltetrahydro-2H-pyran (10c)-



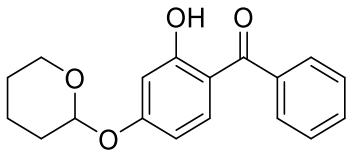
88 % yield, white solid, **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 0.50-2.35 (m, 64H), 3.45-3.61 (m, 2H), 3.84-3.96 (m, 1H), 4.11 (q, J = 7.2 Hz, 1H), 4.70 (s, 1H), 5.29-5.35 (m, 1H), **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 11.90, 12.02, 18.82, 19.07, 19.43, 19.68, 19.81, 19.87, 20.07, 20.14, 21.09, 23.08, 24.35, 25.38, 25.50, 26.04, 28.03, 28.31, 29.15, 29.19, 29.74, 30.72, 31.00, 31.33, 31.94, 31.98, 33.96, 36.19, 36.80, 36.83, 37.25, 37.49, 38.81, 39.81, 40.29, 42.35, 43.70, 45.84, 50.19, 50.23, 56.07, 56.79, 62.39, 62.83, 62.95, 63.41, 67.01, 76.03, 76.86, 77.17, 77.49, 94.63, 96.85, 96.99, 98.59, 98.92, 140.90, 121.55, 121.63, 141.08.

2-Phenoxytetrahydro-2H-pyran (10d)



Colourless liquid, 79 % yield; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 66-2.00 (m, 6H, 3 x C**H**$_2$), 13.60 (s, 1H, -C**H**$_2$-O), 3.92 (d, J = 8.0 Hz, 1H, -C**H**$_2$-O), 5.43 (d, J = 2.5 Hz, 1H, -C**H**-O), 6.98-7.47 (m, 5H, Ar-**H**), **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 18.94, 25.33, 30.50, 62.15, 96.40, 116.54, 121.68, 129.48, 157.15, *m/z* (**ESI-MS): [**M$]^{+}$ 179.2**.**

(2-hydroxy-4-((tetrahydro-2H-pyran-2-yl)oxy)phenyl)(phenyl)methanone (10e)



Transparent yellow syrup (semi solid), sweet smell, 97 %; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 1.47-1.75 (m, 4H), 1.84-2.05 (m, 3H), 3.59-3.65 (m, 1H), 3.81-3.90 (m, 1H), 5.50-5.54 (m, 1H), 6.51 (dd, J = 8.9, 2.5 Hz, 1H), 6.70-6.74 (m, 1H), 7.46-7.69 (m, 6H), **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 200.26, 165.97, 163.72, 138.36, 135.39, 131.59, 128.99, 113.83, 128.39, 108.34, 104.24, 96.18, 77.45, 77.13, 76.81, 62.29, 30.04, 25.07, 18.57, **elemental analysis** $C_{18}H_{18}O_4$ requires: C, 72.47; H, 6.08; O, 21.45; found elemental analysis: C, 73.03; H, 6.65

5.4.4 Protection of monosaccharides

Method A: Acetonation of monosaccharides at room temperature

The D-sugar (30 mmol) and SpEC-SO_3H (1 g, 10 %, 3.07 mmol SO_3H) were stirred in acetone (150 mL, previously dried with potassium carbonate) for 20 hours at room temperature. The mixture was filtered through a pad of Celite® and the SpECs washed with dry acetone (10 mL). The acetone was evaporated from the combined filtrate. The whitish product was dissolved in diethyl ether (30 mL) and allowed to recrystallize by adding n-hexane (50 mL) to the solution. The mixture was kept overnight in the refrigerator. The mixture was carefully decanted and the white solid recovered was vacuum dried in the desiccator over calcium chloride for 24 hours to a constant weight.

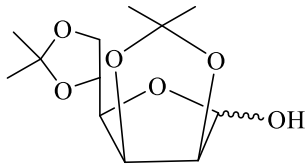
Method B: Acetonation of monosaccharides at reflux

The D-sugar (30 mmol) was stirred under reflux in dry acetone (dried with potassium carbonate before use, 5 mL) in the presence of sulfonated sporopollenin SpEC-SO_3H (1 g, 10 %, 3.07 mmol SO_3H) for 3 hours. The mixture was then filtered through a pad of Celite® and the SpEC-SO_3H washed with dry acetone. The combined filtrates were evaporated to obtain a crude product. The crude product was dissolved in diethyl ether (5 ml) and allowed to crystallize by addition of hexane (~20 ml). The recrystallized solid was dried in a vacuum desiccator to constant weight.

Method C: Isopropylidation of D-sugar with 2,2-dimethoxypropane (DMP) at room temperature

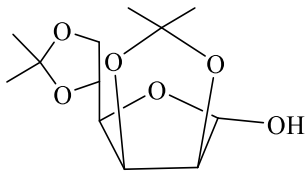
A mixture containing D-sugar (30 mmol), 2,2-dimethoxypropane (5 ml, 4.2 g, 41 mmol) and sulfonated sporopollenin SpEC-SO_3H (1 g, 10 %, 3.07 mmol SO_3H) in acetone (dried with potassium carbonate before use, 60 mL) was stirred at room temperature for 2 hours. Then the mixture was filtered through a pad of Celite® and the SpEC-SO_3H washed with dry acetone. The combined filtrates were evaporated to obtain the crude product. The crude product was dissolved in diethyl ether (5 ml) and allowed to crystallize by addition of hexane (~20 ml). The recrystallized solid was dried in a vacuum desiccator to constant weight.

2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose (14a) from mannose using method A as compared to Rajput *et al*,[50] Maddani *et al*[62] and Cristau *et al*[85]



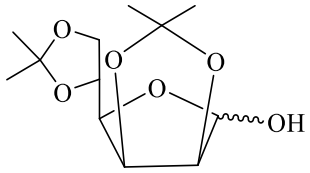
84 % white solid; **^{1}H-NMR (400 MHz,** $CDCl_3$, α:β-anomers = 4:1) δ 1.31-1.52 (m, 16H), 3.21-2.96 (1H), 4.01-4.08 (m, 3H), 4.16 (q, J = 3.7 Hz, 1H), 4.39 (dd, J = 11.3, 6.5 Hz, 1H), 4.60 (d, J = 5.9 Hz, 1H), 4.79 (dd, J = 5.7, 3.7 Hz, 1H), 5.36 (s, 1H) **^{13}C-NMR (101 MHz,** $CDCl_3$, α:β-anomers = 4:1) δ 24.5, 24.9, 25.2, 25.8, 25.9, 26.9, 27.1, 66.6, 67.2, 73.0, 73.3, 76.2, 78.6, 79.4, 79.7, 80.2, 85.5, 97.2, 101.3, 109.2, 109.4, 112.7, 113.5.

2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose (14a) from mannose using method A, but with 5 % SpEC-SO_3H, as compared to Maddani *et al*[62] and Cristau *et al*[85]



α-anomers

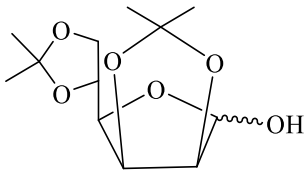
64 % white solid, mp 106 – 109 °C; **^{1}H-NMR (400 MHz,** $CDCl_3$, **)** δ 1.30 (s, 3H), 1.36 (s, 3H), 1.44 (s, J = 2.8 Hz, 6H), 3.25 (d, J = 2.3 Hz, 1H), 4.01-4.08 (m, 2H), 4.16 (q, J = 3.5 Hz, 1H), 4.36-4.41 (m, 1H), 4.59 (s, J = 6.0 Hz, 1H), 4.79 (dd, J = 3.2 Hz, 1H), 5.35 (d, J = 2.8 Hz, 1H,); **^{13}C-NMR (101 MHz,** $CDCl_3$**)** δ 24.49, 25.20, 25.89, 26.87, 66.56, 72.93, 79.68, 80.17, 85.53, 101.25, 109.20, 112.18**.**



^{13}C of the longer crystallization (mixture of α- and β-anomers)

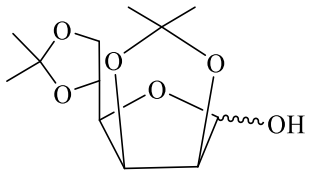
White solid, 95 %; **^{1}H-NMR (400 MHz,** $CDCl_3$, α: β-anomers = 4:1) δ 1.11-1.73 (22H), 2.82-3.26 (1H), 3.82-4.26 (5H), 4.29-4.47 (2H), 4.48-4.69 (2H), 4.70-4.89 (2H), 5.30-5.37 (1H), 5.37-5.44 (1H), **^{13}C-NMR (101 MHz,** $CDCl_3$, α: β-anomers = 4:1) δ 24.50, 25.21, 25.90, 26.90, 27.11, 31.06, 66.59, 67.18, 70.29, 73.35, 76.23, 78.62, 79.20 , 72.99, 79.33, 79.68, 80.19, 85.22, 85.53, 97.07, 101.04, 101.26, 109.20, 109.39, 112.70, 113.48, **elemental analysis** $C_{12}H_{20}O_6$ requires: C, 55.23 %; H, 8.05 %; found C, 53.22 %; H, 8.12 %.

2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose (14a) from mannose using method B as compared to Maddani *et al*[62] and Cristau *et al*[85]



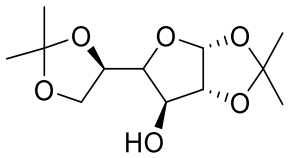
White solid, 91 %; **^{1}H-NMR (400 MHz,** $CDCl_3$, α: β-anomers = 4:1) δ 1.31 (d, J = 5.0 Hz, 3H), 1.35-1.38 (m, 5H), 1.44 (dd, J = 9.6, 6.4 Hz, 7H), 1.52 (s, 1H), 2.97 (s, 1H), 4.01-4.11 (m, 3H), 4.17 (q, J = 3.5 Hz, 1H), 4.35-4.41 (m, 1H), 4.60 (t, J = 5.3 Hz, 1H), 4.79 (q, J = 3.2 Hz, 1H), 5.36 (s, 1H), **^{13}C-NMR (101 MHz,** $CDCl_3$, α:β-anomers = 4:1) δ 24.5, 24.9, 25.2, 25.8, 25.9, 26.9, 27.1, 66.6, 67.2, 73.0, 73.3, 76.3, 78.6, 79.4, 79.7, 80.3, 85.5, 97.2, 101.3, 109.2, 109.4, 112.7, 113.5.

2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose (14a) from mannose using method C as compared to Maddani *et al*[62] and Cristau *et al*[85]



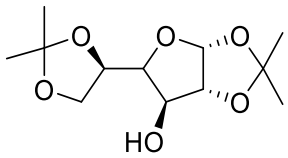
White solid, 95 %; **^{1}H-NMR (400 MHz,** $CDCl_3$, α: β-anomers = 4:1) δ 1.31 (d, J = 4.1 Hz, 3H), 1.36 (d, J = 6.9 Hz, 4H), 1.42-1.44 (m, 6H), 1.52 (s, 1H), 2.16 (s, 2H), 3.35-2.89 (1H), 4.03-4.08 (m, 2H), 4.16 (q, J = 3.7 Hz, 1H), 4.36-4.41 (m, 1H), 4.59 (d, J = 5.9 Hz, 1H), 4.78-4.80 (m, 1H), 5.36 (d, J = 6.4 Hz, 1H), **^{13}C-NMR (101 MHz,** $CDCl_3$, α:β-anomers = 4:1) δ 24.5, 24.9, 25.2, 25.8, 25.9, 26.9, 27.1, 27.6, 66.6, 67.2, 73.0, 73.3, 76.2, 78.6, 79.4, 79.7, 80.2, 85.5, 97.2, 101.3, 109.2, 109.4, 112.7, 113.5.

Crude product of 1,2:5,6-Di-*O*-isopropylidene-D-glucofuranose (14b) from glucose from method B as compared to Maddani *et al*[62] Jha *et* al[86] and Kaitsubata *et al*[87]



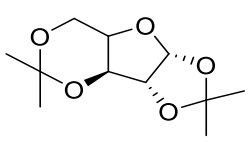
^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.23-1.48 (m, 6H), 1.86 (s, 3H), 2.14 (dd, J = 15.3, 11.2 Hz, 9H), 3.46-4.52 (m, 3H), 6.07 (s, 1H), **^{13}C-NMR (101 MHz,** $CDCl_3$**)** δ 20.7, 25.2, 26.2, 26.8, 27.7, 29.3, 31.0, 31.8, 53.8, 67.7, 73.5, 75.2, 81.2, 85.1, 104.9, 105.3, 109.7, 111.9, 124.3, 155.3, 198.9

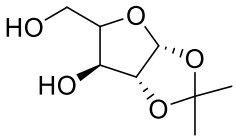
1,2:5,6-Di-*O*-isopropylidene-D-glucofuranose (14b) from glucose from method C as compared to Maddani *et al*[62] Jha *et* al[86] and Kaitsubata *et al*[87]



Transparent semi-solid, 54 %; **^{1}H-NMR (400 MHz,** $CDCl_3$) δ 1.07-1.68 (19H), 1.95-2.28 (4H), 5.99 (1H), 2.38-2.93 (1H), 3.91-4.23 (4H), 4.24-4.42 (3H), 4.44-4.60 (2H),5.88-5.96 (1H); **^{13}C-NMR (101 MHz,** $CDCl_3$**)** δ 25.4, 26.7, 26.8, 27.0, 27.3, 27.4, 55.4, 67.9, 72.7, 76.8, 80.0, 82.2, 104.4, 109.9, 110.0, 111.4,

1,2:3,5-Di-O-isopropylidene-D-xylofuranose (14c) from method A B & C as compared to Rajput *et al*[50]and Maddani *et al*[62]



and


Light yellow semi-solid, 70 %; **^{1}H-NMR (400 MHz,** $CDCl_3$**)** δ 1.65-1.21 (m, 22H), 3.42 (d, J = 3.4 Hz, 2H), 4.11-3.85 (m, 6H), 4.27 (d, J = 2.3 Hz, 1H), 4.49 (d, J = 3.7 Hz, 1H), 5.98 (d, J = 3.9 Hz, 1H), **^{13}C-NMR (101 MHz,** $CDCl_3$) δ 111.7, 110.3, 109.6, 105.3, 104.9, 97.6, 84.7, 78.2, 73.3, 71.7, 66.2, 60.3, 56.1, 53.9, 29.0, 27.2, 27.1, 26.8, 26.4, 26.2, 25.9, 18.7

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Chapter 6: Glycosylation reactions in the presence of SpEC-SO_3H

Carbohydrates are biomolecules that are abundant in living organisms,[1,2] and serve as energy-providing food for animals. Polysaccharides are composed of different units of monosaccharide building blocks[3] such as glucose, mannose, galactose, etc. Monosaccharides themselves are biologically active,[1,3,4] but are also used as starting materials or as precursors in the synthesis of useful chemicals[5-7] and therapeutic drugs.[8-10]

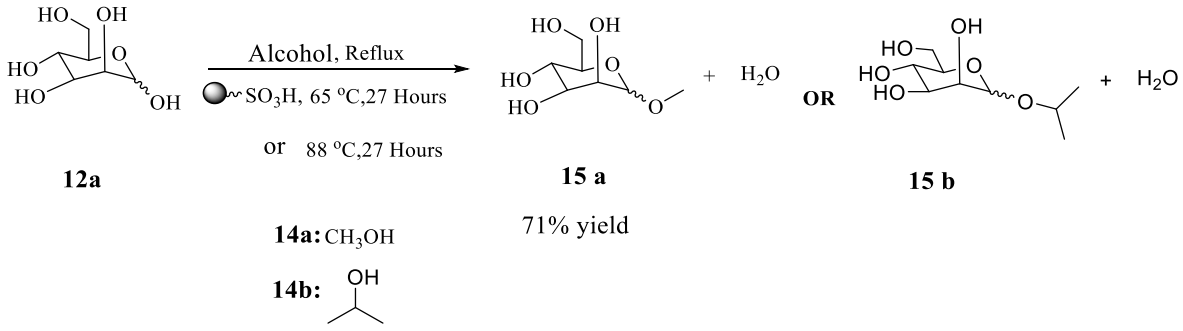
To achieve a high yield in a short period of time without degradation of its structure, catalysts have been investigated for the conversion of monosaccharides into derivatives.[11,12] For example, several catalysts have been reported in the literature for the protection, deprotection and or conversion of monosaccharides into bioactive compounds. Some catalysts investigated were either found to be ineffective or difficult to recover or cause hazardous pollution to the environment and also, some are corrosive, especially the homogeneous catalysts, for example H_2SO_4.[8,13] In recent times, researchers have introduced the use of sulfonated solid acid catalysts, such as Amberlyst 15 etc., into the reactions involving sugar and other molecules.[10-12,14] Sulfonated sporopollenin exine capsules (SpECs-SO_3H) have already proved to be an efficient heterogeneous catalyst in the dehydration of D-xylose to furfural in a microwave aided reaction.[15] There is, however, a need to further investigate its efficiency as an acid catalyst in glycosylation reactions.

6.1 *O*-glycosylation

6.1.1 Fischer glycosylation

Glycosides of unprotected simple sugars are very important in the synthesis and the study of biological macromolecules.[16] The chemical methods are most often carried out through a Fischer glycosylation or a Koenigs Knorr reaction (using a glycosyl halide).[17-19] Despite the long reaction time and the use of large quantities of alcohol, Fischer glycosylation has been reported as the most effective method to synthesis the simple glycosides.[16,20,21] Fischer glycosylation is a simple and less expensive method that involves the direct application of an acid catalyst to the reaction of sugar and an alcohol.[19]

A wide range of catalysts have been used for glycosylation reactions,[18,22] with examples using both homogeneous and heterogeneous catalysts. Roy *et al* reported the efficiency of sulfuric acid immobilized on silica as catalyst for Fischer glycosylation of primary alcohols of various types (both aliphatic and aromatic).[16] In addition, Bornaghi *et al* reported the use of Amberlite IRN 120H$^+$ for the glycosylation of methanol,[20] a useful comparator example for the use of solid sulfonated acid catalyst. SpEC-SO_3H was therefore investigated as a possible replacement to the aforementioned heterogeneous catalyst in Fischer glycosylation reactions. D-Mannose (1.0 g, 5.6 mmol) was heated in methanol in the presence of SpEC-SO_3H (0.5 g, 1.66 mmol equivalent SO_3H) for 27 hours (**Scheme 6-1**). The mixture was filtered, the solvent evaporated from the filtrate and the solid obtained was recrystallized with ethanol. The product, which was confirmed by NMR analysis, gave 71 % yield after the recrystallisation. The product is mostly the α-pyranoside anomer but with a trace of the β-anomer. The reaction was repeated with Amberlyst 15 of equal mmol equivalent amount similar to that of SpEC-SO_3H to catalyse the glycosylation reaction, the yield of the product obtained from Amberlyst 15 was as low as 35 %. Even when 2.4 mmol equivalent SO_3H of Amberlyst 15 was used, the yield obtained was only 43 %.



Scheme 6-1: **SpEC-SO_3H catalysed Fischer glycosylation of alcohol**

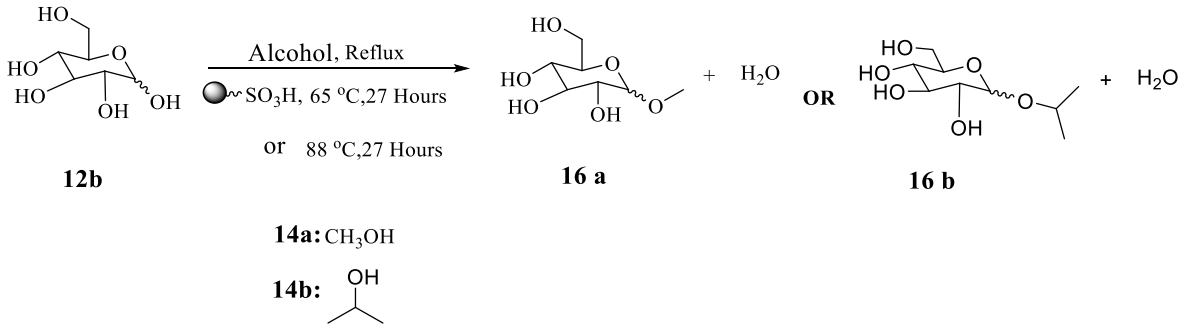
Fischer glycosylation of methanol using anhydrous glucose or glucose monohydrate also proved successful (**Error! Reference source not found.**). Though, the yield from the Fisher glycosylation of methanol with the use of glucose monohydrate was lower than anhydrous from the reagent. The same procedure used for mannose in the glycosylation of methanol was also used in this reaction process. The first attempt was thought not to be successful because of the nature of glucose. The crude product was semi liquid and syrup-like in nature and was difficult to recrystalise. The NMR spectrum of the crude product was complex. However, when the reaction was repeated with monitoring by TLC, the NMR spectrum of the crude product had a better resolution than the first experiment.

The reaction was stopped after 27 hours and the mixture was filtered through a small pad of Celite® to remove the catalyst. The mixture, after being concentrated to the crude product on a rotatory evaporator, was subjected to NMR analysis to confirm the formation of the methyl glucoside. The NMR spectra (both ^{1}H-NMR and ^{13}C-NMR), in agreement with the TLC observation, showed the formation of the glycosylated product. Therefore, the crude product was recrystalised from ethanol and the mixture was kept in the refrigerator for four days. The solid product observed was filtered and dried in the desiccator in order to remove the ethanol that was trapped in the product. A yield of 34 % was obtained containing a mixture of α- and β- glucopyranose anomers in a ratio of 15: 85 % respectively. in order to examine the influence of reaction time on the glycosylation process of the reaction involving glucose, the reaction time was increased to 48 hours while keeping the mole ratio of the catalyst and reactants of the previous reaction. The increase in reaction time did influence the crude yield giving a new yield of 98 %. The first and the second reaction produced the crudes of 1.23 g and 1.21 g respectively. Due to the low yield of the recrystalised product and the long time required for recrystallisation the previous reaction (27 hours), the crude product of the raw reaction was subjected to column chromatography. The purified product was concentrated and a total of 98 % yield was obtained. After subjecting the solid to NMR analysis, the spectra was compared to the literature[23,24] and it was revealed that the product contained a mixture of α- and β-glucopyranose anomers in 2:3 ratio i.e. 40: 60 %. The chemical shifts of the anomeric α-carbon appeared at 100.18 pp while that of the β-anomer appeared at 104.42 ppm in the ^{13}CNMR spectrum.[25]

Glycosylation of the secondary alcohol *iso*-propanol in the presence of SpEC-SO_3H and mannose yielded a product that was difficult to recrystallize. Isopropyl mannopyranoside (**Scheme 6-1**) is a semi-solid or syrup-like compound. Therefore, column chromatography was employed to isolate the pure compound from the crude. The yield was 85 % after purification and the product was subjected to NMR spectrum to confirm its anomeric form. The NMR data revealed the product was a mixture of α- and β-mannopyranoside, in 85:15 ratio, when compared with the reports of Tanaka *et al*[26] and Schmalisch *et al*[27] who reported that α- and β-anomers of isopropyl mannopyranoside product were found at 97.6 ppm and 97.7 ppm on the ^{13}C-NMR spectrum and the ratio was 80:20 respectively. The two mannopyranoside anomers could not be separated from each other because of their R_f values by TLC. This is in agreement with the report of

Tanaka *et al* that the α- and β- anomers of isopropyl mannopyranoside could not be separated from each other.[26] The ^{1}H-NMR chemical shift of the α-anomeric proton of the product obtained from the glycosylation of isopropyl alcohol in the presence of SpEC-SO_3H was between 4.77−4.86 ppm while that of the β-anomer was in the midst of the hydroxy proton signals at 4.69 – 4.71 ppm. Schmalisch *et al*[27] had reported that the anomeric hydrogen of the α-pyranose isopropyl mannoside was at 4.97 ppm while that of the β-pyranose anomer was at 4.76 ppm. Tanaka *et al*[26] reported that the chemical shifts of α- and β-anomers were found at 4.90 and 4.69 ppm respectively. Therefore, due to the reports of these two different workers, it was ascertained that the product obtained was a mixture of α- and β-pyranose anomers.

Glycosylation of isopropyl alcohol was also extended to the use of anhydrous D-glucose (**Error! Reference source not found.**). Here, D-mannose was replaced by D-glucose in the same procedure used for the glycosylation of isopropyl alcohol above. The crude product was purified by column chromatography and a yield of 88 % was isolated which indicated a mixture of α- and β- anomers of isopropyl glucopyranose in 2:1 respectively.



Scheme 6-2: **Fischer glycosylation of alcohol using glucose in the presence SpEC-SO_3H**

Investigation into the Fischer glycosylation of phenol with mannose proved difficult. Phenol, unlike methanol, is a solid, just like the mannose. Carrying out the reaction required making a solution of the reactants by solvent dissolution. Using a solution-method for glycosylation reaction would dilute the concentration of phenol and slow down the reaction rate. A melt glycosylation method was therefore attempted for the reaction of mannose and phenol, but the ^{1}H-NMR spectrum of the isolated 'product' indicated only the peaks of mannose in the monosaccharide peak ranges. Further investigation of the reaction was therefore carried out using dimethyl sulfoxide (DMSO)

as a solvent. However, DMSO has a very high boiling point of 189 °C, therefore, separation of the product from the solvent through evaporation led to the degradation of sugar and the product could not be analysed. Therefore, it was difficult to proceed with further experimentation on this work.

6.1.2 Glycosylation of long chain aliphatic alcohols / fatty alcohols

Long chain alkyl glycosides occur naturally.[28] They are low or non-toxic,[17,29-31] biodegradable,[18,28] non-ionic surfactants.[17,18,29,32] The biodegradability of alkyl glycosides is due to the presence of hydrolysable hemiacetal linkage that bond the sugar with alcohol substrate.[29,30] Also, they have higher stability in alkaline medium than their corresponding esters.[28,33] Long chain alkyl glycosides possess a hydrophobic alkyl end, while the sugar end is hydrophilic (**Figure *6.1-1***).[28,33] Due to these properties and because of their abilities to interact with various solvent, they can therefore be transformed to renewable and other useful compounds.[33,34]

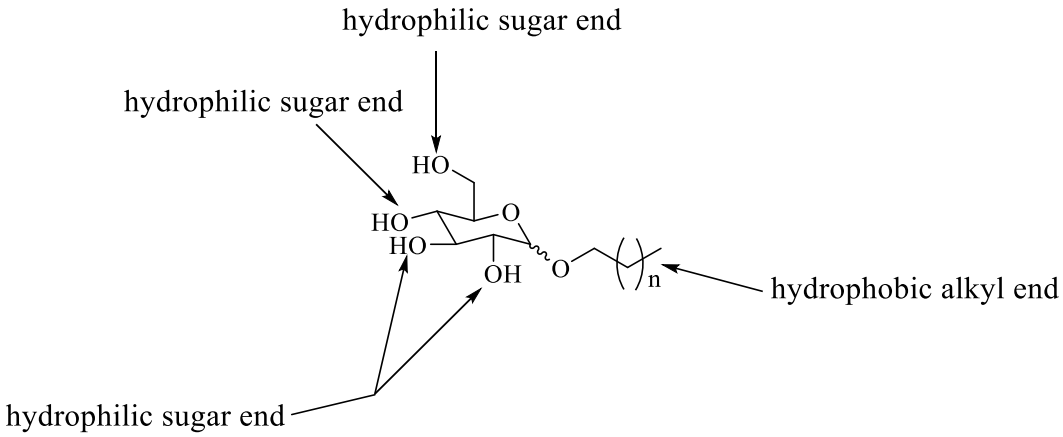


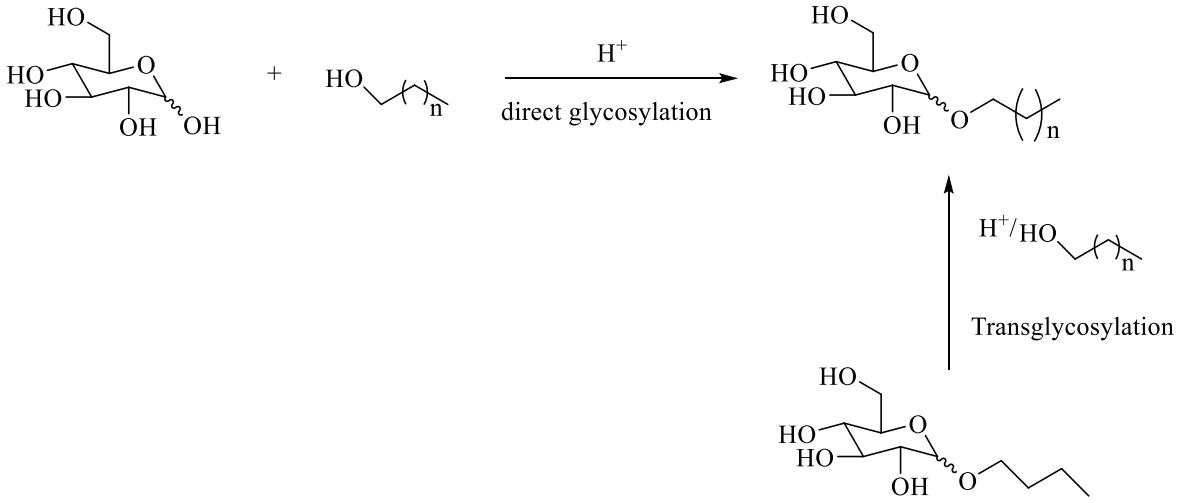
Figure 6.1-1: The structure of glycosylated long chain alcohol

The precursors for the synthesis of alkyl glycosides are renewable, abundant and eco-friendly,[28,33] which make alkyl glycosides to have gained more attention in the last few years[29] as useful materials in food, detergent, cosmetics[32,33] agrochemicals[35] and pharmaceutical industries[17,28,32,36] as well in the production of biofuels.[37-39] Alkyl glycosides have also found use in medicine because of their antimicrobial nature.[33] Some of the long alkyl glycosides are use as drug carriers due to their anti-adhesive properties against pathogens.[33,34,40,41]

Long chain / fatty alkyl glycosides can be synthesized through either chemical or enzymatic processes.[17,35] The enzymatic process is a green process and the most recent

method reported.[17] However, some of the enzymatic methods require the use of expensive sugar nucleotides and thereby make the method economically unsuitable for the large scale synthesis of glycosylated long chain compounds.[19]

A new method for synthesizing long alkyl glycosides has used a transglycosylation process in the presence of an acid catalyst (**Scheme 6-3**).[17,42-44]



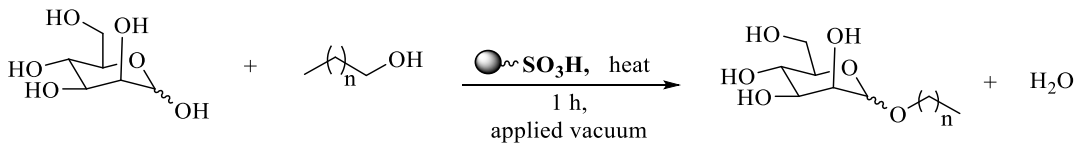
***Scheme 6-3*: Comparison of direct glycosylation and transglycosylation reactions**[22]

Chemical methods are the traditional ways of synthesizing long alkyl glycosides.[17,45] These processes may sometimes involve protection and de-protection of the active hydroxyl groups in order to avoid a mixture of products or obtain a defined product.[17,19,45,46] The process of protection and de-protection leads to an increase in the number of reaction steps and thereby increases the likelihood of impurities in the final product. Sometimes, de-protection is difficult, and it can cause degradation of the sugar if the use of heat is involved. Not only that, it is not economically viable as it leads to an increase in the cost of production and the overall time require. However, direct glycosylation has also been explored since it does not require the use of additional reagents outside of the main precursors for the glycosylation.

Different researchers have applied acid catalysts such as zeolites,[47] ion exchange resins,[20] mesoporous materials, amorphous silica-alumina, organic and mineral acid[16] catalysts to synthesize long chain alkyl glycoside.[48] Karam *et al* applied Aquivion PFSA (perfluorosulfonic acid) PW98 as a catalyst to obtain n-butyl glucoside and other glycosides of fatty alcohols through direct glycosylation or transglycosylation reactions.[22] An 85 % conversion was achieved with a 70 % yield of dodecyl glucoside at the reaction

period of 70 minutes when Aquivion PFSA PW90 was applied as a catalyst to the glycosylation of n-dodecanol with glucose.[22] Use of Aquivion PFSA PW98, a polymeric sulfonic acid, for the glycosylation of long chain alcohols,[22] coupled with the effectiveness and stability of $SpECSO_3H$ in our previous glycosylation reactions, prompted us to investigate the use of $SpEC\text{-}SO_3H$ as a catalyst for the synthesis of the glycosides of longer chain alkyl alcohols.

In the glycosylation of long chain alcohols, the mixture of monosaccharide (6.00 mmol), alcohol (18.00 mmol) and the $SpEC\text{-}SO_3H$ (0.5 g, 1.31 mmol) was heated under vacuum at 80 °C or above for an hour. The reaction flask was connected to the vacuum line to remove the water so avoiding the reverse reaction. The temperature for each reaction was varied depending on the boiling point of each alcohol. The reaction was monitored by thin-layer chromatography (methanol: ethyl acetate, 3:7) to ascertain the completion of the reaction. The catalyst was filtered off and the catalyst was thoroughly washed with the substrate alcohol, except for the substrates with high boiling point. The solvent was evaporated using a rotary evaporator to obtain a crude product. The crude product was purified with column chromatography using a gradient of 100 % ethyl acetate to 100 % methanol as eluent at 10 % intervals.



12a

n = 2, **17a**
n = 8, **17b**

n = 2, **18a,** Alpha:Beta of 3:2
n = 8, **18b**

***Scheme 6-4*: *O*-glycosylation of long chain alkyl alcohol with mannose**

The temperature for this type of glycosylation reaction depended on the range of boiling point of each alcohol substrate. The length of the alkyl chain of the alcohol determines the boiling points therefore the degree of heat for the reaction increases with increase in the number of carbon atoms of a straight chain alcohol. For example, the glycosylation of n-butanol was carried out at 80 °C while n-octanol was glycosylated at 120 °C (**Scheme 6-4**).

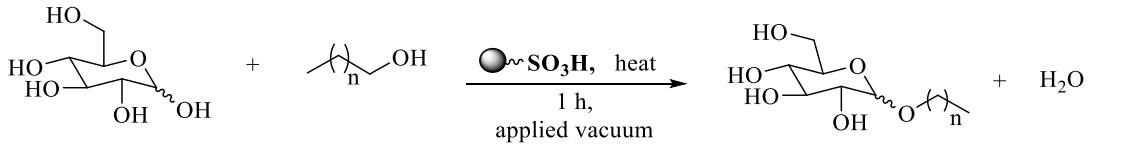
Both mannose and glucose were used in the glycosylation of n-butanol. Both resulted in a product that contained mixtures of the two pyranose anomers, as well degraded sugar. The crude mannoside product was subjected to column chromatography using a mixture

of ethyl acetate and methanol (8:2 to 7:3) resulting in a 46 % product yield. The product was subjected to NMR, elemental and mass spectrometry analysis. The NMR data results were the same as those reported by Lancefield *et al.*[49] The observed m/z 471.28 ($[2M]^+$) in the mass spectrum result confirms that the product was mixture of two anomers because the ^{13}C-NMR spectrum showed the anomeric carbon 99.85 ppm and 100.27 ppm in a ratio of α:β of 3:2. The elemental analysis showed C = 51.69 %, H = 9.03 % compared to the calculated values of C, 50.84; H, 8.53.

The 46 % yield was poor, but it showed that the SpEC-SO_3H could serve as a good acid catalyst. The poor result was a result of the degradation of some of the monosaccharide substrate at higher temperatures, in the presence of the SpEC-SO_3H acid catalyst.

The purification process was tedious as the glycosylated product made from glucose had four anomeric signals at 109.113, 103.373, 98.984 and 97.413 ppm in the ^{13}C-NMR spectrum. This indicated that there was α- and β-furanose as well as α- and β-pyranose-forms of the product in the crude mixture. The crude product provided 1.691 g but when purified through column chromatography, gave only the α- and β-pyranose-forms of the products in 48 % yield, from the butyl glucoside.

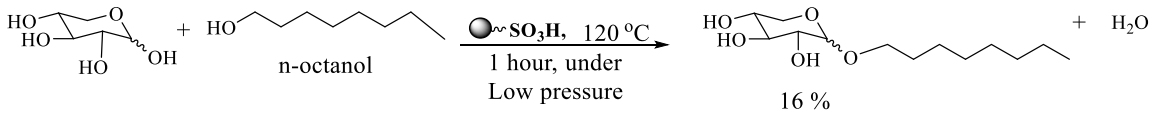
Mannose, glucose and xylose were separately used to glycosylate n-octanol at 120 °C using SpEC-SO_3H catalyst under vacuum. NMR analysis confirmed the formation of the desired products but all of them contained a mixture of anomers. The mannoside (**Scheme 6-4**) gave a crude of 1.939 g (111 %), glucoside gave a crude yield of 3.153 g which is equivalent to 180 % (**Scheme 6-5**) and xyloside (**Scheme 6-6** gave a crude yield of 2.412 g (153 %). There were lots of peaks in the ^{1}H-NMR spectra result which could indicate the formation of different anomers, as well different ring sizes. The crude glucoside of n-octanol was subjected to column chromatography. The expected glucoside compound eluted from the column using 40-50 % methanol in ethyl acetate, and concentration of the sugar fractions gave 45 % product yield. ^{1}H-NMR spectra of the pure product showed a mixture of α- and β-pyranose-forms. When the octyl mannoside was purified by a column chromatography a yield of 45 % was obtained.



12b n = 2, **17a**; n = 8, **17b** n = 2, **19a,** Alpha:Beta of 3:2; n = 8, **19b**

***Scheme 6-5*: *O*-glycosylation of octanol with glucose**

There was evidence of the formation of glycosylated products when the various monosaccharides were used, but there was mixture of products with different constitutional and anomeric forms. For example, ^{1}H-NMR spectra of the product from the glycosylation with D-xylose (**Scheme 6-6**) showed four anomeric hydrogen peaks (presumably pyranoside and furanoside). The xyloside of n-octanol was subjected to column chromatography in an attempt to purify the product and separate the different anomers. A product was eluted from the column using ethyl acetate: methanol (2:8). The solvent was evaporated from the product fractions and the product was subjected to the NMR analysis. The data obtained from the ^{13}C NMR analysis were similar and consistent to those obtained by Hricovíniová,[33] which showed that the chemical shift of α- and β-anomers carbon atoms were found at 98.39 and 102.93 respectively. ^{1}H-NMR data was very complex to interpret because of the different anomers in the product. Since the yield was low (16%), it was concluded that the use of MS/HPLC would be more expedient for the separation process to be able to get individual components from the mixture.



12c **17 b** **20**

***Scheme 6-6*: *O*-glycosylation of n-octanol with xylose**

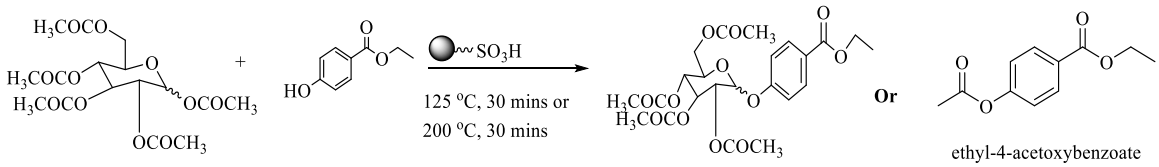
The low yield observed in this glycosylation process is due to the likely formation of humins by the monosaccharides in the presence of acid catalyst. Humins are reported to have the potential of decreasing the catalytic selectivity process which often results in poisoning or deactivating the active sites of solid acid catalysts.[50-52] Also, poor solubility of monosaccharide sugar in long chain alcohols is another factor that could have led to low yield of the glucoside.[53] Though the water by-product was removed by pressure but there could be some remnant which may have either caused reversibility of the reaction through hydrolysis or solvation of the sulfonic acid group (-SO_3H) of the catalyst which often deactivates the activity of acid catalyst.[50,53]

6.1.3 Attempted glycosylation of sterols and phenols

6.1.3.1 Attempted glycosylation of ethyl-4-hydroxybenzoate with pentaacetylglucose

A large number of natural products with biological activity are phenolic glycosides.[54,55] Phenols have antioxidant activities that inhibit free radicals in biological systems.[56]

Different catalysts have been reported in the condensation of phenolic compounds and a monosaccharide derivative.[56] For example, Ponticelli *et al*[56] reported the application of *p*-toluenesulfonic acid to the condensation of glucose pentaacetate and phenolic compounds. The catalytic ability of sulfonated sporopollenin (SpEC-SO_3H) was therefore investigated in the attempted glycosylation of some phenolic compounds and cholestanol using glucose pentaacetate. Glucose pentaacetate and ethyl 4-hydroxybenzoate (3:1 mol ratio) were melted in the presence 55 mole % of SpEC-SO_3H (0.5 g, 1.662 mmol equivalent SO_3H) at 125 ºC for 30 minutes. The progress of the reaction was monitored by NMR spectroscopy and thin layer chromatography (TLC). The crude product was dissolved in dichloromethane and the mixture was filtered. The filtrate was concentrated to determine the weight of the crude extract. The crude extract was dissolved in dichloromethane (100 mL) and washed with 0.5 M NaOH (50 mL) and then with deionised water (50 mL), to remove any hydrolysed sugars. The organic layer was dried with anhydrous sodium sulfate, filtered and was concentrated to a solid (**Scheme 6-7**).



21 **22** **23 a** **24**

***Scheme 6-7*: *O*-glycosylation of ethyl-4-hydroxybenzoate**

^{13}C-NMR data showed the signal thought to be that of the product (tetraacetylglucosylated-4-ethylbenzoate) anomeric carbon at 93.78 ppm, but also a signal for the unreacted precursor in the sugar region on the NMR. TLC (2:3, ethyl acetate-petroleum ether) showed four spots under the UV lamp and potassium permanganate solution (**Figure *6.1-2***).

The sample was subject to further purification with the use of column chromatography using a gradient of 1:9 of ethyl acetate-petroleum ether to 6:4 of the same solvent mixture. Spot "A" was identified to be unreacted ethyl-4-hydroxylbenzoate. Spot B was the

expected product. Spots "C" and "D" were the derivatives of sugars or the unreacted sugars (**Figure *6.1-2***). In order to confirm the formation of the product, the purified product was further investigated using ES-MS which confirmed the formation of the product but the yield of the purified product was very small (0.2 %)

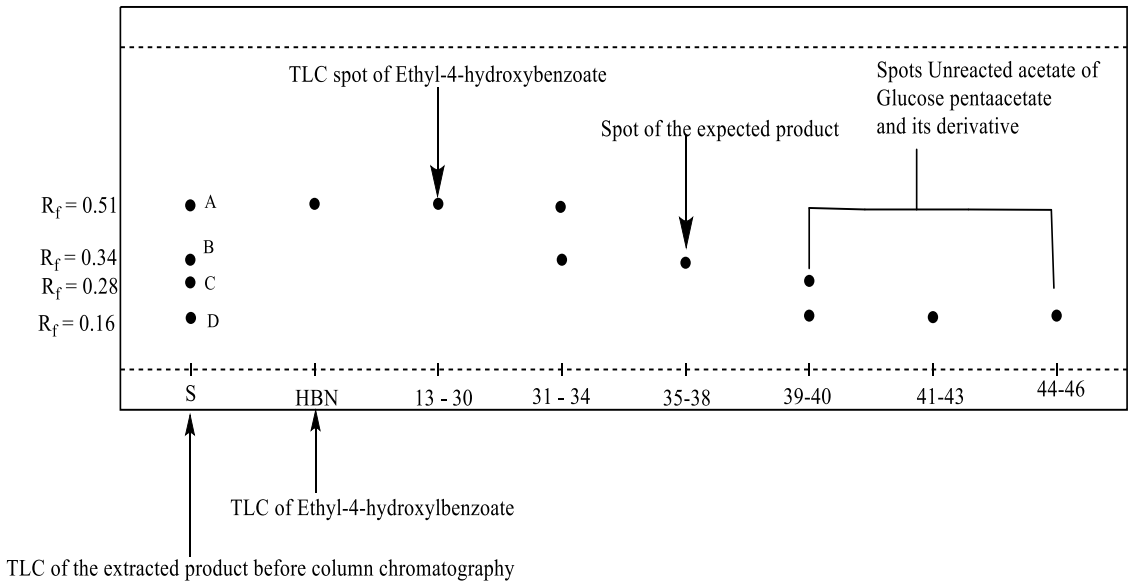


Figure 6.1-2: Thin layer chromatography of O-glycosyl phenol compound

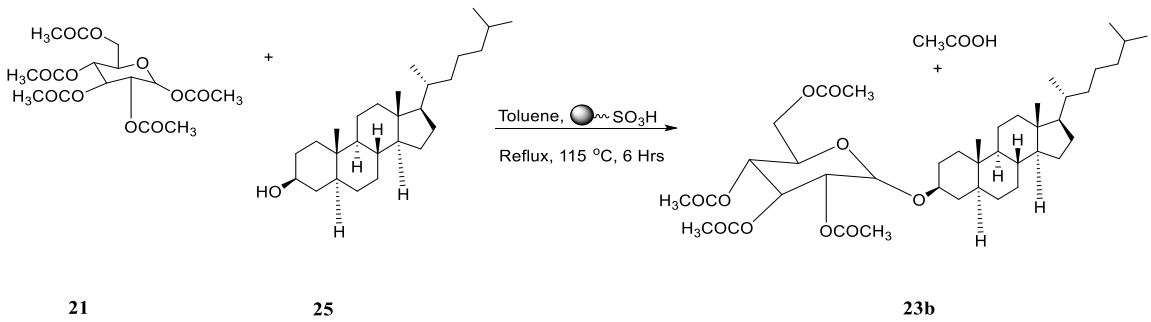
An attempt was made to improve the yield of the product by increasing the reaction time and temperature. The temperature of the melt condensation reaction of glucose pentaacetate and ethyl-4-benzoate in the presence SpEC-SO_3H (as a catalyst) was increased to 200 ºC and the reaction was left for 1 hour. In this reaction, glucose pentaacetate was degraded and only ethyl-4-acetoxybenzoate was obtained as the product (**Scheme 6-7**) with the chemical shifts of the signals on the ^{1}H-NMR spectrum showed δ 8.04-8.13 (1H), 7.87-8.00 (3H), 7.32-7.72 (1H), 6.75-7.04 (4H), 4.19-4.69 (4H), 1.18-1.67 (6H).

Melt condensation conditions caused the degradation of sugar at higher temperatures (200 ºC) and for reaction times longer than 30 minutes. The attempt resulted in the acetylation of the hydroxy group of ethyl-4-hydroxylbenzoate (**Scheme 6-7**). Using a solvent was therefore attempted with the hope of improving the yield of product. Glucose pentaacetate and ethyl-4-hydroxylbenzoate were heated at reflux for 6 hours with SpEC-SO_3H (1.65 mmol) in dichloromethane, but the attempt was not successful. Only the

glucose pentaacetate was recovered at the end of the whole process of the reaction. Dichloromethane was later replaced with toluene and the reactants were heated reflux with SpEC-SO_3H at 115 ºC, but the product could not be identified after the alkali work up. Glycosylation in different solvent media stated above did not yield any product even with increased amounts of catalyst.

6.1.3.2 Attempted glycosylation of 5α-cholestan-3β-ol with pentaacetylglucose

The glycosylation of 5α-cholestan-3β-ol with pentaacetylglucose was attempted using the procedure stated in **6.3.2.3.** Glucose pentaacetate (1.8 mmol) and 5α-cholestan-3β-ol (5.1 mmol), in the presence of 55 mole % SpEC-SO_3H (0.3 g, 0.993 mmol equivalent SO_3H), were heated at reflux in toluene through a column containing 4Å molecular sieves (15 g) for 6 hours at 115 °C (**Scheme 6-8**). The mixture was filtered, and the filtrate was treated with 0.5 M NaOH and deionised water to remove the unreacted alcohol. The organic layer was dried with sodium sulfate and concentrated to a solid. The product was analysed by TLC using visualisation by oxidation with potassium permanganate solution. A mixture of the products and reactants were found; therefore, the product was purified by column chromatography. The yield of the expected product, after column chromatography was very small (1.5 %, 15 mg), and could only be tentatively identified from the mass spectrum (ES-MS).



21 **25** **23b**

***Scheme 6-8*: *O*-glycosylation of 5α-cholestan-3β-ol**

When dichloromethane was used as the solvent for the reaction, no reaction was observed at 40 °C after 6 hours.

6.2 *N*-glycosylation

6.2.1 Glycosylation of urea in the presence or absence of NH_4Cl

Glycosyl urea is an integral part of glycocinnamoylspermidine, an antibiotic also known as cinodine (**Figure *6.2-1***).[57,58]

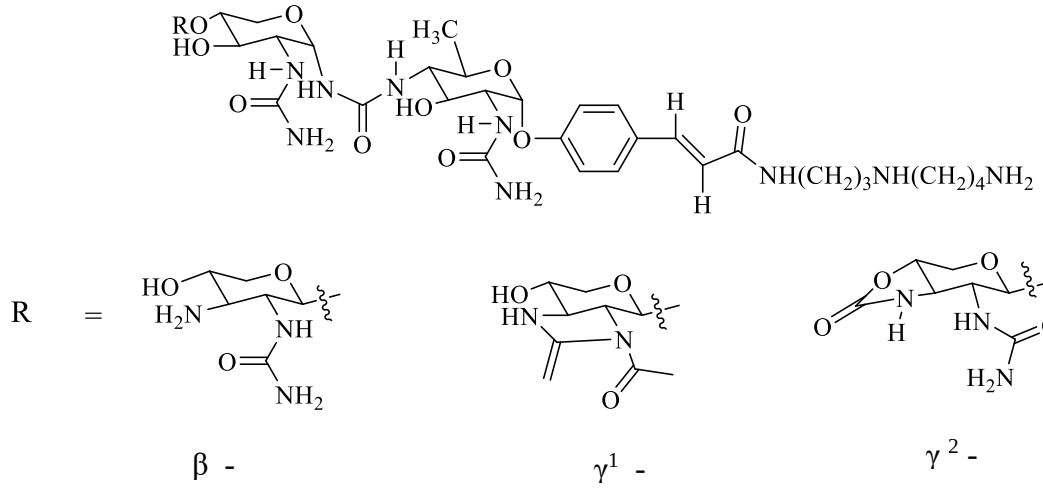


Figure 6.2-1: The Structure of cinodine (glycocinnamoylspermine) [57,58]

It is also an important and excellent adhesive when mixed with phenol and water.[14] Glycosyl urea has also found use in the production of glycosylthymines, and it is also used as lyophilisation stabiliser for enzymes.[14] Amberlyst 15 has been reported to be the most successful catalyst in the condensation of glucose and urea, using either a melt method or a solution phase reaction.[14] The melt method has several advantages over solution phase reactions. The method does not require separation of the product from the solvent used in the reaction process. The concentration of the reaction medium is always high, thereby leading to an increased rate of reaction.

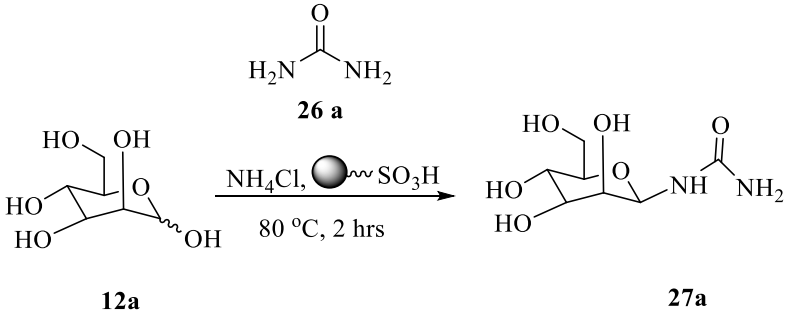
Sulfonated SpECs (SpEC-SO_3H) are quite resistant to high temperature, due to the underlying sporopollenin polymer base.[59,60] SpECs have been found stable at temperatures as high as 400 ºC.[59,61,62] Therefore, the use of SpEC-SO_3H was applied as a catalyst in the melt condensation of anhydrous glucose and urea following the procedure described above without any modification. This initially resulted in a very low yield of hygroscopic solid, which rapidly absorbed moisture from the atmosphere. The attempt to subject glucose and ethyl carbamate to condensation in the presence of SpEC-SO_3H as an acid catalyst using the same method was not successful.

Melting anhydrous D-glucose without decomposition was difficult, therefore, the anhydrous glucose was replaced with glucose monohydrate. Again, the reaction was not successful. This may be due to the ability of the urea glycoside to undergo a reversible reaction in the presence of water. Also, the hydroxyl group attached to the carbon-2 of glucose is in the axial position. This hydroxy group is liable to interact, through intramolecular hydrogen bonding, with the anomeric hydroxy group[63] which may cause steric hindrance and stop the reaction from progressing to the formation of the expected

product. Further investigation may be conducted on this reaction process in the near future.

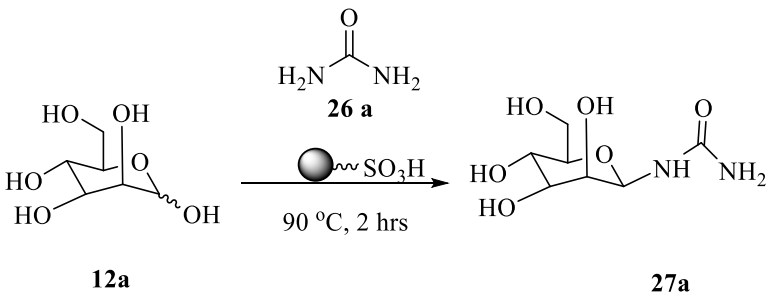
Due to the unsuccessful reaction of glucose and urea, the attention was shifted to the use of mannose in the glycosylation of urea. Mannose is an epimer of glucose with hydroxyl group on the carbon-2 atom in equatorial position. Mannose is more stable, in solution, in its α-anomer form. Therefore, the inversion of the hydroxy group attached to carbon-2 of mannose away from that of glucose decreases its formation of hydrogen bonding with the α-anomeric hydroxy group and thereby reduced and / or eliminates the problem, caused by steric hinderance, that may occur in the reaction process. Ruß *et al*[14] had catalysed the reaction of mannose and urea with either Amberlyst 15 (solid sulfonic acid catalyst) or *p*-TsOH (homogeneous catalyst) in a solvent-free reaction and obtained a good yield of 75 % or 64 % mannose ureide respectively. However, the glycosylation reaction by Ruß *et al*[14] required the use of NH_4Cl in addition to the catalyst and other substrates. So, we studied the reaction of anhydrous D-mannose with urea in the presence of SpEC-SO_3H, as an acid catalyst, and NH_4Cl (**Scheme 6-9**). It was revealed that NH_4Cl was added as an additive. Munirathinam *et al*[64] reported that the use NH_4Cl, as an additive in an organic synthesis, is to coordinate the regioselectivity of the reaction in which the formation of the product with a particular configuration is favoured above the other (while the other is blocked) in the reaction of epoxide, but this was not reported in the work of Ruß *et al*. The glycosylation of urea with D-mannose in the presence of SpEC-SO_3H with the incorporation of NH_4Cl was very successful. A yield of 95 % was obtained after recrystallization using methanol and diethyl ether in 4:1 ratio (v/v). The experiment was repeated three times with similar results. Both the ^{1}H- and ^{13}C-NMR spectra indicated the formation of D-mannosyl urea, as comparing with the data obtained by Ruß *et al,*[14] all the chemical shifts were closely matched except the glycosidic anomeric peak. The chemical shifts data of mannosyl urea by Ruß *et al*[14] was reported in DMSO-D and the anomeric carbon was found at 157.53 ppm. Therefore, Ruß *et al*[14] reported that the compound is a β-anomer. Though, the glycosidic anomeric carbon from the ^{13}CNMR spectra of this experiment was found at 160.17 ppm, it can be concluded as well that the mannosyl urea produced in the presence of SpEC-SO_3H is a β-anomer because the chemical shifts were reported in D_2O. Two other attempts were made at this reaction, in which the mixture during recrystallization was not allowed to stand at room temperature, rather cooled directly to 0 ºC. This resulted in yields above 100 % with ^{13}C-NMR results showing that

unreacted urea recrystallized along with the product. It has been reported[65-67] that such products may be treated with urease to degrade and remove the urea, but this was not attempted.



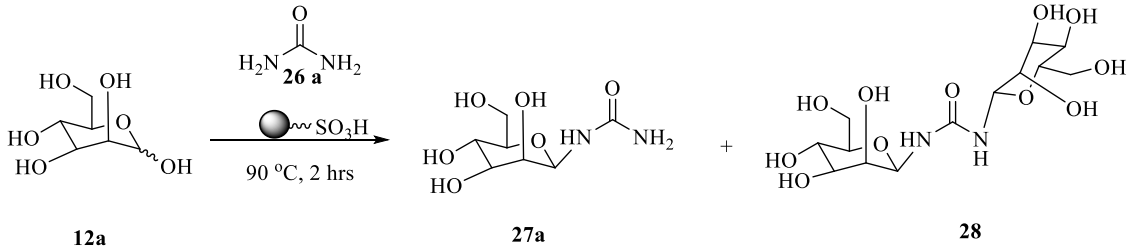
***Scheme 6-9*: Reaction of mannose with urea**

An investigation was conducted to confirm that the catalytic activity of SpEC-SO_3H was not aided by the ammonium chloride (NH_4Cl) used in the previous glycosylation method.[14] Another solvent-free reaction was conducted in the absence of NH_4Cl (**Scheme 6-10**) and a yield of 97 % was still obtained. The 97 % yield was recorded with the third experiment, for which the recrystallization process took two weeks. The first and the second experiments yielded 50 and 51 % respectively as shown in the **Table 6.2-1**, entries **1** & **2**. Here, D-mannose (0.6 g, 3.3 mmol) and urea (1.4 g, 23.3 mmol) were melted to a clear solution at 90 °C (temperature of the oil bath) before the addition of the SpEC-SO_3H to the reaction mixture (**Scheme 6-10**). The reaction was stirred for 2 hours and the catalyst was recovered at the end of the reaction through filtration. The crude product was obtained by concentration on rotary evaporator and allowed to crystallize overnight (**Table 6.2-1**, entries **1** & **2**) or beyond a night to obtain a pure product (**Table 6.2-1**, entry **3**). The result and the high yield of the product showed that NH_4Cl has little or no effect on the catalytic activity of sulfonated sporopollenin as far as using mannose was concerned.



***Scheme 6-10*: Melt reaction of urea and mannose in the absence of NH_4Cl**

The mass spectrometric analysis of products indicated the formation of small amounts of di-glycoside of urea; i.e. glycosylation at both ends of the urea (**Scheme 6-11**).



***Scheme 6-11*: Formation of dimer in the absence of NH_4Cl**

The first and the second glycosylation experiments may have resulted in the formation of mono-glycosylated products (**Table 6.2-1**, **entries 1 & 2**). Their recrystallisation were left overnight.

Table 6.2-1: Glycosylation of urea in the absence of NH_4Cl

Entries	Sugar	Products	Rxn condition	Temp (°C)	Rxn Time (h)	% yield
1	**12 a**	**27a**	Melt	90	2	50
2	**12 a**	**27a**	Melt	90	2	51
3	**12 a**	**27a**	Melt	90	2	97
4	**12 a**	**27a**	Water	90	$2^1/_4$	53

Also, apart from the solvent-free or melt reaction, the investigation was conducted on the catalytic performance of SpEC-SO_3H, without the addition of NH_4Cl, in water. Though it was reported that the yield from sulfuric acid-catalysed reaction of mannose and urea in water was poor and the reaction period was too long[14,68] but this can be improved in with a change of catalyst. Though, sulfuric acid is a homogeneous catalyst with Brønsted acid sites the use of a heterogeneous catalyst with Brønsted acid sites to investigate the reaction might influence or increase the yield. Therefore, the was carried out in water in the presence of SpEC-SO_3H at the same temperature using the same method used in the melt reaction and without NH_4Cl (**Table 6.2-1**, **entry 4**) yielded 53 %. Leaving the product in the refrigerator for a longer time caused an increase in yield, though there was a trace amounts of urea in the product (the carbonyl peak of compound **26 a** to that of compound **27a** was about a 1:8 ratio). The yield is better than the yield reported by Badawi *et al*[68] but the yield was not as good as the one obtained from the solvent-free reaction. The concentration of the substrates in the melt method might have played an important role on the rate of the reaction and may have as well influenced the

yield of the end product. The introduction of solvent will reduce the concentration of the reaction carried out in water, hence the rate reaction will become slower and this will definitely affect the yield.

Also, the low yield from the reaction in water may possibly have resulted from the reversibility potential of the reaction involving monosaccharides. The reaction can go forward and backward in the presence of water and more so, the presence of solvent, which slowed down the reaction process, may have aided the backward reaction to be more favoured.

Other monosaccharides such as D-glucose and D-xylose were also used in the glycosylation of urea using the same conditions as for mannose. Though the crude products showed evidence of the formation of glycosides, it was difficult to isolate the pure products through a crystallization method. This may be due to mixtures of constitutional and anomeric structure of the sugars, as well as the limited differences in solubility of these hydrophilic compounds.

6.2.2 Glycosylation of ethyl carbamate in the presence or absence of NH_4Cl

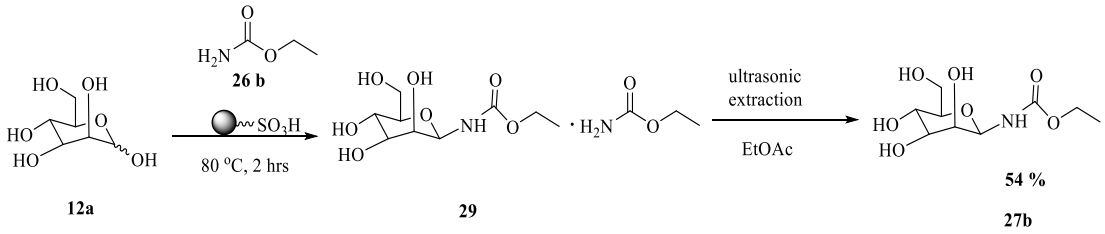
6.2.2.1 Glycosylation of ethyl carbamate in the presence of NH_4Cl

Investigation into the glycosylation of ethyl carbamate with D-mannose in the presence of SpEC-SO_3H and ammonium chloride (NH_4Cl), using the same method used for the glycosylation of urea with the same mole ratios of the reactants as well as mole % of SpEC-SO_3H, gave in a low yield, a white powder. However, the NMR spectrum did not confirm the formation of the desired product, the ethyl carbamate mannoside. The excess ethyl carbamate may have constituted hindrance to recrystallisation of the product. An attempt to investigate the glycosylation reaction of ethyl carbamate with glucose proved unsuccessful. Similarly, no product was recovered from the condensation of glucose monohydrate with ethyl carbamate.

6.2.2.2 Glycosylation of ethyl carbamate in the absence of NH_4Cl

The melt method for the glycosylation of ethyl carbamate was modified (**Scheme 6-11**). The reaction was carried out without the addition of ammonium chloride. Using information obtained from Ruß *et al*,[14] the product was purified by the use of ultrasonic solvent extraction. Ultrasonic extraction using EtOAc (5 mL) was used to remove the excess ethyl carbamate that constituted a hindrance to crystallisation of the product in the

previous attempt. This left a pure, glycosylated compound (54 % yield) behind. The product was shown to probably be the β-pyranose anomer as the glycosidic carbon in the ^{13}C-NMR spectrum was at 78.85 ppm. This result has similarity with that of Ruß *et al*,[14] who showed that the glycosidic carbon of β-mannopyranoside urea was at 78.46 ppm.



Scheme 6-12: **Melt reaction of ethyl carbamate and mannose in the absence of NH_4Cl**

6.2.3 Attempted glycosylation of heterocyclic compounds, unsaturated

Due to the success from the glycosylation of urea in the presence of SpEC-SO_3H, attempts were made to catalyse the glycosylation of a range of heterocyclic compounds and primary amides: imidazole, 2-methylimidazole, 6-chloropurine, acrylamide and benzamide.

Melt reaction and solvent aided reaction methods were used in the glycosylation attempts following the method used for the glycosylation of urea. The sugar degraded and in the reaction process, leaving behind products that were entirely different from the targeted glycosylated products.

It was impossible to achieve the glycosylation of acrylamide in the presence of SpEC-SO_3H, as an acid catalyst. The attempt led to self-condensation reaction of acrylamide to form an insoluble polymer. The ^{1}H-NMR spectrum of the soluble portion of the mixture only revealed the chemical shifts of the proton peaks of acrylamide when the spectrum was compared to the result obtained by Tomás-Mendivil *et al*[69] (**Figure 6.2-2**)

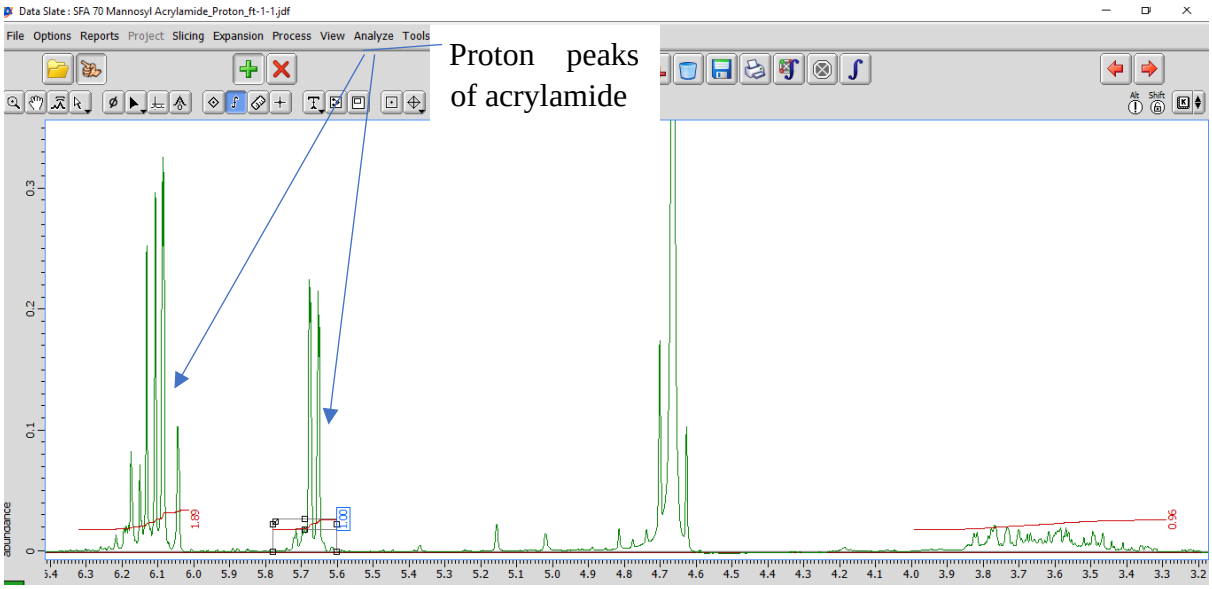


Figure 6.2-2: The ^{1}H-NMR spectrum of the soluble part of the crude product from glycosylation of acrylamide

Glycosylation of benzamide was not successful even when heated at 120 °C. D-mannose (0.6g, 3.3 mmol) and benzamide (2.824 g, 23.3 mmol) were melted at 120 °C until a clear mixture was obtained. SpEC-SO_3H was then added to the melt and the total mixture was stirred at the same temperature for another two hours. Water was immediately added after 2 hours of the reaction but the mixture solidified, and the solid was insoluble in water. After the removal of water, the substance was dissolved in methanol which allowed the SpEC-SO_3H to be filtered off. The filtrate was concentrated, recrystallized with water and methanol and kept in the refrigerator. The recrystallization led to the formation of finger-like crystals after 24 h. The crystals, only showed the ^{1}H-NMR peaks of benzamide (**Figure 6.2-3**). The following chemical shifts; 168.4, 134.8, 131.7, 128.7, 128.0 ppm that showed on ^{13}C-NMR spectrum, leaving the mannose region without any peaks. The NMR spectrum was compared with that of Zhang *et al*, Kuwabara *et al*, Zhao *et al*, Qi *et al* and Peng *et al*.[70-74] Another possible cause of the unsuccessful reaction between benzamide and monosaccharide is the low nucleophilicity of the amide nitrogen due to lone pair conjugation into carbonyl group.

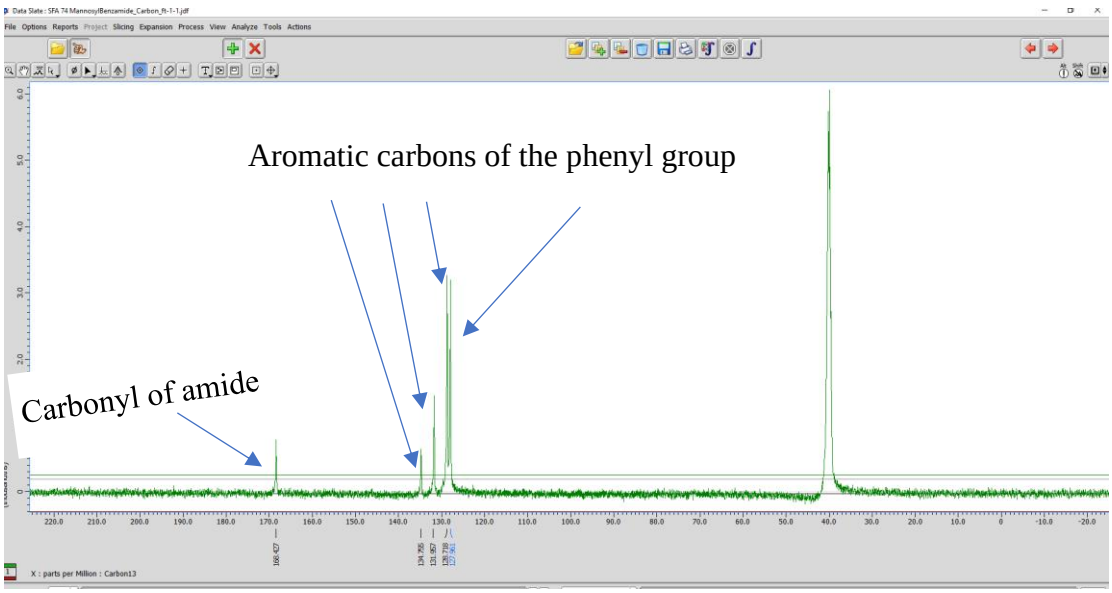
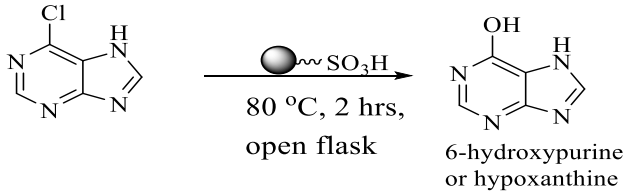


Figure 6.2-3*:* The ^{13}C-NMR spectrum of benzamide recovered from the attempted glycosylation of benzamide

An attempt to melt the mixture of 6-chloropurine and D-mannose at 80 °C was not successful. The heating was left for 2 hours before the addition of SpEC-SO_3H but the reactants remain unmelted even when left for another 2 hours. Water (5 mL) was added, in order to turn the mixture into solution. The reaction vessel was left opened for evaporation of water, and the mixture left for another 2 hours.

The glycosylation reaction was not successful but the addition of water led to the transformation of 6-chloropurine (**30a**) to 6-hydroxypurine (hypoxanthine) (**Scheme 6-13**). The chlorine atoms from the un-oxidised 6-chloropurine was replaced by the hydroxyl group from water molecule that was later introduced to aid the dissolution of the reactants.



30a **31**

Scheme 6-13*:* Formation of hypoxanthine

It was presumed that the monosaccharide was either degraded during the heating period or dissolved and was removed during the recrystallizations process. The solid

substance that was crystallised out was subjected to NMR spectroscopy which confirmed the formation of hypoxanthine.

The most surprising unsuccessful reaction was the reaction attempted between imidazole and D-mannose in the presence of SpEC-SO_3H acid catalyst (**Figure 6.2-4**). The presence of two nucleophilic nitrogen atoms sandwiched by methylidene in a five-member aromatic ring was meant to be a plus for easy reaction. The reaction was not successful despite the several attempts that were made to get the reaction to work. Parameters like temperature were varied, and sometimes the reaction was performed in the open reaction vessel to assist in water removal, but there was no indication that the reaction ended in the formation of a glycosylated product. The melt reactions were attempted both in the presence and absence of NH_4Cl. Other unsuccessful glycosylation reactions included attempted glycosylation of 2-methylimidazole and 2,5-dibromo pyridine. Glucose and xylose were also used in the attempted glycosylation of the substrates 2-methylimidazole and 2,5-dibromo pyridine, but in both cases the processes yielded no products.

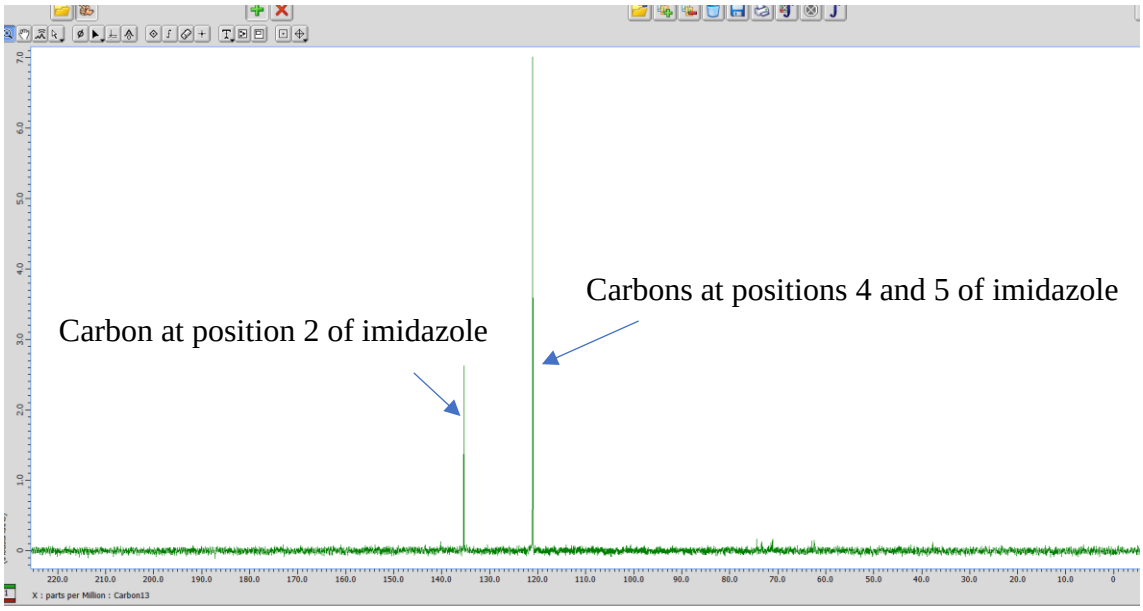


Figure 6.2-4: ^{13}C-NMR of the compound recovered from glycosylation of imidazole

6.3 Experimental

6.3.1 General

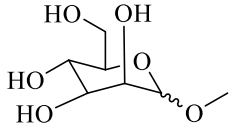
All the chemicals, reagents, and solvents were purchased from either Sigma Aldrich or Fisher Scientific, United Kingdom and used as obtained. Spores of *L. clavatum* used for making sulfonated sporopollenin was donated by Sporomex, UK. All the spectroscopic and elemental analyses were carried out in the Department of Chemistry in the University of Hull. ^{1}H and ^{13}C NMR spectra were obtained on JEOL JNM 400 MHz instrument at 400.3 and 100.6 MHz respectively. Melting points were determined on a Fischer-Johns electrical melting point apparatus with an open capillary and are uncorrected. Precoated Merck aluminum TLC plates were used for thin layer chromatography. ESI-Mass spectra of the compounds were recorded on a HCT ultra ETD II mass spectrometer. The visualizations of the TLC plates were carried out with use of either ultra violet (UV) lamp or potassium permanganate. The TLC plates stained with potassium permanganate were first air-dried and further dried by a gentle heating with use of Steinel HL 1810S hot air gun.

6.3.2 *O*-glycosylation

6.3.2.1 Fischer glycosylation of alcohols and phenols with monosaccharides

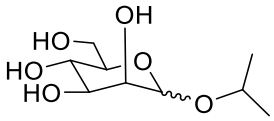
D-Mannose (1.0 g, 5.6 mmol) was added to a reaction flask containing alcohol (371 mmol) and heated at reflux in the presence of SpEC-SO_3H (1.66 mmol equivalent) for 27 hours. The mixture was filtered, at the end of the reaction, through a small pad of Celite® in a sinter funnel (porous grade 4) and the filtrate was concentrated to dryness on rotary evaporator to obtained crude product. The product was recrystallized from ethanol (5 mL).

Methyl mannopyranose (15 a) compared to the NMR data from Tanaka *et al*[26] (α - anomer with a trace of β-anomer)



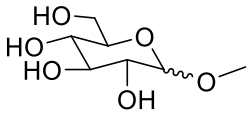
White crystal solid compound, 71 %, **^{1}HNMR** (400 MHz, D_2O) δ 3.19 (d, J = 23.3 Hz, 3H), 3.35-3.47 (m, 2H), 3.55-3.58 (m, 2H), 3.72 (d, J = 15.6 Hz, 2H), 4.57 (s, 1H), 4.66 (s, 4H), **^{13}CNMR** (101 MHz, D_2O) δ 54.7, 56.8, 60.9, 66.7, 69.9, 70.5, 72.5, 76.2, 100.8.

Isopropyl mannopyranose (15 b) compared with NMR data from Tanaka *et al*[26] and Schmalisch *et al*[27] (mixture of α and β-anomers)



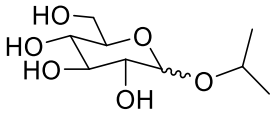
Semi solid, 85 %; **^{1}HNMR** (400 MHz, D_2O) δ 0.92-1.20 (m, 8H), 3.35-4.02 (m, 9H), 4.68 (d, J = 14.7 Hz, 8H), 4.82 (d, J = 1.6 Hz, 1H), **^{13}CNMR** (101 MHz, D_2O) δ 20.1, 20.8, 22.0, 22.2, 60.9, 61.1, 61.7, 66.9, 69.8, 70.4, 70.5, 71.1, 72.0, 72.7, 73.1, 76.2, 97.5, 97.7.

Methyl glucopyranose (16 a)



White solid, 98 %; **^{1}HNMR (400 MHz**, DMSO-D6) δ 2.89 (dd, J = 7.9, 4.9 Hz, 1H), 2.97-3.13 (m, 4H), 3.21 (s, 3H), 3.31-3.34 (m, 6H), 3.39 (q, J = 5.9 Hz, 1H), 3.60-3.65 (m, 2H), 3.98 (d, J = 7.8 Hz, 1H), 4.44-4.50 (m, 2H), 4.69-4.74 (m, 1H), 4.84 (d, J = 5.5 Hz, 1H), 4.90 (dd, J = 13.4, 4.9 Hz, 2H), 5.03 (d, J = 4.8 Hz, 1H), **^{13}C-NMR (101 MHz,** DMSO-D6) δ 54.8, 56.5, 61.4, 61.6, 70.5, 70.8, 72.5, 73.1, 73.9, 77.1, 77.4, 100.2, 104.4.

Isopropyl glucopyranose (16 b)



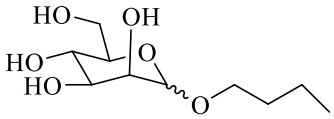
Semi solid, 89 %; **^{1}H-NMR (400 MHz**, DMSO-D6) δ 1.01-1.13 (m, 11H), 3.02-3.13 (m, 4H), 3.28-3.40 (m, 9H), 3.55 (s, 1H), 3.76 (t, J = 6.2 Hz, 1H), 4.12 (d, J = 7.8 Hz, 1H), 4.37-4.44 (m, 2H), 4.65-4.68 (m, 2H), 4.79 (d, J = 5.5 Hz, 1H), 4.83-4.86 (m, 1H)**, ^{13}C-NMR (101 MHz,** DMSO-D6) δ 21.94, 22.25, 23.87, 24.06, 61.54, 61.62, 69.09, 70.32, 70.64, 70.95, 72.36, 73.27, 73.76, 74.00, 77.25, 77.35, 97.32, 101.52.

6.3.2.2 Glycosylation of long chain aliphatic alcohol / fatty alcohol

D-Sugar (6 mmol) was mixed with n-alcohol (18 mmol) in a 50 mL round bottom flask, equipped with a magnetic stirrer bar. Then SpEC-SO_3H (1.31 mmol) was added and the solution was heated in an oil bath at 120 °C under vacuum (15 mmHg) for the desired reaction time. The reaction was monitored by TLC. In the SpEC-SO_3H was filtered off at

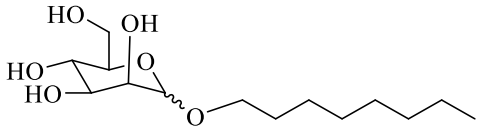
the end of the reaction and the excess n-butanol was removed under vacuum. Filtration was carried out immediately to avoid the solidification of glycosylated products. The glycosylated products were isolated from the mixture through column chromatography with methanol/ethyl acetate as the eluent solvent.

n-Butyl mannopyranoside (18a) as compared to Lancefield *et al*[49]



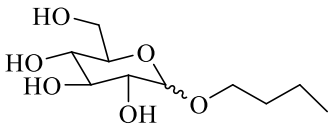
46 %, **^{1}H-NMR (400 MHz,** $CDCl_3$**)** δ 0.87-0.92 (m, 3H), 1.24 (t, J = 7.3 Hz, 1H), 1.30-1.38 (m, 2H), 1.48-1.58 (m, 2H), 2.03 (s, 1H), 2.77 (s, 1H), 3.37 (dd, J = 16.0, 6.4 Hz, 1H), 3.49 (d, J = 9.2 Hz, 1H), 3.62 (dd, J = 16.5, 6.9 Hz, 1H), 3.73-3.94 (m, 5H), 4.10 (q, J = 7.2 Hz, 1H), 4.49 (d, J = 17.9 Hz, 1H), 4.79 (s, 1H), 5.04 (d, J = 45.4 Hz, 3H), **^{13}C-NMR (101 MHz,** $CDCl_3$**) δ** 13.71, 14.47, 19.40, 21.04, 31.54, 60.40, 61.19, 66.20, 67.63, 70.77, 71.72, 72.48, 99.85, 100.27, **elemental analysis:** $C_{10}H_{20}O_6$ requires C, 51.69 %, H, 9.03 %; found: C = 50.84 %, H = 8.53 %, ***m/z*** **(ES-MS):** $[M + H]^+$= 235, $[2M + H]^+$=471.28

n-Octyl mannopyranoside (18 b) compared with NMR data from Mizutani *et al*[75] and Augé *et al*[19] (mixture of α and β-anomers)



Syrup-like liquid, 45 %; **^{1}H-NMR (400 MHz,** DMSO-D6) δ 0.87 (t, J = 6.9 Hz, 3H), 4.35-4.73 (2H), 1.13-1.28 (d, J = 12.8 Hz, 10H), 1.29-1.39 (2H), 2.96-3.79 (23H), 4.28-4.34 (1H), **^{13}C-NMR (101 MHz**, DMSO-D6) δ 14.5, 22.6, 26.0, 26.3, 29.3, 29.5, 31.8, 33.1, 61.2, 61.8, 66.6, 67.5, 70.8, 71.5, 74.5, 98.5, 100.2.

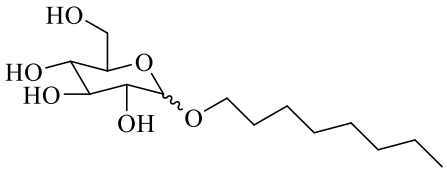
n-Butyl glucopyranoside (19 a)



48 %, **^{1}H-NMR (400 MHz, DMSO-D6) δ** 0.65-0.96 (3H), 0.87-1.25 (1H), 4.80-5.02 (1H), 0.94-1.69 (14H), 2.53-2.86 (1H), 2.67-3.83 (10H), 4.46-4.62 (1H), **^{13}C-NMR (101**

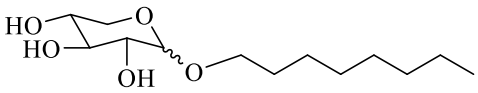
MHz, DMSO-D6) δ 14.5, 22.6, 26.1, 26.2, 29.2, 29.4, 29.7, 29.8, 31.8, 32.8, 61.2, 62.3, 66.4, 67.6, 69.0, 69.9, 70.5, 72.4, 73.8, 77.2, 99.4, 104.0.

n-Octyl glucopyranoside (19 b) compared with NMR data from Griswold *et al,*[76] Gervay-Hague *et al,*[77] and Matin *et al*[78] (mixture of α and β-anomers)



Semi-solid, 45 %; **^{1}H-NMR (400 MHz,** $CDCl_3$**)** δ 0.86 (t, J = 6.6 Hz, 6H), 1.26 (d, J = 3.7 Hz, 20H), 1.59 (d, 4H), 3.28-4.28 (m, 22H), 4.66 (s, 1H), 4.83 (s, 1H), 4.95 (s, 1H), 5.15 (d, J = 16.0 Hz, 1H), 5.40 (s, 1H), **^{13}C-NMR (101 MHz, DMSO-D6)** δ 14.2, 22.8, 26.0, 26.1, 29.4, 29.4, 29.6, 29.6, 32.0, 61.2, 61.3, 68.6, 69.3, 69.4, 70.6, 71.6, 72.0, 74.2, 75.7, 76.4, 98.7, 102.9.

n-Octyl xylopyranoside (20) (mixture of α and β-anomers)



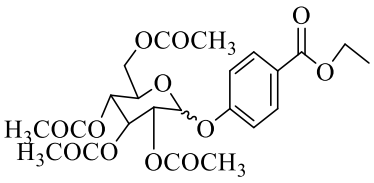
Yellow solid, 16 %; **^{1}H-NMR (400 MHz, DMSO-D6)** δ 0.84 (td, J = 7.3, 3.2 Hz, 6H), 1.31 (d, J = 7.3 Hz, 3H), 1.47 (q, J = 7.2 Hz, 4H), 2.95-2.82 (0H), 3.01-3.12 (m, 4H), 3.28-3.40 (m, 22H), 3.56 (d, J = 9.1 Hz, 3H), 3.71 (s, 1H), 4.05 (d, J = 7.8 Hz, 1H), 4.42 (q, J = 5.6 Hz, 2H), 4.56-4.59 (m, 2H), 4.69 (d, J = 5.0 Hz, 1H), 4.81-4.89 (m, 2H), 4.91 (d, J = 5.0 Hz, 1H), **^{13}C-NMR (101 MHz, DMSO-D6)** δ 14.4, 19.3, 19.5, 31.7, 31.9, 61.5, 61.6, 67.0, 68.7, 70.6, 70.9, 72.5, 73.3, 73.8, 74.0, 77.2, 77.3, 99.0, 103.4.

6.3.2.3 Glycosylation of phenolic compound and 5α-cholestan-3β-ol

SpEC-SO_3H 11 mole % was added to a mixture of glucose pentaacetate (2.5 g, 6.0 mmol) and EITHER ethyl-4-hydroxybenzoate (3.0 g, 18 mmol) OR 5α-cholestan-3β-ol (7.0 g, 18 mmol) in toluene (100 mL). The mixture was stirred and heated at reflux through a column containing molecular sieves 4Å (15 g) for 6 hours at 125 °C. At the end of the reaction, the mixture was allowed to cool to room temperature and was filtered through a filter funnel (porosity grade 4). The filtrate was concentrated to obtain the crude product. The crude product was dissolved in toluene (100 mL) and was washed with 0.5

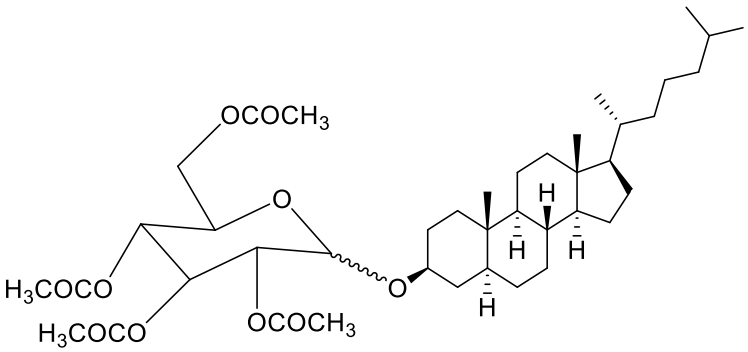
M NaOH (50 mL) and then with water (50 mL). Toluene was removed by rotatory evaporation to obtain a crude product.

Glycosylated ethyl 4-hydroxybenzoate (23a)



light yellow oil, 0.167 %; **^{1}HNMR (400 MHz,** $CDCl_3$) δ 7.95-8.21 (m, 2H), 7.04-7.16 (m, 2H), 5.76-5.90 (m, 1H), 5.62-5.71 (m, 1H), 5.10-5.21 (m, 1H), 4.96-5.07 (m, 1H), 4.32-4.42 (m, 2H), 4.17-4.25 (m, 1H), 4.00-4.13 (m, 2H), 1.98-2.11 (m, 12H), 1.32-1.44 (m, 3H), 1.19-1.33 (m, 1H) **^{13}C-NMR (101 MHz,** $CDCl_3$**)** δ 170.61, 170.09, 169.66, 166.22, 159.47, 131.53, 125.35, 115.91, 93.78, 70.51, 69.97, 68.23, 61.53, 60.82, 20.68, 14.19; ***m/z*** **(ESI-MS):** $[M+H_2O]^+$, 514.11, $[Glu(ACO)_4]^+$, 331.03; $[Glu(ACO)_3]^+$, 270.87; $[Glu(ACO)_2]^+$, 210.91, $[Glu(ACO)_2 – CH_3CO]^+$, 168.90

Glycosylated 5*α*-cholestan-3β-ol (23 b)



Colourless Oily liquid, 3 %; **^{1}H-NMR (400 MHz,** $CDCl_3$**)** δ 7.12-7.17 (m, 2H), 5.14-5.29 (m, 1H), 4.91-5.04 (m, 2H), 4.44-4.61 (m, 1H), 4.00-4.28 (m, 3H), 3.50-3.68 (m, 2H), 2.30-2.36 (m, 2H), 1.97-2.11 (m, 11H), 1.75-1.87 (m, 1H), 1.62-1.71 (m, 1H), 1.41-1.54 (m, 2H), 1.17-1.37 (m, 7H), 1.06-1.15 (m, 2H), 0.94-0.99 (m, 1H), 0.76-0.91 (m, 12H), 0.58-0.63 (m, 3H), 0.13 (m, 1H); ***m/z*** **(ES-MS):** $[Glu(ACO)_4]^+$, 330.98; $[Glu(ACO)_3]^+$, 270.87; $[Glu(ACO)_2]^+$, 210.90.

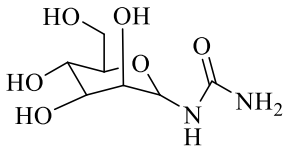
6.3.3 N-glycosylation

6.3.3.1 Melt glycosylation of urea in the presence of NH_4Cl

Anhydrous *D*-mannose (3 g, 16.7 mmol), urea (7 g, 116.6 mmol), NH_4Cl (1 g, 18.7 mmol) and water (0.3 mL) were placed into a reaction flask and thoroughly mixed together. The content was melted with continuous stirring to a clear melt at 80 °C (5

minutes). SpEC-SO_3H (6.62 mmol equivalent of SO_3H) was added and the mixture was stirred for 2 hours at that temperature. At the completion of the reaction, water was added to the molten mixture whilst still warm. The mixture was filtered to remove the catalyst and the filtrate was concentrated by rotatory evaporation. The deep yellow solid recovered was dissolved in methanol (4 mL) and diethyl ether (1 mL) was immediately added. The mixture was allowed to cool down to room temperature and later kept at 0 °C overnight in the refrigerator for a better yield for slow re-crystallization. The mixture was filtered and washed with chilled methanol and diethyl ether (4:1).

β-Mannosyl urea (29) as compared with NMR data from Tanaka *et al*[26] and Ruß *et al*[14]

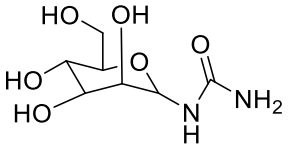


White (powdery) Solid, 95 %, melting point: 144-150 °C; **^{1}H-NMR** (400 MHz, D_2O) δ 3.24-3.32 (m, 1H), 3.38-3.45 (t, J = 9.6 Hz, 1H), 3.51-3.59 (m, 2H), 3.66-3.99 (m, 2H), 4.90 (d, J = 10.5 Hz, 1H), **^{13}C-NMR** (101 MHz, D_2O) δ 60.84, 66.52, 70.76, 73.30, 77.63, 78.85, 160.28; **elemental analysis:** $C_7H_{14}O_6N_2$ requires: C, 37.84 %; H, 6.35 %; N, 12.61 %, found: C, 33.91 %; H, 6.55 %; N, 19.42 %.

6.3.3.2 Melt glycosylation of urea in the absence of NH_4Cl

Anhydrous *D*-mannose (3 g, 16.7 mmol), urea (7 g, 116.6 mmol) and water (0.3 mL) were placed into a reaction flask and thoroughly mixed together. The content was melted with continuous stirring to a clear melt at 80 °C (5 minutes). SpEC-SO_3H (6.62 mmol equivalent of SO_3H) was added and the mixture was stirred for 2 hours at that temperature. At the completion of the reaction, water was added to still melt whilst still warm. The mixture was filtered to remove the catalyst and the filtrate was concentrated by evaporation. The deep yellow solid recovered was dissolved in methanol (4 mL) and diethyl ether (1 mL) was immediately added. The mixture was allowed to cool down to room temperature and later kept at 0 °C overnight in the refrigerator for a better yield for slow re-crystallization. The mixture was filtered and washed with chilled methanol and diethyl ether (4:1).

β-Mannosyl urea (27a) as compared with NMR data from Tanaka *et al*[26] and Ruß *et al*[14]

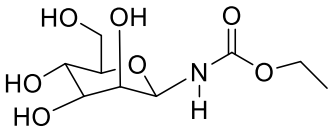


97 %, White (powdery) Solid, melting point: 144-150 °C; **^{1}H-NMR** (400 MHz, D_2O) δ 3.24-3.32 (m, 1H), 3.38-3.45 (t, J = 9.6 Hz, 1H), 3.51-3.59 (m, 2H), 3.66-3.99 (m, 2H), 4.90 (d, J = 10.5 Hz, 1H), **^{13}C-NMR (101 MHz, D_2O)** δ 60.96, 66.49, 70.48, 73.47, 77.39, 78.70, 78.82, 160.07, 160.42, ***m/z* (ESI-MS): [**M^+ + Na] 245, **[**M^+ + Na] 407; **elemental analysis:** $C_7H_{14}O_6N_2$ requires: C, 37.84 %, H, 6.35 %; N, 12.61 %; found**:** C, 33.69 %; H, 6.32 %; N, 19.17 %.

6.3.3.3 Melt glycosylation of ethyl carbamate in the absence of NH_4Cl

Anhydrous D-mannose (3 g, 16.7 mmol) and ethyl carbamate (7 g, 116.6 mmol) were placed into a reaction flask and thoroughly mixed together. The content was heated with continuous stirring to a clear melt at 80 °C (5 minutes). SpEC-SO_3H (6.62 mmol equivalent of SO_3H) was added and the mixture was stirred for 2 hours at that temperature. At the completion of the reaction, water was added to still melt whilst still warm. The mixture was filtered to remove the catalyst and the filtrate was concentrated by evaporation. The deep yellow solid recovered was dissolved in methanol (4 mL) and diethyl ether (1 mL) was immediately added. The mixture was allowed to cool down to room temperature and later kept at 0 °C overnight in the refrigerator for a better yield for slow re-crystallization. The mixture was filtered and washed with chilled methanol and diethyl ether (4:1).

β-Mannosyl ethyl carbamate (27b) as compared with NMR data from Tanaka *et al*[26] and Ruß *et al*[14]



White powder, 53 %; **^{1}H-NMR (400 MHz, D_2O) δ** 1.05 (dt, J = 27.4, 7.0 Hz, 5H), 3.18 (t, J = 14.9 Hz, 2H), 3.26-3.30 (m, 1H), 3.36-3.44 (m, 1H), 3.48-3.58 (m, 2H), 3.69-3.78

(m, 2H), 3.91-4.01 (m, 3H), 4.89 (s, 1H), **^{13}C-NMR (101 MHz, D_2O)** δ 13.65, 60.86, 62.36, 66.40, 70.49, 73.39, 77.43, 79.91, 158.05.

6.3.3.4 Attempted Glycosylation of nitrogenous heterocyclic compounds, unsaturated aliphatic and aromatic amides

D-Mannose (1 mmol) and the amine containing compound (3 mmol) were melted at a specific temperature until a clear mixture was obtain. SpEC-SO_3H (0.662 mmol equivalent of SO_3H) was then added to the melt and the total mixture was stirred at the same temperature for another two hours. Water was immediately added at the completion of the reaction and the catalyst was filtered off. The filtrate was concentrated, and recrystallization of the resulting solid in a suitable solvent was attempted.

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Chapter 7: Application of sulfonated sporopollenin exine capsules (SpEC-SO_3H) in miscellaneous organic reactions

The transformations of many general organic molecules into another useful and valuable chemicals / products, require catalytic processes. Catalysts are employed to increase the rate of reaction, reduce the reaction time and also to minimise the formation of by-product(s) and undesirable products. Most importantly, catalysts aid the reaction to end in the formation of a targeted product with a minimal complexity. It is desirable to employ catalysts in forcing reversible reactions, such as transesterification, hydrolysis, isomerisation reactions, the process that lead to dehydration of organic compounds, rearrangements reactions etc, to completion. The SpECs used in the production of SpEC-SO_3H, as earlier stated, have various advantages that indicate that SpEC-SO_3H has promise as a sustainable catalyst. In this chapter, SpEC-SO_3H is used as a solid acid catalyst in the dehydrative cyclisation of monosaccharides, Beckmann rearrangement and transesterification of sunflower oils.

7.1 Dehydration of sugars

Over the decades, the search for the production of sustainable chemicals through the transformation of biomass to bio-based chemicals has dominated the interest of researchers and the industrial sector.[1] The growing demand for energy fuels, due to the increasing world population and the depletion in the generation of fossil materials couple with environmental pollution[2,3] caused by non-biodegradability of some petroleum products, has propelled the shifting of the interest of researchers to the alternative carbon-based compounds that can serve as an alternative in the replacement of the materials with petroleum source.[4,5] Monosaccharides with six carbon atoms are the most abundant carbohydrate building block in nature,[6] and they are also a biomass resource renewable over a short period of time.[7] They possess the potential of being used or transformed to several valuable miscellaneous organic chemicals.[1]

Some monosaccharides, such as fructose,[1,6,8] glucose[2,5,9] and others,[8] have previously been transformed catalytically into 5-hydroxymethylfurfural (HMF).[7] This chemical is of

importance to the chemical industry. HMF is an aromatic compound with more than two functional groups,[4,7] therefore its usefulness is also as a feedstock or as an intermediate used, for example, in the production of polymers, as a solvents, fuel, pharmaceutical material and source of other fine chemicals.[1,5,8,10]

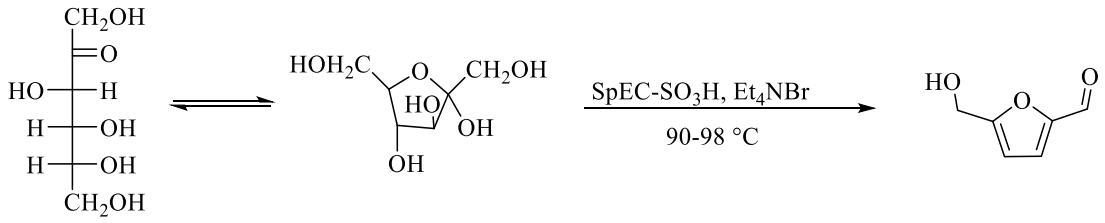
The catalytic transformation of biomass to HMF involves a dehydration reaction if fructose is the starting material. However, the use of glucose or glucose polymers, such assucrose, cellulose etc., as a starting material requires isomerisation and / or hydrolysis prior to their dehydration reaction.[1,6] The possibility of transforming fructose to HMF in the absence of catalyst has been reported in the literature,[3,8,11] but several catalysts, both homogeneous and heterogeneous, have been investigated in the conversion of sugars, especially fructose, to obtain a high yield of HMF.[4,8] Some of the catalysts employed are expensive or not environmental friendly, others are toxic, and difficult to separate from the product and lack reusability.[8]

Tang *et al* reported that Brønsted acid catalysts are more effective in the dehydration of fructose to HMF.[2] SpEC-SO_3H has proved, in our previous studies, to be a good sulfonated solid acid catalyst in organic synthesis with similar properties to Amberlyst 15 and similar resins. The success recorded by Simeonov *et al*[10] in the use of Amberlyst 15 as solid acid catalyst in the production of HMF in water therefore spurred our interest in applying SpEC-SO_3H as replacement for Amberlyst 15 in the same process.

7.1.1 Application of SpEC-SO_3H to dehydration of monosaccharides

Following the general method of Simeonov *et al,*[10] Amberlyst 15 solid acid catalyst was replaced with SpEC-SO_3H. In this work, fructose (5.6 mmol) was dissolved with tetraethylammonium bromide (21.4 mmol) in distilled water (2 mL). The mixture was stirred on a pre-heated oil bath at 100 °C, and the internal temperature was monitored until it reached 90 °C. At this temperature, SpEC-SO_3H (0.498 mmol equivalent SO_3H) was added to the mixture and the stirring of the reaction was continued for 15 minutes after, during the time which the internal temperature reaches 98 °C and the colour of the reaction mixture turned brown (**Scheme 7-1**). At the end of 15 minutes, the reaction was stopped and the hot mixture was filtered through a pad of Celite®. The SpEC-SO_3H was washed thoroughly with absolute ethanol (3 mL), and the combined mixture was concentrated on a rotary evaporator. The crude mixture was dissolved in hot absolute

anhydrous ethanol (2.5 mL) and stirred for a few minutes at the boiling point of the ethanol. Excess ethyl acetate (100 mL) was added to the mixture under vigorous stirring to precipitate the tetraethylammonium bromide out of the mixture. The mixture was cooled to room temperature and the precipitate was removed by filtration. The residual tetraethylammonium bromide was rinsed with ethyl acetate (10 mL). The product was filtered through a pad of silica gel (30 g) using a sinter funnel, and the silica gel was rinsed with ethyl acetate (10 mL). The product was concentrated by rotary evaporation to obtain an oily crude product. The product was obtained as a deep orange liquid, which was purified using flash column chromatography to obtain the product as an orange liquid (**Scheme 7-1**).



12d **32** **33**

***Scheme 7-1*: Sulfonated sporopollenin (SpEC-SO_3H) catalysed conversion of fructose to 5-(hydroxymethyl) furfural (HMF)**

Tetraethylammonium bromide (Et_4NBr) is reportedly an additive which was added with the purpose of easing the separation process of HMF from the crude mixture.[4] The separation of HMF from the crude in a reaction in the absence of Et_4NBr was difficult, despite the indication of the formation of HMF by TLC. The catalytic degradation of fructose in water, by the acid catalyst (SpEC-SO_3H) was very fast; the colour of the crude product became darker (black). This proved that Et_4NBr has synergic effect with the separation of the product from the reaction mixture. With 8.9 mol% of SpEC-SO_3H relative to fructose, the catalytic conversion of fructose in the presence of Et_4NBr yielded 89 % of HMF (**Figure *7.1-1***) after purification by column chromatography. The remaining fructose may have been degraded to levulinic acid or converted to a soluble polymer through aldol condensation or to insoluble humins[4,6] which were likely removed during separation and purification process. The reaction was performed for two more times to confirm the efficiency of SpEC-SO_3H in catalysation of the conversion process. The yield obtained from the reactions were approximately 89±0.33 %. The yields, in summary, were with some negligible differences that were in two decimal places.

Amberlyst 15 ion exchange resin catalyst was reported to have an active site with sulfonic acid groups of about 4.7 mmol/g.[12] The yield obtained from our reaction involving SpEC-SO_3H was compared to that recorded by Simeonov *et al*[10] in a reaction performed with almost equivalent mole % amounts of Amberlyst 15. It was found that the yields were almost the same. 8.9 mol% of SpEC-SO_3H, though a little lower in terms of mol% than Amberlyst 15 (9.4 mole %), gave 89±0.33 % yield while Amberlyst 15 gave a yield in the range of 83 – 87 %.[10]

7.1.1.1 Effect of catalyst loading on the conversion of fructose to 5-(hydroxymethyl) furfural (HMF)

In order to assess the catalytic performance of SpEC-SO_3H, the reaction was carried out with a lower amount of catalyst using the same reaction conditions as reported above in section **7.1.1**. The mol % of SpEC-SO_3H was successively halved and each employed in the conversion of fructose to HMF. The results of the reactions are shown in **Figure *7.1-1***. It was observed that the yield of the product reduced with a decrease in mol% amount of SpEC-SO_3H used. The difference between the yield of the reaction catalysed with 8.9 mol% SpEC-SO_3H (i.e. 0.498 mmol compared to X mmol fructose) and the one catalysed with 4.4 mole % SpEC-SO_3H is 36 %. But the difference in yield obtained from the reaction involving 4.4 mole % SpEC-SO_3H and its half is just 9 %. The increase in the load of sulfonic group on the SpEC-SO_3H catalyst has great effect on the conversion rate of fructose to HMF.

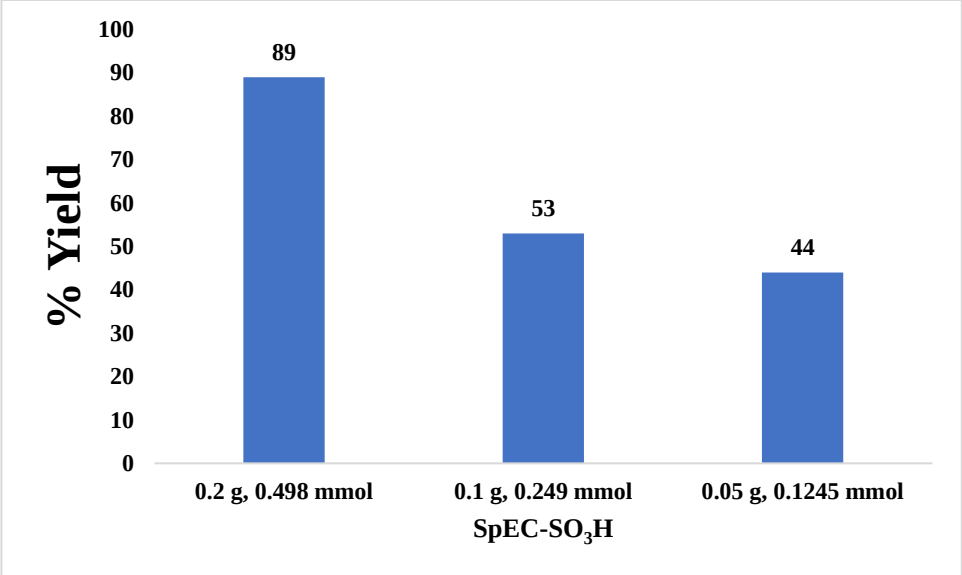
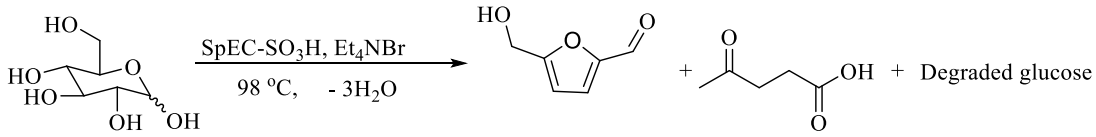


Figure 7.1-1: Conversion of fructose (5.6 mmol) to 5-(hydroxymethyl) furfural by differing levels of sulfonated SpECs (SpEC-SO_3H)

If the mol% of SpEC-SO_3H was increased beyond 8.9 %, it might have increased the yield of HMF beyond 89 % but Tang *et al* reported that large increases in the amount of catalyst caused a lot of side reactions. These included degradation and polymerisation of the desired product (i.e. HMF) and possibly leading to the production of by-products such as humin.[2]

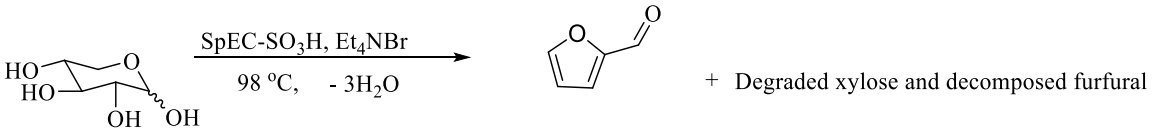
7.1.1.2 The transformation or dehydration of some other monosaccharides to furfural and derivative

The dehydration of D-glucose and D-xylose with SpEC-SO_3H were only successful with low yields. Several different attempts were made which later results in formation of mixture of degraded products of the sugars and transformation of D-glucose / D-xylose to other dehydrated products even after purification with column chromatography (**Scheme 7-2** and **Error! Reference source not found.**).



12b **33** **34**

Scheme 7-2: **Transformation of D-glucose to HMF in the presence of SpEC-SO_3H**



12c **35**

Scheme 7-3: **Attempted formation of furfural from D-xylose in the presence of SpEC-SO_3H**

The same procedure used in the transformation of fructose to HMF was also employed in the dehydration of D-glucose using SpEC-SO_3H (0.498 mmol, 8.75 mol %) as an acid catalyst. The first attempt to transform glucose to HMF was carried out at the temperature between 90 °C to 98 °C but no product was recovered at the end of 15 minutes (**Table *7.1-1*, entry 1**). However, when the reaction temperature was increased to 110 °C and the reaction was monitored with TLC, up to 5 spots were seen on the TLC plate under the UV lamp. The reaction was stopped at the end of 3 hours and a yield of 0.380 g was recovered, from the 1 g of glucose injected into the reaction, and this was purified with column chromatography. After column chromatography, a yield of 31 % of HMF was

obtained (**Table *7.1-1*, entry 2**). Due to the closeness in the retention time of those spots sighted on the TLC under the UV lamp, the product was not pure. Carbon-13 NMR analysis indicated the formation of levulinic acid, due to the appearance of ketone carbonyl chemical shift peak at 207.1 ppm, two carbonyl chemical shift peaks i.e. 177.9 ppm and 176.8 ppm, in the carboxylic acid region and aliphatic peaks 37.8 ppm, 29.9 ppm, and 27.5 ppm,[13,14] as well as the peaks (underlined) associated with the chemical shifts of HMF (i.e. 207.1, 177.9, 176.8, 160.7, 152.511, 123.2, 110.0, 60.6, 57.5, 37.8, 29.9, 27.5, 21.1, 14.2),[10,13] which were more prominent. The observation of levulinic acid is a confirmation of the report of Atanda *et al*[15] that HMF, in polar protic solvent, rehydrates to form levulinic acid and formic acid. Leaving the reaction temperature at 110 °C, the reaction time was increased to 6 hours and the yield after purification declined to 29 % (**Table *7.1-1*, entry 3**). The product, like in the previous reaction, showed that the mixture of HMF and levulinic acid was identify by NMR spectra. Though there was an indication that the crude products contained water soluble polymer which were removed by silica gel during the purification with column chromatography. The soluble polymer seems to be an oligomer whose formation may have resulted from the etherification of HMF formed towards the start of the with 2,5-dioxo-6-hydroxyhexanal, which is one of the intermediates formed in the conversion process.[16] Because of the formation of polymers, degradation of the product and resultant low yield of the expected product i.e. HMF, the temperature was reduced back to 98 °C and the reaction was properly monitored with TLC for 5 hours (**Table *7.1-1*, entry 4**). The reaction resulted in minimal degradation of the sugar and no decomposition of the expected product as there was no indication on the formation of levulinic acid in the NMR spectrum of the crude. After purification, the yield was a bit better than the those obtained in the previous reactions, increasing to 34 %.

Table 7.1-1: Effect of temperature reaction time on catalytic performance of SpEC-SO_3H in the conversion of D-glucose to HMF

Entries	SpEC-SO3H (mmol)	Reaction Time (Hours)	Temp (°C)	Yield (%)
1	0.498	0.25	98	0
2	0.498	3	110	31
3	0.498	6	110	29
4	0.498	5	98	34

It has been reported that formation of an enol is the rate-determining step in the conversion of monosaccharide sugar or carbohydrate to HMF.[3,5,17] In the case of glucose, it has also been reported that the pyranose ring structure is very stable and that glucose is rarely in its ring-open form in solution. The latter is required to allow enolisation and then formation of fructose.[3,5] The slow opening of the ring structure of glucose, therefore, was reported to be responsible for slowing down the rate of its conversion to HMF[3] resulting in the low yields that were often observed in such reaction.[3] Furthermore, it was reported that HMF can be strongly adsorbed on the active sites of Brønsted acid catalyst and thereby reduce or block the catalytic activity of the catalyst[15] which might later have resulted in the formation of low yield of the desired product. In addition, possible degradation of D-glucose, rehydration of HMF and probability of the formation of humins through the self-condensation of HMF and/or condensation with either intermediate or unreacted glucose are other factors that can culminate to reduce the yield in the conversion of D-glucose.[15,18]

The structure of fructose, on the other hand, favours the formation of an enol because fructose is less stable when compared to glucose and also, the acyclic configuration of fructose contributed to its ease enolisation.[3,5,19] Fructose always results into the formation of the mixture of dianhydrides and difructose through an equilibrium which often block the internal active sites and thereby force its conversion to HMF quickly to completion and with a high yield, also minimising the formation of by-product.[3] Therefore, to obtain a higher yield of HMF from glucose, it is necessary to incorporate the mechanism that will first transform the glucose to fructose. Conversion of glucose to HMF was reported to involve the isomerisation of glucose to fructose as one of the intermediates before its dehydration to HMF.[2,3,7] Driving the glucose to fructose through isomerisation process requires the use of catalyst to overcome the high energy barrier.[3,20] The isomerisation of glucose to fructose was reported to be effected by Lewis acid catalysts[2,7,9] and since SpEC-SO_3H is a Brønsted acid, the catalytic action of the SpEC-SO_3H only activated the active sites which in turn led to the polymerisation and degradation of the sugar, as reported by Rosatella *et al*,[3] rather than its transformation to HMF. This showed that application of SpEC-SO_3H to catalyse the conversion of glucose to HMF may require a two-pot reaction or a Lewis acid catalyst in conversion of glucose to fructose and then dehydration of fructose to HMF by SpEC-SO_3H.

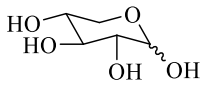


Figure 7.1-2: Chemical structure of D-xylose

Dehydration of D-xylose into a furfural (**Error! Reference source not found.**), just l ike that of D-glucose, proved difficult because their stabilities are enhanced by chair conformation and also, both of them have similar chair-structure, except that D-xylose is devoid of the sixth carbon atom and the hydroxyl group (hydroxymethylene) (**Figure *7.1-2***). At first, with the use of the procedure employed in the transformation of fructose, and retaining the mole equivalent of SpEC-SO_3H at 8.9 mol%, tetraethylammonium bromide was replaced with tetraethylammonium chloride. However, as the latter is a hygroscopic solid, it could not be crystallised or separated from the mixture. Therefore, tetraethylammonium bromide was finally used in an attempt to dehydrate xylose. At the same reaction condition and reaction time, as in the dehydration of fructose, together with the mole equivalent of SpEC-SO_3H remaining at but the dehydration of xylose was a bit difficult because it was reported in the previous study that temperature and reaction time play major roles in its conversion to furfural.[21] If the reaction exceeds the normal optimised period, there will be reduction of the yield and the selectivity of the reaction.[21] However, lower temperature below the optimized reaction temperature slows the conversion of the substrate and therefore lead to loss of the yield to condensation and fragmentation.[18,21] As a result of those stated findings, several unidentified degraded products were observed as indicated by the NMR spectroscopy and TLC. When the internal temperature of the reaction was raised to 110 °C, the ^{13}C-NMR spectrum indicated the likely formation of furfural (δ 190.5, 186.2, 178.1, 177.2, 175.7, 153.1, 148.2, 123.5, 122.4, 120.2, 112.7, 107.3, 102.8, 98.2, 81.9, 80.7, 77.6, 73.0, 69.7, 65.5, 64.0, 61.9, 31.1, 29.6, 26.4, 22.4, 21.8, 21.3, 15.2 ppm) and other decomposed compounds of furfural, like γ-butyrolactone (δ 190.5, 186.2, 178.1, 177.2, 175.7, 153.1, 148.2, 123.5, 122.4, 120.2, 112.7, 107.3, 102.8, 98.2, 81.9, 80.7, 77.6, 73.0, 69.7, 65.5, 64.0, 61.9, 31.1, 29.6, 26.4, 22.4, 21.8, 21.3, 15.2 ppm), as shown by the underlined chemical shifts which were compared to the literature.[22-25] The yield was too low. Surprisingly, when the reaction time at 110 °C was increased from 15 minutes to 25 minutes, the peaks of the chemical shift of sugar and other degraded xylose were prominently observed in the ^{13}C-NMR spectrum. This confirmed the report of Zhu *et al*[21] on the influence of reaction time on the production of furfural. As a result of degradation of xylose and slow transformation

to furfural, the internal temperature of the reaction was reduced to 100 °C and the reaction was left for 25 minutes, but the outcome was not different from the reaction that was carried out at 110 °C. Wang *et al* reported microwave dehydration of D-xylose into furfural but from the above explanation, the conventional method for SpEC-SO_3H catalysed conversion of xylose to furfural is not yet clearly formulated.

7.2 Beckmann rearrangement

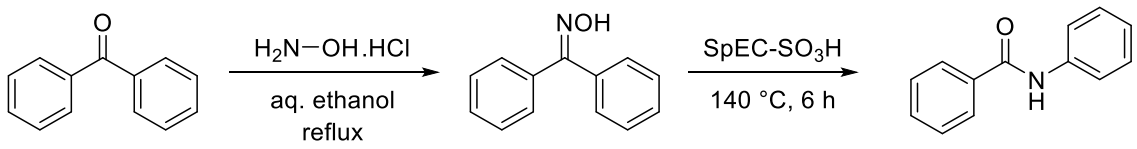
The Beckmann rearrangement is very important in the chemical industry.[26] It is a transformation of ketones to an isomeric / rearranged amide. This rearrangement is a stereospecific reaction and involves the rearrangement of the intermediate oximes in the presence of acid catalyst to yield amide or lactam corresponding to the starting carbonyl compound.[26-33] The resulting amides from the rearrangement are very important starting materials, e.g. the use of caprolactam for the production of nylon-6, and other intermediates used in the chemical industries. Their importance range from the commercial preparation of polyamides, agrochemicals, natural products, fragrances, medicines, synthetic fibres and other polymers.[27,32]

The Beckmann rearrangement, in previous reports, has been accelerated by the use of many different catalysts, of both Brønsted and Lewis acid types.[26-34] Many of them are expensive to produce, require toxic solvents, lead to long reaction times, or result in environmental hazards by producing large amount of by-products and waste.[30,32]

However, the use of environmentally benign catalysts is a current goal in synthetic chemistry to tackle environmental degradation. The use of *para*-toluene sulfonic acid in Beckmann rearrangements by Ponnusamy *et al*,[28] PEG-SO_3H by Wang *et al*,[30] and a sulfonic copolymer by Li *et al*,[32] coupled with the effectiveness of sulfonated spropollenin in the previous reactions, gave motivation to apply sulfonated sporopollenin to the Beckmann rearrangement reaction. A solvent-free (melt) reaction was chosen initially as the option for this rearrangement reaction because of its simplicity, to avoid use of solvents, and take advantage of the robust nature of SpEC-SO_3H.[35]

In this work, the freshly produced benzophenone oxime was rearranged to benzanilide (*N*-phenylbenzamide), by melted method, in the presence of sulfonated SpECs (SpEC-SO_3H). The benzophenone oxime was produced by heating benzophenone and $NH_2OH.HCl$ in aqueous ethanol until formation of the oxime was complete (**Scheme**

7-4). The formation of oxime was monitored by TLC until the appearance of a single spot associated with oxime. SpEC-SO_3H of 48.6 mole % was employed to catalyse the rearrangement of benzophenone oxime to the amide and the rearrangement reaction, which was monitored by TLC, was stopped at the end of 6 hours when the benzophenone oxime has reacted and there was an observation of another spot which was confirmed to be its ketone (**Scheme 7-4**). At first, benzanilide was separated from the mixture by recrystallization from aqueous ethanol (5:1 water-ethanol) and kept in the refrigerator overnight. The white solid crystal recovered by filtration was obtained in to be 80 % yield. However, when the solid crystal obtained was subjected to NMR, the spectrum did not only showed the signals of benzanilide but also showed the peaks of the chemical shifts of benzophenone. Therefore, the recrystallized product was further purified with the aid of column chromatography and a pure product of 60 % amide with approximately 20 % ketone were obtained. The ketone formation was resulted from the hydrolysis of oxime by water in the presence of an acid catalyst. When the mole % of the catalyst was reduced by half, the rearrangement was very slow and the expected product could not be obtained, even, when the reaction was left for 7 hours. An attempt to leave the reaction for 8 hours in the presence of 24.3 mole % SpEC-SO_3H only resulted in the production of benzophenone via hydrolysis. Optimisation of the procedure by fitting the reaction flask with calcium chloride guard tube to regulate the amount of water involved in the reaction and to ensure that excess water did not affect the reaction step leading to Beckmann rearrangement but rather, it was also led to the hydrolysis of the oxime back to its ketone.



5f **36** **37**

Scheme 7-4: **Beckmann rearrangement of benzophenone oximes**

An attempt to apply the method above to the rearrangement of the oximes of cyclopentanone, cyclohexanone and acetophenone in the presence of SpEC-SO_3H was not successful but rather they were hydrolysed to their respective ketones. The water molecule produced in the reaction process, which was meant to help in the rearrangement reaction, most likely initiated the hydrolytic reaction. Therefore, the hydrolysis of benzophenone oxime led to the loss of hydroxylamine. The hydroxylamine may have

probably reacted with the sulfonic group of the SpEC-SO_3H and escaped in the form of ammonia from the reaction, otherwise it may have stuck to the surface of the catalyst. Several attempts were made, even with optimisation of the procedure, since the reaction process involved equilibrium reaction, backward reaction - which is hydrolysis of oxime back to their respective ketone, was more favoured.

In the presence of acid catalyst, oximes can either rearrange to the amide or be hydrolysed to their corresponding carbonyl compounds. The rearrangement reaction seems to depend on the relative stability of the oxime.

Due to difficulties in rearranging the oxime in the presence of SpEC-SO_3H to their corresponding amide; especially the oximes of cycloketone, sulfated sporopollenin (SpEC-OSO_3H) was opted to catalysed the Beckmann rearrangement. The same melt procedure was employed, only with the replacement of SpEC-SO_3H with SpEC-OSO_3H. it worth noting that the application SpEC-OSO_3H in catalysing the rearrangement of the oxime of cycloaliphatic ketone was also unsuccessful. Therefore, SpEC-OSO_3H was also employed in the rearrangement of benzophenone oxime. The reaction, which was monitored by TLC, was stopped at the end of 7 hours when it was discovered that the rearrangement reaction was too slow and hydrolysis was more favoured than rearrangement. The crude extract was later purified and approximately 40 % amide was obtained when the recrystalised product was subject to further purification via column chromatography. With this effort, it was discovered that SpEC-SO_3H was more effective than SpEC-OSO_3H in catalysing the Beckmann rearrangement in term of reaction time and the yield of the product.

7.3 Transesterification reactions

Due to population increase in the world, the demand for energy drastically increased[36,37] and thereby mounted pressure on the current main source of the energy, which is largely tapped from the fossil fuels.[37,38] Researchers' attention has switched to investigating sustainable alternatives to the use of fossil fuel.[38-40]

Production of diesel from plant and animal oils, because of their proximity and availability, has been an important research theme in recent times. Biodiesel was claimed to have reduced the emissions from carbon dioxide by 78 %, particulate matter by 66.7

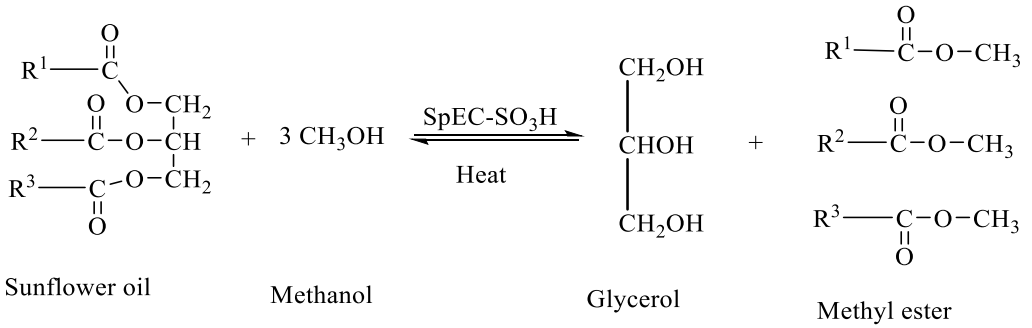
%, carbon monoxide by 46.7 % and unburned hydrocarbons by 45.2 %, thereby, has the potential to reduce the emissions from greenhouse effect.[41,42]

There are several ways of producing biodiesel from plant or animal oils, such as thermal cracking and transesterification with the most prominent among them is trans-esterification.[36] Transesterification of oil triglycerides is achieved by heating animal or plants oils with an alcohol in the presence of a catalyst. Both homogeneous and heterogeneous alkali and acid catalysts have been successfully employed in such transesterifications. Homogeneous catalysts are very cheap and the most often used industrially, but there are disadvantages and drawbacks incurred from their use. For example, difficulties in getting the product separated from the reaction mixture, damage to equipment resulting from corrosion and environmental destruction, have forced researchers to investigate employing heterogeneous catalysts to provide solutions to the problems associated with homogeneous catalysts.[43]

Heterogeneous catalysts, such as Amberlyst 15, have reportedly proven to have good reusability and stability to transesterification reaction, and also they are easy to separate from the reaction mixture.[44] However, some of the heterogeneous catalysts, used in the previous work, are not cost effective, especially the metal-supported catalysts.[45] The catalytic activities of SpEC-SO_3H, in our previous work reported above is similar to those of Amberlyst 15 and other Brønsted acid catalysts, therefore the catalytic performance of SpEC-SO_3H was investigated in the *in-situ* transesterification of sunflower oil. The composition of sunflower oil depends significantly on the environment of the source of sunflower seeds from which the oil is extracted. Sunflower oil was reported to contain triglycerides largely made up of oleic and linoleic acids in 1:2 ratio.[46]

7.3.1 Application of sulfonated SpECs (SpEC-SO_3H) to the formation of fatty acid methyl esters from sunflower triglycerides

In this work, the method used by Chellappan *et al*[47] was used with a little modification to suit the use of SpEC-SO_3H.



Scheme 7-5: **SpEC-SO_3H catalysed transesterification of sunflower oil to its methyl ester**

The transesterification reaction was done in a three-necked round bottom flask. A condenser was fitted to one of the necks, and all the reactor joints were sealed using Teflon tape to prevent the leakage of methanol vapours. A mixture of sunflower oil and methanol (1:70 mole ratio) with SpEC-SO_3H (13.165 mmol, 44 mol %) was added to the flask equipped with magnetic bar. Excess methanol was used in order to drive the forward reaction to completion. As methanol is highly volatile, slow losses through evaporation also needed to be considered.

The mixture was heated at an internal temperature of 68 °C for the times given in **Table *7.3-1***. The reaction was monitored by ^{1}H-NMR spectrometry, at four hour intervals, to know the progress about the conversion of sunflower oil. At the completion of the reaction, or the time the reaction was stopped, the mixture was transferred to a separating funnel and allowed to settle for 24 h. Sometimes it was centrifuged to remove the catalyst. The biodiesel (fatty acid methyl esters, FAMEs) upper layer was separated from the reaction mixture by decantation. The biodiesel was washed with deionized water (three times) to remove unreacted methanol. The percentage conversion of the sunflower oil triglyceride to methyl ester was calculated according to **Equation *3*** shown below. The reaction scheme is as shown in the **Scheme 7-5**

$$\% \text{ Conversion} = 100\left(\frac{\left(\frac{Y}{9}\right)}{\left(\frac{X}{4}\right)+\left(\frac{Y}{9}\right)}\right)\% \quad \text{.....1}$$

Equation 3: Percentage conversion of sunflower oil

Where X is the ^{1}H-NMR integral value of the multiplet of the -CH_2O- groups of the glycerol unit of the of the triglyceride starting material (δ 4.05-4.18 ppm and 4.21-4.34

ppm) as shown in **Figure *7.3-1*** and Y is the integral value of the -OCH_3 singlet of the methyl ester product (δ 3.62-3.67 ppm) as shown in **Figure *7.3-2***.

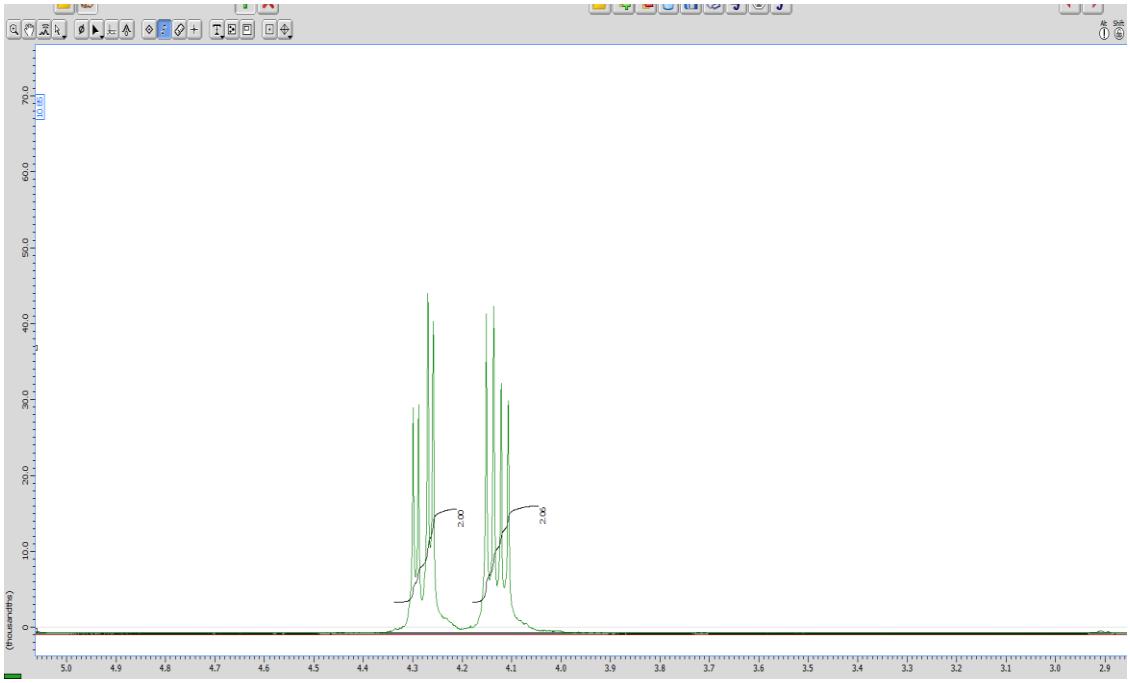


Figure 7.3-1: The ^{1}H-NMR spectrum of raw sample of sunflower oils before transesterification

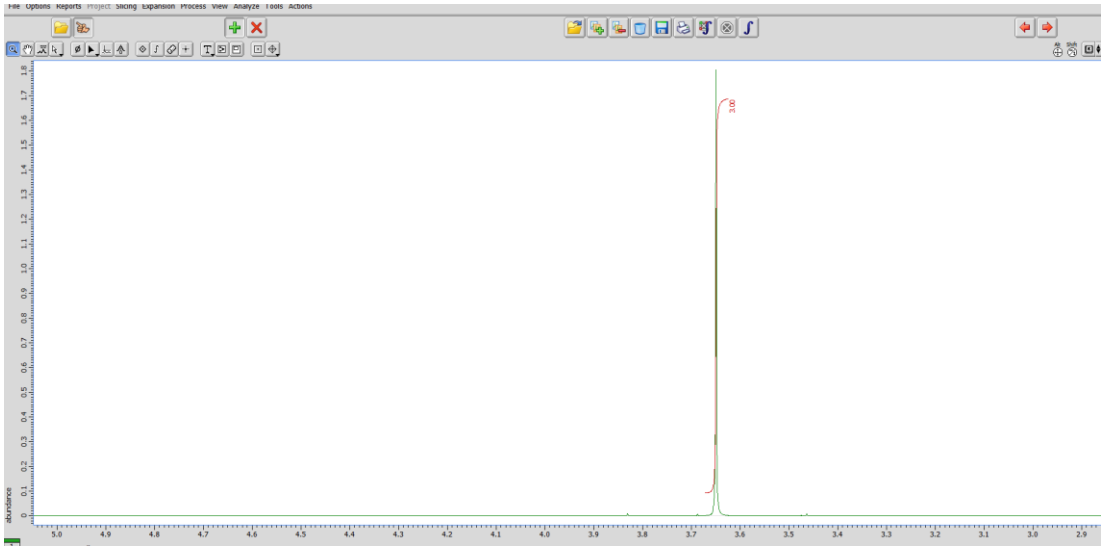


Figure 7.3-2: An extract from the NMR spectra of the sample from the product after transesterification of sunflower oils

Employing 44 mol% of SpEC-SO_3H to catalyse the transesterification reaction between sunflower oil and methanol, the rate of conversion was seen to increase slowly

over time. After four hours of reaction, only 3 % of sunflower oil had been converted to methyl ester but when the reaction was left for 8 hours, the conversion increased to 8 %. The whole triglycerides were converted totally to its methyl esters (**Figure *7.3-3***) after 58 h. It is worth noting that transesterification, in the presence of an acid catalyst, is a reversible reaction[48] as shown in the reaction **Scheme 7-5**. When the reaction was, therefore, left for 60 hours, the % converted methyl ester dropped to 99 %. This, perhaps, might which likely resulted from inaccuracy of integrations of the methyl ester peak or lack of proper drying of the esters sample before its preparation for ^{1}H-NMR spectroscopy. Also, the reduction observed in the % conversion may have probably been due to the sampling collection. The sample collected may not have been totally converted to the esters.

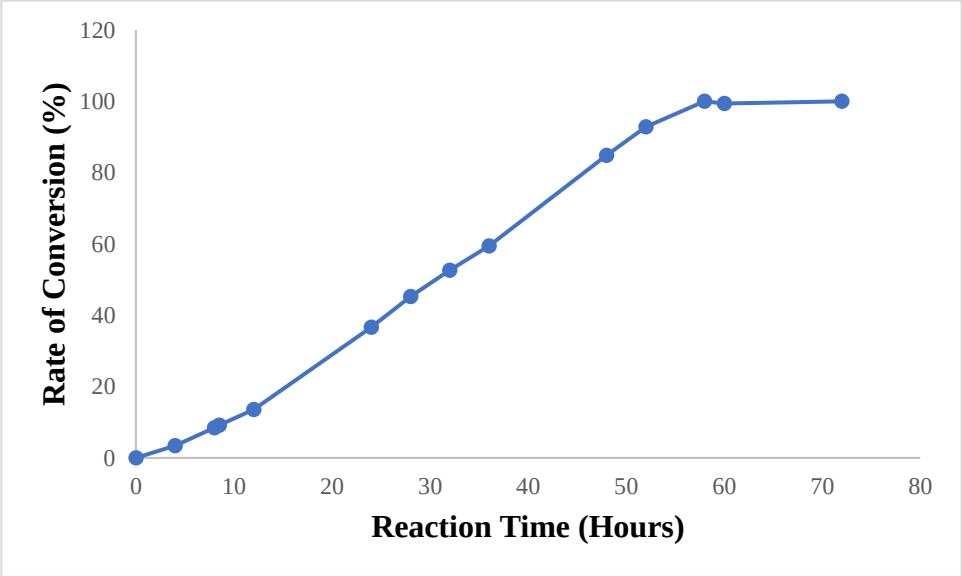


Figure 7.3-3: Conversion of Sunflower oil to biodiesel in the presence of 44 % SpEC-SO_3H

After 60 h the reaction mixture was left for another 12 h to ensure all the glycerides present in sunflower oil were converted, and to check to see there was no subsequent hydrolysis of the product. Another sample collected at the end of 72 h indicated a total conversion of the oil and without the trace of the characteristic -CH_2O- peak of the triglyceride in the ^{1}H-NMR spectrum.

Due to the claim by Hidayat *et al*[48] that transesterification of fatty acid triglyceride with methanol is reaction temperature dependent, the internal temperature of the reaction

was increased to 85 °C. The increase in the internal temperature, however, did not have much effect on the rate of conversion of triglyceride in sunflower oil to methyl ester. The indication of the -CH_2O- and - OCH_3 peaks as shown in the ^{1}H-NMR spectrum gave almost the same result as those revealed by the reaction carried out at an internal temperature of 68 °C. The progress of transesterification was within the same range, this may be associated to the boiling point of methanol - 65 °C. Mardhiah *et al* and other researchers reported that increasing the temperature beyond the desirable or optimum condition will cause the reversibility of the transesterification, especially when the temperature is very close to the boiling point of water.[38,45]

7.3.2 Effect of SpEC-SO_3H loading on the conversion of the triglyceride of sunflower to their corresponding methyl ester

Investigation of the catalytic activity of SpEC-SO_3H was performed in the transesterification reaction of sunflower triglycerides with methanol at different mol % of SpEC-SO_3H, ranging from 8 to 44 mole % (i.e. 2.50 mmol to 13.16 mmol to, assuming that the oil contains pure triglyceride (26.319 g, 28.3 mL, 30 mmol of the triglyceride). The maximum reaction time was fixed at 58 h and the conversion was monitored by NMR spectroscopy as described above. As expected, a reduction in the mol % of SpEC-SO_3H added resulted in a slower conversion of the reactants into products. The rate of conversion by both 44 mol % and 19 mol % (5.58 mmol) of SpEC-SO_3H during the initial stages of the reaction (first 12 hours) were similar, but as the reaction time progressively increases, there was great disparity in the conversion (**Figure *7.3-4***). The conversion did not go beyond 64 % when 19 mol % of SpEC-SO_3H was employed in the reaction, compared to the 100 % conversion, for the reaction period of 58 hours, when 44 mol % catalyst was added. This is largely due to the number of the available sulfonic active acid sites accessible for the reaction. There are simply more acid sites available if more of the SpEC-SO_3H is added, increasing the rate of conversion of the sunflower oil triglyceride to its corresponding methyl ester.

Decreasing the mol % added of the SpEC-SO_3H further lowered the rate of conversion even more (**Figure *7.3-4***). For example, when the mol % was decreased to 12 mol % (3.52 mmol), the conversion did not exceed 43 % by the end of the fixed 58 h (**Figure *7.3-4***). The final mol % of SpEC-SO_3H investigated was 8 mol %. At this dosage of catalyst, a similar % conversion with 12 mol % in the first 8 hours of the

transesterification reaction was observed (**Figure *7.3-4***). However, when the reaction was stopped at 58 h the conversion did not exceed 30 % (**Figure *7.3-4***).

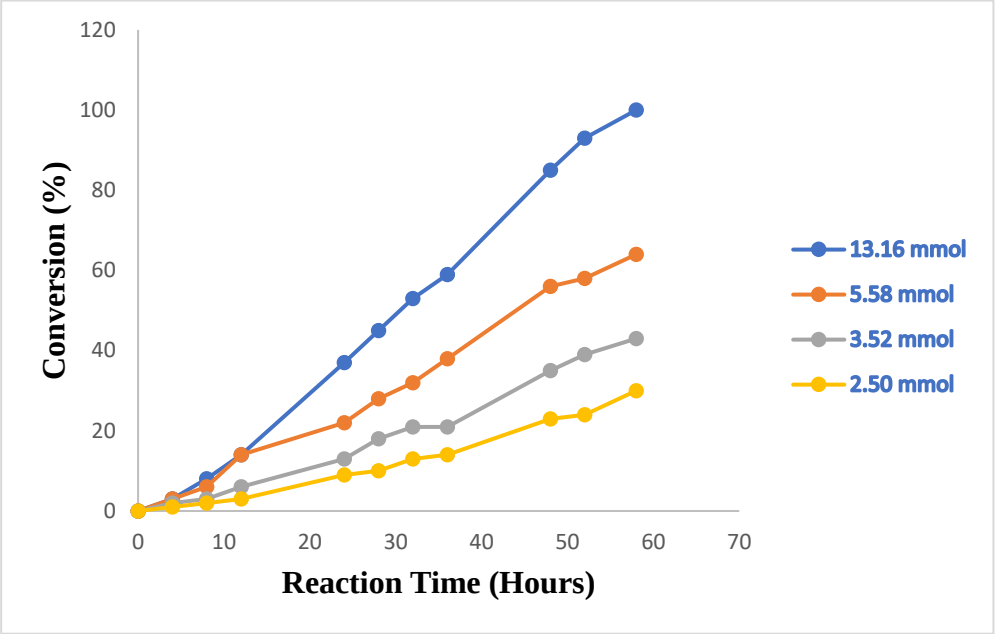


Figure 7.3-4: Conversion of sunflower triglycerides to methyl esters at different mol% of SpEC-SO_3H

Despite the size of the SpECs, SpEC-SO_3H catalyst has been shown to be activity in the production of biodiesel. The catalytic performance of SpEC-SO_3H in the production of biodiesel may likely be due to the contribution from the hydrophilic carboxylic acid and phenol groups which often have strong affinity to attach to the carboxyl part of the triglyceride[39,49] while the hydroxy part of the methanol substrate attached itself to the carbon of the catalyst which in return aided the dispersion of the catalyst in methanol.[39,50] This allowed the methanol to access the interior part of the catalyst and interact with the hydrophobic part of sunflower oil within the catalyst and also increase its interaction with the surface of the catalyst.[45] In addition, the hydrophobic part on the surface of the catalyst contributes to the enhancement of the stability of its activity [45,51] and its selectivity[51,52] in the reaction.

7.3.3 Effect of reaction time on the catalytic performance of SpEC-SO_3H

Keeping the reaction for a long period, at the optimum temperature, will allow the reactants to get in contact with active sulfonic acid site and thereby steadily increase the % conversion of sunflower oil triglyceride to the biodiesel. The catalyst (SpEC-SO_3H) generates the free hydrogen ion (H^+), when in solution, to initiate the conversion of

sunflower oil to its methyl ester. Therefore, if the amount of the catalyst is small, the amount of hydrogen ion would be reduced and limited in catalysing the ester formation. The limited generation of hydrogen ions later resulted in the reduction of the conversion process of the triglyceride to its methyl esters. However, in order to increase the % conversion of sunflower oil to biodiesel, the reaction was left for a longer period to allow more interaction between the sunflower, methanol and the hydrogen ion generated by the catalyst (SpEC-SO_3H) and also to enhance the accessibility of the molecules to have enough contact with H^+ at the active acid site. Therefore, the longer the interaction of the reactants with SpEC-SO_3H, the more possibility of the substrates to access and contact H^+ generated at the active acid site and that will be responsible for an increase in the catalytic conversion of the triglyceride to its ester. When 2.50 mmol SpEC-SO_3H was employed in the conversion of sunflower oil triglyceride to its methyl esters, the conversion was slower than when the higher amount was employed and it took 148 hours to obtain 98 % conversion (**Table *7.3-1***).

Table 7.3-1: Transesterification of sunflower oil with methanol in the presence of different % mole ratio of SpEC-SO_3H

Time (Hour)	Conversion (%)			
	44 mmol %	19 mmol %	12 mmol %	8 mmol %
0	0	0	0	0
4	3	3	2	1
8	8	6	3	2
8.5	9	-	-	-
12	14	14	6	3
24	37	22	13	9
28	45	28	18	10
32	53	32	21	13
36	59	38	21	14
48	85	56	35	23
52	93	58	39	24
58	100	64	43	30
60	99	67	46	34
72	100	-	-	43
76	-	80	56	44
80	-	-	-	45
84	-	-	-	51
96	-	-	-	61
100	-	-	-	68
104	-	-	-	71
108	-	-	-	71
120	-	-	-	90
124	-	-	-	90
128	-	-	-	91
132	-	-	-	91
148	-	-	-	98

Note: Sunflower Oil: 30 mmol (28.3 ml), Methanol: 2096 mmol (85 ml)

Sunflower oil: Methanol = 1:70

It might be tempting to assume that the percentage conversion would be doubled by doubling the amount or the mole % of SpEC-SO_3H, but with the use of 5.58 mmol, it was a 80 % conversion that could be obtained after 76 hours.

The conversion, therefore, can be concluded to have depended on the contact of triglyceride with the H^+ generated at the active sites. Though, the number of active sites in the catalyst may also play a major role but if those active sites are not accessible, the

rate of conversion may still be slower or the catalyst may not be effective in the conversion of triglyceride to the methyl ester. Due to the viscosity of sunflower oil and its low solubility in methanol, agitation of the reaction mixture may also have played a major role in the transesterification of methanol to sunflower oil triglyceride. Despite the size of the SpECs support in SpEC-SO_3H, the SpEC-SO_3H has, therefore, demonstrated to be an active catalyst in the production of biodiesel.

7.4 Experimental

7.4.1 General

Generally, the chemical used and spectrometry analysis are in line to those reported in **heading 3** and **heading 4.**

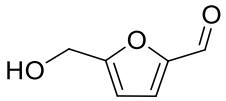
7.4.2 Dehydration of sugars to hydroxymethylfurfural (HMF)

The method used by Simeonov *et al*[10] was adopted in this reaction. A round-bottomed flask, equipped with magnetic stir bar, was charged with tetraethylammonium bromide (4.5 g, 21.4 mmol), distilled water (2 mL) and fructose (1.0 g, 5.6 mmol). The reaction flask was placed in a pre-heated oil bath (at 100 °C) and mixture was stirred until the internal temperature of the reaction reached 90 °C. The internal temperature of the reaction mixture was monitored by placing the thermometer inside the reaction mixture. When the internal temperature reached 90 °C, SpEC-SO_3H (0.2 g, 0.498 mmol) was added. After the addition of SpEC-SO_3H, the stirring of the mixture continued for next 15 min, during which the internal temperature reached 96–98 °C and the colour of the reaction mixture turned brown. At the end of 15 minutes, the flask was removed from the oil bath and the hot mixture was filtered through a pad of Celite®. The SpEC-SO_3H was thoroughly washed with absolute ethanol (2–3 mL). The reaction mixture was concentrated on a rotary evaporator. The crude mixture was dissolved in hot absolute anhydrous ethanol (2.5 mL) and stirred for a few minutes at the boiling point of the ethanol. Excess ethyl acetate (100-150 mL) was added to the mixture under vigorous stirring to precipitate the tetraethylammonium bromide out of the mixture. The mixture was cooled to room temperature and the precipitate was removed by filtration. The residual tetraethylammonium bromide was rinsed with ethyl acetate (10 mL). The product was filtered through a pad of silica gel (30 g) using a sinter funnel, and the silica gel was rinsed with ethyl acetate (10 mL). The product was concentrated by rotary evaporation to obtain an oily crude product. The product is obtained as a deeply orange liquid (90-92% purity). The product was purified using flash column chromatography to obtain the product as an orange liquid and recorded in **Table *7.4-1***.

Table 7.4-1: HMF from fructose

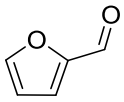
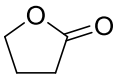
Entry	Fructose (mmol)	SpEC-SO_3H (mmol SO_3H)	SpEC-SO_3H (mol%)	yield of HMF (%)
1	5.6 mmol	0.498 mmol	8.90	77
2	5.6 mmol	0.498 mmol	8.90	89
3	5.6 mmol	0.249 mmol	4.45	53
4	5.6 mmol	0.1245 mmol	2.22	44

Hydroxymethylfurfural (33)



Syrup-like lquid, 89 %; **^{1}H-NMR (400 MHz, $CDCl_3$)** δ 3.71-3.16 (1H), 4.66 (d, J = 5.9 Hz, 2H), 6.46-6.48 (m, 1H), 7.17-7.19 (m, 1H), 9.50 (d, J = 5.7 Hz, 1H), **^{13}C-NMR (101 MHz, $CDCl_3$)** δ 57.5, 110.1, 123.5, 152.3, 161.1, 177.9

NMR data for dehydration of D-Xylose

 and  from the crude product

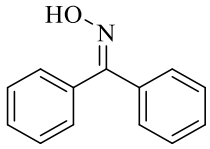
^{13}C-NMR (101 MHz, $CDCl_3$) δ 15.2, 21.3, 21.8, 22.4, 26.4, 29.6, 31.1, 61.9, 64.0, 65.5, 69.7, 73.0, 76.8, 77.1, 77.4, 77.6, 80.7, 81.9, 98.2, 102.8, 107.3, 112.7, 120.2, 122.4, 123.5, 148.2, 153.1, 175.7, 177.2, 178.1, 186.2, 190.5.

7.4.3 Beckmann rearrangement

7.4.3.1 Preparation of benzophenone oxime (36)

A mixture of benzophenone (9.11 g, 0.050 mol), $NH_2OH.HCl$ (0.074 mol, 5.13 g), CH_3COONa (0.125 mol, 10.26 g), ethanol (20 mL) and water (60 mL) were placed in a reaction flask with a reflux condenser. The flask was heated to reflux in the oil bath and the progress of the reaction was monitored by TLC until its completion. At the end of the reaction, the mixture was poured into beaker and allowed to cool down to room temperature. After cooling, the precipitate was filtered with suction, the precipitate was thoroughly washed with water, dried under vacuum, then recrystallized with ethanol to obtain a pure solid.

7.4.3.2 Benzophenone oxime (36)



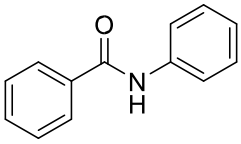
White crystal solid, 10.06 g, 98 %; **^{1}H-NMR (400 MHz, $CDCl_3$)** δ 7.35 (q, J = 6.9 Hz, 3H), 7.46 (t, J = 3.0 Hz, 7H), 9.42 (s, 1H); **^{13}C NMR (101 MHz, $CDCl_3$)**: δ 128.00, 128.36, 128.49, 129.27, 129.37, 129.67, 132.67, 136.26, 158.00.

7.4.3.3 Rearrangement of benzophenone oxime to benzanilide (37)

Benzophenone oxime (1.0 g, 5.06 mmol) was mixed intimately with sulfonated sporopollenin (SpEC-SO_3H, 1.0 g, 2.46 mmol eq. SO_3H) in a reaction flask fitted with a condenser and heated at an oil bath temperature of 140 ºC for 4 hours. At 30 minutes interval, the sample was drawn out of the reaction pot and dissolved in chloroform in order to monitor the progress of the reaction by TLC (ethyl acetate-petroleum ether, 1:9 v/v). At the completion of the reaction, the product was extracted from the reaction mixture with hot ethanol and the solvent evaporated to obtain a crude product. The crude product was purified through column chromatography.

The reaction was also performed using the sulfated sporopollenin catalyst (SpEC-OSO_3H, 0.39 mmol equivalent) at same temperature stated above, but the reaction period was extended to 7 hours.

Benzanilide (37): NMR data were compared to Li *et al*[29] and **Liu *et al***[53]



White solid, 80 %; **^{1}H-NMR (400 MHz, $CDCl_3$)** δ 7.12-7.16 (m, 1 H), 7.34-7.38 (m, 2 H), 7.44-7.56 (m, 3 H), 7.64 (d, J = 7.8 Hz, 2 H), 7.80-7.93 (m, 2 H); **^{13}C-NMR (101 MHz, $CDCl_3$)** δ 120.31, 124.68, 127.14, 128.89, 129.21, 131.97, 135.05, 137.99, 165.94, *m/z* (ESI-MS): $[M]^+$ 197.92; **elemental analysis** $C_{13}H_{11}O_1N_1$ requires: C, 79.17 %; H, 5.62 %; N, 7.10 %; found C, 78.80 %; H, 5.75 %; N, 7.14 %.

7.4.4 Transesterification reaction of sunflower oil triglycerides with methanol.

A mixture of sunflower oil (282.6 mL, 30 mmol) and methanol (85 ml, 2096 mmol) with SpEC-SO_3H (44 mole % relative to the oil) was added to the flask equipped with magnetic bar. The mixture was heated at 68 °C for the period stated in **Table *7.3-1***. At the completion of the reaction, the reaction mixture was transferred to a separating funnel and allowed to settle for 24 hours or centrifuge to remove the catalyst. The mixed fatty acid methyl esters were separated by decantation. The fatty acid methyl esters were washed with deionized water to remove any unreacted methanol. The percentage conversion was calculated according to the NMR spectroscopy method described in Section 7.3.1.

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Chapter 8: Conclusions and Recommendation for Future Work

8.1 Summary of results obtained

Sporopollenin exine capsules (SpECs) that are successively extracted from *Lycopodium clavatum* spores using acetone, potassium hydroxide and orthophosphoric acid, have been developed into a heterogeneous Brønsted acid catalyst through derivatisation with chlorosulfonic acid to make sulfonated SpECs. The typical loading levels of sulfonic acid groups (-SO_3H) found in this supported Brønsted acid catalyst ranged from 2.46 – 3.31 mmol/g. Another method to make SpEC-SO_3H was developed through the conversion of a thiol derivative of SpECs and subsequent oxidation. The conversion was successful because the sulfonic acid loading on SpEC, after oxidation, was between the range obtained in the sulfonation by chlorosulfonic acid. It is believed, based on the latest proposed structure of sporopollenin,[1] that the sulfonation was likely possible because of the presence of phenol groups in the structure of SpECs and it is most likely resulted from the electrophilic substitution of the proton *ortho* to the hydroxyl group of phenol, although, sulfonation at the *para* position is not impossible, but no evidence has been obtained to make this more than speculation. It was discovered that pre-washing the SpEC-SO_3H with the organic solvent to be used for a particular reaction was important to protect the products formed from unnecessary colouration resulting from leaching of materials from the catalyst. This was seen to be preferable than using excessive volumes and ranges of solvents in the washing of the catalyst after preparation.

The use of SpEC-SO_3H as an acid catalyst in various organic reactions was shown to be successful. The exploration of the catalyst in different reaction media showed that SpEC-SO_3H is highly effective natural polymer-based heterogeneous catalyst that is capable of competing with commercially available ones such as DOWEX 50W, Amberlyst 15, Amberlite 120(H) etc. One advantage envisaged for SpEC-SO_3H compared to Amberlyst 15, for example, is that the capsules may be heated up to 400 °C without physical degradation[2-5] meaning it has potential to be a good catalyst in melt (solvent-free) reactions where a high temperature is required. It worth noting that commercially available solid acid catalysts such as Amberlyst 15 was do not have the ability to withstand heat beyond 120 °C.[6]

Application of SpEC-SO_3H in heterocyclic syntheses led to good yields that can be compared favourably to those from commercially available acid catalysts. Thus, SpEC-SO_3H catalysed reactions that led to the formation of disubstituted benzimidazoles from *ortho*-phenylenediamine and aromatic aldehydes giving products in the range of 61-90 % yield. The microwave assisted SpEC-SO_3H-catalysed reaction was faster and gave yields in the same range as the conventional heating. It was found that having an *ortho* substituent in the aromatic aldehyde affected the yield of products formed. For example, a yield of only 6 % product was obtained from the SpEC-SO_3H catalysed reaction of *ortho* phenylenediamine and 2-methoxybenzaldehyde. This was thought to be largely due to steric hindrance rather than any electronic effects. Reaction of either salicylaldehyde or 5-chlorosalicaldehyde with *ortho* phenylenediamine, in the presence of SpEC-SO_3H, formed the diimine. The yield of diimine was high, in the range of 75-90 %, and it was considered that the ortho OH in each case prevented further reaction by forming stable intramolecular hydrogen bonds with the nitrogen atom. All attempts to synthesise benzimidazoles from salicylaldehyde, or derivatives, were not successful even using microwave-assisted heating. The reaction of aliphatic aldehydes with *ortho*-phenylenediamine in the presence of SpEC-SO_3H gave fair yields of the corresponding disubstituted benzimidazoles. SpEC-SO_3H-catalysed reactions of primary aliphatic aldehydes were better than reactions involving a secondary aldehyde, and the yields for both were lower compared to those involving most of the aromatic aldehydes. This was rationalised due to the propensity of aliphatic aldehydes to self-condense through aldol reaction, though no direct evidence for this was sought. The catalyst loading was investigated in the benzimidazole syntheses. It was found that product yields increased with a decrease in the mole percentage of catalyst compared to the reactants. Using a loading of SpEC-SO_3H beyond 13 mol% involving aromatic aldehydes led to very low yield, and it was thought this was due to the product sticking to the surface of the catalyst meaning it could not efficiently be extracted at the end of the process.

Protection of carbonyl compounds with ethylene glycol in the presence of SpEC-SO_3H yielded ketal products in yields ranging from 62-94 %. SpEC-SO_3H-catalysed formation of spiro compounds from cyclopentanone or cyclohexanone was faster and it took shorter reaction times than ketal formation from aromatic ketones. The protection of acetophenone and two other derivatives yielded products in 68 % and above. Ketalisation of the carbonyl group in 2'-hydroxyacetophenone proved very difficult. Despite

optimisation and many attempts, the product and the starting carbonyl could not be separated from each other. Overall, SpEC-SO_3H-catalysed protection of the carbonyl of phenacyl chloride (2-chloroacetophenone) was the highest yielding of all the acetophenones. The protection of benzophenone, in the presence of SpEC-SO_3H, gave the highest yield (94 %).

SpEC-SO_3H has shown a potential to be a catalyst for the protection of different classes of alcohols. SpEC-SO_3H was used as heterogeneous catalyst in the tetrahydropyranylation of primary alcohols, sterols and phenol. The protection of benzyl alcohol was completed in two hours, whilst other alcohols took three hours each. Protection of the two sterols investigated, cholestanol and sitosterol, gave similar yields (85 % and 88 % respectively). The *para*-hydroxy group of 2,4-dihydroxybenzophenone was selectively protected over the *ortho*-hydroxy group, despite doubling the mole ratio of 3,4-dihydropyran required in the reaction, because Intramolecular H bond of the 2'-OH and the carbonyl group (C=O). The yield obtained from the protection of phenol was the lowest at 79 %.

Sulfonated sporopollenin exine capsules (SpEC-SO_3H) proved to be an efficient heterogeneous catalyst for the protection of D-mannose with acetone, giving a yield of 84 % of 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose when the reaction was performed at room temperature. However, the yield was greater (91 %) when the reaction was heated. Catalysing the reaction of mannose and 2,2-dimethoxypropane (DMP) with SpEC-SO_3H yielded 95 % of the product, and the white solid product obtained was confirmed to be a mixture of the α- and β- anomers of bis(isopropylidene)furanose. Similar protection of glucose and xylose gave semi-solid compounds, unlike the crystalline solid obtained from mannose. The semi solids were harder to purify by recrystallisation, but when subjected to column chromatography, the NMR spectra indicated the formation of a mixture of α- and β- forms of bis(isopropylidene) furanose as well. The NMR spectra of the isopropylidene product from xylose also showed 1,2- isopropylidene furanose in addition to the bis(isopropylidene) furanose product. Yield of 54 % and 70 % were respectively obtained for glucofuranose and xylofuranose.

Sulfonated sporopollenin (SpEC-SO_3H) has also shown promising potential for Fischer glycosylation of methanol, but more work on glycosylation of higher alcohols is required. Fischer glycosylation of methanol using sulfonated sporopollenin catalyst led

to a 71 % yield of methyl mannopyranose that is a mixture of α- and β- anomers. The SpEC-SO_3H catalysed Fischer glucosylation of methanol yielded 98 % of the α- and β-glucopyranose anomers in 2:3 ratio, and similar reaction of isopropyl alcohol yielded an 88 % mixture of α- and β- anomers of isopropyl glucopyranose in 2:1 ratio respectively. The catalytic activities of SpEC-SO_3H was not limited to the glycosylation reaction using mannose but also the catalytic performance SpEC-SO_3H, as a solid phase heterogeneous catalyst, for the glycosylation using D-glucose and glucose monohydrate was recorded with a great success. Glycosylation of higher alcohols 1-butanol and 1-octanol with mannose gave 46 % and 45 % respectively, and with glucose the yields were 48 % and 45 % respectively. Glycosylation of 1-octanol with xylose yielded only 16 %.

SpEC-SO_3H catalysed *N*-glycosylation of urea and urethane with mannose yielded 97 % and 53 % respectively, however attempts to replace mannose with other monosaccharides was unsuccessful. Modification and further research on the glycosylation of urea and urethane have shown that ammonium chloride has little or no effect on the catalytic activities of sulfonated sporopollenins (SpEC-SO_3H).

Miscellaneous reactions were investigated using the SpEC-SO_3H catalyst. In the field of sugar chemistry, SpEC-SO_3H was shown to be able to dehydrate fructose giving a high yield of HMF. Dehydration of glucose, *via* isomerisation to fructose, and xylose has however not so far been successful. Other reactions investigated included the Beckmann rearrangement of benzophenone oxime, which in the presence of 49 mol% SpEC-SO_3H catalyst yielded 80 % of *N*-phenylbenzamide in a melt reaction. The transesterification reaction of fatty acid triglyceride with methanol, catalysed by SpEC-SO_3H, was slow. This reaction using 44 mol % SpEC-SO_3H converted 30 mmol of sunflower oil to its methyl ester in 58 hours in a 100 % conversion and 98 % yield was obtained.

8.2 Recyclability of SpEC-SO_3H in organic reactions

Recyclability of solid catalyst is very important for its usefulness in industrial applications. As described above SpEC-SO_3H was applied to various organic reaction ranging from heterocyclic synthesis, protection of carbonyl compounds, isopropylidation of monosaccharides, Fischer glycosylation alcohols, *N*-glycosylation of urea and ethyl carbamate, dehydration of monosaccharides, Beckmann rearrangement and in catalysing the formation of biodiesels from fatty acid triglycerides. In selected reactions, the recyclability of SpEC-SO_3H was investigated.

In general SpEC-SO_3H was found to be recyclable for at least four cycle of reaction before the yields of the products were observed to decline. In some of the reactions, the product yield declines with each cycle of the reaction, while with others, the catalytic activities of SpEC-SO_3H remains intact even after the fourth cycle. For example, the yield declined significantly between the third and fourth reaction cycle in the synthesis of benzimidazole derivatives, while the change in yield was negligible when then SpEC-SO_3H was recycled in the isopropylidation of mannose. However, after each cycle the SpEC-SO_3H needs to be thoroughly washed and dried before been used for the next or the successive cycle of the reaction to avoid the blockage of the active acid site by unextracted products, or to avoid the possible transfer of product from one reaction cycle to the next.

8.3 Recommendation for future work

Further investigation is warranted on the application of SpEC-SO_3H to the synthesis of heterocyclic compounds. For example, it might be possible to synthesise monosubstituted benzimidazoles using a 1:1 ratio of aromatic aldehydes and *ortho*-phenylenediamine or an equivalent precursor. Naturally, there are many other acid-catalysed syntheses which could be investigated in this general field.

The world is tending towards the use of renewable products to reduce the impact of current hazards posed on the environment by synthetic products. Further work on the SpEC-SO_3H-catalysed glycosylation of long chain alkyl alcohols would offer an integrated green route towards bio-derived surfactants.

Also, there is possibility of being able to dehydrate glucose or even cellulose *via* fructose into the important platform molecule HMF in the presence of SpEC-SO_3H.

More extensive work needs to be done on the melt-phase Beckmann rearrangements, most especially investigation on the rearrangement of alicyclic and aliphatic ketoximes. The use of processes which do not involve solvents are likely to be closer to the ideals of green chemistry, and the heating of physically robust SpECs.

The effectiveness of SpEC-SO_3H also need to be investigated in the hydrolysis of the protected compounds, most especially; the hydrolysis of the ketals from carbonyl compounds and acetal of carbohydrates.

Lastly, SpEC-SO_3H is a highly promising bio-sourced solid support catalyst whose use poses reduced environmental impact. The development of SpEC-SO_3H to the standard as applied to industrial processes in the near future would therefore be of interest. First, however, further work is required to be able to improve/control the loading of SO_3H groups per gram of SpECs. In addition, there needs to be a better process for monitoring the uniformity of the particles in the production of SpEC-SO_3H.

8.4 Conclusions

In some cases, it was found that reactants or product could get stuck to the surface of catalyst if too much SpEC-SO_3H was used in to catalyse the reaction. In these cases, isolation of the products from the reaction was harder and a lower yield was obtained. However, if the products are soluble in the solvent used for the reaction, then this is less of an issue, but may not be so 'green' as when using water or melt conditions. For example, the synthesis of 1-(4-hydroxybenzyl)-2-(4hydroxyphenyl)-1H-benzimidazole in the presence of 0.78 mmol SpEC-SO_3H, in water at 70 °C, yielded 43 % but when the number of moles of the catalyst was reduced to 0.26 mmol, the yield increased to 79 %. A good amount of ethanol was required to remove the product from the surface of SpEC-SO_3H. This example explains the influence of the quantity of the catalyst on the catalytic performance of SpEC-SO_3H. Another example was on the production of 1-(4-hydroxybenzyl)-2-(4hydroxyphenyl)-1H-benzimidazole in the presence of 0.13 mmol SpEC-SO_3H; when the reaction was performed in water, a product of 70 % yield was obtained while the yield was 82 % when the solvent was aqueous ethanol. Furthermore, 1-benzyl-2-phenyl-1H-1,3-benzimidazole in the presence of 0.52 mmol SpEC-SO_3H yielded 43 % in water while with the lower amount of catalyst (0.26 mmol), a product of 69 % was obtained in ethanol. Also, ethylene glycol was stuck to the surface of SpEC-SO_3H in the ketalisation reaction leading to the formations of 1,4-dioxaspiro[4.4]nonane from cyclopentanone and 1,4-dioxaspiro[4.5]decane from cyclohexanone thereby producing a reduced product yield, even when the length of the reaction was extended by an hour, to 37 % and 44 % respectively.

Finally, it is worth noting that the commercially available polystyrene resins have the disadvantage of experiencing physical damage when exposed to harsh reaction conditions such as high temperature or vigorous stirring of reactions. Some cannot withstand very strong acids or bases at high temperature (e.g. Amberlyst 15 cannot withstand heat beyond

120 °C)[6] without losing their morphology. Also, they possess variable dispersibility because the dispersability depends on the swelling ability of the beads.[7] SpEC-SO_3H, on the other hand, is relatively cheap to produce, are monodisperse, and resistant to high temperature (e.g. up to 400 °C), many harsh reagents as long as they are not oxidising and strong mechanical stirring / shear conditions.[2-5] Therefore, SpEC-SO_3H can be used to catalysed reactions under harsh condition and high temperature without experiencing any form of damage.

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