Preparation of pelargonic acid vesicles and sustained drug release

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Abstract: This study has selected the medium-chain saturated fatty acid pelargonic acid as the raw material for the first time, and added the surface catalyst Tween-80 to the aqueous solution to form pelargonic acid-Tween-80 composite vesicles by a surface tension method. The compound vesicle was used to encapsulate five heterocyclic drugs (ceftazolin sodium, chlorpromazine hydrochloride, gemcitabine hydrochloride, metronidazole, and isoniazid), all of which have disadvantages when used directly, and the effects of the vesicles on the five kinds of drugs was evaluated in terms of encapsulation rate and the *in vitro* sustained-release in a simulated artificial intestinal fluid environment. The results revealed that the pelargonic acid-Tween-80 composite vesicles exhibit an encapsulation efficiency above 45%, and that the encapsulated drugs achieve a cumulative release effect of 6-10 hours in a simulated artificial intestinal fluid environment, and the cumulative release rate is above 35%. This study proves that pelargonic acid-Tween-80 composite vesicles can be used as a carrier for encapsulated drugs, and this can extend the time of action of the drugs. This research illustrates the potential for application of pelargonic acid in the field of medicine.

Keywords: Pelargonic acid; Vesicles; Tween-80; Drug sustained release.

Introduction

Pelargonic acid is a medium-chain saturated fatty acid widely found in animals and plants, and it mostly exists in the form of fat in plants. Pelargonic acid is cheap and easy to obtain, and is often used as a raw material in organic synthesis. Given its light fat content and coconut flavor, it can be used to make coconut and berry flavored foods, which are safe and non-toxic. At present, research on pelargonic acid is mostly focused on its application as a plant production regulator to stimulate the germination of seeds and the growth and development of seedlings and as a herbicide [1]; other applications are less well explored.

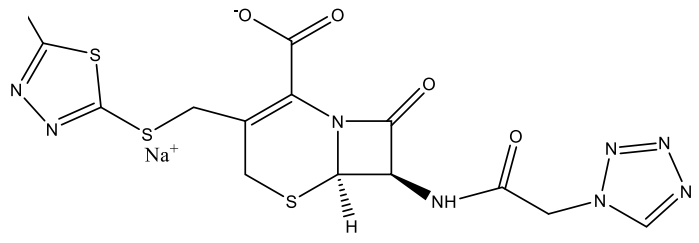
Since Gebicki discovered oleic acid vesicles in 1973, fatty acid vesicles have attracted wide attention. Among them, oleic acid vesicles are the most studied [3-5], whilst Walde *et al*. have explored the vesicle self-assembly of the oleic acid system [6]. When controlling the pH range from 8.2 to 10.0, multiple micron-sized vesicles appeared in the oleic acid solution [7]. Following addition of oleic acid monoglyceride, and adjustment of the ratio of oleic acid monoglyceride in the oleic acid solution, the self-assembled product appeared as unilamellar or even multilamellar vesicles [8]. Linoleic acid aqueous systems were found to self-assemble and form vesicles over the pH range 7.9 ~ 9.2 [9]. Indeed, research on fatty acid vesicles mostly focuses on the preparation of unsaturated long-chain fatty acid vesicles. There are few studies on medium-chain saturated fatty acids, and only thermodynamics and kinetics studies of caprylic acid and capric acid have been reported [10], while corresponding sustained release studies of drugs have not been carried out.

Fatty acid vesicles have a hollow shell structure, which is similar to that of cell membranes. Therefore, they possess good biocompatibility and the ability to improve drug efficacy. Compared with the traditional method of using liposomes to encapsulate and release drugs, fatty acid vesicles have simpler components, are cheaper and utilize more readily available raw materials. They also exhibit a higher degree of safety, which can overcome the limitation of complex composition of liposomes and difficulty when forming high-quality phospholipid molecules. Therefore, fatty acids can be used as drug carriers [11-13] to deliver drugs in the human body, and have excellent development prospects for medical applications. However, fatty acid vesicles have a significant dependence on pH, and their pH window is narrow, with most of the vesicles found in the alkaline range [14-15], which limits their application in the food and pharmaceutical industries. Studies have shown that

surfactants can adjust the pH range of vesicles. In 1980, Regen *et al*. [16] added a polymerizable surfactant, namely cetyl dimethyl ammonium bromide, to vesicles, which enhanced the stability of the vesicles. Moreover, Lee *et al*. [17] added a divinylbenzene crosslinker (pH window 6.0 ~ 8.0) to 10-undecylenic acid to form polymer vesicles with enhanced stability.

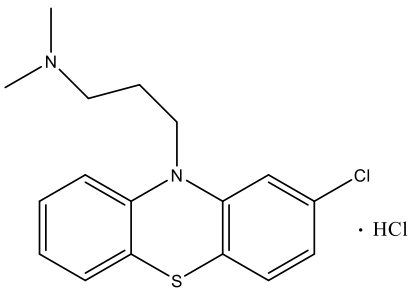
In order to expand the application of pelargonic acid, we have initiated research on the preparation of pelargonic acid in fatty acid vesicles, and have examined the adjustment of the pH window by adding surfactants, and the encapsulation of five heterocyclic drugs (ceftazolin sodium, chlorpromazine hydrochloride, gemcitabine hydrochloride, metronidazole, and isoniazid) for sustained release. The direct effect of the selected drugs on the human body is accompanied by adverse reactions and defects in the use of large doses. In order to improve the drawbacks of the direct effect of the drugs, we selected pelargonic acid-Tween-80 as a self-assembly template to synthesize pelargonic acid-Tween-80. The approach involved utilizing compound vesicles, encapsulating heterocyclic drugs as encapsulated molecules, and then exploring the encapsulation and sustained release of pelargonic acid compound vesicles to drugs. Experiments have shown that pelargonic acid-Tween-80 composite vesicles can be used as carriers to encapsulate drugs, which can fully prolong the time of drug action and improve the disadvantages caused by direct action of the drugs. Such research provides valuable insight into the application of medium-chain saturated fatty acids in the sustained release of vesicle drugs.

(a)

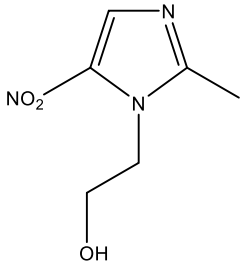


Cefazolin Sodium, Cefamezin, CEZ

(b) (c)

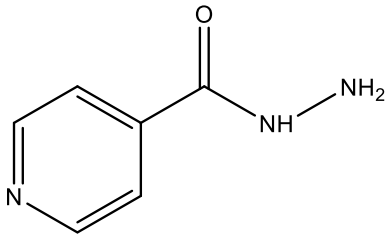


Chlorpromazine hydrochloride, L-B-Q



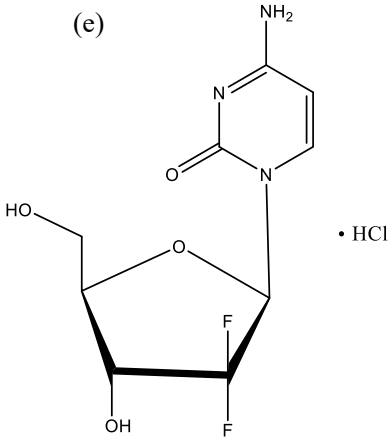
Metronidazole, MNZ

(d)



Isoniazid, IN

(e)



Gemcitabine hydrochloride, dFdC

Chart 1. The five heterocyclic drugs (ceftazolin sodium, chlorpromazine hydrochloride, gemcitabine hydrochloride, metronidazole, and isoniazid) studied herein.

2 Materials and methods

2.1 Materials

Hydrochloric acid, sodium chloride and sodium hydroxide were purchased from Tianjin Jinhui Atayal Chemical Reagent Co., Ltd. Pelargonic acid, cefazolin sodium, chlorpromazine hydrochloride, gemcitabine hydrochloride, metronidazole and isoniazid were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Potassium dihydrogen phosphate was purchased from Tianjin Hengxing Chemical Reagent Company. Dipotassium hydrogen phosphate and Tween-80 were purchased from Sinopharm Group, whilst a JEM-F200 transmission electron microscope, a DF-101S type thermostatic magnetic stirrer, a TECNIAG2 SPIRIT BIOTWIN type dynamic light diffuser, a PHS-3C type pH meter were employed during these studies. UV-Vis spectra were recorded on a UV-2700 UV spectrophotometer. An MD34 dialysis bag, a dialysis bag special clip, a JD200-4 electronic balance, a TC-15 constant temperature heating mantle, self-made bubble meter, a QYC-2102C shaker were also utilized.

2.2 Measurement of surface tension

In this experiment, the maximum bubble pressure method [18] was used to measure the corresponding surface tension σ and concentration c of the pelargonic acid saturated fatty acid aqueous solutions at different pH values and concentrations at 37 ℃. The surface tension values were measured three times in parallel (data error <0.001) and averaged, using σ as the ordinate and lgc as the abscissa. The σ-lgc curve was drawn, at the inflection point of the curve, the critical aggregation concentration is the critical micelle concentration CAC for the formation of pelargonic acid vesicles. In order to ensure that the solution can form vesicles to the greatest extent, the concentration of the solution should be selected to be a value greater than the critical aggregation concentration. In our process, sodium hydroxide, potassium dihydrogen phosphate and potassium dihydrogen phosphate and dipotassium hydrogen phosphate buffer solutions were used to control the pH of the system, and NaCl solution was used to adjust the ionic strength of the system [19].

2.3 Determination of pH window range by the acid-base titration curve method

Pelargonic acid was dissolved in a 0.1 mol/L sodium hydroxide solution to prepare a saturated fatty acid sodium salt solution, which was diluted to a concentration of 0.05 mol/L, and used as the mother liquor; the pH of the solution was adjusted to 13. The above solution was divided into 10 mL portions (≥25 portions), and each portion of the sodium pelargonate solution was titrated against 0.2 mol/L hydrochloric acid to obtain a series of sample solutions with different pH values. During the process, the concentration of the saturated fatty acid sodium salt solution remained unchanged. After the sample solution was allowed to stand undisturbed for 3 days at room temperature, the pH value of the solution was measured.

2.4 Characterization of self-assembled products

One of the vesicle solutions of a certain pH value of pelargonic acid within the self-assembly pH window was randomly selected, filtered with a 0.45 μm filter membrane, and dynamic laser light scattering was performed using a laser with wavelength 632.8 nm to

obtain the corresponding light intensity (SI) and particle size (Rh) distribution.

A similarly selected vesicle solution was also randomly selected and dropped onto the copper mesh. The sample was stained with a dye, and the excess liquid absorbed by the filter paper, and naturally dried, and transmission electron microscope (TEM) was employed to observe the morphology.

2.5 Determination of surface tension of the pelargonic acid-Tween-80 composite solution

Using the controlled variable method, the molar ratio of the surfactant Tween-80 and pelargonic acid in the pelargonic acid composite solution was determined to be 1:1. The composite aqueous solution was accurately prepared with a series of solutions at different concentrations and pH 6.8. At 37 ℃, the maximum bubble pressure method was used to determine the relationship between the surface tension σ and the concentration c, and a σ-lgc curve was drawn. The critical aggregation concentration obtained at the turning point using this method is the critical micelle concentration cc for the formation of the pelargonic acid composite vesicles.

2.6 DLS and TEM studies to verify the pH window for the composite vesicles

Using 0.1 mol/L sodium hydroxide solution as the solvent, and taking the concentration of pelargonic acid at 0.03 mol/L, a composite solution with a molar ratio of pelargonic acid and surfactant Tween-80 at 1:1 was prepared. This was then divided into multiple portions, and 7 mL of each portion was transferred into a transparent Erlenmeyer flask for acid-base titration. The titrated solution was allowed to stand at room temperature for 3 days. During this time, the structure of the self-assembled composite vesicle was basically stable, and the pH change was measured. At the same time, the sample of the composite solution under different pH conditions (pH 5.5, 6.5 and 7.5) was filtered through a 0.8 μm filter membrane, and then dynamic laser light scattering was employed. The laser wavelength was 632.8 nm and the scanning time was 180 s. The corresponding light intensity (SI) and particle size (Rh) distribution were measured. According to the pH change and light intensity, the SI-pH regression curve was drawn, and the pH window of the composite vesicle was judged by the peak width at half-peak height.

2.7 DLS and TEM characterization to verify the rationality of the pH window range of the composite vesicles

The pH window of the above-mentioned composite vesicles is 5.5 ~ 7.5, which improves on the narrow pH window associated with pelargonic acid vesicles and the dependence on pH. After the pH window of the composite vesicles migrated to 5.5 ~ 7.5, dynamic light scattering was used with the laser wavelength at 632.8 nm, and a scanning time of 180 s. The corresponding light intensity (SI) and particle size (Rh) distribution were measured (three parallel measurements). The solution of pelargonic acid-Tween-80 composite vesicles was dropped onto a copper mesh with a carbon film, and was treated with the dye phosphotungstic acid. Following drying, the filter paper absorbed the remaining liquid at the edge. After natural drying, it was passed through a transmission electron microscope (TEM) in order to observe its shape.

2.8 Establishment of the drug standard curve

10 mg each of cefazolin sodium, chlorpromazine hydrochloride, gemcitabine hydrochloride, metronidazole, and isoniazid were weighed out, and PBS buffer added to a 100 mL volumetric flask. Then 1 mL, 2 mL, 4 mL, 5 mL, 6 mL, and 8 mL was measured out of the prepared drug solution into a 10 mL volumetric flask. After obtaining a constant volume and shaking, a series of different drugs with increasing concentration relationship can be obtained. Using a PBS buffer (simulated artificial intestinal fluid) as a blank control, a series of prepared solutions were scanned under an ultraviolet spectrophotometer to obtain the absorption spectra and absorption wavelength data of the five heterocyclic drugs. The absorbance of the wavelength at the maximum absorption peak was recorded for each group. Finally, with the concentration (c) of different drugs as the abscissa and the absorbance (A) of the different drugs as the ordinate, the standard curves of five heterocyclic drugs were obtained.

2.9 Evaluation of drug vesicle embedding/sustained release

7 ml of compound vesicles containing cefazolin sodium, chlorpromazine hydrochloride,

gemcitabine hydrochloride, metronidazole, and isoniazid drugs respectively were put into the dialysis bag, and then the dialysis bag was placed in a 150 mL pH = 6.8 environment for 14 h. In the first two hours, samples were taken every 10 minutes, and then samples were taken at 30-minute intervals, each with a sample volume of 3 mL. The absorption value of the pelargonic acid-Tween-80 composite vesicles containing different drugs at the maximum absorption peak was measured with an ultraviolet spectrophotometer, and the encapsulation efficiency of the vesicles calculated by subtracting the absorption value of the blank vesicles. The formula for calculating the encapsulation rate is as follows:

Encapsulation rate (wt %)=$(m_1-m_2)/m_1*100\%$

m_1 is the total mass of drugs before dialysis; m_2 is the mass of drugs in the sustained-release solution outside the dialysis bag after 14 h of dialysis to reach equilibrium.

7 mL of compound vesicles containing cefazolin sodium, chlorpromazine hydrochloride, gemcitabine hydrochloride, metronidazole, and isoniazid drugs respectively, were taken and placed in a dialysis bag, which was placed in a 150 mL pH = 6.8 buffer solution. After standing for 5 h to release the free drug molecules that are not contained in the vesicles, the dialysis bag containing the drugs was transferred to a fresh 200 mL simulated intestinal fluid environment, set to 37 ℃, with the speed at 60 r/min, for drug release determination. A sample of the solution was taken every 20 minutes, 3 mL each time, and placed on an ultraviolet spectrophotometer and the absorption value at the maximum absorption peak measured to obtain the cumulative drug release.

Calculation formula of cumulative release rate: Cumulative release rate Q(%)=(cumulative drug concentration in release fluid at each time × release medium volume/vesicle drug content)×100%.

2.10 Infrared spectroscopy and thermogravimetric analysis of the isoniazid inclusion compound in the composite vesicles

The pure vesicle solution with drug molecules embedded in the vesicles and the clathrate solution with drug molecules embedded in the vesicles were respectively determined, and then freeze-dried to obtain dry powder samples. They were characterized by infrared spectroscopy (IR) and thermogravimetric (TG) analysis.

Characterization by infrared spectroscopy: FTIR measurements of dry samples were

carried out at room temperature. The wave number range is 500 ~ 4000 cm^{-1}, the resolution is 2 cm, and the scanning frequency is 128 scans every 4 cm^{-1}.

Thermogravimetric analysis: Under a nitrogen atmosphere, about 5 mg of sample sealed in an aluminum pan was heated from room temperature to 500 °C at a nitrogen rate of 50 mL/min. It was stored at this temperature for 5 min, and was then cooled to 40 °C at a rate of 10 °C, and finally reheated to 500 °C at the same rate.

3. Results and discussion

3.1 The pH response range of the self-assembly of pelargonic acid solution

By use of acid-base titration, the surface tension σ and concentration c of a pelargonic acid solution at 37 ℃ was measured, and a σ-lgc curve (Figure 1) was drawn.

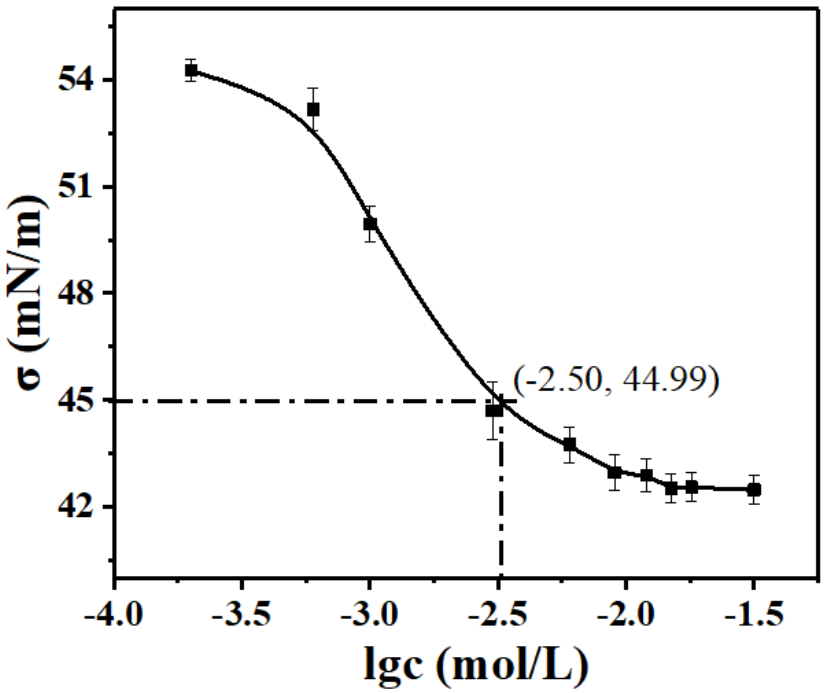


Figure 1. σ-lgc curve of the pelargonic acid solution at 37 ℃.

The inflection point value is converted to the corresponding concentration value by the power of 10 formula. It can be seen from Figure 1 that the critical micelle concentration value of the pelargonic acid solution is 0.0031 mol/L. In order to make the structure and morphology of pelargonic acid vesicles more regular and closer to the pH range of human life activities, the solution was selected for acid-base titration, the concentration of the selected solution for acid-base titration should be greater than the critical micelle concentration. Therefore, 0.03 mol/L

pelargonic acid solution was used for the acid-base titration experiment.

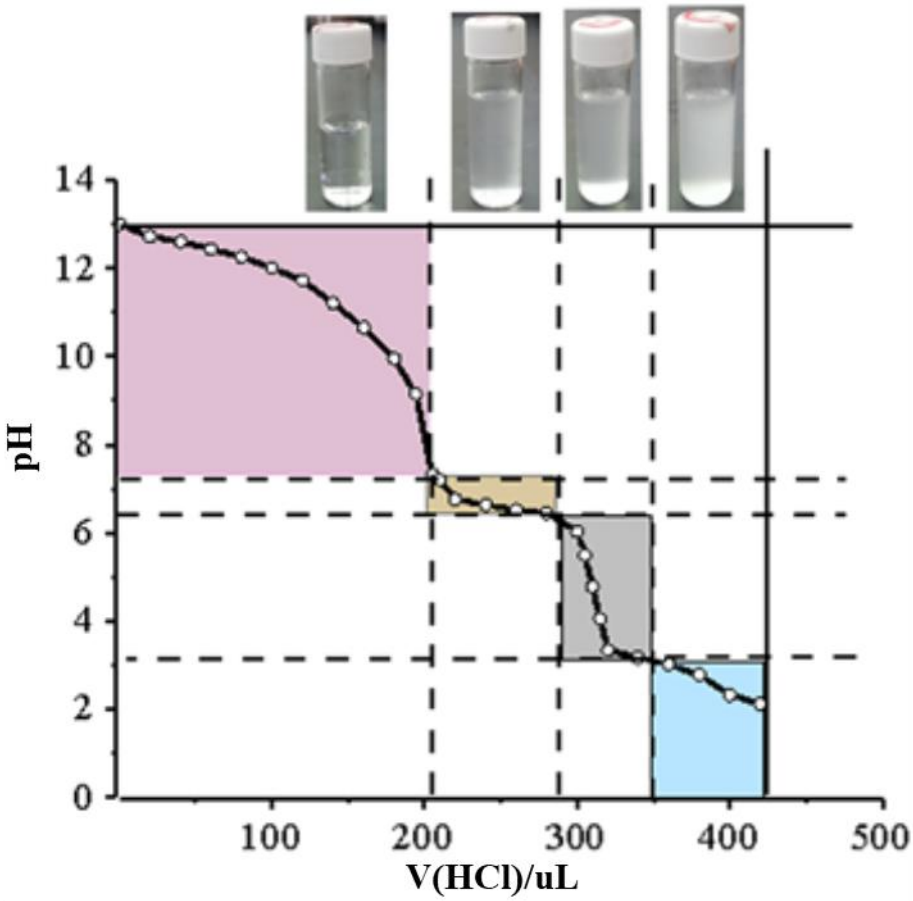


Figure 2. Acid-base titration of the pelargonic acid solution.

As shown in Figure 2, when the pH is in the range 7.5 to 13, the appearance of the pelargonic acid sample is clear and transparent, and basically does not change with change of pH. The reason for this phenomenon is that most of the carboxyl groups in the pelargonic acid solution system exist in the form of ions, and the electrostatic repulsive force between the carboxylates induces the formation of a self-assembled body with a smaller curvature, that is, a micellar state. On decreasing the pH, the pH of the pelargonic acid solution exhibits a titration jump at 7.5 and 6.6, and in the range of 6.6 ~ 7.5, the system reveals a strong opalescence phenomenon. The number of carboxylic acid molecules and carboxylate ions present is almost the same, and the former mainly exist with hydrogen bonds. The electrostatic repulsion with the carboxylate ions is weakened, which enables the sample to self-assemble to form an assembly with a larger radius of curvature. According to related literature [19-20], according to the change of the solution state before and after the acid-base

titration, the pelargonic acid solution may self-assemble to form pelargonic acid vesicles in the pH range 6.6 ~ 7.5. On continued dropwise addition of HCl to the pelargonic acid solution, the pH value drops sharply, and the system undergoes a phase separation and oil droplets form. This is a manifestation of the disintegration of the vesicles caused by the presence of a large amount of protonated fatty acids. The jump point of the acid-base titration can verify the change of the vesicle state and provide a reference for further determining the pH range of the vesicle.

In order to verify the reliability of the results, we employed TEM to characterize the morphology of the self-assembled pelargonic acid vesicles at pH 7.1 and 6.8.

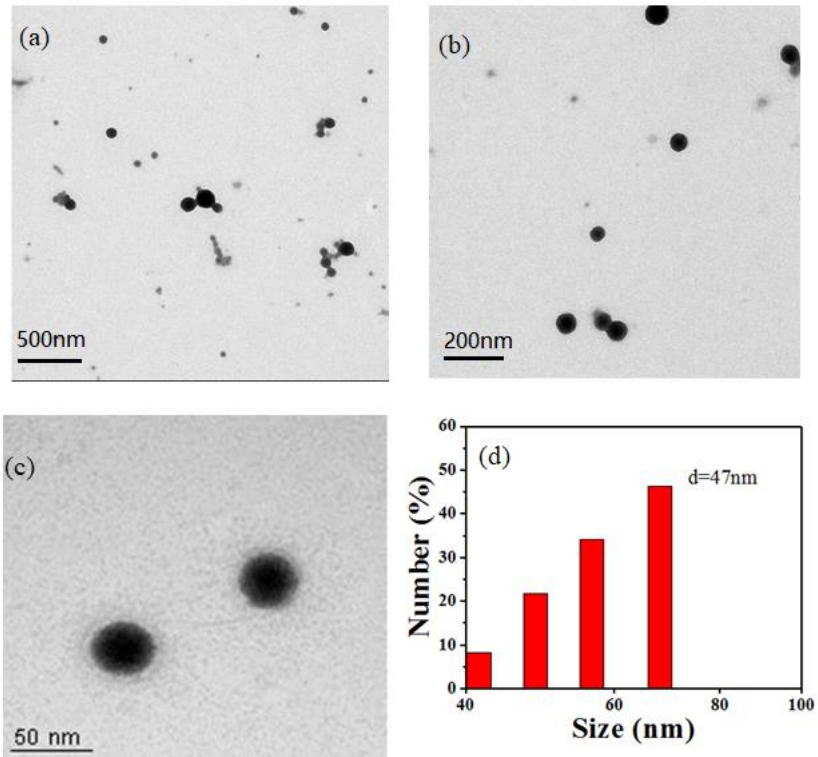


Figure 3. Vesicle morphology and particle size of pelargonic acid at pH=7.1 (a) – (c) are vesicle morphology of pelargonic acid; (d) is the vesicle particle size of pelargonic acid.

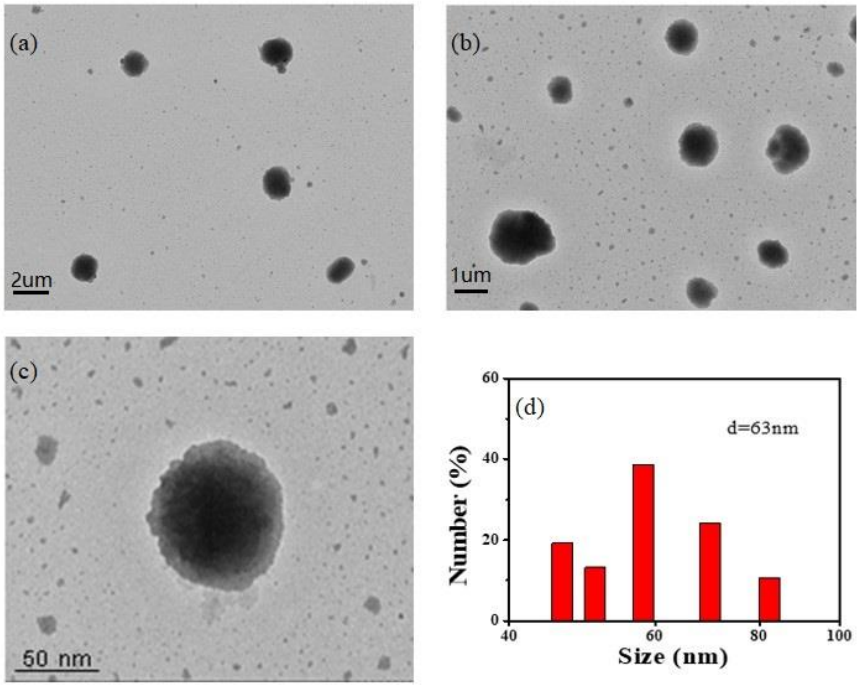


Figure 4. Vesicle morphology and particle size of pelargonic acid at pH=6.7; (a) – (c) are vesicle morphology of pelargonic acid; (d) is the vesicle particle size of pelargonic acid.

As shown in Figures 3 and 4, the self-assembled system contains carboxylate ions and carboxylic acid molecules, and the interaction occurs between them, so that pelargonic acid forms a regular vesicle structure at pH=7.1 and pH=6.7. This further illustrates that as the pH of the self-assembly system decreased, the average diameter of the vesicles of the system increased. The reason may be the coexistence of carboxylate ions and carboxylic acid molecules in the self-assembled system. At high pH values, the carboxylate ions mainly play an electrostatic repulsion effect in the system, which makes the molecular arrangement loose and manifests as a smaller particle size. When the pH is lowered, the carboxylate ion is gradually protonated. At this time, a more stable hydrogen bond is formed between the carboxylate ion and the carboxylic acid group, which is conducive to the close arrangement of the molecules, and is manifested by an increase in particle size. According to the TEM results, the pelargonic acid can self-assemble to form vesicles. The pH window of the pelargonic acid vesicles is in the range 6.6 ~ 7.5, *i.e.* the neutral and weakly alkaline range. It is noteworthy that this pH window range is the closest to the pH range of human life activities, and the vesicle structure and morphology are regular. Research on the preparation and application of pelargonic acid vesicles has not yet been reported. In order to expand the

application of pelargonic acid, our experiments select pelargonic acid vesicles as carriers for sustained drug release.

3.2 Establishment of a DLS method to determine the pH range of the vesicles

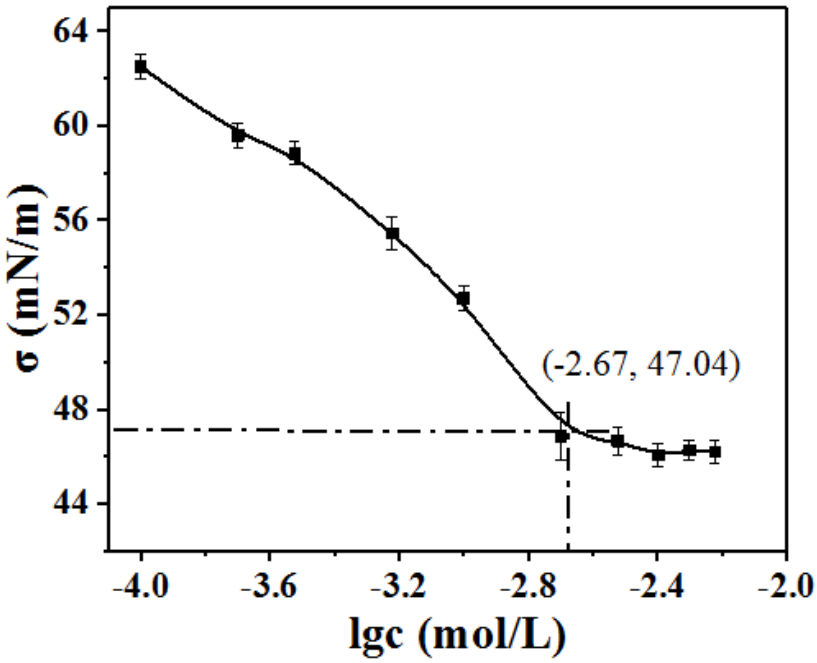


Figure 5. Surface tension diagram of the composite system with the non-ionic surfactant Tween-80.

Figure 5 is a graph of the surface tension of the composite solution with a 1:1 molar ratio of pelargonic acid and Tween-80 surfactant. From the turning points in the figure, the critical micelle concentration of the composite solution with Tween-80 surfactant is obtained; the values are 0.0021 mol/L. Since the concentration required for vesicle formation is usually greater than the value of the micelle concentration [19], the composite vesicle concentration value is generally selected to be higher than the critical micelle concentration value. In this work, it takes about 10 times the critical micelle concentration value. Therefore, the concentration of the composite vesicles is selected to be 0.03 mol/L and the molar ratio is 1:1. It was found through experiments [22] that when the system appears opalescent and partially transparent, use of dynamic laser scattering can measure the corresponding pH. The light intensity data in the corresponding pH range appears at a maximum value, mainly because the corresponding light intensity value of the vesicle is greater than the micellar light intensity value. Therefore, this paper uses the SI-pH regression curve method to judge

the migration and expansion range of the fatty acid pelargonic acid-Tween-80 surfactant composite vesicles, and conservatively takes the corresponding pH range at half-peak height as the fatty acid pelargonic acid -The pH window of Tween-80 surfactant complex vesicles; the results are shown in Figure 6.

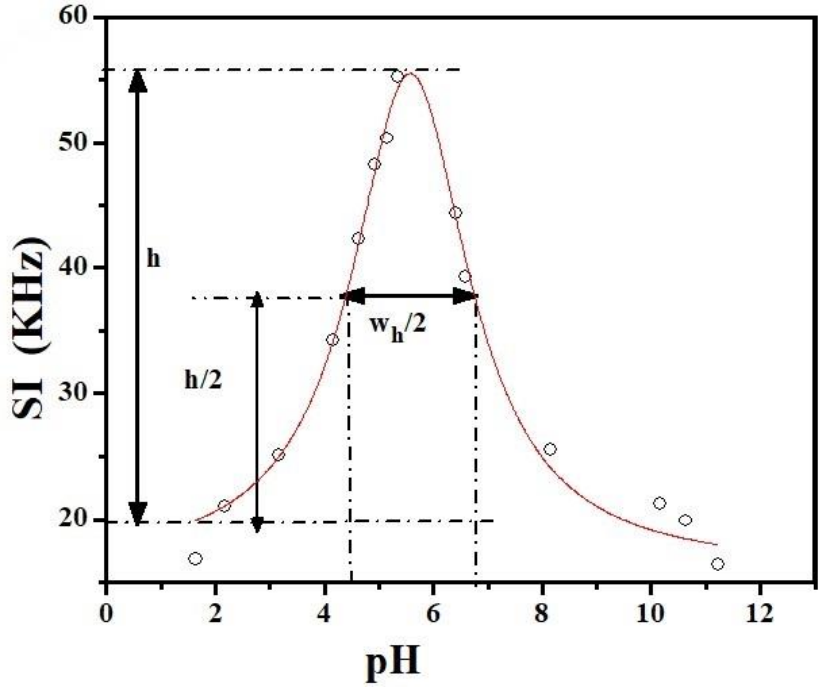


Figure 6. The SI-pH curve of the pelargonic acid-Tween-80 composite vesicles (c = 0.03 mol/L, molar ratio 1:1).

It can be seen from Figure 6 that the peak width at half the peak height is taken as the pH window. After adding Tween-80, the pH window of the vesicles moved to the weakly acidic region, so that the pH window of the pelargonic acid-Tween-80 composite vesicles has expanded from 6.6 to 7.5 to pH 5.5 to 7.5. It can be concluded that the Tween-80 can make the pH window of the vesicles migrate to encompass the biological environment range, and Tween-80 is a green and safe surfactant that is non-irritating to human epidermis and can be used in the food industry. Therefore, we choose the pelargonic acid-Tween-80 composite as the vesicle system, the particle size and light intensity of the vesicles were characterized by DLS, and the morphology of the composite vesicles were characterized by TEM, to verify the rationality and feasibility of the SI-pH method to explore the window of

the composite vesicles.

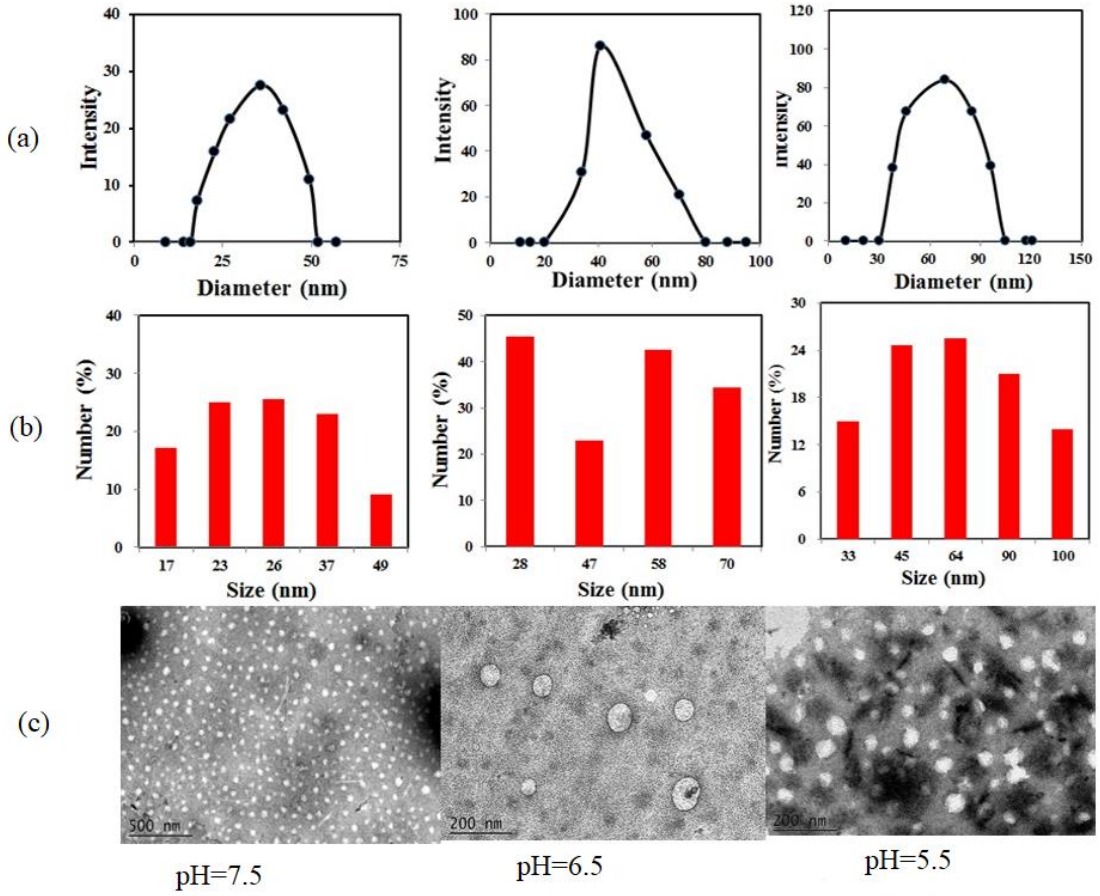


Figure 7. TEM morphology of pelargonic acid-Tween-80 composite vesicles (c=0.03mol/L, molar ratio 1:1) at different pH, TEM morphology and dynamic light dispersion intensity and particle size distribution. (a) is the dynamic light dispersion light intensity of pelargonic acid-Tween-80 composite vesicles; (b) is the particle size distribution of pelargonic acid-Tween-80 composite vesicles; (c) is pelargonic acid-Tween-80 composite vesicles TEM morphology of the bubble).

It can be seen from the TEM images in Figure 7(c) which have carboxylic acid molecules in the pelargonic acid complex vesicle system over the range of pH 5.5 ~ 7.5, with polyethylene oxide (PEO) in Tween-80, and acid ion, that they interact to form regular spherical vesicles, and the vesicles near pH=6.5 have the closest morphology to a sphere. At this time, the stability of the vesicles was relatively high, and after three days of standing, the color of the solution basically did not change. From the DLS light intensity diagram and particle size distribution diagram in Figure 7(a and b), it can also be seen that the particle

size of the pelargonic acid-Tween-80 composite vesicles increases on decreasing the pH. The reason for this is that with the decrease of pH, the basic units that self-assemble to form vesicles in the pelargonic acid complex vesicle system are different, and the different forces between the units cause differences in the particle size of the vesicles. At low pH, most of the pelargonic acid complex vesicle system consists of carboxylic acid molecules. At this time, hydrogen bonds are formed between the carboxylic acid molecules and between the carboxylic acid and the polyethylene oxide (PEO) in Tween-80 to generate intermolecular hydrogen bonds. Such bonding forces the intermolecular arrangement closer, and the vesicle size increases. On increasing the pH, there are more carboxylic acid molecules and acid radical ions in the pelargonic acid composite vesicle system, which then mainly relies on the electrostatic repulsion between the molecules. The hydrogen bonding makes the arrangement of molecules loose, and most of the products are unilamellar vesicles, which reduces the particle size of the vesicles. When the carboxylic acid molecule and the acid radical ion are equivalent, the vesicles formed are more stable, and the particle size of the vesicles is mid-range. According to the report of Walede *et al*. [6], the solution changes uniformly after adding the surfactant, which is of significance for our subsequent experiments.

Therefore, through the data analysis by TEM and DLS, it is shown that the SI-pH method is reasonable and feasible for exploring the composite vesicle window. The addition of Tween-80 not only enables the pelargonic acid complex vesicles to form vesicles in the simulated human intestinal fluid (pH 6.8), but also enhances the stability of the fatty acid vesicles, increasing the possibility of application in living organisms.

3.3 Analysis of the principle of migration and expansion of Tween-80 in the pH window of the vesicles

The pH range of the self-assembled pelargonic acid vesicles is 6.6 ~ 7.5, which is relatively narrow . The self-assembled pelargonic acid vesicles are very dependent on the pH value, which is not conducive to their application in sustained-release drugs. In order to improve this situation, we studied the addition of Tween-80 during the preparation of the fatty acid vesicles and their self-assemble to form the composite vesicles. By changing the

intermolecular forces between vesicles, the pH window of the self-assembled vesicles can be changed.

Since Tween-80 contains 20 polyoxyethylene (PEO) structures, the addition of Tween-80 causes the pH window of the vesicles to migrate in the acidic direction (from pH 6.6 - 7.5 to pH 5.5 - 7.5). These structures can simultaneously form hydrogen bonds with multiple surrounding carboxyl groups [23]. Under acidic conditions, the hydrogen bonding between PEO and pelargonic acid is enhanced. This bonding partly replaces the hydrogen bonding between the original mixed fatty acid or the ionic dipole interaction between the sodium salt. This condition promotes the stability of the vesicle structure and increases the solubility of the pelargonic acid molecules. This allows access to vesicles which are stable over a large pH range and in a low pH environment. These results provide a basis for future application in the sustained release of drugs.

3.4 Evaluation of pelargonic acid-Tween-80 composite vesicles on drug entrapment/sustained release

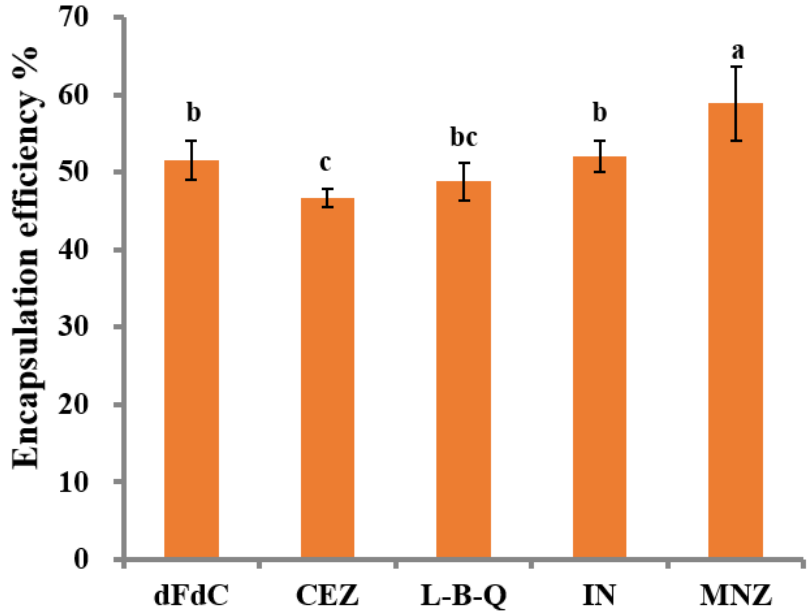


Figure 8. Encapsulation efficiency of composite vesicles with different drugs in pH = 6.8 buffer solution (a, b, bc, c represents the encapsulation efficiency, indicating a>b>bc>c).

Shown in Figure 8 is the histogram for the encapsulation efficiency of the different drugs in buffer solution at pH 6.8 by the composite vesicles; the drugs evaluated were gemcitabine hydrochloride (dFdc), cefazolin sodium (CEZ), niacin (IN), metronidazole

(MNZ) and chlorpromazine hydrochloride (LBQ). The encapsulation efficiency of niacin (IN) and metronidazole (MNZ) by the composite vesicles were 51.63%, 46.70%, 48.84%, 52.06% and 58.90%, respectively. A two-sample t-test was used to analyze the results between groups. The encapsulation rate of metronidazole, gemcitabine hydrochloride/isoniazid, and cefazolin sodium were significantly different, and revealed that the encapsulation result for metronidazole was the best.

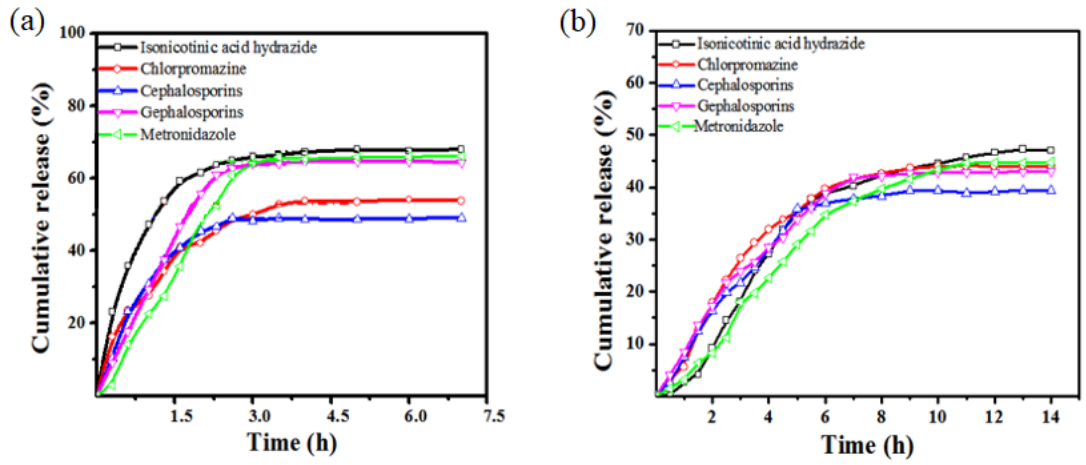


Figure 9. (a) The release diagram of the pure drug; (b) the release diagram after the drug is encapsulated by the vesicle.

It can be seen from Figure 9a that in the simulated artificial intestinal fluid environment, the free drug reaches a constant release rate in about 3 h. Among them, isoniazid has the fastest release rate, with a cumulative release rate of about 65%; cefazolin sodium has the lowest cumulative release rate of about 49%. It can be seen from Figure 9b that when the drugs are encapsulated by pelargonic acid composite vesicles, the release rates of the five heterocyclic drugs are significantly slower, and all drugs can achieve the cumulative release effect of at least 6 hours in the simulated human intestinal environment. Among them, cefazolin sodium is the worst at about 6 hours, and isoniazid has the best effect at about 10 hours. The cumulative release rate of all drugs was above 35%. It shows that through vesicle embedding, all five drugs have achieved a sustained release effect. Among them, isoniazid has the best effect, followed by metronidazole, and cefazolin sodium is the worst. On

comparing the structures of the heterocyclic drug molecules, we infer that the drug release rate may be related to intermolecular forces. The weak van der Waals forces mainly exist between chlorpromazine hydrochloride and cefazolin sodium and vesicle molecules, while gemcitabine hydrochloride, isoniazid and metronidazole all generate strong hydrogen bonding forces with vesicle molecules, and the stronger the interaction force, the slower the release. Comparing the cumulative release results of the five drugs, it was found that the cumulative release rates of isoniazid and metronidazole were higher than those of the other three drugs, and the cumulative release rate of cefazolin sodium was the lowest. We reasoned that the cumulative release results might be related to the size of the drug molecule. The space and release channel for drug encapsulation by pelargonic acid complex vesicles are fixed, while the molecular space structure of cefazolin sodium is the largest, and the cumulative release result is the lowest; the molecular space structure of isoniazid and metronidazole is small, which led to the highest corresponding cumulative release rate. Therefore, we infer that the cumulative release rate is related to the size of the molecular space structure.

3.5. Infrared spectroscopic analysis of the complex vesicles and isoniazid inclusion complex

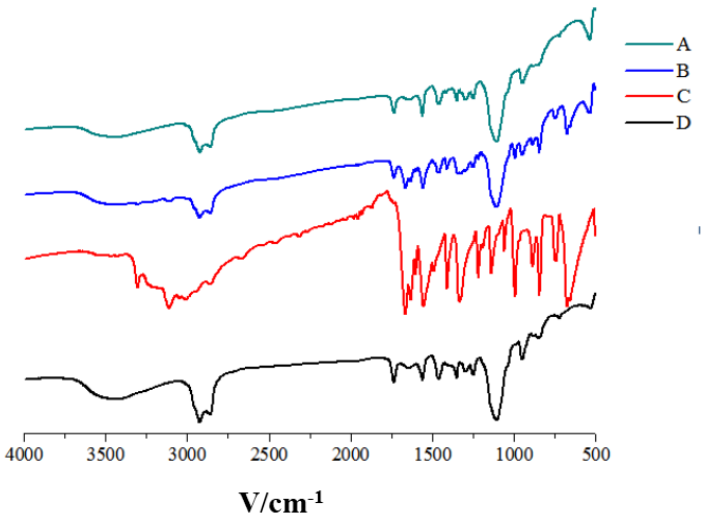
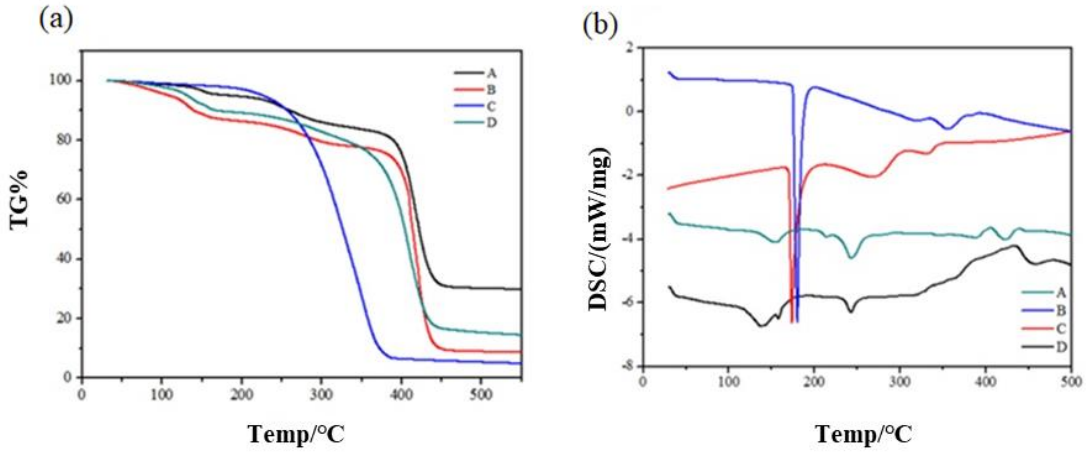


Figure 10. (A) Inclusion compound; (B) physical mixing; (C) isoniazid; (D) and composite vesicle formed by composite vesicle and drug embedding.

As shown in Figure 10, isoniazid has the most obvious absorption peaks at 3111cm^{-1} and 1666cm^{-1}, which are attributed to the υNH bond and υC=O bond of isoniazid [23]. The

454 composite vesicle is made of pelargonic acid. Self-assembled pelargonic acid possesses a
455 stretching vibration for C-O-C of -COOH in the region 1240 ~ 1289 cm^{-1}, and a stretching
456 vibration peak for the -CO bond in the region 1710 ~ 1760 cm^{-1} [24]. On comparing the spectra
457 of the physical mixture, isoniazid and composite vesicles, it can be seen from the figure that
458 the spectrum of the physical mixture is a simple superposition of the pure drug isoniazid and
459 the composite vesicles, indicating that there is no interaction between them. Compared with
460 isoniazid, the absorption peaks of the inclusion compound disappeared at 3111cm^{-1} and
461 1666cm^{-1}, indicating that the drug molecules were coated by the vesicles.
462
463 **3.6 Thermogravimetric analysis diagram of complex vesicles and isoniazid inclusion**
464 **complex**
465



466
467 **Figure 11**. (A) Thermogravimetric spectra and thermograms of complex vesicles; (B) isoniazid; (C)
468 physical mixing; (D) inclusion complexes formed by complex vesicles and drug entrapment (a is a
469 thermogravimetric spectrum, b is a thermogram).
470
471 Figure 11 shows the corresponding TG and DSC graphs of the inclusion compound,
472 isoniazid, physical mixture and pure vesicles. Isoniazid has an obvious endothermic peak at
473 172.82°C. The endothermic peak of the physical mixture of vesicles and isoniazid is
474 consistent with the peak of the single substance. There are three new endothermic peaks in
475 the vesicles and drug inclusion complexes [25], which are 138°C, 242°C and 434°C,
476 respectively. The vesicle shell around the isoniazid began to decompose at 138°C, then the
477 vesicle shell and isoniazid molecules began to decompose at 242°C, and the isoniazid

completely decomposed at 434°C. The decomposition process of the vesicles and isoniazid inclusion compounds is obviously different from their physical mixing, which also provides strong evidence for the successful entrapment of drugs in the vesicles instead of adsorption.

Conclusion

The pH range of vesicles formed by the self-assembly of fatty acids is mostly alkaline, which deviates from the weakly acidic to neutral range of human drugs, which is not conducive to the stable application of fatty acid vesicles in drug release[7,9,26].Pelargonic acid can self-assemble in aqueous solution to form pelargonic acid vesicles with a pH window of 6.6 ~ 7.5. Tween-80 surfactant can be added to the pelargonic acid solution to obtain pelargonic acid-Tween-80 composite vesicles. Compound vesicles can migrate and expand the pH window of vesicles to pH 5.5 ~ 7.5. Adding Tween-80 can reduce the dependence of vesicles on pH, which is more conducive to its application in sustained-release drugs. We use pelargonic acid-Tween-80 composite vesicles for the encapsulation and sustained release of the drugs gemcitabine hydrochloride, cefazolin sodium, chlorpromazine hydrochloride, isoniazid and metronidazole. The results showed that five water-soluble heterocyclic drugs were encapsulated by the pelargonic acid-Tween-80 composite vesicles, and the encapsulation efficiency was above 45%. Put it into a buffer solution of pH 6.8 for sustained release experiment. It was found that pelargonic acid-Tween-80 composite vesicles could be used as carriers to encapsulate five drugs and all had sustained release performance, which can reach the simulated human intestinal fluid environment. After 6-10 hours of release, the cumulative release rate is above 35%, which can fully extend the time for the drug to take effect. This work lays the foundation for further exploration of medium-chain saturated fatty acid vesicles for sustained-release drugs.

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Declaration

The authors declare that there are no competing interests.

Data availability

Data generated or analyzed during this study are provided in full within the published article.

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