

Composite Liquid Marbles as a Macroscopic Model System Representing Shedding of Enveloped Viruses *via* a Budding Mechanism

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Paper contains ESI (10 movies)

Abstract

A model macroscopic system imitating entering of viruses into living cells is suggested. The system represents the contact of a composite (core-shell) liquid marble with hydrophobic/hydrophilic particles. Composite liquid marbles are water droplets coated with silicone oil armored with nanometric hydrophobic particles and served as an interfacial model of a living cell. Composite marbles swallowed hydrophilic polymer particles, but prevented hydrophobic particles from entering their core. Swallowing of hydrophilic particles by composite marbles resembles the budding of living cells by viruses. The interfacial mechanism of swallowing is suggested.

Keywords: bi-liquid composite marbles; fumed fluorosilica particles; oil coating; enveloped viruses; coronavirus; budding.

Viruses are the most abundant biological entities on earth, more than single-celled organisms and bacteria together.¹ They also represent the largest and most genetically diverse reservoir of nucleic acid that can infect all forms of life. Currently, 1.67 million unknown viruses are estimated to be circulating in animal reservoirs, demonstrating the potential for epidemics.¹ Coronavirus belongs to the so-called enveloped viruses.²⁻⁴ Enveloped viruses have a lipid-based membrane surrounding the protein capsid.²⁻⁴ This envelope is partly composed of the cell membrane within which the virus replicated, and it contains proteins and carbohydrates.⁴ Viruses are tiny objects with a characteristic dimension of *ca.* 100 nm.¹ Thus, their experimental

exploration is extremely challenging. In particular, the experimental study of the physics of the process of viral shedding occurring *via* viral budding⁵ is a complicated task.

We suggest a macroscopic system imitating viral budding by living cells, namely we explored swallowing of solid particles by bi-liquid composite marbles. Liquid marbles are non-stick water droplets coated with nano- or micron-sized particles.⁶⁻⁸ Liquid marbles are not hermetically coated by solid particles. The respirability of liquid marbles makes them suitable for the cultivation of microorganisms and cells.⁹⁻¹² Liquid marbles may be used as mini-reactors and bio-reactors.^{9,13-14} Use of liquid marbles for non-traditional computing was reported recently.¹⁵⁻¹⁶ Liquid marbles may be actuated by UV and IR light¹⁷ and Marangoni flows.¹⁸⁻¹⁹ Liquid marbles demonstrate obvious potential for micro-fluidics applications.²⁰⁻²¹ Bi-liquid, core-shell or composite marbles, *i.e.* water marbles coated with a thin layer of oil comprising solid hydrophobic particles, were introduced recently.²²⁻²⁴ The structure of the external interface of a composite liquid marble resembles that of living cell membranes, built of lipid bilayers (including cholesterols) and containing intrusions of globular proteins and fragments of the cytoskeleton, as depicted in **Figure 1 a-b**. In both of the composite marbles and living cells water is enveloped by a hydrophobic layer filled by heterogeneities. We do not exaggerate the physical similarity of the composite marbles and living cells, however we demonstrate that the core-shell marbles enable us to exemplify the budding of enveloped viruses within a macroscopic system. Thus, composite marbles supply an *interfacial model* of a living cell.

We studied the contact of composite marbles with four kinds of solid object of two different millimetrically-scaled sizes, namely: hydrophobic particles, hydrophilic particles,

hydrophobic and hydrophilic particles coated with oil. Three very different scenarios of contact were observed: (i) pristine hydrophobic and oil-coated hydrophobic particles were attached to the surface of the liquid marble, (ii) hydrophilic particles were swallowed by the composite marbles and penetrated its **core**, as occurs when a living cell “absorbs” the enveloped virus; (iii) oil-coated hydrophilic particles accomplished the coating layer of the composite marbles, **becoming** attached to the marble in the nearest vicinity of its surface. All of these scenarios will be addressed below in detail. We pose a following general question: how **do** soft objects filled with water and coated with armored oil layers contact with entities possessing different hydrophilicity?

Composite marbles prepared as shown in **Figure 2** and **Movie S1** and described in detail in the **Method section** were transferred to hydrophobized glass slides. **An** image of the composite marble is **shown** in **Figure 3**. Objects possessing very different wetting properties (in other words various surface energies) were placed in the vicinity of the **composite** marble, as shown in **Figure 4** and pushed gently with a spatula towards the surface of **it**. Let us start **with** the **millimetre-sized** hydrophobic (polypropylene, PP) particles which were attached to the surface of the composite marble, as shown in **Figures 4a, 5a** and **Movie S2**. Let us introduce the spatial scales, inherent for this experimental situation, according to Eq.1:

$$\frac{R}{a} \gg 1; \frac{a}{b} \gg 1, \quad (1)$$

where $R \cong 4.0 \text{ mm}$, $a \cong 0.5 \text{ mm}$ and $b \cong 50 \text{ nm}$ **primary diameter more like 30 nm** are the radii of the marble, PP and fumed fluorosilica particles respectively (see **Figure 1**). Thus,

hydrophobic PP particles are small relative to the marble **but** large relative to the fumed fluosilica particles. For **the** purposes of brevity we label **these** PP particles as “small” ones, and the fumed fluosilica particles as the “colloidal” ones. The same spatial inter-relation generally keeps for “budding” of living cells by viruses, if R , a and b represent the characteristic dimensions of a living cell, virus **particles** and globular proteins **respectively** (see **Figure 1**).

The experimental findings evidence that the small hydrophobic PP particles behave as colloidal ones and do not penetrate into the marble volume, **instead** adhering to the **composite** marble surface. The physics of this attachment was explained in refs. 6-8, when a smooth spherical particle of radius r comes to the liquid surface from air, the energy gain is given by:

$$\Delta G_{air \rightarrow interface} = -\pi r^2 \gamma (1 + \cos \theta_Y)^2$$

(2)

where θ_Y is the Young contact angle inherent to the particle/liquid/air system and γ is the surface tension of the liquid/air interface. It is seen from Eq.2, that the entire system containing the marble and particle lowers its energy by the particle adhering to the interface regardless of the radius of the particle. However, it is thermodynamically unfavorable for the hydrophobic PP particle to enter into the water volume, and it remains attached the composite marble surface as depicted in **Figures 4a, 5a**.

The behavior of hydrophilic (plasma treated) PP particles contacting the composite marble was rather different. The marble swallowed the particle as shown in **Figures 4b, 5b** and **Movie S4, S5**. Hydrophilic particles penetrated into the **core** of the **composite** marble, thus

resembling the behavior of enveloped viruses under their budding of the living cell, as shown in **Figures 4b, 5b** and **Movie S4**. The characteristic time of the “swallowing” was established experimentally as $\tau \cong \tau_{PRITAM}$. The mechanism of the “swallowing” is suggested in **Figure 6**: interface with an area S separates a hydrophilic particle from water. The particle is hydrophilic, thus wetting of this surface is thermodynamically favorable and results in the free energy gain:

$$\Delta\Phi = (\gamma_{s/w} - \gamma_{s/a})S = -\gamma S \cos\theta_Y,$$

(3)

where $\gamma_{s/w}$ and $\gamma_{s/a}$ are the interfacial tensions at the solid/water and at the bare solid/air interfaces, respectively. The free energy gain $\Delta\Phi$ is increased with the increase of the wetted area S . Thus, the force $|\vec{F}| = \left| \frac{\partial\Phi}{\partial r} \right|$ (where r is the radial coordinate of the center of mass of the hydrophilic particle) emerges, pushing the particle into the water core (see **Figure 6**). The force \vec{F} acts until the wetted area is increased, in other words until complete wetting of a hydrophilic particle by water. It seems reasonable to suggest that the similar interfacial mechanism governs entering of viruses into living cells. However, the study of the exact mechanism of virus budding of living cells remains a challenging experimental and theoretical task. It is noteworthy that the study of our model system only sheds light on the interfacial aspect of this mechanism.

In the case of oil-coated hydrophobic and hydrophilic particles the wetting scenario was different. Both of these particle types adjusted themselves to the coating layer of liquid marbles, thus accomplishing wrong word- not sure what you are trying to say? it as demonstrated in

Figure 4c-d and **Movies S6, S7**. It was observed that oil-coated hydrophobic particles are attached to the **composite** marble surface outside of the marble, whereas oil-coated hydrophilic particles are adhered to the composite marble, **keeping** the PP particles connected to the oil layer from inside.

We also studied the contact of large $a \approx 2.5 \text{ mm}$ hydrophobic and plasma hydrophilized PP particles by the composite marble, illustrated in **Figures 6a, b** and **c, d** and the supporting **Movies S8, S9** and **S10**. In this case the spatial scales are given by Eq. 4:

$$\frac{R}{a} \cong 1; \frac{a}{b} \gg 1$$

(4)

The nature of the interfacial processes remained the same irrespective **of** the change in the spatial scales, namely: hydrophobic PP polymer particles did not penetrate into the composite marble **core**, whereas plasma hydrophilized PP particles were swallowed by the composite marble, as shown in **Figure 7**. The characteristic time of the “swallowing” was established experimentally as $\tau \cong ??? \text{ PRITAM}$.

We conclude that the composite (core-shell) marbles swallowed hydrophilic polymer particles, thus supplying the interfacial model of the processes occurring under budding of living cells by viruses. At the same time, the composite marbles prevented hydrophobic particles from entering their **core**. This conclusion is true for particles of various sizes.

Materials and Methods

Materials

The following materials were used in order to prepare the sandwich liquid marbles **make full sentences:**

- microscope glass slides (3 mm × 2 mm);
- poly(dimethylsiloxane) (PDMS) Sylgard 184, supplied by Dow Corning, USA;
- deionized water (DI) from Millipore SAS (France) (specific resistivity $\hat{\rho} = 18.2 \text{ M}\Omega \text{ cm}$ at 25 °C, surface tension $\gamma = 72.9 \text{ mN/m}$; viscosity $\eta = 8.9 \times 10^{-4} \text{ Pa s}$);
- Poly(dimethylsiloxane), bis(hydroxyalkyl) terminated supplied by Sigma-Aldrich (molecular weight 5600 g/mol, surface tension $\gamma = 19.2 \text{ mN/m}$, viscosity $\eta = 0.1 \text{ Pa s}$ (100 cSt), volume magnetic susceptibility $\chi_{\text{oil}} = -7.5 \times 10^{-6}$ **not needed?**, boiling point $449.7 \pm 45.0 \text{ }^\circ\text{C}$ at 760 mmHg);
- fumed fluorosilica particles were synthesized according to the protocol reported in ref. 26; the primary particle diameter is **approx.** 30 nm.

A Nikon 1 v3 camera was used to capture images, movies of composite liquid marbles and the process of swallowing of different particles.

Polypropylene (PP) particles of two diameters (1 mm and 5 mm), supplied by Sigma-Aldrich, were used as hydrophobic particles. The apparent water contact angle of pristine PP was $\theta = ???$ *Pritam*

Methods

Hydrophobic glass substrates were prepared by coating of the cold plasma-treated microscope glass slides with a very thin layer of PDMS oil as described in our earlier work [35]. The slides were coated with silicone oil with a spin coater (Laurell Technologies, WS-650MZ-23NPPB Spin Processor) at 5000 rpm. A 5 μ L water drop was deposited using a micropipette on the oil-coated hydrophobic glass. The spreading parameter describing wetting of the silicone oil/water system is positive (for the detailed analysis of the spreading parameter see the discussion in ref. 25); thus, the silicone oil covers the water drop. Tilting of the glass substrate forced the water drop covered with silicone oil to roll to the Petri dish containing fumed fluorosilica particles, as shown in **Figure 2**. The particles adhere to the drop giving rise to a stable **composite** liquid marble as depicted in **Figure 3**. The process of **composite** liquid marble preparation, carried out under ambient conditions, is illustrated in the supplementary **Movie S1**. The weight ratio of the components of the **composite** marbles, namely water/oil/particles was established by weighing as 1/0.054/ 0.102.

Polypropylene (PP) particles were plasma treated using an inductive radio frequency air plasma discharge with the following parameters: frequency was 13.56 MHz; air pressure was 0.5 Torr; power of the plasma discharge was 18 W; time span of irradiation was 300 s. The apparent water contact angle of plasma treated PP was $\theta = ???$ *Pritam*.

Apparent contact angles were measured with a Rame-Hart goniometer at ambient conditions.

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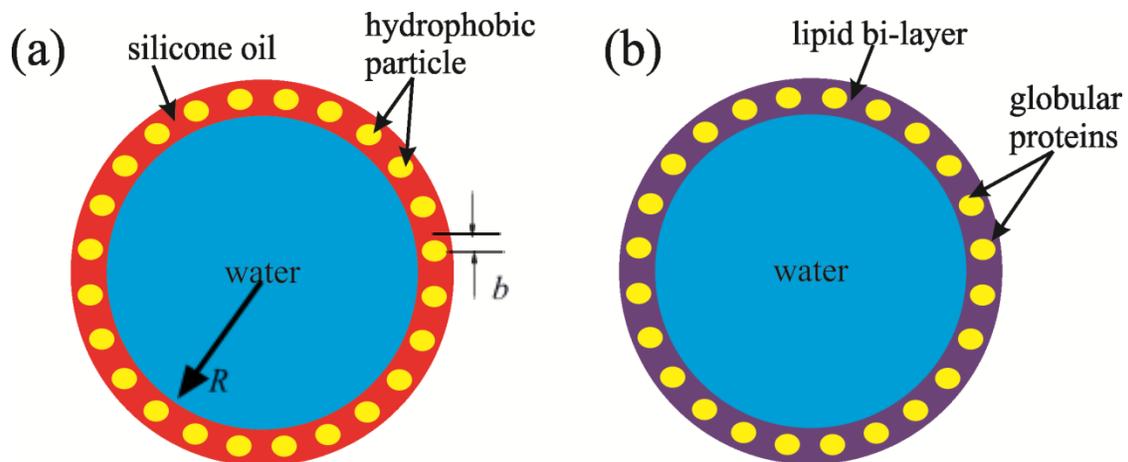


Figure 1. Sketch demonstrating the interfacial resemblance of a composite liquid marble (a) and living cells (b); the radius of the marble $R \cong 4.0 \text{ mm}$; the primary diameter of the hydrophobic fumed fluorosilica particles $b \cong 30 \text{ nm}$.

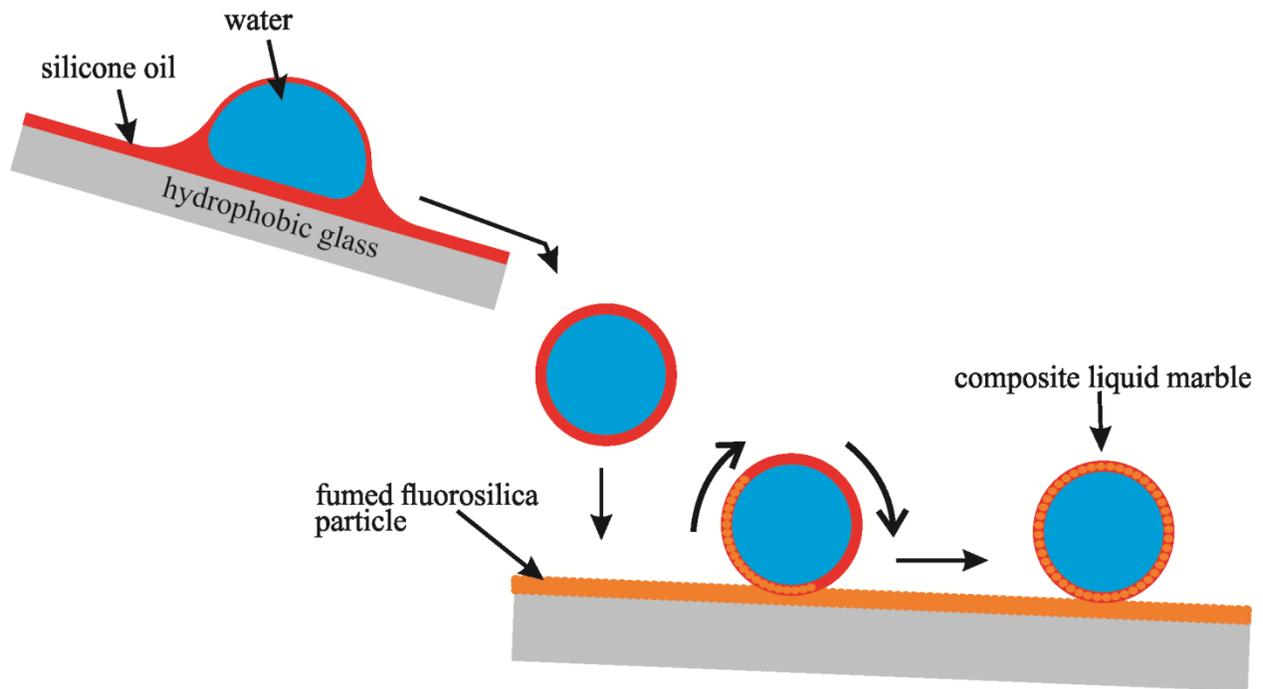


Figure 2. Preparation of a composite liquid marble is shown. A water droplet coated with silicone oil rolls along a hydrophobic substrate and falls onto a solid substrate containing fumed fluorosilica particles. The process results in the formation of a composite (core-shell) marble.

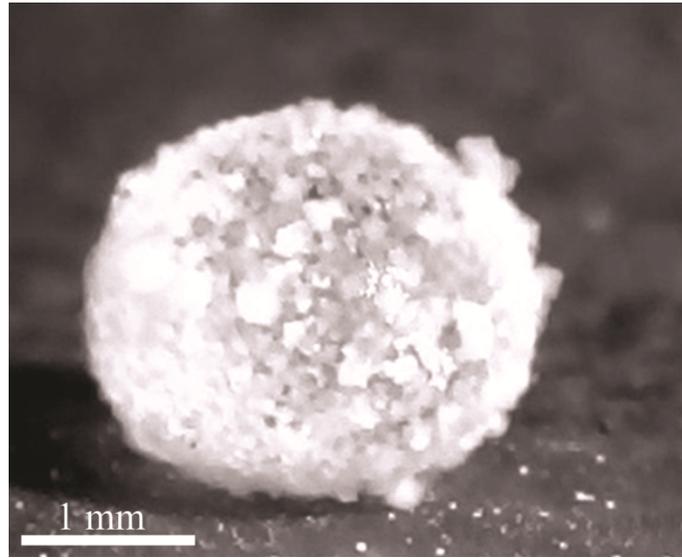


Figure 3. Optical image of a composite liquid marble ($V=???$ Pritam) is presented.

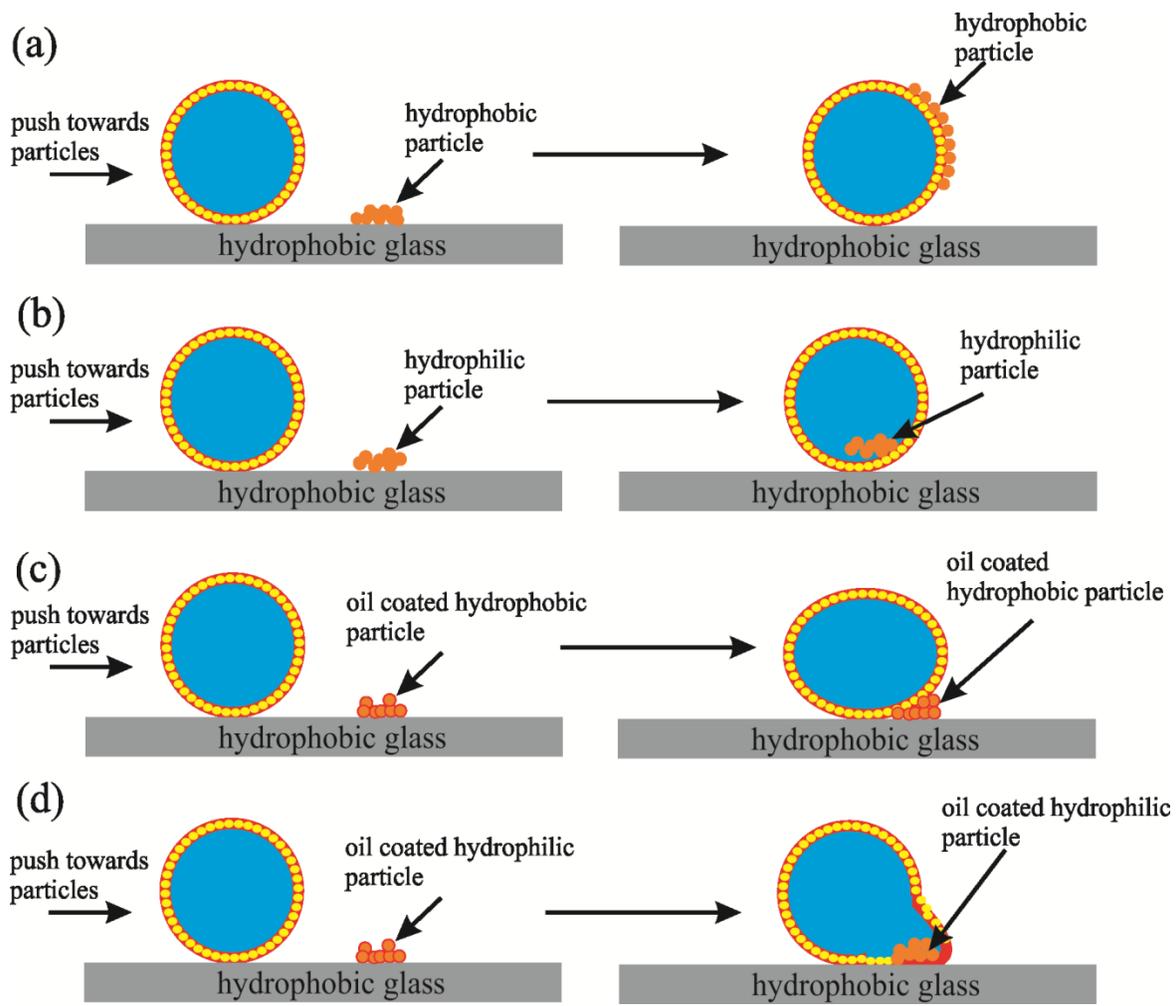


Figure 4. Scheme illustrating the contact between hydrophobic (a), hydrophilic (b), oil-coated hydrophobic (c) and oil-coated hydrophilic (d) particles with a composite liquid marble.

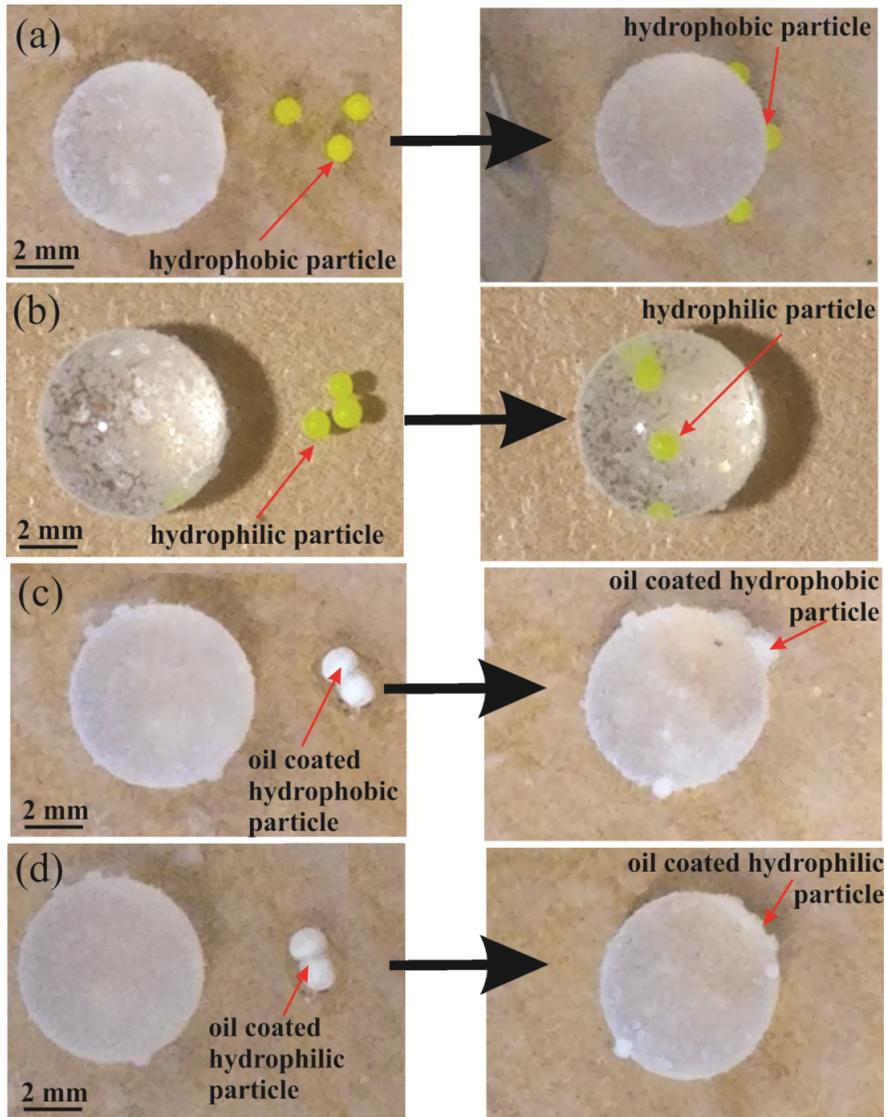


Figure 5. Optical images show contact between hydrophobic (a), hydrophilic (b), oil-coated hydrophobic (c) and oil-coated hydrophilic (d) particles with a composite liquid marble.

in (b) the original marbles appears to have bare patches on its surface: is this why particles enter?
 in (b), how can you prove that particles are actually in the core water? some could argue they are on the surface...

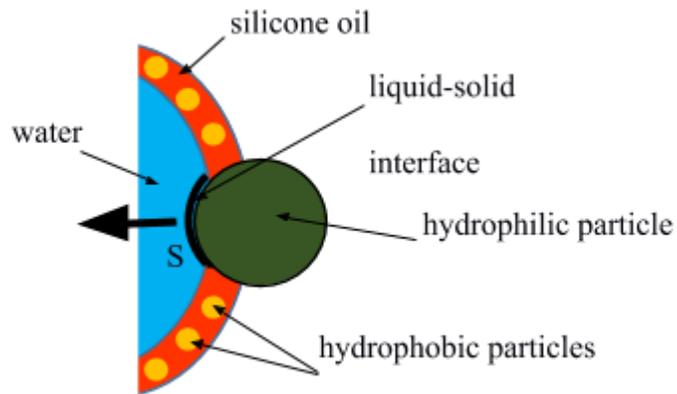


Figure 6. Mechanism of penetration of hydrophilic particles into the bulk of a composite liquid marble. Interface of area S separates a hydrophilic particle from water. Force \vec{F} pushes particle? into the water core.

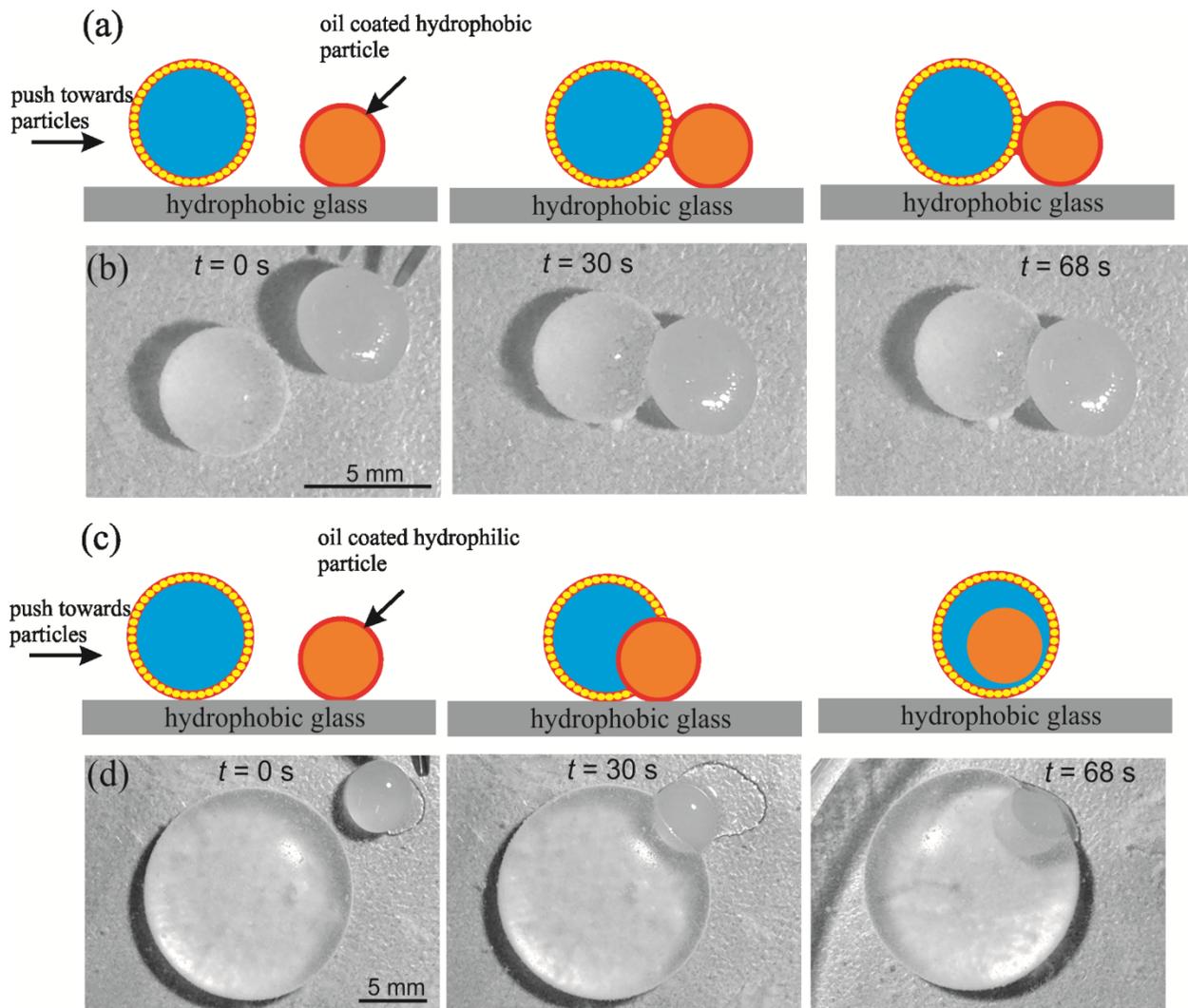
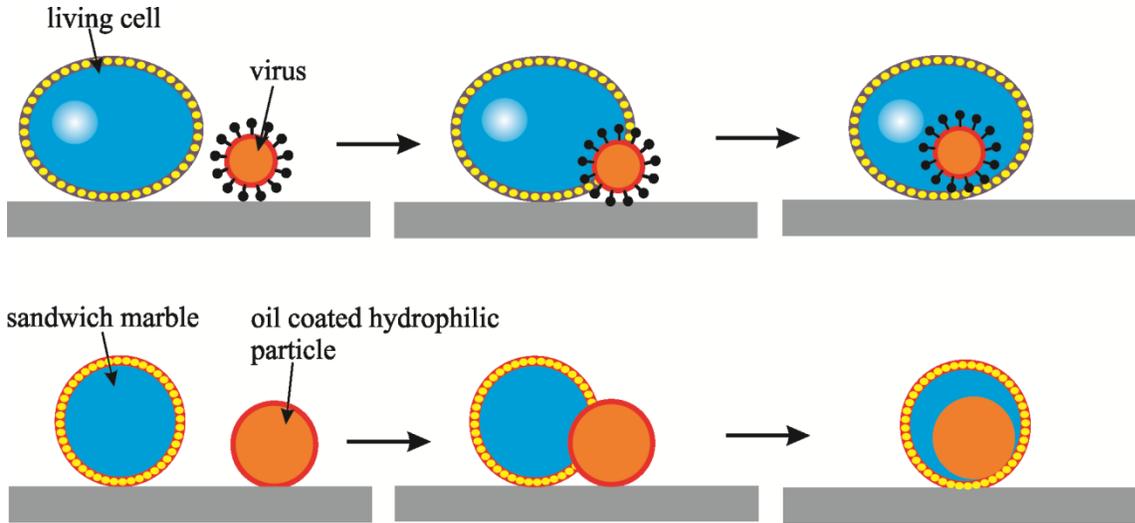


Figure 7. Attachment of oil-coated hydrophobic particle to a composite liquid marble: (a) scheme, (b) sequence of images illustrating particle attachment. Swallowing of oil-coated hydrophilic polystyrene particle by a composite liquid marble: (c) scheme, (d) sequence of images representing the swallowing.

TOC image



change 'sandwich' to composite