Edible Liquid Marbles and Capsules Covered with Lipid Crystals

Yuki Kawamura¹, Hiroyuki Mayama² and Yoshimune Nonomura^{1*}

¹ Department of Biochemical Engineering, Graduate School of Science and Engineering, Yamagata University (4-3-16 Jonan, Yonezawa 992-8510, JAPAN)

² Research Institute for Electronic Science, Hokkaido University (N21W10, Sapporo 001-0021, JAPAN)

Abstract: Liquid marbles are water droplets covered with solid particles. Here we show a method for the preparation of edible liquid marbles and capsules covered with fatty acid crystals and triacylglycerol crystals. We prepared liquid marbles using a simple method; namely, a water droplet was rolled on lipid crystals in petri dishes. The resulting marbles were converted to capsules covered with a lipid shell by heating. These marbles were stable not only on glass surfaces but also on water surfaces because they had rigid hydrophobic exteriors. The lifetime of the liquid marbles on water depended on the alkyl chain length of the lipid molecules and the pH of the water. These findings are useful for applying liquid marbles to food, cosmetic, and medical products.

Key words: Liquid marble, Hydrophobic material, Fatty acid, Triacylglycerol

1 INTRODUCTION

Liquid marbles and dry water are water droplets covered with solid particles such as hydrophobic silica and fluorine resin particles; here, liquid marbles are macroscopic single water droplets, while dry water is a white powder containing water droplets surrounded by solid particles¹⁻⁷. They have received the attention of material scientists because of certain useful applications; for example, dry water have been applied to some cosmetic products^{8,9}. Capsules containing drug compounds and stimulus-responsive liquid marbles were prepared by covering marbles with a solid particles based on a copper substrate or polystyrene latex^{10,11}. Wang et al. reported a gas storage system in which methane molecules were absorbed in the water of the marbles^{12,13}. Bormashenko et al. showed that liquid marbles could be used effectively for the detection of water pollution from oils and petroleum¹⁴. However, there have been few examples of food products or oral formulations consisting of liquid marbles because of the toxicity of these hydrophobic particles; the thermal degradation products and fine particles of polytetrafluoroethylene are toxic and cause acute inflammatory responses in the lung^{15,16}. Although amorphous silica has low toxicity, its use is limited in many countries^{17,18}. If liquid marbles are stabilized with edible solid particles having low toxicity, a facile encapsulation technology for the preparation of food products and

oral formulations becomes possible.

Here, we propose a method for the preparation of liquid marbles covered with fatty acid crystals and triacylglycerol crystals because these lipid crystals are suitable stabilizing agents for edible liquid marbles. The advantages of such crystals are as follows: first, they can be adsorbed on air-liquid interfaces and form stable liquid marbles because they are hydrophobic particles, i.e., the contact angles of water droplets on these lipid crystals are 90°–150°¹⁹. Second, the toxicity of these lipids is low enough to allow their use in food products or oral formulations, e.g., 50% of the lethal dose was 4640 and 22 mg kg⁻¹ when stearic acid was given to rats by oral or intravenous administration, respectively²⁰⁻²². In the present study, we prepared liquid marbles covered with fatty acid crystals and triacylglycerol crystals and checked their stability on glass and water surfaces. Furthermore, we evaluated the effect of aqueous pH on their stability because the ionization state and solubility of fatty acids vary with the pH.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

Five kinds of fatty acids [lauric acid (CH₃(CH₂)₁₀COOH, LUNAC L-98, length of alkyl chain = C12, melting point =

*Correspondence to: Yoshimune Nonomura, Department of Biochemical Engineering, Graduate School of Science and Engineering, Yamagata University, 4-3-16 Jonan, Yonezawa 992-8510, JAPAN
E-mail: nonoy@yz.yamagata-u.ac.jp

Accepted March 28, 2012 (received for review January 31, 2012)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

321–324 K), myristic acid ($\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$, LUNAC MY-98, C14, 330–332 K), palmitic acid ($\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$, LUNAC P-95, C16, 339–340.5 K), stearic acid ($\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$, LUNAC S-98, C18, 346–349 K), behenic acid ($\text{CH}_3(\text{CH}_2)_{20}\text{COOH}$, LUNAC BA, C22, 359–360 K)] were obtained from Kao Corporation. Three kinds of triacylglycerols [trilaurin ($(\text{CH}_3(\text{CH}_2)_{10}\text{COO})_3(\text{CH}_2\text{CHCH}_2)$, 324.5 K), trimyristin ($(\text{CH}_3(\text{CH}_2)_{12}\text{COO})_3(\text{CH}_2\text{CHCH}_2)$, 334–335 K) and tripalmitin ($(\text{CH}_3(\text{CH}_2)_{14}\text{COO})_3(\text{CH}_2\text{CHCH}_2)$, 343.5 K)] were purchased from Tokyo Chemical Industry Co., Ltd. Tristearin ($(\text{CH}_3(\text{CH}_2)_{16}\text{COO})_3(\text{CH}_2\text{CHCH}_2)$, 351.5 K) was purchased from Wako Pure Chemical Industries Co., Ltd.

2.2 Preparation

The procedures for the preparation of liquid marbles and capsules are shown in Fig. 1. Initially, the lipid crystals were ground using an agate mortar and were separated into large particles (particle size = 355–500 μm) and small particles (particle size = less than 180 μm) using sifters. The large particles (1.0 g) and small particles (1.0 g) with amorphous shape were mounted on petri dishes A and B (inside diameter = 3.3 cm), respectively. A droplet of water (5 μL) containing 0.05 wt% rhodamine 6G (reagent grade; Kanto Chemical Co.) was dripped onto petri dish A from a micro-syringe and was rolled with a spatula for several seconds. Rhodamine 6G stained the water phase and facilitated the identification of the mixed states. From the results of preliminary studies, we had determined that rhodamine 6G did not affect the condition of the binary systems. Next the formed droplet was moved to petri dish B and was again rolled with a spatula. To obtain capsules, the liquid marbles were heated at about melting point of the lipid for 30–40 min. In this process, the marbles were introduced into a sealed vehicle with water to maintain a high level of humidity.

2.3 Measurements

We observed the liquid marbles with a microscope (Dino-lite Digital Microscope, ANMO Electronics Co., Ltd, Hsinchu, Taiwan). We checked the stability of the marbles on glass when they were put in the sealed petri dishes with water under a high-humidity environment. Furthermore,

we estimated the lifetimes of the liquid marbles on water surfaces when the marbles were placed onto 5 g of aqueous solutions of hydrochloric acid or sodium hydroxide with pH 2–12. We measured the contact angle using a DM-501 contact angle meter (Kyowa Interface Science, Tokyo, Japan) following the application of 2 μL of a water droplet on the liquid marble surface.

3 RESULTS

3.1 Preparation of liquid marbles and capsules covered with lipid crystals.

We succeeded in preparing liquid marbles using a simple method; namely, a water droplet was rolled on lipid crystals in petri dishes. An image of the liquid marble covered with behenic acid crystals is shown in Fig. 2a. The surface of the water droplet (diameter $\approx$ 3 mm) was covered with both the large and small particles of behenic acid. If the marble was stabilized using only the large particles, it was unstable owing to the presence of vacant spaces between the particles. On the other hand, when the water droplets were covered with only the small particles, these marbles collapsed several tens of minutes after their preparation. The film consisting of small fatty acid crystals was too fragile to hold the marble state against mechanical stimuli or gravity force. In the simple rolling method, all lipid crystals that were examined in the present work formed liquid marbles. The resulting marbles were converted to capsules covered with a lipid shell by heating (Fig. 2b). Systematic tests predicted that near spherical capsules could be obtained when the marbles were preserved at 358 K for 30–40 min. Deformed capsules were obtained by heating at higher temperatures. At temperature extremely higher than the melting point, for example at 368 K for behenic acid, although their capsule structure was maintained, the marbles were deformed by their own weight because the lipid film was melted rapidly. At lower temperature, the evolution from marbles to capsules was not observed.

3.2 Stability of the liquid marbles on glass.

Figure 3 shows the lifetimes of the liquid marbles on

Fig. 1 Method for the preparation of liquid marbles and capsules covered with lipid crystals.

Fig. 2 Microscopic images of a liquid marble (a) and a capsule (b) covered with behenic acid crystals.

Fig. 3 Lifetime of liquid marbles on glass surfaces.

glass. The marbles covered with stearic acid (C18), behenic acid (C22), trimyristin, tripalmitin, and tristearin were maintained for more than 10 days (8.64×10^5 s). The lifetimes of the marbles covered with lauric acid (C12), myristic acid (C14), and trilaurin lasted only several tens of minutes, and at the collapse time, the water content leaked out through the cracks on the surface film consisting of solid particles. These results suggest that the lifetime of the marbles depends on the length of the alkyl chain of the lipid molecules, i.e., the marbles covered with lipids having longer alkyl chains are more stable. The capsules covered with behenic acid (C22) were also maintained for more than 10 days on glass. The mechanical strength of lipid film was kept after heating at 358 K.

3.3 Hydrophobicity and stability on water surfaces.

The hydrophobicity of the marbles depended on the types of lipids covering the water droplets. The marbles covered with lauric acid (C12) and myristic acid (C14) crystals absorbed a droplet of water immediately following the application of 2 μ L of water to their surfaces. In contrast, the marbles covered with other lipids repelled water, which formed a droplet with a $120^\circ - 140^\circ$ contact angle (θ), as shown in Fig. 4a. Relation to their hydrophobicity, floating ability of these hydrophobic marbles and capsules were investigated. First, it was found that the marbles showed stable floating on water with pH 7 (Fig. 4b), while the capsules obtained from the liquid marbles collapsed just after contact with the water.

Such floating ability was observed for the liquid marbles covered with lipids whose alkyl chains were longer than C16, while the marbles covered with lauric (C12) or myristic (C14) acid collapsed just after contact with water. The lifetimes of the marbles covered with palmitic (C16), stearic (C18), and behenic (C22) acid crystals were 2, 67, and 699 s, respectively. These results show that the stability of the liquid marbles on water surfaces also depends on the alkyl chain length of the lipid molecules. Additionally, the stability of the liquid marbles on water depended on the pH of the water (Fig. 5). The lifetime of the marbles covered with behenic acid crystals was over 10 days at pH 2, but it was 16 s at pH 12. The marbles covered with fatty

Fig. 4 Photograph of liquid marbles covered with behenic acid crystals following the application of a water droplet on the marble (a) and the application of the marble on water surface (b).

Fig. 5 Dependences of lifetime of the liquid marbles of lauric (○), myristic (●), palmitic (□), stearic (■), behenic acids (▲), and trilaurin (◇).

acid crystals were more stable at a lower aqueous pH. On the other hand, the stability of the liquid marbles covered with triacylglycerols did not depend on the pH. For example, the lifetime of marbles covered with trilaurin was several tens of seconds, while that of those covered with tristearin was more than 10 days, irrespective of the pH.

4 DISCUSSION

In this study, we present a mechanism for the adsorption of lipid particles on water droplets. In general, solid particles are adsorbed at air/water interfaces when they have a suitable affinity for both fluids^{23–27}. Levine et al. showed that the adsorption energy (F), which is the energy change with adsorption from a fluid phase at an interface, is derived as follows²⁸:

$$F = \pi R^2 \gamma_w (1 - \cos \theta_s)^2 \quad (1)$$

where R is the radius of a solid particle, γ_w is the surface tension of water, and θ_s is the contact angle of the solid particle at the three-phase contact line. For example, the energy F of a behenic acid particle ($R = 250 \mu\text{m}$) was estimated to be $2.76 \times 10^{-8} \text{ J}$ when $\gamma_w = 72.8 \text{ mN/m}$ and $\theta_s = 113^\circ$ ¹⁹. This result suggests that desorption of the adsorbed particle is limited because F of solid particles is much larger than that of surfactant molecules²³.

We further discuss the mechanism of water repellency based on Cassie-Baxter model, which phenomenologically describes the contact angle on heterogeneous surface^{29, 30}. The Cassie-Baxter equation is valid when a liquid drop on a rough surface is much larger than the roughness scale. In the present case, the size of the water droplet on liquid marble was about 10 times larger than that of lipid crystals. We can roughly discuss a possible scenario to change the

contact angle based on the model. The contact angle θ_{CB} is

$$\cos \theta_{CB} = \sum_i f_i \cos \theta_i \quad (2)$$

where the suffix i denotes the material i , f_i and θ_i are the surface fraction and the contact angle on flat surface of material i , respectively. Now, the surface of the liquid marble is binary system. Assuming that the material 1 and 2 are lipid and air, respectively, then

$$\cos \theta_{CB} = f_1 (1 + \cos \theta_1) - 1 \quad (3)$$

where $f_1 + f_2 = 1$ and $\theta_2 = 180^\circ$. Now $\theta_1 = 100 - 120^\circ$ and $\theta_{CB} = 120 - 140^\circ$, therefore, we obtain $f_1 = 0.47 - 0.61$. Roughly speaking, the interface between water and the floating liquid marble is 50% lipid crystal and 50% air in surface fraction.

We further considered the reasons for the variance in the stability of the marbles on water with the length of the alkyl chain and pH of the water, and speculated that the solubility of the lipids is the most important factor. In general, limited amounts of lipids are solubilized in water owing to strong intermolecular interactions between the lipid molecules (i.e., van der Waals interactions and hydrogen bonding). The small amount of solubilized lipid can confer wettability to the lipid particle and induce a reduction in the adsorption energy F and the propensity to collapse. The shorter lifetime of the liquid marbles covered with the lipids having a shorter alkyl chain is caused by their higher solubility. On the other hand, the marbles covered with liquid having longer alkyl chains are more stable due to their lower solubility to water. The dependence of the lifetime on the pH for the marbles covered with fatty acid particles is also substantiated by a similar argument; namely, fatty acids exhibit high solubility in alkaline solutions because of the ionization of their carboxyl groups^{31, 32}. The lifetime of the marbles covered with triacylglycerol particles is independent of the pH because the fatty acid carboxyl groups combine with the hydroxyl groups of glycerol to form esters.

5 CONCLUSION

We prepared liquid marbles covered with fatty acid crystals and triacylglycerol crystals. We examined the stability of these marbles on glass and water surfaces and determined that their stability depended on the alkyl chain length and solubility of the fatty acids and the pH of water (i.e., the fatty acid liquid marble was pH responsive). These findings are useful for the preparation of hydrophobic or pH-sensitive capsules for food or health care products.

ACKNOWLEDGMENT

This research was supported by Grant-in-Aids for Scientific Research on Innovative Area (Nos. 21106504 and 23106704) and Scientific Research (C) (No. 23540473) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT).

References

- 1) Bormashenko, E. Liquid marbles: Properties and applications. *Curr. Opin. Colloid Interface Sci.* **16**, 266-271 (2011).
- 2) McHale, G.; Newton, M. I. Liquid marbles: principles and applications. *Soft Matter* **7**, 5473-5481 (2011).
- 3) Aussillous, P.; Quéré, D. Liquid marbles. *Nature* **411**, 924-927 (2001).
- 4) Binks, B. P.; Murakami, R. Phase inversion of particle-stabilized materials from foams to dry water. *Nat. Mater.* **5**, 865-868 (2006).
- 5) Aussillous, P.; Quéré, D. Properties of liquid marbles. *Proc. R. Soc. A* **462**, 973-999 (2006).
- 6) Nonomura, Y.; Ikeda, T.; Sugimoto, M.; Taniguchi, T. Phase inversion of Pickering foam and dry water stabilized by microbowls. *Chem. Lett.* **40**, 136-137 (2011).
- 7) Yildirim, A.; Budunoglu, H.; Daglar, B.; Deniz, H.; Bayindir, M. One-Pot Preparation of Fluorinated Mesoporous Silica Nanoparticles for Liquid Marble Formation and Superhydrophobic Surfaces. *ACS Appl. Mater. Interfaces* **3**, 1804-1808 (2011).
- 8) Kashimoto, A.; Nonomura, Y.; Kurita, K.; Hotta, H.; Suzuki, T.; Kaneko, Y.; Iitaka, K. Soft press: The ultra-low pressure molding technology for powder cosmetics. *IFSCC Magazine* **7**, 183-187 (2004).
- 9) Igarashi, K.; Sagara, K.; Egawa, J. A new water-in-powder technology: a novel structure for creating unique cosmetic products. *IFSCC Magazine* **11**, 31-34 (2008).
- 10) McEleney, P.; Walker, G. M.; Larmour, I. A.; Bell, S. E. J. Bell, Liquid marble formation using hydrophobic powders. *Chem. Eng. J.* **147**, 373-382 (2009).
- 11) Dupin, D.; Armes, S. P.; Fujii, S. Stimulus-Responsive Liquid Marbles. *J. Am. Chem. Soc.* **131**, 5386-5387 (2009).
- 12) Wang, W.; Bray, C. L.; Adams, D. J.; Cooper, A. I. Methane Storage in Dry Water Gas Hydrates. *J. Am. Chem. Soc.* **130**, 11608-11609 (2008).
- 13) Carter, B. O.; Wang, W.; Adams, D. J.; Cooper, A. I. Gas Storage in "Dry Water" and "Dry Gel" Clathrates. *Langmuir* **26**, 3186-3193 (2010).
- 14) Bormashenko, E.; Musin, A. Revealing of water surface pollution with liquid marbles. *Appl. Surf. Sci.* **255**, 6429-6431 (2009).
- 15) Oberdörster, G. Toxicology of ultrafine particles: in vivo studies. *Phil. Trans. R. Soc. Lond. A* **358**, 2719-2740 (2000).
- 16) Williams, S. J.; Baker, B. B.; Lee, K.-P. Formation of acute pulmonary toxicants following thermal degradation of perfluorinated polymers: Evidence for a critical atmospheric reaction. *Food Chem. Toxicol.* **25**, 177-185 (1987).
- 17) Lewinson, J.; Mayr, W.; Wagner, H. Characterization and toxicological behavior of synthetic amorphous hydrophobic silica. *Regulat. Toxicol. Pharmacol.* **20**, 37-57 (1994).
- 18) Merget, R.; Bauer, T.; Küpper, H.; Philippou, S.; Bauer, H.; Breitstadt, R.; Bruening, T. Health hazards due to the inhalation of amorphous silica. *Arch. Toxicol.* **75**, 625-634 (2002).
- 19) Minami, T.; Mayama, H.; Nakamura, S.; Yokojima, S.; Shen, J. W.; Tsujii, K. Spontaneous Formation of Super Water-Repellent Fractal Surfaces in Mixed Wax Systems. *Soft Matter* **4**, 140-144 (2008).
- 20) Elder, R. L. 13th Report of the cosmetic ingredient review expert panel. Safety assessment of cosmetic ingredients. *J. Am. Coll. Toxicol.* **6**, 321-401 (1987).
- 21) Orö, L.; Wretling, A. Pharmacological Effects of Fatty Acids, Triolein and Cottonseed Oil. *Acta. Pharmacol. Toxicol.* **18**, 141-152 (1961).
- 22) Connor, W. E.; Hork, J. C.; Warner, E. D. Massive thrombosis produced by fatty acid. *J. Clin. Invest.* **42**, 860-866 (1963).
- 23) Binks, B. P. Particles as surfactants-similarities and differences. *Curr. Opin. Colloid Interface Sci.* **7**, 21-41 (2002).
- 24) Nonomura, Y.; Sugawara, T.; Kashimoto, A.; Fukuda, K.; Hotta, H.; Tsujii, K. Self-assembly of surface-active powder at the interfaces of selective liquids 1: Multiple Structural Polymorphism. *Langmuir* **18**, 10163-10167 (2002).
- 25) Nonomura, Y.; Fukuda, K.; Komura, S.; Tsujii, K. Self-assembly of surface-active powder at the interfaces of selective liquids 2: Behavior of the organic crystalline powder. *Langmuir* **19**, 10152-10156 (2003).
- 26) Nonomura, Y.; Komura, S.; Tsujii, K. Adsorption of disk-shaped Janus beads at liquid-liquid interfaces. *Langmuir* **20**, 11821-11823 (2004).
- 27) Nonomura, Y.; Kobayashi, N.; Nakagawa, N. Multiple Pickering emulsions stabilized by microbowls. *Langmuir* **27**, 4457-4562 (2011).
- 28) Levine, S.; Bowen, B. D.; Partridge, S. J. Stabilization of Emulsions by Fine Particles 1. Partitioning of Particles Between Continuous Phase and Oil/Water Interface. *Colloids Surf.* **38**, 325-343 (1989).
- 29) Bormashenko, E.; Bormashenko, Y.; Musin, A.; Barkay, Z. On the Mechanism of Floating and Sliding of Liquid Marbles. *Chem. Phys. Chem.* **10**, 654-656 (2009).
- 30) Bormashenko, E.; Pogreb, R.; Musin, A.; Balter, R.;

- Whyman, G.; Aurbach, D. Interfacial and conductive properties of liquid marbles coated with carbon black. *Powder Technol.* **203**, 529-533 (2010).
- 31) Kanicky, J. R.; Poniatowski, A. F.; Mehta, N. R.; Shah, D. O. Cooperativity among Molecules at Interfaces in Relation to Various Technological Processes: Effect of Chain Length on the pKa of Fatty Acid Salt Solutions. *Langmuir* **16**, 172-177 (2000).
- 32) Kanicky, J. R.; Shah, D. O. Effect of Degree, Type, and Position of Unsaturation on the pKa of Long-Chain Fatty Acids. *J. Colloid Interface Sci.* **256**, 201-207 (2002).
-