Journal of Industrial and Engineering Chemistry xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

journal homepage: www.elsevier.com/locate/jiec

Formation of stable adhesive water-in-oil emulsions using a phospholipid and cosurfactants

Hyunjun Kim[a,1], KyuHan Kim[b,1], Hyun-Ro Lee[b], HyeongChan Jo[c], Dae-woong Jeong[c], Jiheun Ryu[a], Dae-Gab Gweon[a,*], Siyoung Q. Choi[b,*]

[a] Department of Mechanical Engineering, KAIST, Daejeon 305-701, Republic of Korea
[b] Department of Chemical and Biomolecular engineering, KAIST, Daejeon 305-701, Republic of Korea
[c] Department of Bio and Brain Engineering, KAIST, Daejeon 305-701, Republic of Korea

ARTICLE INFO

Article history:
Received 23 March 2017
Received in revised form 19 June 2017
Accepted 25 June 2017
Available online xxx

Keywords:
Water-in-oil emulsion stability
Phospholipid emulsion
Adhesive emulsion
Lipid bilayer
DHA
SPAN 80

ABSTRACT

The use of adhesive water-in-oil (W/O) emulsions covered with phospholipids have been limited due to their poor stability. We suggest a new and simple method to create adhesive W/O emulsions stabilized by 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) with two different cosurfactants (docosahexaenoic acid (DHA) and sorbitan oleate (SPAN 80)). Although an adhesive W/O emulsion with DOPC is typically unstable because of its molecular structure, we demonstrate that the addition of cosurfactants whose molecular shapes could be complementary to that of DOPC far better stabilizes W/O emulsions and indeed leads to the production of adhesive emulsions by the formation of a bilayer between two monolayers of each droplet surface.

Introduction

Emulsions systems where a disperse liquid phase is distributed in a continuous liquid phase [1], have been used in numerous applications such as pharmaceuticals [2,3], food [4–7], and the cosmetic industry [8]. Despite their importance, emulsions are inherently unstable due to the large energy penalty by the newly created surfaces, but, they can be stabilized kinetically, for example, by adding a small amount of surfactant to either phase of the fluid. The added surfactant spontaneously covers a newly created surface, playing two distinct roles: it reduces permeability, thus preventing Oswald ripening, and it provides an energy barrier that prevents coalescence of droplets, either by steric hindrance or electrostatic repulsion [1,9,10]. The HLB (hydrophilic–lipophilic balance) of a surfactant could determine approximately whether it stabilizes oil-in-water (O/W) or water-in-oil (W/O) emulsions [11]. A surfactant with a small HLB number, which is soluble in oil, could stabilize W/O emulsions while a large HLB number, which is soluble in water, could create stable O/W emulsions [12].

Among various species of surfactants for covering emulsion droplets, phospholipids could be beneficial over simple surfactants because of their good biocompatibility [13–18] and many biological applications, such as excellent model systems of lung surfactants [19,20], lipid droplets [21,22], and even cell membranes [23,24]. Especially, the successful formation of adhesive phospholipid emulsions would be highly required for establishing the artificial protocell systems to understand biological functions, such as cell division [25], uptake of small molecules [26], and protein synthesis [27], as well as for constructing complex electronic devices whose signals could be transferred across the phospholipid layers [28–30].

However, frequently used phospholipids, such as 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) etc., could not act as a stabilizer themselves due to their molecular shape whose HLB value is moderate, ~7 [31,32], thereby resulting in the poor stability of both W/O and O/W emulsions. Nevertheless, there have been consistent efforts to achieve stable adhesive emulsions containing phospholipids, and finally, this could be possible by forming stable droplet bilayers. This droplet bilayer has been developed using several different

* Corresponding authors.
E-mail addresses: dggweon@kaist.ac.kr (D.-G. Gweon), sqchoi@kaist.ac.kr, yssmnz@gmail.com (S.Q. Choi).
[1] These authors are equally contributed.

http://dx.doi.org/10.1016/j.jiec.2017.06.046
1226-086X/

strategies. For example, dispersed water droplets in an oil phase that contain phospholipids could be attached to each other by changing the solvent quality of a continuous phase [29,33,34] or by using an appropriate species of phospholipid [28,30] or using a hydrogel support [35,36], resulting in the formation of bilayers from two monolayers of each droplet surface. Furthermore, a network of droplet bilayers has been also developed to achieve a simple system for electronic signal transport [28–30]. These emulsion bilayers could be stable for a long time based on specialized methods and equipment, but this still requires the development of a much simpler way to achieve emulsion bilayers with good stability.

In this article, we report a simple and novel method to achieve a stable *adhesive* water-in-oil emulsion with the formation of bilayers between the monolayers at the surface of dispersed droplets. Here, our main route to achieve this goal is to add another surfactant, called a cosurfactant [37,38]. This cosurfactant typically plays a role in producing better packed surfactant layers to create more stable emulsions of desired kinds, although there might be a variety of interactions between the newly added cosurfactant and existing components. In our system, we use two different cosurfactants (docosahexaenoic acid (DHA) and sorbitan oleate (SPAN80)) whose molecular shapes could be complementary to DOPC [38], and we investigate the effect of these cosurfactants on the stability of a water-in-squalene emulsion containing DOPC and the formation of bilayers between dispersed droplets. To assess the stability of emulsions with varying concentrations of DHA/SPAN80, we have used two different methods: (i) observation of the macro-/micro-structures of emulsions that are vigorously emulsified for 24 h, and (ii) a single drop experiment, where a single drop is covered with surfactants on the planar interface in which surfactants are laden to only consider the coalescence process, ruling out sedimentation instability. Furthermore, we also measure the interfacial tension between water and oil that dissolve the mixture of the phospholipid/cosurfactant, and correlate this to the stabilization mechanism.

Experimental

Materials

1,2-Dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) was purchased from Avanti Polar Lipids. Docosahexaenoic acid (DHA), sorbitan oleate (SPAN 80), and squalene were purchased from Sigma–Aldrich. All other chemicals were of analytical grade and used without further purification.

Emulsion preparation and observation

Prior to emulsification of the squalene–water mixture, a squalene solution containing DOPC and SPAN 80/DHA was prepared. DOPC and SPAN 80/DHA, which were initially dissolved in chloroform. were dried, and then dissolved by 0.75 ml of squalene. In this case, the concentration of DOPC and SPAN 80/DHA in squalene was varied from 0 mg/ml to 30 mg/ml.

The squalene solution of DOPC (0.75 ml) and Span 80/DHA (0.75 ml) was mixed first, and this mixture (1.5 ml) was then emulsified with an aqueous solution (0.5 ml). In each case, the final mixture of squalene and water was homogenized by a vigorous vortexing (Vortex mixer, DAIHAN Scientific), and after 24 h, the remaining emulsion was observed macroscopically by a camera (Canon EOS-100d) on a steady table at room temperature. Microscopic images of water droplets in the emulsion were also observed by optical microscopy (Upright type, Olympus). These macroscopic and microscopic images were used for determining the emulsion stability during a day time scale, as shown in Figs. 1 and 2.

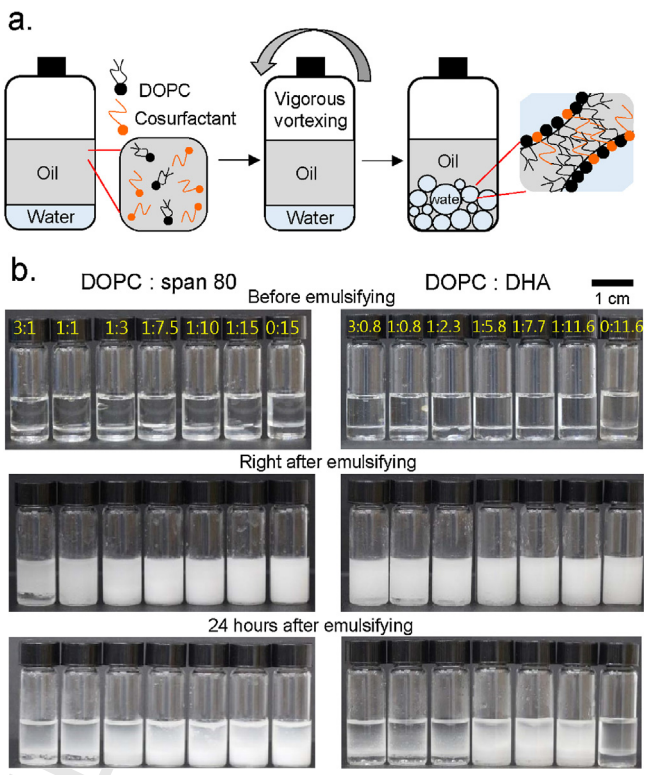

Fig. 1. Formation of water-in-oil emulsions and their macroscopic images. (a) Simple schematic describing the emulsification process of adhesive water-in-oil emulsions using a vigorous vortexing method. (b) Macroscopic images of emulsions that contain various concentrations of DOPC and cosurfactant in squalene. (e.g. 3 mg/ml of DOPC and 1 mg/ml (0.8 mg/ml) of Span 80 (DHA), 1 mg/ml of DOPC and 1 mg/ml (0.8 mg/ml) of Span 80 (DHA), 1 mg/ml of DOPC and 3 mg/ml (2.3 mg/ml) of Span 80 (DHA), 1 mg/ml of DOPC and 7.5 mg/ml (5.8 mg/ml) of Span 80 (DHA), 1 mg/ml of DOPC and 10 mg/ml (7.7 mg/ml) of Span 80 (DHA), 1 mg/ml of DOPC and 15 mg/ml (11.6 mg/ml) of Span 80 (DHA), 0 mg/ml of DOPC and 15 mg/ml (11.6 mg/ml) of Span 80 (DHA), respectively). Here, a slightly lower concentration of DHA solution has been used to match the number density of DHA molecules to that of Span 80. Left: DOPC and span 80, Right: DOPC and DHA. Macroscopic images are taken at three different stages, before emulsifying (top), right after emulsifying (middle), and 24 h after emulsifying (bottom), respectively.

Single drop stability test on the planar oil/water interface

A squalene solution with the same composition as that used in the emulsion formation was also used in the single drop stability test. First, 1 ml of the solution was carefully transferred to quartz cuvettes (Sigma–Aldrich, 3.5 ml) that were initially filled with 1.7 ml of distilled water. The cuvettes were then left for 5 min to stabilize the flat squalene/water interface.

To place a water drop on the flat squalene/water interface, a microsyringe (Hamilton, 710SNR, 100 μl) filled with distilled water was used. It was first brought into the squalene phase that contains phospholipids and cosurfactants, and a water droplet was then formed at the end of the syringe. This droplet was stabilized for 5 min, and was brought to the flat squalene/water interface. To visualize the adhesion process, 0.5 μg/ml of Texas-red DHPE (Invitrogen) was contained in the squalene. All of these events were observed and recorded through an inverted-typed optical microscope (Olympus), and the resting time of the drop before coalescing with the underlying phase was analyzed afterwards, based on the recorded video.

Please cite this article in press as: H. Kim, et al., Formation of stable adhesive water-in-oil emulsions using a phospholipid and cosurfactants, J. Ind. Eng. Chem. (2017), http://dx.doi.org/10.1016/j.jiec.2017.06.046