Available online at www.sciencedirect.com
ScienceDirect
Colloids and Surfaces A: Physicochem. Eng. Aspects 297 (2007) 211–220
COLLOIDS AND SURFACES A
www.elsevier.com/locate/colsurfa

O/W/O double emulsions stabilized with WPI–polysaccharide conjugates

Axel Benichou [1], Abraham Aserin, Nissim Garti *

Casali Institute of Applied Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Received 19 June 2006; received in revised form 19 October 2006; accepted 21 October 2006
Available online 26 October 2006

Abstract

Protein/polysaccharide conjugates were used to stabilize oil-in-water (O/W) emulsions and oil-in-water-in-oil (O/W/O) double emulsions. By properly selecting the type of protein (WPI) and the polysaccharide (xanthan gum, fenugreek gum), and by using specific ratios of the two biopolymers and their solution concentrations, amphiphilic biopolymer adducts were formed.

A synergism in the emulsification properties was observed in WPI/polysaccharide conjugates compared to each of the biopolymers alone.

Submicron droplets of oil-in-water were obtained by applying a high-pressure homogenization process during the first step of the double emulsion preparation. It was also demonstrated that double-emulsion globules could be formed with a very high yield of addendum entrapment (above 95%) during the second step of the emulsification process.

The differentiation between the two types of oils, O_1 (the inner) and O_2 (the outer), in the double emulsions enabled high entrapment capacity of the addendum in the inner oil phase. In addition, when the inner oil phase (O_1) was a 1:1 (w/w) mixture of MCT/triacetin and the external oil phase (O_2) was a silicone oil, it was possible to slow the release of the entrapped matter while the solubility of the inner phase in the external oil phase remained constant. The addendum solubility in the external oil phase was not a limiting factor in the release process. The presence of hydrophobic additives (i.e., 1 wt.% glycerol monooleate) in the inner oil phase helped to better control the transport to the external oil phase. In the best case, the addendum leakage to the external oil phase was only *ca.* 0.2 wt.% during a period of 28 days at 25 °C.

WPI/xanthan gum adducts served as thick and efficient barriers against release of flumethrin® (a veterinary drug model) entrapped in the core of the O/W/O multiple globules.

Keywords: O/W/O double emulsions; Xanthan gum; Fenugreek gum; Whey protein isolate (WPI)/polysaccharide hybrids; Sustained release

1. Introduction

Double emulsions, also known as emulsions of emulsions, consist of droplets of one dispersed liquid that is further dispersed in another liquid. The inner dispersed globule/droplet in the double emulsion is separated (compartmentalized) from the outer liquid phase by a layer of a third phase [1–3]. The internal droplets can serve as an entrapping reservoir for active ingredients that can be released by a controlled transport mechanism, thus conferring significant potential in many applications to these multi-compartment liquid dispersions. In practice, double emulsions consist of large and polydispersed droplets that are thermodynamically unstable with a strong tendency for coalescence, flocculation, and creaming. The most common double emulsions are water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) types. Oil-in-water-in-oil double emulsions have been less extensively studied. However, some applications for O/W/O double emulsions in food [4–7], cosmetic [8–12], or drug delivery applications [11,13–15] have been mentioned. Modulated release of triterpenic compounds from an O/W/O double emulsion formulated with dimethicones studied with infrared spectrophotometric and differential calorimetric approaches is one of these examples [10]. The effects of different dimethicones incorporated within double emulsions were studied through *in vitro* penetration. The incorporation of silicones within O/W/O double emulsions seems to be an efficient means of modulating the penetration and distribution of drugs in the skin.

In another study, the stability of retinol (Vitamin A alcohol) in three different emulsions was compared: oil-in-water (O/W), water-in-oil (W/O), and oil-in-water-in-oil (O/W/O) [8];

* Corresponding author. Tel.: +972 2 658 6574/5; fax: +972 2 652 0262.
E-mail address: garti@vms.huji.ac.il (N. Garti).
[1] The results presented in this paper were included in A. Benichou's doctoral dissertation in Applied Chemistry at The Hebrew University of Jerusalem, Israel.

0927-7757/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfa.2006.10.048

stability in the O/W/O emulsion was the highest among the three types of emulsions.

Orange oil-in-water emulsions were encapsulated in another oil phase to form a double emulsion having orange oil inside its inner compartment [9]. Although the yield was only 44.5%, it is a promising area for further research in preventing air oxidation of the oil. Spray drying of the double emulsion can provide a secondary coating to secure maximum protection of orange oil and to afford a free-flowing flavored powder. This method may have potential applications in various food or pharmaceutical products where maximum protection against air oxidation is required. A secondary coating was applied to flavor oil already encapsulated in a double emulsion. Spray drying technique includes spraying a flavor emulsion into a stream of hot air; the water phase evaporates rapidly leaving the flavor material locked in the carrier. Most of the O/W/O double emulsions have found applications in the food industry and have been patented [4–7]. Generally, the differentiation between a liquid inner oil phase and a liquid outer oil phase permits the encapsulation of food additives. This method can also provide enhanced protection of the entrapped active matter against oxidation [8] or flavor release [16].

Double emulsions are usually not empty. Soluble active materials are entrapped during the emulsification in the inner oily phase. Because of the osmotic pressure gradient, the active matter tends to diffuse and migrate from the internal phase to the external interface [5,17]. The dilemma that researchers faced, was how to control the diffusion of oil molecules, as well as the emulsifier molecules and the active matter, from the internal phase to the outer phase [18]. It seemed almost impossible to retain the active material within the inner phase upon prolonged storage, mainly because the monomeric emulsifiers tend to migrate from both the inner interface and the outer interface to the intermediate layer where they aggregate to form micelles capable of solubilizing the entrapped active matter.

During years of investigation to improve stability and to control sustained and prolonged release of active materials, monomeric surfactants have been progressively replaced by polymeric emulsifiers [3,19–21]. Polymeric amphiphilic molecules, both synthetically tailor-made and naturally occurring ones, are known to improve the interfacial coverage during emulsification [20–22] and so insure better encapsulation and controlled release of the addenda entrapped in the internal core of the double emulsion droplets [23].

The use of macromolecular amphiphiles and stabilizers, such as proteins and polysaccharides, has long been adopted by scientists exploring the stability of W/O/W double emulsions [20–28]. Whey proteins [22–24], bovine serum albumin (BSA) [24], cyclodextrins [14,15], pectin [24] and chitosan [25] were mentioned and evaluated. The proteins were usually used in combination with other monomeric emulsifiers. A significant improvement in the stability of the emulsions was shown when these macromolecules were encapsulated onto the external interface. In most cases, the macromolecule was used in low concentrations (max. 0.2 wt.%) and in combination with a large excess of non-ionic monomeric emulsifiers. Furthermore, from the release curves, it seems that the marker transport is more controlled. Dickinson and co-workers [22,23,27,28] concluded that proteins or other macromolecular stabilizers are unlikely to completely replace lipophilic monomeric emulsifiers in double emulsions. However, proteins in combination with stabilizers do have the capacity to confer some enhanced degree of stability on a double emulsion system and, therefore, the lipophilic emulsifier concentration is substantially reduced.

Recently, in our lab [20,21] WPI/polysaccharide conjugates were envisaged to stabilize the outer interface of W/O/W double emulsions and found significant improvement both in the stability and in the release of markers (glucose, Vitamin B_1) compared to the use of the protein in the external phase only. These new amphiphilic adducts serve as good steric stabilizers, improve stability and shelf-life, and slow the release of the markers. They therefore play a double role in the emulsions: film formation and barrier to the release of small molecules at the internal interface, and steric stabilizers of the inner oil–water interface.

From all the scattered information that is available in the literature, we learned that the best entrapment capacities, along with best stabilities of the globules and the best retention of the addenda in the inner phase, can be achieved if the oil in the inner phase differs from that of the outer phase and the surfactants are strongly anchored to the interface; the polymeric adducts are therefore excellent candidates for such requirements.

To the best of our knowledge, it is the first time that biopolymeric conjugates of protein and polysaccharide have been used in stabilizing O/W/O double emulsions. These hybrids form new structures in aqueous solutions that have specific molecular hydrophobic or electrostatic charge interactions [20,21,29]. Such molecular adducts (hybrids or conjugates) will stabilize the double emulsions, serving as thick and efficient barriers against release of the addendum entrapped in the core of the double emulsion droplets.

2. Materials and methods

Medium chain fatty acid triglycerides (MCT, C8–C10) and Neobee M5 were purchased from Stepan, Anaheim, CA, USA. Linear alkanes, decane, dodecane, tetradecane, are from Sigma, St. Louis, MO, USA. Silicone oils, decamethylcyclopentasiloxane, SF 1202 (99%), viscosity 4 cP at 25 °C, is from GE Bayer Silicones, Leverkusen, Germany. ST-cyclomethicone 5-NF decamethylcyclopentasiloxane (95%), viscosity 4 cP at 25 °C, Q7-9120 20 Cs and Q7-9120 100 Cs polydimethylsiloxane mixtures with viscosities of 20 and 100 cP, respectively, at 25 °C, are from Dow Corning, Midland, MI, USA. Triacetin (USP grade) 1,2,3-propanetriol-triacetate (3Ac) is from Aldrich, Milwaukee, WI, USA.

Abil EM90: polyether polysiloxane block copolymer and Abil EM97: α,ω-polyether siloxane are from Goldschmidt, Essen, Germany. Distilled monoglyceride oleate (GMO, 90% monooleate), Dimodan MO-90, was purchased from Danisco, Brabrand, Denmark. Dow Corning 3225C: octamethylcyclotetrasiloxane (66%) decamethylcyclopentasiloxane (20%) block copolymer, and Dow Corning 5225C: decamethylcyclopentasiloxane (86%) are from Dow Corning, Seneffe, Belgium.