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Macro- vs. micromolecular stabilisation of W/O/W-emulsions

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ABSTRACT

Water-in-Oil-in-Water (W/O/W) double emulsions (DEs) were prepared with high molecular weight gelatin as hydrophilic emulsifier and gelling agent of the external water phase and their stability was compared to DEs prepared with the hydrophilic emulsifier polysorbate 80. The Water-in-Oil (W/O)-emulsions were prepared with corn oil and polyglycerol polyricinoleate (PGPR) as the lipophilic emulsifier. Tartrazine, a synthetic dye, was added to the inner water phase and the encapsulation efficiency of the marker was measured. A gelled DE was successfully obtained and its rheological properties were possible to adjust without affecting the DE stability to a large degree. During a 90-days stability study, DEs stabilised by gelatin showed a high initial encapsulation yield (98.5–96.5%), with no further release of tartrazine measured after the first day.

Release of tartrazine was observed from the DEs prepared with polysorbate 80 during the first days after preparation, whereafter no further release was observed for the remaining 90 days period. Contrary to the DEs stabilised by gelatin, the encapsulation efficiency of tartrazine was highly dependent on the concentration of PGPR. By addition of a counter solute (NaCl) into the external water phase, osmotically adjusted DEs (Δ −0.14, −0.07, 0.00 and 0.14 Osm) were prepared for the polysorbate 80 stabilised W/O/W-emulsion. Higher encapsulation yields were obtained with increasing amounts of NaCl, with the highest amount of tartrazine retained for the hyperosmotic DE (Δ 0.14 Osm) formulation.

1. Introduction

A Water-in-Oil-in-Water (W/O/W) emulsion is a type of double emulsion consisting of water droplets dispersed into oil droplets. The presence of an inner reservoir separated from a continuous phase by an immiscible liquid enables many potential applications. Within drug delivery, W/O/W-emulsions have been tested for controlled and prolonged release of active ingredients (Higashi & Setoguchi, 2000; Okochi & Nakano, 2000). Additionally, they have also been investigated for usage within drug overdose treatment (Hamoudeh et al., 2006), biochemical separation (Thien & Hatton, 1988), enzyme immobilisation (Scheper et al., 1989), cosmetics (Raynal, Grossiord, Seiller, & Clausse, 1993) and foods (Frasch-Melnik, 2011). Even with many potential applications, to our knowledge, no products based on this technology have reached the market. This is mainly due to destabilisation of the W/O/W-emulsion with time, resulting in release of the active ingredient (inner solute) and/or physical breakdown of the double emulsion structure. An applicable double emulsion should ideally have a high intrinsic stability, which after application causes desired release or uptake of active ingredient.

Different studies have tried to elucidate the mechanisms of permeation of solute and solvent. Five main mechanisms of permeation have been proposed. 1) Transport by reverse micelles (Matsumoto, Kita, & Yonezawa, 1976), 2) formation of transient pores with following release (Paula, Volkov, VanHoek, Haines, & Deamer, 1996; Pays, Giermanska-Kahn, Pouligny, Bibette, & Leal-Calderon, 2002), 3) coalescence between the internal and external water phase, 4) transport of water by hydrated surfactants (Thien & Hatton, 1988) and 5) permeation by dissolution of the active ingredient in the oil phase with following diffusion (Matsumoto et al., 1976). It seems likely that different mechanisms of release may occur simultaneously and vary depending on type and concentration of emulsifiers, oil and inner solute. Permeation occurring by either of these mechanisms should be possible to overcome by the use of optimised stabilising agents. Macromolecular emulsifiers have shown promising results, with extensive focus on biopolymers because of their inherent biocompatibility (Dickinson, 2011). Contrary to low molecular weight surfactants,

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biopolymeric emulsifiers are not known to be able to form reverse micelles. They are also less dynamic and may thereby potentially reduce the formation of transient pores. Since transient pores can lead to coalescence between the inner water droplets and external water phase, such degradation may hence be reduced. Finally, biopolymeric emulsifiers are not soluble in the oil phase, i.e. they are not able to mediate water transport by diffusion between the external and internal water phase.

In addition to using surface active macromolecules as hydrophilic emulsifiers, polymers may also be used as a texture modifier of the external water phase. This may reduce coalescence between the oil globules, eliminate gravitational separation and also give a convenient delivery system for solid dosage form double emulsions intended for oral administration (Haug et al., 2011; Seternes, Draget, & Haug, 2010). A gelled double emulsion has earlier been prepared by introducing a combination of alginate and Ca^{2+} into the outer water phase. A slower release was measured from the gelled double emulsion compared to release of inner marker from an alginate gel (Weiss, Scherze, & Muschiolik, 2005). Addition of different polysaccharides (scleroglucan, carrageenan and a mixture of xanthan and locust bean gum) into the external water phase of a W/O/W-emulsion has also been reported. This was performed in order to reduce the creaming of a double emulsion sample, by the formation of a weak gel. W/O/W-emulsions with a reduced gravitational separation were successfully prepared with scleroglucan and with the mixture of xanthan and locust bean gum (Benna-Zayani, Kbir-Ariguib, Trabelsi-Ayadi, & Grossiord, 2008). None of the texture modifiers mentioned above are able to act as an emulsifier in their own right and must therefore be combined with alternative stabilising agents in order to prepare a W/O/W-emulsion.

The presence of an inner and external water phase separated by an immiscible oil phase may give rise to an osmotic pressure gradient. This driving force may lead to swelling/shrinking of the W/O/W-emulsion, which potentially promote destabilisation of the double emulsion. This may be regulated by adjusting the concentration of solutes in either phase. Although this seems trivial, additional factors need to be taken into consideration. First of all, the high Laplace pressure for the small inner water droplets may give rise to an outwards flow. In order to reduce this destabilising effect an opposing osmotic pressure gradient must be applied. Secondly, to obtain sufficient payload there is often a requirement for a high solubility of the inner solute/active ingredient. This restricts the use of W/O/W-emulsion delivery units to highly hydrophilic inner solutes, e.g. ionic substances. The osmotic pressure of such solutions, especially at higher concentration of solute, may be difficult to estimate due to non-ideal behaviour. Both of these effects complicate the osmotic tailoring of DEs.

The present paper examines the use of gelatin as a hydrophilic emulsifier and its efficiency compared with polysorbate 80, a commonly used low molecular weight emulsifier. Since gelatin is an amphiphilic biopolymer and is able to form a thermo reversible gel, it should represent an excellent stabilising agent, both in terms of a texture modifier and as a hydrophilic emulsifier of W/O/W-emulsions. Tartrazine, a synthetic dye, is used as an inner solute/marker in order to evaluate the encapsulation efficiency of W/O/W-emulsions during 3 months of storage. Polyglycerol polyricinoleate (PGPR) was used as a lipophilic emulsifier in order to prepare the primary emulsion (W/O-emulsion). PGPR is an emulsifier which has been shown to give high stability of W/O-emulsions in combination with triglyceride based oils (Garti, Binyamin, & Aserin, 1998; Surh, Vladisavljevic, Mun, & McClements, 2007). Since PGPR is of synthetic origin and has a low limit of acceptable daily intake, effort has been put into finding natural substitutes for PGPR or reducing its concentration (Dickinson, 2011; Fechner, Knoth, Scherze, & Muschiolik, 2007). Thus, the stability of the DEs stabilised by gelatin or polysorbate 80 is evaluated at varying concentration of PGPR.

2. Materials and methods

2.1. Materials

A type B gelatin with a reported bloom value of 150 was kindly supplied by Gelita and used without further processing. The lipophilic emulsifier Polyglycerol Polyricinoleate (PGPR, Grindsted PGPR 90) was supplied by Danisco (Copenhagen, Denmark). Tartrazine (Dye content ≥85%) was purchased from Sigma Aldrich. Corn oil and polysorbate 80 (Tween 80) were also purchased from Sigma Aldrich. Sodium azide (NaN_3) was supplied from BDH Laboratory Supplies Poole (BH15 1TD, England). All experiments were performed using deionised water (MQ-water).

2.2. Preparation of W/O/W-emulsion

The inner aqueous phase was prepared by dissolving tartrazine (20 mg/mL) in MQ-water containing sodium azide (0.02 wt.% NaN_3). Since NaN_3 was used as a preservative agent in the external water phase containing gelatin, it was also added to the inner water phase in order to avoid differences in osmotic pressure. The oil phase was finalised by dissolving PGPR (2, 4, 6, 8 or 10 wt.%) into corn oil. A W/O-emulsion (20 wt.% aqueous phase, 80 wt.% lipid phase) was manufactured by dispersing the inner aqueous phase throughout the oil phase using a VDI 12 homogeniser (VWR International, Darmstadt, Germany) equipped with a dispersing element (type S12N-12S, VWR International, Darmstadt, Germany) at a mixing speed of 30 000 RPM at room temperature. The tartrazine solution (10 mL) was added continuously during mixing, at a rate of 5 mL/min. After adding the inner aqueous water phase, the homogenisation procedure was continued for an additional 3 min. The external aqueous phase was prepared by dissolving the emulsifier (2 wt.% gelatin or polysorbate 80) into the external aqueous phase (0.02 wt.% NaN_3).

Two different methods were evaluated for the preparation of double emulsions with gelatin: 1) Homogenisation of the W/O/W-emulsion in the presence of surplus of gelatin (20 or 30 wt.%). 2) Homogenisation of the W/O/W-emulsion in the presence of only 2 wt.% gelatin, with additional gelatin (18 or 28 wt.%) dissolved into the external water phase after the emulsification. The latter method gave W/O/W-emulsions with larger droplets and higher fraction of tartrazine (higher encapsulation yield) retained within the double emulsion (data not included). Due to the higher encapsulation yield, the second method was chosen as a standard preparation procedure for the W/O/W-emulsions with gelatin.

The W/O/W-emulsions were prepared by mixing the W/O-emulsion (20 wt.%) throughout the external water phase (80 wt.%) at a mixing speed of 12 500 RPM for 4 min. Additional gelatin (18 or 28 wt.%) was dissolved only into the W/O/W-emulsions already containing gelatin by gentle stirring at 50 °C. DEs were stored at 20 °C and 60 %RH during the time span of the study.

2.3. Encapsulation and release of tartrazine from W/O/W-emulsions

One of the main parameters used for evaluation of double emulsion stability is the amount of encapsulated marker (active ingredient) retained within the inner water phase as a function of