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Release of electrolytes from double W/O/W emulsions stabilized by biopolymers

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Abstract — Water-in-oil-in-water (W/O/W) double emulsions were prepared by xanthan gum (XG) in presence of sodium caseinate (SC) using a two-step emulsification process and the kinetics of release of NaCl ions from the internal to the external water phase was investigated. It was noticed that release rate of the electrolyte encapsulated in the internal aqueous phase of the double emulsion stabilized by SC-XG at natural pH depends strongly on the protein concentration; however the effect of XG concentration was found less important. Adjustment of pH of emulsion near the isoelectric point of the protein improved the kinetics release of NaCl. Interfacial film at pH 5 is rigid than that at natural pH which explains the decrease of the release rates at pH 5. Microscopic analysis of formulated emulsions, after formulation and after one month at room temperature, showed that the release of the encapsulated electrolyte in the internal aqueous phase is made by diffusion through the lipid membrane.

Keywords: double emulsions, biopolymers, sodium caseinate, release, xanthan gum

Introduction

Double emulsions are complex systems in which the droplets of the dispersed phase themselves contain small dispersed droplets. There are two types of double emulsions, water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) [1]. Due to their multi-compartment structure, these systems have many applications in the pharmaceutical, cosmetic, and food industries [2, 3].

The globules are permeable to many different chemical species which can migrate from the internal phase to the external one and vice-versa, without film rupturing. The diffusion processes always involve a concentration gradient exerted by the whole set of various molecules (surfactant, electrolytes, osmotic regulators and encapsulated compounds). However, several possible mechanisms have been proposed for the transport of the encapsulated compounds: a) direct solubilisation of the entrapped species in the oil phase (for neutral molecules), b) transport *via* the hydrophilic surfactant polar head group in the case of water [4,5], c) transport through the oil phase into reverse micelles [6], d) formation of thermally-activated transient holes in the thin liquid films separating the internal droplets and the globule surface [7-9]. As a result of one or a combination of these mechanisms (coalescence and diffusion), the release of the encapsulated compound occurs at various rates and in an uncontrolled way. This is why the use of multiple emulsions as commercial products is restricted, although much attention has been paid to their potential practical applications in various domains [10].

Various parameters influence the release kinetics of the encapsulated compounds: the type of the surface-active species (monomeric or polymeric) and their concentrations **[**11,12], the osmotic pressure mismatch between the aqueous compartments [13], the average diameter and volume fractions of the inner droplets and of the oil globules [14], the oil chemical nature [15]. Among these, the surfactant type plays a major role. Pioneering studies on double emulsions were performed in presence of short surfactants [16]. However, the intrinsic instability (fast coalescence and diffusion) of such materials did not allow viable technological developments. Recent studies in presence of amphiphilic polymers, proteins, and solid colloidal particles

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[17-20] reveal that coalescence can be inhibited and that diffusive transport may be extremely slow.

In the present study, W/O/W double emulsions were formulated using a mixture of sodium caseinate (SC) and XG by using two-step emulsification process. NaCl ions were initially dissolved in the internal droplets and their kinetics of release into the external aqueous phase was followed. This investigation was focused on the impact of SC and XG concentration on the rate of NaCl leakage. The influence of the pH of the external phase aqueous was also studied.

2. Materials and Methods

2.1. Materials

The organic phase is olive oil; it was purchased from a local supermarket (Medea, Algeria). The used lipophilic surfactant is Dehymuls E (dicocoyl pentaerythrityl distearyl citrate, sorbitan sesquioleate, Cera alba, and aluminum stearates); it has a hydrophilic-lipophilic balance (HLB) of 4 and it was supplied by Henkel (Algeria). SC, obtained from bovine milk, is produced by Sigma Life Science (New Zealand). XG was obtained from Rhodia (Algeria). Sodium benzoate E211 was used as a preservative agent; it was purchased from Sigma-Aldrich (Switzerland). NaCl, encapsulated agent, was obtained from Sigma-Aldrich.

2.2. Preparation of Biopolymer Solutions

SC solutions were prepared by dispersing SC powder in distilled water containing sodium benzoate using magnetic stirring. SC solutions were stored for 24 hours at room temperature to complete their hydration. XG was dissolved in distilled water containing sodium benzoate at room temperature using vigorous magnetic stirring for 2 hours. SC–XG solution was prepared by mixing the two biopolymer solutions using moderate magnetic stirring for 1 hour at natural pH [21].

2.3. Preparation of W/O/W Emulsions

Double emulsions were prepared by the two-step emulsification process. This method consists first to produce simple W/O emulsion based on lipophilic surfactant which is itself encapsulated in an external aqueous phase containing SC-XG mixture. The primary W/O emulsion was prepared by adding the aqueous phase (distilled water) to the oil phase (olive oil) containing lipophilic surfactant (Dehymuls E) using an agitator at 1000 rpm for 45 minutes. The two phases were previously heated to 75 °C. The primary W/O emulsion containing 20% olive oil and 3% Dehymuls E was then added gradually to the external aqueous phase containing a mixture of SC–XG at room temperature using moderate magnetic stirrer. The mixture was then homogenized by a homogenizer (model T25, Janke Kunkel, IKA Labortechnik, Staufen, Germany) at 8000 rpm for 5 minutes to reduce the droplet size.

In order to investigate the influence of protein and polysaccharide concentrations on the release of electrolyte encapsulated on double emulsion, different formulations were prepared (Table 1). The percentage of 20% of the simple emulsion was chosen because, as it is low, it favors the creaming. This will allow the studying of the effect of polysaccharide in the external phase in absence of creaming [22].

It must be noted that the adsorption of SC at the interface of oil droplets is affected by the pH and the concentration of polysaccharide about its IP [23-25]. For this reason, we have chosen to prepare double emulsions by the same process of two-step emulsification, but adjusting the pH of the external phase at 5 (pH near the PI of SC) using HCl 0.1% solution.

Tableau 1 *Compositions (wt%) of multiple W/O/W emulsions prepared by SC–XG mixture at pH natural* (1% NaCl)

	F1	F2	F3	F4
1st Emulsion	20	20	20	20
SC/XG	1/3	2/3	3/3	2/2
Sodium benzoate	0.2	0.2	0.2	0.2
water	78.8	78.55	78.3	78.8

2.4. W/O/W emulsion characterization

The conductivity of emulsions was measured with a LF 191 conductimeter (WTW, Germany). The pH was measured directly in the emulsion using microprocessor pH/ion meter pMX 2000 (WTW, Germany).The observation of the double emulsions was made using an optical microscope (Olympus1 BX-51, Zeiss, Germany) provided with a camera.

2.5 Quantification of NaCl release from W/O/W emulsions

Eq. (1) represents the percentage release of double emulsion stabilized by a mixture of SC–XG [26],

$$\text{Release (\%)} = (EC_{DE} / EC_{EM,\ t=0}) \times 100 \qquad (1)$$

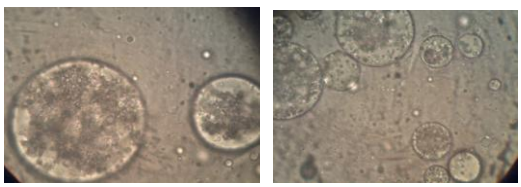
Where EC_{DE} is the electrical conductivity of the measured double emulsion, and $EC_{EM,\ t=0}$ is the electrical conductivity of the emulsion if all the salt was released immediately after preparation.

3. Results and Discussion

3.1. Characteristics of Double Emulsions

All emulsions made at natural pH and pH 5 presented a milky and homogeneous aspect with a variable consistency which depends on biopolymer concentration and pH of the external phase.

The structure and morphology of the emulsions are characterized by analysis of water droplets in the internal emulsion, and the fat globules in the double emulsion by microscopic observation. After 30 days at room temperature, internal droplets and blood cells show no change (instability phenomenon) as shown in Figure 1.



a/ directly after formulation b/ after one month at room temperature

Fig.1. Microscopy of double emulsion (F3: SC/XG = 3/3) directly after formulation and after one month storage at room temperature.

3.2. NaCl release as a function of SC and GX concentration in the external aqueous phase

The fraction of NaCl released versus time for three double emulsions made by various concentrations of SC are shown in Figure 2.

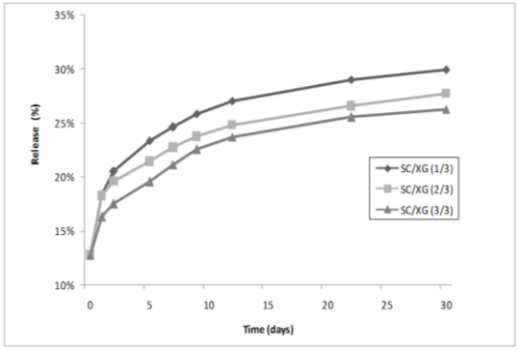


Fig.2. NaCl release for W/O/W emulsions stabilized by SC-XG mixture as a function of SC concentrations at natural pH.

The analysis of this figure showed that the rate of release of NaCl encapsulated in the internal aqueous phase of the double emulsions stabilized by SC-XG at natural pH depends strongly on the protein concentration. At natural pH, SC with negative charge adsorbs at the external O/W interface and forms an interfacial film around oil globules loaded with water droplets. The thickness and rigidity of the interfacial film is function of SC concentration. More the concentration of SC for the same concentrations of GX increases, more the thickness of the interfacial O/W film increases, which slows the diffusion of NaCl from the internal phase to the external aqueous phase.

From microscopic analysis, after formulation and after one month of storage at room temperature and from the NaCl release study, it can be concluded that the release of the encapsulated electrolyte in the internal aqueous phase is made by diffusion through the lipid membrane. This release is a function of the thickness of the outer interfacial film which is a function of SC concentration. These results are in agreement with those observed by measuring the release rate of an electrolyte encapsulated in emulsion stabilized by SC [27].

The variation of XG concentration in the external aqueous phase has a low effect on the release of NaCl as shown in Figure 3.

At natural pH, SC with negative charge adsorbed at the interface while XG with the same negative charge does not adsorb at the interface, therefore XG has no influence on the thickness and stiffness of the interfacial film. In conclusion, the concentration of XG in the outer aqueous phase has no influence on the rate of release of NaCl encapsulated in double W/O/W emulsions stabilized by SC-XG at natural pH.

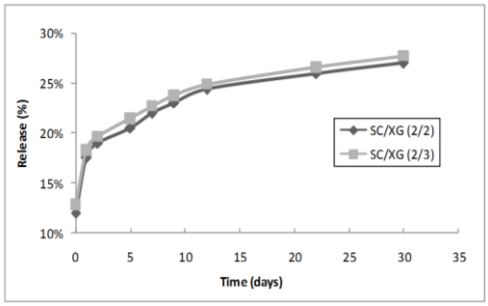


Fig.3. NaCl release for W/O/W emulsions stabilized by SC-XG mixture as a function of XG concentrations at natural pH.

3.3. Effect of the pH of the external aqueous phase on NaCl release

To study the effect of pH of the external aqueous phase on the kinetics of release of NaCl, emulsions (SC/XG = 2/3) were prepared at natural pH and pH 5. The results of measurement of the release of NaCl as a function of time and pH are shown in Figure 4.

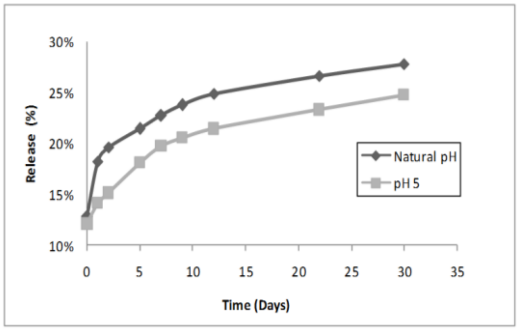


Fig.4. Effect of pH of the external aqueous phase on the kinetics of release of NaCl encapsulated in double W/O/W emulsions stabilized by SC/XG (2/3)

This figure shows that the pH of the external aqueous phase has a significant effect on the release of NaCl. At natural pH the outside interfacial film is formed by the molecules of SC, by against at pH 5 this film is constituted by a complex formed between SC macromolecules adsorbed at the interface and XG ones surrounding due to the decrease of repulsive interactions. At pH 5, SC which is close to its IP is weakly negatively charged, so the repulsive interactions between the two biopolymers decrease. First, protein is adsorbed at the oil/water interface then the polysaccharide through hydrophobic interactions forms a complex which increases the rigidity of the interfacial film covering the oil droplets. So the interfacial film at pH 5 is more rigid than that at natural pH which explains the decrease in NaCl release rates at pH 5 by comparison to natural pH. In previous work using a XG complex and milk protein for stabilizing double emulsions, it was demonstrated that the kinetics of release of vitamin B1 encapsulated in double emulsions is function of the pH of the external phase [28].

Conclusion

The effects of SC and XG concentrations on the release of NaCl electrolyte from double W/O/W emulsions were evaluated. Influence of the pH of the external phase was also studied. The experimental measurements revealed that the release occurred without film rupturing and that the release rate was strongly influenced by the concentration of SC. It was also demonstrated that the adjustment of the pH of the external phase around the isoelectric point (IP) of SC modified the kinetics of release of NaCl from internal to external phase.

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