Journal of Colloid and Interface Science 341 (2010) 101–108

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Effect of calcium salts and surfactant concentration on the stability of water-in-oil (w/o) emulsions prepared with polyglycerol polyricinoleate

Andrés L. Márquez [a,b], Alejandra Medrano [c], Luis A. Panizzolo [c], Jorge R. Wagner [b,*]

[a] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA-CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 116, B1900AJJ La Plata, Buenos Aires, Argentina
[b] Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, B1876BXD Bernal, Buenos Aires, Argentina
[c] Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República, General Flores 2124, 11800 Montevideo, Uruguay

ARTICLE INFO

Article history:
Received 7 July 2009
Accepted 12 September 2009
Available online 18 September 2009

Keywords:
PGPR
W/o emulsions
Calcium
Coalescence
Sedimentation
Interfacial rheology

ABSTRACT

The objective of this work was to obtain water-in-oil (w/o) emulsions with polyglycerol polyricinoleate (PGPR) as emulsifier and to study the effect of the addition of calcium in the dispersed aqueous phase on the stability of these systems. Emulsions were formulated with 0.2, 0.5 and 1.0% w/w PGPR and 10% w/w water containing calcium chloride at varied concentrations or other salts (calcium lactate or carbonate; sodium, magnesium or potassium chloride). The stability of these systems was studied with a vertical scan analyzer during 15 days; coalescence and sedimentation were observed as simultaneous destabilization processes. The increase of PGPR concentration and/or calcium chloride content gave more stable emulsions. The stabilizing effect of calcium salt was attributed to the diminution of the water droplets size, the decrease of the attractive force between water droplets and the increase of the adsorption density of the emulsifier. The viscoelastic parameters of the interfacial film were decreased with increasing calcium and PGPR concentrations. Calcium chloride produced a higher increase of stability than calcium salts with lower dissociation degree. The presence of any assayed salt in the aqueous phase also allowed the stabilization of w/o emulsions with higher water contents.

1. Introduction

Polyglycerol polyricinoleate (PGPR; trade name: ADMUL WOL) is a powerful emulsifier, which can be used for the obtaining of stable water-in-oil (w/o) emulsions. Its high emulsifying properties are attributed to the excellent water-binding capacity of the long hydrophilic polyglycerol chain. In food industry, PGPR is commonly used along with lecithin to diminish the viscosity of chocolate covertures [1,2]. It is also employed as emulsifier in high or low fat content products, such as butter, margarines and salad dressings [3].

The digestibility of PGPR is 98% and it does not interfere with the normal metabolism of lipids [4]. Studies made on rats did not evidence adverse effects on growth and reproduction [5] neither carcinogenic danger [6]. It has been demonstrated that PGPR is tolerated by humans at high doses and in absence of clinical symptoms [7]. PGPR was considered GRAS (Substance Generally Recognized as Safe) by the FDA [8].

W/o emulsions are less common than o/w ones and fewer studies are dedicated to them; butter and margarine are typical examples of w/o emulsions. These systems can be used as an oil phase substitute in food emulsions, obtaining water-in-oil-in-water (w/o/w) emulsions with same amount of dispersed phase but with lower lipid content [9]. W/o/w emulsions are also a potential method to isolate substances in the dispersed aqueous phase of the primary w/o emulsion [10–12].

Some foods are usually fortified with calcium salts for nutritional purposes. Nevertheless, calcium interacts with some food emulsifiers (e.g. soybean proteins and phospholipids) [13–16] affecting the stability of food products; the isolation of this cation in the internal aqueous phase of a w/o/w emulsion could be a solution to obtain calcium-fortified systems stabilized with such surfactants. Thus, the obtaining of a stable primary w/o emulsion containing calcium in the dispersed aqueous phase is required as a first step.

Stability is a very important parameter to know the applicability of an emulsion to produce more complex systems. Because the w/o emulsions studied in this work are wanted to be used in the formulation of reduced lipid content foods fortified with calcium, a relatively high stability is needed to get a product of invariable quality during the required time (transport, storage, etc.). W/o emulsions are usually destabilized by coalescence and sedimentation [17,18]; these destabilization processes should be reduced without using high concentrations of emulsifier, as PGPR should not be used in excess in food systems. The stability of different

* Corresponding author. Fax: +54 11 43657101.
E-mail address: jwagner@unq.edu.ar (J.R. Wagner).

0021-9797/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jcis.2009.09.020

types of emulsions prepared with PGPR has been previously studied by other authors [19–22], but the effect of the inclusion of calcium inside the aqueous phase of w/o emulsions stabilized with PGPR has not been reported. The present study deals with the influence of calcium salts on the stability of w/o emulsions prepared with different concentrations of PGPR, analyzing different potential factors such as droplet size distribution, interfacial tension and interfacial rheology.

The objective of the present work was to study the effect of calcium salts and surfactant concentration on the stability of w/o emulsions prepared with refined sunflower oil, distilled water and PGPR, in order to evaluate these systems as a potential method for calcium fortification and oil phase reduction of foods formulated on base of oil and/or fats.

2. Materials and methods

2.1. Materials

Refined sunflower oil (Molinos Río de la Plata S.A.; Avellaneda, Argentina); distilled water; PGPR 90 (Grindsted-Danisco; provided by CALSA; Lanús, Argentina); anhydrous calcium chloride, sodium chloride, and potassium chloride (Anedra; San Fernando, Argentina); calcium lactate and precipitated calcium carbonate (Parafarm; Buenos Aires, Argentina); 6-hydrated magnesium chloride (Riedel-de Haën; Germany). All salts were of analytical grade.

The solubility in water of the salts was determined at defined concentration (0.25 M) and temperature (25 °C). All chloride salts, which are totally dissociable, were 100% soluble. Calcium lactate, organic salt less dissociable than calcium chloride, was 73% soluble. And calcium carbonate, the least dissociable salt, was 0.025% soluble.

2.2. Emulsion preparation

PGPR and oil were mixed at different proportions to obtain a content of 0.2, 0.5 and 1.0 g of PGPR for 100 g of emulsion. The w/o emulsions were prepared with oil phase containing PGPR and 10% w/w aqueous phase containing different amounts of calcium chloride (0, 10, 100 and 1000 mg Ca/100 g aqueous phase) or other salts (calcium lactate or carbonate; sodium, magnesium or potassium chloride) at a defined concentration (1000 mg Ca/100 g aqueous phase or 0.25 M). For these systems, a standard homogenization was performed with an Ultraturrax T-25 (IKA-Works; Wilmington, USA) using a S25 N-8G rotor (IKA-Works; Wilmington, USA; rotor/stator distance, 0.25 mm; rotor diameter, 6.1 mm) at 24,000 rpm during 2 min (sample weight, 50 g). A higher energy homogenization, using Ultraturrax but with a S25-NK-19G rotor (IKA-Labortechnik; Staufen, Germany; rotor/stator distance, 0.3 mm; rotor diameter, 12.7 mm) at 24,000 rpm during 2 min (sample weight, 70 g) was applied to obtain stable w/o emulsions with 20–40% w/w aqueous phase, 1.0% w/w PGPR and 1000 mg Ca/100 g aqueous phase.

In order to determine whether the continuous phase was oil or water, the emulsions were observed by light microscopy. Moreover, when the continuous phase was water the emulsions had a higher consistency because of the high concentration of oil as dispersed phase.

2.3. Global stability

Global stability of the w/o emulsions was determined by light scattering measurements using a vertical scan analyzer (QuickScan, Beckman Coulter; Fullerton, USA) during 15 days at room temperature. This equipment scans the sample along the tube where it is contained, giving a number of profiles of back-scattering (%BS) and transmission percentages as a function of time and tube length [17]. From the obtained profiles, mean values of %BS were calculated in the 20–50 mm zone ($\%BS_{20-50}$), corresponding to the medium part of the tube. The $\%BS_{20-50}$ value was used as a relative mean size of water droplets of w/o emulsions with same water content: the higher the $\%BS_{20-50}$ value the lower the water droplets size [18]. Measurements were performed at least in duplicate. In order to measure the global stability of the emulsions, it was defined the destabilization percentage (%D), as follows:

$$\%D = [(BS_{in20-50} - BS_{f20-50})/BS_{in20-50}] \times 100 \quad (1)$$

where $BS_{in20-50}$ is the initial $\%BS_{20-50}$ value and BS_{f20-50} is the $\%BS_{20-50}$ value after 7 days.

2.4. Light microscopy

Micrographs were obtained with a Leica DMLB optical microscope (Leica Microsystems, GmbH; Wetzlar, Germany) with an adapted digital camera (Leica DC100, Leica Microsystems, GmbH) operating at 200× magnification.

2.5. Droplet size distribution

Size distributions of water droplets of w/o emulsions were obtained with a particle analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd.; Workcestershire, UK). Samples were dispersed into refined sunflower oil in the dispersion unit (Hydro 2000MU). Sauter mean diameter (d_{32}) and De Brouker mean diameter (d_{43}) were obtained from the droplet size distributions; d_{32} and d_{43} are related to the surface and volume distributions, respectively. Measurements were performed immediately after the emulsion preparation at least in duplicate.

2.6. Interfacial tension and interfacial rheology

Interfacial tension and interfacial rheology data were obtained with a dynamic droplet tensiometer (Tracker, IT-Concept; Saint-Clementtes Places, France). Measurements were performed at room temperature (25 ± 3 °C). The oil phase (containing PGPR) was located in the bucket of the tensiometer and the aqueous phase was included in a syringe from which a droplet of 2 or 7 μL was formed. The time to reach equilibrium conditions was 20 min, after which the interfacial tension and the viscoelastic characteristics of the interfacial film were evaluated during 10 min at constant strain amplitude ($\Delta A/A = 0.1$) and oscillation frequency ($\omega = 200$ mHz). The method consists of subjecting the droplet to an automatically controlled, sinusoidal compression–expansion, at the desired frequency and amplitude. Measurements were performed at least in triplicate. The surface dilational modulus (E) derived from the change in interfacial tension (γ) (Eq. (2)) as a result of a small change in droplet surface (A) (Eq. (3)), according to Eq. (4) [23].

$$\gamma = \gamma_0 \times \sin(\omega\theta + \phi) \quad (2)$$

$$A_0 = A_0 \times \sin(\omega\theta) \quad (3)$$

$$E = \frac{d\gamma}{\frac{dA}{A}} = -\frac{d\pi}{d\ln A} \quad (4)$$

where γ_0 and A_0 are the stress and strain amplitudes, respectively, θ is time, ϕ is the phase angle between stress and strain, $\pi = \gamma^0 - \gamma$ is the interfacial pressure, and γ^0 is the interfacial tension in the absence of emulsifier.

The dilational modulus E is a complex quantity and is composed of real and imaginary parts (Eq. (5)). The real part is referred to the elastic or storage component (εd) and the imaginary part to the

viscous or loss component (ηd). For a perfectly elastic material, the stress and strain are in phase ($\phi = 0$) and the imaginary term is zero. In the case of a perfectly viscous material $\phi = 90°$ and the real part is zero. The loss-angle tangent is defined by Eq. (6). If the film is purely elastic, the loss-angle tangent is zero.

$$E = (\gamma_0/A_0) \times (\cos\phi + i\sin\phi) = \varepsilon d + i\eta d \quad (5)$$

$$\tan\phi = \eta d/\varepsilon d \quad (6)$$

2.7. Statistical analysis

The statistical analysis was performed by analysis of variance (ANOVA) and test of least significant difference (LSD; $p < 0.05$) using the statistical program Statgraphics Plus 7.0.

3. Results and discussion

Fig. 1 shows the %BS profiles for w/o emulsions, with and without added calcium in the aqueous phase, prepared with different PGPR concentrations and measured during 15 days. The decrease of the %BS values along the tube containing the sample evidences an increase of water droplets size due to a coalescence process. On the other hand, the increase of %BS at the bottom of the tube, at the expense of %BS diminution on the top part, indicates a sedimentation process of the same water droplets. Similar results were observed in w/o emulsions stabilized with lecithins [17] and Spans [18]. Although the %BS profiles show that coalescence and sedimentation occurred simultaneously, in emulsions without added salt the former process seemed to start at a faster speed than the latter, according to observations until 1 day. During that time, accumulation of water droplets at the bottom of the tube was not

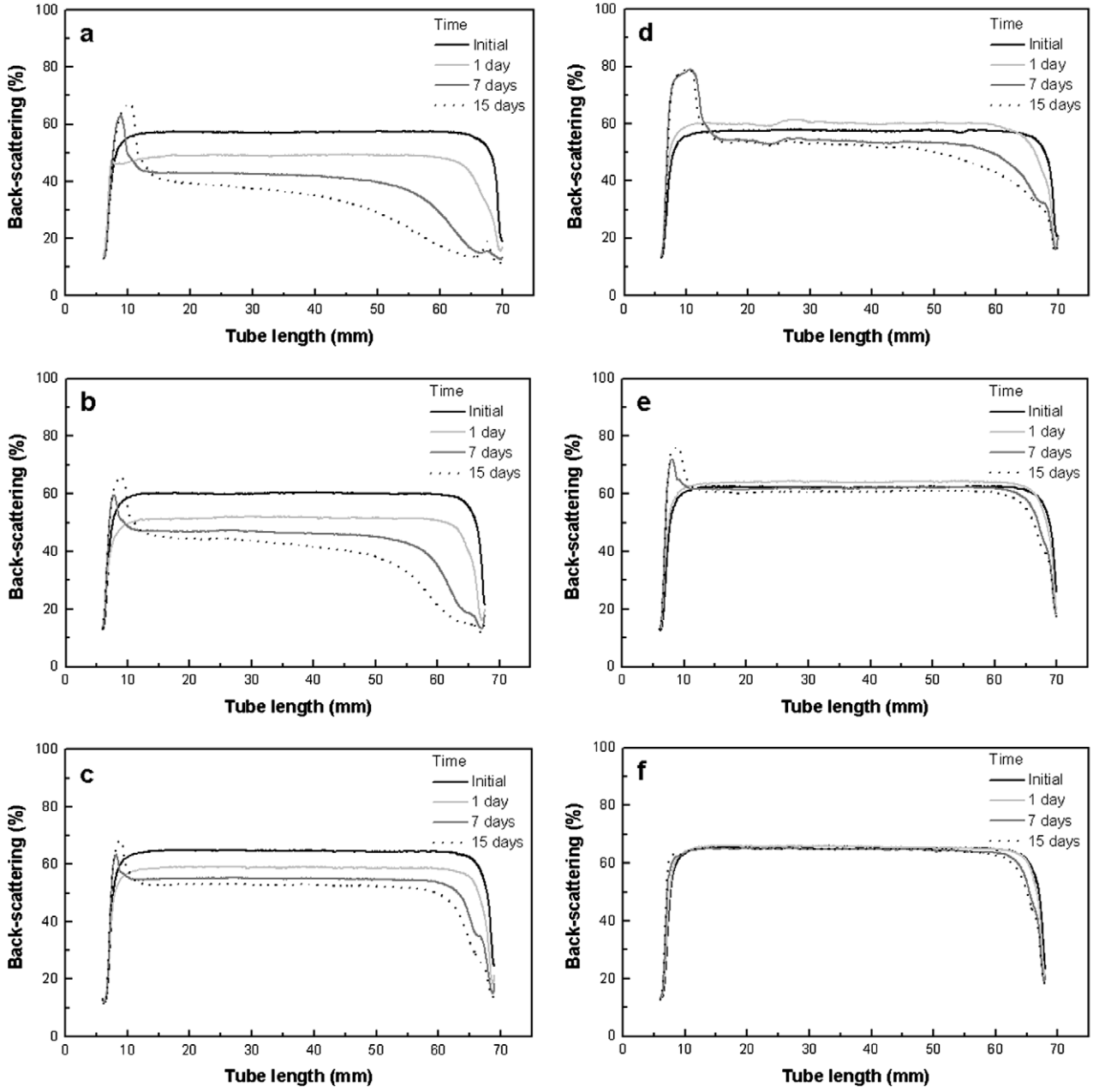

Fig. 1. Back-scattering (%BS) profiles of w/o emulsions with 10% aqueous phase as a function of time and tube length. Without added salt: (a) 0.2% PGPR; (b) 0.5% PGPR; (c) 1.0% PGPR. With added $CaCl_2$ (1000 mg Ca/100 g aqueous phase): (d) 0.2% PGPR; (e) 0.5% PGPR; (f) 1.0% PGPR.

observed, despite the evident %BS diminution at the top part (Fig. 1a–c). However, during the next days coalescence was slowed while sedimentation was accelerated. These results indicate that sedimentation became faster when water droplets size was increased by coalescence, inducing the gravitational separation [24].

With regard to the effect of PGPR concentration, an increase of stability to both destabilization processes with the increase of the emulsifier amount was observed in those emulsions with no added salt. This effect of PGPR increase on the stability of w/o emulsions was previously reported in systems prepared with soybean oil and homogenized with a two stage valve homogenizer [20]. This result was attributed to the lower initial size of water droplets produced by the higher surfactant content, according to observations by optical microscopy (Fig. 2) and manifested by a higher initial $\%BS_{20-50}$ value ($p < 0.05$; Table 1) and lower mean droplet diameters (d_{32} and d_{43}; $p < 0.05$; Table 2). This droplet size diminution with the increase of PGPR concentration was also evidenced by the droplet size distributions (Fig. 3a). A lower droplet size reduces the coalescence process as a consequence of a lower collision efficiency [24]. Moreover, increasing PGPR concentration increases the viscosity of the emulsion [20], which may lead to reduced rates of coalescence of water droplets. These w/o emulsions with PGPR were much more stable than those prepared under the same conditions with different types of Span as emulsifiers [18], which demonstrates the higher capability of PGPR to obtain these systems.

The addition of calcium chloride in the aqueous phase produced a notorious effect on the stability of these systems: the w/o emulsions became more stable. This is evidenced by comparing the %BS profiles of the emulsions with no added salt (Fig. 1a–c) against the emulsions with 1000 mg Ca/100 g aqueous phase (Fig. 1d–f). The coalescence process was highly reduced as it can be appreciated by the slower %BS diminution as a function of time. Sedimentation was also slowed, as a direct consequence of the coalescence inhibition. The emulsion with 0.2% w/w PGPR (Fig. 1d) and, in a lower

Table 1
Mean initial back-scattering ($\%BS_{in20-50}$) of w/o emulsions with 10% aqueous phase as a function of PGPR and calcium (as $CaCl_2$) concentrations.

PGPR (%)	Calcium (mg/100 g aqueous phase)			
	0	10	100	1000
0.2	57.7 ± 0.5 a	57.5 ± 1.3 a	58.6 ± 0.4 a	58.3 ± 0.6 a
0.5	62.4 ± 1.9 b	62.6 ± 0.7 b	61.9 ± 0.8 b	62.3 ± 0.5 b
1.0	65.4 ± 0.6 c	65.4 ± 1.0 c	65.7 ± 0.6 c	65.5 ± 0.2 c

Mean values ± S.D., $n = 3$. Mean values with different letters are significantly different ($p < 0.05$).

Table 2
Mean droplet diameters (d_{32} and d_{43}) for w/o emulsions with 10% aqueous phase without or with added $CaCl_2$ (1000 mg Ca/100 g aqueous phase).

Salt	PGPR (%)	d_{32} (μm)	d_{43} (μm)
No salt	0.2	2.946 ± 0.035 a	6.061 ± 0.117 a
	0.5	2.366 ± 0.036 b	4.067 ± 0.014 b
	1.0	1.275 ± 0.170 d	2.471 ± 0.087 d
Calcium chloride	0.2	3.058 ± 0.003 a	3.340 ± 0.003 c
	0.5	1.811 ± 0.020 c	2.414 ± 0.048 d
	1.0	1.421 ± 0.013 d	1.711 ± 0.025 e

Mean values ± S.D., $n = 2$. Mean values with different letters are significantly different ($p < 0.05$).

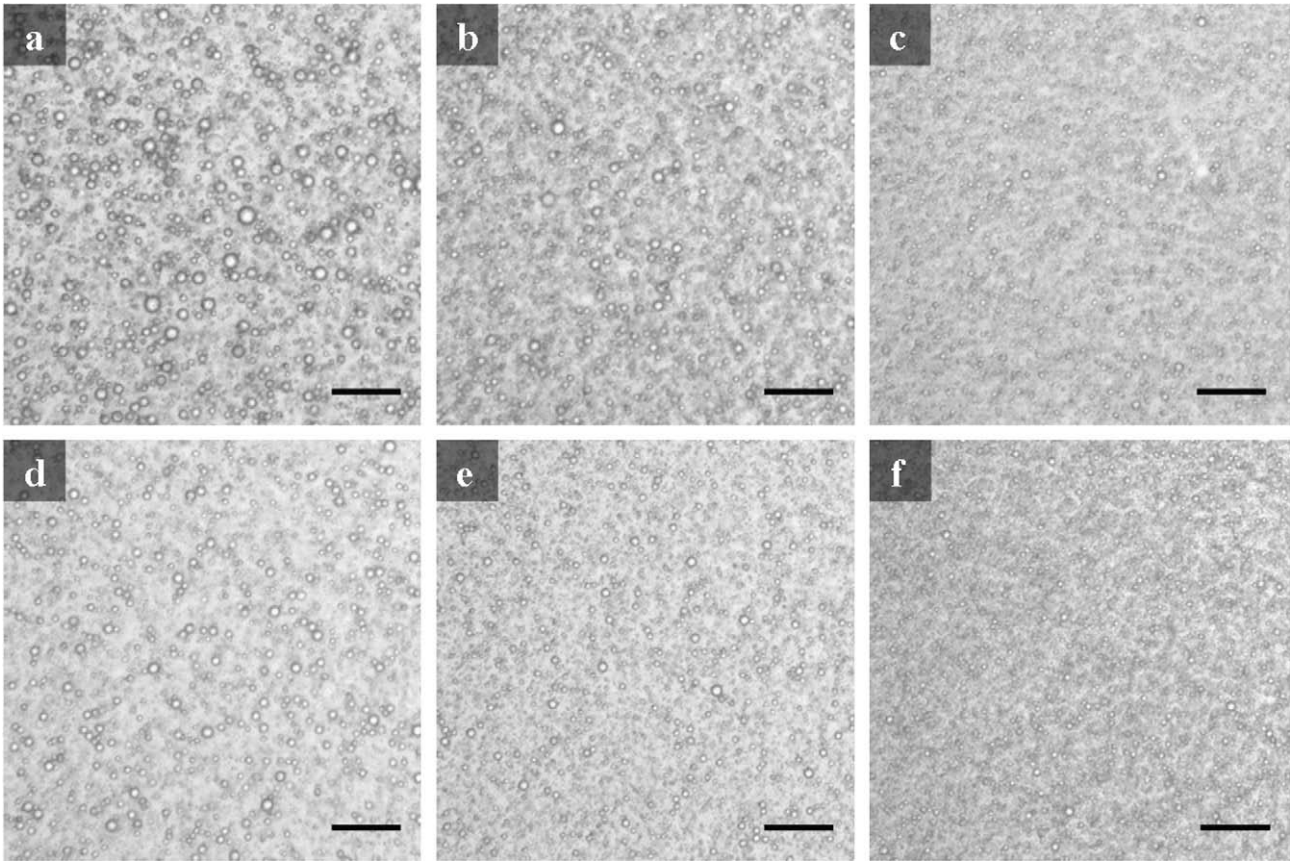

Fig. 2. Optical micrographs of w/o emulsions with 10% aqueous phase, immediately after preparation. Without added salt: (a) 0.2% PGPR; (b) 0.5% PGPR; (c) 1.0% PGPR. With added $CaCl_2$ (1000 mg Ca/100 g aqueous phase): (d) 0.2% PGPR; (e) 0.5% PGPR; (f) 1.0% PGPR. Bar = 5 μm.

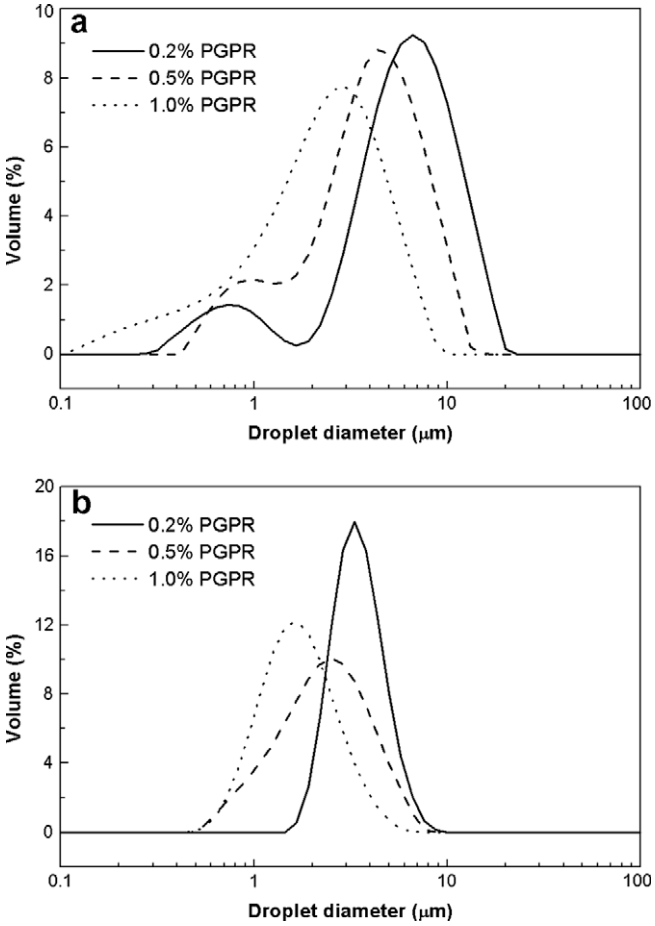

Fig. 3. Volume droplet size distributions of w/o emulsions with 10% aqueous phase. (a) Without added salt. (b) With added $CaCl_2$ (1000 mg Ca/100 g aqueous phase).

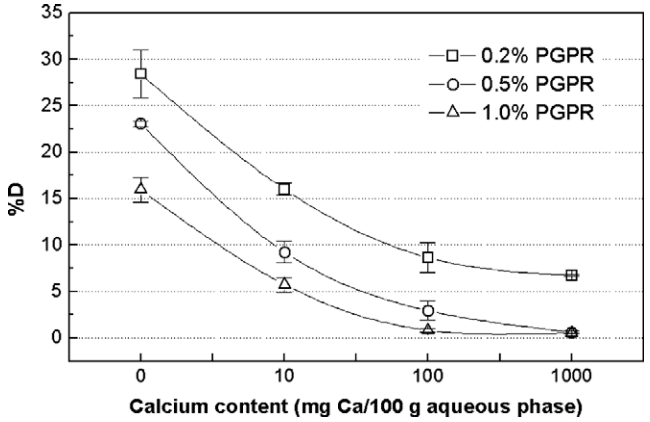

Fig. 4. Effect of PGPR and calcium (as $CaCl_2$) concentrations on the destabilization percentage (%D) of w/o emulsions with 10% aqueous phase. Mean values ± S.D., $n = 2$.

extent, the sample with 0.5% w/w PGPR (Fig. 1e) showed an increase of the %BS values during the first day; this effect could be attributed to the incipient sedimentation process, as water droplets tend to descend to the bottom of the tube while their size is not sufficiently increased. This would also explain the relatively high %BS values at the bottom of the tube after 7 days (Fig. 1d), since relatively small water droplets were accumulated. The higher stability was observed in the emulsion with added calcium and 1.0% w/w PGPR (Fig. 1f).

Fig. 4 summarizes the combined stabilizing effect of PGPR and calcium chloride. It can be observed that the stability of w/o emulsions increased (%D decreased) with increasing PGPR and/or calcium concentrations ($p < 0.05$). The addition of 10 mg Ca in 100 g aqueous phase produced a higher increase of stability than doubling PGPR concentration in emulsions with no added salt. At higher calcium concentrations %D tended to a minimal value; emulsions with 0.5 and 1.0% w/w PGPR reached a similar lowest %D value at 1000 mg Ca/100 g aqueous phase, while 0.2% w/w PGPR was not enough to stabilize the system until that level. It was also detected a significant interaction between PGPR and calcium concentrations ($p < 0.05$), confirming a synergic effect between the emulsifier and the salt.

The stabilizing effect of calcium salt can be partially explained by the droplet size distributions of these emulsions. The addition of relatively high concentrations of calcium chloride in the aqueous phase produced a clear change: the distributions were bimodal with no added salt (Fig. 3a) while they were monomodal with 1000 mg Ca/100 g aqueous phase (Fig. 3b). It was also detected a diminution of the d_{43} values when the salt was added at same PGPR content ($p < 0.05$; Table 2). However, the addition of calcium chloride did not produce a significant change of the d_{32} values at 0.2 and 1.0% w/w PGPR ($p > 0.05$; Table 2); it was neither observed a significant variation of the initial $\%BS_{20-50}$ values at same PGPR content ($p > 0.05$; Table 1), indicating that $\%BS_{20-50}$ correlates better with d_{32} than with d_{43}. The differences between the results given by d_{32} and d_{43} can be explained by the droplet size distributions: in the emulsions with calcium chloride the droplet diameters of the unique population were located between the two populations of the systems with no added salt, whose distributions were wider (Fig. 3). Since d_{43} is more sensitive to the presence of small amounts of large particles than d_{32}, its higher values for the emulsions with no added salt were attributed to the presence of larger water droplets, which can be observed in the optical micrographs, especially at 0.2% w/w PGPR (Fig. 2). The presence of those larger droplets would affect the stability of these systems, increasing the coalescence and sedimentation rates. This would explain the higher stability of the emulsions containing calcium chloride in the dispersed aqueous phase. Nevertheless, other factors must be considered since, for instance, the d_{43} value of the emulsion with 1.0% w/w PGPR and no added salt was not significantly different than the d_{43} value of the emulsion with 0.5% w/w PGPR and 1000 mg Ca/100 g aqueous phase ($p > 0.05$; Table 2), despite the considerably higher stability of the second system (Fig. 4).

Park et al. [25] explained that the addition of electrolytes in the aqueous phase increases the w/o emulsion stability to coalescence because electrolyte lowers the attractive force between the water droplets. According to Israelachvili [26], the attractive force (by van der Waals interactions) between two aqueous droplets in the oil continuous phase is at minimum when the refractive indices and/or the dielectric constants of the two phases are matched. Increasing the electrolyte concentration increases the refractive index of the water phase, and thus decreases the refractive index difference between oil and water phases [25]. Moreover, the dielectric constant of the aqueous phase is decreased with increasing electrolyte concentration [27], reducing the dielectric constant difference between both phases. Thus, the addition of calcium salt into the water phase would decrease the attractive force between water droplets, reducing the collision frequency. In this way, calcium salt would allow the production of w/o emulsions with higher stability to coalescence and, consequently, sedimentation.

An alternative way by which calcium salt could increase stability to coalescence is through its effect on the adsorption density of PGPR at the interfacial film. A necessary condition for this

Table 3
Effect of calcium (as $CaCl_2$) concentration on the interfacial tension (γ), elastic dilational modulus (εd), viscous dilational modulus (ηd), and tan ϕ ($\eta d/\varepsilon d$) of the interfacial film of w/o emulsions with 1.0% PGPR. Droplet volume: 7 μL.

Calcium (mg/100 g aqueous phase)	γ (mN/m)	εd (mN/m)	ηd (mN/m)	tan ϕ
0	4.95 ± 0.41 a	13.29 ± 0.11 a	7.15 ± 0.17 a	0.538 ± 0.008 a
10	4.86 ± 0.24 a, b	13.59 ± 0.18 a	6.79 ± 0.13 a	0.500 ± 0.014 a
100	4.46 ± 0.09 b	13.58 ± 0.15 a	6.17 ± 0.03 b	0.454 ± 0.007 b
1000	3.11 ± 0.04 c	11.24 ± 0.24 b	4.17 ± 0.40 c	0.372 ± 0.042 c

Mean values ± S.D., $n = 3$. Mean values with different letters are significantly different ($p < 0.05$).

mechanism to operate is that calcium salt must reduce the oil/water interfacial tension in the presence of the emulsifier [28]. Table 3 shows that increasing calcium concentration in the aqueous phase decreased the interfacial tension ($p < 0.05$), indicating a higher adsorption density of the emulsifier. Since PGPR is a non-ionic surfactant, this effect of calcium salt could be explained by the presence of ionic surface active components such as free fatty acids from the emulsifier, as it was proposed by other authors in assays made with mineral oil as continuous phase [28]. In our case, the sunflower oil could also contribute free fatty acids. Calcium would bind by those fatty acids reducing their competitive adsorption and favoring the adsorption of PGPR at the interface. The reduction of the interfacial tension would also explain the diminution of the d_{43} values when calcium chloride was added in the aqueous phase (Table 2).

Apart from reducing the interfacial tension, calcium salt could affect the interactions between PGPR molecules adsorbed at the interface by two ways: (a) modifying the ionic strength of the medium (more hydrophilic environment) and, thus, promoting interactions between the hydrophobic chains of PGPR; and (b) the cation could act as a bridge to join the hydrophilic polyglycerol chains, due to the negative charge density of the hydroxyl groups. Both effects should be reflected in an increase of the viscoelastic parameters of the interfacial film. However, the effect of the salt on the interfacial rheology of the studied w/o emulsions was the opposite: the increase of calcium concentration led to decreased elastic and viscous dilational modules ($p < 0.05$; Table 3). Similar results were obtained with increasing PGPR concentration, which also reduced the interfacial tension and the elastic and viscous dilational modules ($p < 0.05$; Table 4). This indicates that the inclusion of calcium salt had an effect comparable to the increase of emulsifier content, explaining its stabilizing action. However, the effect of calcium was more important on the viscous dilational modulus, leading to lower tan ϕ values (more elastic behavior; Table 3), while the effect of PGPR was more important on the elastic dilational modulus, leading to higher tan ϕ values (less elastic behavior; Table 4). These results lead to the conclusion that the increase of stability as a consequence of a higher calcium or PGPR concentration was attributed to the reduction of the interfacial tension rather than to the viscoelastic properties of the film. Although a higher stability to coalescence would be suspected to be attributed to the viscoelasticity of the film, other authors did not find direct correlation between emulsion coalescence stability and the rheological properties of protein adsorption layers [29] and other types of surfactant layers [30]. Moreover, in w/o emulsions stabilized with Span 80, other authors observed a relatively low dilational elasticity at relatively high emulsifier concentration despite the strong stability of the system [31].

Comparative measurements with different calcium and chloride salts were performed. The inclusion of every salt in the dispersed aqueous phase enhanced the w/o emulsion stability in comparison to the systems with no added salt. The addition of equal calcium amount in form of different salt types produced emulsions with different stability. The order of emulsion stability coincided with the order of dissociation degree of the salts ($p < 0.05$): carbonate < lactate < chloride (Fig. 5). Calcium chloride and calcium lactate produced lower interfacial tensions than calcium carbonate ($p < 0.05$; Table 5), explaining the higher stability given by the more dissociable salts because of a higher adsorption density of the emulsifier [28]. However, calcium lactate produced a lower interfacial tension than calcium chloride ($p < 0.05$), despite the higher stability given by the second salt. Thus, the reduction of the attractive force between the water droplets as a consequence of a higher electrolyte concentration [25] would be a more important factor than the reduction of the interfacial tension, since more dissociable salts contribute a higher amount of free electrolytes. On the other hand, the addition of different chloride salts (sodium, magnesium, and potassium) at equivalent concentration in the aqueous phase (0.25 M) did not produce significant differences in stability in comparison to calcium chloride ($p > 0.05$; data not shown), despite the different interfacial tensions given by the different salts ($p < 0.05$; Table 5). This reinforces the theory that the effect of the salts on the attractive force between the water droplets is a more important factor than their effect on the interfacial tension. With regard to the effect of the salts on the rheological properties of the interfacial film, no correlation was observed be-

Table 4
Effect of PGPR concentration on the interfacial tension (γ), elastic dilational modulus (εd), viscous dilational modulus (ηd), and tan ϕ ($\eta d/\varepsilon d$) of the interfacial film of w/o emulsions without added salt. Droplet volume: 7 μL.

PGPR (%)	γ (mN/m)	εd (mN/m)	ηd (mN/m)	tan ϕ
1.0	4.95 ± 0.41 a	13.29 ± 0.11 a	7.15 ± 0.17 a	0.538 ± 0.008 a
2.0	3.07 ± 0.21 b	7.03 ± 0.14 b	4.93 ± 0.12 b	0.702 ± 0.030 b
3.0	3.05 ± 0.22 b	6.70 ± 0.34 b	5.02 ± 0.11 b	0.751 ± 0.022 c
4.0	2.91 ± 0.02 b	4.42 ± 0.14 c	4.23 ± 0.13 c	0.958 ± 0.007 d

Mean values ± S.D., $n = 3$. Mean values with different letters are significantly different ($p < 0.05$).

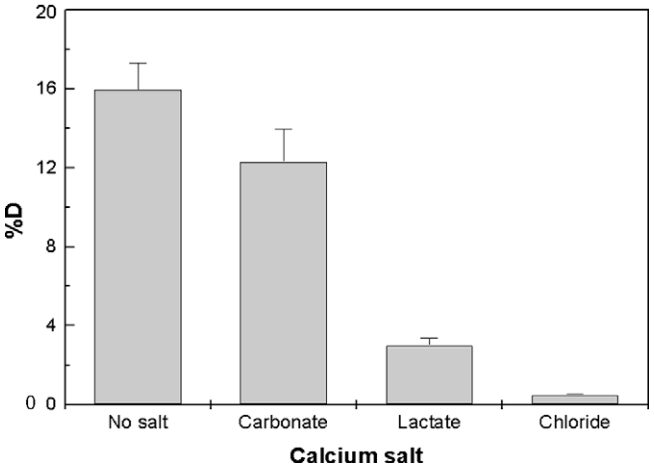

Fig. 5. Effect of the variation of calcium salt types (1000 mg Ca/100 g aqueous phase) on the destabilization percentage (%D) of w/o emulsions with 10% aqueous phase and 1.0% PGPR. Mean values ± S.D., $n = 2$.

Table 5
Effect of added salt in the aqueous phase (at 0.25 M concentration) on the interfacial tension (γ), elastic dilational modulus (εd), viscous dilational modulus (ηd), and tan ϕ ($\eta d/\varepsilon d$) of the interfacial film of w/o emulsions with 1.0% PGPR. Droplet volume: 2 μL.

Salt	γ (mN/m)	εd (mN/m)	ηd (mN/m)	tan ϕ
No salt	3.37 ± 0.06 a	8.20 ± 0.08 a	5.57 ± 0.18 a, b	0.680 ± 0.023 a
Calcium carbonate	2.91 ± 0.06 b	11.90 ± 0.87 d	5.93 ± 0.18 a	0.499 ± 0.025 c
Calcium lactate	1.67 ± 0.06 e	7.93 ± 0.86 a	4.41 ± 0.61 c	0.564 ± 0.127 b, c
Calcium chloride	2.24 ± 0.35 d	8.14 ± 0.31 a	4.82 ± 0.09 c	0.593 ± 0.023 a, b
Sodium chloride	2.16 ± 0.03 d	10.73 ± 0.35 c	5.42 ± 0.01 b	0.505 ± 0.017 b, c
Potassium chloride	2.51 ± 0.09 c	11.41 ± 0.24 c, d	5.97 ± 0.13 a	0.523 ± 0.017 b, c
Magnesium chloride	1.65 ± 0.05 e	9.19 ± 0.35 b	4.91 ± 0.32 c	0.534 ± 0.037 b, c

Mean values ± S.D., $n = 3$. Mean values with different letters are significantly different ($p < 0.05$).

tween the viscoelastic parameters and the stability of the emulsions (Table 5).

Another important effect of the addition of salt on these emulsions was the variation of the inversion point: with no added salt, emulsions were oil-in-water (o/w) with 15% w/w or more water content; the addition of calcium chloride (at concentrations higher than 10 mg Ca/100 g aqueous phase) or the other assayed salts allowed the production of w/o emulsions with higher water contents. However, with the standard homogenization condition the addition of salt only produced relatively stable w/o emulsions with up to 20% w/w aqueous phase; at higher water contents w/o emulsions were formed but rapidly destabilized (during the first minutes after homogenization), even with 2.0% w/w PGPR. When the water content is increased, the number of droplets increases and so their interaction, which favors the coalescence process [24].

The higher energy homogenization allowed the formulation of stable w/o emulsions with up to 40% w/w aqueous phase (1.0% w/w PGPR; 1000 mg Ca/100 g aqueous phase; %D ≈ 0), due to the formation of smaller water droplets. This condition produced initial $\%BS_{20-50}$ values higher than 70 in w/o emulsions with 10% w/w aqueous phase (1.0% w/w PGPR), which were higher than those obtained with the standard homogenization (Table 1). The increase of stability was limited by the amount of emulsifier, since at relatively low PGPR concentrations (0.2% w/w) w/o emulsions prepared with higher energy were even more unstable than with standard energy, because there was not enough emulsifier to cover the produced interfacial area. These results indicate the need of reducing water droplets size when more water is wanted to be stabilized; since emulsifiers like PGPR should not be used in excess for the elaboration of food products, the right equilibrium between homogenization energy and emulsifier concentration must be found to produce stable w/o emulsions.

4. Conclusions

The stability to coalescence and sedimentation of w/o emulsions prepared with PGPR was enhanced with the increase of emulsifier concentration due to the diminution of water droplets size. The addition of calcium salt in the aqueous phase of these emulsions allowed the production of more stable systems. This result would be partially explained by the presence of larger water droplets in the systems with no added salt. The higher stability in presence of calcium salt could also be attributed to a lower attractive force between water droplets and a higher adsorption density of the emulsifier manifested by a lower interfacial tension. The stabilizing effect of the salt and the emulsifier was not attributed to the rheological properties of the interfacial film because its viscoelastic parameters were decreased with increasing calcium and PGPR concentrations. Higher stability was obtained with more dissociable calcium salts, while the presence of different chloride salts produced similar stability. The addition of salts allowed the production of stable w/o emulsions with higher water content.

This work contributes to the knowledge on the influence of the addition of electrolytes in w/o emulsions, which are a field not usually approached in colloid and interface science. The obtained results lead to the conclusion that calcium not only contributes its nutritional property, but it also acts functionally by allowing the obtaining of w/o emulsions with higher stability and lower emulsifier content. These systems could be utilized as lipid substitutes in the production of foods based on oil and/or fats, working as the dispersed phase for the formulation of w/o/w emulsions; thus, products such as reduced fat creams and mayonnaises highly fortified with calcium could be obtained.

Acknowledgments

The authors acknowledge the financial support from the Agencia Nacional de Promoción Científica y Tecnológica (FONCyT, PICTO 2006 – 36473), Universidad Nacional de Quilmes (Program I+D PUNQ 53/1007), and Plan de Desarrollo Tecnológico (PDT) del Ministerio de Educación y Cultura (Project S/C/O/66/12). J.R. Wagner and A.L. Márquez are researcher and fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), respectively; and L.A. Panizzolo and A. Medrano are researchers of the Sistema Nacional de Investigadores and Plan de Desarrollo de las Ciencias Básicas (PEDECIBA).

References

[1] H.F. Banford, K.J. Gardiner, G.R. Howat, A.F. Thomson, Confect. Prod. 36 (1970) 359.
[2] B. Schantz, H. Rohm, Lebensm. Wiss. Technol. 38 (2005) 41.
[3] R. Wilson, B.J. van Schie, D. Howes, Food Chem. Toxicol. 36 (1998) 711.
[4] D. Howes, R. Wilson, C.T. James, Food Chem. Toxicol. 36 (1998) 719.
[5] R. Wilson, M. Smith, Food Chem. Toxicol. 36 (1998) 739.
[6] M.R. Smith, R. Wilson, P.A. Hepburn, Food Chem. Toxicol. 36 (1998) 747.
[7] R. Wilson, M. Smith, Food Chem. Toxicol. 36 (1998) 743.
[8] Food and Drug Administration (FDA). GRAS Notice No. GRN 000179. CFSAN/Office of Food Additive Safety January 20, 2006.
[9] B. de Cindio, D. Cacace, Int. J. Food Sci. Technol. 30 (1995) 505.
[10] C. Laugel, P. Chaminade, A. Baillet, M. Seiller, D. Ferrier, J. Controlled Release 38 (1996) 59.
[11] M.L. Cole, T.L. Whateley, J. Controlled Release 49 (1997) 51.
[12] G.M. Tedajo, S. Bouttier, J. Fourniat, J.-L. Grossiord, J.P. Marty, M. Seiller, Int. J. Pharm. 288 (2005) 63.
[13] A.G. Appu Rao, M.S. Narasinga Rao, Cereal Chem. 52 (1975) 21.
[14] J.M. Whittinghill, J. Norton, A. Proctor, J. Am. Oil Chem. Soc. 77 (2000) 37.
[15] J.R. Wagner, M.C. Tomás, in: C.E. Lupano (Ed.), Functional Properties of Food Components, Research Signpost, Kerala, 2007, p. 23.
[16] P. Pathomrungsiyounggul, A.S. Grandison, M.J. Lewis, J. Food Sci. 72 (2007) 428.
[17] L.G. Pan, M.C. Tomás, M.C. Añón, J. Surfactants Deterg. 5 (2002) 135.
[18] A.L. Márquez, G.G. Palazolo, J.R. Wagner, Colloid Polym. Sci. 285 (2007) 1119.
[19] C.-J. Cheng, L.-Y. Chu, R. Xie, J. Colloid Interface Sci. 300 (2006) 375.
[20] J. Su, J. Flanagan, Y. Hemar, H. Singh, Food Hydrocolloids 20 (2006) 261.
[21] A. Fechner, A. Knoth, I. Scherze, G. Muschiolik, Food Hydrocolloids 21 (2007) 943.
[22] M. Bonnet, M. Cansell, A. Berkaoui, M.H. Ropers, M. Anton, F. Leal-Calderon, Food Hydrocolloids 23 (2009) 92.
[23] J. Lucassen, M. Van den Tempel, Chem. Eng. Sci. 27 (1972) 1283.
[24] D.J. McClements, Food Emulsions: Principles, Practice and Techniques, CRC Press, New York, 1999.

[25] C.I. Park, W.-G. Cho, S.J. Lee, Korea-Aust. Rheol. J. 15 (2003) 125.
[26] J. Israelachvili, Intermolecular and Surface Forces, second ed., Academic Press, London, 1992.
[27] M. Paunovic, M. Schlesinger, Fundamentals of Electrochemical Deposition, second ed., Wiley-Interscience, New York, 2006.
[28] M.P. Aronson, M.F. Petko, J. Colloid Interface Sci. 159 (1993) 134.
[29] S. Tcholakova, N.D. Denkov, I.B. Ivanov, B. Campbell, Adv. Colloid Interface Sci. 123–126 (2006) 259.
[30] D. Georgieva, V. Schmitt, F. Leal-Calderon, D. Langevin, Langmuir 25 (2009) 5565.
[31] E. Santini, L. Liggieri, L. Sacca, D. Clausse, F. Ravera, Colloid Surf. A 309 (2007) 270.