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Synergistic performance of lecithin and glycerol monostearate in oil/water emulsions

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ABSTRACT

The effects of the combination of two low-molecular weight emulsifiers (lecithin and glycerol-monostearate (GMS)) on the stability, the dynamic interfacial properties and rheology of emulsions have been studied. Different lecithin/GMS ratios were tested in order to assess their impact in the formation and stabilization of oil in water emulsions. The combination of the two surfactants showed a synergistic behaviour, mainly when combined at the same ratio.

The dynamic film properties and ζ-potential showed that lecithin dominated the surface of oil droplets, providing stability to the emulsions against flocculation and coalescence, while allowing the formation of small oil droplets. At long times of adsorption, all of the mixtures showed similar interfacial activity. However, higher values of interfacial pressure at the initial times were reached when lecithin and GMS were at the same ratio. Interfacial viscoelasticity and viscosity of mixed films were also similar to that of lecithin alone. On the other hand, emulsions viscosity was dominated by GMS.

The synergistic performance of lecithin-GMS blends as stabilizers of oil/water emulsions is attributed to their interaction both in the bulk and at the interface.

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1. Introduction

The formation of stable colloidal dispersions (emulsions, foams, etc.) is important in the food industry. To this end, the use of specific surface-active substances (emulsifiers) is required for their formation and stabilization. The stability and mechanical properties of emulsions depend on the way in which the constituent emulsifiers, low-molecular weight emulsifiers (LMWE) and biopolymers adsorb and interact at fluid interfaces. The physico-chemical properties of the surface-active molecules are of great interest because they determine the colloidal stability of emulsions [1]. The optimum use of the emulsifiers depends on the knowledge of their physico-chemical characteristics (such as surface activity, structure, miscibility, interfacial viscosity, etc.) and the kinetics of the film formation at fluid interfaces [2,3]. The distribution of the emulsifiers in emulsions is determined by the competitive and co-operative adsorption between them at the fluid-fluid interfaces and also by the nature of their interactions, both at the interface and in the bulk phase [4].

Phospholipids (lecithin) and monoglycerides (MG) are the most common examples of LMWE used in the stabilization of different food products [5], as they are very surface active compounds [6,7] that can rapidly cover the interface, by the Gibss-Marangoni mechanism [8]. The understanding of these phenomena is a key factor in the development of strategies for controlling food dispersions, formation and stabilization [2,3].

Phospholipids are ionizable emulsifiers which are needed to increase colloidal stability and provide interfacial interactions between food components [4,9,10], that are important factors to further improve emulsion stability and shelf life in many foods. These applications include traditional food formulations (such a bakery, confectionery or meat products, ice-cream, dressings, etc.) or new formulations (low fats and instant foods, high- or low-alcohol food formulations, functional foods, etc.). The increasing demand for low-fat products, convenience, instant, and functional foods has placed a greater importance upon the use of phospholipids as emulsifiers in foods. Thus, the production of industrial foods requires a complete understanding of the behaviour of emulsifiers used as processing aids, both at interface and in the bulk phases, at equilibrium and under dynamic conditions [11]. Phospholipids at interfaces have been reviewed by Pichot et al. [7].

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Phospholipid emulsifiers were proved to provide stability to emulsions by acting as both a mechanical and electrostatic barrier to coalescence [12]. Rydhag and Wilton investigated how the composition of PC affects the emulsion stability [13].

Due to its amphiphilic properties, MG are used to stabilize oil/water (O/W) emulsions. MG can develop different forms of liquid crystalline in these systems and structure the emulsions with modified properties (termed as structured emulsions) [14]. For this reason, MG are used in low-fat spreads, baked products (cakes and breads), creams and toppings (whipping and cream substitutes), and fat substitutes in low calories food, among others. Most of these food applications are related to the organoleptic and textural properties that MG confer to the final product [15].

Surfactants used in commercial applications typically consist of a mixture of surfactants because they can be produced at a lower cost than pure surfactants and because these mixtures can also exhibit better properties than each one alone [16]. Interactions between the adsorbed molecules of the emulsifiers could affect the structure, topography and dynamic characteristics (relaxation phenomena and viscoelasticity) of the mixed interfacial films [17,18]. The knowledge of the static (adsorption, structure, topography, etc.) and dynamic (dynamic surface tension and surface rheology) characteristics of surfactants mixtures adsorbed at the O/W interface is a key factor for the formation and stability of optimized food emulsions.

Emulsion structure, stability and rheology depend on the composition, thickness and viscoelasticity of the adsorbed stabilizing interfacial layer, as well as on the properties of the continuous phase. The interfacial rheology is a very sensitive technique to monitor the interfacial structure upon competitive adsorption and the magnitude of interactions between different emulsifiers at the interface [19,20]. Competitive adsorption can lead to different film compositions and structures, depending on the surface activity of each component and their concentration. When present at low subphase concentration, they can both adsorb at the interface as space is available. Nevertheless, at higher concentration, the most surface active component forming the most viscoelastic film would prevail in the film, at least initially [21].

The knowledge of the relationship between the interfacial properties of the surfactants and their emulsifying properties (emulsifying capacity and stability) is a very interesting challenge; nevertheless, the understanding of such relationship is not always simple.

The aim of the present work was to investigate the impact of the combination of lecithin and MG, specifically glycerol-monostearate (GMS), in the formation and stability of food emulsions. For both, pharmaceutical and food applications, attempts have been made to use mixtures of phospholipids and other emulsifiers to enhance emulsion stability. In this way, Klang et al. [22] evaluated submicron emulsions with mixtures of piroxicam and poloxamer to identify the optimal experimental conditions of its fabrication; Yu et al. [23] studied the formulation of intravenous emulsions with anti-tumor compounds in an O/W emulsion to achieve the administration of these compounds in anti-tumor therapy; Donsi et al. [24] evaluated the effect of the presence of two emulsifiers (lecithin and starch) with two different bioactive additives in order to design better nanoemulsion formulations and Pichot et al. [25] investigated the effect on the combination of different surfactant (Tween 60, lecithin and sodium caseinate) and their mixtures on the stability of emulsions. The observed improvements were attributed to the mixture of surfactants that, once adsorbed at the interface, resulted in a reduction of the dispersed phase droplet size.

The emulsifying characteristics of lecithin and GMS, as well as of lecithin/GMS mixed systems (evaluating different lecithin/GMS ratios), will be studied in the present work. To this end, parameters such as the formation and stability of the emulsions, as well as oil droplets size, ζ-potential and the viscosity of each emulsion will be determined. Finally, the relationship between these properties and the interfacial properties will be explored.

2. Materials and methods

2.1. Materials

Lecigran 1000P (de-oiled, powdered soy lecithin, a mixture of polar (phosphor- and glycol-) lipids and a small amount of carbohydrates, dispersible in water, soluble in fats/oils and partly soluble in ethanol. Corresponds with E-322 (EC regulation) for food additive: lecithin) was acquired from Cargill Texturizing Solution US, LLC (Decatur, United States) and a commercial GMS (glycerol ester of stearic acid, $C_{21}H_{42}O_4$) was purchased from Química Oeste S.A. (Buenos Aires, Argentina) [26]. A commercial chia oil (a valuable source of *ω-3 fatty acids*) without further purification was used as the oil phase in order to study the performance of lecithin and GMS in a real interface, because they are used without purification in the manufacturing of food products. Millipore water was used to prepare the different emulsions.

2.2. Emulsion preparation

The oil and lecigran/GMS solutions at total concentration of 1.8% (w/w) [27] were emulsified at a 10:90 O/W ratio. Lecigran and GMS were weighed, and the oil phase was added and stirred for 5 min with a vortex. Next, the water phase was added and the final solutions were pre-emulsified with an Ultra-Turrax for 3 min at 25000 rpm. Then, the samples were emulsified for 10 min using an ultrasonic processor Vibra Cell Sonics, model VCX 750 (Sonics & Materials Inc., Newtown, Connecticut, United States) at a frequency of 20 kHz and amplitude of 20%. A 13 mm tapered microtip was used to sonicate 10 mL of samples in a 15 mL glass tube reactor that was glycerine-jacketed at 60 °C with a thermostated bath (Polystat, Cole-Parmer) [28].

2.3. Droplet size determination

The particle size distribution of the emulsions was measured using a light scattering instrument. The droplet size of emulsions was measured using a Mastersizer 2000 with a Hydro 2000MU as dispersion unit, both from Malvern Instruments (Malvern Instruments, Worcestershire, United Kingdom). The emulsion was dispersed in recirculating water in the Hydro 2000MU with the pump speed set in 1800 rpm [29]. The refractive index (RI) of the dispersed phase (1.458) and its absorption parameter (0.001) were used in droplet size determination. The information about emulsion particle size was then obtained via a best fit between light scattering theory (Mie) and the measured light scattering pattern. The droplet size is reported as the volume-surface mean diameter or Sauter diameter ($D_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$) and the equivalent volume-mean diameter or De Broucker diameter ($D_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$), where n_i is the number of droplets of diameter d_i.

D_{32} was used to estimate the specific surface area of the emulsions and D_{43} was used to monitor the changes in the droplet size distribution. D_{43} is a surface diameter and is a parameter more sensitive than D_{32} to analyse the destabilization process (flocculation and coalescence) [30,31]. The interpretation of these two diameters will allow to analyse the stability of the different emulsions.

The droplet sizes were reported as the average and standard deviation of ten readings made on the sample. The measurements were carried out in duplicate of emulsion samples.

2.4. Interfacial measurements

All the interfacial experiments were carried out in an automatic drop tensiometer PAT-1 (Sinterface Technologies, Berlin, Germany). Emulsifier-oil solutions were prepared at 0.003% (w/w) that was a concentration equivalent to that used in emulsions formation (1.8%) considering the surface concentration resulting from total adsorption of the emulsifiers. Solutions were stirred at 60 °C for 30 min to ensure emulsifiers dissolution.

A water droplet was formed at the tip of a stainless steel capillary (constant volume: 12 μL) immersed in a glass cuvette filled with the emulsifier-oil solution. This cuvette is covered by a compartment which is thermostatized (37.0 ± 0.1 °C) by circulating water from a thermostat. Measurements were done until steady state adsorption was reached (around 180 min) [32]. The glass materials in contact with the solutions were properly cleaned in order to avoid any contamination by surface-active substances.

2.4.1. Dynamic interfacial pressure

Time-dependent interfacial pressure (π) of adsorbed lecigran, GMS and lecigran/GMS mixed films at the O/W interface was determined. The interfacial pressure is $\pi = \gamma^{\circ} - \gamma$, where γ° is the subphase interfacial tension (27 mN/m), in the absence of the emulsifiers, and γ the interfacial tension of solution at each time (θ). The interfacial tension (γ) was calculated thorough the analysis of the droplet profile [33]. The average accuracy of the interfacial tension is roughly 0.1 mN/m. However, the reproducibility of the results, for at least two measurements, was better than 1%.

2.4.2. Dilatational rheology

The interfacial viscoelastic parameters (interfacial dilatational modulus, *E*, and its elastic, *E′*, and viscous, *E″* E (iω) = −dπ/dlnA = E′ (ω) + iE″ (ω) (E′) and a loss part (E″). E′ is the interfacial elasticity and η (E″/ω) is the interfacial viscosity θ = arc tg (E″/E′) elasticity and η (E″/ω) were measured as a function of time. These parameters were obtained from the change in π induced by an interfacial area perturbation (sinusoidal interfacial compression and expansion, by decreasing and increasing the drop volume at a 3% deformation amplitude ($\Delta A/A$) and an angular frequency of 0.05 Hz) [32,34,35]. As indicated previously, the interfacial dilatational modulus derived from the change in π, resulting from a small change in the interfacial area (Eq. (1)) [36]

$$E(i\omega) = -d\pi/d\ln A = E'(\omega) + iE''(\omega) \quad (1)$$

where E (dilatational modulus) is a complex parameter, which is composed of a storage (E′) and a loss part (E″). E′ is the interfacial elasticity and η (E″/ω) is the interfacial viscosity, where ω is the angular frequency of the area modifications (ω = 2πf). The phase angle (θ) can be defined as: θ = arc tg (E″/E′).

2.5. ζ-potential measurements and particle size

ζ-potential of each emulsion was measured using a Dynamic Laser Light Scattering instrument (Potential Nano-ZS, Malvern Instruments, Worcestershire, United Kingdom) provided with a He-Ne laser (633 nm) and a digital correlator, Model ZEN3600. The emulsions were diluted with Millipore water at 1:1000 (emulsion:water), as described in Camino et al. [29]. The ζ-potential was determined by measuring the direction and velocity of the droplet movement when subjected to an applied electric field at 25 °C. ζ-potential values are reported as the average and standard deviation of measurements made on two samples, with ten readings made per sample. The size of lecithin, GMS particles and their mixtures in water were also determined by Dynamic Laser Light Scattering.

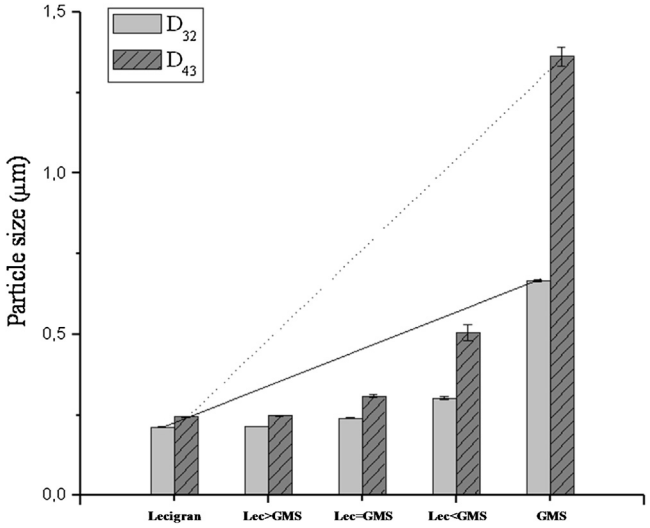

Fig. 1. D_{32} (μm) and D_{43} (μm) values of emulsions once formed. Errors bars represented the standard deviation (SD) of duplicates (n = 2).

For particle size distribution, the CONTIN algorithm [37] was used. The assay was performed in triplicate.

2.6. Viscosity analysis

Viscosity measurements of lecigran, GMS and mixed lecigran/GMS emulsions were carried out in a cone and plate (cone spindle CP-41) LVT Brookfield Viscometer at 25 °C at increasing and decreasing shear rates in the range 70–180 s^{-1}. Flow curves were fitted with the power law (Eq. (2)):

$$\tau = k \cdot \gamma^{n} \quad (2)$$

where τ is the shear stress, γ the shear rate, *n* the flow behaviour index and *k* the consistency index. Results were expressed according to the values of *k* and *n* calculated from the equation of the power law. Average values of duplicated assays were considered.

3. Results

3.1. Emulsion formation and stability

The role of emulsifiers, which are surface-active molecules that adsorb at the surface of freshly formed oil droplets during homogenization, is to facilitate further droplet disruption by lowering the interfacial tension, thereby reducing the size of the droplets produced during homogenization. Emulsifiers also reduce the tendency for droplets to aggregate by forming protective films and/or generating repulsive forces between the droplets. A good emulsifier should rapidly adsorb at the surface of oil droplets formed during homogenization, rapidly lower the interfacial tension by a significant amount and protect the droplets against aggregation during emulsions processing, storage, and utilization [38,39]. Emulsion instability is a complex process and may involve a combination of different mechanisms such as creaming or sedimentation, flocculation and coalescence.

Lecigran, GMS and lecigran/GMS mixtures were emulsified at 10:90 O/W ratio, using the same amount of total surfactant in all cases (1.8%) that ensured saturation of emulsions interface [40,41]. Different lecigran/GMS ratios, Lec > GMS (85:15), Lec = GMS (50:50) and Lec < GMS (15:85) (w/w), were tested in order to assess their impact in the formation and stability of the emulsions. Fig. 1 shows the values of the droplets size (D_{32} and D_{43}) for the lecigran and GMS O/W emulsions, as well as for their mixtures at different con-

centration ratios. Lecigran formed emulsions with a much lower droplet size than GMS as indicated by the D_{32} value. Lecigran emulsions once formed exhibited a D_{43} value (0.244 μm) similar to D_{32} value (0.212 μm). Contrarily, GMS emulsions exhibited a D_{43} value (1.363 μm) twice the D_{32} value (0.667 μm) according to its higher polydispersity. All the mixed emulsions showed droplets sizes (D_{32}) much lower than what would be expected from the simple mixing ratio of the two emulsifiers, being the size of droplets closer to single lecigran emulsion. Nevertheless, the Lec < GMS emulsion exhibited a higher degree of flocculation. Thus the presence of lecigran in the mixture favours the formation of smaller droplets, as well as a lower initial degree of flocculation.

The stability of emulsions after 21 days of storage at 25 °C was evaluated from the evolution of droplets sizes (data not shown). Destabilization parameters: % of coalescence and% of flocculation (Table 1) were calculated for each sample [31,42], based on the D_{32} and D_{43} values:

$$\%\text{ofcoalescence} = [(D_{32f} - D_{32i})/(D_{32i})] * 100 \quad (3)$$

$$\%\text{offlocculation} = [(D_{43f} - D_{43i})/(D_{43i})] * 100 \quad (4)$$

where D_{32i} and D_{43i} were initial values for the fresh emulsions and D_{32f} and D_{43f} were final values, measured 21 days after the emulsions formation.

When analysing these parameters (Table 1) it can be observed that lecigran emulsion exhibited some flocculation and coalescence. GMS emulsion exhibited even more coalescence and a very high degree of flocculation. The degree of coalescence of Lec > GMS was similar to single lecigran emulsion but with a decreased degree of flocculation. The Lec = GMS emulsion resulted even much more stable than lecigran emulsion as it showed half the degree of coalescence and a five times lower degree of flocculation. The Lec < GMS emulsion exhibited a degree of coalescence similar to GMS emulsion, but half the degree of flocculation. These results suggest that the presence of the lecithin inhibited the flocculation and coalescence even if present in a small proportion, improving the stability of the emulsions.

In an oil–water emulsion GMS will develop into a highly hydrated crystalline lamellar phase, covering oil droplets, and form a mesomorphic gel with some fat-like characteristics [43,44]. Compared to water gel and monoglycerides–oil solution, where monoglycerides crystallization commences soon after preparation, in emulsions of low oil concentration (10–30%) where the emulsion droplets are reduced to the sub-micrometer range due to the strong mechanical force during emulsification, the formation of GMS crystalline structure would start gradually after emulsion formation [6]. The crystals will pack in lamellar, hexagonal and orthorhombic styles, depending on storage temperatures and time. It seems that in O/W emulsions crystalline structure can exist both at interface and in oil phase [6]. The crystalline monoglycerides at the oil–water interface are connected from one droplet to another, causing droplets flocculation. In very concentrated systems it forms a continuous solid network giving the gel fat-like properties [45]. The presence of other emulsifiers as Tween or milk proteins, delays the crystallization of monoglycerides and the polymorphic transition of crystals [6].

Therefore, the strong decrease of flocculation and coalescence in the mixed emulsions may be attributed, at least partially, to the inhibition of time dependent crystallization of interfacial GMS in the presence of lecithin.

3.2. ζ-potential

The ζ-potential (Table 1) is a good parameter that may help to better understand the mechanism underlying the stability of the emulsions. A minimum ζ-potential value of ±30 mV is needed to obtain physically stable emulsions [46,47]. All the emulsions exhibited highly negative values of ζ-potential. The values for Lec = GMS and Lec > GMS emulsions were similar to the emulsions formed solely by lecithin (ζ-potential ≈ −53 mV) and they presented higher absolute values than GMS emulsions. So, it can be concluded that lecithin predominated in the surface of oil droplets, further contributing to their stabilization.

3.3. Viscosity

The flow behaviour of fresh emulsions was evaluated by plotting the shear stress (τ) as a function of the shear rate (γ) within 70–180 s^{-1} (data not shown). The rheological parameters (k and n) that were determined from the power law model equation (Eq. (2)) are shown as a function of GMS ratio in the emulsions, in Fig. 2(a, b). The GMS emulsion performed as a Newtonian fluid and exhibited a very low consistency coefficient (k). Mao et al. [6] also reported that GMS (1%) stabilized soybean oil emulsions show Newtonian behaviour.

Contrarily lecithin emulsion exhibited a high pseudoplasticity (n = 0.29) and consistency. Bhattachary et al. [48] also reported that lecithin oil in water emulsions exhibit pseudoplastic behavior. Phospholipids in o/w emulsions are adsorbed at the oil droplet surface forming a multilayer lamellar structure, with viscous behaviour [7]. Moreover, deoiled lecithin has been reported to promote a high emulsion viscosity as compared to other lecithins [49].

The factors that affect the rheology of emulsions are the viscosity of the continuous phase, the concentration and size distribution of droplets, deformability, internal viscosity and the nature of particle–particle interactions which are determined by the emulsifier. The differences in the rheological parameters of GMS and lecithin emulsions are mainly reflecting differences in interactions and in the particles structure. Moreover, the increase of the viscosity of the aqueous phase, through the formation of self bodying mesophase structures by the unadsorbed emulsifiers, may be also important.

Addition of GMS, even at a low ratio (Lec > GMS) or at equal ratio (Lec = GMS) induced a strong decrease of pseudoplasticity and of the consistency coefficient. The Lec < GMS emulsion exhibited rheological parameters similar to those of single GMS emulsion. Thus GMS mostly dominated the flow behaviour of mixed emulsions.

3.4. Interfacial pressure and viscoelastic properties for lecithin, GMS and mixed films at the oil-water interface

3.4.1. Interfacial activity

The behaviour of lecigran, GMS and lecigran/GMS mixtures at the O/W interface was analysed in order to assess the possible interactions that occur between the surfactants, thus allowing a better explanation of emulsions behaviour.

Fig. 3 shows the interfacial pressure (π) evolution with the adsorption time for lecigran, GMS and lecigran/GMS mixed films. The behaviour of π is associated with the adsorption of the surfactants to the O/W interface. At long adsorption times, a final value of $\pi \sim 24$ mN/m was obtained for GMS, that resulted slightly higher than that obtained for the lecigran film ($\pi \sim 22$ mN/m). Higher differences were observed at short adsorption times where lecithin adsorbed faster than GMS.

At shorter adsorption times the interfacial activity of the Lec > GMS and Lec < GMS mixed films was dominated by the lecithin, as a similar trend in the increase of π with time was observed. Nevertheless higher initial values of π, even higher than those observed for single emulsifiers, were reached when the lecigran and GMS were present at the same ratio (Lec = GMS), that reveal a cooperative adsorption behaviour between the surfactants.

Table 1
Degree of coalescence and flocculation (%) for the different emulsions, and ζ -potential values. Error values of Z-potential represent the standard deviation (SD) of triplicates (n = 3).

Emulsion	Lecigran	GMS	Lec > GMS	Lec = GMS	Lec < GMS
% Coalescence	22.64	41.68	24.77	10.00	44.70
% Flocculation	47.54	2171.24	36.03	9.74	1300.99
Z-potential (mV)	−52.7 ± 0.6	−45.9 ± 0.5	−53.4 ± 0.7	−52.8 ± 0.4	−42.9 ± 0.5

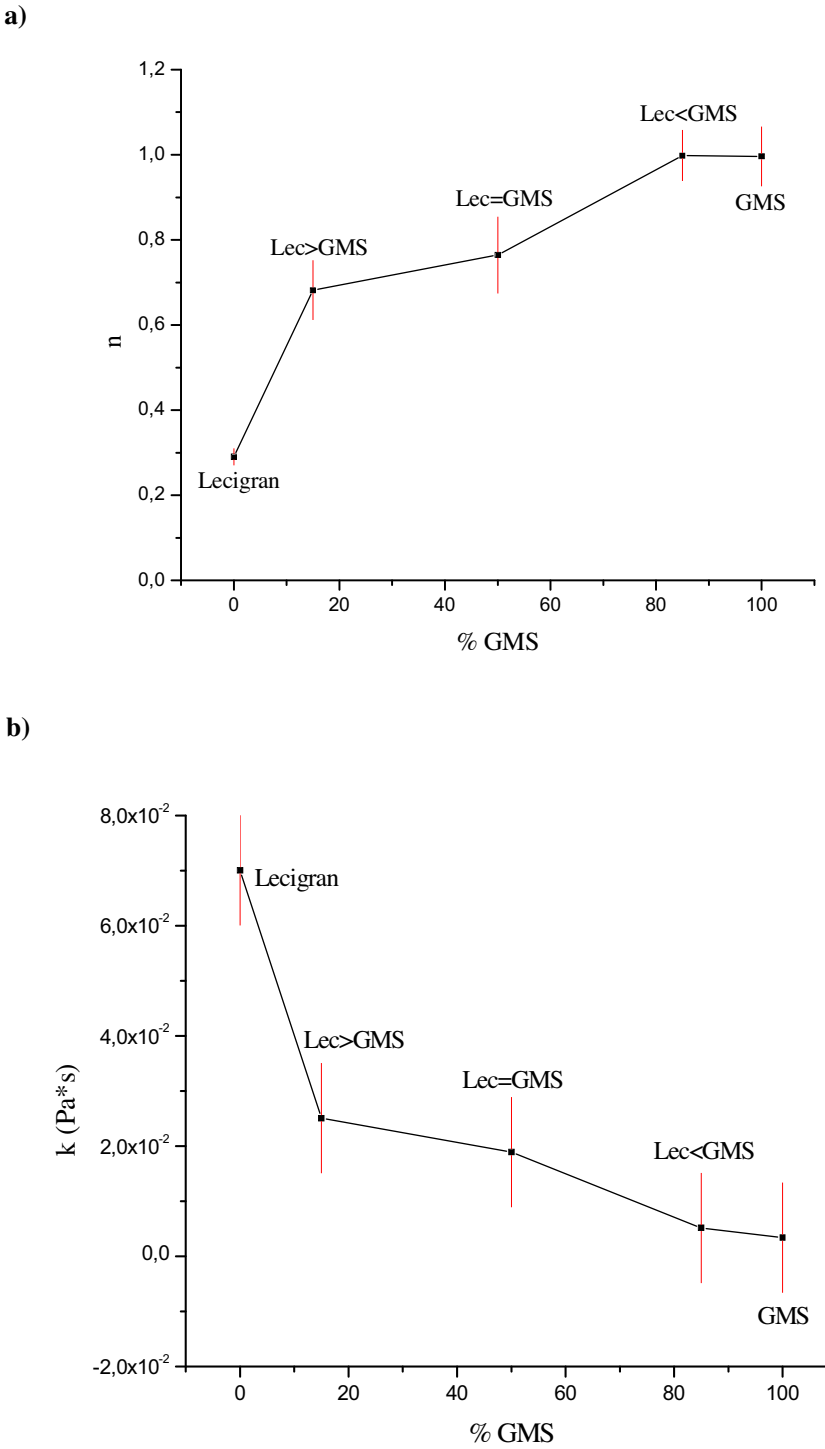

Fig. 2. (a) Effect of surfactant ratio on the emulsion behaviour (Newtonian or pseudo-plastic), and (b) Effect of surfactant ratio on the consistency coefficient (*k*) of emulsions. Errors bars represented the standard deviation (SD) of duplicates (n = 2).

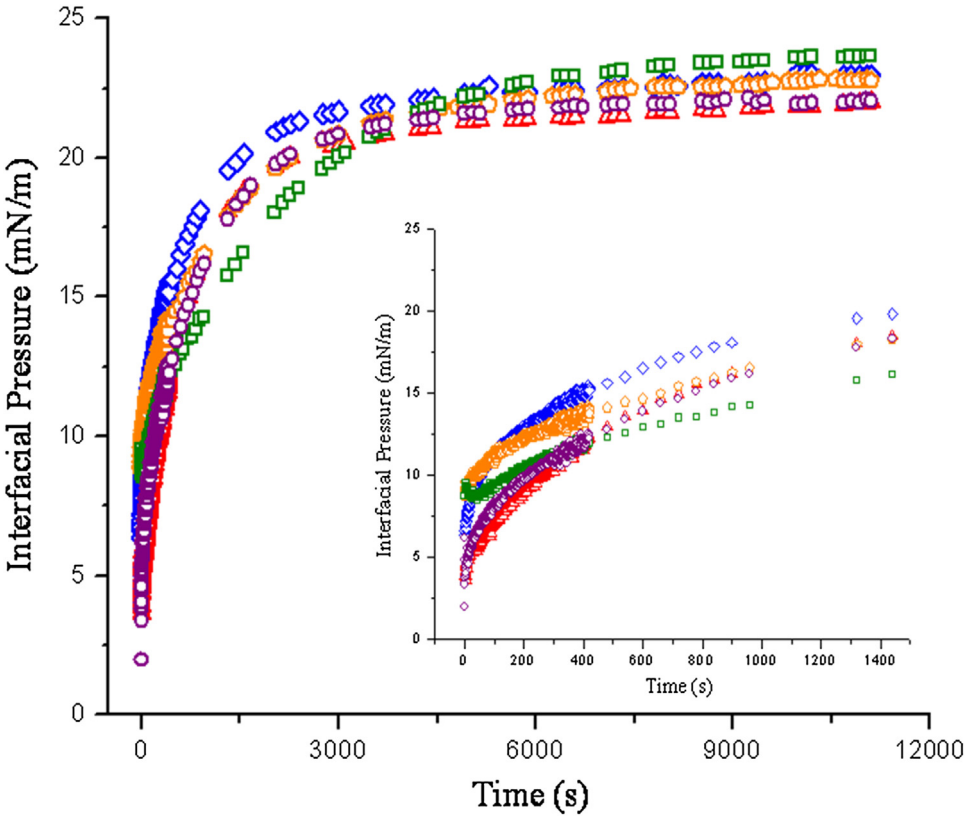

Fig. 3. Surface pressure (π) versus the time (s) for lecigran, GMS and lecigran/GMS mixed films at the oil-water interface at 37 °C. Symbols: (□) GMS, (○) lecigran, (Δ) Lec > GMS, (◊) Lec = GMS and (⌓) Lec < GMS.

During competitive adsorption between both surface active molecules at high bulk concentrations (as is the case), the surface pressure is initially controlled by the component which adsorbs more rapidly (lecithin) and then by GMS [21]. Moreover, the competitive adsorption of mixed lecithin/GMS systems, may be affected by their interaction in the bulk phase.

In order to determine the adsorption rate of these components, the following empirical equation was used to fit the curves in Fig. 3 (Eq. (5)):

$$\pi - \pi_0 = \Delta\pi_\infty t/(B + t) \quad (5)$$

where π and π_0 are the interfacial pressure at the time t and at t = 0, respectively; $\Delta\pi_\infty$ is the final interfacial pressure, t is the time and B is the time required to reach half $\Delta\pi_\infty$ [50].

Fig. 4 shows the correlation between the surfactants composition and the time required to reach half $\Delta\pi_\infty$. The higher the B value the lower the rate of adsorption. Lecigran adsorbed at the O/W interface about ten times faster than GMS that can be related to its ability to form initial smaller oil droplets in the emulsion (Fig. 1). For all the mixed systems, B values were lower than those expected from the mixing ratio of the single components, showing that the adsorption rate is dominated by the lecithin.

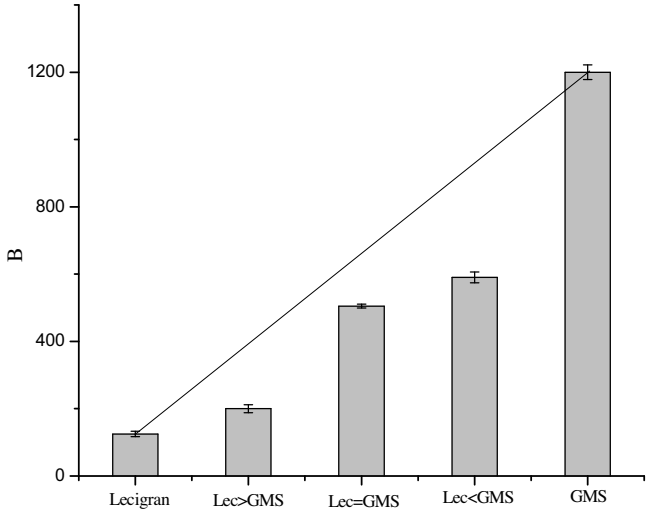

Fig. 4. Correlation between surfactant ratio and time required to reach half $\Delta\pi_\infty$ (B). Errors bars represented the standard deviation (SD) of duplicates (n = 2).

3.4.2. *Interfacial dilatational rheology*

The evolution of several interfacial dilatational parameters with time is shown in Fig. 5. All the interfacial films showed a rapid increase of phase angle and interfacial viscosity, indicating the occurrence of rapid interactions between the adsorbed components at the O/W interface. The viscoelasticity of the lecigran film (Fig. 5a) was lower than that of GMS film, as shown by the higher phase angle value for lecigran. All the mixed films, even that with the smaller lecigran concentration, showed a viscoelasticity similar to single lecigran film.

Fig. 5b shows that the interfacial viscosity of the lecigran film was higher than that of GMS film, and the mixed films showed viscosities closer to lecigran film. These results suggest that lecigran also dominated the viscosity of the mixed interfacial films.

Film rheology can affect the stability of the emulsions [51], as higher viscoelastic films may be more resistant to the coalescence of the oil droplets, therefore increasing emulsion stability [52]. Nevertheless, GMS, that formed a film with significant higher

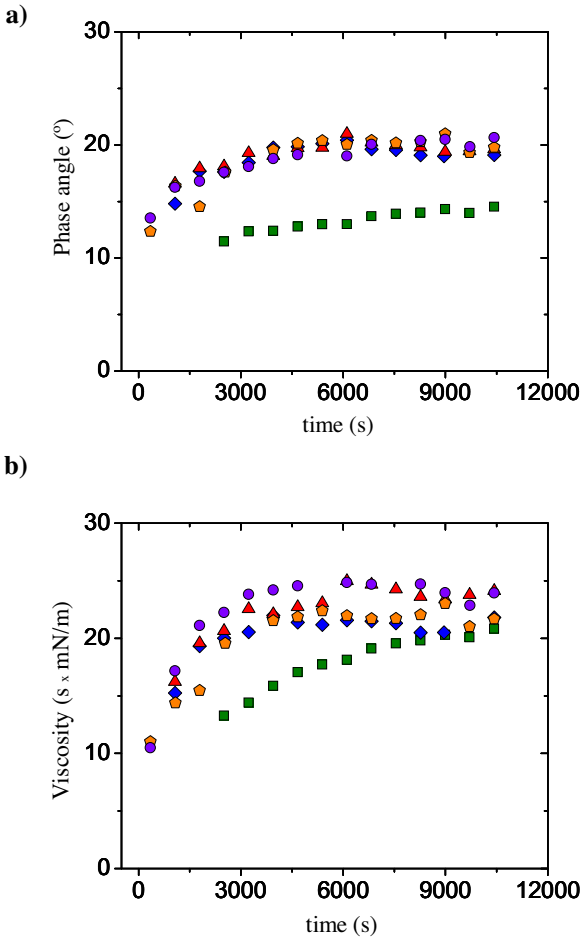

Fig. 5. Time evolution of (a) phase-angle (°) and (b) viscosity for lecigran, GMS and mixed films at the oil-water interface at 37 °C. Symbols: (□) GMS, (○) lecigran, (Δ) Lec > GMS, (◊) Lec = GMS and (⌂) Lec < GMS.

viscoelasticity (low phase angle values), formed the more unstable emulsions against coalescence (Table 1), showing that other factors, such as surface charge, steric factors and bulk viscosity further affect the emulsions stability [53]. In fact, lecigran emulsions exhibited higher ζ-potential values and lower initial droplets size that could account for by its higher stability.

3.4.3. Lecithin-GMS interactions in the bulk

In order to get a deeper understanding of possible interactions between lecithin and GMS, the size of particles in water dispersions were determined for single components and for the Lec = GMS mixture. As shown in Fig. 6, lecithin dispersed in water by ultrasounds, formed particles of 100 nm (they ranged between 20 and 300 nm). Lecithin in water creates vescicles (700–1000 nm) where it self-organizes into liquid crystalline structures consisting of several bimolecular layers. The size of the vescicles may be further modified by ultrasounds treatment that can decrease their size. GMS formed in water particles of 200 nm (ranging from 40 to 500 nm). GMS can also self-assemble into different lyotropic liquid crystalline structures in water [6].

In the mixed system (Lec = GMS) the particle size distribution is similar to single GMS indicating that lecithin vescicles are mostly disintegrated by the presence of GMS which suggests an interaction between the two emulsifiers. According to Almgren [54] surfactants added into an aqueous solution containing lecithin vescicles promoted disintegration of the lamellar structure, resulting in a formation of mixed micelles. Nevertheless this is a smooth transition

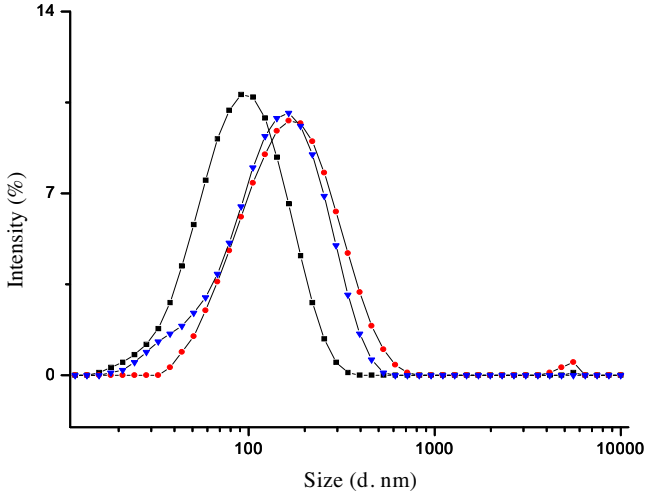

Fig. 6. Size particle of lecigran, GMS and lecigran/GMS mixtures dispersed in water. Symbols: (■) lecigran, (●) GMS and (▼) lecigran/GMS mixture.

that commences when a saturation of lamellae with the adsorbed surfactant is achieved.

4. Discussion

The dynamic film properties and ζ-potential of emulsions showed that lecithin dominated the surface of oil droplets, providing to the emulsions stability against flocculation and coalescence, while allowing the formation of small oil droplets. A synergism on the increase of interfacial pressure at short adsorption times was observed when lecithin and GMS were present at the same ratio. The mixture with the same lecigran/GMS ratio also formed the most stable emulsion against coalescence and flocculation, showing low oil droplets diameters. On the other hand, emulsion viscosity was dominated by GMS.

The synergistic performance of lecigran and GMS mixtures to form and stabilize oil/water emulsions, mainly at equal ratio, is consistent with the formation of mixed structures in the bulk and at the interface. The obtained results (dynamic film properties and ζ-potential of emulsions) suggest that at the interface, GMS would be pushed to the oil phase and lecithin would prevail at the surface of oil droplets. As GMS is more hydrophobic (HLB 3.8) than lecithin (HLB 7), when forming the emulsion (from the emulsifiers dissolved in the oil phase), GMS could remain mostly in the oil phase and lecithin in the surface interacting with water throughout phosphocholine head groups.

The origin of synergy between lecithin and GMS may arise from hydrophobic interactions between the hydrophobic tails and hydrogen bonds between hydrophilic groups of both molecules. Whenever the kind of interactions deserve future studies, the main roles of the phospholipid in these mixed structures would be: (a) to enhance the rate of interfacial adsorption favouring the formation of emulsions with a low oil droplet size, (b) to enhance the charge of oil droplets, thus improving electrical stabilization (c) to inhibit GMS interfacial crystallization that promotes flocculation and coalescence of emulsions, (d) to increase emulsion viscosity, thus enhancing stability.

On the other hand, GMS could (a) anchor lecithin in the interfacial film. Surfactants exist in dynamic equilibrium between the O/W interface and the continuous phase. Surfactants with relatively high water solubility can desorb from one droplet, diffuse through the aqueous phase, and adsorb onto a different droplet exposing bare interfacial regions on the droplets, promoting their coales-

cence [55] and (b) increase the structuring of the oil layer involved in the interfacial film, thus increasing its resistance to coalescence.

In conclusion, the synergism between lecigran and GMS at the same ratio could be used in the formulation of new emulsions with an outstanding stability. Nowadays, there is an important interest in the formulation of delivery systems of bioactive compounds, that could be improved their transport inside the gastrointestinal tract and inside the bloodstream, increasing their bioactive effect [56]. As biocompatible and cheap, this blend may have industrial impact.

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