

Linseed oil miniemulsions stabilized by a polyelectrolyte complex (gum Arabic-chitosan)

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ABSTRACT

An linseed oil miniemulsion was improved using the microfluidization process and stabilizing by a polyelectrolyte complex. Variables such as gum Arabic/oil ratio (GA/O), oil volume fraction (ϕ), homogenization pressure and steps of homogenization were studied. The results show that microfluidization is an effective process to produce primary emulsions characterized by monomodal distribution between 440 and 826 nm and ζ potential values of -12.1 and -44.6 mV. Afterwards, a selected emulsion was titrated with a chitosan (Ch) solution. The droplet size and charge of the secondary emulsion (multilayer) changed from 585 to 428 nm and -34 to $+56$ mV with the increase of Ch concentration. A bridging flocculation process was observed in a biopolymer mass ratio ($R_{GA/Ch}$) of R_5 , because the net charge was close to zero. This study provides useful information on the formation of miniemulsion with droplet size, and charge specific which may increase the rate of lipid digestion.

Keywords: miniemulsions, polyelectrolyte complex, gum Arabic, chitosan.

1 INTRODUCTION

Today we know that fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer [1]. Linseed oil contains 55% of α -linolenic acid (Omega-3) and 16% of linoleic acid (Omega-6) [2]. Commonly, these fatty acids are marketed as emulsions, and their therapeutic efficacy depends on their appropriated design. An emulsion is a mixture of two immiscible liquids, in which one liquid is dispersed in the form of droplets in another liquid that forms the continuous phase. Based on the emulsion droplet size, emulsions can be divided into micro- (10–100 nm), mini (nano)- (100–1000 nm) and macro-emulsions (0.5–100 μm) [3]. Recently, developments in droplet microfluidics enable the generation of monodisperse and size-controlled single/multiple emulsion droplets [3-5]. These are frequently used as a precursor to fabricate particles or capsules for industrial applications [5]. Actually, polyelectrolyte complexes (PEC) membranes are

widely used as potential carrier systems in the development of new products (emulsions, capsules, or gels), because these provide specific flow behaviors, textures, and appearances, tactile and oral properties [6]. PEC formation is observed when the two oppositely charged polyelectrolyte are mixed in aqueous solution under accurate conditions [6, 7].

Gum Arabic (GA) is one of the world's most common gums, widely used in the production of beverage and flavor emulsions and meal replacers. Recently, it was reported the PEC formation between GA and chitosan (Ch) [8]. The aim of this study was to develop and characterize linseed oil emulsions stabilized by a polyelectrolyte complex (gum Arabic/chitosan).

2 METHODOLOGY

2.1 Materials

Gum Arabic (GA) and linseed oil were purchased in Natural Products of Mexico SA de CV (Morelos, Mexico) and chitosan (Ch, degree of deacetylation 92.2%) in Sigma Aldrich (State of Mexico, Mexico).

2.2 Emulsions

To obtain a fine emulsion with desirable properties (lower particle sizes and higher electric charge), twenty emulsions were produced using an M110 PS microfluidizer (Microfluidics, USA) according with a 2^4 factorial design with 4 central points: Pressure: 34.5 to 206.8 MPa, steps (cycles) 1 to 5, oil volume fraction (ϕ) 0.05 to 0.1, and GA/O ratio 1 to 3. Coarse emulsions were prepared using a high shear dispersion equipment (Silverson, England) at 5000 RPM for 10 minutes. The charge and size of the emulsions were evaluated.

2.3 ζ potential

The ζ -potential was determined using a light scattering equipment Zetasizer Nano ZS90 (Malvern Instrument, UK). The emulsions were diluted in desionized water (1/100) to make the appropriate measurements. The ζ potential was calculated using the Smoluchowsky mathematical model

measuring the direction and velocity of the emulsions as they moved along the applied electric field at 20°C.

2.4 Average particle size and distribution

The hydrodynamic size (Z-average diameter, d_h) and distribution of the emulsions was estimated using a Zetasizer Nano ZS90. The d_h was calculated by the Stokes–Einstein equation: $d_h = k_B T / 3\pi\eta_s D$; where k_B is the Boltzmann constant, T is the absolute temperature, η_s is the dynamic viscosity of the solvent and D is the z-average translational diffusion coefficient.

2.5 Colloidal titration

One selected emulsion was used to obtain the multilayer emulsions. A titration experiment was performed adding a Ch solution (10 mg/mL) into a 10 mL of the emulsion (1/100) above the isoelectric point of the system [8]. The charge and the d_h of the system was determined. The titration measurements are reported as the average \pm standard deviation of measurements made on three independent samples with three measurements made per run at 20 °C.

2.6 Formation of multilayer emulsions

Electrodeposition technique layer by layer [9] was used for the formation of multilayer emulsions. Based on the charge of the emulsions (cationic, neutral, and anionic), three selected biopolymers mass ratios ($R_{GA/Ch}$) were prepared. Similarly, the charge and d_h of the emulsions were monitored.

2.7 Statistics

All data were reported as mean values and standard deviations of three measurements. Statgraphics Centurion XV version 2.15.06 was used for analysis of variance with a confidence level of 95%.

3 RESULTS

3.1 Microfluidization

All factors (ϕ , GA/O, pressure and steps) showed a significant effect ($p > 0.05$) on the d_h and charge of the emulsions.

3.1.1. Particle size and distribution

The pre-emulsions showed a d_h up to 1500 nm. In general, the d_h of the emulsions decreased and a monomodal distributions with hydrodynamic droplet sizes between 440 and 720 nm was observed. These emulsions are classified as “mini-emulsions” and showed a better stability to gravitational separation and aggregation than

conventional emulsions. Into the microfluidizer chamber, the emulsion droplets are subjected to high shear and collision that reduce the average size of the emulsion allowing a narrow droplet size distribution [10].

A d_h decrement was observed with the increment GA/O ratio and with the decrement of the ϕ (Figure 1). Higher emulsifier concentrations were required to produce a kinetically stable emulsion against coalescence and flocculation [10].

When the pressure of microfluidization increased from 34.5 to 206.8 MPa, an increased of the d_h and distribution of particle sizes were observed. This is known as over-processing of the emulsion, at it is related to the phenomena of coalescence and rupture of some droplets during extreme conditions inside the microfluidizer chamber. Similar results were reported by Mahdi Jafari, He [11]. They found that the optimum conditions to produce fine emulsions of *d*-limonene with whey protein isolated by microfluidization were moderate pressure (42-63 MPa) and 2 cycles.

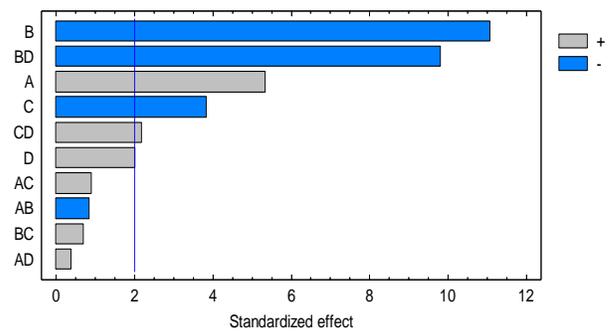


Figure 1. Standardized Pareto chart of d_h ($p > 0.05$): A (ϕ), B (GA/O), C (cycle) & D (pressure).

3.1.2. ζ potential

In general, droplets with ζ potential outside the range -30 mV to +30 mV are considered stable to the flocculation [12]. The charge of the pre-emulsion droplets was around -14.6 mV. After microfluidization steps, the ζ potential of the emulsions decreases in some cases significantly showing values between -12.1 and -44.6 mV. A positive effect of the GA/O ratio was observed, an increase of the amount of emulsifier, the particle size decreases, increasing the electrostatic repulsion between the emulsion droplets (Figure 2). Meanwhile, an increase of the ϕ , increase the particle size of the emulsion, decreasing the repulsive force between oil droplets. This behavior may favor the flocculation and coalescence of droplets [13]. Finally, an increase in the pressure and cycle causes a decrease in the net charge of the system, possibly due to an increase of the collisions between particles. Similar results were obtained by Salvia-Trujillo, Rojas-Graü [14] in the formation of

microfluidized emulsions of essential oil of lemongrass with alginate.

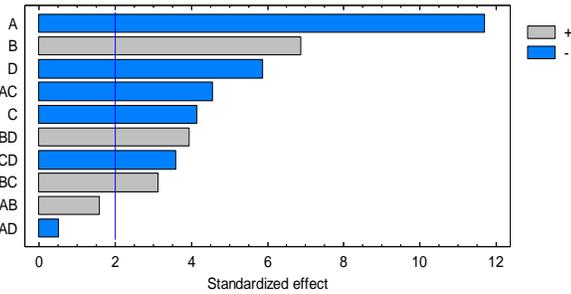


Figure 2. Standardized Pareto chart of ζ potential ($p > 0.05$): A (ϕ), B (GA/O), C (cycle) & D (pressure).

3.2 Formation of multilayer emulsions

In the electrodeposition technique layer by layer, anionic emulsifier is used to produce a “primary” emulsion containing small droplets (anionic), then an oppositely charged polyelectrolyte is added to the system that adsorbs to the droplet surfaces and produces a “secondary” emulsion containing droplets coated with a two-layer interface (cationic) [9].

Based on the results, an emulsion with 5% disperse phase, GA/O 3:1, 2 cycle at 172 MPa was produced. Figure 3 shows the evolution of the ζ potential and d_h as the primary miniemulsion (anionic) was titrated with the Ch (cationic) dispersion.

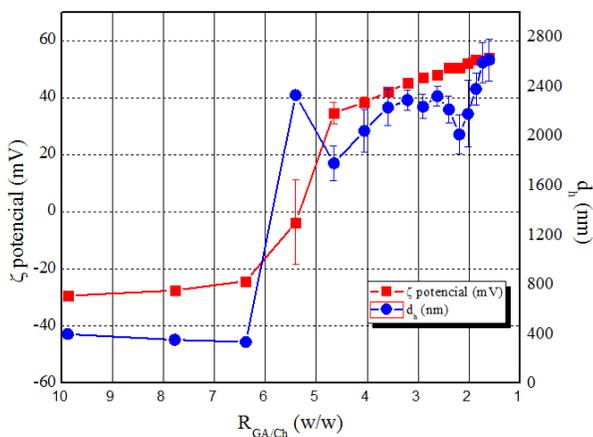


Figure 3. Evolution of the ζ potential (■) and d_h (●) as the emulsion was titrated with the chitosan.

In biopolymers ratio greater than R_{10} , the miniemulsions were decorated with Ch molecules, the electric charge of the systems keep the droplets without flocculation, but the d_h decrease significantly from 573.2 ± 11.8 to 432.6 ± 4.6 nm with the increase of Ch concentration.

At a $R_{GA/Q} \sim 5$ a stronger electrostatic interaction between biopolymers favors that, the Ch segments are adsorbed on multiple adjacent miniemulsions, bridging them together to form a complex coacervation miniemulsion. As a consequence, the d_h increase abruptly and the net charge close to zero. At a relatively high biopolymers ratio ($>R_{6.5}$), the net charge of the miniemulsion trends to +50 mV. However, through the flocculation process a highly viscous coacervate emulsion was formed inside the cuvette, therefore the d_h values are not trustworthy at all. Similar behavior was observed in the biopolymer alone by Espinosa-Andrews, Enríquez-Ramírez [8].

Based on these results, three miniemulsions with different charge were prepared (Table 1): cationic (R_{20}), neutral (R_5), and anionic (R_1).

Biopolymer matrix	ζ potential (mV)	d_h (nm)
GA	-33.5 ± 0.2^a	573.2 ± 11.7^a
R_{20}	-21.3 ± 0.4^b	554.5 ± 6.2^b
R_5	0.4 ± 0.4^c	–
R_1	55.0 ± 1.5^d	432.6 ± 4.5^c

^{a, b, c, d} The mean values (\pm SD, $n = 3$) that share the same letter in the same column are homogeneous groups ($p > 0.05$) according to Fisher LSD test.

Table 1. Physicochemical parameters of multilayer miniemulsions.

The ζ potential results agree with the titration results. In R_{20} , Ch addition decrease significantly the charge and d_h of the miniemulsion, without macroscopic phase separation. In R_5 , a complex coacervation miniemulsion was formed, the net charge was close to zero and a high flocculate viscous emulsion was observed. Finally, R_1 did not show phase separation, it was characterized by a cationic emulsion droplet, where the d_h decreased as a consequence of PEC formation.

Droplet charge approached to electroneutrality at the biopolymers coacervation, negatively increased with a GA excess and positively with a Ch excess that inhibiting bridging flocculation by keeping the droplets emulsion separated by electrostatic effects. If the droplets in an emulsion have a positive charge in the small intestine, they may also become trapped within the mucous layer (which consists of anionic biopolymers) [15]. Therefore, positive miniemulsion may improve bioavailability of lipophilic components. In general terms, the average droplet size of the secondary miniemulsion decreased with increasing the Ch content. It is known that the bioavailability of lipophilic functional components encapsulated is increased when the droplet size is decreased. Some possible reasons discussed in the literature are that small droplets have a large surface area and may therefore be digested faster by digestive enzymes so that their contents can be released and absorbed easily. The small droplets may penetrate into the mucous

layer, thereby increasing their residence time and bringing them closer to the site of absorption. The water-solubility of highly lipophilic components increases as the droplet size decreases, which may enhance absorption [15].

CONCLUSIONS

Microfluidization technique was useful for producing linseed oil miniemulsion. Gum Arabic/oil ratio, oil volume fraction, homogenization pressure and cycles of homogenization showed a significant effect ($p > 0.05$) on the Z-average diameter and charge of the miniemulsions.

The biopolymers mass ratio between gum Arabic/chitosan showed significant influence ($p > 0.05$) on the hydrodynamic size and ζ potential due conformational changes caused by the attractive interaction between the macromolecules. Polyelectrolyte complexes of gum Arabic and chitosan were attributed special features to miniemulsions that can be manipulated for specific applications as carriers for bioactive lipid.

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