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Preparation of Ethyl Cellulose Nanoparticles by Solvent-Displacement Using the Conventional Method and a Recirculation System

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Abstract. Ethyl cellulose polymeric nanoparticles (NPs) were prepared using the solvent-displacement technique with ethanol as the solvent. Optimization of the method included evaluating stirring rate and stabilizer type. NPs of 142.1 to 226.5 nm were obtained in a reproducible and efficient way (95% process efficiency) and with good stability (at room temperature). Moreover, a recirculation device was used in order to obtain concentrated NPs dispersions by a continuous process with potential scale-up. This method was challenged to encapsulate a hydrophilic antiviral model molecule (glycyrrhizinic acid) resulting in low entrapment efficiencies (approximately 1%).

The results indicate that NPs are obtained using this simple, economical process that offers the possibility to transport different agents for applications in food-processing, cosmetics production or pharmaceutical products.

Key words: ethyl cellulose, polymeric nanoparticles, solvent-displacement method, optimization, recirculation device.

Resumen. Se prepararon nanopartículas poliméricas (NPs) de etilcelulosa por la técnica de desplazamiento de disolvente usando etanol como disolvente. La optimización del método incluyó evaluación de la velocidad de agitación y el tipo de estabilizante. Se obtuvieron NPs de 142.1 a 226.5 nm en un modo reproducible y eficiente (95% eficiencia del proceso) y con buena estabilidad (a temperatura ambiente). Además, se utilizó un dispositivo de recirculación con la finalidad de obtener dispersiones concentradas por un proceso continuo con potencial de escalamiento. Este método se retó para encapsular una molécula hidrofílica antiviral modelo (ácido glicirricínico) teniendo como resultado bajas eficiencias de encapsulamiento (aproximadamente 1%).

Los resultados indican que se obtienen NPs de etilcelulosa por este método simple y económico que ofrece la posibilidad de transportar diferentes agentes para su aplicación en alimentos, cosméticos o productos farmacéuticos.

Palabras clave: Etilcelulosa, nanopartículas poliméricas, método de desplazamiento de solvente, optimización, dispositivo de recirculación.

Introduction

For at least the past three decades, polymeric NPs have been extensively studied as carriers for drugs and other substances. The preferred polymers are biodegradable, but some non-biodegradable ones also have important pharmaceutical characteristics that can be exploited according to the route of administration.

In 1987, Fessi et al., developed and patented a method to prepare nanoparticles called solvent-displacement, or nanoprecipitation [1]; which has the advantages of being simple, economical, reproducible and fast [2, 3]. Moreover, the amounts of organic solvent and surface-active agents needed are small, and the resulting particles are also small, generally with narrow polydispersity indexes. Furthermore, mechanical or thermal stress is minimal because prolonged shearing/stirring times, sonication, or very high temperatures are not required, which lowers energy consumption. Additionally, solvents with low toxic potential could be used [1, 2, 3].

In general, this method requires two miscible phases: one solvent, the other non-solvent. The polymer and the drug selected are dissolved in the solvent phase (eg. acetone, ethanol, methylene chloride, etc.), while a stabilizer is incorporated into

the non-solvent phase (usually water). The injection of the organic phase into the aqueous phase under magnetic stirring leads to immediate formation of NPs due to displacement of the solvent. The pre-formed polymers most commonly used in this technique are biodegradable polyesters (eg. poly (ε-caprolactone), poly (D-lactic-*co*-D-glycolic) acid) and, to a lesser extent, cellulose derivatives; however, the first are quite expensive. Another option to form polymeric nanoparticles is ethyl cellulose, a water-insoluble, non-biodegradable, ether cellulose polymer that forms strong, tough films with good adhesion at low concentrations [4].

Different studies have proposed using ethyl cellulose as the matrix polymer to formulate drug NPs. Tachaprutinun et al. (2009) [5] attempted to encapsulate astaxanthin in ethyl cellulose NPs using the solvent-displacement method through a dialysis bag, but their results were disappointing, since using this modality to obtain NPs makes process scale-up very difficult. A high-loading encapsulation of six fragrances was achieved with a polymer blend (ethyl cellulose, hydroxypropyl methyl cellulose and polyvinyl alcohol) using the solvent-displacement method. Because all the fragrances were lipophilic, high encapsulation efficiency was achieved; but, once again, the dialysis bag system was an important drawback in terms of obtaining and encapsulating the oils efficiently [6]. Arias et al. [7] developed a magnetic colloid with iron in the core of the particle, surrounded by a polymeric shell of ethyl cellulose. The process followed to prepare the magnetic NPs was emulsion solvent evaporation. In addition, two methods of drug-loading were studied: addition and adsorption. High drug-loading was obtained with both methods, but the main drawback of this research was the use of benzene and decane in the process, since both substances are toxic (ICH Class 1).

On the other hand, Piñón-Segundo et al., [8] proposed a novel recirculation system (Fig. 1b) to prepare poly(ε-caprolactone) NPs by solvent-displacement to obtain dispersions with high polymer concentrations. This device represents a considerable industrial advantage and offers the possibility of scaling-up the solvent-displacement method, while also helping to improve efficiency and avoid polymer-aggregation as the ethanol diffuses. The device they designed permits constant changes of water after first contact with the organic solution.

In the literature, there are two mechanisms to explain NP formation by this technique. The first one proposed that the interfacial turbulence at the interface of the solvent and non-solvent phases (which are governed by the Marangoni effect) [3] together with diffusion and flow phenomena, lead to polymer aggregation from stabilized emulsion droplets. As the two solvents are mutually miscible, when they are mixed a violent spreading occurs because of the difference in surface tension between them. Thus, a constant formation of eddies of solvent at the interface of the two phases causes the polymer present in the solvent droplets to aggregate and form NPs as a consequence of the lack of a non-solvent medium and continuous solvent diffusion which are rapidly stabilized by the surfactant [2, 9, 10, 11]. Fig. 1a shows the process of nanoparticle formation using this mechanism.

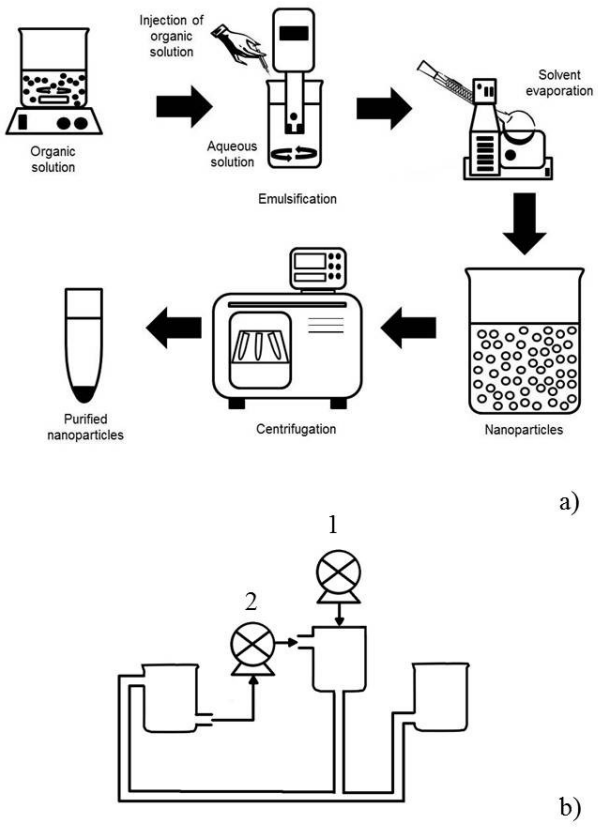

Fig. 1. Solvent-displacement method to obtain Ethocel^{TM} nanoparticles. a) conventional method; b) recirculation system; pump 1 controls the organic solution injection rate (ethyl cellulose/ethanol), and pump 2 controls the recirculation rate.

More recently, some publications have explained nanoparticle formation based on the "ouzo effect" [12, 13], which occurs in a ternary system composed of a hydrophobic solute, a solvent, and a non-solvent; specifically in the so-called ouzo region between the bimodal (miscibility-limit) and spinodal (stability-limit) curves, called the metastable region where the hydrophobic solute is introduced rapidly to cause super-saturation and then nuclei formation [3, 12, 14]. The small particles that form then increase in size due to Ostwald ripening (nucleation and growth process). In this process, polymer molecules act as the hydrophobic solute molecule with the ability to grow and generate polymeric NPs (Fig. 2). This is why droplet or particle size will depend on the polymer concentration in the organic solution [15]. On the right side of the ouzo boundary (spinodal curve) the polymer precipitates spontaneously because of solution instability and large fluctuations in the solute concentration, yielding macroscopic aggregates [16].

One issue with the solvent-displacement technique is that it is limited to water-miscible solvents and, as a result, only drugs that are soluble in these solvents can be incorporated. Then, one of the goals of this research was to encapsulate a model drug (specifically, glycyrrhizinic acid, GA, in its non-ionized form) by the solvent-displacement method to challenge this system which is usually used to carry lipophilic

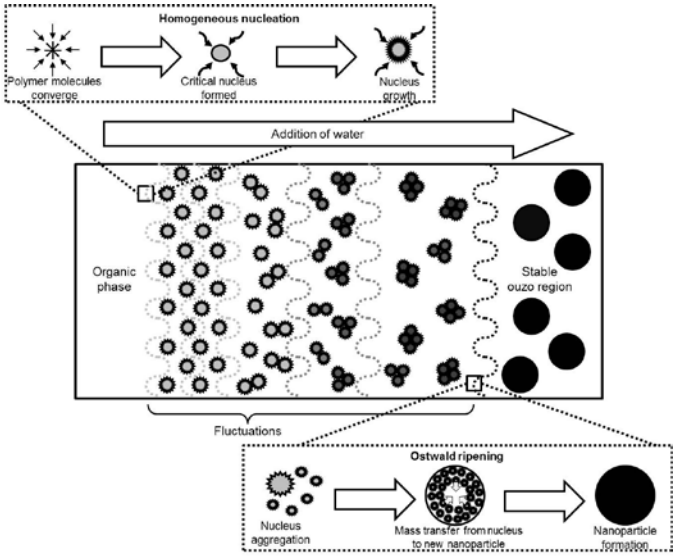

Fig. 2. Mechanism of the formation of nanoparticles explained by the ouzo effect.

drugs, but holds the appealing potential of being able to transport water-soluble drugs as well.

GA is a water-soluble molecule comprised of a hydrophilic part (two moieties of glucuronic acid) and a hydrophobic fragment (a moiety of glycyrrhetinic acid, known as the aglycone moiety) (Fig. 3) [17]. The acid form is easily soluble in alcohol (methanol and ethanol). This drug is a weak acid with five hydroxyl and three carboxyl groups and presents three pK_a values (pK_{a1}= 2.76; pK_{a2}= 2.81; pK_{a3}= 4.71) [18]. FDA has included this molecule in the list of GRAS (*Generally Recognized as Safe*) substances [19]. For many years, glycyrrhizinic acid (GA) has been used as anti-ulcer, anti-inflammatory, anti-tumor, and anti-viral [17, 20] and is the most important saponin of licorice root.

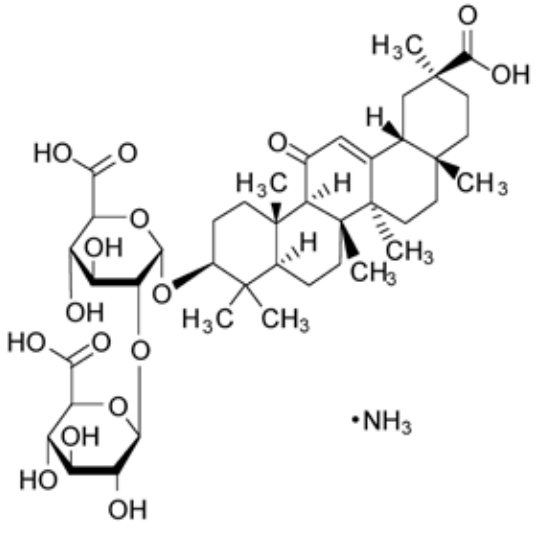

Fig. 3. Molecular structure of glycyrrhizinic acid (GA) (ammonium salt).

Finally, the main objective of this research was to produce NPs of ethyl cellulose in a reproducible way by the solvent-displacement technique using ethanol as solvent, and following the conventional method or the recirculation system, in order to determine if NPs can be formed efficiently and with the desired characteristics of size and electrical charge. The selection of this particular polymer (Ethocel^{TM} Std 4 Premium or Std 10 Premium FP) was based on its lower cost compared to biodegradable polymers; also, since it is pharmaceutical grade, it is already approved and accepted worldwide; furthermore they present different viscosity ranges (3-5.5 and 9-11 cP), which is a feature that impacts nanoparticle formation. The use of ethanol as a solvent has additional advantages, since it is classified as ICH Class 3, has low toxic potential, and is relatively inexpensive and polar, which could allow it to encapsulate polar drugs. Therefore, the solvent-displacement technique proposed in this research is a simple, economical, one-step process for producing nanoparticles. Stirring rates and stabilizer type were examined as the main preparative variables. The recirculation system was evaluated in order to obtain a continuous process which helps produce concentrated nanoparticle dispersions. No homogenization process was required in either case, which means additional savings in energy and other costs for industrial applications.

Results and Discussion

As expected, mean particle size was inversely- proportional to stirring rate: *i.e.*, the higher the stirring rate, the smaller the particle size (Fig. 4).

The reduction in particle size at higher velocities is explained due to the increase in kinetic energy on the solvent front that caused a higher degree of polymer/solvent-droplet dispersion in the aqueous phase, thus reducing local saturation of the polymer/solvent droplets in the aqueous phase [3].

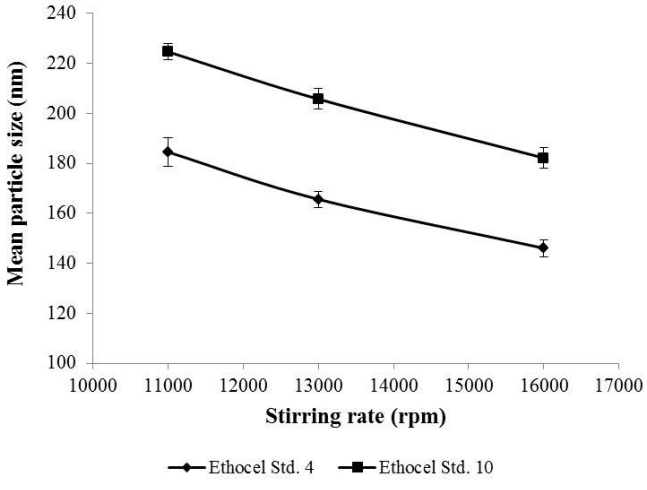

Fig. 4. Mean particle size of batches prepared with Ethocel^{TM} Std 4-PVAL at 5% w/v and Ethocel^{TM} Std 10- SLS at 2% w/v as a function of stirring rate. The bars show the standard deviation, n= 3.

Table 1. Mean particle size and Z-potential of Ethocel™ Std 4 and Ethocel™ Std 10 NPs obtained by the conventional method.

Batch	Ethocel™ Std 4, PVAL at 5%						Ethocel™ Std 10, LSS at 2%					
	11,000 rpm		13,000 rpm		16,000 rpm		11,000 rpm		13,000 rpm		16,000 rpm	
	MPS (nm)[a]	Z-Pot (mV)[a]	MPS (nm)[a]	Z-Pot (mV)[a]	MPS (nm)[a]	Z-Pot (mV)[a]	MPS (nm)[a]	Z-Pot (mV)[a]	MPS (nm)[a]	Z-Pot (mV)[a]	MPS (nm)[a]	Z-Pot (mV)[a]
1	191.3 ± 3.9 [0.254]	-27.9 ± 0.5	168.6 ± 1.2 [0.201]	-28.1 ± 1.7	149.4 ± 1.2 [0.214]	-29.7 ± 0.1	226.5 ± 2.4 [0.168]	-48.1 ± 1.0	209.8 ± 2.0 [0.235]	-48.0 ± 0.8	183.9 ± 1.7 [0.326]	-50.3 ± 0.7
2	180.1 ± 1.1 [0.183]	-27.9 ± 0.6	166.8 ± 1.1 [0.213]	-28.2 ± 1.4	142.7 ± 2.7 [0.230]	-29.8 ± 0.2	226.3 ± 1.8 [0.177]	-48.2 ± 1.0	202.1 ± 1.5 [0.208]	-48.2 ± 0.6	177.7 ± 4.2 [0.196]	-50.1 ± 0.7
3	182.3 ± 2.3 [0.249]	-28.0 ± 0.6	161.7 ± 0.5 [0.156]	-28.3 ± 2.0	146.1 ± 2.2 [0.175]	-29.6 ± 0.2	221.2 ± 2.4 [0.123]	-48.1 ± 1.3	205.5 ± 4.5 [0.312]	-48.0 ± 0.6	185.0 ± 1.2 [0.269]	-50.3 ± 0.5

[a] Reported as mean ± standard deviation; n = 3. Numbers in square brackets indicate polydispersity indexes.

With all stirring rates it was possible to obtain sub-micron sizes for both polymers; ranging from 180-226 nm, 160-209 nm, and 142-185 nm at 11,000, 13,000 and 16,000 rpm, respectively (Table 1); with no need for a homogenization process (a factor of economic interest for industry as it allows energy and money savings).

According to the mechanism of particle formation due to interfacial turbulences (Marangoni effect), when globules of the emulsion are finer and homogeneous, the polymer-saturated region becomes thinner; thus facilitating the stabilization and formation of smaller particles [21, 22, 23]. The process of mixing the organic phase that contains the polymer with the aqueous phase is an important factor that strongly impacts final particle size.

We found that the particle size obtained with Ethocel™ Std 4 was smaller than that produced with Ethocel™ Std 10 at the same stirring rates. This can be explained by the higher viscosity achieved with the latter due to the higher number of ethoxy groups in its structure. The higher the viscosity of the polymer solution, the lower the solvent diffusion rate, but the NPs formed from the turbulent flow of the solvent will be larger [3, 23, 24]. Upon analyzing the "ouzo effect", we found that Ostwald ripening has a great impact on final particle size, mainly due to the diffusive transport of dissolved matter through the dispersion medium. Thus, it is to be expected that in more viscous solutions the rate of diffusion will be lower and will exert an effect on final nanoparticle size. In addition to the viscosity of the phase containing the polymer, that of the solvent *per se* also plays an important role in nanoparticle formation.

According to some experiments, increasing the rate of diffusion allow obtaining smaller particles. In this regard, upon studying three different solvents (acetone, acetonitrile and tetrahydrofuran, with diffusion coefficients in water of 1.28, 1.26 and 1.08 $x10^{-5}$ cm^2/s, respectively) size decreased in the following order: acetone < acetonitrile < tetrahydrofuran [3] for PLGA nanoparticles. Ethanol has a diffusion coefficient of 1.21 $x10^{-5}$ cm^2/s but, as can be seen in Table 1, some batches would fix in size between the sizes reported using acetonitrile and tetrahydrofuran. However, size depends not only on diffusion velocity but also, as explained above, on the polymer concentration and stirring rate [3, 22].

Two types of stabilizers were tested, PVAL and SLS with both it was possible to obtain stable dispersions with Ethocel™ Std 4 and PVAL at 5% w/v and Ethocel™ Std 10 and SLS at 2% w/v. Two different concentrations of SLS were tested (1.5% and 2% w/v), and the best results were obtained with 2% of SLS, because an absence of agglomerates or sediment characterized these systems during at least 15 days. Also, particle size was under 300 nm. According to the mechanism of NP-formation under the method used, when solvent-displacement occurs because of the miscibility between ethanol and water, droplets of the solvent are rapidly stabilized by the surfactant when they are torn from the solvent's interface. Hence, if there are more molecules of the stabilizing agent at the interface, the resulting particle will be protected against aggregation more efficiently than in a system that contains a lower concentration of stabilizer [22, 23]. SLS is an ionic surfactant, while PVAL is non-ionic. In this respect, SLS exerts its function through charge repulsion, while PVAL acts as a barrier among NPs [9, 23]. Quintanar-Guerrero et al. concluded that PVAL chains are strongly-attached to the NP surface, forming a stable layer which exerts sufficient steric stabilization-producing dispersions that remain stable for long periods of time [25].

For practical purposes, the following batches were prepared at a constant stirring rate of 11,000 rpm because while particles of nanometric size were obtained at all stirring rates tested (Table 1), the separation procedure proved to be quite difficult with smaller sizes as they required longer ultracentrifugation times.

Table 1 shows the results of mean particle size and Z-potential for Ethocel™ Std 4 and 10 NPs. Z-potential suggests that stable dispersions were obtained with SLS at 2% w/v (Table 1) because the value of those batches was above $|30$ mV$|$ [26]. Colloids in aqueous media present an electrical charge, and their stability is determined by adding the contribution of van der Waals attractive forces and electrical double-layer

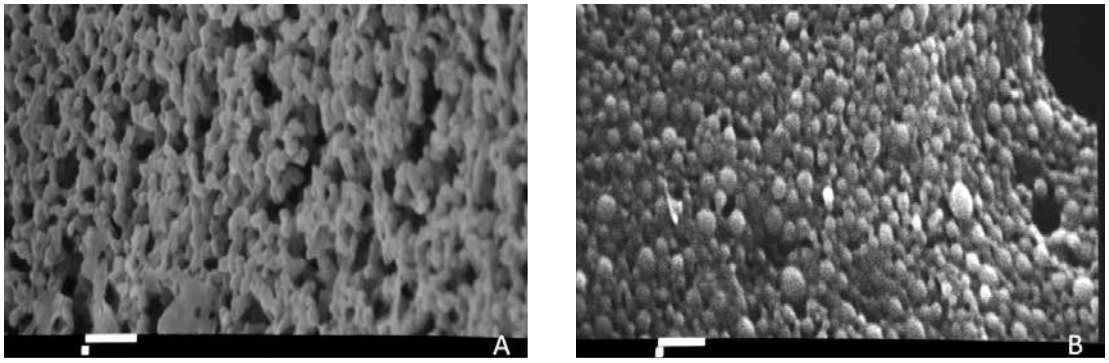

Fig. 5. Scanning electron micrographs of: A) Ethocel™ Std. 4 Premium-PVAL at 5%; and, B) Ethocel™ Std. 10 Premium FP-SLS at 2% (bar = 1 μm).

repulsive forces. SLS is an anionic surfactant that provides a negative charge to the nanoparticle which acts as an energy barrier that prevents two particles from adhering or coalescing. Batches with PVAL had a Z-potential close to 30 mV. Although these values are not as high as those obtained for NPs with SLS, they are also considered stable due to the steric repulsion that PVAL exerts, as discussed above.

With respect to mean particle size, findings indicate that the particles obtained were of the desired size, and these results were corroborated by micrographs (Fig. 5). Microscopic observation revealed spherical particles with a diameter of 250 nm that corresponded to the results obtained by dynamic light-scattering. SEM also revealed spherical particles with a solid matrix structure, but no crystal formation on the surface.

In the conventional solvent-displacement method and in the recirculation system the same polymer and stabilizer concentrations in the organic phase were used. However, in the recirculation system, consecutive injections of 20 mL of organic solution were carried out. The mean sizes obtained with the recirculation system were very similar to those produced by the conventional method (ranged from 160 to 197 nm for both polymers) (Table 2). It is important to point out that after the third injection of the organic phase into the aqueous phase of the device; particle size did not change; suggesting that polymer aggregation take place in the aqueous phase with no deposition on the dispersed particles. This behavior changed with the ensuing injections, when size increased quickly to micrometer range. The recirculation of the aqueous phase into the device allows to continually changing the water having first contact with the organic solution during the process of nanoparticles preparation. For that reason, recirculation system would avoid a possible saturation of the medium as ethanol diffuses, so the process of nanoparticle formation would be more efficient than the traditional process, and a higher quantity of polymer would be transformed into nanoparticles. In this case, the maximum concentration of nanoparticles dispersion was 70 mg/ml.

The batches prepared with the drug increased their particle size (227 nm and 262 nm for Ethocel™ Std 4 and 10, respectively), while Z-potential remained virtually identical (no statistically-significant difference, $p < 0.05$). Additionally, the freeze dried NPs were easily re-suspended and showed a physical stability for 15 days at room temperature.

However, our attempt to encapsulate GA in its non-ionized form resulted in low entrapment efficiency for both polymers (approximately 1%), as determined by HPTLC, *High Performance Thin Layer Chromatography* (data not shown). The reason of modifying the ammonium salt of GA to obtain the molecular form (neutral and more hydrophobic) of it obeys to the fact that as having any electrical charge on the molecule, it will be more easily entrapped. The non-ionized form of GA was obtained under strong acidic conditions, this means at pH values lower than the first pKa of the molecule ($pKa_1 = 2.76$) [18] and has the same therapeutic properties as the ionized form. In contrast, our group encapsulated 4.3% of the same drug using the double-emulsion technique [21]. This was due to the high solubility of the drug in the aqueous phase, which led to diffusion from the water-miscible organic phase into the external aqueous phase. We are currently attempting to enhance drug entrapment by decreasing water-drug solubility through the formation of an ion-pair between GA and a salt which acts as a counter-ion under certain conditions, such as pH and the

Table 2. Mean particle size and Z-potential of NPs prepared with Ethocel™ Std 4 and Ethocel™ Std 10 using the recirculation system.

Batch	Ethocel™ Std 4-PVAL at 5%		Ethocel™ Std 10- LSS at 2%	
	Mean particle size (nm)[a]	Z-potential (mV)[a]	Mean particle size (nm)[a]	Z-potential (mV)[a]
1	159.8 ± 2.4; [0.892]	-27.3 ± 0.649	197.2 ± 1.5; [1.894]	-49.8 ± 0.736
2	160.3 ± 1.4; [0.267]	-29.6 ± 0.910	195.2 ± 0.7; [0.734]	-48.6 ± 0.866
3	160.8 ± 1.4; [1.329]	-26.8 ± 0.472	194.9 ± 1.8; [1.211]	-49.3 ± 0.614

[a] Reported as mean ± standard deviation, n=3. Numbers in parentheses indicate polydispersity indexes.

concentration of this salt. Preliminary results show an improvement in this characteristic.

Ethyl cellulose NPs were obtained using the solvent-displacement method that has the advantages of being a fast and economical technique that employs an affordable, non-toxic polymer. The conventional method and the recirculation system produced NPs successfully; the latter with the attractive potential for scale-up. Additionally, the recirculation system permits the continue production of NPs, the polymer is completely transformed into NPs with no apparent aggregation, the efficiency would be higher than the conventional method, the polidispersity indexes are lower resulting in more homogeneous particle sizes and the scaling-up for an industrial application is simple. Results showed that it is possible to obtain submicronic dispersions up to a maximum concentration of 70 mg/ml without aggregation (size less than 200 nm and Z-potential indicating good stability) for both polymers used. To the best of our knowledge, this is the first time that ethyl cellulose (Ethocel^{TM}) NPs have been obtained with good physical and chemical characteristics by this method. The attempt to encapsulate a hydrophilic model drug resulted in very low encapsulation; however, these carriers may have a wide variety of applications in such areas as food-processing, cosmetics production and pharmaceutics. Also, they can be used as a coating or film-forming material.

Experimental

Materials

Ethyl cellulose polymers (Ethocel^{TM} Std 4 Premium and Ethocel^{TM} Std 10 Premium FP) (Fig. 5) were donated by Colorcon de México, S. de R.L. de C.V. Poly(vinyl alcohol) (PVAL) with a molecular mass of 31,000 (Mowiol® 4-88) was obtained from Hoechst (Frankfurt-am-Main, Germany). Sodium lauryl sulphate (SLS) was provided by Hycel de México, S.A. de C.V. Glycyrrhizinic acid (GA, ammonium salt, ≥95% purity) was purchased from Sigma Aldrich (St. Louis, MO, USA). Absolut Ethanol ACS was supplied by Fermont (Monterrey, Mexico), and the distilled water was obtained from a Milli-Q station (Milli-Q, USA). All other reagents were at least of analytical grade and used without further purification.

Glycyrrhizinic acid in its non-ionized form

Briefly, 5 g of ammonium salt of GA was dissolved in distilled water under magnetic stirring. Then a solution 0.1 N of HCl was added until the formation of a rigid gel. After removing the water from this gel in an oven at 50ºC, the precipitate was filtered and washed with distilled water. Finally, a fine white powder was obtained after drying in a desiccator.

Nanoparticle preparation using the solvent-displacement method

The conventional solvent-displacement method was used [5, 6] to prepare the NPs. An initial screening of the main parameters that commonly have impact on NP formation was performed as the first step of the study. The studied parameters were stirring rate, time of stirring, type and concentration of stabilizer. Briefly, 400 mg of Ethocel^{TM} Std 4 Premium or Std 10 Premium FP were dissolved in 20 ml of ethanol. When the model drug was included, 30 mg were dissolved in this phase. This solution was placed in a syringe and injected into 40 ml of a solution of the stabilizing agent during 4 min under high-speed stirring at room temperature (Ultraturrax® T25, IKA; NC, USA). Finally, the residual ethanol was eliminated by vacuum distillation at 25ºC and 70 mmHg. The remaining stabilizer and un-encapsulated drug was then removed by ultracentrifugation at 35,000 rpm for 40 min (Beckman® Optimal LE-80K, CA, USA).

To determine the effect of the process conditions, NPs were prepared at different stirring rates: 11,000, 13,000, and 16,000 rpm, with a high-speed stirrer (Ultraturrax®, T25, IKA, NC, USA) and two types of stabilizers, PVAL (5% w/v) and SLS (1.5% and 2% w/v). Batches were prepared in triplicate.

Freeze-drying of some batches was performed after removing the excess of stabilizer in a Freezone 6 (Labconco®, United Kingdom) lyophilizer for 24 h at -40 ºC and 100 x 10^{-3} mbar.

Recirculation system

The device used was the one proposed by Piñón-Segundo [16] (Fig. 1b). The organic/aqueous phase ratio and polymer and stabilizer concentrations were kept at the same proportions used in

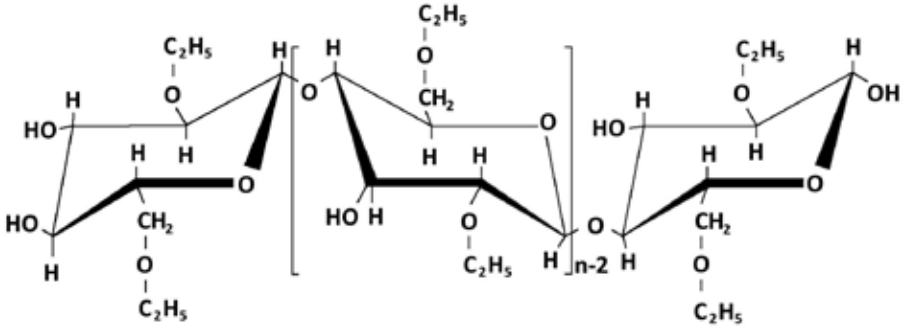

Fig. 6. Chemical structure of ethyl cellulose.

the conventional solvent-displacement method. The organic phase was injected directly into the circulating distilled water at a fixed injection rate of 25 ml/min, using a Masterflex® L/S 7518 pump (Cole Parmer, USA), and the recirculation rate at 55 ml/min was controlled by a Watson Marlow 502 S pump (New Brunswick Scientific, USA). Successive injections of the organic solution were poured into the circulating aqueous phase (three times in total), allowing solvent evaporation between each injection at room temperature. NPs and polymer aggregates were recovered in the recirculated water and were then separated using a mesh (US Std No. 100) and dried in a desiccator until reaching a constant weight.

Particle size analysis

Measurements were performed in triplicate using the dynamic light-scattering technique (Coulter N4, CA, USA) at a 90ºC fixed-angle for 180 s at 25ºC. The laser light wavelength (He/Ne, 10 mW) was set at 678 nm. A digital correlator was used to analyze the scattering intensity data under a unimodal analysis mode.

Z-potential

The electrophoretic mobility of the dispersions was measured and then transformed into Z-potential in triplicate by applying the Smoluchowski approximation (Malvern Instruments NS ZEN 3600, Worcestershire, UK) at 25ºC in a capillary cell. Samples were analyzed in triplicate.

Scanning electron microscopy

After removing the excess stabilizer from the sample by two centrifugations (30,000 rpm/50 min) and following re-suspension in distilled water, a few drops of the dispersion were placed on a glass coverslip and dried at room temperature. The dried samples were then coated with gold (~20 nm thickness) using a Sputter Coater JFC-1100 (JEOL, Tokyo, Japan).

Entrapment efficiency

In order to evaluate the performance of the proposed method, a highly hydrophilic drug (glycyrrhizinic acid, solubility in water = 1 mg/ml) was encapsulated in NPs by both processes. Once lyophilized, 20 mg of the nanoparticles were added to 5 ml of ethanol and stirred for 24 h. This sample was filtered (Millipore® 0.22 μm), and the filtrate was assayed by HPTLC [11]. Fifty microliters of the filtrate were applied on a normal phase plate by means of an Automated TLC Sampler III (ATS3, CAMAG, Muttenz, Switzerland) and developed with a mobile phase composition of ethyl acetate-methanol-acetic acid-water (67:8:8:17). The chamber was equilibrated with 10 ml of mobile phase for 15 min prior to inserting the plate. Then the plate was scanned and measured in the absorbance-reflection mode at λ= 254 nm with a densitometer (CAMAG TLC-Scanner 3, Muttenz, Switzerland). Results were interpolated in a calibration curve which was linear within a range of 56-280 ng, R^2= 0.998. Entrapment efficiency expressed as a percentage was calculated from the percent of drug-loading [amount of drug in NPs x 100/amount of NPs] x 100, divided by the percent of initial drug content in the formulation.

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