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Polymeric colloidal systems containing ethionamide: preparation and physico-chemical characterization

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The association of ethionamide with different colloidal systems was evaluated. Nanocapsules (NC), nanospheres (NS), and nanoemulsions (NE) were prepared by interfacial deposition and spontaneous emulsification techniques. Ethionamide was incorporated before (B) and after (A) preparation of nanoparticles. Ethionamide was assayed by HPLC, the particle size was determined using a Nanosizer®, and the zeta potential using a Zetasizer® 4. Free ethionamide was determined using a combined ultrafiltration-centrifugation technique. The drug release was determined by direct dilution of the nanoparticle dispersion in phosphate-buffer pH 7. All preparations retained acceptable particle size distribution (±300 nm), except the NE. The zeta potential of all formulations was between −36.6 mV and −46.1 mV. Percentages of ethionamide associated were: NC (B: 62.4%, A: 56.2%), NS (B: 53.0%, A: 43.2%), and NE (B: 38.5%). After 45 days, the percentage of drug association with NC increased (B: 66.8%, A: 60.6%). The release profiles demonstrated that associated ethionamide was more readily released from the NC and NS prepared by procedure A rather than B. The ethionamide amount not released (B) was greater in NS than NC. The drug is mainly adsorbed onto the surface of nanoparticles. However, approximately 10% of ethionamide is encapsulated into NC and 20% entrapped into NS, respectively.

1. Introduction

During recent years, colloidal drug delivery systems have attracted considerable interest due to their potential to achieve sustained drug release and to deliver drugs to specific target sites [1]. Colloidal drug carriers involve mainly submicron emulsions, nanoparticles, liposomes and lipid complexes [2]. Nanoparticles, polymeric submicron solid carriers of nanometric size, is a general name used to describe nanospheres and nanocapsules [1]. Nanocapsules have a polymeric wall enveloping a liquid core (oil), whereas nanospheres consist of a polymeric matrix. In general, biodegradable nanoparticles can be prepared by different methods: by polymerization of dispersed monomers [3, 4], by emulsification-diffusion [5, 6] or by precipitation of preformed polymers [7]. The last is a very simple and easy method for the preparation of colloidal systems, which can be employed to obtain nanocapsules, nanospheres and submicron emulsions. Investigations have been made on nanoparticles prepared by this method containing different drugs such as indomethacin [8], metipranolol [9], diclofenac [10], primaquine [11], itraconazole [12] and cyclosporin A [13]. The quantitative determination of drug associated with the colloidal systems may be by an ultracentrifugation technique [14, 15] or by a combined ultrafiltration-centrifugation technique [8, 10, 16]. In general, it is assumed that the difference between of the total amount of drug in the system and the free drug in the clear supernatant (after ultrafiltration) is the amount of drug incorporated into the nanoparticles. According to the literature, drug molecules may be dissolved in the polymeric matrix of nanospheres, encapsulated into nanocapsules or adsorbed on to the surface of nanoparticles [17]. However, few authors have studied the mechanism of the association of drugs with colloidal systems [11, 12, 18, 19].

In the present study, nanocapsules and nanospheres containing the tuberculostatic drug ethionamide, were prepared. In order to elucidate the mechanism by which this drug is associated with nanoparticles, the release profiles of ethionamide-loaded polymeric colloidal systems prepared by two different methods of drug incorporation were compared. The influence of the polymer on the percentage of drug association was evaluated by comparison between nanoparticles and nanoemulsions.

2. Investigations, results and discussion

2.1. Particle size and zeta potential

The results of particle size analysis, shown in Table 1, indicate that the mean sizes of the NC, NS and NE systems are in a sub 400 nm range. In the presence of ethionamide, similar results were obtained for NC and NS (B or A). The preparation NE (B) showed coalescence of oil droplets after 45 days of storage. NE (A) was not prepared because the structure of submicron emulsions (micelles) is very different from that of polymer colloids (nanoparticles) [18] and permeation of drug through the surfactant layer is possible.

Regarding zeta potential measured after 15 days of storage (Table 1) all formulations had a negative charge between -36.6 ± 0.5 mV and -46.1 ± 0.5 mV. The surface charge of NC, NS and NE was just slightly affected by the presence of ethionamide. The zeta potential values suggest that the surface charge of colloid was influenced by the composition of the formulation (NC -46.1 ± 0.5 mV, NS -39.4 ± 0.7 mV and NE -40.6 ± 0.7 mV). Calvo et al. [18] reported that the presence of oil and lecithin may confer a higher negative charge on nanocapsules of poly-ε-caprolactone and submicron emulsions than on nanospheres, due to the presence of free acids or negatively charged phospholipids in those raw materials. From these observations they deduced that the polymer coating formed around the oil droplets was not a solid polymer wall but an insubstantial polymer film. In the present study, differences in zeta potential cannot be explained

Table 1: Particle size and zeta potential of colloidal systems

Colloidal system	Size (nm) 45 days	Zeta potential (mV) 15 days
NC (without ethionamide)	198 ± 29	-46.1 ± 0.5
NC (B)	218 ± 40	-43.4 ± 0.8
NC (A)	287 ± 98	-41.1 ± 0.6
NS (without ethionamide)	210 ± 105	-39.4 ± 0.7
NS (B)	236 ± 82 (89%) 46 ± 20 (11%)	-36.6 ± 0.5
NS (A)	378 ± 130	-38.3 ± 0.5
NE (without ethionamide)	306 ± 61	-40.6 ± 0.7
NE (B)	unstable	-41.6 ± 0.6

exclusively on the basis of the presence of caprylic/capric triglyceride. Comparing the formulations (without ethionamide) where the oil is present (NC and NE) the zeta potential observed was -46.1 ± 0.5 mV and -40.6 ± 0.7 mV, respectively. In addition, no statistically significant differences were detected in the data obtained from NS (oil free) and NE (-39.4 ± 0.7 mV and -40.6 ± 0.7 mV). According to Chouinard et al. [20] the zeta potential of particles is a complex function of several variables, e.g. particle size, surface roughness and coating thickness among others.

2.2. Partition coefficient

In the literature, the efficiency of the drug association within nanocapsules has been correlated to the partition coefficient [21] that denotes the lipophobicity or hydrophobicity of a compound. In addition to solubility, affinity of the drug for the oil phase is an essential characteristic to improve the encapsulation [22]. Ethionamide partition coefficients between the oil and aqueous phases at different pH values are shown in Table 2. When the partition coefficients were measured at 6 and 12 h, it was observed that they were not statistically different. At pH values lower than 4, the partition coefficient of ethionamide between the oil and 0.05 N HCl was 0.02. On the other hand, at pH values higher than 4 the partition coefficients were 3.63 and 5.48 when measured between caprylic/capric triglyceride and distilled water or buffer pH 7, respectively. The determination of partition coefficients provided evidence for the pH dependence of drug solubility. Ethionamide is a pyridine derivative that forms salt in 0.05 N HCl, which is more soluble in the aqueous medium than in the oil. When distilled water or pH 7 buffer were used, the ethionamide (free base form) was more soluble in the oil. Because of these observations, caprylic/capric triglyceride (oil core) and distilled water (aqueous phase) were chosen to prepare ethionamide-loaded nanocapsules by method B.

2.3. Percentage of associated ethionamide

The percentage of drug incorporation into colloidal systems was affected by the type of formulation (Fig. 1). As described below, the percentage of drug associated was determined by the difference between the total and free ethionamide in the suspensions. Apart from NE (45 days), all formulations were stable and the total amount of ethionamide was recovered. After preparation employing method B, the percentages of ethionamide associated were $62.4 \pm 2.2\%$ (NC), $53.0 \pm 3.1\%$ (NS) and $38.5 \pm 2.9\%$ (NE). After 45 days of storage at 4 °C, the percentages of drug associated were $66.8 \pm 3.0\%$ (NC) and $51.9 \pm 1.8\%$ (NS). NE was not stable. However, when ethionamide was added to the pre-formed nanoparticle suspensions (method A), the percentage association measured as a function of time reached different values in all cases. While for NC (A), the percentage of ethionamide associated after 6 h ($56.9 \pm 0.6\%$) was equivalent to the percentage measured after 24 h ($56.2 \pm 0.5\%$), for the NS (A), the percentage of drug associated after 6 h was $39.8 \pm 0.2\%$, and after 12 h and 24 h the percentages were $43.4 \pm 0.3\%$ and $43.2 \pm 0.2\%$, respectively. After 45 days of storage at 4 °C, the percentages of ethionamide associated were $60.6 \pm 0.5\%$ for NC (A) and $40.4 \pm 2.7\%$ for NS (A) (Fig. 1). Despite a significant difference between the association efficiencies of nanoparticle suspensions prepared using methods B or A, these results suggest that adsorption onto the surface of nanoparticles is the preferred method of drug incorporation. This could indicate that only small amounts of drug are encapsulated into the oil core of NC (B) or included in the matrix network of NS (B). After 45 days of storage, a significant increase in the percentage of drug associated with NC was observed (B: $62.4 \pm 2.2\%$ to $66.8 \pm 3.0\%$ and A: $56.2 \pm 0.5\%$ to $60.6 \pm 0.5\%$). This trend in association was detected only in the case of NC suspensions. The increasing and higher percentage of ethionamide associated with NC rather than with NS could be due to their different total surface areas related to their different structures and/or the concentration of particles.

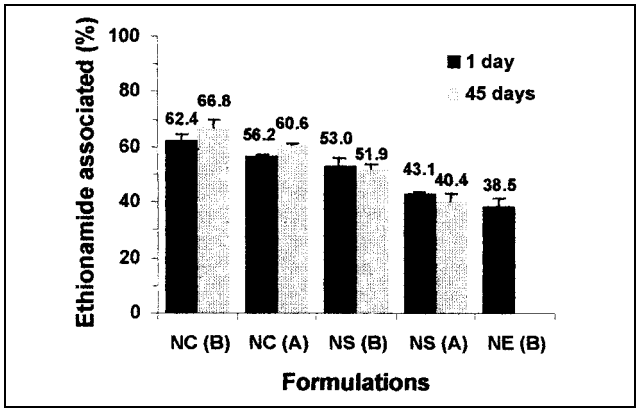

Fig. 1: Percentage of ethionamide associated with colloidal systems after 1 and 45 days of storage

2.4. pH values

In the case of NC without ethionamide, pH decreased during storage (6.9 ± 0.2 to 4.7 ± 0.0) (Table 3). This decline could be attributed to the rate of dissociation of carboxylic groups of the polymer outer surface. The backbone of polymer systems is well known and related to the T_g transition. However mobilities of other segmental parts are related to molecular relaxation processes studied by different techniques like NMR, dielectric measurements, high resolution Raman and light scattering techniques [23]. In this way, we consider the NC nanostructures dispersed in the liquid phase undergo a relaxation process related to

Table 2: Partition coefficients of ethionamide between caprylic/capric triglyceride and aqueous phases

Oil / aqueous phase	Concentrations (mg ml^{-1}/mg ml^{-1})	Partition coefficient (P)
Oil/buffer pH7	1.75/0.32	5.48
Oil/water	1.63/0.45	3.63
Oil/buffer pH4	1.28/0.96	1.33
Oil/0.05N HCl	0.04/2.40	0.02

Table 3: pH Values of colloidal systems

Colloidal system	pH after 1 day	pH after 45 days
NC (without ethionamide)	6.9 ± 0.2	4.7 ± 0.0
NC (B)	5.2 ± 0.0	4.9 ± 0.1
NC (A)	5.1 ± 0.1	4.9 ± 0.0
NS (without ethionamide)	5.4 ± 0.2	4.8 ± 0.1
NS (B)	5.6 ± 0.1	5.1 ± 0.1
NS (A)	5.2 ± 0.1	5.4 ± 0.1
NE (without ethionamide)	5.9 ± 0.0	5.0 ± 0.2
NE (B)	6.8 ± 0.1	5.1 ± 0.1

the mobility of poly(*t*-butyl methacrylate-co-acrylic acid) segmental and main chain.

In the case of NC (B or A) the pH values are similar at 1 and 45 days (5.2 ± 0.0 and 4.9 ± 0.1, 5.1 ± 0.1 and 4.9 ± 0.0, respectively). After 1 day, the difference in pH values of NC without ethionamide and NC (B or A) (6.9 ± 0.2, 5.2 ± 0.0 and 5.1 ± 0.1, respectively) can be explained by the adsorption of the drug. The ethionamide-conjugated acid present in the aqueous phase adsorbs onto the particle surface by ionic interaction with the carboxylate anions of outer surface of the nanoparticle (Fig. 2). While in NC (B or A) this neutralization can facilitate dissociation of the adjacent carboxylic groups of the polymer, for NC without ethionamide the rate of dissociation (−COOH) depends on a relaxation process.

As far as the NS suspensions (B, A and without ethionamide) were concerned, all the formulations had pH values between 5.2 ± 0.1 and 5.6 ± 0.1 after 1 day. After 45 days of storage, the pH values measured for NS without ethionamide and NS (B) were 4.8 ± 0.1 and 5.1 ± 0.1, respectively. For NS (A) the pH value after 45 days (5.4 ± 0.1) was not statistically different from the pH value measured after 1 day (5.2 ± 0.1). These results lead to the conclusion that the rate of dissociation of carboxylic groups of the outer surface of the nanoparticles was neither influenced by the presence of ethionamide nor by the NS nanostructure resulting from the method used.

After 1 day, NE (B) had pH 6.8 ± 0.1 compared with pH 5.9 ± 0.0 for NE without ethionamide. The presence of caprylic/capric triglyceride and the absence of a polymeric wall could explain these results. The caprylic/capric triglyceride contains traces of free carboxylic acids [18]. The pH value (5.9 ± 0.0) could be due to the orientations of the carboxylic groups at the dispersed phase and consequently their dissociation in aqueous medium [24]. After 1 day, the pH value (6.8 ± 0.1) measured for NE (B) may be due to the drug remaining in the aqueous medium forming a salt by a neutralization process between ethionamide and free carboxylic acids at the outer surface of micelle.

2.5. Drug release

Up to this point, the model of ethionamide association with nanoparticles was based on the adsorption of ethionamide conjugated-acid by salt formation with carboxylate anions at the polymer surface. In order to support this hypothesis and to evaluate the different percentages of drug associated by the two methods of incorporation employed, the drug release profiles of NC (B and A) and NS (B and A) were determined. The ultrafiltration-centrifugation technique allows only small amounts of filtrate to be separated from the suspension. In addition, each formulation had a different amount of ethionamide associated which was less than 100%. Therefore, to plot the release profiles (Fig. 3) the percentages of ethionamide released were calculated from the quantities of drug obtained in the filtrate after dilution, determined by HPLC, subtracted from the amount of free ethionamide detected in the filtrate of each suspension before dilution. The release profiles of nanoparticles showed a similar behavior for NC (B and A) and NS (B and A). The ethionamide associated was totally released (40 min) from the NC and NS prepared by method A. Since the percentages of ethionamide released after 40 and 210 min were not statistically different, one can presume that steady state was attained for all formulations. Thus, it was assumed that the ethionamide adsorbed on to the polymer surface by ionic interaction with the carboxylate groups (Fig. 2) was totally released at this time. From the percentages of total free drug determined by HPLC (Fig. 4) at 210 min it is possible to estimate the percentage of ethionamide associated with the colloids (method B) by mechanisms other than adsorption (dissolution in the oil or dispersion in the polymer layer). For NC (B) the total free drug at 210 min was $90.3 \pm 3.4\%$. So it can be estimated that approximately 10% of the ethionamide is dissolved in the oil or in the polymer wall. In the case of NS (B), this value was $81.3 \pm 3.4\%$, suggesting that approximately 20% is entrapped in the polymer matrix.

In conclusion, this work demonstrated that the association of ethionamide with polymeric colloidal systems (NC and NS) preferentially follows an adsorption mechanism. The model that we propose to explain this adsorption is salt formation between the ethionamide-conjugated acid and the carboxylate anions of the polymer outer surface. The oil/water partition coefficient is not the only parameter to be examined in order to predict the efficiency of association when heterocyclic drugs with a basic nitrogen group

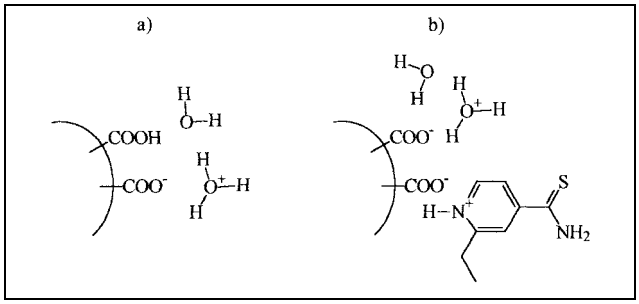

Fig. 2: a) Model of polymer outer surface. b) Model of ethionamide adsorption

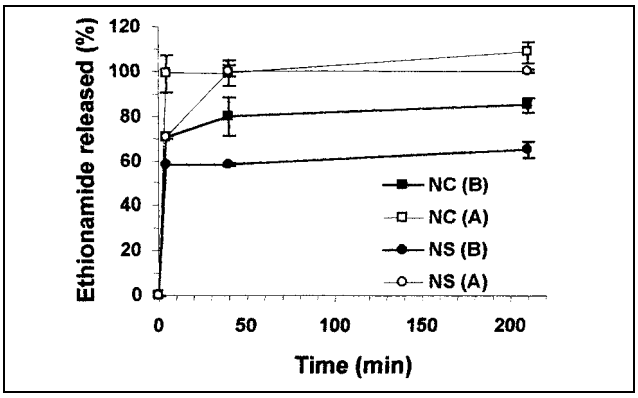

Fig. 3: Percentage of ethionamide released from NC and NS

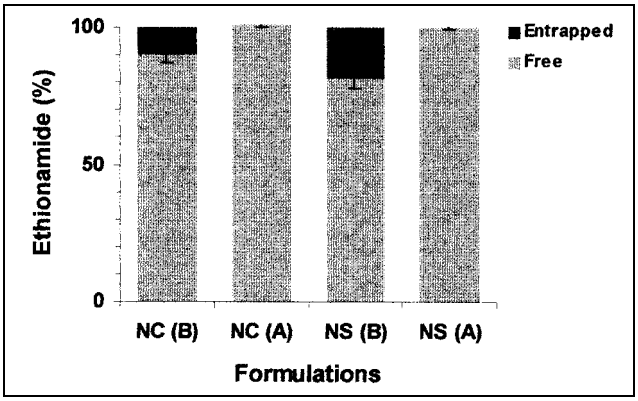

Fig. 4: Percentages of free ethionamide and ethionamide entrapped in NC and NS formulations prepared by methods B and A

Table 4: Composition (mg/ml of suspension) of nanocapsules (NC), nanospheres (NS) and nanoemulsions (NE)

Composition (mg/ml)	NC	NS	NE
Ethionamide	0.3	0.3	0.3
poly(*t*-Butyl methacrylate-co-acrylic acid)	10.0	10.0	–
Sorbitan monostearate	7.6	7.6	7.6
Caprylic/capric triglyceride	30.0	–	30.0
Poloxamer 188	7.6	7.6	7.6

are employed in formulations containing acid substances. Analysis of the release profile of ethionamide as well a comparative study of different formulations was necessary to clarify the mechanism of the association of the drug to the colloidal systems. Finally, we can also conclude that an isolated determination of the percentages of drug associated in a colloidal suspensions by the ultrafiltration-centrifugation method is incapable of differentiating encapsulation (drug dissolved in the oil) from the adsorption of the drug.

3. Experimental

3.1. Materials

Ethionamide was obtained from Sigma (St. Louis, EUA); poly(*t*-butyl methacrylate-co-acrylic acid) (Eudragit® S90) was purchased from Röhm Tech. Inc. (Altmann, São Paulo, Brazil), sorbitan monostearate was supplied by Aldrich (Strasbourg, France) and poloxamer 188 (Synperonic PE/F68) by ICI (Clamart, France). Caprylic/capric triglyceride (Miglyol® 810) was purchased from Hulls (Puteaux, France). Acetone and acetonitrile used were of pharmaceutical grade and analytical grade, respectively.

3.2. Preparation of the colloids

Nanocapsules (NC), nanospheres (NS), and nanoemulsions (NE) were prepared according to the method reported by Fessi et al. [7] (Table 4). To prepare the nanocapsules suspensions, the lipophilic solution consisted of caprylic/capric triglyceride, sorbitan monostearate, poly(*t*-butyl methacrylate-co-acrylic acid) and acetone. This organic solution (40 ml) was poured into the aqueous phase (80 ml) containing poloxamer 188, under moderate magnetic stirring, at room temperature. The acetone and some water were evaporated under reduced pressure and final volume adjusted with water to 15 ml. Nanoemulsions were prepared as for nanocapsules, omitting the polymer, and nanospheres were also prepared as for nanocapsules, omitting the oil. Formulations were made in triplicate.

3.3. Drug incorporation

Method B: Following the procedure for preparation of colloids described above, the drug (ethionamide) was previously dissolved in the organic phase (acetone) containing the oil, sorbitan monostearate, and the polymer. Nanoprecipitation was achieved by pouring this solution into the aqueous phase containing poloxamer 188 and the nanoparticles or nanoemulsions obtained were designated NC(B), NS(B) or NE(B).

Method A: NC or NS were prepared according to the method for preparation of colloids (see 3.2.). Then, ethionamide was added to the pre-formed suspensions and maintained under stirring for 24 h at room temperature. From these nanoparticles systems designated NC(A) and NS(A), samples were collected at 6 h, 12 h, 24 h and 45 days.

3.4. Colloidal systems characterization

The size of nanocapsules was estimated by laser light scattering N4 Counter Nanosizer® (USA). Each suspension sample was diluted to the appropriate concentration with filtered distilled water. The zeta potential was measured using a Zetasizer®4, with a Series 7032 Multi8Correlator (France). The results presented were all normalized to value of $\zeta = -55$ mV for the standard solution (a carboxylated polystyrene latex supplied by Malvern, Orsay, France). Measurements were made in triplicate.

3.5. Partition coefficient

Ethionamide was dissolved in caprylic/capric triglyceride to give a concentration of 2.66 mg/ml. Two milliliters of this solution were added to 2 ml of each one of the following aqueous media: distilled water, 0.05 N HCl, acetate buffer pH 4 or phosphate buffer pH 7. Each vial was shaken for 12 h at room temperature. Concentrations of ethionamide (mg/ml) in each lipophilic or aqueous phase were determined by HPLC according to the procedure described belows. Measurements were made in triplicate. The partition coefficients (P) were calculated by dividing the concentration of ethionamide in the oil phase by the drug concentration in each of the aqueous phases.

3.6. Determination of the association of ethionamide with the colloids

Ethionamide was assayed by HPLC. The system consisted of a Waters 510 (Millipore) pump using reversed-phase Nova-Pak C_{18} 4 μm as column, and a reversed-phase LiChrosorb RP-18 10 μm as pre-column, a Rheodyne injector and a Waters 484 (Millipore) UV detector. The mobile phase consisted of acetonitrile/water (30 : 70 v/v). The total sample volume injected was 20 μl. Ethionamide was detected by absorption at 290 nm at an approximate retention time of 4,03 min. The linear response was 0,1–4,0 μg/ml with a correlation coefficient of $r = 0{,}99988$. Free ethionamide was determined in the ultrafiltrate obtained by a combined ultrafiltration-centrifugation technique (Ultrafree® MC, Millipore). Total ethionamide was determined after dissolution of nanoparticles in acetonitrile. The ethionamide associated with the colloidal systems was calculated from the difference between the total and the free drug concentrations measured in the colloidal suspensions and the ultrafiltrate, respectively.

3.7. Drug release [11]

In order to investigate the ethionamide released from the nanoparticles suspensions (methods B and A), colloidal suspensions were diluted in phosphate-buffer pH 7 (1 : 10 v/v). Aliquots (0.4 ml) were collected 5, 40 and 210 min after dilution and filtered by centrifugal ultrafiltration process using Ultrafree® MC units (Millipore) [11]. The ultrafiltrate obtained was finally assayed for ethionamide by HPLC.

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