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Diffusion and mathematical modeling of release profiles from nanocarriers

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Abstract

The aim of this work was to establish models and to differentiate the kinetic release behavior of drug models from nanocapsules, nanoemulsion and nanospheres by physico-chemical characterization and release experiments. SAXS analysis showed that the polymer is organized in the nanocapsules, while in the nanospheres the sorbitan monostearate is organized and acts as an impurity of the poly(ε-caprolactone) suggesting that constituents in these nanocarriers are differently organized. Formulations presented particle sizes ranging from 178 to 297 nm, probe content from 0.981 to 0.997 mg/mL, pH values from 4.90 to 5.10 and zeta potential from −37.9 to −51.9 mV. The kinetic experiments showed that the nanostructures present similar behaviors when the probe is adsorbed on the nanocarriers (indomethacin-loaded formulations). However, when the probe is entrapped within the nanocarriers (indomethacin ethyl ester-loaded formulations), nanocapsules, nanospheres and nanoemulsion presented different kinetic behaviors. Mathematical modeling of the release profiles was conducted, showing that the presence of the polymer increases the half-lives of the burst phases (5.9, 4.4 and 2.7 min) while the presence of the oil increases the half-lives of the sustained phases (288.8, 87.7 and 147.5 min) for nanocapsules, nanospheres and nanoemulsion, respectively.

Keywords: Nanoparticles; Nanocapsules; Indomethacin; Alkaline hydrolysis; Poly(ε-caprolactone); Indomethacin ethyl ester

1. Introduction

Polymeric nanoparticles have been studied as drug carriers in the past 20 years (Magenheim and Benita, 1991; Brannon-Peppas, 1995; Couvreur et al., 1995; Ponchel and Irache, 1998; Montasser et al., 2000; Ravi Kumar, 2000; Soppimath et al., 2001; Couvreur et al., 2002; Schaffazick et al., 2003; Vauthier et al., 2003). Different methods and a wide range of active substances and models of drugs have been studied in this field for intravenous, oral, ophthalmic and topical administrations (Couvreur et al., 1995; Schaffazick et al., 2003; Alvarez-Román et al., 2004). Nanoparticle is a generic term to refer nanospheres (NS) and nanocapsules (NC), which are polymeric nanocarriers presenting matricial and vesicular structures, respectively (Couvreur et al., 1995). Nanoemulsion, prepared without polymer, is a submicrometric emulsion. The control to produce one or other system is reached either by the selection of the preparation method or by the qualitative composition of the formulations (Schaffazick et al., 2003).

Among others, the objective in developing these nanocarriers for intravenous administration is drug targeting, improving selective action of antibiotics (Puisieux et al., 1994; Fresta et al., 1995; Pinto-Alphandary et al., 2000) or antitumorals (Brasseur et al., 1991; Couvreur et al., 1995; Puisieux et al., 1994; Yoo et al., 2000). Furthermore, the main advantages of nanoparticles for oral administration of drugs are the decrease of drug toxicity, including anti-inflammatory drugs (NSAIDs) such as diclofenac (Guterres et al., 1995, 2001) and indomethacin (Ammoury et al., 1993; Chasteigner et al., 1995), and the increase of drug stability in the gastrointestinal tract, focusing hormones, proteins and peptides (Couvreur et al., 1995; Hillery et al., 1996; Allémann et al., 1998; Jung et al., 2000; Vila et al., 2002). In addition, the development of nanoparticles for ocular administration has received great attention (Losa et al., 1993; Calvo et al., 1996; Ding, 1998), aiming toward the control of drug release, increase in drug ocular bioavailability and/or decrease of drug side effects due to systemic absorption.

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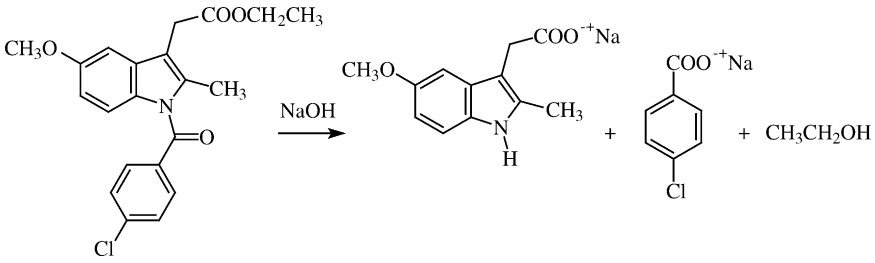

Fig. 1. Alkaline hydrolysis of indomethacin ethyl ester.

The different methods to prepare nanoparticles include in situ polymerization (Couvreur et al., 1995) by dispersion of monomers or by interfacial polymerization and precipitation of pre-formed polymers by nanoprecipitation, interfacial deposition (Fessi et al., 1989), solvent evaporation (Desgouilles et al., 2003) or by the emulsification–diffusion technique (Quintanar-Guerrero et al., 1997). Nanoemulsions (NE), submicronic emulsions, are also proposed as nanocarriers and can be obtained by spontaneous emulsification (Calvo et al., 1996).

Drug release from nanoparticulated systems depends on dessorption, diffusion, particle erosion or the combination of these factors (Soppimath et al., 2001). Different methods are employed for in vitro drug release evaluations based on dialysis (Losa et al., 1993; Calvo et al., 1996), ultracentrifugation (Seijo et al., 1990; Fontana et al., 1998; Gref et al., 2001) or ultrafiltration–centrifugation (Bapat and Boroujerdi, 1992; Lopes et al., 2000). Nevertheless, according to Washington (1990) an experimental sink condition is not achieved using any of these methods. In fact, they are useful to determine the drug partition coefficient between the nanoparticles and the continuous phase. Moreover, the determination of drug partition coefficient between nanocarrier and continuous phase (for any system) or between oil and water (for nanoemulsion and nanocapsules) does not provide any information about the mechanism of drug encapsulation (Lopes et al., 2000; Pohlmann et al., 2004). In fact, Ammoury et al., 1990 prepared different indomethacin-loaded nanocarriers containing or not containing phospholipids, poly(±-lactide) and benzyl benzoate. The in vitro indomethacin release profiles were determined by dialysis sac experiments, and the mathematical modeling of profiles showed an incomplete drug release probably due to the presence of poloxamer inside the sac. Furthermore, since the solubility of indomethacin is pH dependent, the authors suggested that the release is due to the drug partitioning from the colloidal suspension phases to the external sink solution.

Recently, our research group has developed a new tool to determine the release profile of an organic molecule from polymeric NC, in which a sink condition is simulated by the use of an interfacial reaction (Pohlmann et al., 2004). This strategy was used to compare differently loaded polymeric NC: indomethacin-loaded NC and indomethacin ethyl ester-loaded NC. The results showed that the mechanism of association was different depending on the substance entrapped in the NC. Indomethacin was adsorbed, while its ethyl ester was predominantly entrapped within the NC.

The reaction of the entrapped probe occurs at the particle/water interface. The hydrolysis of the probe is shifted to the products that are soluble in alkaline aqueous medium (Fig. 1). This approach simulates a sink condition because each new molecule of probe that reaches the interface is degraded, resulting in concentration gradient of the probe in the particle. Indomethacin ethyl ester was chosen as a probe because it is unable to give ionizable forms in aqueous solution before its decomposition by the alkaline hydrolysis at the particle/water interface. The reaction only occurs at the interface because of the solubility of the reactants. Indomethacin ethyl ester is insoluble in water, entrapped in the nanocarriers, and the hydroxyl anions are water soluble. Since this approach permits the release of each molecule of the probe from the NC in a medium free from this probe, no separation technique is required.

In this work, the objective was to differentiate the structural organization at a molecular level and the kinetic release behavior of NC, NE and NS by means of a physico-chemical characterization, using dynamic light scattering, zeta potential, probe content (HPLC), small angle X-ray scattering (SAXS) and a kinetic release experiment followed by mathematical modeling.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (PCL) ($M_w = 65{,}000$) was supplied by Aldrich (Strasbourg, France). Dicyclohexylcarbodiimide (DCC), 4-(*N*,*N*-dimethyl)aminopyridine (DMAP) and indomethacin were obtained from Sigma (St. Louis, USA). Caprilic/capric triglyceride mixture was delivered from Brasquim (Porto Alegre, Brazil). Span 60®, sorbitan monostearate, and Tween 80®, polysorbate 80, were obtained from Delaware (Porto Alegre, Brazil). All other chemicals and solvents used were of analytical or pharmaceutical grade. All reagents were used as received.

2.2. Synthesis of indomethacin ethyl ester

As described by Kalgutkar et al., 2000, the synthesis of the indomethacin ester was carried out under argon by preparing a solution of indomethacin (5 mmol) in ethanol (20 mL) to which was added of DMAP (0.2 mmol). The solution was stirred for 10 min at 0 °C following the addition of DCC (5 mmol). After 30 min, the temperature was raised to 25 °C and the

reaction was followed by thin layer chromatography (TLC). After 16 h, the solvent was evaporated under reduced pressure. Dichloromethane (30 mL) was added to the residue and the suspension was filtered. The filtrate was extracted by saturated $NaHCO_3$ aqueous solution (3× 10 mL), and the organic phase was dried with anhydrous $MgSO_4$, filtered and evaporated. The product was purified by column chromatography (Silica gel 60, 70–230 mesh) using ethyl acetate and cyclohexane (1:1, v/v) as eluent. The isolated product was obtained as a solid (78% of yield) presenting a melting point (uncorrected) of 82–83 °C.

^{1}H NMR 200 MHz (δ, ppm) $CDCl_3$: 7.66 and 7.46 (AB, 2H and 2H, ArH *p*-chlorobenzoyl), 6.97 (d, 1H $J = 2.5$ Hz, H-4), 6.87 (d, 1H $J = 9.0$ Hz, H-7), 6.67 (dd, 1H $J = 9.0$ and 2.5 Hz, H-6), 4.16 (q, 2H $J = 7.1$ Hz, OCH_2), 3.84 (s, 3H, OCH_3), 3.65 (s, 2H, CH_2), 2.38 (s, 3H, CH_3), 1.27 (t, 3H $J = 7.1$ Hz, CH_3CH_2O).

^{13}C NMR 75 MHz (APT, δ, ppm) $CDCl_3$: 170.9 (CO-ester), 168.3 (CO-amide), 156.0, 139.2, 135.9, 134.0, 130.8, 130.7 and 112.7 (7× Cq), 131.1 and 129.1 (4× CH *p*-chlorobenzoyl), 114.9, 111.6 and 101.3 (3× CH indol), 61.0 (OCH_2), 55.7 (OCH_3), 30.4 (CH_2), 14.2 and 13.3 (CH_3 and CH_3CH_2).

2.3. Analytical procedures

The HPLC system consisted of a Perkin-Elmer S-200 with injector S-200, detector UV–vis, a guard-column and a column (Nova-Pak C18, 150 mm, 3.9 mm, 4 μm, Waters). The mobile phase (0.7 mL/min) consisted of acetonitrile/water (70:30, v/v) adjusted to pH 5.0 ± 0.5 with 10% (v/v) acetic acid. After injection of 20 μL, the indomethacin ethyl ester was detected at 267 nm with a retention time of 7.8 min. The co-injection of the indomethacin ester and the indomethacin (acid) showed two peaks at 7.8 and 3.4 min, corresponding to the products, respectively. The HPLC method was validated following the ICH (1996). Linear calibration curves for the ester and for indomethacin could be obtained in the range of 1.00–25.00 μg/mL presenting correlation coefficients higher than 0.9995 (indomethacin ester) and 0.9992 (indomethacin). Inter- and intraday variability were determined for different indomethacin or ester concentrations (3.00, 12.00 and 17.00 μg/mL) and for each different concentration of the calibration curves (1.00, 2.00, 5.00, 10.00, 15.00, 20.00 and 25.00 μg/mL). Inter- and intraday variability did not exceed 6.82% (indomethacin ester) or 1.68% (indomethacin) and accuracy was 96.8% (indomethacin ester) or 99.0% (indomethacin). The limits of quantification were 1.00 μg/mL for both indomethacin and its ester.

2.4. Preparation of nanocarriers

NC suspensions were prepared by nanoprecipitation (Fessi et al., 1989). At 40 °C, indomethacin or indomethacin ethyl ester (0.010 g), poly(ε-caprolactone) (0.100 g), capric/caprylic triglyceride (0.33 mL) and sorbitan monostearate (Span 60®) (0.077 g) were dissolved in acetone (27 mL). In a separate flask, polysorbate 80 (Tween 80®) (0.077 g) was added into 53 mL of water. The organic solution was poured into the aqueous phase under magnetic stirring at room temperature. After 10 min, the acetone was eliminated and the aqueous phase concentrated under reduced pressure. The final volume was adjusted to 10 mL. NS suspensions and NE were prepared as described above omitting the oil, and the polymer, respectively. For comparative experiments, a dispersion of surfactants was also prepared omitting the oil and the polymer and it was called ND, as well as a nanosphere formulation containing only the polymer and the polysorbate 80 was prepared and called NS-b. Formulations were made in triplicate.

2.5. SAXS measurements

Small angle X-ray scattering (SAXS) measurements were carried out on the bending magnetic beamline D11A of the Laboratório Nacional de Luz Síncrotron (LNLS, Campinas, Brazil). The liquid samples were placed in a stainless steel sample holder closed by two mica windows of 20 μm thickness and thermostatizated at 20 °C with an accuracy of ±0.1 °C. An exposure time of about 10 min was required for all the samples. The wavelength of the incident beam was 1.605 Å and a linear detector (Princeton Instruments) was used at 73.0 cm from the sample. Silver beonate was used for calibration purposes. The intensities were corrected for the detector response and the dark current signals, as well as for sample transmission and background scattering. The characteristic lengths d of the lamellae arrangements in the nanoparticles were determined through the well-known Bragg relation (Eq. (1)):

$$q = n(2\pi/d) \quad (1)$$

where q is the scattered wave vector, n an integer and d is the distance between crystallographic planes.

2.6. Probe content in formulations

The total concentration of ester or indomethacin in the formulations was measured by HPLC as described above. Each suspension (100 μL) was treated with acetonitrile (10 mL), the solution was filtered (Millipore 0.45 μm) and injected (20 μL). The concentration of the non-associated probe with nanocarriers was determined by HPLC in the ultrafiltrate (ultrafiltration–centrifugation technique, Ultrafree-MC GPMC 10 kDa, NMWL, Millipore). The concentration of associated probe with nanocarriers was calculated by the difference between the total and the non-associated concentrations.

2.7. pH measurements

After preparation, the pH values of nanocarrier suspensions were determined using a potentiometer (Micronal B-474).

2.8. ζ-Potential measurements

The zeta potential of nanocarrier suspensions was determined after dilution of samples in 1 mM NaCl using a Zetasizer® (Malvern).

2.9. Particle sizes, size distribution and polydispersity

The particle sizes, size distributions and polydispersity were determined by photon correlation spectroscopy (PCS) by analysis of the polarized scattered light at 90° in diluted samples. Measurements were made at room temperature (20 °C) using a Brookheaven Instruments (New York, USA) standard setup (BI-200M goniometer, BI-9000AT digital correlator and a BI9863 detection system). A coherent Spectra Physics He-Ne laser (35 mW, $\lambda_0 = 632.8$ nm) was used as light source.

2.10. Alkaline hydrolysis

Chemical hydrolyses of the indomethacin or its ester (1 mg/mL) were carried out at 37 °C (Ika EH4) by addition of 1 mL of probe-loaded nanocarrier suspension (NC, NS or NE) to 4 mL of 0.05 M NaOH aqueous solution. For indomethacin hydrolyses, the samples (300 μL) were collected between 0 and 2 min and for indomethacin ethyl ester the samples (300 μL) were collected between 0 and 1440 min. At pre-determined time intervals, each sample was treated with 2.5 M HCl (5 μL) and acetonitrile (1.2 mL) to stop the reaction and to dissolve all components. After centrifugation (5 min, 12,000 rpm), the supernatants were analyzed by HPLC as described above. Reactions were made in triplicate.

2.11. Mathematical modeling

Mathematical modeling (MicroMath Scientist®) was used to analyze the probe disappearance profiles. The model-dependent approaches monoexponential (Eq. (2)) and biexponential (Eq. (3)) were employed. The selection of the model was based on the best correlation coefficient, the best model selection criteria (MSC), both provided by the software, and the best graphic adjustment.

$$C = C_0\, e^{-kt} \tag{2}$$

$$C = a\, e^{-k_1 t} + b\, e^{-k_2 t} \tag{3}$$

The observed rate constants are k, k_1 and k_2 and the initial probe concentrations are C_0, a and b.

3. Results and discussion

3.1. Synthesis of indomethacin ethyl ester

The ester was obtained by the condensation of ethanol with the acid activated indomethacin. After purification, the product (78% of yield) was analyzed by nuclear magnetic resonance (NMR) and HPLC. NMR spectra showed signals at 4.16 and 1.27 ppm (^{1}H NMR) corresponding to ethoxyl moiety and 170.9 and 61.0 ppm (^{13}C NMR, APT) attributed to CO-ester and OCH_2. The purity was determined as 99.24 ± 0.04% (HPLC).

3.2. Preparation of nanocarrier suspensions

After preparation, all formulations presented a macroscopic homogeneous appearance, like a milky white bluish opalescent liquid. Unloaded-nanocarriers or indomethacin-loaded or indomethacin ethyl ester-loaded formulations were obtained without any subsequent step of filtration or centrifugation.

3.3. SAXS analyses

Theoretically, NC are described as lipophilic vesicles (Puisieux et al., 1994), in which the polymer is surrounding an oil core, while the NE formulation, prepared without polymer, consist of dispersed oil in water. The NS, which are prepared omitting only the oil in the formulation, are matrices of polymer stabilized by surfactants (Pohlmann et al., 2002). In order to access the molecular organization of the polymer or other component in the nanocarriers, small angle X-ray scattering analyses were carried out.

Analyses were performed for drug-unloaded formulations (Fig. 2). The peak at $q \cong 0.035$ Å^{-1} in the NC spectrum indicated that a component is organized in these nanocapsules. This result reflects the crystalline region of the poly(ε-caprolactone) (Jeong et al., 2003). For NC formulation, the lamellae (d) was 18.0 nm. The absence of this peak in the NS spectrum could be due to the presence of sorbitan monostearate in the formulation. Additionally, the NS spectrum showed a peak at $q \cong 0.10$ Å^{-1}. In order to determine the nature of this peak, another formulation was prepared by emulsifying only the surfactants in water omitting the oil and the polymer. This formulation, called nanodispersion (ND), was composed of nanoparticles of sorbitan monostearate stabilized with polysorbate 80. The SAXS spectrum for this formulation showed a similar peak ($q \cong 0.10$ Å^{-1}) as observed for NS. The calculated values of d for NS and ND were 6.0 and 6.1 nm, respectively. On the other hand, the peak at $q \cong 0.10$ Å^{-1} was not present in NC and NE spectra. Based on DSC analyses, a previous model considered sorbitan monostearate to be dissolved in the oil core (capric/caprilic triglyceride)

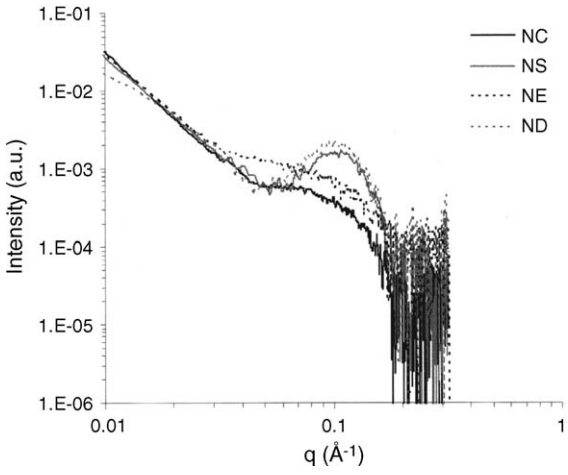

Fig. 2. SAXS spectra of nanocapsules (NC), nanospheres (NS), nanoemulsion (NE) and nanodispersion (ND).

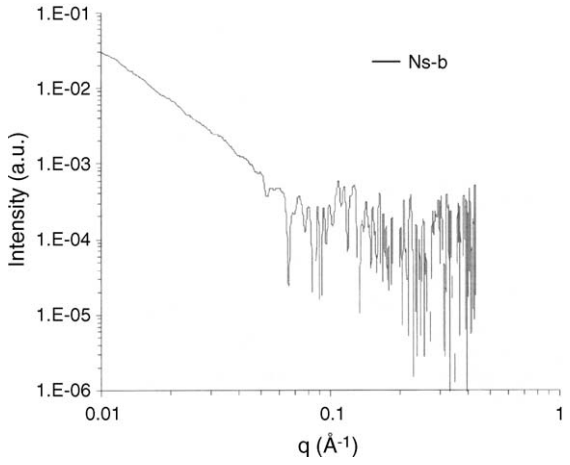

Fig. 3. SAXS spectrum of nanospheres prepared with polymer and polysorbate 80 and without sorbitan monostearate (NS-b).

of nanocapsules (Müller et al., 2001). So, for NC and NE the results obtained by SAXS corroborate with this model if the peak at $q \cong 0.10\ \text{Å}^{-1}$ is attributed to the sorbitan monostearate. In order to confirm this hypothesis, a different NS formulation was prepared. In this case, the nanoparticles were composed only of the polymer, omitting the sorbitan monostearate. This formulation, called NS-b, was analyzed by SAXS (Fig. 3). The absence of peak at $q \cong 0.10\ \text{Å}^{-1}$ in this spectrum confirmed that it corresponds to sorbitan monostearate in NS and ND formulations. For NS, the sorbitan monostearate would act as an impurity affecting the crystallinity of the polymer. In this way, the model of molecular organization for NS in this study is in agreement with a previous model, proposed for a similar NS formulation regarding DSC and PCS analyses, that considered the sorbitan monostearate and poly(ε-caprolactone) matrix as heterogeneous nanoparticles (Müller et al., 2001; Pohlmann et al., 2002).

3.4. Particle sizes, probe contents, ζ-potential and pH values

The probe-loaded and unloaded NC, NS and NE suspensions presented particle sizes below 300 nm (Table 1) and size distribution width lower than 60 nm (polydispersity <0.2). Furthermore, the formulations presented indomethacin content of 0.985 ± 0.010 (NC), 0.979 ± 0.002 (NS) and 0.997 ± 0.005 (NE) mg/mL and indomethacin ethyl ester contents of 0.981 ± 0.008, 0.985 ± 0.009 and 0.992 ± 0.012 mg/mL, respectively. The ultrafiltration–centrifugation technique was used to separate the continuous phase from the nanocarriers showing the absence of probe in the ultrafiltrate for all systems. Taking into account that this technique is widely described in the literature (Magenheim and Benita, 1991) to determine the association of drugs with nanoparticles, in the present work it was assumed that, for all investigated systems, the probes were 100% associated with the nanocarriers.

Considering all formulations, the pH values varied between 4.90 ± 0.03 and 5.20 ± 0.09 (Table 1). Regarding zeta potentials, the unloaded formulations presented values of −44.6 ± 0.7 mV (NC), −49.9 ± 0.7 mV (NE) and −40.7 ± 1.2 mV (NS), while the indomethacin-loaded formulations presented −50.7 ± 1.4, −51.9 ± 1.2 and −37.9 ± 1.2 mV, respectively. Indomethacin-loaded NS, which correspond to matricial particles, presented a slightly higher value than the unloaded-NS formulation. The values observed for indomethacin-loaded NC and indomethacin-loaded NE are in accordance with a previous report (Calvo et al., 1996) in which the zeta potentials for NC and NE formulations were found to be similar. The authors in that work suggested that the polymer coating of NC is not a consistent polymer wall but a slight polymer film. On the other hand, our previous studies (Pohlmann et al., 2004) of indomethacin-loaded NC suspension suggested drug adsorption as the mechanism of probe association to the NC. The zeta potential values determined for the indomethacin ethyl ester-loaded suspensions were −46.9 ± 0.7 mV (NC), −49.3 ± 1.4 mV (NE) and −45.3 ± 1.7 mV (NS). In this case, the NC and NE showed a smaller influence due to the probe presence in the nanocarriers than the indomethacin-loaded NC and NE. The ester-loaded NS suspension presented a value lower than the unloaded-NS formulation. The results suggest that the association mechanism of these two probes to the nanocarriers could be different from one another.

3.5. Release behavior by interfacial reaction

The alkaline hydrolyses of probes were carried out with the objective of comparing NC, NE and NS. The use of both

Table 1
Physico-chemical characteristics of unloaded nanocapsules (NC), nanoemulsion (NE) and nanospheres (NS), indomenthacin-loaded nanocapsules (IndOH-NC), indomenthacin-loaded nanoemulsion (IndOH-NE) and indomenthacin-loaded nanospheres (IndOH-NS), and indomethacin ethyl ester-loaded nanocapsules (IndOEt-NC), indomethacin ethyl ester-loaded nanoemulsion (IndOEt-NE) and indomethacin ethyl ester-loaded nanospheres (IndOEt-NS)

Nanocarrier	Particle diameter[a] (nm)	Probe content (mg/mL)	pH	ζ-Potential (mV)
NC	288 ± 13	–	5.08 ± 0.08	−44.6 ± 0.7
NE	217 ± 23	–	5.02 ± 0.10	−49.9 ± 0.7
NS	186 ± 19	–	5.01 ± 0.03	−40.7 ± 1.2
IndOH-NC	297 ± 15	0.985 ± 0.010	5.17 ± 0.12	−50.7 ± 1.4
IndOH-NE	236 ± 20	0.997 ± 0.005	4.90 ± 0.03	−51.9 ± 1.2
IndOH-NS	178 ± 16	0.979 ± 0.002	4.97 ± 0.05	−37.9 ± 1.2
IndOEt-NC	279 ± 26	0.981 ± 0.008	5.12 ± 0.15	−46.9 ± 0.7
IndOEt-NE	247 ± 14	0.992 ± 0.012	5.10 ± 0.07	−49.3 ± 1.4
IndOEt-NS	180 ± 21	0.985 ± 0.009	5.20 ± 0.09	−45.3 ± 1.7

[a] Polydispersity lower than 0.2.

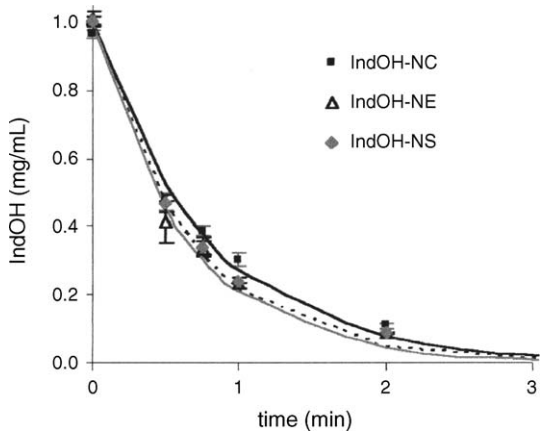

Fig. 4. Indomethacin consumption from probe-loaded nanocapsules (IndOH-NC), probe-loaded nanoemulsion (IndOH-NE) and probe-loaded nanospheres (IndOH-NS).

probes, indomethacin and its ester, which present similar molecular geometry and molecular weights (357.78 and 385.83 g/mol, respectively), but different acid–base characters, can supply valuable information about their release behavior from the nanocarriers.

The total consumption of indomethacin from IndOH-NC, IndOH-NE and IndOH-NS was observed before 2 min (Fig. 4). On the other hand, the total consumption of the ester in each reaction was 1440 min (24 h), 720 min (12 h) and 480 min (8 h) (Fig. 5) from the indomethacin ethyl ester-loaded nanocarriers (IndOEt-NC, IndOEt-NE and IndOEt-NS, respectively).

Once all the components of nanocarrier formulations are also ester derivatives, they could be degraded by the NaOH. In order to verify the integrity of the nanocarriers during the hydrolysis, the particle sizes and polydispersity were determined. For all systems, the particle sizes and polydispersity remained constant during the alkaline hydrolysis, suggesting that neither swelling nor erosion took place. These results showed that the alkaline medium did not essentially affect the nanocarrier structures during the time required for the total disappearance of each probe.

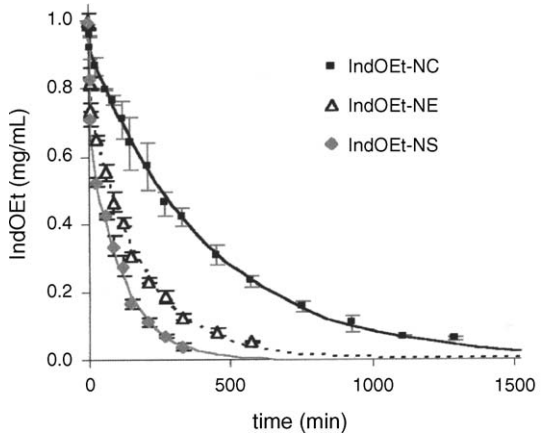

Fig. 5. Indomethacin ethyl ester consumption from probe-loaded nanocapsules (IndOEt-NC), probe-loaded nanoemulsion (IndOEt-NE) and probe-loaded nanospheres (IndOEt-NS).

Thus, the use of indomethacin did not differentiate the kinetic behavior of nanocarriers. On the other hand, the indomethacin ethyl ester was a useful probe showing that each system (NC, NE or NS) presented different reaction profiles.

The nanocarriers containing the probes are hydrophobic particles, and the hydroxyl anions are solvated in the continuous phase (aqueous medium). Thus, the hydrolysis reaction can only occur at the particle/water interface, where both reactants can interact. The probe and the hydroxyl anion must diffuse, respectively, from the nanocarrier and from the aqueous medium to the interface. In this case, the different magnitudes of time required for the total consumption of indomethacin (2 min) and for its ester (8–24 h) suggested that the mechanisms of probe association with the nanocarriers are different.

The indomethacin-loaded formulations (IndOH-NC, IndOH-NE and IndOH-NS) may present similar release behaviors because the probe is adsorbed on the nanocarriers. After reaching the interface, the hydroxyl anions react with the carboxylic acid moiety of indomethacin giving the corresponding salt, which is soluble in the continuous phase. Then, the salt in solution reacts with hydroxyl anions and its amide function is hydrolyzed. In this case, the hydrolysis is not dependent on the diffusion and follows a pseudo-first order chemical kinetic expression due to the excess of hydroxyl anions. On the other hand, the consumption of the indomethacin ethyl ester is a consequence of its diffusion from the nanocarrier, in which it is entrapped. A certain amount of the ester can be adsorbed on the nanocarriers, but the main mechanism of the ester association with nanocarriers is the entrapment. In order to confirm or refuse these hypotheses, the mathematical modeling of the experimental data was carried out for all reactions.

3.6. Mathematical modeling

Each reaction was modeled using monoexponential (Eq. (2)) and biexponential (Eq. (3)) equations. The alkaline hydrolysis of indomethacin or its ester in acetonitrile solution were previously reported (Pohlmann et al., 2004). The monoexponential model best described the experimental data for these reactions, determining observed rate constants of $2.7080 \pm 0.0125\ \text{min}^{-1}$ (indomethacin) and $1.6400 \pm 0.0061\ \text{min}^{-1}$ (indomethacin ethyl ester), respectively. The half-life of the hydrolyses of these probes in acetonitrile/50 mM NaOH aqueous solution (1:4, v/v) were 0.26 min (indomethacin) and 0.42 min (indomethacin ester).

For indomethacin-loaded nanocarriers, the best fitting was also observed using the monoexponential model (Table 2) showing the observed rate constants of $1.2880 \pm 0.0636\ \text{min}^{-1}$ (IndOH-NC), $1.5380 \pm 0.0128\ \text{min}^{-1}$ (IndOH-NE) and $1.5620 \pm 0.0874\ \text{min}^{-1}$ (IndOH-NS). These reactions took place at a similar rate as observed for indomethacin chemical hydrolysis in solution. The respective half-lives ranged between 0.44 and 0.54 min. The slight delay for the reactions of indomethacin associated with nanocarriers could be explained by the necessity of hydroxyl anions to reach the particle/water interface, which presents negative zeta potential (Table 1). These results suggest that the similar release behavior of indomethacin from NC,

Table 2
Observed rate constants, correlation coefficients and MSC from monoexponential modeling of indomethacin alkaline hydrolyses (acetonitrile solution, IndOH-NC, IndOH-NE and IndOH-NS) and indomethacin ester alkaline hydrolyses in solution

Parameters	IndOEt solution	IndOH solution	IndOH-NC	IndOH-NS	IndOH-NE
k (min^{-1})	1.6400 ± 0.0061	2.7080 ± 0.0125	1.2880 ± 0.0636	1.5620 ± 0.0874	1.5380 ± 0.0128
r (range)	0.9983 ± 0.0013	0.9975 ± 0.0008	0.9968 ± 0.0009	0.9965 ± 0.0005	0.9941 ± 0.0046
MSC (range)	4.0155 ± 0.4936	3.8678 ± 0.1210	3.8654 ± 0.3053	3.8728 ± 0.2825	4.3026 ± 1.2933

NE or NS is a consequence of its mechanism of association. The indomethacin is only adsorbed on the nanocarriers independently on the type of nanostructure evaluated.

In parallel, for the ester-loaded nanocarriers, the best data fitting was observed with the biexponential model (Table 3). The observed rate constants for the burst phases (k_1) were $0.1176 \pm 0.0132\,min^{-1}$ (IndOEt-NC), $0.2528 \pm 0.0768\,min^{-1}$ (IndOEt-NE) and $0.1581 \pm 0.0552\,min^{-1}$ (IndOEt-NS) and the observed rate constants for the sustained phase (k_2) were $0.0024 \pm 0.0002\,min^{-1}$ (IndOEt-NC), $0.0047 \pm 0.0001\,min^{-1}$ (IndOEt-NE) and $0.0079 \pm 0.0006\,min^{-1}$ (IndOEt-NS).

The initial concentrations of indomethacin ethyl ester for the burst phases (a) ranged between 0.05 and 0.16 mg/mL, while the initial concentrations for the sustained phases (b) varied between 0.84 and 0.93 mg/mL. These values showed that at least 84% of the ester was entrapped within the nanocarriers. The half-lives for hydrolyses of the ester associated with the nanocarriers were calculated for each burst and sustained phases (Table 4). The presence of the polymer increased the half-life of the burst phase, while the presence of the oil increased the half-life of the sustained phase. Comparing the data, IndOEt-NC presented the highest half-lives for both phases.

Table 3
Observed rate constants, correlation coefficients and MSC obtained by fitting of indomethacin ethyl ester alkaline hydrolyses (IndOEt-NC, IndOEt-NE and IndOEt-NS)

	IndOEt-NC	IndOEt-NE	IndOEt-NS
Monoexponential			
k (min^{-1})	0.0027 ± 0.0003	0.0060 ± 0.0000	0.0093 ± 0.0004
r (range)	0.9978 ± 0.0004	0.9945 ± 0.0014	0.9972 ± 0.0007
MSC (range)	4.4392 ± 0.5279	3.1561 ± 0.0457	4.1172 ± 0.4468
Biexponential			
k_1 (min^{-1})	0.1176 ± 0.0132	0.2528 ± 0.0768	0.1581 ± 0.0552
k_2 (min^{-1})	0.0024 ± 0.0002	0.0047 ± 0.0001	0.0079 ± 0.0006
a (mg/mL)	0.0503 ± 0.0260	0.1630 ± 0.0139	0.1259 ± 0.0078
b (mg/mL)	0.9337 ± 0.0274	0.8394 ± 0.0007	0.8717 ± 0.0267
r (range)	0.9981 ± 0.0004	0.9989 ± 0.0001	0.9990 ± 0.0001
MSC (range)	5.1533 ± 0.2790	5.4201 ± 0.2130	5.9207 ± 0.7435

Table 4
Half-lives of indomethacin ethyl ester consumption in the alkaline hydrolyses of IndOEt-NC, IndOEt-NE and IndOEt-NS

Formulation	$t_{1/2}$ burst (min)	$t_{1/2}$ sustained (min)
IndOEt-NC	5.9	288.8
IndOEt-NE	2.7	147.5
IndOEt-NS	4.4	87.7

In conclusion, the NC, NS and NE present different organization at a molecular level as depicted by SAXS analyses. The presence of the oil in the formulations (NC and NE) causes the dissolution of the sorbitan monostearate (low hydrophilic–lipophilic balance surfactant). On the other hand, in the case of NS this component is organized and acts as an impurity of the poly(ε-caprolactone).

Furthermore, as a general rule all formulations (unloaded, indomethacin-loaded or indomethacin ethyl ester-loaded nanocapsules, nanospheres or nanoemulsion) presented similar particle sizes, probe content (loaded formulations), pH values and zeta potentials. The kinetic experiments demonstrated that the NC, NS and NE present similar behavior, likely due to the probe being is adsorbed on the nanocarriers (indomethacin-loaded formulations). On the other hand, when the probe is likely entrapped within the nanocarriers (ester-loaded formulations), NC, NS and NE presented different kinetic behaviors. In this case, the presence of the polymer (NC and NS) prolonged the ester burst release, while the presence of the oil prolonged the ester sustained release.

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