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Determining the simultaneous presence of drug nanocrystals in drug-loaded polymeric nanocapsule aqueous suspensions: A relation between light scattering and drug content

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ABSTRACT

The encapsulation of lipophilic drugs in polymeric nanoparticles can form simultaneously both polymeric nanoparticles and drug nanocrystals. The objective was to detect the presence of nanocrystals in the nanoparticle suspensions using a simple methodology, and to determine if the nanocrystals are formed during preparation or by drug leakage from the particles during storage. Indomethacin was chosen as drug model. Unloaded and drug-loaded (1 mg/mL) nanocapsules showed diameters close to 280 nm and polydispersity lower than 0.20, remaining constant after 120 days. Comparing indomethacin loaded (3 mg/mL) and unloaded formulations, variations in the scattered light depolarization degree indicated the simultaneous presence of nanocrystals and nanocapsules in the suspensions. A relation between the scattered light intensities and the drug precipitation was established. As a function of time, when the decrease in the Rayleigh ratios occurred, the drug contents decreased due to precipitation. On the other hand, when the Rayleigh ratios slightly increase, the drug contents are constant. The nanocrystals formed in the oversaturated formulations, agglomerate and precipitate during storage. When the drug is adsorbed on the nanocapsules, but the system is not oversaturated, no nanocrystal was formed and the formulation is physico-chemically stable at least for 150 days of storage.

1. Introduction

The pharmacological response to a drug is directly related to its concentration at the required site of action. However, the distribution of a substance in the body is essentially determined by its physico-chemical properties. An unspecific distribution causes great drug concentration in healthy organs, tissues and cells leading to drug toxicity (Couvreur et al., 2002; Soppimath et al., 2001). An alternative to drive the drug to the required site is to associate it with a carrier system (Couvreur et al., 2002; Vauthier and Couvreur, 2007).

Among the different nanocarrier systems, the polymeric nanoparticles have been designed to encapsulate lipophilic drugs in order to target organs or tissues, to avoid drug degradation, to improve drug efficacy or to circumvent drug toxicity (Allemann et al., 1998; Pinto-Alphandary et al., 2000; Yoo and Park, 2000; Guterres et al., 2001; Couvreur et al., 2002; Vila et al., 2002). The polymeric nanoparticles are, respectively, named nanocapsules or nanospheres depending on the presence or absence of oil in their formulations. The nanocapsules and nanospheres can be prepared by *in situ* polymerization or by precipitation of preformed polymers (Couvreur et al., 2002; Schaffazick et al., 2003; Moinard-Checot et al., 2006; Vauthier and Couvreur, 2007). The supramolecular structure models proposed for nanocapsules and nanospheres are, respectively, a polymeric wall enveloping an oil core and a polymeric matrix stabilized by surfactants (Pisani et al., 2006; Pohlmann et al., 2002, 2007). In the past few years, our group has been dedicated to study and propose supramolecular models for polymeric nanocapsules prepared with poly(ε-caprolactone), sorbitan monostearate, polysorbate 80 and different oil cores (Guterres et al., 2000; Cruz et al., 2006a,b; Poletto et al., 2008; Jäger et al., 2007). When capric/caprylic triglyceride is used as oil, the sorbitan monostearate is manly dissolved in the core and the

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semi-crystalline polymer is located at the particle/water interface (Muller et al., 2001; Cruz et al., 2006a).

The encapsulation of lipophilic drugs by the dispersion of an organic polymeric solution in an external aqueous medium can simultaneously furnish polymeric nanoparticles and drug nanocrystals, both stabilized by the surfactants in the aqueous phase (Calvo et al., 1996). Hypothetically, when the drug concentration is higher than the saturation, nanocrystals of drug could be formed simultaneously with the nanocapsules during preparation. So, after storage the drug could precipitate as a result of the agglomeration of those nanocrystals. This hypothesis was formulated when diclofenac-loaded nanocapsules were prepared with poly(D,L-lactide) and triglycerides (Guterres et al., 1995). After preparation, the formulation showed 100% of drug encapsulation (diclofenac at 2 mg/mL) presenting an adequate granulometric profile as determined by dynamic light scattering (DLS). However, after 30 days, the drug concentration in the samples decreased in 50% and, after 8 months of storage, diclofenac crystals have been observed on the walls of the recipient. Nanocrystals were probably stabilized by the surfactants after preparation, and, within the storage, they agglomerated and, consequently, precipitated.

More recently, diclofenac-loaded nanocapsules (1 mg/mL) showed reductions in the drug recovery after 105 days of storage (Schaffazick et al., 2002). For the nanocapsules prepared with poly(ε-caprolactone), the recovery was 90%, while for those prepared with Eudragit® S90 the drug recovery was 65%. In this case, the decrease in the diclofenac concentration was also theoretically attributed to the presence of drug nanocrystals simultaneously with the nanocapsules in the suspension, which agglomerate and precipitate within the period of storage.

Flurbiprofen-loaded poly(ε-caprolactone) nanospheres showed similar release profiles after comparing the dissolution of free flurbiprofen (1 mg/mL) to the profile of the drug released from nanospheres (1 mg/mL) (Lacoulonche et al., 1999). The authors suggested that the initial drug release (burst) from nanospheres could be due to either the presence of drug at the nanoparticle surface or the presence of drug crystals in suspension. In addition, our group studied the encapsulation mechanism of indomethacin (1 mg/mL) for drug-loaded nanocapsules prepared with poly(ε-caprolactone), capric/caprylic triglyceride, sorbitan monostearate and polysorbate 80 (Pohlmann et al., 2004; Cruz et al., 2006a). The results showed that the most probable mechanism of indomethacin encapsulation was the adsorption on the particles because the drug was released in 2 min using an interfacial hydrolysis to simulate a sink condition of release. However, the rapid consumption of the drug could be related to the presence of nanocrystals in suspension. To determine if the burst release is a consequence of drug adsorption or drug nanocrystals is a very difficult task, and it was not verified in those studies.

Since the ultrafiltration-centrifugation technique is unable to separate the nanoparticles from the drug nanocrystals, a selective methodology to detect the simultaneous presence of drug nanocrystals in this kind of suspension has to be applied. It is worth to mention, the homogeneity of these formulations is strongly related to their successful application. In addition, the knowledge if the agglomeration followed by the precipitation is a consequence of the simultaneous presence of nanocrystals in suspension during preparation, or if the drug leakage from nanocarriers is sufficient to promote the formation of nanocrystals, is necessary to design and obtain optimized formulations. Taken those considerations into account, the objective of this work was to verify the simultaneous presence of drug nanocrystals in drug-loaded polymeric nanocapsule aqueous suspensions by light scattering techniques, and, furthermore, to establish a relation between the scattered light intensities and the physical instability of the drug determined by HPLC. Indomethacin was chosen as drug model because it is not internalized in the nanocapsules (Pohlmann et al., 2004), but the indomethacin-loaded nanocapsules showed selective cytotoxicity after treating glioma cell lines (U138-MG and C6) (Bernardi et al., 2008).

Table 1
Qualitative and quantitative composition of nanocarriers

Sample[a]	Polymer (mg/mL)	Oil (mg/mL)	Indomethacin (mg/mL)	Sorbitan monostearate (mg/mL)	Polysorbate 80 (mg/mL)
NC^0	10	30	–	7.7	7.7
NC^1	10	30	1	7.7	7.7
NC^3	10	30	3	7.7	7.7
ND^0	–	–	–	7.7	7.7
ND^1	–	–	1	7.7	7.7
ND^3	–	–	3	7.7	7.7

[a] Final concentration after eliminating the acetone and concentrating the suspension to 10 mL.

2. Experimental

2.1. Materials

Indomethacin was obtained from Sigma (St. Louis, USA), poly(ε-caprolactone) (MW 65,000) from Aldrich (Strasbourg, France) and sorbitan monostearate and polysorbate 80 were supplied by Delaware (Porto Alegre, Brazil). Caprylic/capric triglyceride (Miglyol® 810) was purchased from Hulls (Puteaux, France). Acetone and acetonitrile presented analytical and chromatographic grades, respectively.

2.2. Preparation of nanoparticles

For nanocapsule preparations, the organic solution consisted of Miglyol® 810, indomethacin (0, 1 or 3 mg/mL), sorbitan monostearate and poly(ε-caprolactone) dissolved in acetone (23 mL) and for nanodispersions it was composed of the surfactant and the drug (Table 1) in acetone. Each solution was poured into an aqueous phase containing polysorbate 80 (Table 1) dissolved in water (53 mL) under moderate magnetic stirring. After the nanoprecipitation, the acetone was removed and the suspension concentrated under reduced pressure to a final volume of 10 mL. Nanocapsules were called NC and nanodispersions ND. The superscripts 0, 1 and 3 indicate unloaded and indomethacin-loaded formulations (1 and 3 mg/mL). As control formulation in the density studies, a nanosphere suspension (NS^0) was also prepared using the polymer (10 mg/mL), sorbitan monostearate (7.7 mg/mL) and polysorbate 80 (7.7 mg/mL). Formulations were made in triplicate.

2.3. Light scattering experiments

The measurements were performed at room temperature (20 °C ± 1) using a Brookhaven Instruments standard setup (BI200M goniometer, BI9000AT digital correlator) with a vertically polarized Coherent He–Ne Laser (λ = 632.8 nm) as light source. The scattering volume was minimized for dynamic light scattering experiments using a 0.4 mm aperture before the entrance of the photomultiplier. All the measurements were carried out at 20 °C.

The suspensions were diluted separately with water (10%, v/v; MilliQ®) giving the respective suspension *Y*, which has been re-diluted (500–5000 times) to furnish the following suspensions: *A*: 1.00 mL of *Y* in 50 mL; *B*: 0.77 mL of *Y* in 50 mL; *C*: 0.59 mL of *Y* in 50 mL; *D*: 0.50 mL of *Y* in 50 mL; *E*: 0.33 mL of *Y* in 50 mL; *F*: 5.00 mL of *D* in 10 mL; *G*: 3.00 mL of *D* in 10 mL; *H*: 2.00 mL of *D* in 10 mL.

The diluted suspensions (*A* to *H*) obtained from each formulation were filtered (Millipore®, 0.45 μm) directly into the optic cells in order to determine the scattered light depolarization degree. On the other hand, for the analysis of the scattered light intensity and for the DLS measurements only the water used to dilute the samples has been filtered to avoid sample selection.

2.4. Particle diameter and size distribution

The particle diameters and the particle size distributions were determined using the respective dilutions from each formulation. The scattered light was observed at 90° using the equipment described above.

Normalized electric field correlation functions $g_1(t)$, calculated from the intensity autocorrelation functions $g_2(t)$, were analyzed by using the well-known cumulant method.

The obtained relaxation frequency is associated to a diffusion coefficient *D*.

$$D = \frac{\Gamma}{q^2} \tag{1}$$

The scattering vector *q* takes into account the refractive index of the solvent (n) and the scattering angle (θ) as given in the following equation:

$$q = \frac{4\pi n}{\lambda} \text{sen}\left(\frac{\theta}{2}\right) \tag{2}$$

The hydrodynamic radii (R_h) were then calculated from the diffusion coefficient using the well-known Stokes–Einstein.

$$D_0 = \frac{k_b T}{6\pi\eta_0 R_h} \tag{3}$$

wherein k_B is the Boltzmann constant, T is the absolute temperature and η_0 is the viscosity of the solvent.

2.5. Scattered light depolarization degree (ρ)

The scattered light depolarization degree (Pereira et al., 2002) supplies information about the anisotropy degree of particles in the suspensions. Based on a static light scattering (SLS) experiment, the polarized scattered light (I_{vv}) is separated from the depolarized scattered light (I_{vh}) by using a polarizer. Changes in the I_{vv} intensity generally are related to variations in the sample concentrations, while changes in the I_{vh} intensity are due to the anisotropy of the particles. The ratio of I_{vh} and I_{vv} (Eq. (4)) gives the scattered light depolarization degree (ρ).

$$\rho = \frac{I_{vh}}{I_{vv}} \tag{4}$$

2.6. Rayleigh ratio (da Silveira et al., 1996)

For the different samples, the analysis of the scattered light intensities by SLS can be used to obtain information about the presence of aggregates of high molar mass or the formation of crystalline structures in suspension. The scattered light intensities are plotted as Rayleigh ratio following equation:

$$R_s = \left(\frac{I_{v_s}}{I_{v_{tol}}}\right)\left(\frac{n_s}{n_{tol}}\right) R_{tol} \tag{5}$$

where R_s is the Rayleigh ratio of the suspension, I_{v_s} is the average light scattering intensity of the suspension, $I_{v_{tot}}$ is the average light scattering intensity of toluene, n_s is the refractive index of the suspension, n_{tol} is the refractive index of toluene and R_{tol} is the Rayleigh ratio of toluene.

After removing the influence of the solvent, the Rayleigh ratio of the particles in suspension can be obtained using the following equation:

$$R_p = R_s - [(1-\varphi)R_a] \tag{6}$$

where R_p is the Rayleigh ratio of the particles, φ is the volumetric fraction of the scattering particle and R_a is the Rayleigh ratio of water used as solvent. Since the samples were diluted 500 times for analysis, the volume fraction (φ) was disregarded.

2.7. Density studies

Separation of the particles to determine the presence of nanospheres in the nanocapsule formulation was carried out on a colloidal silica gradient (Percoll® 54%, v/v in NaCl 0.15 M, initial density: 1.074 g cm^{-3}) formed *in situ* during ultracentrifugation in a rotor model PS28T (Hitachi CP70 MX, Japan) at 20 °C and 30,000 × *g* for 90 min. Percoll® (19.6 mL) was added to 0.4 mL of NC^0 without previous concentration. For external calibration of the bands, in a separate tube, Density marker Beads® of different pre-determined densities were added at the same conditions used for the samples. Particle densities were calculated from the curves plotting distance to the top *versus* the density of each band of marker. Two control formulations were also analyzed, the ND^0 and the NS^0.

2.8. Quantification of indomethacin

NC^1, NC^3 and ND^1 (0.1 mL) were separately diluted with acetonitrile to 10 mL in order to dissolve all components. Each solution was filtered (0.45 μm, Millipore) and injected for HPLC analysis. To determine, respectively, the physical and the chemical stability of indomethacin in the formulations, equal volumes of each sample was separated in two different flasks (A and B), kept at room temperature and protected from light. The samples from flask A were not shaken before analyses, while the samples from flasks B were shaken before HPLC analysis.

The HPLC system consisted of a PerkinElmer 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 apparent pH 5.0 ± 0.5 with 10% (v/v) acetic acid. After the injection of 20 μL, indomethacin was detected at 267 nm with a retention time of 3.45 min. The HPLC method was validated considering the linearity, inter- and intraday variability, selectivity, accuracy, limit of quantification and recovery (Pohlmann et al., 2004). Linear calibration curves for the indomethacin were obtained in the range of 1.00–25.00 μg/mL presenting correlation coefficients higher than 0.9992. Inter- and intraday variability values were determined for different indomethacin 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 values did not exceed 1.68% and the accuracy was 99.0%. The limit of quantification was 1.00 μg/mL. The hydrolysis products of indomethacin showed retention time at 2.03 min (5-methoxy-2-methylindol-3-acetic acid) and 2.39 min (*p*-chlorobenzoic acid). For the stability studies, each flask was storage at room temperature and protected from light.

2.9. Statistical analyses

The statistical analyses were carried out for static light scattering experiments and for the indomethacin quantification analyses using ANOVA and test-*t* ($\alpha = 0.05$).

Table 2
Depolarization degree ($\rho_{c\to0}$) values for NC^0 and NC^1 in function of storage time

Storage time (days)	$\rho_{c\to0}$ to NC^0	$\rho_{c\to0}$ to NC^1
0	$0.01296 \pm 6.6 \times 10^{-4}$	$0.01362 \pm 1.0 \times 10^{-3}$
25	$0.01092 \pm 6.9 \times 10^{-4}$	$0.01035 \pm 3.7 \times 10^{-4}$
45	$0.01015 \pm 1.0 \times 10^{-3}$	$0.01449 \pm 1.5 \times 10^{-3}$
70	$0.01421 \pm 6.2 \times 10^{-4}$	$0.01402 \pm 9.9 \times 10^{-4}$
95	$0.01327 \pm 7.8 \times 10^{-4}$	$0.01262 \pm 1.3 \times 10^{-3}$
120	$0.01493 \pm 6.4 \times 10^{-4}$	$0.01321 \pm 2.0 \times 10^{-3}$

3. Results and discussion

3.1. Nanoparticle diameter and scattered light depolarization degree (ρ)

The nanocapsules containing or not indomethacin were called NC^1 (1 mg/mL) and NC^0, respectively. The mean diameters were about of 280 ± 20 nm and polydispersity indexes ranged between 0.03 and 0.20. The particle diameters and the polydispersity indexes were not influenced by the dilution of the formulation (*A* to *H*). After 120 days of storage at room temperature, the mean diameters and the polydispersity indexes remained constant.

As a first approach, the anisotropy of the nanocapsules in suspension was examined. In this way, the scattered light depolarization degree (ρ) (Table 2) was determined for NC^0 using Eq. (4). After preparation and after 120 days, NC^0 showed $\rho_{c\to0}$ lower than 0.015 and constant. The results indicated that NC^0 formulation present very low anisotropy. The same analysis was carried out for NC^1. Comparing the results obtained for NC^1 and NC^0, no significant difference ($p > 0.05$) in $\rho_{c\to0}$ values were detected, indicating that both systems, NC^0 and NC^1, can be considered spherical shaped.

To study the influence of the indomethacin concentration on the physical stability of the suspensions, NC^1 (1 mg/mL) was compared to another nanocapsule formulation containing 3 mg/mL of indomethacin (NC^3). This concentration was chosen considering that two formulations of nanocapsules prepared with 1.5 mg/mL of indomethacin showed significant decreases in the drug recoveries after 3 months of storage (Pohlmann et al., 2002). In this way, a nanocapsule formulation prepared with 3 mg/mL of drug should be oversaturated. After preparation, NC^3 presented mean diameters of 295 ± 18 nm with a monomodal distribution after dilution (*A* to *H*) remaining constant after 14 days. The polydispersity indexes ranged between 0.05 and 0.27. After increasing the drug concentration, the mean size and the size distribution for NC^3 were similar to those observed for NC^1. However, NC^3 showed macroscopic drug crystals precipitated after 10 days of storage.

Theoretically, the presence of nanocrystals in the suspension could cause an increase in the anisotropy of the system, reflecting in an augmentation in the scattered light depolarization degree. Indeed, after preparation NC^3 showed $\rho_{c\to0}$ of $0.01821 \pm 7.61 \times 10^{-4}$ and after 14 days $\rho_{c\to0}$ was $0.02265 \pm 1.39 \times 10^{-4}$. The results indicated that this formulation have an anisotropy a little higher than NC^0 and NC^1, which slightly increased after 14 days of storage. NC^3 is probably a suspension formed by a mixture of nanocrystals and nanocapsules. In order to confirm this hypothesis the Rayleigh ratio was determined. To evaluate if nanospheres could be present in the nanocapsule suspension a density study was performed.

3.2. Density study and Rayleigh ratio determination

To determine the nanoparticle composition in the nanocapsule suspension, a density gradient was prepared. After ultracentrifugation, ND^0 and NS^0 samples showed densities of 1.021 and 1.077 g cm^{-3}, respectively, while NC^0 sample showed two bands, one with density ranging from 0.955 to 0.989 g cm^{-3} and another one presenting density of 1.021 g cm^{-3}. No band at 1.077 g cm^{-3} was observed for NC^0. The results indicated that nanospheres were not formed, but this formulation contains sorbitan monostearate particles concomitant with nanocapsules. The concentration of sorbitan monostearate particles in the nanocapsule suspension was not determined, but previous results (SAXS) suggest that this component is mainly dissolved in the oil of those nanocapsules (Cruz et al., 2006a).

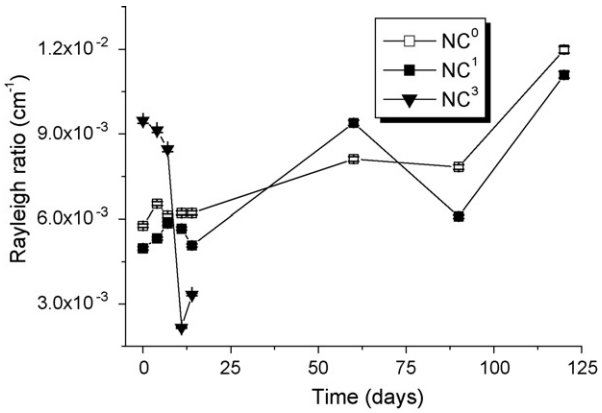

Fig. 1. Rayleigh ratio determined for NC^0, NC^1 and NC^3 as a function of the storage time.

In order to compare the average of the light scattering intensities for the samples NC^0, NC^1 and NC^3 as a function of the storage time, the Rayleigh ratios were determined using Eq. (3). NC^0 and NC^1 showed small fluctuations within 14 days and an increase in the Rayleigh ratio after 120 days of storage (Fig. 1). NC^0 and NC^1 formulations presented similar profiles, while NC^3 showed the highest Rayleigh ratio within 7 days, which rapidly decreased from 7 to 14 days of storage.

For NC^0 and NC^1, the variations of Rayleigh ratio were independent of the drug and dependent on modifications at the particle/water interface of the polymeric nanoparticles. In this way, the interfacial hydrolysis of the polymer (Calvo et al., 1996) and the consequent interaction of those carboxylic groups with the aqueous phase could explain the variations of Rayleigh ratio for NC^0 and NC^1. In addition, the highest Rayleigh ratio observed for NC^3 indicated the presence of different scattering particles in the suspensions beyond the nanocapsules, suggesting that nanocrystals were likely formed during the preparation. Within the storage period, they agglomerated and precipitated. The disappearance of those particles from the suspension because of their precipitation caused the decrease in the Rayleigh ratio.

If drug nanocrystals are dispersed in the suspension, a sample containing only the surfactants and the drug should present similar behavior to that observed for NC^3. So, for comparison, three colloidal suspensions were prepared. The first one was prepared using only the sorbitan monostearate and the polysorbate 80 (ND^0); the other two were prepared, respectively, with 1 (ND^1) and 3 mg/mL (ND^3) of indomethacin and the surfactants (sorbitan monostearate and polysorbate 80).

ND^3 was not physically stable and the indomethacin precipitated just after preparation, making not possible further analysis. ND^0 and ND^1 showed variable diameters depending on both the dilution of the samples (500–2000-folds) and the time of storage (Table 3), as well as ND^1 had drug precipitation after 14 days. Additionally, ND^0 and ND^1 showed different Rayleigh ratios after preparation (Fig. 2). Furthermore, ND^0 presented an increase in the Rayleigh ratio within 14 days, whereas ND^1 showed also an augmentation of the Rayleigh ratio within 11 days of storage, but, after

Table 3
Mean diameters for ND^0 and ND^1 after preparation and after 14 days of storage, diluting the samples 500- and 2000-folds

Sample	Storage (days)	Diameter (nm) dilution 500×[a]	Diameter (nm) dilution 2000×[a]
ND^0	0	151 ± 24	115 ± 62
	14	122 ± 64 (40%); 503 ± 223 (40%)	151 ± 12 (50%); >1500
ND^1	0	108 ± 24	97 ± 81
	14	179 ± 337 (80%); 550 ± 203 (20%)	558 ± 359

[a] Values between parentheses correspond to the distribution rate using CONTIN software.

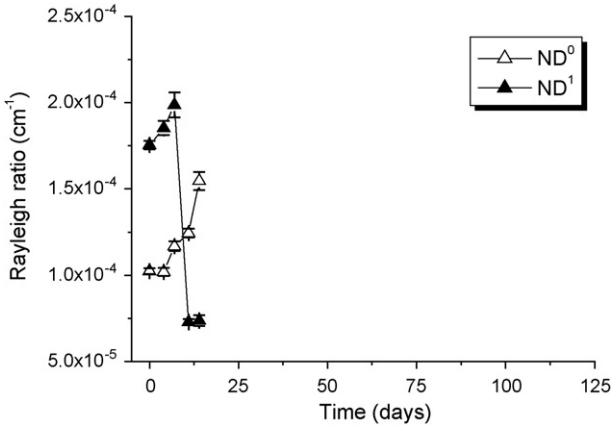

Fig. 2. Rayleigh ratio determined for ND^0 and ND^1 as a function of the storage time.

this point, the sample showed a significant ($p < 0.05$) decrease in this value. The mass transfer between the particles could increase the light scattering intensity during storage. The decrease of the Rayleigh ratio can be explained by the drug precipitation, corroborating with the results obtained for NC^3 (Fig. 3).

3.3. Drug contents as a function of time storage

The drug precipitation after the agglomeration of nanocrystals could cause a decrease of the total drug content in a stored suspension (Guterres et al., 1995). In this way, to establish a relation between the Rayleigh ratio and the drug contents as a function of time storage, the samples were analyzed by HPLC.

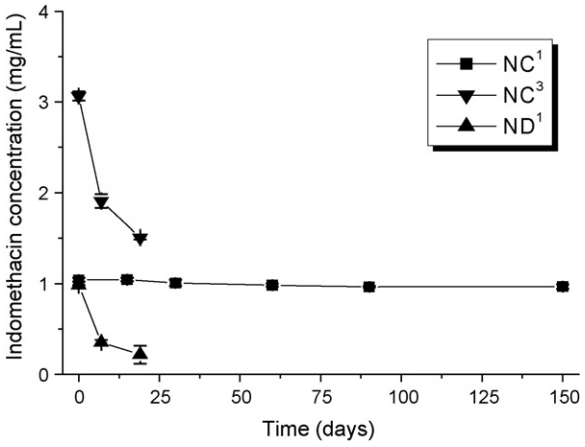

Fig. 3. Indomethacin recovery as a function of the storage time for NC^1, NC^3 and ND^1.

To differentiate if a decrease in the recovery was caused by a physical or a chemical instability of the drug, each sample was separated in two flasks. The first one was kept immobile and the second one was shaken before aliquot withdrawn to dissolution with acetonitrile followed by HPLC analysis. The initial content of indomethacin in NC^1 was 1.01 ± 0.02 mg/mL. This value remained constant ($p > 0.05$) after 150 days of storage for both immobile and shaken samples. The results indicate that NC^1 formulation has not nanocrystals in suspension. In this way, our interpretation for the previous data using the simulated sink condition of release is correct and the drug is located at the particle/water interface (Pohlmann et al., 2004; Cruz et al., 2006a). So, the adsorption is the mechanism of drug encapsulation for NC^1 and indomethacin remained encapsulated during storage.

On the other hand, for the immobile samples NC^3 and ND^1, the analyses showed significant reduction ($p < 0.05$) in their indomethacin contents as a function of the time storage, without the presence of the degradation products. For NC^3, the initial indomethacin content was 3.06 ± 0.16 mg/mL. Even though this value remained constant ($p > 0.05$) for the shaken sample during 150 days, for the immobile sample of NC^3 it was 1.91 ± 0.15 mg/mL after 7 days and 1.51 ± 0.03 mg/mL after 19 days of storage. This formulation presented a precipitate after 10 days of storage by a visual analysis. In a similar manner, ND^1 showed significant reduction ($p < 0.05$) in the indomethacin content after storage. The initial indomethacin concentration was 0.98 ± 0.01 mg/mL, decreasing to 0.35 ± 0.01 mg/mL after 7 days, and finally reaching 0.22 ± 0.02 mg/mL after 19 days of storage.

The results indicated that nanocrystals are formed simultaneously with the nanoparticles when the system is oversaturated (NC^3 or ND^1). There is a correlation between the variation in the scattering intensities and the decrease in the drug contents (for immobile samples), which are related to the presence of nanocrystals, when the drug is chemically stable within the time of storage. When the formulations are oversaturated, the nanocrystals formed during the preparation agglomerate and precipitate during storage.

4. Conclusions

The simultaneous presence of drug nanocrystals in drug-loaded polymeric nanocapsule aqueous suspensions was verified when oversaturated samples were prepared. A relation between the scattered light intensities and the physical instability of the drug was established. As a function of time storage, when the decrease in the Rayleigh ratios is observed the drug contents decreased. On the other hand, when the Rayleigh ratios are constant or slightly increasing the drug contents are constant. The nanocrystals formed in the oversaturated formulations, agglomerate and precipitate during storage. When the drug is adsorbed on the nanocapsules, but the system is not oversaturated, no nanocrystal was formed and the formulation is physico-chemically stable at least for 150 days of storage.

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