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Hydrophilic gel containing nanocapsules of diclofenac: development, stability study and physico-chemical characterization

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The purpose of this work was to develop and to characterize hydrophilic gels containing nanocapsules (NC) of diclofenac (DIC). Nanocapsules suspension of poly-ε-caprolactone containing free acid diclofenac were prepared by nanoprecipitation. The pH value of the nanocapsules suspension was 5.70 ± 0.03 and the mean sizes of the NC were in the sub 300 nm range. Drug incorporated into the nanocapsules was close to 100% and the encapsulation efficiency was 104.1% ± 3.5%. Diclofenac nanocapsules suspension (1 mg/mL) was incorporated in a Carbopol® gel matrix fournishing a formulation with 0.5 mg of DIC/g. The gel stability was evaluated in terms of the macroscopic and microscopic aspect, rheological properties, pH and drug recoveries. As a result, we obtained a suitable formulation for topical use presenting a non-Newtonian behaviour with plastic properties and with intact nanostructures in the gel matrix after 3 months storage at room temperature (freeze-fracture electron microscopy).

1. Introduction

Diclofenac, a potent non-steroidal anti-inflammatory drug, is marketed worldwide for the topical treatment of pain associated with arthrosis and acute soft-tissue injury [1–3]. It has been claimed that the topical application of diclofenac over an inflamed joint results in synovial fluid drug concentrations which exceed plasma concentrations suggesting a direct penetration of the drug into the joint [4]. However, in 1995 Zimmerman et al. [5] encountered four patients where bleeding from a peptic disease was associated with the cutaneous application of diclofenac gel. This was attributed to systemic absorption of diclofenac after administration of this drug as an emulsion gel.

Polymeric nanoparticles are being increasingly investigated for sustained release and to achieve targeted drug delivery. Colloidal drug carriers are interesting in the field of drug delivery systems because of their small size allowing them to permeate through biological barriers [6]. Regarding the mode of action of nanoparticles, one might hypothesize that they are associated with the skin surface, facilitating drug transport by changing the vehicle/stratum corneum partition coefficient [7]. Rolland et al. [8] observed a direct influence of the size of a particle on its penetration into the skin. The percutaneous penetration pathway of polymeric microspheres is size dependent. Particles <3 μm were randomly distributed into the stratum corneum and hair follicles. The main penetration pathway of these microspheres is the transepidermal route because outer surface of the follicular orifice is only 0.1% of the total skin surface area. The largest microparticles (>10–20 μm) do not penetrate the skin and remain on the stratum corneum outer surface [9].

The effectiveness of colloidal drug delivery systems (liposomes and polymeric nanospheres or nanocapsules suspensions) to reduce potential side-effects, such as gastrointestinal ulceration in the case of orally administered anti-inflammatory drugs, was shown in several investigations [10–12]. On the other hand, the nanoencapsulation of diclofenac reduces its local toxicity after intramuscular injection [13]. Two hypotheses can be raised to explain this protective effect: a slow release of the drug, lowering drug concentrations as compared to the initial levels provided by an aqueous solution and/or the lowest irritation potential of diclofenac in its acid form (used to prepare the nanocapsules) as compared with the salt form (used in commercial preparations).

Therefore, the aim of this work was to develop and to characterize a hydrophilic gel containing nanocapsules of diclofenac as well as to evaluate its stability. In order to study the influence of nanostructures on the gel properties, two similar formulations were prepared, one without incorporation of nanocapsules and another the containing diclofenac in its sodium salt form.

2. Investigations, results and discussion

The method employed allowed to obtain colloidal nanocapsules suspensions of diclofenac (1 mg/mL), using poly(ε-caprolactone) like vesicles wall former. The results of particle size analysis (Table 2) indicate that the mean sizes of the NC are in the sub 300 nm range. Regarding

Table 1: Composition of gels used in the study

	Constituents	Formulations		
		HG_C	HG_{DICNa^+}	HG-NC_{DIC}
a	Carbopol 940®	1.0 g	1.0 g	1.0 g
b	Methylparaben	0.1 g	0.1 g	0.1 g
c	Glycerine	5 mL	5 mL	5 mL
d	Triethanolamine	1.05 g	1.05 g	1.05 g
e	Diclofenac sodium (1.0 mg/mL)	–	50 mL	–
f	Suspension of nanocapsules containing diclofenac (1.0 mg/mL)	–	–	55 mL
g	Distilled water	ad 100 g	ad 100 g	ad 100 g
Order of addition		a + b + c + d + g	a + b + c + d + g (10 mL) + e + g	a + b + c + d + g (10 mL) + f + g

pH, the suspension presented an acidic value (pH 5.70 ± 0.03) due to the polymer structure and in agreement with those reported for diclofenac nanocapsule suspensions prepared by nanoprecipitation using this polymer [16, 17]. The percentage of drug incorporated into the nanocapsules was close to 100% and the encapsulation efficiency was 104.1% ± 3.5%. Nanocapsules suspensions containing higher concentrations of diclofenac were not stable presenting components precipitation.

This diclofenac nanocapsule suspension (1 mg/mL) freshly prepared was incorporated in the Carbopol 940® hydrogel (HG-NC_{DIC}) at room temperature (see Table 2) furnishing a formulation containing a dose of 0.5 mg of diclofenac/g. Similar hydrogel formulation containing the same dose of diclofenac salt sodium but without nanocapsules was also prepared in a comparable way ($HG_{DIC\,Na^+}$).

All the gels tested were perfectly homogeneous and stiff, as requested for skin application. To complete the evaluation of the new formulations developed, keeping in mind their suitability for topical application, we performed a long-term stability study. HG-NC_{DIC} and HG_{DICNa^+} remained visually almost unchanged when stored at room temperature for 12 months.

In addition, the rheological behavior of the gels, more precisely their viscosity, was studied since it plays an important role in the mixing and flow of materials, their packaging into containers, physical stability, and even patient acceptability [18]. Furthermore, the viscosity of the gel matrix may affect the absorption rate of drugs through the skin well as their biological availability. On the basis of the results shown in Figs. 1–4, we can deduce that all samples evaluated showed non-Newtonian behaviour, since their viscosities were not constant, but changed as a function of the shear rate (Figs. 1(a), 2(a), 3(a) 4(a)). The rheograms in Figs. 1(b), 2(b), 3(b) and 4(b) also show that all evaluated samples had plastic properties, because they do not flow immediately after being submitted to a shear stress. The values at which the formulations start flowing are called yield values and were obtained from the square root of shear rate, $\sqrt{s}$, plots against the square root of shear stress, $\sqrt{\tau}$, for all the formulations, and an approximately linear relation was obtained in each case. Accordingly, the relation between $\sqrt{s}$ and $\sqrt{\tau}$ fits the equation of Casson [19]:

$$\sqrt{\tau} = k + k\sqrt{s} \qquad (1)$$

where, k is the intercept at $\sqrt{s} = 0$ and k′ is the slope of the line. If the straight line is extrapolated to the shear stress axis, the yield value, which is equal to k in eq. (1), can be obtained. The results in Table 3 indicate that the HG_{DICNa^+} and HG-NC_{DIC} maintained the plastic behavior of the gel matrix what turns all the formulations appropriate for the topical use. Fig. 1 and Table 3 reveal that the incorporation of DIC_{Na^+} into the gel caused no significant ($p < 0.05$) modification in the rheo-

Table 2: Parameters of nanocapsules characterization (mean ± SD, n = 3)

Formulation	Particle size (nm ± s)	pH (± s)	Encapsulated Drug (%)	Encapsulation Efficiency (%)
NC_{DIC} 1 mg/mL	228 ± 57	5.7 ± 0.03	100.0 ± 0.0	104.1 ± 3.5

NC_{DIC}, nanocapsule suspensions of diclofenac

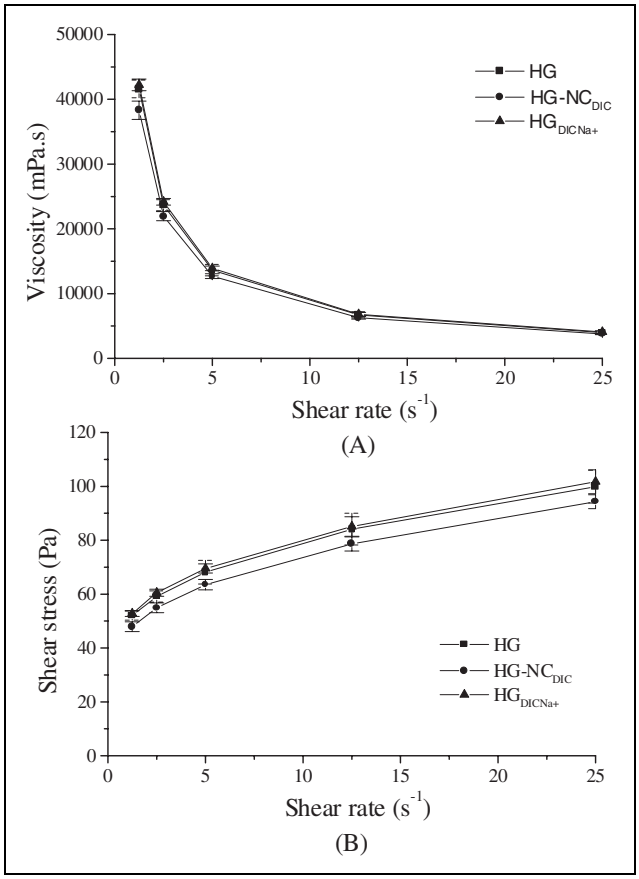

Fig. 1: Effect of diclofenac sodium (DIC_{Na^+}) and nanocapsules containing free acid diclofenac (NC_{DIC}) on the rheograms of hydrogel: (A) viscosity vs shear rate; (B) shear stress vs shear rate

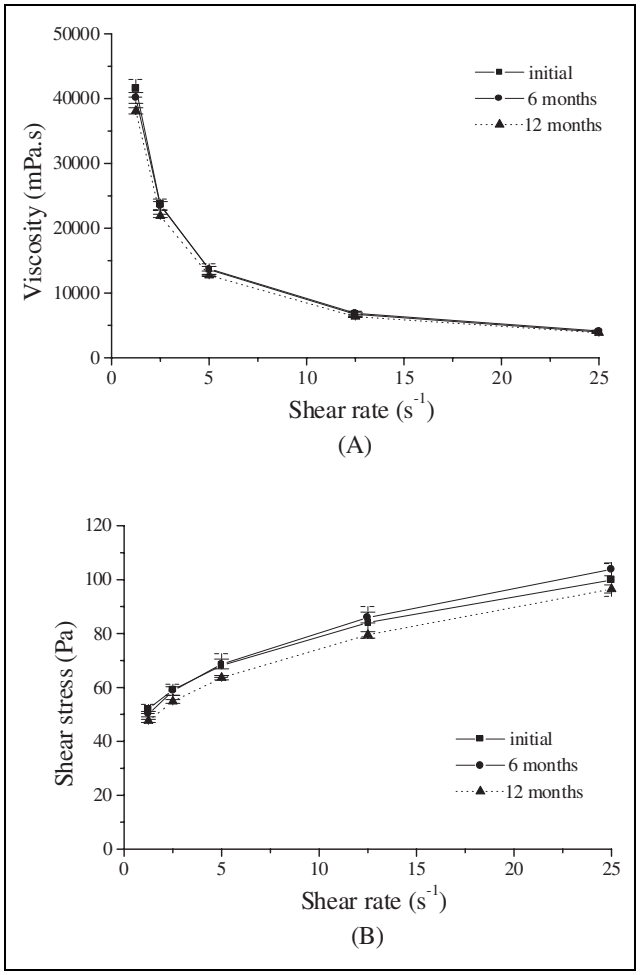

Fig. 2: Rheograms of the hydrogel: (A) viscosity vs shear rate; (B) shear stress vs shear rate.

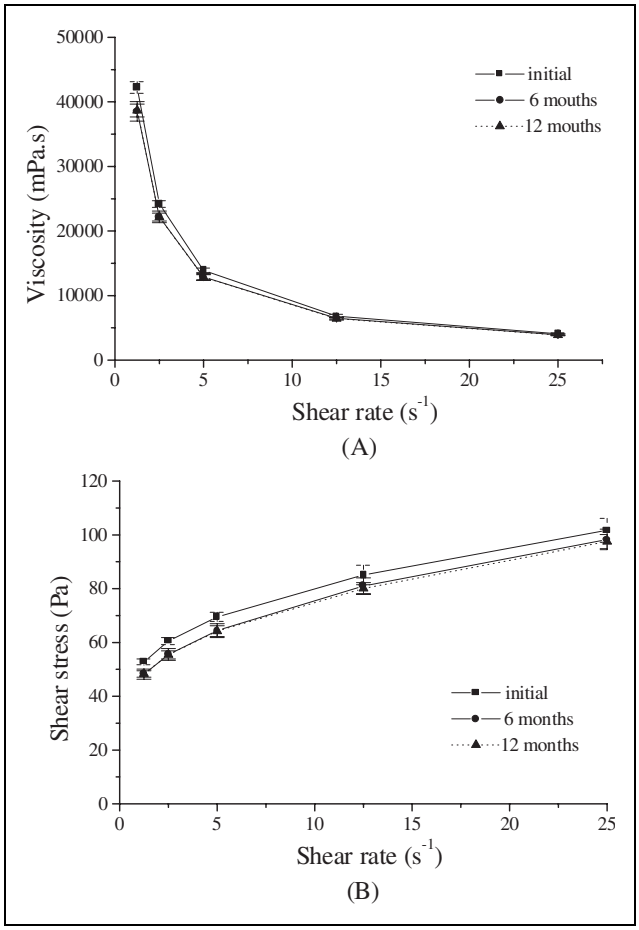

Fig. 3: Rheograms of the hydrogel containing diclofenac sodium (HG_{DICNa^+}): (A) viscosity vs shear rate; (B) shear stress vs shear rate

grams and yield value, while the incorporation of NC_{DIC} produced little, but significant ($p < 0.05$), change in the rheological properties of the hydrogel. Flow curves of the hydrogel were not significantly ($p < 0.05$) changed during storage (6 and 12 months), as shown in Fig. 2(a), 2(b) and Table 3. In contrast, the formulation HG_{DICNa^+} showed a significant ($p < 0.05$) decrease in viscosity, shear stress and yield value after 6 months storage. These alterations stayed constant with elapsing of the time of storage up to 12 months (Fig. 3(a), 3(b) and Table 3). The HG-NC_{DIC} formulation, however, demonstrated a significant increase ($p < 0.05$) in viscosity, shear stress and yield value after 6 months and with elapsing of the time of storage up to 12 months these values decreased markedly, differing significantly ($p < 0.05$) from those obtained after 6 months (Fig. 4(a), 4 (b) and Table 3).

Table 3: Yield values of the gels (mean ± SD), as function of time (n = 9)

Formulations	Yield values (Pa)		
	Initial[a]	6 Months	12 Months
HG	43.0 ± 1.9	40.8 ± 1.1	38.5 ± 0.5
HG-NC_{DIC}	43.8 ± 1.4	38.8 ± 1.7	39.0 ± 1.1
HG_{DICNa^+}	39.3 ± 1.6	43.4 ± 1.9	38.2 ± 1.2

HG, gel; HG-NC_{DIC}, gel containing diclofenac-loaded nanocapsules; HG_{DICNa^+}, gel containing salt sodium diclofenac
[a] 15 days after preparation

Theoretically, the pH value of the vehicle is also an important factor to be considered in the evaluation of drug penetration from a gel dosage form across the skin [20]. The hydrogel without diclofenac (HG) and the formulation containing diclofenac sodium salt (HG_{DICNa^+}) presented pH values close to 7.0, that remained almost unchanged after 12 months of storage (Table 4). On the other hand, the formulation containing diclofenac nanocapsules (HG-NC_{DIC}) showed a significant ($p < 0.05$) decrease in pH (7.11 ± 0.14 to 5.87 ± 0.02). This decline during the stability study could be explained by the presence of diclofenac free acid in the nanocapsule formulation. With time, a leakage of the drug associated in nanovesicles to the dispersing medium could be occured which explains this result. Another possible explanation concerns the degradation of polyesters in aqueous media, which was already shown for nanocapsules prepared with poly(D,L-lactide) and for poly(ε-caprolactone) pseudolatexes [21–23].

Table 4: pH of gels (mean ± SD), as function of time (n = 9)

Formulations	pH		
	Initial[a]	6 Months	12 Months
HG	7.80 ± 0.08	7.33 ± 0.08	7.38 ± 0.14
HG-NC_{DIC}	7.11 ± 0.14	6.60 ± 0.10	5.87 ± 0.02
HG_{DICNa^+}	7.27 ± 0.09	7.08 ± 0.02	7.28 ± 0.02

HG, gel; HG-NC_{DIC}, gel containing diclofenac-loaded nanocapsules; HG_{DICNa^+}, gel containing salt sodium diclofenac
[a] 15 days after preparation

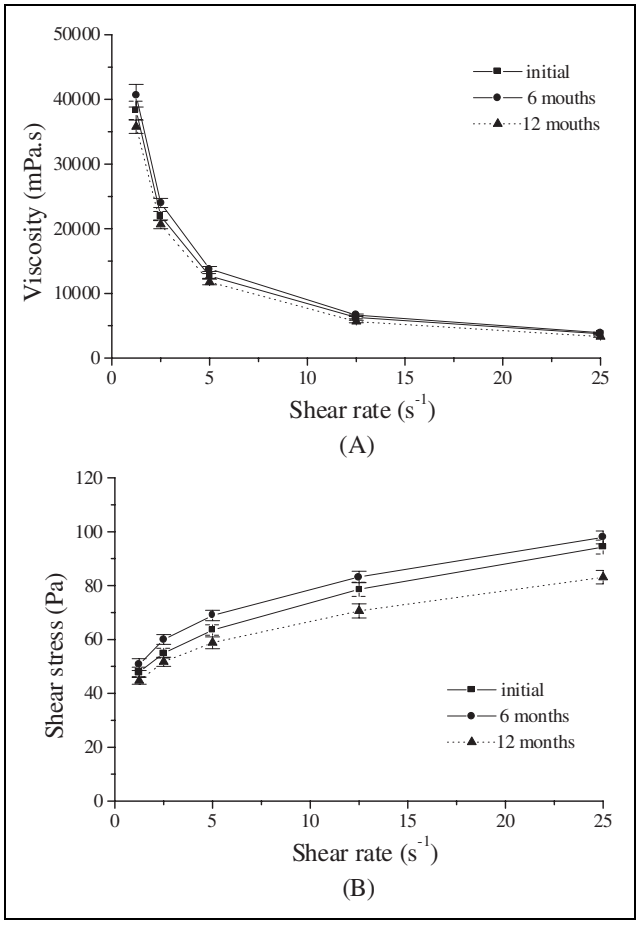

Fig. 4: Rheograms of the hydrogel containing nanocapsules of diclofenac (HG-NC_{DIC}): (A) viscosity vs shear rate; (B) shear stress vs shear rate

Regarding the drug recoveries after 15 days of storage, the formulations presented values of 101.3% ± 3.8% (HG-NC_{DIC}) and 103.4% ± 10.0% (HG_{DICNa^+}) (Table 5). After six months of storage at room temperature, diclofenac recoveries from both formulations did not decrease. However, after 12 months of storage, only the formulation prepared with diclofenac sodium salt remained unchanged. The gel containing diclofenac-loaded nanocapsules presented a declined of about 20% in the drug content (Table 5).

Because of that and to confirm the integrity of the NC_{DIC} incorporated in HG, the HG-NC_{DIC} formulation was evaluated by freeze-fracture electron microscopy (FFEM). As seen in the FFEM pictures shown in Fig. 5(B), the nanocapsules had an almost spherical shape and remained intact in the hydrogel upon storage at room temperature for 3 months. After 12 months (Fig. 5(C)), however, the presence of deformed vesicular structures, previously described in the literature [24], could be observed.

Table 5: Contents of diclofenac (%) (mean ± SD) in the gels, as function of time (n = 9)

Formulations	Diclofenac (%)		
	Initial[a]	6 Months	12 Months
HG-NC_{DIC}	101.3 ± 3.8	112.5 ± 4,7	81.6 ± 4.4
HG_{DICNa^+}	103.4 ± 10.0	117.1 ± 11.5	111.0 ± 4.5

HG-NC_{DIC}, gel containing diclofenac-loaded nanocapsules; HG_{DIC}, gel containing salt sodium diclofenac
[a] 15 days after preparation

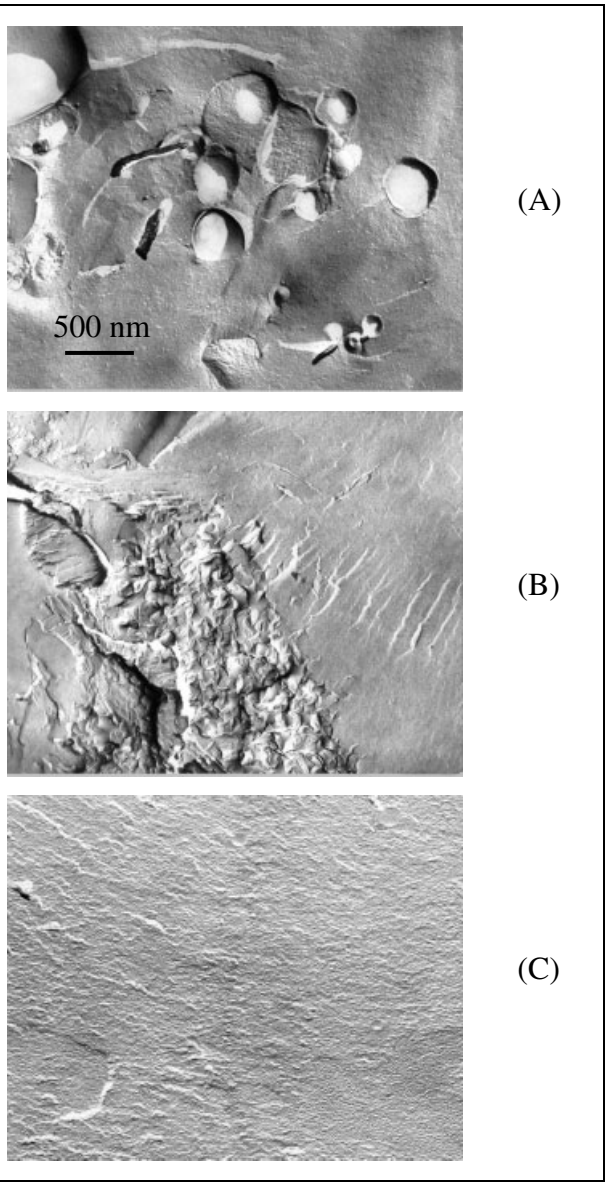

Fig. 5: Electron micrographs of freeze-fracture replicas: HG-NC_{DIC}, gel containing diclofenac-loaded nanocapsules after 3 months (A) and 12 months storage at room temperature (B) and HG, hydrogel (C)

In conclusion, this study showed the technological viability of the development of a hydrogel containing diclofenac-loaded nanocapsules. Intact nanostructures were present in the gel matrix after storage at room temperature for at least 3 months. Work is in progress to evaluate the *in vitro* release profile of diclofenac as well as the topical anti-inflammatory activity of the drug.

3. Experimental

3.1. Materials

Diclofenac (sodium salt) was obtained from Sigma (St Louis, USA); poly(ε-caprolactone) (PEC, MW = 80,000) from Aldrich (Strasbourg, France). Caprilic/capric triglyceride (Miglyol 810®) was obtained from Hulls (Puteaux, France); sorbitan monostearate and polysorbate 80 were supplied by Delaware (Porto Alegre, Brazil). Carbopol 940® (B. F. Goodrich, São Paulo, Brazil), methylparaben, glycerine and triethanolamine were purchased from Delaware (Porto Alegre, Brazil). All other chemicals and solvents used were of pharmaceutical grade. All reagents were used as received.

3.2. Preparation of the free acid form of diclofenac

An aqueous solution of sodium diclofenac was acidified to pH 4.0 with acetic acid 10%. This solution was then extracted three times with chloroform. After filtering through sodium sulfate, the solvent was removed by evaporation under reduced pressure. The diclofenac obtained was characterized by ^{1}H NMR (200 MHz) (Varian).

3.3. Preparation of nanocapsule suspensions

Nanocapsule suspensions of PEC containing free acid diclofenac were prepared as described by Fessi et al. [14]. Briefly, lipophilic solution was consisted of Miglyol 810® (3.3 mL), diclofenac (free acid) (0.100 g), sorbitan monostearate (0.766 g), the polymer (1.000 g) and acetone (267.0 mL). This organic phase was added under moderate magnetic stirring into an aqueous solution containing polysorbate 80 (0.766 g in 533.0 mL of water). Acetone was removed by evaporation under reduced pressure and the final concentration of the suspension was adjusted to 1 mg/mL of diclofenac. Formulations were made in triplicate.

3.4. Characterization of nanocapsule suspensions

The particle size was measured by laser light scattering (Nanosizer®, Coultronics, Andilly, France).
Diclofenac was assayed by HPLC. The system consisted of a SPD-10A Shimadzu detector, LC-10AD Shimadzu pump, SIL-10A Shimadzu injector and Nova-Pak® C18 – 3.9×300 mm Waters column. The mobile phase consisted of acetonitrile/water (65 : 35 v/v) adjusted to pH 4.0 with acetic acid 10%. Diclofenac was detected at 280 nm with a retention time of about 6.7 min. Free diclofenac (non-associated) was determined in the ultrafiltrate after separation of the nanocapsules by ultrafiltration-centrifugation technique (Ultrafree-MC 10.000 MW, Millipore). Total diclofenac was measured using HPLC after dissolution of a colloidal suspension by acetonitrile. The amount of diclofenac associated with the nanocapsules was calculated from the difference between the total and free drug concentrations measured in the nanocapsule suspension and in the ultrafiltrate. The pH values of the nanocapsules suspensions were determined directly in the samples (Micronal B374 potentiometer).

3.5. Gel preparation

The weighed amounts of the gels constituents were placed in a beaker according to the order described in Table 1. Stirring was continued until all ingredients were completely dispersed and/or dissolved. All formulations were prepared in triplicate.

3.6. Gel characterization

3.6.1. Viscosity determination

The rheological study of the gels was carried out at 27 ± 1 °C using a Brookfield rotational viscometer, model DV-I+ serie RV, fitted with an SC4-25 small adapter.

3.6.2. pH Determination

The gels were diluted to 10% (w/v) in distilled water and their pH values were determined in a Micronal B374 potentiometer, at room temperature.

3.6.3. Determination of diclofenac in the gels

The content of diclofenac in the gels was determined by HPLC after the following extraction procedure. Approximately 1.0 g of each formulation was accurately weighed and placed in a 50 mL volumetric flask, acetonitrile was added and the flask was heated to 65 °C until the gel was completely dissolved in the acetonitrile. The solution was cooled to room temperature and diluted to volume with acetonitrile. After filtration through a 0.22 µm hydrophilic membrane (Durepore®), the solutions were injected into the chromatograph under the conditions described above (see characterization of nanocapsules suspensions).

3.6.4. Freeze-Fracture Electron Microscopy (FFEM)

Freeze-fracture was performed on samples sandwiched between copper plates (maintained at the same temperature as the sample) and thermally quenched by plunging into liquid propane. Fracturing and shadowing were performed at −130 °C in a BAF 400D apparatus (BALTEC, Balzers Liechtenstein). The replicas were cleaned with chloroform and examined using a CEM 902A (Zeiss, Germany) electron microscope.

3.6.5. Stability study

The gels were packed in opaque vessels and stored at room temperature (23 ± 2 °C). For each formulation, the parameters previously described were checked at regular time intervals (48 hours after preparation and after 6 and 12 months of storage). Physical evaluation of the samples' stability was carried out by visual inspection and rheological tests. Chemical stability was evaluated by pH and HPLC analysis.

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