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Semisolid topical formulations containing nimesulide-loaded nanocapsules, nanospheres or nanoemulsion: development and rheological characterization

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The objective of this work was to develop and characterize semisolid topical formulations containing nimesulide-loaded nanospheres, nanocapsules or nanoemulsion. The nanoprecipitation and spontaneous emulsification methods were used to prepare the colloidal suspensions and the nanoemulsion. The hydrodynamic diameters were 282 nm for the nanoemulsion, 293 nm for the nanocapsules and 191 nm for the nanospheres containing nimesulide. The encapsulation efficiencies were close to 99% in all cases and pH values ranged between 5.1 and 5.3. Each drug-loaded nanocarrier formulation was incorporated in Carbopol 940® gels. The semisolid dosage forms showed yellowish, glossy and homogeneous aspect after the incorporation of the colloidal suspensions and nanoemulsion. The recovery of nimesulide and the pH values for the gels containing nanoemulsion, nanospheres or nanocapsules remained constant during storage (120 days). For all formulations, the rheograms exhibited a non-Newtonian behavior presenting pseudoplastic characteristics and shear thinning. The rheograms were adjusted to Ostwald's model showing regression coefficients higher than 0.9900. None thixotropic phenomenon was experimentally detected under the test conditions for all formulations.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in therapeutics due to their anti-inflammatory, analgesic or antipyretic activities. However, these drugs may also cause gastrointestinal disorders in some patients, including irritation, bleeding and ulceration (Babar et al. 1990; Curdy et al. 2001; Joseph et al. 2002). In consequence, there is a great interest in the development of topical dosage forms containing NSAIDs, in order to avoid gastrointestinal toxicity observed after oral administration or to promote significant levels of the drug in the application site for prolonged periods (Gürol et al. 1996; Mikulad et al. 1998).

Nimesulide (4-nitro-2-phenoxymethanesulfonanilide) differs from other NSAIDs by the chemical structure and selective inhibition of cyclooxygenase-2 (Kovariková et al. 2003). Several works were carried out with the objective of increasing the properties of dissolution and the bioavailability of nimesulide (Castelli et al. 2003; Meriani et al. 2003). Formulations were also developed including the drug in cyclodextrins (Nalluri et al. 2003), phospholipidic membranes (Ferreira et al. 2003) and in solid lipid nanoparticles (Patravale and Ambarkhane 2003).

Comparing different NSAIDs, some studies have demonstrated that a gel containing nimesulide showed better anti-inflammatory activity than a gel loading diclofenac or piroxicam (Gupta et al. 1996). According to Sengupta et al. (1998), the topical administration of nimesulide may be a safe and effective alternative to oral and rectal routes.

In the past few years, colloidal systems were prepared using biodegradable polymers that have been studied due to their potential for NSAID topical and ophthalmic administration (Couvreur et al. 1995; Yokoyama and Okano 1996; Giunchedi et al. 1999; Pinto-Aphandary et al. 2000; Kim and Lee 2001). These studies showed that the selectivity and efficiency of the drugs can be improved by encapsulating them in to the colloidal systems.

The main advantages of nanoparticles are the increasing of bioavailability in the case of poorly water-soluble drugs, the protection of drugs from inactivation in the gastro-intestinal tract, the protection of mucosa from the toxicity of drugs, the increasing of permeation of drugs through mucosal surfaces and the prolongation of the blood circulation of injected drugs (Legrand et al. 1999). Besides, some investigations showed that nanoparticles and liposomes present the tendency to interact with inflammed tissues. This specificity may represent new possibilities of therapy for several rheumatic diseases (Kreuter 1994) by using the encapsulation of NSAIDs or antibiotics in nanocarrier systems as a strategy.

The literature shows that the efficiency and the adjustment of the cutaneous route may open a therapeutical alternative for NSAID administration (Bernareggi 1988; Foldvari 2000; Güngor and Bergisadi 2003). The objective

of this work was to develop and characterize semisolid topical formulations containing nimesulide encapsulated in nanospheres, nanocapsules and nanoemulsion. Up to now, as far we know, there is no report in the literature which proposes the entrapment of nimesulide in nanoparticles, which are dispersed in gel formulations.

2. Investigations, results and discussion

The nanocapsules, nanospheres, and the nanoemulsion containing nimesulide were obtained as aqueous colloidal formulations. The physicochemical characteristics of the nimesulide-loaded nanocarriers are presented in Table 1. The particle sizes were in the sub 300 nm range. These results are compatible with those reported for similar systems containing diclofenac, another NSAIDs (Guterres et al. 1995; Müller et al. 2001; Milão et al. 2003). The encapsulation efficiencies were close to 99% for all formulations and the pH values were in the range of 5.1 to 5.3. The formulations did not present significant difference ($p > 0.05$) among the pH values.

The nimesulide-loaded nanoemulsion (1.37 mg/ml), nanocapsules (1.41 mg/ml), and nanospheres (1.37 mg/ml), recently prepared, were incorporated in Carbopol 940® gel. This acrylic acid hydrogel has been selected due to its widely use in pharmaceutical formulations (Peppas et al. 2000). The nimesulide concentrations in the gels were limited to its concentration in each nanocarrier formulation, which was used as aqueous phase in each gel product. In this way, gels presented final nimesulide concentrations of 1.30 mg/g (nanoemulsion: GNM-NE), 1.29 mg/g (nanocapsules: GNM-NC) and 1.26 mg/g (nanospheres: GNM-NS). A similar gel (GC-NM) formulation containing 1.5 mg/g of nimesulide but using water instead of a nanocarrier formulation was also prepared for comparison. In parallel, a gel (GC) with neither nimesulide nor nanocarriers was also prepared as control (Table 2).

Table 1: Parameters of nanoemulsion, nanospheres and nanocapsules characterization (mean ± SD, n = 3)

Formulation	Particle size (nm ± s)	pH (± s)	Entrapped drug (mg/mL)	Encapsulation efficiency (%)
NE	282 ± 22	5.3 ± 0.05	1.37 ± 0.05	98.9 ± 0.4
NC	293 ± 9	5.2 ± 0.34	1.41 ± 0.10	99.2 ± 0.4
NS	191 ± 14	5.1 ± 0.13	1.37 ± 0.05	99.3 ± 0.2

NE, nimesulide-loaded nanoemulsion; NC, nimesulide-loaded nanocapsule suspensions; NS, nimesulide-loaded nanosphere suspensions

Table 2: Composition of gel formulations

Components	Formulations				
	GNM-NE	GNM-NC	GNM-NS	GC-NM	GC
Carbopol 940®	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g
Methylparaben	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g
Propylparaben	0.1 g	0.1 g	0.1 g	0.1 g	0.1 g
Sorbitol	5 mL	5 mL	5 mL	5 mL	5 mL
Triethanolamine	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g
Nimesulide	–	–	–	0.15 g	–
Sorbitan monoestearate	–	–	–	0.76 g	–
Polysorbate 80	–	–	–	0.76 g	–
Nimesulide-loaded Nanoemulsion	ad 100 g	–	–	–	–
Nimesulide-loaded Nanocapsules	–	ad 100 g	–	–	–
Nimesulide-loaded Nanospheres	–	–	ad 100 g	–	–
Distilled water	–	–	–	ad 100 g	ad 100 g

Table 3: Nimesulide contents (%) and values of pH in the gels as function of time (mean ± SD) (n = 3)

Formulations	Nimesulide (%)		pH	
	Initial(*)	4 months	Initial(*)	4 months
GNM-NE	102.9 ± 4.3	106.1 ± 5.5	6.82 ± 0.26	6.51 ± 0.12
GNM-NC	98.3 ± 6.0	105.6 ± 10.4	6.84 ± 0.07	6.53 ± 0.30
GNM-NS	97.7 ± 3.8	107.5 ± 8.8	6.98 ± 0.05	6.70 ± 0.27
GC	–	–	7.01 ± 0.11	7.02 ± 0.12
GC-NM	97.7 ± 2.2	99.94 ± 4.7	6.89 ± 0.11	6.87 ± 0.09

GNM-NE, gel containing nimesulide-loaded nanoemulsion; GNM-NC, gel containing nimesulide-loaded nanocapsule; GNM-NS, gel containing nimesulide-loaded nanosphere; GC gel control; GC-NM, gel containing nimesulide
* 48 h after preparation

All the gel formulations showed satisfactory organoleptic characteristics after the incorporation of nanocapsules, nanospheres and nanoemulsion containing nimesulide. When pure nimesulide was incorporated, the gel showed a yellowish color. On the other hand, when nimesulide-loaded nanocapsules, nanospheres or nanoemulsion were incorporated, the gels were white.

Table 3 shows the contents of nimesulide in gels with nanoemulsion (GNM-NE), nanocapsules (GNM-NC), nanospheres (GNM-NS) and gel with nimesulide (GC-NM) after preparation and after 4 months of storage at room temperature. The formulations were stable during storage, presenting no significant decrease of nimesulide contents ($p > 0.05$) by the F-test. Regarding the pH, all gel formulations presented values close to 7, which were stable after 4 months ($p > 0.05$).

Through the analysis of the rheograms (Gupta and Garg 2002) in Fig. 1, it is possible to verify that the flow curves of all gels showed non-Newtonian behavior, since their viscosities were not constant (Radebaugh et al. 1996; Shawesh et al. 2003). The non-Newtonian behavior of this kind of preparation is reflected by the Power Law index (n) (Contreras et al. 2001).

There are several models, which may be used to establish index flow (n) in different non-Newtonian systems (Kim et al. 2003). The most adequate model for a viscoplastic fluid depends on the fluid response to deformation and how well the experimental data fit the model (Briceño 2000). The formulations were analyzed using different models: Bingham (Eq. (1)), Casson (Eq. (2)), Ostwald (Eq. (3)) and Herschel-Bulkley (Eq. (4)).

$$\tau = \tau_o + \eta\dot{\gamma} \quad (1)$$

$$\tau^{0.5} = \tau_o^{0.5} + \eta^{0.5}\dot{\gamma}^{0.5} \quad (2)$$

$$\tau = \kappa\dot{\gamma}^n \quad (3)$$

$$\tau = \tau_o + \kappa\dot{\gamma}^n \quad (4)$$

The τ_o is the yield stress, η is the viscosity, n is the index of flow, K is the index of consistency, τ is the shear stress and $\dot{\gamma}$ is the shear rate (Kim et al. 2003).

The rheograms of this study were better adjusted to the Ostwald's model. The results showed good regression coefficients, proving that the Potency Law reproduced properly the rheological behavior of these systems (Table 4). The gels showed pseudoplastic and shear thinning behavior ($n < 1$) (Table 5). The consistency index (κ) and the flow index (n) were compared between each gel containing nanocarrier formulation (GNM-NE, GNM-NC or

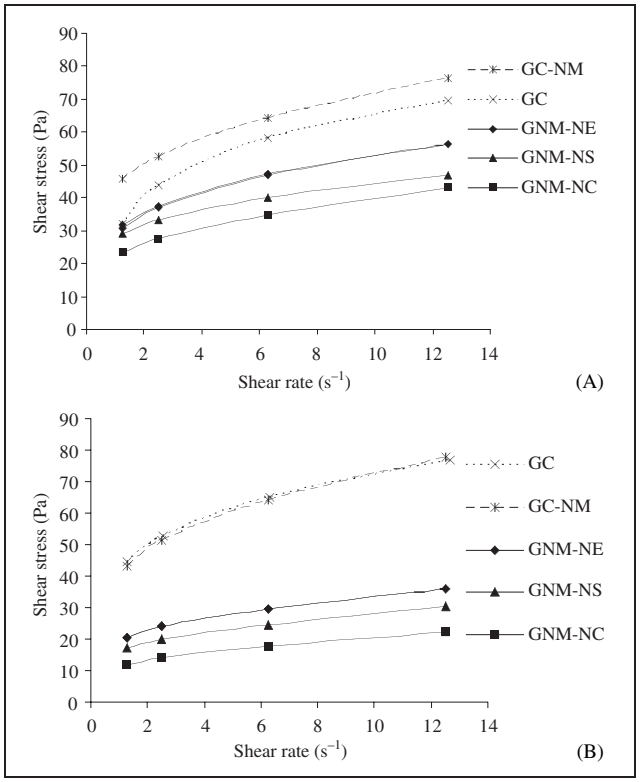

Fig. 1: Rheograms of GNM-NE (gel containing nimesulide-loaded nanoemulsion); GNM-NC (gel containing nimesulide-loaded nanocapsule); GNM-NS (gel containing nimesulide-loaded nanosphere); GC-NM (gel containing nimesulide) and GC (gel control). (A) shear stress vs. shear rate, 7 days after preparation; (B) shear stress vs. shear rate, 4 months after preparation

Table 4: Regression coefficient (R^2) for various flow models in shear rate-shear stress curve

Formulations	Bingham	Casson	Ostwald	Herschel-Bulkley
GNM-NE	0.9720	0.9932	0.9959	0.9406
GNM-NC	0.9716	0.9974	0.9958	0.9409
GNM-NS	0.9745	0.9981	0.9941	0.9421
GC	0.9359	0.9842	0.9995	0.8395
GC-NM	0.9582	0.9884	0.9958	0.9119

GNM-NE, gel containing nimesulide-loaded nanoemulsion; GNM-NC, gel containing nimesulide-loaded nanocapsule; GNM-NS, gel containing nimesulide-loaded nanosphere; GC gel control; GC-NM, gel containing nimesulide

GNM-NS), the nimesulide-loaded gel (GC-NM) and the control gel (GC) (Table 5) after preparation and after 4 months of storage at room temperature. No thixotropic phenomenon was experimentally detected under the test conditions for all formulations. It is important to consider that the validity of this model is limited to the region of the tested deformation conditions.

The results (Figs. 1A, 1B and Table 5) show that the incorporation of the nanocarrier formulations did not modify the kind of flow presented by these systems neither for the initial values nor after the storage period. Considering these results it is possible to conclude that all formulations showed adequate characteristics to be topically applied. In the case of dermatological administration, plastic properties are preferred because the formulation flow resistance is low when it is applied under medium to high shear conditions. On the other hand, the flow is zero under stress caused by gravity (Welin-Berger et al. 2001). Thus, plasticity presents an obvious relevance as a suitable descriptor of the consistency of topical formulations and the viscosity has a dominant effect on the release of the active substance from the vehicle (Welin-Berger et al. 2001). According to the initial values (Table 5), there was no significant difference ($p > 0.05$) between the gel formulations containing nanoemulsion (GNM-NE) or nanocapsules (GNM-NC) in relation to the gel control (GC) concerning the flow index (n). The gel formulations containing nimesulide-loaded nanospheres (GNM-NS) and nimesulide (GC-NM) showed significant difference ($p > 0.05$) in the flow index compared to the gel control (GC) according to Tukey's test. After 4 months there was no significant difference in the flow index (n) among all the formulations tested. It should be noted that the gel control (GC) showed an alteration in the flow index after the storage period. This alteration can be explained by the increase of the consistency (Table 5) of the gel control (GC) and the possible rearrangement of the polymeric network during the storage period. The structure of Carbopol® gels is determined by the level in which the macromolecules connect and form entanglement networks (Contreras et al. 2001). In accordance with the initial results, there was not significant difference ($p > 0.05$) in the consistency index (K) of the gels containing the nanocarrier formulations (GNM-NE, GNM-NC, GNM-NS) compared to the gel control (GC). The gel containing nimesulide (GC-NM) showed a consistency index (κ) different from the gel control (Table 5). After the storage period there was a significant decrease in the consistency index (K) of the gels containing nanocarriers (GNM-NE, GNM-NC, GNM-NS) compared to the gel control (Fig. 1B and Table 5). The decrease in viscosity for semisolid formulations containing nanocarriers has been previously reported in the literature when liposomes containing calcein were added in Carbopol® gel (Pavelic et al. 2001). Similar results about the viscosity of hydrogels based on Carbopol® were also observed after the methyl-β-cyclodextrin incorporation (Booulmedarat et al. 2003).

Table 5: Flow indexes (n), consistency (K) and spreadability (Si) of the gels

Formulations	n		K(Pa · s^n)		Si (mm^2)	
	Initial*	4 months	Initial*	4 months	Initial*	4 months
GNM-NE	0.25^{ab}	0.24^{b}	29.79^{a}	19.40^{b}	2708.2^{a}	3119.3^{abc}
GNM-NC	0.26^{ab}	0.26^{b}	21.97^{a}	11.17^{b}	2771.0^{a}	3172.1^{ab}
GNM-NS	0.20^{b}	0.24^{b}	27.77^{a}	16.08^{b}	2534.2^{a}	3276.44^{a}
GC	0.33^{a}	0.23^{b}	30.77^{a}	42.25^{a}	2649.2^{a}	2530.6^{bcd}
GC-NM	0.22^{b}	0.25^{b}	43.25^{b}	40.80^{a}	2591.9^{a}	2226.5^{d}

GNM-NE, gel containing nimesulide-loaded nanoemulsion; GNM-NC, gel containing nimesulide-loaded nanocapsule; GNM-NS, gel containing nimesulide-loaded nanosphere; GC gel control; GC-NM, gel containing nimesulide

* 7 days after preparation

a-b-c-d: the averages with different letters (inside of the same column) were considered statistically different in agreement with Tukey's test

The efficacy of a topical therapy depends on the way in which the patient spreads the drug formulation in even layers to administer a standard dose. Spreadability is therefore an important characteristic of these formulations and it is responsible for correct dosage transfer to the target site (Garg et al. 2002). Many authors have evaluated the applicability of products using the theoretical equation of laminar flow "plan between parallel plates" (Vennat et al. 1992; Lardy et al. 2000). The parallel plates method is the most used to regulate and quantify the applicability of semisolid preparations (Garg et al. 2002). The results concerning the spreadability of gels are showed in Figs. 2A and B. After preparation (7 days), it was not detected significant differences of spreadability ($p > 0.05$) between each formulation containing nanocarriers (GNM-NE, GNM-NC, GNM-NS) and the gel control (GC) (Fig. 2A). After 4 months of storage the gels GNM-NE, GNM-NC and GNM-NS did not show significant difference ($p > 0.05$) of spreadability amoung their values. On the other hand, the gel containing nimesulide-loaded nanospheres (GNM-NS) showed significant increase of spreadability in relation to the control (GC) according to Tukey's test (Fig. 2B). The correspondent graphic representations of spreadability showed similar profiles for all formulations. Furthermore, these results are in agreement with those presented by the rheograms. The structure of Carbopol® (network extent) has implications on the facility in which the initial deformation occurs and in the flow index proper for each formulation. It is important to take into account the decrease of gel viscosity observed by the consistency (Table 5) after the incorporation of this type of nanocarrier system.

In conclusion, this work showed that different nimesulide-loaded nanocarriers (nanoemulsion, nanocapsules, nanospheres) can be incorporated in semisolid hydrophilic gels. In general, the incorporation of nanoemulsion, nanocapsules or nanospheres did not affect the non-Newtonian behavior and the pseudoplastic character of the gels. These gels presented adequate physico-chemical properties for the topical administration of nimesulide.

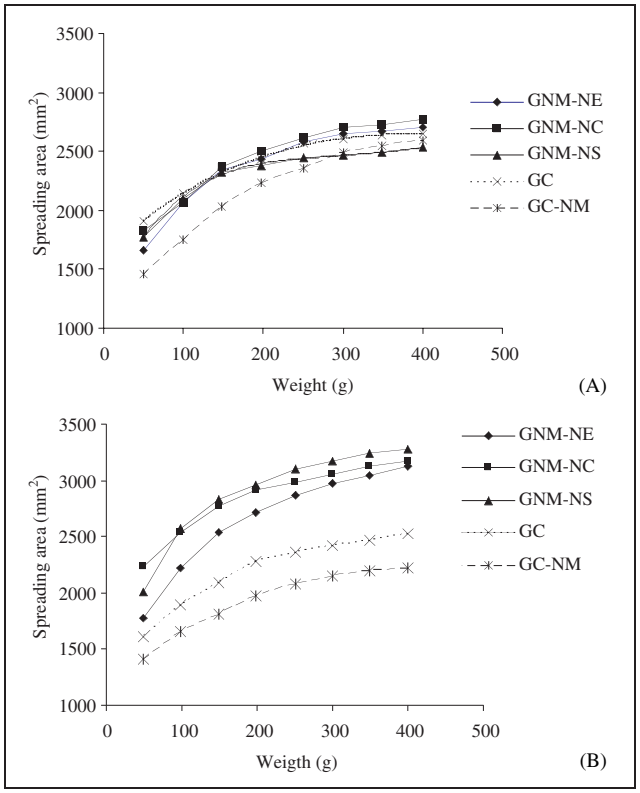

Fig. 2: Spreadability of the gels (A) after 7 days; (B) after 4 months

3. Experimental

3.1. Materials

Nimesulide was obtained from Henrifarma and poly(ε-caprolactone) (PEC, MW = 80,000) from Aldrich (Strasbourg, France). Caprilic/capric triglyceride; sorbitan monostearate; polysorbate 80; methylparaben, propylparaben, sorbitol and triethanolamine were supplied by Delaware (Porto Alegre, Brazil). Carbopol 940® was adquired from B. F. Goodrich, São Paulo, Brazil. All other chemicals and solvents used were of pharmaceutical grade. All reagents were used as received.

3.2. Preparation of formulations

Nanocapsules suspensions of poly(ε-caprolactone) (PCL) containing nimesulide were prepared by nanoprecipitation (Fessi et al. 1988) and spontaneous emulsification. Briefly, the lipophilic solution was consisted of triglyceride (3.1 mL), nimesulide (0.150 g), sorbitan monostearate (0.766 g), the polymer (1.000 g) and acetone (267.0 mL). Nanosphere suspensions were prepared without the addition of the oil. Nanoemulsion was prepared without the addition of the PCL. 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 and water was concentrated by evaporation under reduced pressure and the final formulation was adjusted to 100 mL. Formulations were made in triplicate.

The particle sizes were measured by laser light scattering (Brookheaven Instruments BI-200M, Spectra Physics He−Ne, $\lambda_0 = 632.8$ nm).

Nimesulide was assayed by HPLC. The system consisted of a SPD-10A Shimadzu detector, LC-10AD Shimadzu pump, SIL-10A Shimadzu injector and Lichrospher® 100 RP-18 (5 μm) column. The mobile phase consisted of acetonitrile/water (60 : 40 v/v). Nimesulide was detected at 230 nm with a retention time of about 5.3 min. Free nimesulide (non-associated with nanocarriers) was determined in the ultrafiltrate after separation of the continuous phase from nanoemulsions, nanocapsules or nanospheres by ultrafiltration-centrifugation technique (Ultrafree-MC 10.000 MW, Millipore). Total nimesulide was measured by HPLC after dissolution of all components of the formulations by acetonitrile. The associated nimesulide with the nanocarriers (nanoemulsions, nanocapsules and nanospheres) was calculated from the difference between the total and the free drug concentrations. The pH values of the formulations were determined directly in the samples (Micronal B374 potentiometer).

3.3. Gel preparation

The weight amounts of the gel constituents (Table 2) were placed in a beaker. Stirring was continued until all ingredients were completely dispersed/or dissolved. All formulations were prepared in triplicate.

3.4. Gel characterization

3.4.1. Viscosity

The rheological study of the gels was carried out at 23 ± 1 °C using a Brookfield rotational viscometer, mode LVF, spindle n° 4, with a shear rate interval from 6 to 60 rpm. The data were analyzed by using conventional flow equations such as Bingham, Casson, Ostwald and Herschel-Bulkley. The data obtained were analyzed with Excel for Windows software.

3.4.2. pH Determination

The pH values of the gels were determined directly in the samples (Micronal B374 potentiometer).

3.4.3. Determination of nimesulide in the gels

The content of nimesulide 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. The solution was cooled to room temperature and properly diluted 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 formulations).

3.4.4. Spreading capacity

After preparation (7 days) and after 4 months of storage, the gels were pressed between sequences of weights (g) 49.4, 98.8, 148.0, 197.6, 250.3,

299.8, 348.6, 400.8 respectively, for one minute each, with intervals of 30 s between weights. The spreading areas reached by samples were measured in millimeters in vertical and the horizontal axes. The results were expressed in terms of the spreading area as a function of the applied mass according to the following equation (Eq. (5)) (Münzel et al. 1959; De Paula et al. 1998):

$$S_i = \frac{d^2 \cdot \pi}{4} \quad (5)$$

in which S_i is the spreading area (mm) resulting from the applied mass i(g), and d is the mean diameter (mm) reached by each sample. The spreading area was plotted against the plate weights to obtain the spreading profiles.

3.4.5. Stability

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 (after preparation and after 4 months of storage). Physical stability evaluation of the samples was carried out by visual inspection, rheological tests and spreadability. Chemical stability was evaluated by pH and HPLC analyses.

3.5. Statistics

All the results are expressed as the mean ± the standard deviation of the mean and statistically analyzed using variance analysis (ANOVA). Results presenting $p < 0.05$ were considered statistically different. The comparisons among the averages were performed using Tukey's test.

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