Influence of Oleic Acid on the Rheology and in Vitro Release of Lumiracoxib from Poloxamer Gels

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Abstract – Purpose. Transdermal delivery of anti-inflammatory lumiracoxib (LM) could be an interesting strategy to avoid the side effects associated with systemic delivery, but it is ineffective due to the drug poor skin penetration. We have investigated the effects of oleic acid (OA), a lipid penetration enhancer, on the *in vitro* release of LM from poloxamer-based delivery systems (PBDS). The rheological behavior (shear rate dependent viscosity) and gelation temperature through measurements of optimal sol-gel transition temperatures ($T_{sol-gel}$) were also carried out in these systems. **Methods.** *In vitro* release studies of LM from PBDS were performed using cellulose acetate as artificial membrane mounted in a diffusion system. The amount of LM released was divided by exposition area ($\mu g/cm^2$) and these values were plotted as function of the time (h). The flux of the drug across the membrane (*J*) was calculated from the slope of the linear portion of the plot and expressed as $\mu g/cm^2/h$. The determination of viscosity was carried out at different shear rates (γ) between 0.1- 1000 S^{-1} using a parallel plate rheometer. Oscillatory measurements using a cone-plate geometry rheometer surrounded by a double jacket with temperature varying 4-40°C, was used in order to determine $T_{sol-gel}$. **Results.** Increase of both polymer and OA concentrations increases the viscosity of the gels and consequently reduces the *in vitro* LM release from the PBDS, mainly for gels containing OA at 10.0% (w/w) compared to other concentrations of the penetration enhancer. $T_{sol-gel}$ transition temperature was decreased by increasing viscosity; in some cases the formulation was already a gel at room temperature. Rheological studies showed a pseudoplastic behavior, which facilitates the flow and improves the spreading characteristics of the formulations. **Conclusions.** Taken together, the results showed that poloxamer gels are good potential delivery systems for LM, leading to a sustained release, and also have appropriate rheological characteristics.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the production of primary prostanoids by blocking the access of arachidonic acid to the active site of cyclo-oxygenases (COX). Selective cyclo-oxygenase (COX)-2 inhibitors are promising drugs in the treatment of inflammatory or acute pain and osteoarthritis since their use has been proven to reduce gastrointestinal adverse events while preserving the capacity to control pain and inflammation (1-2). Accumulated data support the idea that an increased expression of COX-2 plays a significant role in different disease processes, including several cancers (3-4). Anticancer effects are also reported for COX-2 inhibitors in adenocarcinoma of the colon, lung cancer, intestinal polyposis (5-6) and in skin cancers (7-8).

Lumiracoxib (**LM**) or 2-[2-[(2-chloro-6-fluorophenyl) amino]-5-methylphenyl] acetic acid is a new selective COX-2 inhibitor developed for the management of chronic pain associated with osteoarthritis, rheumatoid arthritis and acute pain. Due to its chemical structure (**Figure 1**) and pharmacological properties, LM appears to be different from other COX-2 inhibitors (1). When administered orally, this compound produces gastrointestinal benefits in patients with osteoarthritis, who show decreased severe gastrointestinal ulcer complications by more than 70%, compared with treatments with ibuprofen and naproxen (9-10).

An interesting alternative to the oral route, mainly for those high-risk patients on long-term treatment with a COX-2 inhibitor, is the transdermal delivery of LM. Transdermal delivery would allow treatment of acute pain associated to arthritis or arthrosis while minimizing hepatic toxicity and other side effects associated with systemic delivery. To date, there isn't any LM

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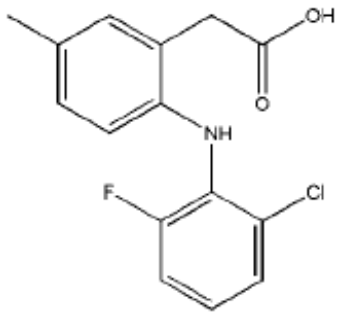


Figure 1. Lumiracoxib chemical structure.

transdermal formulation commercially available; however its ability to penetrate human skin was investigated recently for the first time by our research group (11) using poloxamer-based delivery systems (PBDS) as a novel alternative for LM delivery. Therefore, the present study aims to characterize these PBDS in relation to rheological behavior and the influence of the main adjuvant of the formulation, oleic acid (OA). Poloxamer (Pluronic F 127 or PF-127) is a biocompatible polymer, which shows low toxicity and weak immunogenic properties and has been used for medical and pharmaceutical purposes as a drug delivery system (12-13). Aqueous solutions of PF-127 at concentrations 10-30% w/w show *in situ a* thermo reversible gelation behavior (14) which permits administration in cold solutions (approx. 4-5 °C) and allows an intimate surface contact at the site of application. After topical administration, PF-127 solutions become a semisolid non occlusive gel at room temperature. Since gelation is reversible, removal is facilitated by immersion in, or irrigation with cold water (12). The gel allows a 'depot-like' sustained release that has been applied in drug delivery systems for ophthalmic, (15-16) rectal (17-18) transdermal (11, 19-20) parenteral (21-23) and topical (24) applications. In addition, PF-127 water-based polymeric gels offer several other advantages over traditional bases in terms of ease of application, cosmetic acceptability (colorless and water-washable), good physicochemical stability and desirable drug release characteristics (25). PBDSs have been suggested as potential drug delivery systems for transdermal application of many antinflammatory drugs (26-27) including naproxen, (28) indomethacin, (19, 29) piroxicam (20) and diclofenac (30) due to higher drug retention at the site of action, sustained release and improved therapeutic effectiveness. This means that transdermal PBDSs may provide comparable anti-inflammatory and analgesic activities to oral formulations while significantly reducing systemic side effects and gastrointestinal irritation associated with oral administration (26).

However, skin is widely recognized for its outstanding barrier properties compared with other biological membranes. Therefore, the penetration of LM in/through the skin is not easily achieved due to the barrier-structure of stratum corneum (SC) and the drug high lipophilicity (log P octanol/water= 3.89). In order to optimize the topical delivery of LM it is necessary to use techniques that reduce the diffusional resistance of SC. Oleic acid (OA) is a *cis*-unsaturated free fatty acid generally recognized in various *in vitro* studies as a skin penetration enhancer for several drugs, including anti-inflammatory drugs. (31-34). Besides, OA is the fatty acid most commonly found in nature, and is also found abundantly (~ 68%) among the lipids of human SC (35). As an endogenous constituent of human skin, its use is advantageous for topical or transdermal administration. due to good biocompatibility. OA concentrations ranging from 3% to 30% w/w has been proposed in the literature (33-34, 36-38) as a skin penetration enhancer for several drugs, in part due to the low potential for *in vivo* skin irritation (39) and clinic safety (40) which are also advantageous for topical or transdermal administration.

In the present study, PBDS (PF-127) gels containing the enhancer OA were investigated as to its effect on the *in vitro* release of LM from these gels. The rheology and gel-sol transition temperatures were also examined since the presence of additives may influence the physical-properties of poloxamer gels (41).

MATERIAL AND METHODS

Chemicals

PF-127 was purchased from BASF. Lumiracoxib was obtained by extraction of commercial tablets of Prexige® 100 mg (Novartis, Stein, Switzerland). Oleic acid and cellulose acetate membranes (MWCO 12.000) were obtained from Sigma Chemical (St Louis, MO, USA). Propylene glycol (PG) was from VETEC (Rio de Janeiro, Brazil). HPLC-grade acetonitrile and Tween 20 were obtained from TEDIA (Rio de Janeiro, Brazil). Milli-Q deionized water (Millipore, MA, USA) was used to prepare all solutions for high performance liquid chromatography (HPLC). All other chemicals, purchased from Merck (Darmstadt, Germany), were of analytical grade.

LM extraction

The LM extraction was carried out according to methodology previously described in detail by our research group (42). In brief, ground commercial tablets of Prexige® were extracted into water for 2 hours in a Sohxlet apparatus. The aqueous fraction was discarded and the solid residue was again extracted with ethanol using the Sohxlet for 4 h. The ethanol extract was evaporated in a rotatory evaporation system (100 rpm, at 70 °C) to a crude solid residue, further purified by dissolution in 0.1 M NaOH, followed by filtration on filter paper. The filtrate was acidified with 10% (v/v) HCl to pH 4.0 and after precipitation it was dissolved by addition of a saturated solution of NaCl. The resulting solution was extracted with ethyl acetate, and the organic phase washed twice with NaOH 0.1 M. Compound LM obtained in the aqueous phase was precipitated by adding 10% (v/v) HCl to pH 4.0 in an ice bath and its purity was confirmed by acid-base titration, melting point determination, Infra Red (IR) and Nuclear Magnetic resonance (NMR) spectroscopy.

Preparation of poloxamer gels

PF-127 gels were prepared by processing in the cold. (12) Briefly, appropriate amounts of PF-127 sufficient to yield 20, 25 and 30% (w/w) gels were slowly added to cold water (4-5 °C) with constant stirring to optimize solubilization. Dispersions were kept refrigerated overnight until a clear solution was formed. Gels loaded with LM and OA were prepared (**Table 1**) by adding to the cold Pf-127 solutions a weighed amount of the OA (1.0, 2.5, 5.0 and 10.0% w/w) and 20.0% w/w propylene glycol (PG) necessary to disperse LM in the P-127 solutions. These solutions were then brought to the desired volume with water and thoroughly agitated while cold. Concentrations of PF-127, LM and OA reported in (**Table 1**) are expressed as percentages of weight/weight (% w/w). No insoluble particles were observed after preparation of the PBDSs. Control gel formulations were represented by 20%, 25% and 30% w/w PF-127 solutions containing LM in 20.0% w/w PG (without OA) or the pure gels (without OA, LM or PG). Another control in the *in vitro* release studies was a formulation containing LM (1.0% w/w) in pure PG.

Rheological properties of the gel formulations

The rheological properties of PF-127 gels containing OA in different concentrations were evaluated in order to determine the effects of both, OA and polymer concentrations on gel viscosities. Relationships between gel viscosity and flux of drug release were also investigated in this study. The measuring system involved a rheometer model having a parallel-plate (Rheometric Scientific SR5 NJ, USA) with a diameter of 25 mm and gap of 0.5 mm at 25°C. Samples were sufficient to cover the plate as a film and shear rates (γ) ranging between 0.1 and a 1000 S^{-1}. The results of shear-rate-dependent viscosity (Pas) for gels at different shear rates were presented as averages ± S.D of three experiments (n=3) for each group.

Determination of viscoelastic properties

The analysis was performed using a controlled-stress rheometer model HAAKE Rheostress 600 (Thermo Fisher Scientific - MA, USA) with cone-plate geometry (diameter 20 mm, angle 1°). The apparatus was surrounded by a double jacket with an electric resistance capable to maintain the entire unit at 37 °C. The instrument was used in the oscillatory mode, in which the cone performs dynamic oscillations at a given frequency. To measure the shear steady state properties, the same geometry was used; in this case, the cone rotates at a given angular velocity (ω), which produces a shear rate (γ) gradient through the gap between the cone and plate.

Samples of PF-127 pure gels (20%, 25% and 30% w/w) were previously exposed to the working temperature. Analysis began by fixing a constant frequency (1 Hz) and submitting the samples to a strain rate from 5 to 1200 Pa. To measure linear viscoelastic properties it is necessary to determine the viscoelastic region by measuring *G'* (elastic modulus) and *G''* (loss modulus) as a function of the strain amplitude using the 20%, 25% e 30% (w/w) PF-127 pure gels at 37 °C. The strain amplitude where all the other dynamic measurements were made was chosen when both G' and G'' were constant.

$$G' = G^* . \cos(\delta)$$
$$G'' = G^* . \text{sen}(\delta)$$
$$\eta' = G''/\omega$$

Where ω is the angular frequency and δ is the angle phase.

The use of linear viscoelastic properties to physically characterize a material is advantageous because its internal structure is preserved during the measurements, and deformation is very small.

Table 1. Gel formulations and it controls with their respective concentrations of OA ranging from 1.0-10.0%.

Samples	*PF-127 (% w/w)*	*OA (%w/w)*
[a] Gel formulations	20	1.0
		2.5
		5.0
		10.0
	25	1.0
		2.5
		5.0
		10.0
	30	1.0
		2.5
		5.0
		10.0
[a] Control gels (without OA)	20	----
	25	----
	30	----
[b] Pure gels	20	----
	25	----
	30	----

[a] All gel formulations and control gels (without OA) contain LM (1.0% w/w) and propylene glycol (20.0% w/w) as co solvent for drug incorporation in the gels.
[b] Gels without LM, OA or PG.

Determination of gelation temperature

The gelation temperature is defined as the temperature in which the polymer in solution becomes a gel. Oscillatory experiments were mainly used in order to determine the sol–gel transition temperature ($T_{sol-gel}$). This was possible by measuring the temperature where *G*' underwent a critical variation and characterizing the gel texture beyond the gelation point, by recording the *G*' variations as a function of shear frequency. These latter experiments were carried out under a stress value which belonged to the viscoelastic linear regime where *G*' remained invariant and the sample did not undergo structural modifications. The experiment was carried out with the same instrumental shear stress measuring system described previously. In this case, the apparatus was surrounded by a double jacket with an electric resistance and cooler capable to keep the entire unit between 4 to 40 °C. Samples (PF-127 gels in different concentrations containing LM and OA) as well as control gels were previously exposed to 4 °C. The analysis began by fixing a constant frequency of 1 Hz and submitting the samples to a 10 Pa strain while the temperature increased by 2 °C/min. The $T_{sol-gel}$ graph was determined by sweeping temperatures as a function of *G'*. The transition temperature was defined as the point where the elastic modulus was half way between G' for the solution and G'' for the gel (15, 43). The results were expressed as averages ± S.D of three experiments (n=3) for each group.

HPLC analysis

LM quantification in samples of the *in vitro* release studies was carried out by HPLC according to methodology described previously by our research group (44) using a Shimadzu HPLC system consisting of a photodiode array detector, model SPD M-10AD VP (detector at 278 nm), an LC-10ADVP pump, an auto-injector SIL-10AD VP and a Class VP integrator software. In brief, separation was performed on a C8 reverse phase Shimpack column (Shimadzu) 150mm × 4.6 mm (5 µm) at room temperature (25 °C). Acetonitrile: 0.01 M sodium phosphate buffer, pH 2.5 (50:50) mixtures were used as the mobile phase, at a flow rate of 1 ml/min and 50 µl as the injection volume. LM retention time was 7.6 min and the assay was linear for concentrations between 4 and 200 µg/ml with a correlation coefficient (r) of 0.9998, and injection variability < 1% for intra-day and < 3% for inter-day variation. The HPLC method was validated during 6 days and the coefficients of variation for precision and accuracy were below 3%. Detection limits (DL) were 0.20 µg/ml with a precision of 5% and the quantification limit (QL) of the assay was 0.68 µg/ml. These values are considered adequate for both analytical assays (45). No unidentified peaks were detected by HPLC.

In vitro release studies

In vitro kinetics of drug release from P-127 poloxamer formulations into receptor media by using membrane models may serve as a comparative tool during the development of topical/transdermal formulations for drug diffusion in gel matrices (21, 46). The diffusion system employed in the *in vitro* release studies was mounted on a dissolution test apparatus as previously described in details in a recent paper of our research group (11). It is constituted by a glass water bath (maintained at 37 °C) mounted on a six points stirring plate. Six beakers fitted inside the bath contained 100 ml 0.1 M phosphate buffer (pH 7.4) as acceptor solution with 3.0% of polysorbate 20 (Tween 20) to ensure sink conditions. The solubility of LM in this medium was 340.7 µg/mL. The acceptor solution was maintained at 37°C and stirred with a magnet bar at 500 rpm. Donor compartments, made of stainless steel provided a cylindrical gel compartment over the beakers. The artificial membrane used in these experiments was cellulose acetate exposing a surface area of 1.13 cm^2. Cold PF-127 gel solutions (0.2 g) were introduced into the donor compartment and at specific times (0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hs) the beakers were opened and 1 ml of the acceptor solution was withdrawn, followed by the replacement of an equal volume of the medium. The collected fractions were filtered through a 0.45 mm membrane (filtering unity, Millipore Corporate Headquarters, Billerica, MA, USA) and the filtrates were analyzed by HPLC to determine the amount of LM diffused into the acceptor solution. The amount of LM released was calculated according to the equation: **Q real, t = (C measured, t x Vr + (Va x ∑ n-1 Ca),** where Q= accumulated permeated amount; Q real= real value at time t; C measured, t= concentration measured in the sample at time t; V r= volume of the diffusion cell; Va = volume of the removed sample and Ca = concentration in the removed sample. The amount of released LM was divided by the exposed area (µg/cm^2) and the results plotted as a function of time (hs) and presented as averages ± S.D of six experiments (n=6) for each group. The flux of drug across the membrane (*J*) was calculated from the slope in the linear portion of the plot and expressed as µg/cm^2/h.

Statistical analysis

Results are reported as means ± S.D. Data were statistically analyzed by one-way ANOVA (followed by Tukey´s multiple comparison test). The level of significance was set at $p < 0.05$.

RESULTS

LM extraction from commercial tablets

The yield of LM in the extraction and purification process was 76.8% (w/w) and its melting point between 156 and 158 °C, close to the literature values for this drug, 157- 159 °C (47). Assignments of chemical shifts obtained by analyzing the carbon and hydrogen NMR spectra are described in **Table 2**. The identity of the compound was confirmed by NMR spectra of carbon and hydrogen and infrared spectroscopy (data not shown) and the results are in agreement with the literature (10, 43). In addition, these results suggest the absence of contaminating substances, which confirm both the purity of the extracted drug and the effectiveness of the extraction procedure.

Acid-base titration of the raw material indicated a drug content of 97. 9 ± 0.08%, showing that it is suitable for the development of new pharmaceutical forms.

Determination of rheological properties of PF-127 gels

The linear viscoelastic region can be observed in **Figure 2**, where G' and G'' are both constant for all concentrations in the polymer gels. The viscosity shear rate was measured at 25 °C. and viscosity measurements for the pure PF-127 gels at 20%, 25% and 30% (w/w) and P-127 gels containing LM and OA are shown in **Table 3**. For shear rate of 0.1 S^{-1}, we can observe that in pure gels (without LM or OA) an increase in the polymer concentration (from 20% to 30% w/w) results in increased viscosity at 25 °C. The addition of LM to the 20.0%, 25.0 % or 30.0% (w/w) poloxamer pure gels does increase significantly (***$p < 0.0001$) its viscosities at all shear rates tested. Furthermore, for all shear rates, OA addition (1-10% w/w) to the P-127 gels at 20% w/w increased significantly (***$p < 0.0001$) its viscosities compared to these gels containing only 1% LM (w/w); on the other hand, for P-127 gels at 25% and 30% (w/w) the increases in gel viscosity (***p < 0.0001) were observed by adding OA in the range 2.5%- 10.0% w/w (**Table 3**).

In general, for all gels tested the viscosity decreased as shear rate (γ) increased until 1000 S^{-1}. They were, thus, characterized as pseudoplastic systems, which is a desirable behavior for

Table 2. LM ^{1}H and ^{13}C (200MHz e 50MHz) NMR

	Lumiracoxib NMR
^{1}H NMR (DMSO-d_6)	2,21 (s, 3H, -CH_3); 3,66 (s, 2H, -CH_2-); 6,43 (dd, H_6, J=8,0Hz, J_{H-F}=3,0 Hz); 6,90 (d, H_5, J=8.0Hz); 7,02-7,13 (m, 3H, H_3, H_{11}, -NH-); 7,20 (ddd, H_{10}, J=8,5Hz, 1,5Hz, J_{H-F}=11Hz); 7,34 (d, H_{12}, J=8.5Hz).
^{13}C NMR (DMSO-d_6)	20,1 (-CH_3); 37,8 (-CH_2-); 115.3 (d, H_{10}, J_{C-F} =20Hz); 116,8 (C_6); 123,3 (d, C_{11}, J_{C-F}=9,0Hz); 124,4 (C_2); 125,7 (C_{12}); 127,4 (d, C_{13}, J_{C-F}=4,0Hz); 127,9 (C_5); 129,0 (d, C_8, J_{C-F}=14,0Hz); 130,1 (C_4); 131,3 (C_3); 140,0 (C_7); 155,5 (d, C_9, J_{C-F}=247Hz); 173,4 (CO_2H).

formulations applied topically because they flow easier. In pharmaceutical gels, as ointments or cream tubes, the shear rate is a measure of the velocity in relation to the tube diameter. Therefore, these solutions will flow more easily as the velocity in the tube increases.

Determination of gelation temperature ($T_{sol-gel}$)
The $T_{sol-gel}$ for PF-127 formulations (20%, 25% and 30% w/w) containing 1% (w/w) LM with different concentrations of OA are shown in **Figures 3A, 3B and 3C,** respectively and **Table 4**. In general, the $T_{sol-gel}$ decreases with increasing polymer concentration. The addition of LM to the gels significantly increased (***$p < 0.001$) the $T_{sol-gel}$, affecting the process of gelation for all formulations. However, as OA concentration increased (from 1-10% w/w) the $T_{sol-gel}$ decreased. This was observed for all three polymer concentrations, showing a more pronounced effect when poloxamer was used at 30.0% w/w. In some cases the formulation jellified at very low temperatures (around 4°C) preventing measurements.

***In vitro* release studies**
Figure 4A shows that between 2 to 12 h, a higher amount of LM was released (and a higher flux of the drug was observed) from propylene glycol (PG) containing 1% LM (w/w) compared to the PF-127 gels, demonstrating that the delivery systems are able to promote a sustained release of LM. There were no statistical differences in drug flux from the PF-127 gels containing different concentrations of the polymer ($p > 0.05$). For all P-127 concentrations (**Figures 4B, 4C and 4D**) the addition of 10% (w/w) OA decreased the LM release compared to the other formulations. To understand the mechanism of drug release from these formulations, the data were treated according to zero-order (cumulative amount of drug released vs time), first-order (log of the cumulative amount of drug released vs time) and Higuchi's (cumulative amount of drug released vs square root of time) equations.

In our experiment, the *in vitro* released amounts of LM from all the formulations could be best expressed by Higuchi's equation where plots showed high linearity ($r^2 \geq 0.98$) and the linear regression slope indicated the rate of drug release, or the flux (J) values (μg/cm^2/h) (**Table 5**). The higher flux was verified for the control formulation (LM 1% w/w in pure PG) compared to the P-127 gels. PF-127 gels (20% to 30% w/w) with increasing OA concentration (1 to 10% w/w) showed decreased J values. In general, J values were approximately 2 times smaller in the presence of 10% (w/w) OA for all concentrations of PF-127 gels compared with their respective controls (PF-127 gels without OA).

DISCUSSION

Transdermal delivery of non-steroidal anti-inflammatory drugs (NSAIDs) may be an interesting strategy for delivering these drugs to the diseased site, but it would be ineffective due to low skin permeability. Transdermal penetration of drugs from PBDS may be enhanced when associated to skin penetration enhancers (24, 27, 48). The system improves drug penetration in the skin and it has been explored as an alternative to injecting, oral and transdermal routes. In a recent paper (11) we investigated whether oleic acid (OA) ranging from 1-10% w/w in this PBDS can improve LM (1% w/w) delivery to/through the skin. Both *in vitro* percutaneous absorption and skin retention studies of LM were measured in the presence or absence of OA in PBDS using porcine ear skin.

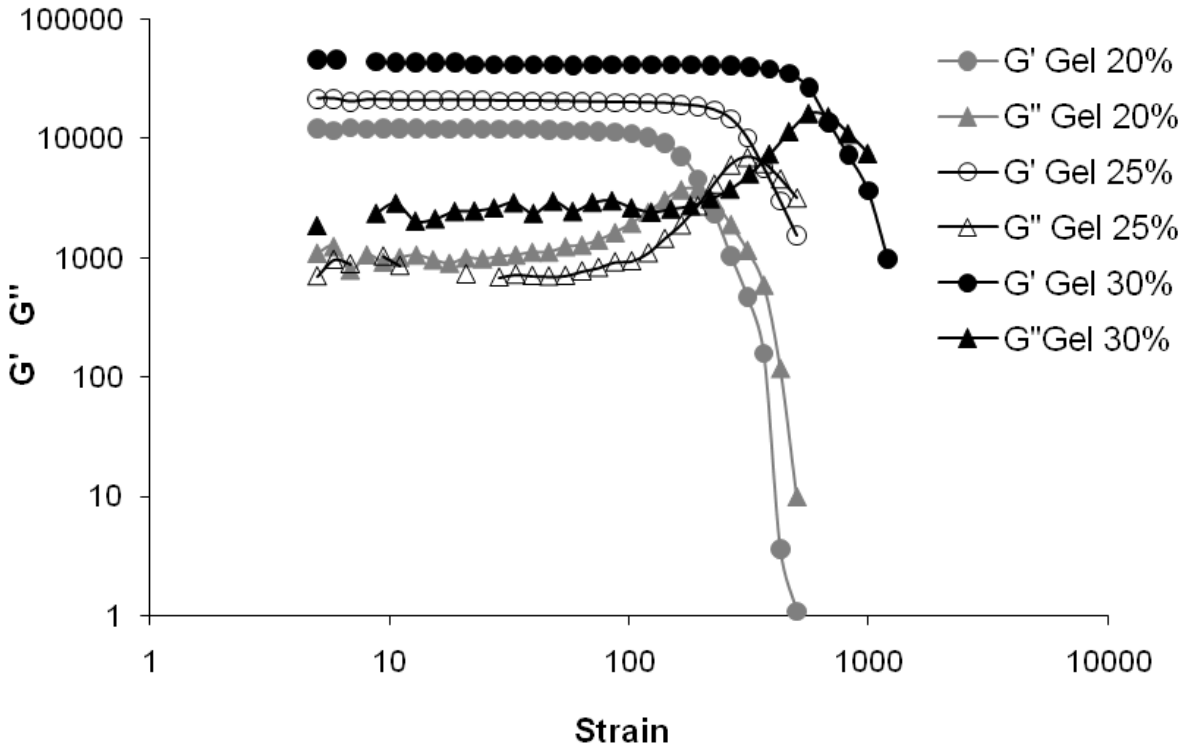
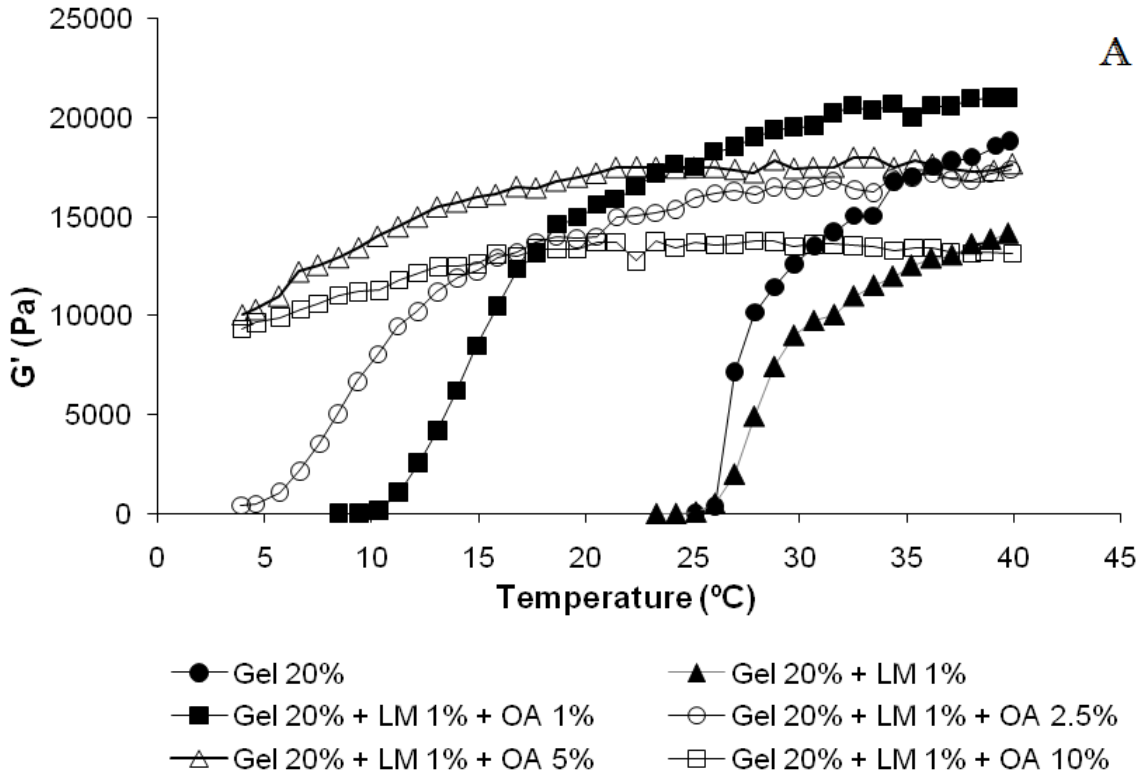


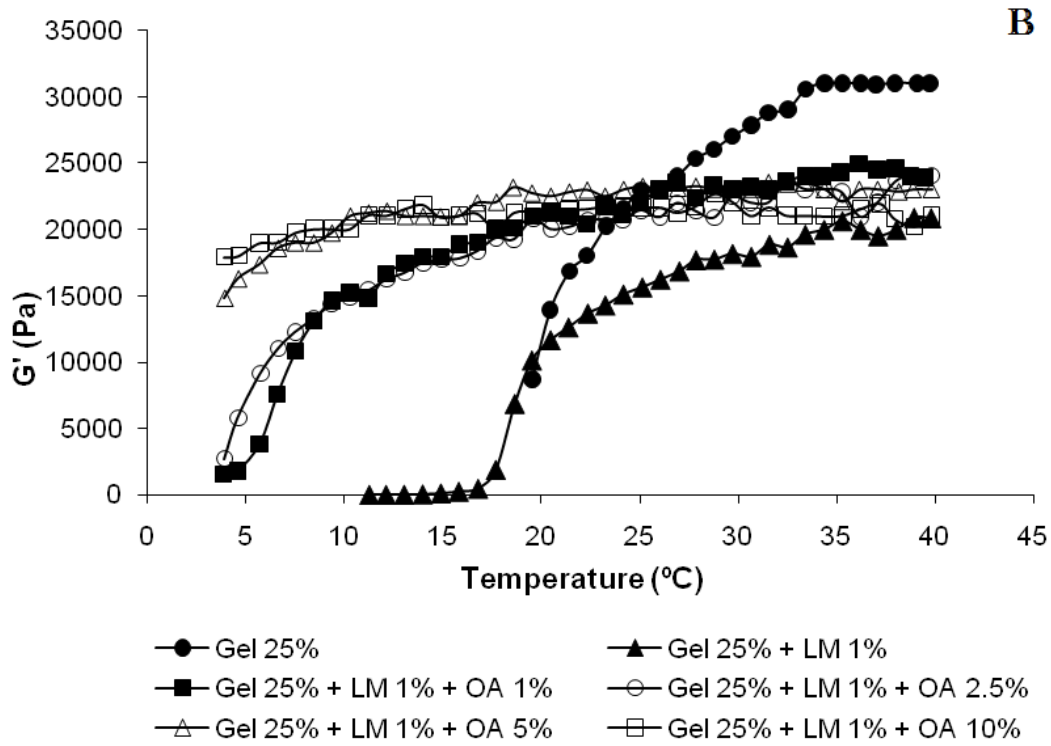
Figure 2. Strain measurements on 20, 25% and 30% w/w PF-127 gels. The elastic modulus (G') and the loss modulus (G'') are plotted versus strain am*p*litude.

Table 3. Shear rate-dependent viscosity (Pas) for Poloxamer 127 gels at 25 °C at different shear rates

Sample (% w/w)	Shear rate (S^{-1}) [a]				
	0.1	1.0	100	400	1000
Gel 20%	2063.95	253.69	3.83	1.08	0.40
Gel 25%	3004.00	343.40	4.49	1.22	0.41
Gel 30%	7430.19	811.90	9.69	2.56	0.98
Gel 20% + LM (1%) [b]	2533.96	270.27	3.07	0.80	0.77
Gel 25% + LM (1%) [b]	5193.98	521.43	5.26	1.32	0.91
Gel 30% + LM (1%) [b]	9602.85	1013.91	11.30	2.92	1.01
Gel 20% + LM (1 %) + OA 1% [c]	3953.67	507.46	8.36	2.43	0.95
Gel 20% + LM (1%) +OA 2.5%	4417.74	711.70	18.47	6.15	1.53
Gel 20% + LM (1%) +OA 5 %	8855.23	1408.96	35.67	11.79	2.08
Gel 20% + LM (1%) OA 10%	10712.73	1979.25	67.56	24.44	4.74
Gel 25% + LM (1%) + OA 1% [d]	4064.43	583.45	12.02	3.74	1.33
Gel 25% + LM (1%) + OA 2.5%	6279.14	956.75	22.21	7.16	2.12
Gel 25% + LM (1%) + OA 5%	9869.61	1625.17	44.07	14.87	2.91
Gel 25% + LM (1%) + OA 10%	15149.56	2627.24	79.01	27.52	5.11
Gel 30% + LM (1%) + OA 1% [e]	6250.29	802.42	13.23	3.84	1.56
Gel 30% + LM (1%) + OA 2.5%	10365.73	1394.44	25.23	7.54	2.74
Gel 30% + LM (1%) + OA 5%	14282.36	2199.38	52.16	16.91	3.08
Gel 30% + LM (1%) + OA 10%	21517.91	3459.39	89.41	29.75	5.63

[a] *Means ± S.D of the results in three experiments are shown (one way ANOVA test).*
[b] *Statistically significant compared to their respective control gels (*** $p < 0.0001$) for all tested shear rates.*
[c, d, e] *Statistically significant (*** $p < 0.0001$) respectively for gels at 20.0% containing 1.0- 10.0% OA and gels at 25.0% and 30.0% containing 2.5% - 10.0% OA (compared to their respective control gels) for all tested shear rates.*


A
G' (Pa)
Temperature (ºC)
Gel 20%
Gel 20% + LM 1%
Gel 20% + LM 1% + OA 1%
Gel 20% + LM 1% + OA 2.5%
Gel 20% + LM 1% + OA 5%
Gel 20% + LM 1% + OA 10%


B
G' (Pa)
Temperature (ºC)
Gel 25%
Gel 25% + LM 1%
Gel 25% + LM 1% + OA 1%
Gel 25% + LM 1% + OA 2.5%
Gel 25% + LM 1% + OA 5%
Gel 25% + LM 1% + OA 10%

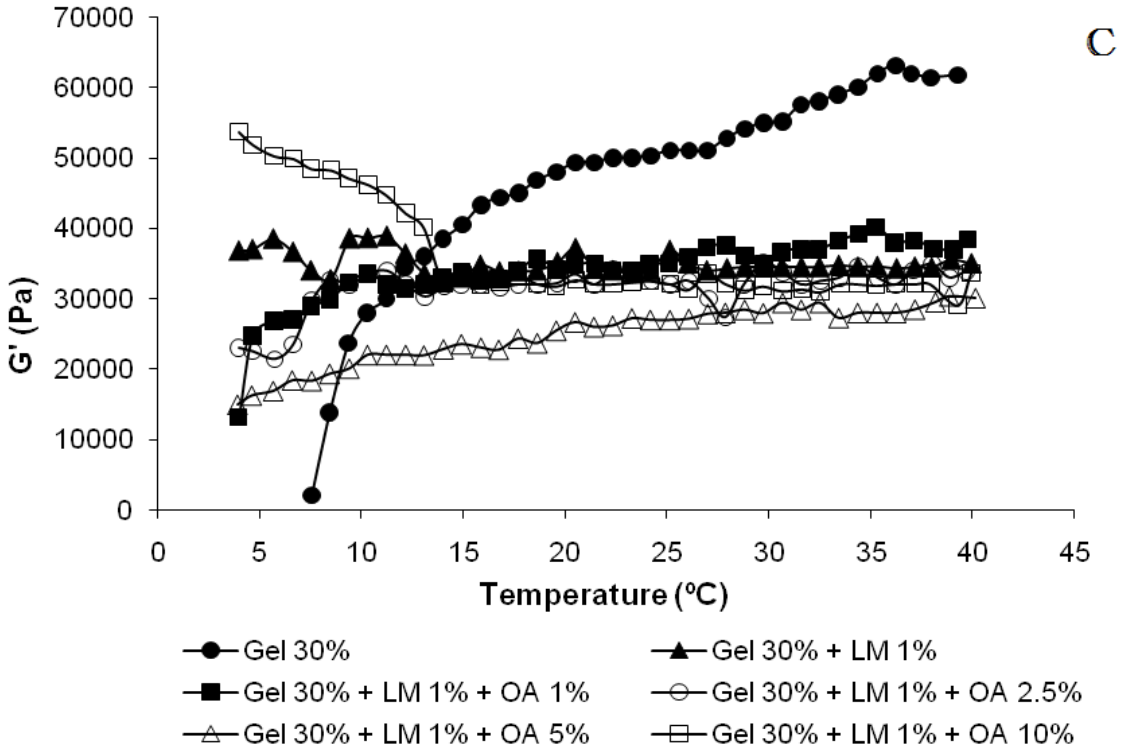


Figure 3. The effect of drug addition (1.0% w/w) and oleic acid on the sol-gel transition temperature for poloxamer PF-127 are shown in concentrations (w/w) (**A**) 20%, (**B**) 25% and (**C**) 30%. The oscillating frequency was 1 rad S^{-1} and the temperature 37 °C represented by Elastic modulus, *G'* (Pa) as a function of temperature at a frequency of 1 rad S^{-1}.

Table 4. Sol-gel transition temperature (°C) for the PF-127 gels

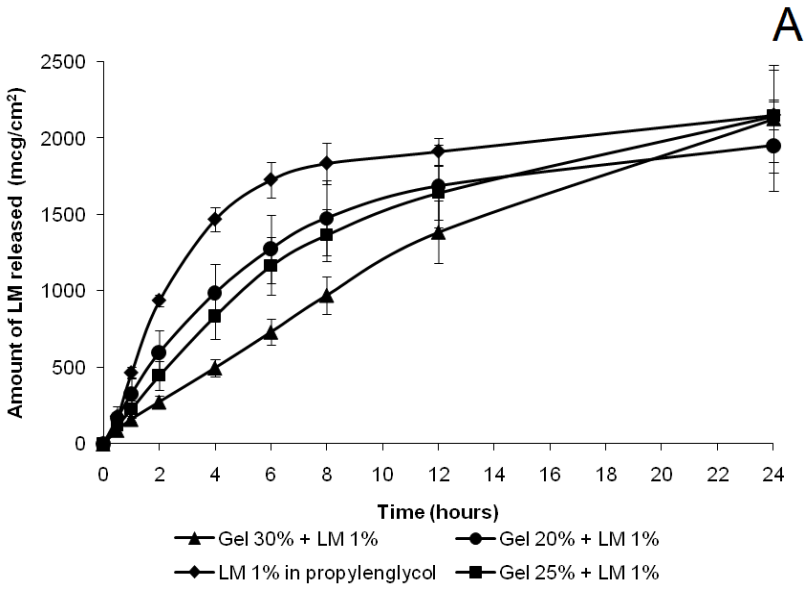
Sample (% w/w)	Sol-gel transition temp. (°C) [b]
Gel 20% [c]	29.25
Gel 20 % + LM (1 %) [d]	30.29
Gel 20% + LM (1 %)+ OA 1 % [e]	18.35
Gel 20% + LM (1 %)+ OA 2,5 %	12.94
Gel 20% + LM (1 %)+ OA 5 %*	-- [a]
Gel 20% + LM (1 %)+ OA 10 %*	-- [a]
Gel 25% [c]	19.33
Gel 25% + LM (1 %) [d]	22.87
Gel 25% + LM (1 %)+ OA 1 % [e]	8.58
Gel 25% + LM (1 %)+ OA 2.5 %	5.71
Gel 25% + LM (1 %)+ OA 5 %*	-- [a]
Gel 25% + LM (1 %)+ OA 10 %*	-- [a]
Gel 30% [c]	12.19
Gel 30 % + LM (1 %) [d]	15.23
Gel 30 % + LM (1 %)+ OA 1 % [e]	4.21
Gel 30 % + LM (1 %)+ OA 2.5 %*	-- [a]
Gel 30 % + LM (1 %)+ OA 5 %*	-- [a]
Gel 30 % + LM (1 %)+ OA 10 %*	-- [a]

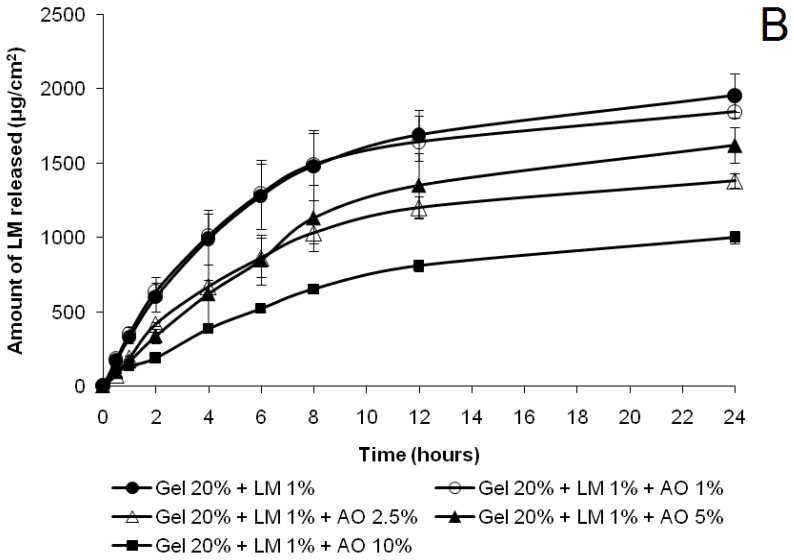
[a] *The $T_{sol\text{-}gel}$ were not measured because these formulations became gel at low temperature.*
[b] *Means ± S.D of the results in three experiments are shown (one way ANOVA test).*
[c] *Statistically significant (*** $p < 0.0001$) among 20.0%, 25.0% and 30.0% pure gels.*
[d] *Statistically significant (*** $p < 0.0001$) among 20.0%, 25.0% and 30.0% gels containing 1.0% LM.*
[e] *Statistically significant (*** $p < 0.0001$) among 20.0%, 25.0% and 30.0% gels containing 1.0% LM and OA (1.0% to 10.0%).*

The flux of *in vitro* percutaneous absorption and *in vitro* retention of LM in viable epidermis increased in the presence of 10.0% w/w OA in 25.0 % w/w poloxamer gel. In addition, *in vivo* cutaneous irritation potential was carried out in rabbits showing that this formulation did not provide primary or cumulative cutaneous irritability in animal model. The results showed that 25.0% poloxamer gel containing 10.0% w/w OA is potential transdermal delivery system for LM.

In the present paper, we carried out the characterization of these systems in terms of rheology and drug release from PBDS considering the influence of crescent OA concentrations in these systems.

The study of rheological characteristics (viscosity measurements and the optimal sol-gel transition temperature) of the gels is important because it affects both the rate of dissolution and release of the contained drug. In addition, it was also evaluated whether and how OA concentrations affect LM release from the poloxamer gels. According to **Table 3** the viscosities of the proposed formulations increased with the increase of poloxamer and OA concentrations. Fixing a shear rate at 0.1 S^{-1} shows that the increase of both, polymer and OA concentrations causes an increase in gel viscosity.





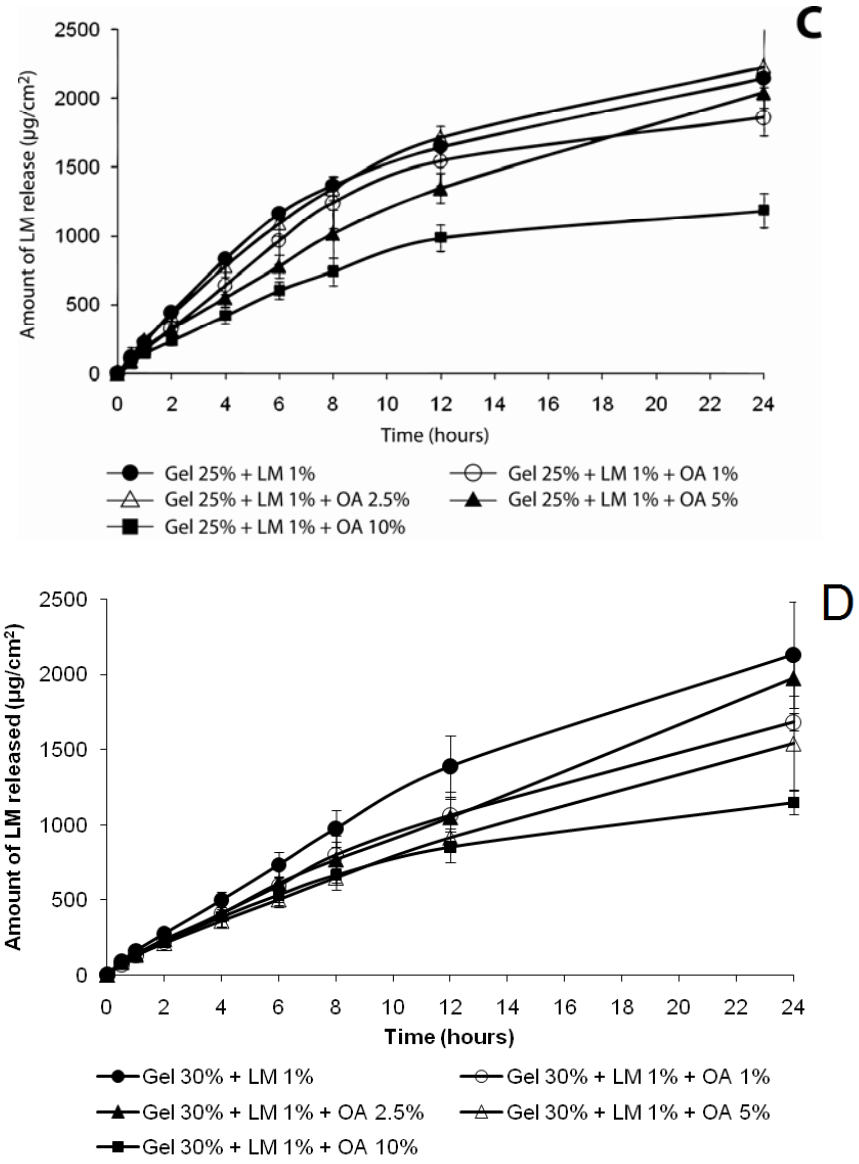


Figure 4. *In vitro* release profile of LM from (**A**) poloxamer 20%, 25% and 30% (w/w) control gels and propylene glycol (PG) with LM 1% w/w. Results are expressed as amount of LM released (µg/cm^2) into acceptor solution versus time (hs) and presented as averages ± S.D of six experiments for each group. (**B**) PF-127 gels at 20% (**C**) PF-127 gels at 25% and (**D**) PF-127 gels at 30% with LM 1% and OA 1%, 2.5%, 5.0% and 10.0%. Statistical analysis of the LM released at 24 h: One-way ANOVA (multiple comparisons Tukey's test) (**A**) Amount released is not considered significant among control gels compared to LM in PG ($p > 0.05$). Values are considered significant (***$p < 0.001$) for (**B**) control gel at 20% compared to gel formulation with OA 2.5%, 5.0% or 10.0%; gel formulation with OA 10.0% compared to formulations with OA 1.0% or 5.0% (**C**) control gel at 25.0% compared to gel formulation with 1.0% or 10.0% OA; formulation with 10.0% OA compared to 1.0%, 2.5% or 5.0% OA and (**D**) control gel at 30% compared to gel formulations with 1.0%, 5.0% and 10.0% OA; formulations containing 10.0% OA compared to 1.0%, 2.5% or 5.0% OA.

The reduction of water content in the formulation due to the OA addition to the system (which is a viscous liquid) could contribute to this effect. Probably, the delivery system promotes the formation of an emulsion-like system, due to micelle formation, thus increasing the rigidity grade of the gel structure. Addition of LM to the pure gels promoted a significant increase (***p < 0.0001) in viscosity compared to the pure poloxamer gel (without LM or OA).

Table 5. Values of the flux (*J*) of the LM (1%) obtained from Higuchi model kinetic of the *in vitro* release profile from poloxamer gels in the presence of different concentrations of OA.

Sample % (w/w)	J ($\mu g/cm^2h^{-1}$) ± SD[a]	Correlation coefficient, r^2
LM (1%) in PG	600.5 ± 39.56[a]	0.9956
Gel 20 % + LM (1 %)	457.41 ± 70.18[b]	0.9712
Gel 20% + LM (1 %)+ OA 1 %	429.48 ± 40.64	0.9872
Gel 20% + LM (1 %)+ OA 2,5 %	325.01 ± 11.86	0.9897
Gel 20% + LM (1 %)+ OA 5 %	379.83 ± 43.07	0.9956
Gel 20% + LM (1 %)+ OA 10 %	226.97 ± 8.93[c]	0.9870
Gel 25% + LM (1 %)	482.54 ± 61.26[d]	0.9858
Gel 25% + LM (1 %)+ OA 1 %	437.43 ± 31.15	0.9905
Gel 25% + LM (1 %)+ OA 2.5 %	503.41 ± 49.43 [b]	0.9946
Gel 25% + LM (1 %)+ OA 5 %	437.45 ± 24.26[c]	0.9995
Gel 25% + LM (1 %)+ OA 10 %	272.95 ± 25.25	0.9831
Gel 30 % + LM (1 %)	450.70 ± 68.74[e]	0.9711
Gel 30 % + LM (1 %)+ OA 1 %	358.79 ± 15.73	0.9988
Gel 30 % + LM (1 %)+ OA 2.5 %	398.70 ± 52.37	0.9870
Gel 30 % + LM (1 %)+ OA 5 %	316.24 ± 30.43	0.9986
Gel 30 % + LM (1 %)+ OA 10 %	254.12 ± 7.33	0.9854

[a] Means ± S.D of the results in six experiments are shown (one way ANOVA test). Statistically significant for control LM in PG compared to control gels at 20.0% ($p < 0.05$) 25.0% (* $p < 0.05$) and 30.0% (** $p < 0.01$).*
*[b] Statistically significant (*** $p < 0.001$) compared with the gel 20% formulation containing 10% (w/w) OA.*
[c] Not statistically significant ($p > 0.05$) compared with the gel 25% and 30% formulation containing 10% (w/w) OA.
*[d] Statistically significant (** $p < 0.01$) compared with the gel 25% formulation containing 10% (w/w) OA.*
*[e] Statistically significant (** $p < 0.01$) compared with the gel 30% formulation containing 10% (w/w) OA.*

Poloxamer P-127 gels are pseudoplastic, and if shear – deformed, their viscosity decreases. Indeed, we can observe that as the shear rate increases (0.1 to 1000 S^{-1}) gel viscosity decreases, the solutions become more fluid, the flow from the container is facilitated and the skin spreading improved (49). This rheological property of poloxamer gels is well known and was reported by other research groups (23, 24, 29).

The thermo reversible behavior of the PF-127 gels is an interesting characteristic of these delivery systems. For topical application, a reversible state-transition (sol-gel) property enables a cool solution to flow on the skin, allowing an intimate contact and generating a non-occlusive gel at body temperature. However, it is known that control of drug release from the poloxamer matrix depends of the "thermogel" final strength, which is proportional to the polymer concentration. In **Figure 2** it may be observed that the strain to rupture the gel matrix increases according to the increase in polymer concentration (and consequently its viscosity). Moreover, to each polymer concentration, *G'* e *G''* are constant between 5 and 80 Pa. Thus, the strain of 10 Pa was chosen because it is a safe viscoelasticity region to work in the gelation temperature assays and oscillatory measurements are conducted in the linear viscoelasticity range (non destructive dynamic conditions). From these different values, two moduli can be calculated, the storage modulus G´ and the loss modulus G", which are characteristics of the stored elastic energy and the viscous dissipated energy, respectively. The transition temperature was defined as the point where the elastic modulus was half way between G' for the solution and G' for the gel, as determined previously by others (15, 23). In **Figures 3A, 3B and 3C**, the G´and G" values were plotted as function of strain amplitude for all formulations.

Recent literature data also show that increasing poloxamer gels concentrations (from 10 to 30%) lead to decreased $T_{sol\text{-}gel,}$ turning the system to semi-solid at body temperature (15, 30, 50). In our studies, similar results were found for pure gels: increasing the percentage of gels (20% to 30% w/w) reduces significantly (***$p < 0.0001$) the T sol-gel (**Table 4)**.

It is well known that the presence of additives may modify some physicochemical characteristics of pure poloxamer gels, such as the gel-sol transition temperature (35, 51, 52). Including drugs (50, 51) or various additives (54-56) have decreased $T_{sol\text{-}gel}$ of poloxamer formulations. These agents interfere in Poloxamer 407 micellization and alter the dehydration of hydrophobic PO blocks (29, 57). Molecules like diclofenac, ethanol and HCl increase Tsol- gel

while others (e.g., NaCl, Na_2HPO_4, NaH_2PO_4) do the opposite (30, 54). In **Table 4** we can observe that the addition of LM (1% w/w) to the poloxamer gels significantly increases (***$p < 0.0001$) the T sol-gel, as the polymer concentration increases. In contrast the addition of OA (1.0% to 10.0% w/w) significantly decreased (***$p < 0.0001$) the $T_{sol-gel}$ in the same conditions. The OA addition to the P-127 gels resulted in increased *G'* values (**Figures 3A, 3B, 3C**) and as expected a decrease in the $T_{sol-gel}$. In some cases, the formulations were gels at low temperature and it was not possible to measure the $T_{sol-gel}$. A thermo reversible gelation behavior of the poloxamer formulations facilitates its administration: at room temperature (lower than the body temperature) would still be in liquid form, what facilitates its application and after that becomes gel on skin surface, allowing its retention in this site. For all gels containing OA was observed that the formulations became gels at room temperature, that is, absence of thermo reversible gelation. However, it is not a limiting factor for the effectiveness of such formulations.

To incorporate lipophilic drugs in the poloxamer gels, it is necessary to add substances as propylene glycol (PG), polyethylene glycol or glycerin, as a co-solvent (26). In this report, for all P-127 gels with incorporated LM or LM/OA, 20% (w/w) PG was used to facilitate the dispersion of the drug. This high concentration of PG can increase gel viscosities and consequently contributed in the reduction of $T_{sol-gel}$ for all formulations in which LM was added. This effect, associated to the addition of 5.0% and 10.0% (w/w) OA had high influence on $T_{sol-gel}$ for all concentrations of the poloxamer-based gels.

Several authors have demonstrated that transdermal controlled delivery systems increase the therapeutic effect of drugs. It has been postulated that PBDS promotes a more sustained release of the drug in the course of time (21-22). For a transdermal system it is interesting that the drug release slowly in an extended time period (sustained release) improving the therapeutic efficacy, since such a behavior reduces the administration frequency and increases patient compliance. Besides, for a transdermal system, sustained release of LM could avoid peaks of plasmatic drug concentration, which could lead to systemic side effects. Therefore, for the drug in question, a sustained release promoted by PBDS presents considerable advantages.

The potential of poloxamer gels to sustain the release of antinflammatory drugs has been suggested in the literature (19-20) but this is the first time that the sustained release of LM from poloxamer gels containing OA is studied. Drug release from semi-solid systems depends on drug transference from gel to the aqueous medium; the more viscous the gel, the slower is drug dissolution and release. In general, increasing the polymer concentration increases the viscosity of the poloxamer gels which can reduce the process of drug release (16, 23, 58). In the present work, LM *in vitro* release studies (**Figure 4A** and **Table 5**) showed that using only propylene glycol (PG) as vehicle can provide a higher flux compared to PF-127 gels. For all poloxamer concentrations without OA (control gels) the LM flux was basically the same. In contrast, the presence of OA at 10.0% w/w (**Figures 4B, 4C** and **4D**) significantly reduced LM release for all polymer concentration (not statistically different among them) and consequently also reduced approximately 2 times the *J* value (**Table 5)** compared to other OA concentrations. It can be related to an increase in the gel viscosities by presence of OA in that concentration. The **Table 3** shows that the viscosity of pure gels increase with increasing concentration of polymer, but this increase is greater with the addition of increasing amounts of OA (1-10% w/w) for all gel concentrations (20 to 30% w/w). Besides the viscosity factor, addition of a lipid component as OA not only improved the solubility of the lipophilic drug in the gels (20, 25 and 30% w/w) but also contributed to a slower LM release from PBDS. Another reason must be assigned by micellar structure of poloxamer and possible micellar entanglements which produce high viscosity, partial rigidity and slow dissolution of the gels. Such properties facilitate incorporation of both hydrophilic and hydrophobic drugs, promote slow release of the incorporated drugs or a sustained drug release (59)**.** In the present work, a more viscous gel (by adding of 10% w/w OA) can release less and more slowly the drug in question (reducing LM release and its flux) possibly due to an increase in the diffusion time of the drug through the gel matrix. Since LM is a highly lipophilic drug it must be dispersed in the PBDS by addition of hydrophilic co-solvent (propylene glycol) used in this case for drug incorporation. Moreover, the addition of a lipidic component like OA increased the solubility of the drug in this system and also contributed to drug release reduction from the PBDS over time. Thus, the sustained release of LM from PBDS containing 10% (w/w) OA should probably be

influenced by these two factors mentioned above, that is; the characteristics of poloxamer gel associated with the presence of OA at high concentration. The results of our *in vitro* release studies confirmed that increasing gel viscosity can modify LM dissolution and release processes from the formulation.

Our *in vitro* release studies were carried out until 24 hours, because it is the maximum time for the total release of the LM contained in the formulations, that is, in 24 hours about 100% of LM has been released from all formulations. Similarly, 24 hours is a suitable and prolonged time release for transdermal administration, so that the gel can be applied to the skin only once a day.

Moreover, the time of 24 hours is considered a long time to release a drug. Overall, the *in vitro* release profiles of controls (Figure 4A) and 20-30% w/w PBDS containing 1% w/w LM and 1.0, 2.5 and 5.0% w/w OA (Figures 4B, 4C and 4D) showed a rapid initial release (burst effect) followed by a slow and gradual release of the drug. For other hand, the PBDS (20-30% w/w) containing OA 10% w/w had lower pronounced initial burst (initial concentration is low), lower amount of LM released/cm^2 until 24 hs and lower flux (J) compared to other formulations. These results indicate a sustained release profile of the drug, ie, the formulations of gel (20-30% w/w) containing 10% OA (w/w) and LM 1% (w/w) released lower LM amount from the beginning (burst was reduced, what avoid peak concentrations) and this amount was maintained for a long time (until 24 hs).

Higuchi kinetics determined for the formulations confirm the hypothesis that the release of LM in the delivery systems is controlled by viscosity. Data in the literature about the cumulative amount of drug released per unit area for poloxamer gels generally approaches the Higuchi square root equation (59-60). When the amount of LM released was plotted against the square root of time (**Table 5**), we found a linear relationship, that is, the *in vitro* dissolution study shows that LM release followed Higuchi-order or a diffusion controlled manner. The Higuchi model describes the release from systems where the solid drug is dispersed in an insoluble matrix, and the rate of drug release is related to the rate of drug diffusion (62-63). Diffusion is related to transport of drug from the dosage matrix to the *in vitro* fluid depending on the concentration. As gradient varies, the drug is released, and the distance for diffusion increases. This could explain why the drug diffuses at a comparatively slower rate as the distance for diffusion increases, which is referred as Higuchi's kinetics. It means that a linear relationship exists between rate of LM released and amount of both poloxamer and OA, indicating that the drug release was controlled basically by the system viscosity.

It can be concluded that formulations containing OA 10% (w/w) could be suitable as delivery systems for LM, firstly for its pseudoplastic characteristic (decrease of viscosity with increasing shear stress) which facilitates the application of such formulations on the skin due to better spreading. Secondly, these formulations have a reduced LM release rate (twice compared to PBDS without OA) and in addition, this release was sustained for an extended period of time (24 hs). This behavior is important for a transdermal delivery system, in order to avoid peak in the plasma concentrations with generation of systemic side effects and to reduce administration frequency, and in return a better patient compliance. As mentioned previously, the PBDS containing 25.0% of poloxamer gel and 10.0% OA also showed the highest LM flux through porcine skin and the highest LM retention for in vitro permeability studies and no cutaneous irritability (11).

CONCLUSIONS

Taken together, these results demonstrates for the first time, that poloxamer gels containing OA can be potential delivery systems for transdermal delivery of LM, giving the sustained release properties and appropriate rheological characteristics. These results suggest that PBDS are promising delivery systems to promote transdermal sustained release of the antinflammatory LM.

Novelty of the work: A transdermal delivery of non-steroidal antinflammatory drugs like lumiracoxib (LM) can be an interesting alternative to the oral route of this drug, since it was recently withdraw of the market due to the liver damage when systemically administered in tablets as dosage form. There are no transdermal formulations of LM and it could be an alternative to treat inflammation caused by arthritis or arthrosis. Then, an adequate delivery system to LM is necessary in order to release the drug properly from the PBDS as well as have good characteristics related to semi-solid preparations

for transdermal application, which were evaluated through *in vitro* release studies and rheological behavior in this paper, respectively.

Keywords: lumiracoxib, oleic acid, poloxamer gels, rheological studies, *in vitro* release studies.

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REFERENCES

1. Mysler E. Lumiracoxib (Prexige): a new selective cox-2 inhibitor. Int J Clin Pract 58: 606–611, 2004.
2. Tannenbaum H, Berenbaum F, Reginster J-Y, Zacher J, Robinson J, Poor G, Bliddal H, Uebelhart D, Adami S, Navarro F, Lee A, Moore A, Gimona A. Lumiracoxib is effective in the treatment of osteoarthritis of the knee: a 13 week, randomised, double blind study versus placebo and celecoxib. Clin Rheumatol 25: 42–53, 2005.
3. Prescott SM. Is Cyclooxygenase-2 The Alpha and The Omega in Cancer**.** J Clin Invest 105: 1511–1513, 2000.
4. M´eric JM, Rottey S, Olaussen K, Soria J-C, Khayat D, Rixe O, Spano J-P. Cyclooxygenase-2 as a target for anticancer drug development. Crit Rev Oncol Hematol 59: 51–64, 2006.
5. Achiwa H, Yatabe Y, Hida T, Kuroishi T, Kozaki K, Nakamura S, Ogawa M, Sugiura T, Mitsudomi T, Takahashi T. Prognostic significance of elevated cyclooxygenase-2 expression in primary, resected lung adenocarcinomas. Clin Cancer Res, 5: 1001–1005, 1999.
6. Jacoby RF, Seibert K, Cole CE, Kelloff G, Lubet RA .The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. Cancer Res 60: 5040–4, 2000.
7. Orengo F, Gerguis J, Phillips R, Guevara A, Lewis AT, Black HS. Celecoxib, a cyclooxygenase- 2 inhibitor as a potential chemopreventive to UV-induced skin cancer: a study in the hairless mouse model. Arch Dermatol 138: 751–5, 2002.
8. Afaq F, Adhami VM, Mukhtar H. Photochemoprevention of ultraviolet B signaling and Photocarcinogenesis Mutat Res 571: 153–173, 2005.
9. Acemoglu M, Allmendinger T, Calienni J, Cercus J, Loiseleur O, Gottfried H, Sedelmeier H, Xu D. Synthesis of new *N*-aryl oxindoles as intermediates for pharmacologically active compounds. Tetrahedron 60: 11571-11586, 2004.
10. Shi S, Klotz U. Clinical use and pharmacological properties of selective COX-2 inhibitors. Eur. J Clin Pharmacol 64: 233–252, 2008.
11. Moreira TS, Sousa, VP, Pierre MBR. A Novel Transdermal Delivery System for the Anti-Inflammatory Lumiracoxib: Influence of Oleic Acid on In Vitro Percutaneous Absorption and In Vivo Potential Cutaneous Irritation. AAPS Pharm Sci Tech. 11 (2): 621-629, 2010.
12. Schmolka IR. Artificial skin. I. preparation and properties of pluronic F-127 gels for treatment of burns. J Biomed Mater Res 6: 571–582, 1972.
13. Johnston TP, Miller S. Toxicological evaluation of poloxamer vehicles for intramuscular uses, Parenter Drug Assoc 39: 83-88, 1985.
14. Moore H, Croy S, Mallapragada S, Pandit N. Experimental Investigation and Mathematical Modeling of Pluronic F127 Gel Dissolution: Drug release in Stirred Systems. J Control Rel 67: 191-202, 2000.
15. Edsman K, Carlfors J, Petersson R. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur J Pharm Sci 6: 105-112, 1998.
16. Ma WD, Xu H, Wang C, Nie SF, Pan WS. Pluronic F127-g-poly(acrylic acid) copolymers as in situ gelling vehicle for ophthalmic drug delivery system. Int J Pharm 350: 247-56, 2008.
17. Barichello JM., Morishita M, Takayama K, Chiba Y, Tokiwa S, Nagai N. Enhanced rectal absorption of insulin-loaded Pluronic® F-127 gels containing unsaturated fatty acids Int J Pharm, 183: 125-132, 1999.
18. Koffi AA, Agnely F, Ponchel G, Grossiord JL. Modulation of the rheological and mucoadhesive properties of thermosensitive poloxamer-based hydrogels intended for the rectal administration of quinine. Eur J Pharm Sci 27: 328-335, 2006.
19. Miyazaki S, Tobiyama T, Takada M, Attwood D. Percutaneous absorption of indomethacin from pluronic F-127 gels in rats. J Pharm Pharmacol 47: 455–457, 1995.
20. Shin SC, Cho CW, Oh IJ. Enhanced efficacy by percutaneous absorption of piroxicam from the poloxamer gel in rats. Int J Pharm 17: 213–218, 2000.
21. Veyries ML, Couarraze G, Geiger S, Agnely F, Massias L, Kunzli B, Faurisson F, Rouveix B. Controlled release of vancomycin from Poloxamer 407 gels. Int J Pharm, 192: 183-193, 1999.
22. Paavola A. Controlled release injectable liposomal gel of ibuprofen for epidural analgesia. Int J Pharm 199: 85-93, 2000.
23. Ricci EJ, Bentley MV, Farah M, Bretãs RE, Marchetti JM. Rheological characterization of Poloxamer 407 lidocaine hydrochloride gels. Eur J Pharm Sci 17: 161-167, 2002.
24. Bentley MVLB, Marchetti JM, Ricardo N, Ali-Abi Z, Collett JH. Influence of lecithin on some physical chemical properties of poloxamer gels:

rheological, microscopic and *in vitro* permeation studies. Int J Pharm 193: 49-55, 1999.
25. Escobar-Chávez JJ, López-Cervantes M, Naïk A, Kalia YN, Quintanar-Guerrero D, Ganem-Quintanar A. Applications of thermoreversible pluronic F-127 gels in pharmaceutical formulations, J Pharm Sci 9: 339-358, 2006.
26. Chi SCh, Do K, Tan HK, Chun HW. Anti-inflammatory and analgesic transdermal gel, *United States Patents.*, Patent number 5,527,832, 1996.
27. Shin SC, Cho CW, Oh IJ. Effects of non-ionic surfactants as permeation enhancers towards piroxicam from the poloxamer gel through rat skins. Int J Pharm 17: 199-203, 2001.
28. Escobar-Chavez JJ, Quintanar-Guerrero D, Ganem-Quintanar A. In vivo skin permeation of sodium naproxen formulated in pluronic F-127 gels: effect of Azone and Transcutol. Drug Dev. Ind. Pharm 31: 447-454, 2005.
29. Shawesh A, Kallioinen S, Hellen L, Antikainen O, Yliruusi J. Pluronic F-127 gels as a vehicle for topical formulations of indomethacin and rheological behavior of these formulations. Pharmazie 57: 186-90, 2002.
30. Yong CS, Choi J, Quan QZ, Rhee JD, Kim CK, Lim SJ, Kim KM, Oh PS, Choi HG. Effect of sodium chloride on the gelation temperature, gel strength and bioadhesive force of poloxamer gels containing diclofenac sodium. Int J Pharm 226: 195-205, 2001.
31. Francoeur ML, Golden GM, Potts, RO. Oleic acid: its effects on stratum corneum in relation to (trans) dermal drug delivery. Pharm Res 7: 621-627, 1990.
32. Yamashita F, Koyama Y, Kitano M, Takakura Y, Hashida M. Analysis of in vivo skin penetration enhancement by oleic acid based on a two-layer diffusion model with polar and nonpolar routes in the stratum corneum. Int J Pharm 117: 173-179, 1995.
33. Larrucea E, Arellano A, Santoyo S, Ygartua P. Combined effect of oleic acid and propylene glycol on the percutaneous penetration of tenoxicam and its retention in the skin. Eur J Pharm Biopharm, 52: 113-9, 2001.
34. Pierre MBR, Ricci EJr, Tedesco AC, Bentley MVL. Oleic Acid as Optimizer of the Skin Delivery of 5-Aminolevulinic Acid in Photodynamic Therapy. Pharm Res 23: 360-366, 2006.
35. Lampe MA, Burlingame AL, Whitney JA, Willians ML, Brown BE, Roitman E, Elias, PM Human stratum corneum lipids: characterization and regional variations**.** J. Lipid Res 24: 120-130, 1983.
36. Escribano E, Calpena AC, Queralt J, Obach R, Doménech J. Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. Eur J Pharm Sci 19 (4):203-210, 2003.
37. Baboota S, Al-Azaki A, Kohli K, Ali J, Dixit N, Shakeel F. Development and evaluation of a microemulsion formulation for transdermal delivery of terbinafine. PDA J Pharm Sci Technol 61 (4): 276-85, 2007.
38. Singh S, Gajra B, Rawat M, Muthu MS. Enhanced transdermal delivery of ketoprofen from bioadhesive gels. Pak J Pharm Sci 22 (2): 193-8, 2009.
39. Boelsma E, Tanojo H, Bodd HE and Ponec M. Assessment of the Potential Irritancy of Oleic Acid on Human Skin: Evaluation In Vitro and In Vivo. Toxicology in Vitro 10*:* 129-142, 1996.
40. Jiang J, Wang RK. Comparing the synergistic effects of oleic acid and dimethyl sulfoxide as vehicles for optical clearing of skin tissue in vitro. Phys Med Biol 49(23): 5283-94, 2004.
41. Vadnere M, Amidon G, Lindenbaum S, Haslam JL. Thermodynamic studies on the gel-sol transition of some pluronic polyols. Int J Pharm 22: 207–218, 1984.
42. Moreira TS, Cid YP, Kummerle AE, Fraga CAM, Pierre MBR, Sousa VP. Extração e purificação de fármacos anti-inflamatórios não esteroidais ciclo-oxigenase 2- seletivos. Quim Nova, 32 (5): 1324-1328, 2009.
43. Bonacucina G, Spina M, Misici-Falzi M, Cespi M, Pucciarelli S, Angeletti, M., Palmieri, G.F Effect of hydroxypropyl β-cyclodextrin on the self-assembling and thermogelation properties of Poloxamer 407. Eur J Pharm Sci, 32: 115-122, 2007.
44. Moreira TS, Pierre MBR, Fraga CAM, Sousa VP. Development and validation of HPLC and UV spectrophotometric methods for the determination of lumiracoxib in tablets. Journal of Basic and Applied Pharmaceutical Sciences 29 (3): 267-275, 2008.
45. Causon R. Validation of chromatographic methods in biomedical analysis viewpoint and discussion. J Chromatogr B 689: 175–180, 1997.
46. Proniuk S, Dixon SE, Blanchard J. Investigation of the utility of an in vitro release test for optimizing semisolid dosage forms. J Pharm Dev Technol 6: 469-476, 2001.
47. Fugimoto RA, Mugrare B, Mcquire LW, Van Duzer JH. Certain 5-alkyl-2 arylaminophenylacetic acids and derivates, NOVARTIS-ERFINDUNGEN VERWALTUNGS-GESELLSHAFT MBH, Switzerland Patents, Patent number WO 99/11605, 1998.
48. Nair V, Panchagnula R. Poloxamer gel as vehicle for transdermal iontophoretic delivery of arginine vasopressin: evaluation of in vivo performance in rats. Pharmacol Res 47: 555-562, 2003.
49. Brummer R, Godersky S. Rheological studies to objectify sensations occurring when cosmetic emulsions are applied to the skin. Coll. Surf. A 152: 89-94, 1999.

50. Dumortier G, El Kateb N, Sahli M, Kedjar S, Boulliat A, Chaumeil JC. Development of a thermogelling ophthalmic formulation of cysteine. Drug Dev Ind Pharm 32 (1):63-72, 2006.
51. Miller SC, Drabik BR. Rheological properties of poloxamer neous absorption of piroxican from the poloxamer gel in rats. Int J Pharm 22: 207-218, 1984.
52. Gilbert JC, Richardson JL, Davies MC, Palin KJ, Hadgraft J. The effect of solutes and polymer on the gelation properties pluronic F-127 solution for controlled drug delivery. J Control Rel 5: 113–118, 1987.
53. Pisal SS, Paradkar AR, Mahadik KR, Kadam SS. Pluronic gels for nasal delivery of Vitamin B12. Part I, preformulation study. Int J Pharm 270: 37-45, 2004.
54. Choi H, Lee M, Kim M, Kim C. Effect of additives on the physicochemical properties of liquid suppository bases. Int J Pharm 190: 13-19, 1999.
55. Ryu JM, Chung SJ, Lee MH, Kim CK, Shim CK. Increased bioavailability of propranolol in rats by retaining thermally gelling liquid suppositories in the rectum. J Control Rel 59: 163-172, 1999.
56. Charrueau C, Tuleu C, Astre V, Grossiord JL, Chaumeil JC. Poloxamer 407 as a thermogelling and adhesive polymer for rectal administration of short-chain fatty acids. Drug Dev Ind Pharm 27: 351-357, 2001.
57. Chang JY, Oh YK, Choi HG, Kim YB, Kim K. Rheological evaluation of thermosensitive and mucoadhesive vaginal gels in physiological conditions. Int J Pharm 241: 155-163, 2002.
58. Zhang L, Parsons DL, Navarre C, Kompella UB. Development and in-vitro evaluation of sustained release poloxamer 407 (P407) gel formulations of ceftiofur. J Control Rel 85 (1-3):73-81, 2002.
59. Dumortier G, Grossiord JL, Agnely F, Chaummeil JC. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Pharm Res 23: 2709-2728, 2006.
60. Miyazaki S, Suzuki S, Kawasaki N, Endo K, Takahashi A, Attwood D. In situ gelling xyloglucan formulations for sustained release ocular delivery of pilocarpine hydrochloride. Int J Pharm 229: 29-36, 2001.
61. Takahashi A, Suzuki S, Kawasaki N, Kubo W, Miyazaki S, Loebenberg R, Bachynsky J, Attwood D. Percutaneous absorption of non-steroidal anti-inflammatory drugs from in situ gelling xyloglucan formulations in rats. Int J Pharm 246: 179-186, 2002.
62. Schwartz JB, Simonelli AP, Higuchi WI. Drug release from wax matrices. Analysis of data with first-order kinetics and with the diffusion-controlled model. J Pharm Sci 57: 274-277, 1968.
63. Lee HJ, Lee MH, Shim CK. Preparation and Evaluation of Ethyl Cellulose Microcapsules of Indomethacin. Arch Pharm Res 7: 33-40, 1984.