

Effect of Vehicle on Diclofenac Sodium Permeation from New Topical Formulations: *In Vitro* and *In Vivo* Studies

Vanna Sanna¹, Alessandra T. Peana² and Mario D.L. Moretti^{2,*}

¹Prion DGN c/o Porto Conte Ricerche, Località Tramariglio, 07041, Alghero, Sassari, Italy; ²Department of Scienze del Farmaco, University of Sassari, via Muroni 23/a, 07100 Sassari, Italy

Abstract: In this study the effect of vehicle on *in vitro* diffusion of diclofenac sodium (DS) from new different formulations such as Carbopol gel (A), Sodium lauryl sulphate cream (B) and Carbopol cream (C) was evaluated with Franz diffusion cells using hydrophilic and hydrophobic synthetic membranes. The commercial formulation Voltaren[®] Emulgel was used as reference.

Furthermore, the *in vivo* efficacy of topical formulations was studied in the carrageenan-induced edema and hyperalgesia, whereas the antinociceptive effect was evaluated on thermal pain threshold in rat paw.

The flux of DS across hydrophilic membranes showed this rank order: Control \approx C > A \approx B. On the other hand, the diffusion rate of DS across hydrophobic membranes resulted in the following order: Control > B > A \approx C; this suggested a lower interaction between the vehicles and these membranes.

The *in vivo* results indicated that the prepared formulations failed in the inflammatory tests to reduce the development of edema.

Nevertheless, treatment with B formulation inhibited the development of acute hyperalgesia induced by carrageenan, and elicited a significant increase in paw withdrawal latencies whereas other formulations were ineffective.

The results obtained in this study suggest that Sodium lauryl sulphate cream might be useful in local pain conditions and may be an effective alternative to the presently used systemic routes.

Keywords: Diclofenac sodium, *in vitro* diffusion, *in vivo* studies.

INTRODUCTION

Diclofenac Sodium (DS) is a potent member of the non-steroidal anti-inflammatory drugs (NSAIDs), widely used because of its strong analgesic, antipyretic and anti-inflammatory effects [1-3].

It is marketed as injections, oral sustained release tablets and topical formulations. After oral administration, DS is extensively metabolized in the liver and because of its short biological half-life, the drug has to be administered frequently [4].

Clinical evidence suggests that topically applied NSAIDs are safer and at least as efficacious as oral NSAIDs in the treatment of rheumatic diseases [5].

The topical application allows for a higher local concentration of the drug at the site of initiation of the pain and lower or negligible systemic drug levels producing fewer or not adverse drug effects [6]. However, some degree of systemic absorption will occur following localized delivery methods, and the degree of systemic absorption needs to be assessed during the development of formulations [5,7].

Topical drugs used to control pain act locally on damaged or dysfunctional soft tissues or peripheral nerves, and

their actions may be on the inflammatory response itself or on sensory neurons [8]. However, there is a considerable interest in the preclinical literature in identifying novel peripheral targets, and on the development of this approach as a viable alternative to systemic therapies [9,10].

As DS is not easily absorbed on dermal application [11], many strategies have been suggested in order to overcome the low permeability of drug through the skin [12-14].

An improved Diclofenac formulation with a high degree of skin permeation could be useful in the treatment of not only locally inflamed skin tissues, but also inflammatory and painful states of supporting structures of the body bones, ligaments, joints, tendons and muscles [15,16].

In developing drug preparations for topical delivery through the skin, the choice of vehicle formulations for a given drug can greatly influence the rate and extent of drug permeation across the skin [17].

The aim of this study was to prepare alternative topical formulations containing Diclofenac Sodium (1% w/w) for enhancing the skin penetration of drug.

The prepared formulations, consisting of Carbopol gel, Sodium lauryl sulphate and Carbopol cream, were characterized in terms of physical examination and rheological properties. In order to study the influence of vehicle composition on the release rates of drug *in vitro* diffusion studies were

*Address correspondence to this author at the Dipartimento di Scienze del Farmaco, University of Sassari, via Muroni 23/a, 07100 Sassari, Italy; Tel: + 39 079 228734; Fax: + 39 079 228733; E-mail: dsfgigi@uniss.it

carried out. A commercial available formulation (Voltaren[®] Emulgel) was used as reference formulation.

To evaluate the interactions between the various vehicles and the membrane, the diffusion tests were performed by using two different synthetic membranes with hydrophilic and hydrophobic characteristics as models.

Moreover, the *in vivo* acute anti-inflammatory, anti-hyperalgesic and antinociceptive activity of all formulations were tested when applied on the skin. Then, the efficacy of DS topical formulations were studied in the carrageenan-induced edema and hyperalgesia in rat paw that are common models of local inflammation and pain inflammatory. In addition, the antinociceptive activity was evaluated on thermal pain threshold in rat paw.

MATERIALS AND METHODS

Sodium Diclofenac, Cetostearyl alcohol, Carbopol 940, liquid paraffin, Polyethylene glycol 40 stearate, Cetiol[®] and Cetomacrogol, were supplied by Cruciani-Prodotti Crual srl (Roma, Italy); Isopropyl alcohol and Sodium lauryl sulphate were obtained from Riedel- De Haen (Hannover, Germany); Sodium hydroxide solution (100 g/L) was purchased from Merck (Darmstadt, Germany); EDTA was supplied by Carlo Erba Reagenti (Milano, Italy); Propylene glycol was obtained from Res Pharma (Vimodrone, Milano, Italy) and Diethyl ammonium and Carragenina λ from Sigma Aldrich srl (Milano, Italy). Voltaren[®] Emulgel marketed in Italy from Novartis, was obtained from a local pharmacy. The synthetic membranes used are Millipore's LCR membrane in Polytetrafluoroethylene (PTFE) hydrophilic and Durapore's membrane in Polyvinylidene fluoride (PVDF) hydrophobic. All membranes were purchased from Millipore S.p.A. (Milano, Italy).

Preparation of Formulations

The examined topical formulations (Table 1) were prepared as follows.

Carbopol hydrogel (A): Carbopol, EDTA and propylene glycol were dispersed under stirring in two-third of water for overnight; then, a solution of DS in the remaining water was added, and sodium hydroxide solution was dropped in order to reach a neutral pH and a suitable gel viscosity.

Sodium Lauryl sulphate cream (B): Cetostearyl alcohol, liquid paraffin, white vaseline and sodium lauryl sulphate were melted, and water at the same temperature was added under slow speed agitation cooling to room temperature. DS dissolved in remaining water was then dispersed into cream by stirring at the last stage.

Carbopol cream (C): the EDTA and polyethylene glycol stearate were dissolved in a dispersion of Carbopol in purified water heated to 70°C under stirring at a high speed. Next, the oily phase (vaseline, paraffin and cetostearyl alcohol), previously melted at 60°C, was incorporated with continuous stirring. Sodium hydroxide was added to reach a neutral pH and the mixture was stirred until cooled to room temperature. Then, the drug was dispersed into cream.

One hundred gram of commercial topical Voltaren[®] Emulgel (Novartis Farma SpA, VA, Italy), used as reference formulation, contains 1.16 g of diclofenac diethyl ammonium (equivalent to 1 g DS), isopropyl alcohol, propylene glycol, perfume, Cream 45, and other additives.

A formulation without DS, and with the same excipients composition of Voltaren[®] Emulgel, was prepared as follows: propylene glycol (4 g) and isopropyl alcohol (20 g) were dissolved under stirring into a dispersion of Carbopol (1 g) in water (5 g). Diethyl ammonium (about 0.5-0.8 g) was added

Table 1. Percentage Composition (w/w) of the Formulations

INGREDIENTS (% w/w)	FORMULATIONS		
	A	B	C
Diclofenac sodium	1	1	1
Cetostearyl alcohol		8	8
Carbopol 934	0.8		0.5
EDTA	0.2		0.1
Propylene glycol	5		
Liquid paraffin		6	10
Polyethylene glycol			3
Sodium Lauryl sulphate		1	
Sodium hydroxide solution (100 g/L)	1.1		1.2
White vaseline		15	10
Purified water to	100	100	100

until a neutral pH to give a suitable gel that is then incorporated in the mixture containing liquid paraffin (5 g), Cetiol® (5 g) and Cetomacrogol (4 g) to give the emulgel.

Characterization of Formulations

The prepared formulations were visually inspected for their color, homogeneity, consistency, spreadability, phase separation and pH and compared with the commercial formulation [18].

The viscosity of the different formulations was carried out using a rotational viscometer (Viscotester® VT 181, Haake, Karlsruhe, Germany) equipped with a sensor system E1000 (diameter 7.0 mm, length 17.7 mm). About 50 g of each formulation were transferred at $20 \pm 1^\circ\text{C}$ in a measuring cylinder, and viscosity of the formulations was performed at a rotational speed of 1 and 4 (corresponding to about 6.0 and 180 rpm, respectively). Under the same conditions, the viscosity of the control formulation (Voltaren® Emulgel) was examined in triplicate.

In Vitro Release Studies

The *in vitro* release experiments were carried out by using Franz-type modified diffusion cells apparatus with side-arm and external jacket Erweka HD/T6 (Erweka, GmbH-Heusenstamm, Germany).

An exact amount of formulations (1.0 g) was spread out on membrane positioned between the donor and receptor chambers with an available diffusion area of 0.694 cm^2 .

The permeation tests were performed using Polytetrafluoroethylene (PTFE) hydrophilic and Polyvinylidene fluoride (PVDF) hydrophobic membranes. The membranes were first hydrated by soaking in the receptor medium for 24 h.

The receptor compartment was filled with 4.5 ml of PBS (phosphate buffer saline, pH 7.4) and kept at $32 \pm 0.5^\circ\text{C}$. A peristaltic pump was used to allow the complete mixing of the receiving phase during the test instead of magnetic stirring.

Samples of 1 ml were withdrawn from the receptor medium at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5 h, and replaced with the same volume of PBS. Sink conditions were met in all cases.

The samples were analyzed for DS content at a wavelength of 276 nm by UV-Vis spectrophotometer Hitachi-U-2001UV (Hitachi Instruments, USA) [19].

All experiments were performed in triplicate and the results were expressed as mean \pm SD.

Cumulative amounts of drug (μg) permeating the unit diffusion surface (cm^2) were plotted against time (h). The flux (J) was determined from the slope of the steady-state portion of the amount of drug released divided by effective diffusion area versus time, according to literature [20]. The lag time, Tlag (min), was determined from the x-intercept of the slope at the steady state [21].

In Vivo Studies

The *in vivo* studies were carried out in accordance with the Italian law (D.L. n° 116/1992), which allows experi-

ments on laboratory animals only after submission of a research project to the competent authorities, and in accordance to the "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985).

The experiments were performed on male albino Wistar rats weighing 150-170 g each (Harlan, Italy). They were maintained under controlled environmental conditions (temperature $22 \pm 2^\circ\text{C}$; humidity 60-65%; 12-h light-dark cycle). All animals received standard laboratory diet and water ad libitum.

All topical formulations (100 g) were applied to the plantar surface of the hind paw of rats (n=6) by gently rubbing 50 times with finger. Control rats (n=6) received only the base formulations by the same mode of application.

Anti-Inflammatory Activity

Anti-inflammatory activity was evaluated on the basis of inhibition of carrageenan-induced hind paw edema in rat, [22, 23]. Three hours after the treatment with the different formulations, 100 μl of carrageenan solution (1%, w/v) were injected subplantarily into the treated paw. The increase in paw volumes was measured with a water plethysmometer (Basile, Comerio, Italy), at time zero (basal) and 3 h after carrageenan administration. The results are expressed as the mean paw volumes (ml) \pm S.E.M.

Antihyperalgesic Activity

Thermal hyperalgesia was evaluated by the paw withdrawal test using a Plantar Test (Basile, Comerio, Italy) modelled as described by Hargreaves *et al.* [24]. Rats were placed individually in clear plastic chambers positioned on the glass floor of the testing apparatus. Following acclimation, a radiant heat source was aimed at the plantar surface of each hind paw. A photoelectric cell automatically stops the heat source when the reflected light beam is interrupted (i.e. when the animal withdraws the paw) and records the paw withdrawal latency (PWL).

A cut-off latency of 30 s was employed. After baseline paw withdrawal latencies were recorded, the plantar surface of the left hind paw (ipsilateral) was treated with each DS formulation and after three hours, injected subcutaneously with 1 mg of Carrageenan (1 mg in 100 μl of saline). Three hours later, three measurements of paw withdrawal latency at 5 min intervals were collected and averaged. All control groups received only the formulations without drug (base formulations).

Antinociceptive Activity

The antinociceptive activity of each formulation was evaluated as thermal pain threshold in rat paw by measure of the paw withdrawal latency to radiant heat source using the plantar test. The paw withdrawal latency was recorded three hours after topical treatment into the paw. Paw withdrawal latency was also recorded at the contralateral side (untreated paw). All control groups received only the base formulations.

Observers performing all the experiments were unaware of group allocation of the subjects.

Statistical Analysis

Viscosity and *in vitro* diffusion data were subjected to one-way analysis of variance (ANOVA) (Origin[®], version 7.0 SR0, OriginLab Corporation, MA, USA). In all cases, individual differences between formulations were evaluated using a non-parametric post hoc test (Tukey's test).

All *in vivo* data were expressed as the mean \pm S.E.M. (n=6 per group). Carrageenan-induced edema and hyperalgesia tests data were analyzed by two-way analysis of variance (ANOVA), while for the antinociceptive test one way ANOVA was used. Consecutive post hoc comparisons, using Fisher's LSD test, were performed when appropriate.

The differences were considered to be statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Characterization of Formulations

The prepared formulations shared a smooth and homogeneous appearance. The Carbopol hydrogel (A formulation) showed a slightly opalescence aspect while the Carbopol and Sodium Lauryl sulphate creams (B and C formulations) have a white color.

All preparations were easily spreadable, with acceptable bioadhesion and fair mechanical properties. The pH values ranged from 6.2 to 7.1, which are considered acceptable to avoid the risk of irritation after skin application [25].

Viscosity is an important physical property of topical formulations, which affects the rate of drug release; in general, an increase of the viscosity vehicles would cause a more rigid structure with a consequent decrease of the rate of drug release [26, 27].

To investigate the influence of vehicle rheological properties on DS release, the viscosity of prepared formulations was evaluated and compared to reference Emulgel (Table 2).

At the low speed, the viscosity of Control formulation (Voltaren[®] Emulgel)(11.54 \pm 1.3 Pa*s) resulted significantly lower ($P < 0.05$) than that of A (27.88 \pm 3.8 Pa*s) and C (44.50 \pm 3.9 Pa*s) formulations containing Carbopol polymer.

Carbopol is a hydrophilic polyacrylic acid polymer and its carboxyl groups become highly ionized after neutralization, forming a gel due to electrostatic repulsion among charged polymer chains interconnected by crosslinks [28, 29]. The viscosity and bioadhesive strength of Carbopol

formulations is highly dependent upon the pH, showing the elevated viscosity when neutralized [30].

On the other hand, not significant differences were found from the comparison between Voltaren Emulgel and B formulation containing Sodium Lauryl sulphate (7.62 \pm 1.8 Pa*s).

At the higher rotational speed, the commercialized Emulgel was characterized by a viscosity of 4.9 \pm 1.1 Pa*s that resulted significantly lower only when compared to C formulation, but is not significant different with respect to A and B formulations (10.1 \pm 2.3 and 1.7 \pm 0.7 Pa*s, respectively). The differences observed in the viscosity values of formulations containing Carbopol were related to ratio of solid to liquid fraction of ingredients used.

For all formulations the viscosity was found to be lower from 2.4 to 4.4 times as the rotational speed increased. In fact, when the speed increases, the normally disarranged molecules of the vehicle are caused to align their long axes in the direction of flow. Such orientation reduces the internal resistance of the material and hence decreases the viscosity [31].

In Vitro Release Results

To evaluate the effect of vehicle composition on the DS release from formulations and the influence of the possible interaction vehicle-membrane, the diffusion studies were carried out using two model membranes with different characteristics.

For the topical formulations prepared, the tests were performed for 1.5 h and the commercial Voltaren[®] Emulgel was used as reference formulation.

Cumulative amount of DS permeated through PTFE hydrophilic membrane were reported as a function of square root of time (Fig. 1).

It was observed that all formulations are characterized by similar release profiles and facilitated drug delivery with very short lag-times.

The slopes representing fluxes ($\mu\text{g}/\text{cm}^2/\text{h}^{1/2}$), correlation coefficients and the drug amount ($\mu\text{g}/\text{cm}^2$) permeated after 1.5 h were calculated (Table 3).

The flux of DS from Control Emulgel (288.3 \pm 5.9 $\mu\text{g}/\text{cm}^2/\text{h}^{1/2}$) resulted significant higher ($P < 0.05$) compared with A formulation (147.8 \pm 16.9 $\mu\text{g}/\text{cm}^2/\text{h}^{1/2}$) and B formulation (150.2 \pm 4.8 $\mu\text{g}/\text{cm}^2/\text{h}^{1/2}$), while was similar ($P > 0.05$) to this obtained from C formulation (285 \pm 16.5 $\mu\text{g}/\text{cm}^2/\text{h}^{1/2}$).

Table 2. Viscosity (in Pa*s) of DS Formulations Measured at Low and High Rotation Speeds (mean \pm SD, n = 3)

FORMULATIONS	VISCOSITY	
	Low speed	High speed
Control	11.5 \pm 1.3	4.9 \pm 1.1
A	27.9 \pm 3.82	10.1 \pm 2.3
B	7.6 \pm 1.8	1.7 \pm 0.7
C	44.5 \pm 3.82	15.4 \pm 3.3

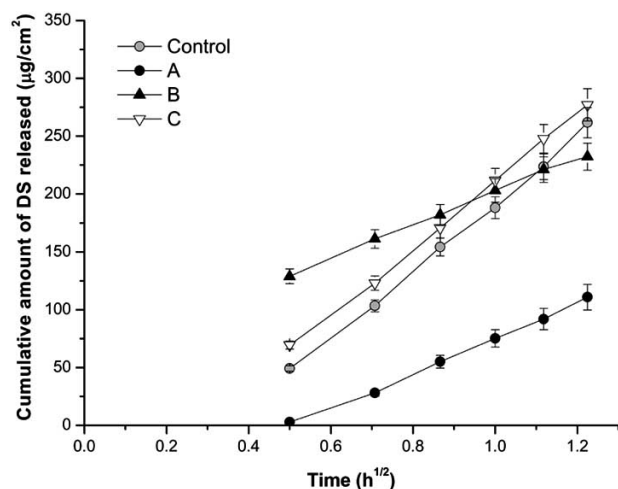


Fig. (1). *In vitro* release profiles of DS from various formulations through PTFE hydrophilic membrane.

In particular, in the case of Sodium lauryl sulphate cream (B formulation), the diffusion rate of drug is higher at the first two points and leveled off at the late stage of the test, resulting comparable to Control and C formulations.

The drug release from all investigated formulations followed the Higuchi diffusion model [32], as confirmed from the good correlation coefficients ranging from 0.980 to 0.992. This finding indicates that the rate-controlling stage in the release process was the diffusion of the dissolved drug through the vehicle network to the external medium [33, 34].

Besides, these results demonstrates that the viscosity of the polymer matrices did not show a direct relationship with the drug flux.

Statistical analysis of the cumulative amount of drug permeated after 1.5 h showed that the DS released from Control formulation ($258.5 \pm 13.1 \mu\text{g}/\text{cm}^2$) is higher ($P < 0.05$) than the A formulation ($110.9 \pm 10.6 \mu\text{g}/\text{cm}^2$), while was not sig-

nificant different ($P > 0.05$) when compared to B and C formulations that released $239.0 \pm 10.2 \mu\text{g}/\text{cm}^2$ and $277.2 \pm 12.7 \mu\text{g}/\text{cm}^2$ of DS, respectively.

The release profiles of DS across PVDF hydrophobic membranes are illustrated in Fig. (2).

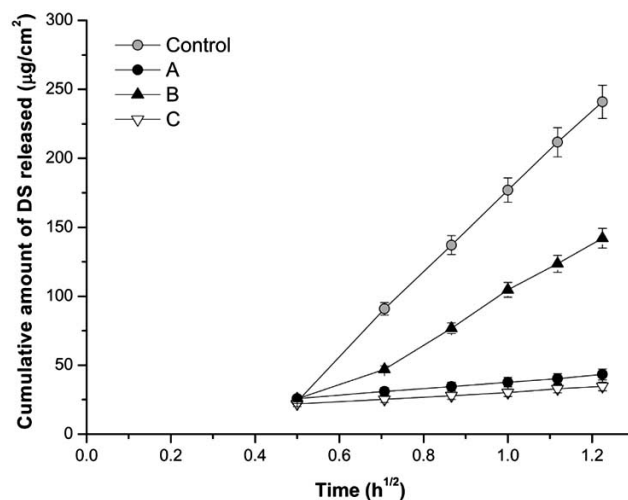


Fig. (2). *In vitro* release profiles of DS from various formulations through PVDF hydrophobic membrane.

The behaviors of emulgel and B formulations resulted comparable to those obtained across hydrophilic membranes, while important differences were observed for A and C formulations, that were characterized by very slow release and superimposed profiles.

The commercialized emulgel showed the higher flux values of DS ($302 \pm 12.6 \mu\text{g}/\text{cm}^2/\text{h}^{1/2}$) followed by B formulation ($169.7 \pm 15.3 \mu\text{g}/\text{cm}^2/\text{h}^{1/2}$) and finally by A and C formulations that exhibit the lower diffusion rates ($23.6 \pm 2.7 \mu\text{g}/\text{cm}^2/\text{h}^{1/2}$ and $20.6 \pm 7.2 \mu\text{g}/\text{cm}^2/\text{h}^{1/2}$, respectively) (Table 3).

Table 3. Effect of Formulation on Diffusion Rates, Correlation Coefficient and Cumulative Amount of DS Released After 1.5 h Through Different Synthetic Membranes

Formulations	Flux ($\mu\text{g}/\text{cm}^2/\text{h}^{1/2}$)	Correlation Coefficient	Released Drug after 1.5 h ($\mu\text{g}/\text{cm}^2$)
PTFE hydrophilic			
Control	288.3 ± 5.9	0.987	258.5 ± 13.1
A	147.8 ± 16.9	0.980	110.9 ± 10.6
B	150.2 ± 4.8	0.992	239.0 ± 10.2
C	284.6 ± 16.5	0.990	277.2 ± 12.7
PVDF hydrophobic			
Control	302.4 ± 12.6	0.999	244.3 ± 14.6
A	23.6 ± 2.7	0.983	43.3 ± 2.5
B	169.7 ± 15.3	0.984	145.4 ± 12.6
C	20.6 ± 7.2	0.945	37.9 ± 7.0

Similarly, the cumulative amount of drug recovered after 1.5 h from all formulations resulted significant different ($P > 0.05$); the rank order was found to be Control ($244 \pm 15 \mu\text{g}/\text{cm}^2$) $>$ B ($145 \pm 12.6 \mu\text{g}/\text{cm}^2$) $>$ A ($43 \pm 2.5 \mu\text{g}/\text{cm}^2$) \approx C (37.9 ± 7.0 ($43 \pm 2.5 \mu\text{g}/\text{cm}^2$)).

The comparison between the diffusion parameters of DS obtained through the two different membranes clearly showed that the release process was strongly influenced from both the compositions of formulations and the interactions between the vehicle and membrane that make a important contribution to the overall diffusion rate [35].

The commercial formulation was characterized by a higher drug release that revealed to be similar with hydrophilic and hydrophobic membranes, therefore suggesting a good interaction of Emulgel with both membranes. This findings can be explained considering the components properties in the different formulations. In particular, the presence of Carbopol and emulsifying agent in high levels, having hydrophilic characteristics, promotes the interaction with PTFE membrane. On the other hand, formulations with high amount of isopropyl alcohol, which is known as a potent penetration enhancer [36], should have the interaction and drug diffusion through hydrophobic membrane improved.

The Sodium lauryl sulphate cream (B formulation) exhibited a slower drug release and not significant differences in the drug diffusion through the two membranes were observed. Due to the absence of Carbopol polymer, this formulation is unable to strongly interact with membranes, however, the high content of the oily phase would promote the drug diffusion out of hydrophobic vehicle in which is weakly soluble, and its dissolution in the release medium.

In contrast, the drug release from Carbopol gel and cream (A and C formulations) appeared strictly related to the characteristics of membrane used. In fact, these formulations have been shown to promote the drug diffusion only through PTFE membrane. The hydrophilic nature of the polymer ensures a good interaction with hydrophilic membranes; thus, the release medium could easy penetrate into the swollen polymeric matrix, leading to the drug dissolution. On the other hand, the poor interaction with hydrophobic membranes determines a decrease of the medium penetration causing the drug to remain trapped in the network polymeric structure.

The higher flux values observed for cream with respect to Carbopol gel could be related to the presence of emulsifying agent (Cetostearyl alcohol) and hydrophilic polyethylene glycol that, with a moderate penetration enhancing activity, can promote the drug diffusion from this formulation [37].

In Vivo Results

To compare the *in vivo* efficacy of the prepared formulations with the reference Emulgel, the acute anti-inflammatory, antihyperalgesic and antinociceptive activity of all formulations were tested after application on the skin in rats.

The carrageenan-induced edema and hyperalgesia in rats paw are common models of local inflammation and pain

inflammatory [16]; in addition, the antinociceptive effect was evaluated on thermal pain threshold in rat paw.

Statistical analysis relative to the commercialized emulgel (E) showed a significant effect in the carrageenan-induced paw edema both of group ($F(1,10)=6.85$, $P=0.026$) and of time ($F(1,10)=239.27$, $P=2.6 \times 10^{-8}$) compared with the respective control group (Ec), treated with base formulations without drug (data not shown).

On the other hand, the topical administration of A, B and C formulations did not reveal any significant effects when compared to the control groups (Ac, Bc and Cc). Post-hoc test, performed to investigate the differences between the groups, demonstrated that only the reference formulation produced a reduction of the carrageenan-induced edema than the value exhibited by rats in the control group, Control c ($P=0.019$).

With regard to carrageenan-induced thermal hyperalgesia in rats, the B formulation showed a significant effect both of group ($F(1,10)=18.39$, $P=0.0016$) and of time ($F(1,10)=13.38$, $P=0.004$), increasing the paw withdrawal latency with respect to the control group (Bc) ($P=0.0016$). In contrast, there were not significant effects between the E, A, and C formulations compared to the control groups (Ec, Ac and Cc) (data not shown).

However, the statistical comparison of data related to the antinociceptive activity of the untreated group and the groups treated with B and Bc formulations (Fig. 3), revealed a significant effect of group ($F(2,15)=3.61$, $P=0.04$). Post-hoc test demonstrated that the local administration of Sodium lauryl sulphate cream produced a significant increase in thermal pain threshold compared to the value exhibited by rats paw in the untreated group ($P=0.0347$), and in the paw base control group Bc ($P=0.034$). Other different formulations (Control, A and C) not showed a significant effect with respect to the respective controls (Control c, Ac and Cc).

As reported in literature, after topical administration, the diclofenac acts directly upon ongoing inflammatory hypersensitization, while in the antihyperalgesic effects of drug not only peripheral but also central mechanisms are involved [38].

Collectively, the *in vivo* results suggest that commercialized formulation is able to attenuate the development of inflammatory edema produced by carrageenan but does not prevent the development of hyperalgesia and not increase thermal pain threshold in rats paw. This behavior may reflect an insufficient systemic absorption or, more probably, the difficulty of increasing the high pain threshold of these paws.

On the other hand, the antihyperalgesic and antinociceptive properties of Sodium lauryl sulphate cream suggest that this formulation promotes a more rapid penetration of diclofenac through the skin and improves its systemic absorption.

Nevertheless, the topical B formulation applied into the paw elicited an antinociceptive effect that was demonstrated to be local, since this formulation did not produce an antinociceptive effect in the contralateral paw (increase of thermal paw threshold).

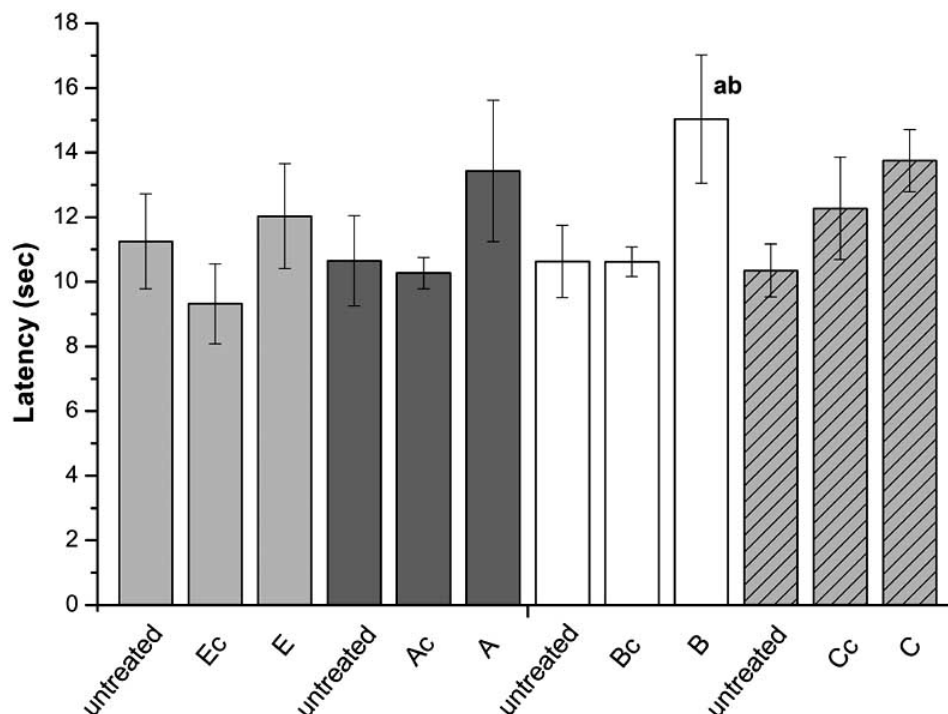


Fig. (3). Effect of topical formulations in the antinociceptive test in rats. Data represent mean values of paw latency (second), 3 h after treatment. Significant differences from respective untreated paw group are indicated by ^a while from formulations without diclofenac by ^b (^{a,b} $P < 0.05$, one way ANOVA followed by Fisher's LSD test).

CONCLUSIONS

The results here presented clearly show that the *in vitro* diffusion of Diclofenac Sodium from the new topical formulations used in this study is strongly related to the vehicle composition and its interaction with different membranes.

From the *in vivo* results, among the tested formulations, Sodium lauryl sulphate cream seems to be the most appropriate formulation and may be effective in treatment of cutaneous pain and hyperalgesia conditions.

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