Acta Poloniae Pharmaceutica – Drug Research, Vol. 63 No. 5 pp. 461–467, 2007 ISSN 0001-6837
Polish Pharmaceutical Society

FORMULATION AND *EX VIVO* EVALUATION OF ROFECOXIB GEL FOR TOPICAL APPLICATION

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Abstract: The potential gastrointestinal disorders associated with oral administration of rofecoxib can be avoided by delivering the drug to the inflammation site at a sustained, concentrated level over an extended period of time. Hydroxypropylmethylcellulose (HPMC), sodium alginate and Carbopol 940 were used in an attempt to develop topical gel formulations of rofecoxib. The effects of polymer composition on the rate of drug release from the gel formulations were examined through cellulose membrane mounting on a Keshary-Chien diffusion cell. The effects of initial drug concentration and viscosity on the permeation rate of rofecoxib from the gel formulations were evaluated using rat epidermis at 37 ± 0.5°C. The anti-inflammatory activity of the rofecoxib gel formulation was evaluated using the rat hind paw edema model. The gel formulation consisting of 4% w/w sodium alginate-Carbopol 940 at 3:1 ratio was found to be suitable for topical application based on *in vitro* evaluation and *ex vivo* permeation studies. The drug permeation rate increased with an increase of the initial drug concentration in gels up to 25 % w/w. An inverse relationship was observed between the *in vitro* drug release rate/*ex vivo* permeation rate and viscosity of the gel formulations. The anti-inflammatory activity of 4% w/w sodium alginate-Carbopol 940 gel containing 25% w/w rofecoxib in the rat hind paw edema model reveals that the drug was delivered to the inflammation site at a controlled level over a period of 6 h. These results suggest the feasibility of the topical gel formulation of rofecoxib.

Keywords: rofecoxib, sodium alginate, Carbopol 940, topical gel, anti-inflammatory activity, rat hind paw edema model

The use of non-steroidal anti-inflammatory drugs is well recognized for regional inflammatory disorders such as muscle pain, osteoarthritis and rheumatoid arthritis. Rofecoxib, a specific COX2 inhibitor, is one of the most potent non-steroidal anti-inflammatory agents. The drug was approved by FDA in the year 1999 (1, 2) for the treatment of pain and inflammation associated with musculoskeletal disorders, primary dysmenorrhea, rheumatoid arthritis and osteoarthritis. The oral bioavailability of rofecoxib is about 93 % and the steady state plasma concentration is reached at 3-4 days with multiple dose oral administration. However, its use has been associated with a number of gastrointestinal disorders (3). These potential side effects may be overcome by the topical administration of the drug.

The present research has been undertaken with the aim to develop a transdermal gel formulation of rofecoxib, which would attenuate the gastrointestinal related toxicities associated with oral administration. The log (P) value of rofecoxib is about 1.705 indicating its lipophilicity for the development of transdermal formulation. Also, rofecoxib having molecular weight of 314.36 Da and melting point in the range of 204 – 208˚C can be considered ideal for transport through the skin (4). However, rofecoxib has not been investigated for potential administration via transdermal route except one research paper that reported the enhancement of skin permeation of rofecoxib using topical micro emulsion (5).

In the present study, transdermal gel formulations for rofecoxib were prepared using HPMC, sodium alginate and Carbopol 940 alone and/or in combination, as gel forming polymers. After *in vitro* evaluation of the gel formulations, *ex vivo* permeation of rofecoxib from it was evaluated across rat epidermis. Further, the *in vitro* anti-inflammatory activity of the gel formulation was studied using the rat model.

MATERIALS AND METHODS

Materials

Rofecoxib was a gift sample from Alembic Pharmaceutical Ltd. (Vadodara, India). Sodium alginate, Carbopol 940 (Loba Chemie Pvt. Ltd., Mumbai, India), glycerol (Qualigens Fine

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Chemicals, Mumbai, India), methyl paraben (Himedia Laboratories Pvt. Ltd., Mumbai, India), triethanolamine (Central Drug House Ltd., Mumbai, India), Polyethylene glycol 400 (Merck Ltd., Mumbai, India), cellulose membrane (Sigma Chemical Company, USA), sodium bromide (Loba Chemie Pvt. Ltd., Mumbai, India) and chloroform (Thomas Baker Chemicals Pvt. Ltd., Mumbai, India) were procured and used in this investigation.

Animals

All experiments with animals were approved by the Institutional Animal Ethics Committee of Dibrugarh University, India. The Wistar male albino rats were obtained from M/S Ghosh Enterprises, Kolkata, India and maintained under controlled conditions of temperature as well as humidity and the rats had free excess to water and food.

Analytical method

The estimation of drug in the samples collected from *in vitro* release and *ex vivo* permeation studies was performed by a UV spectrophotometric method. In order to generate calibration curve, an accurately weighed amount of rofecoxib was solubilized in the solution of 40% v/v PEG 400 in water to obtain a primary standards in the concentration range of 10 – 80 µg/mL and the calibration curve was obtained by measuring their absorbance at predetermined λ_{max} of 257 nm with a Hitachi U-2001 UV-VIS spectrophotometer. The concentration of rofecoxib in test samples was calculated using the linear regression equation of the calibration curve (Absorbance = 0.0252 + 0.0117 × Concentration, r^2 = 0.9973). The high value of correlation coefficient (r^2) indicates the linearity of the calibration curve and the curve did not deviate significantly from the origin as indicated by its low value of intercept. The method was validated for accuracy and precision. When a standard drug solution was assayed repeatedly (n = 6), mean standard error (accuracy) and RSD (precision) were found to be 0.5% and 0.7%, respectively.

Preparation of rofecoxib gel

Gels were prepared by dispersing the polymers in a mixture of water and glycerol with methylparaben as preservative and varying amount of rofecoxib, being kept under magnetic stirring until homogeneous dispersion was formed. The dispersion was then neutralized and made viscous by the addition of triethanolamine. The compositions of different gel formations are listed in Table 1.

***In vitro* evaluation**

The physical appearance and homogeneity of the prepared gels were tested by visual observations. The spreadability of the gel formulations was determined at 24 h after permeation, by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm × 20 cm) after one min. The standardized weight tied on the upper plate was 125 g (6). The Voveran Emulgel (Novartis Pharma) was considered as reference standard.

The pH of the gel formulations was determined by using a pH meter (TOSHNIWAL, Model CL 54). The measurement was performed at 1, 30 and 60 days after preparation to detect any pH fluctuation with time.

For assay of the drug in gels, rofecoxib was extracted from 1 g of each gel formulations with 20 mL of methanol for 30 min. The resultant mixture was filtered through membrane filter (pore size 0.45 mm). The absorbance of the sample was determined spectrophotometrically at 257 nm (Hitachi U-2001 UV-VIS spectrophotometer) after appropriate dilu-

Table 1. Compositions of gels.

F.N.Code	Sodium alginate (%w/w)	Carbopol (%w/w)	HPMC (%w/w)	Rofecoxib (mg)	Glycerol (mL)	Methyl paraben (mg)
F1	-	-	12	50	0.5	10
F2	4	-	-	50	0.5	10
F3	-	2	-	50	0.5	10
F4	3	1	-	50	0.5	10
F5	3.5	0.5	-	50	0.5	10
F6	2.5	1.5	-	50	0.5	10
F7	3	1	-	10	0.5	10
F8	3	1	-	20	0.5	10
F9	3	1	-	75	0.5	10

tion with aqueous solution of PEG 400 (40% v/v). The concentration of rofecoxib was estimated from the regression equation of the calibration curve.
The viscosity of the gel formulations was determined using Brookfield viscometer with spindle no. 6 at 10 rpm at the temperature of 31°C.

The *in vitro* drug release from gel formulations was studied across cellulose membranes using Keshary-Chien diffusion cell (7) with effective diffusional surface area of 1.54 cm^2 and a receptor cell volume of 19.5 mL. The receptor compartment was filled with the solution of PEG 400 (40% v/v) in water and maintained at 37 ± 0.5°C with constant magnetic stirring. 1 g of gel was placed on the donor compartment and covered with a piece of aluminium foil to prevent drying out. The samples (1 mL) were collected from the receptor compartment at predetermined time interval for 6 h period and replaced by equal volume of fresh prewarmed receptor solution to maintain constant volume allowing sink condition throughout the experiment. The amounts of rofecoxib in the samples were assayed spectrophotometrically at 257 nm against appropriate blank.

***Ex vivo* evaluation**

The abdominal hair of Wistar male albino rats, weighing 150 – 200 g, was shaved using an electric razor after sacrificing with excess chloroform inhalation. The abdominal skin was surgically removed and adhering subcutaneous fat was carefully cleaned. The epidermis was then separated from dermis by soaking the full thickness skin in 2 M sodium bromide solution in water for 6 – 8 h (8). The epidermis was thoroughly washed with water, dried at 25% RH, wrapped in aluminium foil and stored in freeze until further use.

For *ex vivo* permeation studies, skins were allowed to hydrate for 1 h before being mounted on the Keshary-Chien diffusion cell with the stratum corneum (SC) facing the donor compartment. The receptor compartment was filled with aqueous solution of PEG 400 (40% v/v) and receptor phase was maintained at 37 ± 0.5°C. 1 g of the gel was placed on the SC side in the donor compartment and covered with aluminium foil to prevent drying out. The amount of drug permeated was determined spectrophotometrically at 257 nm by removing 1 mL aliquot through a hypodermic syringe fitted with a 0.22 mm membrane filter, at designated time intervals for 8 h. The volume was replenished with the same volume of prewarmed receiver solution to maintain sink conditions. Blanks are run for each set as described above with placebo gel and calculated accordingly.

***In vitro* anti-inflammatory activity**

The *in vitro* anti-inflammatory activity of the gel formulation was performed using carrageenan induced rat hind paw edema model (9). The Wistar albino male rats weighing 150 – 210 g were fasted overnight, but water was allowed *ad libitum*. The animals were divided into three groups of four animals each. Group 1 (control) received placebo gel, group 2 received 1.2 mg/mL of rofecoxib suspension in water and the group 3 received 1.2 mg/kg equivalent to rofecoxib in gel formulation. Immediately after drug administration 0.05 mL of 1% w/w solution of carrageenan was injected into the planter surface of the hind paw. The hind paw volume was measured at different time intervals for 6 h after carrageenan treatment using a plethysmograph. The percent inhibition in hind paw edema volume was calculated using the following formula and compared with those recorded for control group.

$$\text{Anti-inflammatory activity } (\%) = (1 - D/C) \times 100$$

where D is the change in paw volume in the treated

Table 2. Physicochemical evaluation of gel formulations.

F.N.Code	Spreading diameter after 1 min (mm)	% Rofecoxib content (mean ± SD)	Physical appearance	Homogeneity	pH (mean ± SD)
F1	72	86.76 ± 5.23	White	Homogeneous	6.70 ± 0.03
F2	64	87.28 ± 4.25	Opaque	Homogeneous	7.09 ± 0.04
F3	48	90.16 ± 6.52	White	Homogeneous	6.56 ± 0.03
F4	54	88.03 ± 5.01	Opaque	Homogeneous	6.35 ± 0.04
F5	52	87.03 ± 4.04	Opaque	Homogeneous	6.39 ± 0.01
F6	60	86.87 ± 3.58	Opaque	Homogeneous	6.54 ± 0.02
Voveran Emulgel (Novartis Pharma)	53	–	Opaque	Homogeneous	6.90 ± 0.05

Table 3. Release parameters of rofecoxib from F1 to F6 gel formulations through cellulose membrane.

F.N.Code	Amount release at 6 h (μg/cm²) (mean ± SD)	Release rate (μg/cm²/h) (mean ± SD)	Best fit regression equation for release plot	r^2
F1	1857.10 ± 80.25	491.73 ± 24.19	Q = 491.73 t +141.11	0.9216
F2	2912.96 ± 101.52	579.08 ± 23.24	Q = 579.08 t + 132.02	0.9870
F3	2149.01 ± 88.26	510.21 ± 22.99	Q = 510.21 t + 118.64	0.9257
F4	3016.44 ± 104.23	632.54 ± 25.83	Q = 632.54 t + 181.57	0.9776
F5	1750.72 ± 78.55	512.50 ± 24.33	Q = 512.50 t + 88.79	0.8825
F6	1635.01 ± 85.66	487.97 ± 15.69	Q = 487.97 t + 110.52	0.8588

Table 4. Permeation parameters of rofecoxib from various gel formulations across rat epidermis.

F.N.Code	Amount permeated at 8 h (μg/cm²) (mean ± SD)	J (μg/cm²/h) (mean ± SD)	T_L (h) (mean ± SD)	K_P ($cm^2/S \times 10^{-6}$) (mean ± SD)	Best fit regression equation for permeation plot	r^2
F2	1748.96 ± 78.35	229.28 ± 10.12	0.41 ± 0.12	6.36 ± 0.21	Q = 229.28 t – 80.19	0.9932
F3	1397.11 ± 84.55	179.14 ± 8.52	0.37 ± 0.03	4.97 ± 0.15	Q = 179.14 t – 55.44	0.9933
F4	1948.32 ± 91.45	254.63 ± 10.98	0.40 ± 0.01	7.07 ± 0.42	Q = 254.63 t – 107.16	0.9934
F7	453.66 ± 40.12	88.12 ± 5.09	0.31 ± 0.02	2.45 ± 0.12	Q = 88.12 t – 43.39	0.9636
F8	1005.80 ± 70.15	141.31 ± 5.63	0.34 ± 0.03	3.92 ± 0.08	Q = 141.31 t – 11.32	0.9795
F9	1732.36 ± 83.25	249.10 ± 10.57	0.43± 0.08	7.21 ± 0.17	Q = 249.10 t – 120.18	0.9848

Table 5: Percent inhibitions of hind paw edema.

Formulations	Percent inhibition					
	1 h	2 h	3 h	4 h	5 h	6 h
Rofecoxib oral	16.66	45.00	52.21	65.51	63.13	62.89
Rofecoxib gel	9.44	35.10	46.34	55.45	56.22	58.93

group and C is the change in paw volume in the control group.

Data and statistical analysis

The steady state flux (J, μg/cm²/h) was calculated from the slope of the linear plot of the cumulative amount permeated per unit area (μg/cm²) as a function of time (t, h). The lag time (t_L, h) was determined from the x-intercept of the slope at the steady state. The permeability coefficient (K_P, cm²/S) was calculated from the flux and donor drug concentration.

Data are represented as mean ± SD (n = 3). Statistical comparisons were made using Student's *t*-test at a significance level of $p < 0.05$ using MS-Excel software.

RESULTS AND DISCUSSION

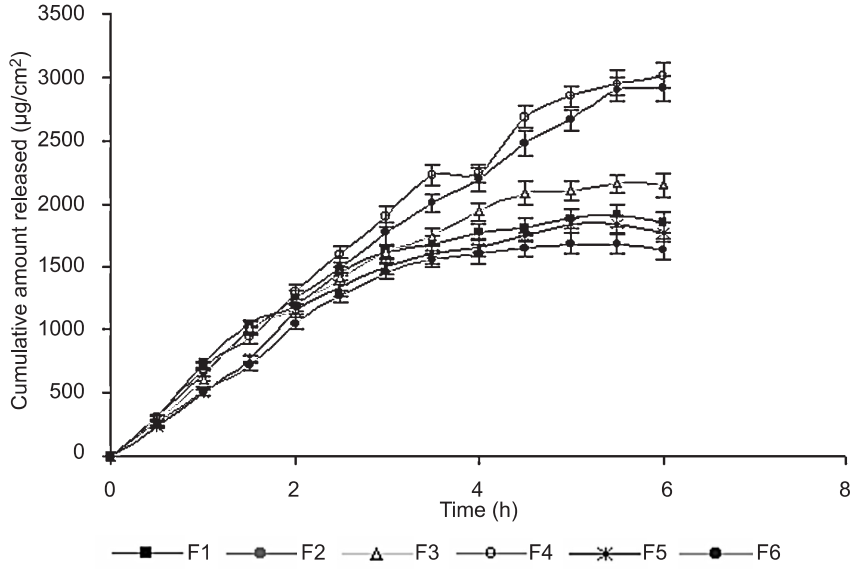
***In vitro* evaluation**

The physicochemical properties of the gel formulations are shown in Table 2. From the results it is clearly evident that all the gel formulations showed good homogeneity and spreadability. The physical appearance of the gel formulations was white or opaque in nature. The drug content of the gel formulations was in the range of 86.76 ± 5.23 to 90.16 ± 6.52%, showing content uniformity. The pH of the gel formulations was in the range of 6.35 ± 0.04 to 7.09 ± 0.04, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values (varied from 0.01 to 0.16) as a function of time for all formulations. The physicochemical properties of the prepared gel formulations were in good agreement with those of a marketed product namely Voveran Emulgel from Novartis Pharma.

The viscosity of the gel formulations generally reflects its consistency. The consistency of F2 (4% w/w sodium alginate), F3 (2% Carbopol 940) and F4 (4% sodium alginate-Carbopol 940 at 3:1 ratio) gel formulations can be ranked according to their viscosity values as follows: F3 (viscosity 89000 cps) > F2 (viscosity 28000 cps) > F4 (viscosity 21000 cps).

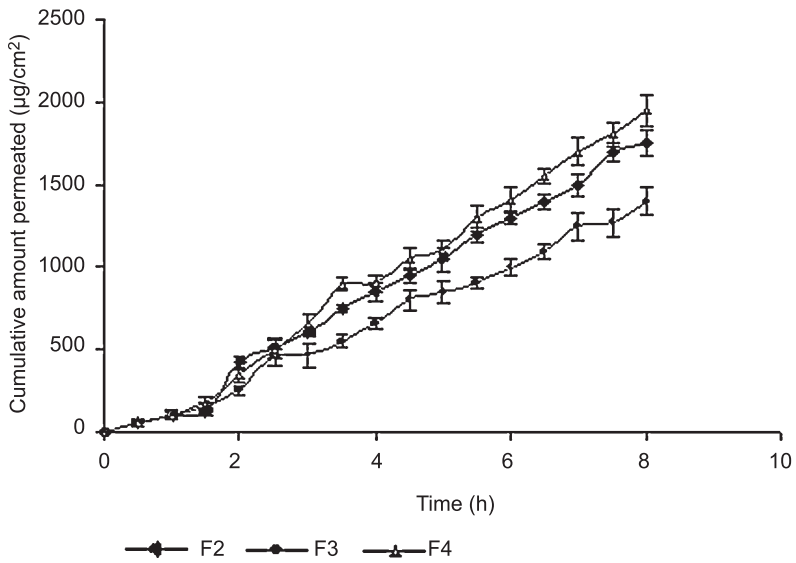
The experiments for *in vitro* release of rofecoxib from gel formulations through cellulose mem-

brane were carried out to select appropriate polymer composition for gel formulation having suitable consistency for topical application. Figure 1 depicts the release profile of rofecoxib from various gel formulations across the cellulose membrane. The release parameters are tabulated in Table 3. A linear relationship [$r^2 > 0.9$ (0.94 – 0.97)] was obtained between the amount released and the square root of time as proposed by the Higuchi's theory (10), indicating the diffusion controlled mechanism of drug release. The results reveal that the total amount of drug release at 6 h for the formulations F1 to F6 was



F1 (12% w/w HPMC gel), F2 (4% w/w sodium alginate gel), F3 (2% w/w Carbopol 940 gel), F4 (4% w/w sodium alginate-Carbopol 940 at 3:1 ratio gel), F5 (4% w/w sodium alginate-Carbopol 940 at 7:1 ratio gel), F6 (4% w/w sodium alginate-Carbopol 940 at 5:3 ratio gel)

Figure 1. Release profiles of rofecoxib from various gel formulations across cellulose membrane. Bar represents mean ± S.D (n = 3).



F2 (4% w/w sodium alginate gel), F3 (2% w/w Carbopol 940 gel), F4 (4% w/w sodium alginate-Carbopol 940 at 3:1 ratio gel)

Figure 2. Permeation profiles of rofecoxib from various gel formulations across rat epidermis. Bar represents mean ± S.D (n = 3).

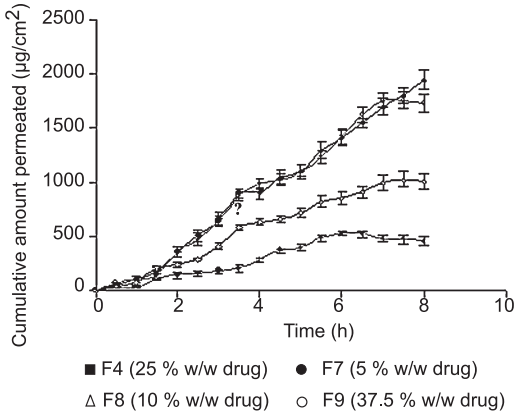


Figure 3. Effect of initial drug concentration on permeation of rofecoxib from sodium alginate-Carbopol 940 (3:1 ratio) gel across rat epidermis. Bar represents mean ± S. D (n = 3)

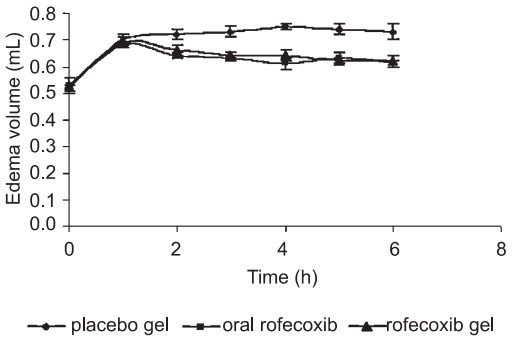


Figure 4. Change in edema volume with rofecoxib oral, placebo gel and rofecoxib gel after carragenan treatment. Bar represents mean ± S.D (n = 4).

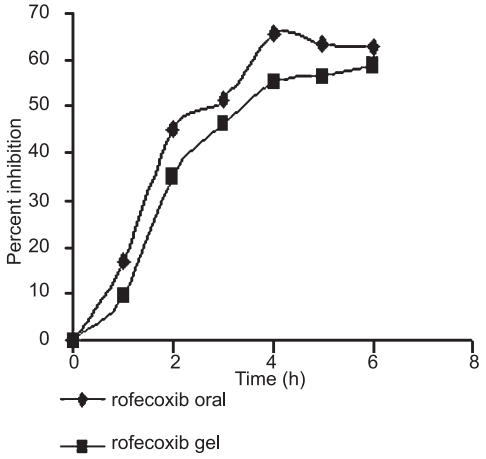


Figure 5. Percent inhibition of hind paw edema after oral administration of rofecoxib and application of rofecoxib gel.

dependent on the nature of the polymer used and viscosity, as well as the consistency of the formulations. The drug release was inversely proportional to the viscosity of the gel formulations. The gel formulations consisting of the mixture of sodium alginate and Carbopol showed good physicochemical characteristics for topical application. The drug release increased significantly ($p < 0.05$) when the amount of Carbopol in the gel formulation was increased from 0.5% (formulation F5) to 1% w/w (formulation F4), beyond which a significant decrease ($p < 0.05$) in drug release was observed (formulation F6). The maximum drug release was observed with the formulation F4 (4% w/w sodium alginate-Carbopol 940 at 3:1 ratio) followed by the formulation F2 (4% w/w sodium alginate) and F3 (2% w/w Carbopol) (Table 3). However, no statistically significant difference in drug release was observed between F2 and F4 ($p < 0.05$). It may be due to the softness and less viscous nature of the formulations. The rank order of the various gel formulations based upon their maximum drug release is F4 > F2 > F3 > F1 > F5 > F6. Based on the physicochemical properties and drug release, the formulation F4 was found to be suitable for topical application.

Ex vivo evaluation

Figure 2 and 3 depict the *ex vivo* skin permeation profile of rofecoxob from gels across rat epidermis. The skin permeation profile showed the same pattern as that of the *in vitro* release profile across the cellulose membrane. A linear relationship [$r^2 > 0.9$ (0.96 – 0.99)] was observed between the cumulative amount permeated and time, indicating zero order permeation kinetics and the permeation of rofecoxib was based on diffusion controlled mechanism. The various permeation parameters are tabulated in Table 4. The steady state flux was observed after a small lag time in the range of 0.31 – 0.43 h. An inverse relationship was observed between permeation rate and the viscosity of the gel formulation. The results are presented in Table 4. Figure 3 depicts the effect of initial drug concentration on permeation of rofecoxib from 4% w/w sodium alginate-Carbopol 940 gels at 3:1 ratio. The cumulative amounts permeated at 8 h were 453.66 ± 40.12, 1005.8 ± 70.15, 1948.32 ± 91.45 and 1732.36 ± 83.25 µg/cm^2 from the gel formulations containing 5% (F7), 10% (F8), 25% (F4) and 37.5% (F9), respectively (Table 4). The results reveal that there is a significant increase in permeation flux ($p < 0.05$) with increasing the drug concentration from 5% to 25% w/w, further that no significant increase in permeation rate was observed ($p > 0.05$) (formulation

F9 containing 37.5% w/w drug). This could be attributed to the increased viscosity of the gel formulations at higher drug concentration.

***In vitro* anti-inflammatory activity**

Figure 4 represents the change in edema volume after carrageenan treatment with rofecoxib oral suspension, rofecoxib gel and control gel. As shown in Table 5 and Figure 5, the maximum 65.51% inhibition of edema was observed with oral rofecoxib at 4 h after carrageenan treatment and maximum 58.93% inhibitions of edema was observed with rofecoxib gel formulation at 6 h after carrageenan treatment. It may be due to the initial slower release of drug from the gel formulation. The better anti-inflammatory activity found with the rofecoxib gel treatment may be accelerated for controlled drug release and protection of drug from first-pass hepatic metabolism which is encountered in the oral route.

CONCLUSION

The rofecoxib gel for topical application was developed using sodium alginate and Carbopol 940 as gel forming polymers. The percutaneous delivery of rofecoxib from the topical application of the prepared gel formulations across rat epidermis was found feasible based on *ex vivo* permeation studies. The formulation F4 consisting of 4% w/w sodium alginate-Carbopol at 3:1 ratio was found to be suitable for topical application based upon its physicochemical properties. The anti-inflammatory activity of this gel formulation in rat hind paw edema model reveals that rofecoxib was delivered to the inflammation site at a controlled level over a period of 6 h. These results suggest the feasibility of the topical gel formulation of rofecoxib.

REFERENCES

1. Jacson L.R., Marrow J.D.: in The Pharmacological Basis of Therapeutics, Hardman J.E., Limound L.E., Gilman A.G. Eds., p. 714, McGraw-Hill Inc., New York 2001.
2. Soniwala M.M., Patel P.R., Mansuri N.S., Prikh R.K., Gohel M.C.: Ind. J. Pharm. Sci. 67, 61 (2005).
3. Layton D., Riley J., Wilton L., Shakir S.A.W.: Int. J. Pharm. Pract. 10, R 13 (2002).
4. Ahmed A.B.: M. Pharm. Thesis, Dibrugarh University, India (2006).
5. Desai K.G.H.: Drug Develop. Res. 63, 33 (2004).
6. Vennat B., Gross D., Pourrat A., Pourrat H.: Drug Develop. Ind. Pharm. 17, 2083 (1991).
7. Keshary P.R., Chien Y. W.: Drug Develop. Ind. Pharm. 10, 1663 (1984).
8. Thomas N.S., Panchagnula R.: Eur. J. Pharm. Sci. 18, 71 (2003).
9. Vyas S.P., Gogoi P.J., Jain S.K.: Drug Develop. Ind. Pharm. 17 (8), 1047 (1991).
10. Higuchi W.I.: J. Pharm. Sci. 51, 802 (1962).

Received: 23.02.2007