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DEVELOPMENT AND CHARACTERIZATION OF PSEUDOLATEX BASED TRANSDERMAL DRUG DELIVERY SYSTEM OF DICLOFENAC.

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ABSTRACT

A transdermal drug delivery system of diclofenac was developed for prolonged and controlled release of diclofenac. The designed system essentially based on polymeric pseudolatex dispersion. The formulation variables that could effect the formulation stability vis a vis drug release were studied. To achieve the desired release rate, different combination of hydrophilic and hydrophobic polymer were used for the preparation of pseudolatex system. The designed system exhibited linear relationship between drug release (Q) V/s square root of time ($t^{0.5}$). The product having skin permeability rate 0.188 mg/h/cm^2 was selected for the *in vitro* anti-inflammatory activity and *in vivo* evaluation. The system could maintained a constant and effective plasma level for 24 hours. The effective drug plasma concentration was monitored periodically. The study revealed that designed pseudolatex transdermal drug delivery system of diclofenac could be used successfully with improved performance and hold promise for further studies.

INTRODUCTION

Diclofenac is a potent non-steroidal anti-inflammatory agent. It is used in the treatment of rheumatoid arthritis and other rheumatic disorder (1). Inspite of good bioavailability after oral administration contra-indicative manifestations are associated with diclofenac therapy. It is extensively metabolized in the liver and mainly excreted in urine. It has also a narrow therapeutic index. Because of its short biological half life the drug has to be given quite frequently (2). Diclofenac is highly permeable through skin due to its greater lipophilicity. It's partition coefficient is 13.4 (n-octanol/phosphate buffer, pH 7.4) (3). The high transdermal permeability of diclofenac was recently recognized and therapeutic system to provide a transdermal delivery was explored by Nishihata et al.(4). In this investigation diclofenac was selected for topical application because no toxicity or topical irritation has been reported. Takamura and coworkers developed an o/w emulsion of diclofenac for topical application and studied for its physico-pharmaceutical characteristics (5).

Present work is aimed at development of a controlled release transdermal drug delivery system of diclofenac based on pseudolatex dispersion. The system was designed and developed to release the drug at a defined and controlled rate over an extended period for 24 hours. The designed system was evaluated for in vitro drug release and permeation across the freshly excised cadavar skin and finally tested for anti-inflammatory activity and in vivo performance.

EXPERIMENTAL

A. Materials

Diclofenac (Lupin Labs. Pvt. Ltd., Aurangabad, India), Voltrarol® 50 (Diclofenac 50 mg,enteric coated tablet; Ciba-Geigy, Pharmaceuticals, Macclesfield, Ches., Great Britain); Eudragit RL-100 (Polyacrylic and methacrylic acid ester with low content

of quaternary ammonium group, Rohm Pharm, West Germany); Polyvinyl-pyrrolidone (BDH Chemicals Ltd., Poole, England); Tween-80 (Poly-oxy ethylene (80) sorbitan mono oleate, Kochlight Chemical Lab. England). Dibutyl phthalate (Sigma Chemical Co., St. Louis, USA); Liquid Paraffin (Loba Chemie Ind. Co. Bombay), other chemicals and reagents were used as obtained.

B. Drug Permeability Studies

The drug permeability through excised human cadavar skin was determined by placing solution of diclofenac in phosphate saline buffer (PSB) of pH 7.4 which contained 5% v/v methanol into the donar compartment of franz diffusion cell (Crown Glass Co., N.J., USA). The contents of donar and receptor compartment were separated by placing freshly excised cadavar skin in between the two compartments. The skin was mounted in such a way that stratum corneum side of the skin continuously remained in an intimate contact with the content of the donar compartment. The receptor compartment contained phosphate saline buffer of pH 7.4. The sink condition was maintained by using 40% v/v polyethylene glycol 400 in the receptor compartment at $37\pm1^{o}C$.

Samples (0.5 ml) were withdrawn periodically for four hours and assayed for drug content spectrophotometrically (Double Beam Spectrophotometer Shimadzu UV-150-02, Japan) using the method reported by Sterlin et al.(6).

C. Dose Designing

To achieve an effective plasma concentration of diclofenac (2 mcg/h) the required absorption rate through the skin was calculated to be 0.178 mg/h. The absorption rate was calculated using pharmacokinetic parameters by the equation discussed by Sanvordekar et al.(7).

D. Preparation of Pseudolatex

The drug and polymer solution in chloroform containing 10.0% w/w polymer (Eudragit RL-100 alone and with polyvinyl pyrrolidone in different combinations : 9:1, 8:2, 7:3, 6:4) 3.4% w/w drug,

TABLE I.

Composition of Pseudolatex Formulations

Ingredient	Per cent concentration (w/w)				
	Formulation				
	A	B	C	D	E
Diclofenac	3.4	3.4	3.4	3.4	3.4
Eudragit RL-100	10.0	9.0	8.0	7.0	6.0
Polyvinyl Pyrrolidone	0.0	1.0	2.0	3.0	4.0
Liquid Paraffin	2.0	2.0	2.0	2.0	2.0
Dibutylphthalate	4.0	4.0	4.0	4.0	4.0
Tween 80	10.0	10.0	10.0	10.0	10.0

2.0% w/w liquid paraffin and 4.0% w/w dibutylphthalate was emulsified with an aqueous solution of Tween-80 (10% w/w) (Table I). The prepared emulsion was well stirred and kept in a vacuum oven at 45°C for 8-10 hours to evaporate organic solvent in internal phase completely and water of external phase partially (30-40% of originally incorporated).

E. Physical and Chemical Characterization

The physical parameters evaluated include sedimentation height, particle size, viscosity and pH. The samples were allowed to equilibriate with room temperature prior to their evaluation for any of the related parameter.

i) Sedimentation height : The sample were kept in graduated measuring cylinder and the height of sediment were measured periodically and recorded in Table II.

ii) Particle size : The particle size distribution of pseudolatex was measured using an optical light microscope with a stage micrometer (Erma,Tokyo,Japan, 0.01 mm) at X100 magnification at room temperature and recorded in Table III.

TABLE II

Sediment height of Pseudolatices

Formulation.	Sedimentation height (in cm) Time in Weeks 1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3
B	0	0	0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3
C	0	0	0	0.0	0.0	0.0	0.1	0.1	0.2	0.2	0.3	0.3
D	0	0	0	0.0	0.0	0.1	0.1	0.2	0.2	0.3	0.4	0.4
E	0	0	0	0.1	0.1	0.1	0.2	0.2	0.3	0.4	0.4	0.5

TABLE III

Average Particle size (mcm) of Pseudolatices

Formulation	Initial	6th Week	12 week
A	13.464	14.091	16.302
B	14.586	15.213	16.263
C	14.930	18.142	18.260
D	17.782	17.150	20.691
E	19.642	19.800	20.690

TABLE IV

Viscosity of Pseudolatices

Formulation.	Viscosity (Cps) Time in Weeks 0	2	4	6	8	10	12
A	28.5	29.5	30.5	31.0	32.3	33.7	36.3
B	29.3	30.4	32.3	34.9	35.8	37.2	38.5
C	29.8	30.9	31.1	33.2	35.3	36.8	39.2
D	32.1	33.6	33.9	34.7	35.5	38.4	41.8
E	33.7	35.2	36.5	37.5	38.2	39.3	42.4

iii) Viscosity : Following equilibriation of the samples with room temperature the viscosity was determined using a Brook field viscometer (Brook field Eng. Lab., USA) and recorded in Table IV.

iv) pH : pH measurement were made on a digital pH meter (Elico Pvt. Ltd., India). Each product was allowed to equilibriate for 10 min.

F. Determination of Drug Concentration in the Pseudolatex

The drug concentration in the pseudolatex was determined spectrophotometrically. The pseudolatex was dried to a constant weight under vacuum and dissolved in methanol. The absorbance was measured at 276 nm using Shimadzu Spectrophotometer UV 150-02 (6).

G. In vitro drug release

In vitro drug release of drug from pseudolatex preparations was determined to quantify the in vitro availability of drug using Franz diffusion cell. The procedure was same as outlined above for drug skin permeation studies except that treated cellophan membrane was sandwitched between donar and receptor compartment. The preparations were kept in the donar compartment. In this study the receptor solution was completely withdrawn and replaced with the fresh phosphate saline buffer of pH 7.4 (containing 40% v/v Polyethylene glycol 400) at each scheduled sampling time. The diclofenac in the samples was assayed spectrophotometrically at 276 nm (6).

H. In vitro Skin Permeation

In vitro drug permeation through cadavar skin from pseudolatices was studied using Franz diffusion cell. The preparations was placed on the skin mounted in donar compartment of the Franz diffusion cell. The same procedure was adopted as described for drug skin permeation studies.

0.5 ml of sample was withdrawn at each sampling time from the receptor compartment at a regular time interval for 28 hours and assayed for diclofenac content spectrophotometrically at 276 nm (6).

I. In vitro anti-inflammatory activity

Anti-inflammatory studies were performed using a plethysmometer to measure carrageenan induced paw volume following the method of Winter et al.(8).

Twelve Adult male, wistar albino rats 125-165 gm were fasted for 18 hours but had free access to water. Each treatment i.e. plain drug oral administration and transdermal system application was given to the rats of each group (six rats in each group).

Abdominal portion of the rat was cleaned and shaved and transdermal preparation containing 0.139 mg of drug was applied on 1 cm^2 area.

One hour following the treatment 0.05 ml of 1% w/v solution of carrageenan was injected intraplanterly. One hour after the treatment with carrageenan the volume of the hind paw was measured at an hourly intervals for six hours and compared with those recorded for control group (applied transdermal preparation without drug). The percent inhibition in carrageenan induced oedema was calculated (Table VI, Fig 4).

J. In vivo performance

On the basis of the in vitro skin permeation studies the formulation 'C' (Eudragit RL-100:PVP::8:2), that permeated the drug through skin at desired rate i.e. 0.188 mcg/hr was selected for in vivo evaluation.

The in vivo absorption study was carried out on ten male human volunteers (age 22±2 yrs, height 162±3 cm, weight 50±4 kg) who signed the consent forms. Subjects showed normal haemotological and urinary biochemical data. None received medication for

at least 14 days prior to the study. To each subject Voltraro(R)50 (treatment 1) administered orally and pseudolatex transdermal preparation (treatment II) were applied topically at the forearm region at least one week apart following overnight fasting.

Blood samples (5 ml) were collected periodically at the time intervals indicated in Fig 4 via a cannula inserted into the forearm vein. Plasma was separated from the sample and the diclofenac content was determined by gas liquid chromatography method reported by Geiger et al. using a Hewlett-Packard 5710A gas chromatograph (9). Mean plasma levels were computed and drug plasma profiles following treatment I and II was constructed (Fig 4).

RESULTS AND DISCUSSION

The drug permeability through excised cadavar skin determined to be 0.260 mg/h/cm^2 was indicative of good permeant nature of the drug across the skin (Fig 1).

The selection of best pseudolatex preparation was made on the basis physicochemical stability. The various parameters studied include sedimentation height, particle size, viscosity, pH and drug content. It was found that with increasing concentration of hydrophilic polymer in the pseudolatices the sedimentation height was increased (Table II). Particle size of the pseudolatex was studied for 12 weeks. It was noted that the presence of higher proportion of hydrophilic polymer in dispersion phase causes change in particle size on keeping for longer period (Table III). Similarly, the viscosity of the pseudolatices was recorded to increase with increasing concentration of hydrophilic polymer (Table IV). The hydrophilic polymer in latex particle owing an affinity to dispersion vehicle (water) and particles in its vicinity could have acted as structural vehicle and as a result retarded the sedimentation of latex particles. Moreover, rheological characteristics of hydrophilic polymer may be attributed to the increased viscosity with its increasing concentration in polymeric

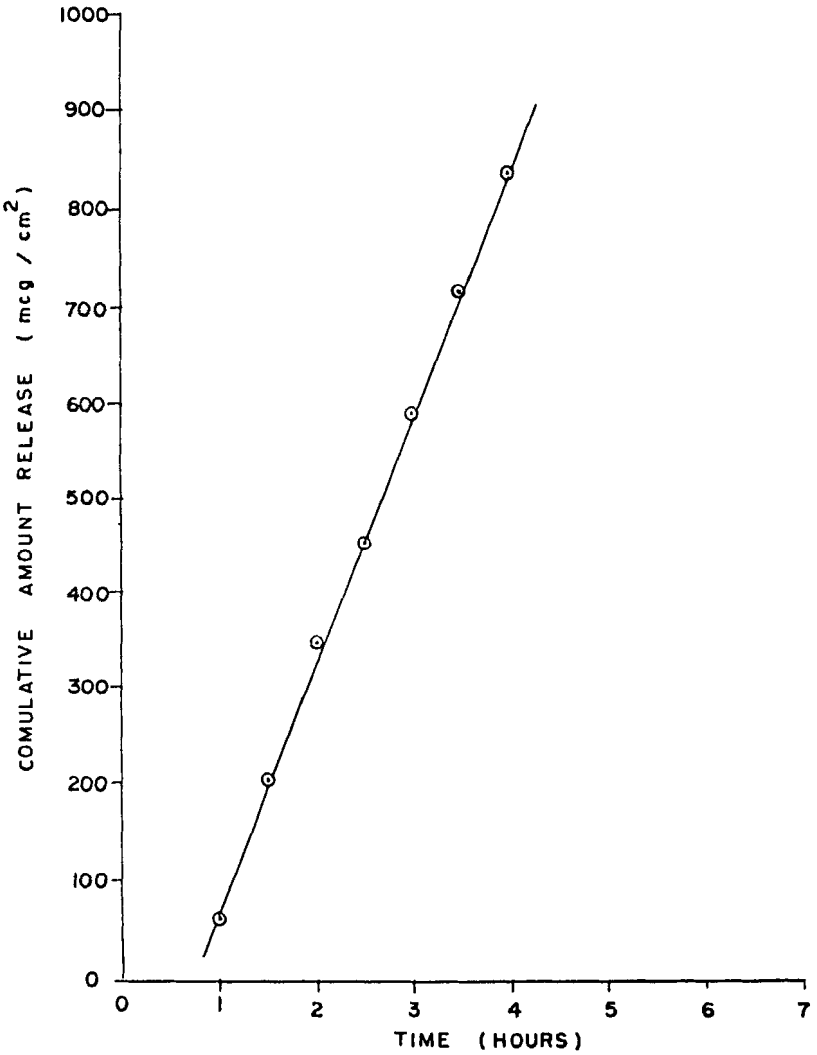

Figure 1 : In vitro drug skin permeation profile of diclofenac through cadavar skin.

mix of dispersed phase (latex particles). With increasing concentration of hydrophilic polymer in latex particles the comparatively increased percent swelling of an individual particles could be accounted for increased particle size. The pH of all the polymeric combination was found to be stable that is indicative of non-ionizing characteristic of the used polymers.

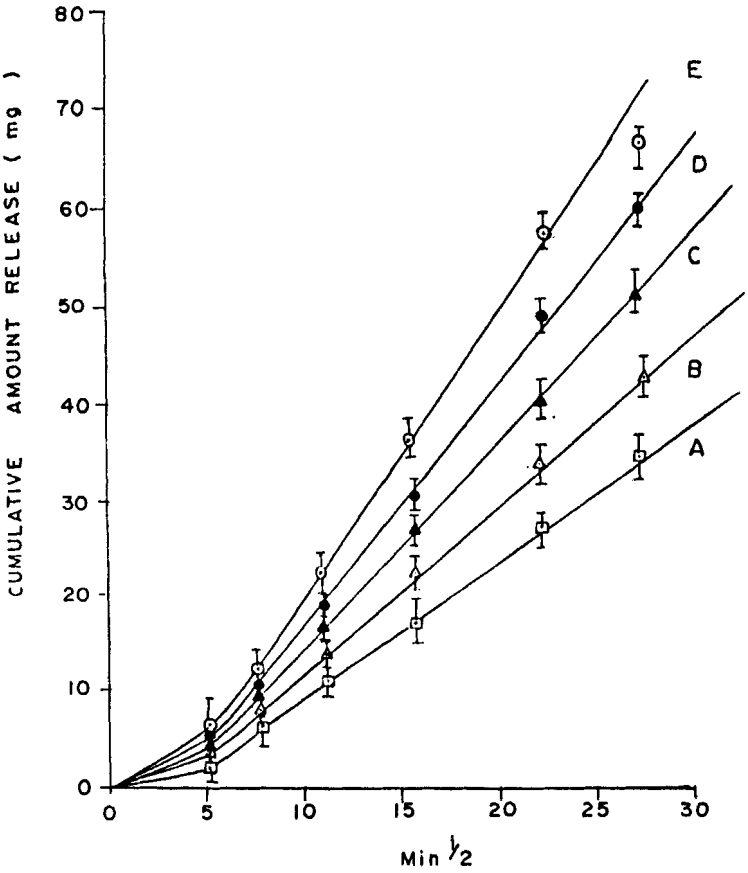

Figure 2 : In vitro drug release rate profile of diclofenac from different pseudolatices (15 cm^2); bars at data points indicates standard deviation (±S.D.).

The pseudolatices were studied for their in vitro drug release. Figure 2 shows that the linear relation could be established between cumulative amount released (Q) V/s square root of time ($t^{0.5}$) indicative of matrix diffusion controlled profile. The slope of the linear portion of the plot was used for the calculation of the release rate constant of diclofenac. It was observed that with the increasing concentration of polyvinyl pyrrolidone from 10 to 40% w/w in the total polymer matrix the in vitro release rate of the drug increased from 0.224 $mg/h/cm^2$

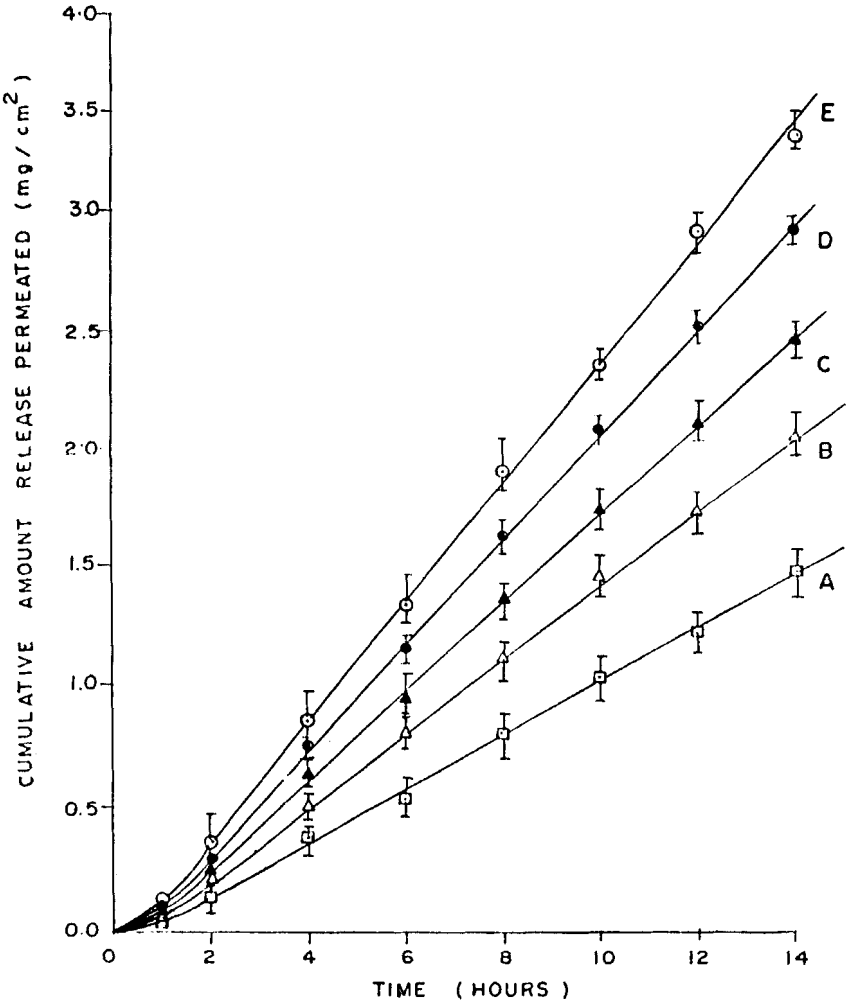

Figure 3: In vitro drug skin permeation rate profile of diclofenac through cadavar skin from different pseudolatices; bars at data points indicates standard deviation (±S.D.).

to 0.464 mg/h/cm^2 (Table V). It is due to high lipophilicity of the drug. The maximum concentration of PVP that could be incorporated in the pseudolatex dispersion was 40% w/w based on total polymer weight. The preparation prepared using EuRL-100:PVP (6:4) released 92% of the incorporated drug in 8 hours. The uniform and fast release recorded in the case of pseudolatices could be

TABLE V

Release rate and Permeability rate constant of different formulations

Formulation	Release rate constant mg/h/cm^2	Permeability rate constant mg/h/cm^2
A	0.224	0.111
B	0.288	0.146
C	0.384	0.188
D	0.416	0.223
E	0.464	0.251

attributed to the uniform distribution of drug at molecular level rather sub crystalline as discussed by Buyukyaylaci et al.(10) and Jain et al.(11).

Product A to E were studied for in vitro skin permeation. Figure 3 indicates that diclofenac penentrated through the cadavar skin following zero-order kinetics. It may observed that as the concentration of hydrophilic polymer content increased from 10 to 40% w/w (based on total polymer weight), the permeation rate also increases from 0.111 mg/h/cm^2 to 0.251 mg/h/cm^2 (Table V). The cumulative amount of drug permeated (Q) across the skin was plotted as a function of time (h). The linear portion were obtained after a lag period of 30-40 minutes. The linear relationship indicated zero-order permeation of drug through the skin. The better skin permeation of pseudolatex could be due to the uniform and molecular dispersion of the drug in the system and possibly due to permeability enhancing effect of surfactant (used in the preparation of pseudolatex) as well (12).

As given in Figure 4 and Table VI formulations A to E shows higher percentage inhibition of oedema as compared to drug given orally (plain drug). The better anti-inflammatory activity recorded with the pseudolatices treatment, could be accounted for controlled drug release and protection of drug from first hepatic pass metabolism i.e. incounter in oral route.

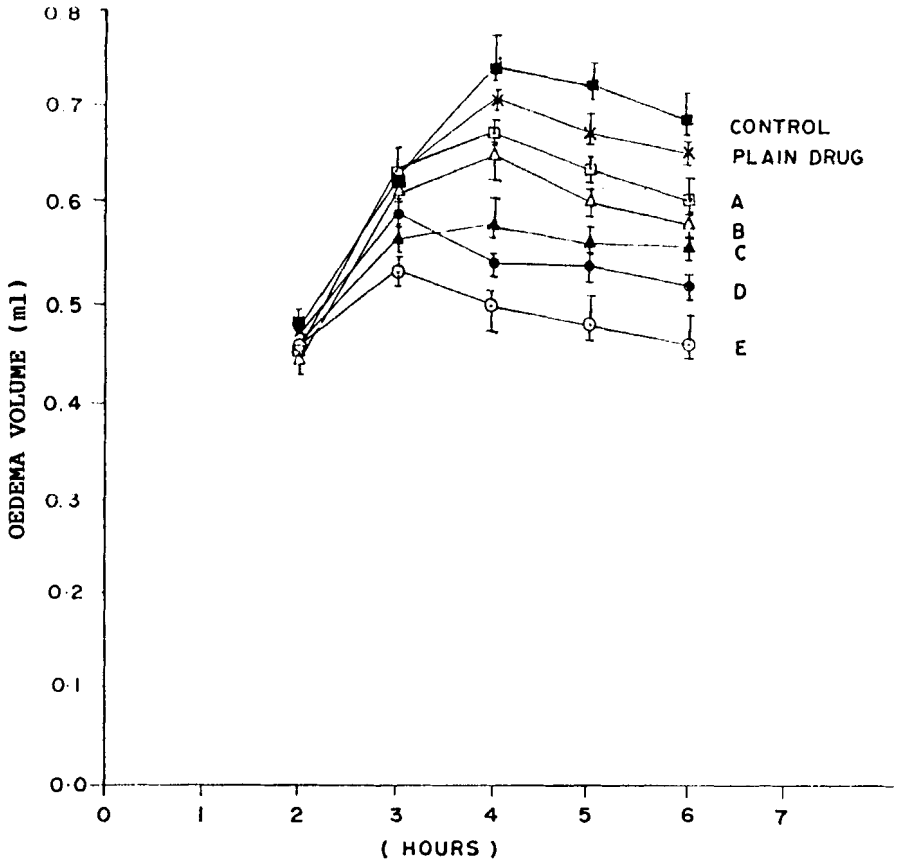

Figure 4 : Effect of pseudolatex preparations on carrageenan treated hind paw volume in rats; bars at data points indicates standard deviation (±S.D.).

TABLE VI

Percent inhibition of Oedema

Formulation	Percent inhibition
Diclofenac (oral)	8.12
A	9.45
B	12.16
C	21.62
D	27.02
E	32.43

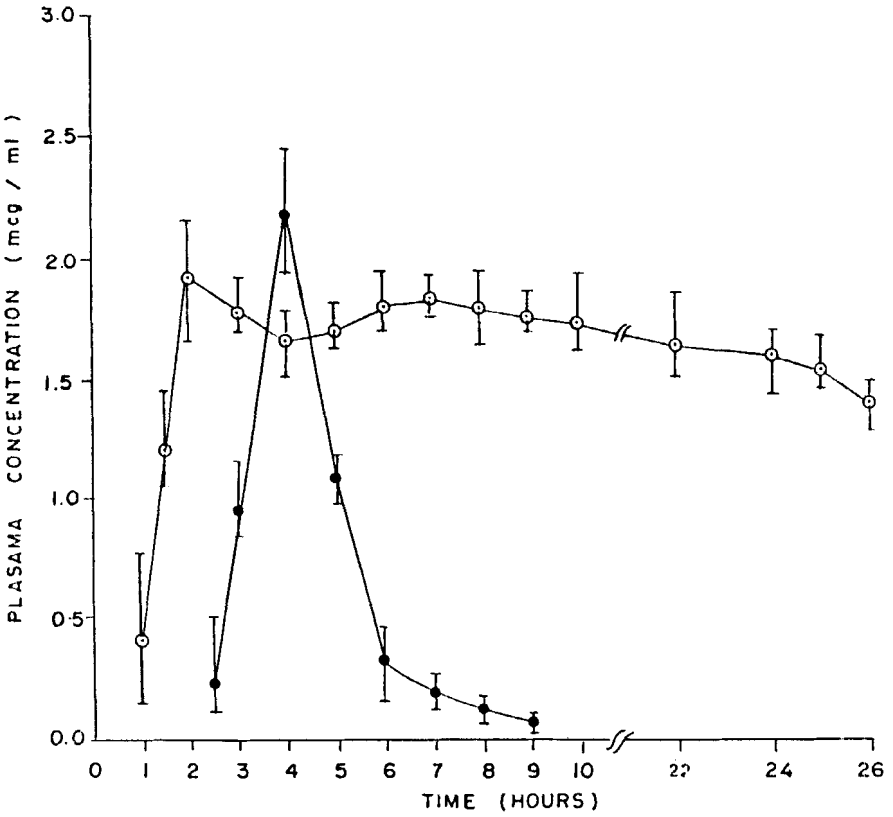

Figure 5 : Mean plasma level of diclofenac following (I) Oral administration of voltarol® 50 tablet ●—● (II) Transdermal preparation application o—o; bars of data points indicates standard deviation (±S.D.).

On the basis of *in vitro* skin permeation studies and anti-inflammatory studies the product C, i.e. Eudragit RL-100:PVP::(8:2) was selected for *in vivo* studies. The *in vivo* performance of the transdermal product (treatment II) was compared with orally administered tablet, Voltarol® (treatment I).

Figure 5 is a plot of the mean plasma concentration of diclofenac as a function of time for treatment I (with voltarol® 50) and treatment II (with pseudolatex transdermal preparation). To examine intersubject variation resulting from either treatment,

TABLE VII

Pharmacokinetic Parameters for each Diclofenac treatments.

Treatment	C_{max} ±S.D. (mcg/ml)	t_{max} ±S.D. (h)	AUC[a] ±S.D. (mcg.h/ml)	t_{lag} ±S.D. (h)
I Voltarol® 50	2.18±0.52	4.0±0.35	4.68±0.5	2.5±0.45
II Pseudolatex transdermal preparation.	1.92±0.50	2.0±0.28	43.02±1.10	1.0±0.24

a area under the curve calculated by trapazoidal rule.

the peak plasma level values were normalized at C_{max} and for all other sampling time the concentration were related to this value as described by Willis et al.(12). Treatment I resulted in a significant inter-subject variation in plasma concentration at different sampling time ($p < 0.05$ student 't' test). This could be due to delayed absorption of the drug from Voltarol® 50, which is enteric coated.

In the case of treatment II (pseudolatex preparation), the inter-subject variation in plasma level was significantly different over the entire time of treatment ($p < 0.05$, student 't' test). The drug reached a peak plasma level within 1 hour after the application. The observed plasma profile following treatment II is indicative of an initial slow absorption followed by a controlled absorption of the drug from the pseudolatex preparation.

Finally, the pharmacokinetic parameters i.e. C_{max}, t_{max}, AUC and t_{lag} were calculated from the plasma drug profile of transdermal treatment and oral administration of Diclofenac (Table VII).

Across the skin absorption of diclofenac following the application of pseudolatex polymeric dispersion of diclofenac could affected at a controlled rate approximating zero-order

kinetics. The plasma level of diclofenac following the topical application could be maintained for 24 hours, however, with significant inter-subject variation. Compared with the Voltarol® 50 treatment, the transdermal preparation of diclofenac resulted in low peak plasma levels and considerably low values of t_{lag} and t_{max}. The performance is indicative of satisfactory therapeutic action of the drug for a prolonged time and possibly better than conventional multiple oral dose which could produce troughs and peaks in drug plasma level particularly between the doses. Thus diclofenac possesses potentiallity for transdermal application and holds promise for clinical evaluation.

CONCLUSION

It is concluded that pseudolatices of diclofenac can successfully be prepared using Eudragit RL-100:PVP::8:2 containing 3.4% w/w drug for transdermal drug delivery. The antiinflammatory activity as well as plasma profile of diclofenac found to be better, controlled and prolonged than orally administered drug.

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REFERENCES

1. Martindale- The Extra Pharmacopoeia, 29th Ed., The Pharmaceutical Press, London, (1989) p. 12.
2. R. Menasse, R. Hodwall, P.R. Kractz, J. Pericin, C. Riester, L. Sallmann, A. Ziel and R. Jaque, Pharmacological properties of diclofenac sodium and its metabolites, **Scand. J. Rheumatol.** Suppl. **22**, 5-16 (1978).

3. J.V. Willis, M.J. Kendall, R.M. Flinn, D.P. Thunhill and P.G. Welling, The Pharmacokinetics of diclofenac sodium following intravenous and oral administration, **Eur. J. Clin. Pharmacol., 16,** 405-410 (1979).

4. T. Nishihata, A. Kamada, K. Saki, K. Takahasi, K. Matsumoto, K. Shinozaki, Y. Tabata, M. Keigami, T. Miyagi and N. Tatsumi, Percutaneous absorption of diclofenac in rats and human : aqueous gel formulation, **Int. J. Pharm., 46,** 1-7 (1988).

5. A. Takamura, F. Ishii, S. Noro and M. Koishi, Physico-pharmaceutical characteristics of an oil in water emulsion-type ointment containing diclofenac sodium, **J. Pharm. Sci., 73** 676-681 (1984).

6. H. Stierlin and J.W. Faigle, Biotransformation of diclofenac sodium (Voltarol®) in animal and man, **Xenobiotica, 9,** 601-610 (1979).

7. D.R. Samvordekar, J.G. Cooney and R.C. Wester, Transdermal nitroglycerine pad. U.S. Patent No.4, 336, 243, June 22, 1982.

8. C.A. Winter, E.A. Risley and G.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for inflammatory drugs, **Proc. Soc. Exp. Biol. Med., 162,** 544 (1963).

9. U.P. Geiger, P.H. Degen and A. Siofli, Quantitative assay of diclofenac in biological material by gas liquid chromatography, **J. Chromatogr., 111,** 293-298 (1975).

10. S. Buyukyaylaci, Y.M. Joshi, G.E. Peck and G.S. Banker in "Recent Advances in Drug Delivery Systems" (James M. Anderson and Sung Wan Kim, Eds), Plenum Press, New York and London, 291-307 (1984).

11. S.K. Jain, S.P. Vyas and V.K. Dixit, Effective and Controlled transdermal delivery of ephedrine, **J. Contr. Rel.** (In press).

12. K.A. Walter, O. Olejinik, and S. Harris, Influence of non-ionic surfactant on the permeability of ionized molecules through hairless mouse skin, **J. Pharm. Pharmacol., 36,** 78 (1984).

13. J.V. Willis, M.J. Kendall and D.B. Jack, The influence of food on the absorption of diclofenac after single and multiple oral dose, **Eur. J. Clin. Pharmacol., 19**, 33-37 (1981).