Research Article

PREPARATION AND EVALUATION OF MONOLITHIC TRANSDERMAL THERAPEUTIC SYSTEMS CONTAINING FENOPROFEN FOR THE TREATMENT OF ARTHRITIS

Manjula D*[1], Shabaraya AR[2], Somashekar Shyale[3], Josephine Leno Jenita[1]

1. Dayananda Sagar College of Pharmacy, Bangalore, Karnataka, India.
2. Srinivas College of Pharmacy, Mangalore, Karnataka, India.
3. V.L. College of Pharmacy, Raichur, Karnataka, India.

***Corresponding author's E-mail:** manjulad21@rediffmail.com

Accepted on: 02-02-2012; **Finalized on:** 20-03-2012.

ABSTRACT

Monolithic transdermal films of Fenoprofen were prepared to avoid hepatic first pass effect by using hydroxy propyl methyl cellulose (HPMC) and ethyl cellulose (EC) by solvent casting method. Dibutyl phthalate (DBP) was used as plasticizer. D-limonene and oleic acid were used as permeation enhancers. The formulations were evaluated for physical appearance, thickness uniformity, weight uniformity, drug content uniformity and water vapour transmission rate. In further study the effect of permeation enhancers on *in vitro* drug release from the films was studied by using Keshary-Chein diffusion cell. Permeation parameters like diffusion rate, permeability coefficient, flux, enhancement ratio and permeability rate were determined. The films containing HPMC showed more permeation than films with EC. The monolithic film made up of HPMC with 10%w/w d-limonene showed better *in vitro* permeation through rat skin. *In vitro* permeation of fenoprofen from the films was diffusion controlled and followed zero order kinetics. The transdermal film that showed better permeation was subjected for *in vivo* studies and it showed anti-inflammatory and analgesic activity statistically significant at $P<0.05$. It was concluded that the above transdermal drug delivery system could be useful to treat chronic pain and inflammation in arthritis.

Keywords: Fenoprofen, Monolithic transdermal film, Permeation enhancer, *In vivo* studies.

INTRODUCTION

Arthritis is a major syndrome affecting majority of the geriatric patients and generally NSAID'S are advised to reduce pain and inflammation[1]. The medications prescribed for the relief of inflammation and associated pain are available as conventional dosages like tablets and capsules. The conventional medications cause GI disturbances and drug level fluctuates considerable making the patient to suffer or the patient is burdened with over-dosage[2]. To minimize the GI disturbances and to improve the bioavailability of the drug certain novel dosage forms are being investigated. In the past two decades there has been a commendable growth both in size and to improve bioavailability and patient compliance. Transdermal drug delivery systems (TDDS) are designed to support the passage of drug substances from the surface of skin, through its various layers, into the systemic circulation. Their advantages over conventional dosage forms include improved patient compliance, avoidance of gastric irritation and first-pass effect and controlled therapeutic responses[3].

Fenoprofen, is a non-steroidal drug used as anti-inflammatory, analgesic and antipyretic. It is used in the treatment of osteoarthritis, rheumatoid arthritis and ankylosing spondilytis[4]. Though it is rapidly being absorbed after oral administration, it undergoes significant first-pass metabolism. It has a very short half life of about 2-3 h and is associated with gastro-intestinal side effects like nausea, gastric irritation etc. To extend drug action, to improve delivery of drug into systemic circulation, the present study was undertaken with the aim to develop and evaluate transdermal films of fenoprofen using various polymers like HPMC and EC, plasticizer like DBP[5]. Further the *in vitro* drug release was studied using d-limonene and oleic acid as permeation enhancers which belong to diterpene and fatty acids respectively. The formulation that showed promising result was further subjected for *in vivo* studies.

MATERIALS AND METHODS

Chemicals and polymers

Fenoprofen was procured from D. K. Pharma, Mumbai, HPMC E-15 (Supra labs), EC (Himedia labs), d-limonene (Rolexchemical industries), Oleic acid (Qualigens fine chemicals) and all chemicals obtained were of analytical grade. The institutional animal ethical committee approved the experimental protocol bearing registration number 557/02/C/CPCSEA under its strict compliance *In vitro* and *in vivo* experiments were carried out.

Preformulation Studies of the drug

The solubility[6] of the selected drug was determined in distilled water, ethanol and phosphate buffer of pH 7.4, according to the method proposed by Diez *et al.* The partition coefficient[7] was performed using n-octanol as oil phase and phosphate buffer pH 7.4 as aqueous phase. Possible drug and polymer interaction was studied using FT-IR[8] (Perkin-elmer 1600 series USA), at 400cm^{-1} to 4000 cm^{-1} and DSC[9] thermograms using Perkin-elmer 6 DSC (Perkin Elmer 1600 series USA). The heating rate selected was from 50°C to 300°C at an increase of 10°C per minute.

Preparation of Monolithic Transdermal Films

Transdermal films were prepared by solvent casting method using mercury substrate according to the method of Mundane *et.al*[10]. In which the required amount of drug was dissolved in ethanol and other solvents were added to it (table-1). Dibutyl phthalate used as plasticizer and stirred well to get a homogeneous solution and poured into glass rings placed on mercury surface, dried for 24h and rate of evaporation was controlled by inverting a funnel over the petridish.

Table 1: The detail formulae of different monolithic transdermal systems containing Fenoprofen

Ingredients	Formulation codes (mg/ml)					
	F1	F2	F3	F4	F5	F6
Fenoprofen	50	50	50	50	50	50
HPMC	200	200	200	-	-	-
EC	-	-	-	200	200	200
Dibutyl phthalate (35%w/w of polymer)	-	-	-	0.0575	0.0575	0.0575
Glycerine	0.0585	0.0585	0.0585	-	-	-
d-limonene (10% w/w of drug)	-	0.0029	-	-	0.0029	-
Oleic acid (20 % w/w of drug)	-	-	0.0111	-	-	0.0111
Alcohol	2	2	2	2	2	2
Dichloromethane	1	1	1	1	1	1
Chloroform	2	2	2	2	2	2

Thickness and weight uniformity test

The thickness[11] of the films were measured at 5 different points of the film by using screw gauze (Mitutoyo, Japan).

Water Vapour Transmission (WVT)[12] Rate

Glass vials of equal diameter were used as transmission cells. These cells were washed and dried in an oven. About 1gm of fused calcium chloride was taken in the cells and patch of area equivalent to brim of vial (1.36 cm^2) was fixed with the help of an adhesive. The cells were weighed accurately and kept in a close desiccator containing saturated solution of potassium bromide (200ml). The humidity inside the desiccator was measured by a hygrometer and it was found to be 84% RH. The cells were taken out and weighed after 1, 2, 3, 4, 5, 6 and 7 days of storage. The WVT was calculated by taking the difference in the weight of the patches before and at regular intervals of 24 hr for a total period of seven days.

Drug Content Uniformity[13]

Transdermal films of 1sq.cm area was cut into small piece and transferred into 100ml volumetric flask. 25ml of methanol was added and shaken for 4hr to extract the drug. Finally, suitable dilutions were made using phosphate buffer pH 7.4 and absorbance was measured at 272nm.

In vitro permeation across the rat abdominal skin

Preparation of the skin[14]

The swiss albino rats were sacrificed by decapituation. The fresh abdominal skin was excised from swiss albino rats weighing 170-190gm. The abdominal skin of excised hairless rat skin was separated along the epidermal junction and was kept under a steam of 60°C water for exactly 50 seconds. The heat-treated skin was cleared of its subcutaneous fatty substances and immediately kept in normal saline solution to flatten and smooth. This step maintains the integrity and viability of the skin.

Permeation studies

Vertically assembled Keshary-Chien[15] diffusion cells having downstream volume of 50ml were used. The above skin was mounted on the diffusion cell and receiver compartment was filled with 50ml phosphate buffer of pH 7.4 and the temperature was maintained at 37°C. The samples were withdrawn every hour (replaced with 1ml fresh buffer to maintain sink condition) and their concentrations were measured in UV-spectrophotometer at 272nm. The permeation studies were carried out up to 24hr.

Data and statistical analysis

The Fenoprofen concentration was corrected for sampling errors by using equation: C'n = Cn (Vt / Vt-Vs) (C'n-1 / C n-1)[16]. The cumulative amounts of Fenoprofen released and permeated per unit area (µg/cm^2) were plotted against time (h) and the slope of the linear portion of plot was estimated as steady state flux (µg/cm2/h)[17]. Data was expressed as mean ± SEM. Statistical evaluation was performed by one-way analysis of variance. When a statistically significant difference ($P<0.05$) was observed, student t-test was performed to evaluate statistical differences between individual means.

In Vivo Studies: Hypersensitivity Study[18]

The studies were carried out by patch testing method on Swiss albino rats. The animals were kept under observation to check flushing, papules, wheals, erythema and oedema for seven days.

Anti-Inflammatory Activity[19]

It was determined by formalin induced paw oedema method in albino rats (170-200g) using mercury plethysmograph. The paw volume of control and test groups were measured by using plethysmograph at selected interval of time up to 24h and percentage reduction in oedema volume was calculated.

Analgesic Activity[20]

This was determined by acetic acid induced writhing method using albino mice. The writhings produced by mice were observed for 15 min. The activity of the formulation was statistically analyzed by student "t" test.

Stability Studies[21]

For all the monolithic systems (F1-F6) the stability studies was conducted for four weeks at temperature 37°C and 45°C at 65% RH.

RESULTS AND DISCUSSION

The solubility of drug in water, ethanol and phosphate buffer pH7.4 was 8.3 mg/mL, 15.3mg/mL and 11.5 mg/mL respectively. The melting point, partition coefficient were found to be 118 ^{0} C and 5.33 respectively. The λmax of the selected drug fenoprofen was found to be 272 nm. The IR spectra of pure drug and its physical mixtures with polymers (HPMC and EC) has showed replication of characteristic peaks of N-H stretch, C-H aromatic stretch, NO_2 stretch, C-O-C stretch, C-H aliphatic stretch and OH stretch respectively as in IR of pure drug revealed no interaction between drug and polymers hence indicated their compatibility. Further DSC studies were also carried out to check drug polymer interactions, shows DSC thermograms of drug alone, drug with polymer (HPMC) showed endothermic peaks at 98.349°C and 101.847°C respectively. There is no appearance of new peak, no change in peak shape which showed compatibility of the fenoprofen with polymer.

A total of 6 formulations were prepared as per formulae given in table-1. All the films were evaluated for physical parameters and results given in table-2. Films were found to be flexible, smooth and transparent. The drug content (DC) analysis of the films has showed that the process employed to prepare films was capable of giving uniform DC and minimum batch variability. The formulations with HPMC showed good WVT than EC films (table-2), the reason may be attributed to hygroscopic nature of HPMC.

Table 2: Physical parameters, Drug content, WVT rate constant of Transdermal systems

Formulation codes	Physical appearance	Weight (mg)	Thickness (µm)	Drug content (mg)	WVT rate constant (mg/24hr/cm^2)
F1	++	272.2 ± 0.49	0.167 ± 0.0067	98.5 ± 0.25	3.307x10^{-2}
F2	++	275.1 ± 0.32	0.147 ± 0.0044	99.1 ± 0.35	4.137x10^{-2}
F3	++	279.5 ± 0.35	0.129 ± 0.0022	98.9 ± 0.37	3.900x10^{-2}
F4	++	291.7 ± 0.42	0.149 ± 0.0044	97.2 ± 0.27	2.600x10^{-2}
F5	++	315.1 ± 0.34	0.149 ± 0.0102	98.1 ± 0.36	2.820x10^{-2}
F6	++	325.1 ± 0.44	0.167 ± 0.0067	97.8 ± 0.41	2.671x10^{-2}

Table 3: Diffusion rate, Permeability co-efficient, flux, Enhancement ratio and Permeability rate of various transdermal films

Formulation codes	Permeability co-efficient mg.mm/hr	Flux mg/cm^2/hr	Enhancement ratio	Diffusion rate constant mg/hr/cm^2
F1	2.56x10^{-2}	6.13x10^{-2}	-	0.230
F2	4,001x10^{-2}	9.52x10^{-2}	1.55	0.358
F3	3.632x10^{-2}	8.68x10^{-2}	1.45	0.326
F4	1.927x10^{-2}	4.59x10^{-2}	-	0.173
F5	3.402x10^{-2}	8.10x10^{-2}	1.32	0.305
F6	3.326x10^{-2}	7.77x10^{-2}	1.26	0.292

The *in-vitro* drug release profiles from HPMC and EC (F1 and F4) were 57.66% and 43.19% respectively, results indicated that HPMC films showed better release than EC films, which may be attributed to the reason of high water vapour permeability of HPMC films than EC films (figure-1). The *in vitro* release from F1 and F2 formulations showed moderate drug permeation through rat skin which might be attributed due to tough barrier, the stratum corneum of skin contributes to low diffusivity. To overcome the problem and to improve flux, diffusion rate of drug through rat skin, d-limonene and oleic acid were incorporated as permeation enhancers in the HPMC and EC films since they showed better release. Among various concentrations 10% w/w of d-limonene (F2, F5) and 20%w/w of oleic acid (F3, F6) showed good release 89.65%, 76.23% (F2, F5) and 81.71% , 73.71% respectively (figure-2). The reason for increased drug release by d-limonene might be due to partial extraction of skin lipids from the stratum corneum, which in turn decreases the barrier property of stratum corneum. Various permeation parameters were calculated and results given in table-3.

The *in vitro* drug release profiles of all monolithic systems were fairly linear with their Correlation coefficients of 0.8980 to 0.9995. The results confirmed that, all the systems followed zero order kinetics, which was desired for controlled delivery of drugs. The Higuchi's plots were linear with their correlation coefficients of 0.9352 to 0.9617. Hence the results showed that, the mechanism of drug release from all the monolithic systems was diffusion mediated.

Among all the formulations F1 to F6, the F2 formulation showed good flux and was selected for *in vivo* studies.

The animals subjected for hypersensitivity studies did not show any signs of erythema, oedema, flushing and papules during 7 days study. Anti-inflammatory activity of F2 formulation showed 64% reduction of formalin induced paw oedema at the end of 24 h. The data was analyzed by using P-STAT package where F2 showed a significant anti-inflammatory activity at $p < 0.05$. Same formulation F2 was subjected for analgesic activity by acetic acid induced writhing method showed analgesic activity for 24 h. The data analyzed with P-STAT package was found to be significant at $p<0.05$ and 0.001. A bar graph of number of writhes per 15mins plotted against time as shown in figure-3. Stability studies of all the films showed no significant change in their physical appearance and drug content parameters.

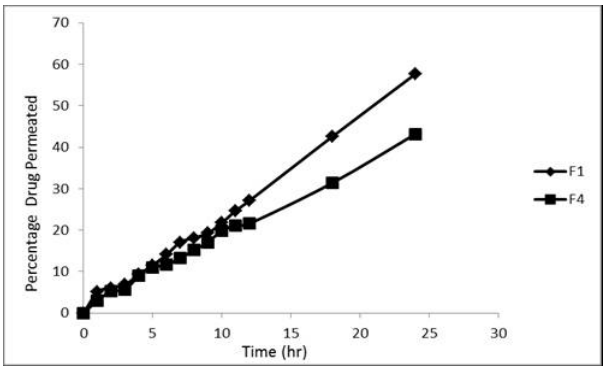

Figure 1: *In Vitro* Drug Release from Formulations F1 and F4

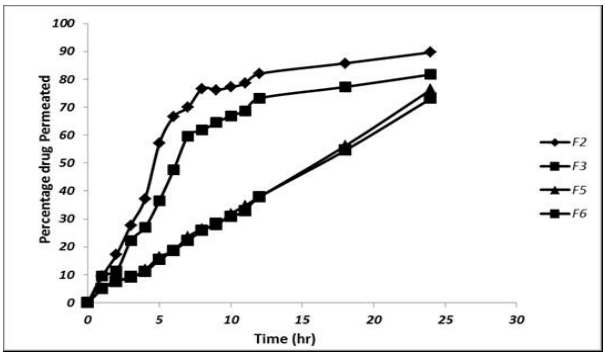

Figure 2: *In Vitro* Drug Release from F2, F3, F5 and F6

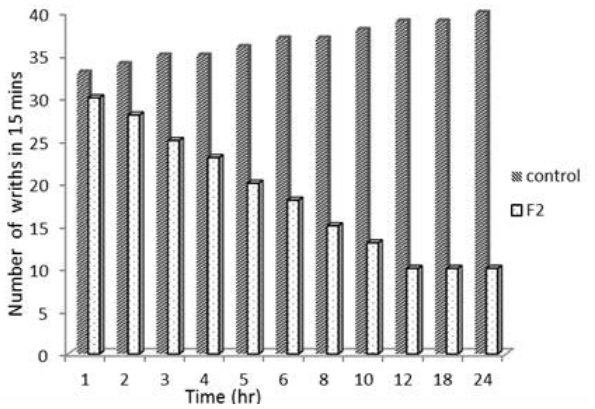

Figure 3: Analgesic Activity of the Selected Formulation F2

CONCLUSION

The fabricated polymeric films containing fenoprofen had shown uniform thickness, folding endurance, weight variation and drug content. *In vitro* skin permeation profile of the films was fairly uniform. Among two permeation enhancers d-limonene showed significant enhancement of drug permeation than oleic acid. The results of the study indicates the feasibility of formulating rate controlled transdermal therapeutic systems of fenoprofen for effective management of chronic pain, inflammation associated with rheumatoid and osteoarthritis conditions.

Acknowledgement: The authors are thankful to Vision group on science and technology for the grants provided to carry out this project and also thankful to management Dayananda Sagar College of Pharmacy, Bangalore, V.L.College of Pharmacy, Raichur.

REFERENCES

1. Robert L Jackson, Marrow D Jason. Analgesic-antipyretic, anti-inflammatory agents and drugs used in the treatment of gout, 10th Edn., Mcg raw Hill publication., New York, 2003.
2. Chien Yie W. Concepts and system design for rate controlled drug delivery, 2nd Edn, Marcel Dekker Inc, 1992.
3. Chien YW, In; Robinson, J.R and Lee, V.H.Controlled drug Delivery: Fundamentals and Applications, 2nd Edn., Marcel Dekker Inc., Newyork, 1987; 523 - 524.
4. Alfonso RG, Remington: The Science and Practice of Pharmacy, 19th Edn, Mac publishing Company, Pennsylvaia, 1985.
5. Pandey S, Praveen SH and Udupa N: Formulation and evaluation of Nimesulide Transdermal drug delivery systems. Indian J. Pharm. Sci. 62(5): 2000; 376-378.
6. Robert V Smith, James T Stewart. Procurement and characterization of standard reference materials. 4th Edn. Philadelphia, 1981.
7. Priyanka Arora, Biswajit Mukherjee. Design, development, physicochemical and *in vitro, in vivo* evaluation of transdermal patches containing diclofenac diethylammonium salt. J. Pharm. Sci. 91(9): 2002; 2078-88.
8. Fregany A, Mohammed, Hussin Khedr. Preparation and in vitro/in vivo evaluation of the buccal adhesive properties of slow-release tablets containing miconazole nitrate. Drug. Dev. Ind. Pharm. 29(3): 2003; 321-37.
9. Rama Rao N and Sudhakar Rao G: Compatibility study between pregelatinized starch as excipients and various commonly used drugs using differential scanning calorimetry. Int. J. Pharm. Excip. 1(3): 2002; 103-106.
10. Munden BJ, Dekay HG and Banker GS: Evaluation of polymeric materials and screening of film coating agent. J. Pharm. Sci. 53: 1964; 395-401.
11. Raghavendra K, Doddayya H, Marshal SC, Patil CC and Habbu PV: Comparative evaluation of polymeric films for transdermal application. The Eastern Pharmacist. XLIII (516): 2000; 109-111.

12. Kusum Devi V, Saisivam S, Maria GR and Deepti PU: Design and evaluation of matrix diffusion controlled transdermal patches of Verapamil hydrochloride. Drug. Dev. Ind. Pharm. 29(5): 2003; 495-503.

13. Kusum Devi V, Saisivam S, Maria GR and Deepti PU: Design and evaluation of matrix diffusion controlled transdermal patches of Verapamil hydrochloride. Drug. Dev. Ind. Pharm. 29(5):2003; 495-503.

14. Flynn GL, Durrheim H and Huguchi WI: Permeation through hairless mouse skin II: membrane sectioning techniques and influence on alkanol permeabilities. J. Pharm.Sci., 70(1): 1981; 52-56.

15. Chien YW., Systemic drug delivery of pharmacologically active molecule across the skin. New York; 1991;100 - 102.

16. Hayton W.L. and Chen T. J. Pharm. Sci. 71: 1982; 820-837.

17. Julraht K., Keith A.P. and James A.W. Drug Develop. Ind. Pharm. 21: 1995; 1377 - 1386.

18. Narasimha Murthy S, Hamsa V and Shyamala Bhaskaran: Formulation and evaluation of transdermal films of terbutalin sulphate. Indian J. Pharm. Sci. 57(5): 1995; 207-209.

19. Loganathan V, Senthilkumar B, Reddy MVS, Sreekanth N and Ubaidulla U: Compatibility studies between ofloxacin and tablet excipients through differential scanning calorimetry. Int. J. Pharm. Excip. 2(4): 2003; 661-664.

20. Ghosh MN. Statistical Analysis: 2 Edn. Calcutta: Scientific Book Agency; 1984; 66 -75.

21. Sridhar SK, Sreenivasulu M, Somasundaram, Ramachandran, Kishor Gnanshyam S and Mundiyandi Saravanan: Synthesis, analgesic and anti-inflammatory activity of N-mannich bases of (41-substituted)-2-phenylindoles. Indian Drugs. 38(10): 2001; 531-534.

22. H.Gerhard Vogel and Wolfgang H.Vogel: Drug discovery and evaluation-Pharmacological Assay. Springer-verlag Berlin Heidelberg, Germany: 1997; 716-761.

23. Wolfgang Grinn. Extension of international conference on harmonization tripartite guideline for stability testing of new drug substances and products to countries of climate zones III and IV. Drug. Dev. Ind. Pharm. 24(4): 1998; 313-325.

****