Research Article

PREPARATION OF MONOLITHIC TRANSDERMAL DRUG DELIVERY SYSTEM FOR ARTHRITIS TREATMENT AND EFFECT OF PERMEATION ENHANCERS ON RELEASE KINETICS

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ABSTRACT

Monolithic transdermal films of Nimesulide (NIM) were prepared to avoid hepatic first pass effect by using hydroxy propyl methyl cellulose (HPMC), ethyl cellulose (EC) alone and in combination with co-polymer poly vinyl pyrollidone (PVP) 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 co-polymer and permeation enhancers effect 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 PVP showed more permeation than films with HPMC and EC alone. The monolithic film made up of HPMC and PVP with 10%w/w d-limonene showed better in vitro permeation through rat skin. *In vitro* permeation of NIM from the films was diffusion controlled and followed zero order kinetics. The transdermal film 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: Nimesulide, Monolithic transdermal film, permeation enhancer, *In vivo* studies.

INTRODUCTION

Transdermal therapeutic systems (TTS) are defined as self-contained discrete dosage forms when applied to the intact skin, deliver the drugs through skin at a controlled rate to the systemic circulation for the input of drugs to maintain prolonged plasma drug levels. Transdermal drug delivery avoids hepatic first pass metabolism, potentially decreases side effects and improves patient compliance[1].

NIM is an effective non-steroidal drug used as anti-inflammatory, analgesic and antipyretic[2, 3]. It is used in the treatment of osteoarthritis and rheumatoid arthritis. Though rapidly absorbed following oral administration, it undergoes significant first-pass metabolism. Its half-life is about 1.8 to 4.73 h. Hepatitis and other gastrointestinal side effects like Epigastralgia, heartburn were reported[4]. Nimesulide is generally well tolerated in short-term treatments, but for long-term treatments which require higher doses (200mg/d) as in osteoarthritis, the incidence of adverse effects was greater. To minimize adverse effects, to extend drug action, to improve delivery of drug in to systemic circulation, the present investigation was under taken with aim to develop and evaluate transdermal films of Nimesulide using various polymers like HPMC, EC and PVP as co-polymer, Plasticizer like DBP [5]. Further the *in vitro* drug release was investigated under the influence of diterpene d-limonene and fatty acid oleic acid as permeation enhancers. The formulation showed promising results was further subjected for *in vivo* studies.

MATERIALS AND METHODS

Chemicals and polymers: Nimesulide was procured from Apex drugs and intermediates, Hyderabad, HPMC E-15 (Supra labs), EC (Himedia labs), d-limonene (Rolex chemical industries), Oleic acid (Qualigens fine chemicals) and all chemicals obtained were of analytical grade. The university 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, phosphate buffer of pH 7.4, methanol and methanol: phosphate buffer of pH 7.4 (1:1). The partition co-efficient[7] was performed using n-octanol as oil phase and phosphate buffer pH 7.4 as aqueous phase. FTIR[8] by using FT-IR (Perkin-elmer 1600 series USA), DSC[9] thermograms using Perkin-elmer 6 DSC (Perkin Elmer 1600 series USA) at 400cm^{-1} to 4000 cm^{-1} range obtained to know the possible interaction of drug and polymer.

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] 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 petridish.

Table 1: Formulation Table of various monolithic transdermal drug delivery systems

Formulation Ingredients	Formulation Codes								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Nimesulide (mg)	50	50	50	50	50	50	50	50	50
HPMC K4M (mg)	150	137.5	125	112.5	100	-	-	100	100
Ethyl cellulose (mg)	-	-	-	-	-	150	137.5	-	-
Poly vinyl Pyrrolidone(mg)		12.5	25	37.5	50	-	12.5	50	50
Di butyl pthalate (ml)	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
(35%w/w of polymer)	-	-	-	-	-	-	-	-	-
d-limonene (ml)	-	-	-	-	-	-	-	0.0059	-
(10%w/w of drug)	-	-	-	-	-	-	-	-	-
oleic acid (ml)	-	-	-	-	-	-	-	-	0.0111
(20%w/w of drug)	-	-	-	-	-	-	-	-	-
Ethyl alcohol (ml)	2	2	2	2	2	2	2	2	2
Dichloromethane (ml)	1	1	1	1	1	1	1	1	1
Chloroform (ml)	2	2	2	2	2	2	2	2	2

Thickness and weight uniformity test: The thickness[11] of the films was measured at 5 different points of the film by using screw gauze (Mitutoyo, Japan.).

Water Vapour Absorption (WVA) Rate[12]: The films of 3.14 cm^2 area were weighed and placed in a desiccator at 84% RH by using saturated potassium bromide solution. The films were taken out and weighed after 1, 2, 3, 4, 5, 6 and 7 days of storage.

Water Vapour Transmission (WVT) Rate[13]: The WVT rate was calculated by using the following formula WVT = WL / S Where W is water vapour transmitted in g, L is thickness of film in cm and S is exposed surface area in cm^2.

Drug Content Uniformity[14]: Transdermal film of 1cm^2 areas was cut and transferred into a 25 ml volumetric flask containing 25 ml methanol and shaken for 4 h to extract the drug. The filtrate was diluted suitably and analyzed at 339.5 nm on UV spectrophotometer (double beam) Hitachi U.V. 2000.

***In Vitro* skin permeation study:** In vitro release studies were performed in Keshary-Chien[15] diffusion cell with downstream value of 50ml. The release studies were performed using rat abdominal skin which was prepared according to Flynn etal[16] method. The rat abdominal skin was mounted onto Keshary-Chein diffusion cell in such a way that stratum corneum side of the skin continuously remained in intimate contact with the transdermal film in donor compartment and the dermis side was in constant contact with the receptor solution methanol: phosphate buffer of pH 7.4 (1:1) at 37±1^0, being stirred magnetically. The samples were withdrawn (1ml each time) over a period of 24h and were analyzed at 339.5nm.

Data and statistical analysis: The NIM concentration was corrected for sampling errors by using equation: $C'_n = C_n (V_t / V_t - V_s) (C'_{n-1} / C_{n-1})$ [17] The cumulative amounts of NIM 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/cm^2/h)[18]. Data were 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[19]:** 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[20,21]: 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[22,23]: 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[24]: For all the monolithic systems (F1-F9) were conducted for four weeks at temperature 37^0 and 45^0 at 65% RH.

RESULTS AND DISCUSSION

The solubility of drug in water, buffer pH7.4, methanol and methanol: buffer pH 7.4 (1:1) was 0.150mg/ml, 0.217mg/ml, 11.901mg/ml and 1.776mg/ml respectively. The melting point, partition coefficient and permeability coefficient were 150^{0}C, 6.064 and -4.025 respectively. The λ_{max} of the selected drug nimesulide was found to be 339.5 nm. The IR spectra of pure drug and its physical mixtures of drug with polymers and co-polymer (HPMC+PVP and EC+PVP) of Spectra of pure drug with polymers and co-polymer 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 and co-polymer (HPMC+PVP) showed endothermic peaks at 152.35º and 150.37º, peak onset at 149.60º and 148.54º respectively. There is no appearance of new peak, no change in peak shape which showed compatibility of NIM with polymers and co-polymer.

A total of 9 formulations were prepared as per formulae given in table-1. All the films were evaluated for physical parameters results was given in table-2 and films were flexible, smooth and yellowish in colour. 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+PVP showed good WVT than EC+PVP films table-2. WVT increased with increased PVP content in the film due to hygroscopic nature of PVP.[25]

Table 2: Physical Parameters, Drug Content, WVA and WVT Rate Constants of transdermal systems

Formulation codes	Physical appearance	Weight (mg)	Thickness (µm)	Drug content	WVA rate constant (gm/24h/ cm^2)	WVT rate constant (gm/24h/cm^2)
F1	++	182.57 ± 0.28	88.57 ± 0.40	97.36 ± 0.28	---	---
F2	++	182.99 ± 0.35	92.88 ± 0.20	97.58 ± 0.32	1.89×10^{-4}	4.13×10^{-3}
F3	++	182.70 ± 0.37	96.46 ± 0.28	98.30 ± 0.23	1.90×10^{-4}	4.58×10^{-3}
F4	++	183.63 ± 0.42	101.74 ± 0.2	97.83 ± 0.34	1.95×10^{-4}	4.61×10^{-3}
F5	++	184.32 ± 0.29	106.49 ± 0.3	98.77 ± 0.30	1.97×10^{-4}	4.71×10^{-3}
F6	++	183.71 ± 0.40	91.02 ± 0.27	97.41 ± 0.27	1.99×10^{-4}	5.01×10^{-3}
F7	++	184.16 ± 0.30	95.52 ± 0.32	97.12 ± 0.44	1.13×10^{-4}	2.66×10^{-3}
F8	++	184.44 ± 0.32	106.53 ± 0.2	98.46 ± 0.36	1.16×10^{-4}	2.88×10^{-3}
F9	++	183.48 ± 0.37	106.78 ± 0.3	96.82 ± 0.29	2.11×10^{-4}	5.16×10^{-3}
F10	++	185.04 ± 0.31	106.53 ± 0.3	98.79 ± 0.24	2.05×10^{-4}	5.09×10^{-3}

Table 3: Diffusion rate, permeability coefficient, flux, enhancement ratio and permeability rate of transdermal systems

Formulation codes	Diffusion rate mg/h	Permeability coefficient cm/h	Flux $mg/cm^2.h$	Enhancement ratio	Permeability rate mg/h.cm
F1	0.169	7.515×10^{-3}	8.487×10^{-2}	–	14.427×10^{-3}
F2	0.183	8.531×10^{-3}	9.189×10^{-2}	1.082	15.621×10^{-3}
F3	0.196	9.482×10^{-3}	9.833×10^{-2}	1.158	16.716×10^{-3}
F4	0.211	10.738×10^{-3}	10.556×10^{-2}	1.243	17.945×10^{-3}
F5	**0.233**	**12.406×10^{-3}**	**11.650×10^{-2}**	**1.372**	**19.805×10^{-3}**
F6	0.120	05.470×10^{-3}	06.012×10^{-2}	_	10.220×10^{-3}
F7	0.134	06.399×10^{-3}	06.758×10^{-2}	1.124	11.488×10^{-3}
F8	**0.274**	**14.599×10^{-3}**	**13.706×10^{-2}**	**1.614**	**23.300×10^{-3}**
F9	0.251	13.416×10^{-3}	12.568×10^{-2}	1.480	21.365×10^{-3}
F10	**0.285**	**15.184×10^{-3}**	**14.256×10^{-2}**	**1.679**	**24.422×10^{-3}**

The *in-vitro* drug release profiles from HPMC and EC films with PVP (F2, F3, F4, F5 and F7) were 60.82%, 64.67%, 69.70%, 76.18% and 44.95% Results indicated that HPMC films with PVP showed better release than EC films with PVP, which may be attributed due to high water vapour permeability of HPMC in the films and due to hygroscopic nature of copolymer PVP (figure-1). The *in vitro* release from all the formulations showed a moderate drug permeation through rat skin which might be attributed due to tough barrier, the stratum corneum of skin contributes to low diffusivity.[26] 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+PVP film (F5) since they showed better release than EC+PVP film. Among various concentrations 10% w/w of d-limonene (F8) and 20%w/w of oleic acid (F9) were showed good release 89.96% and 82.76% 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[27] Various permeation parameters were calculated and results given in table-3.

Fig. 1: In-vitro drug release profile of nimesulide from monolithic systems of F1,F2,F3,F4,F5,F6 and F7

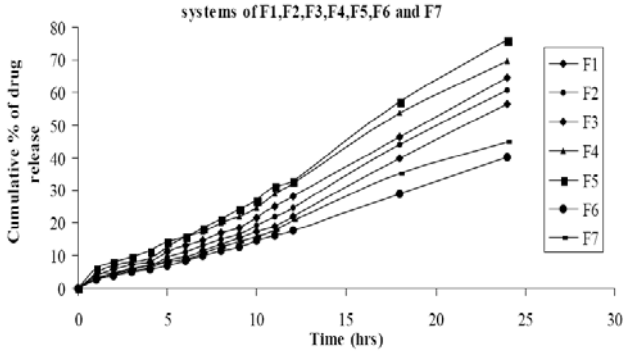
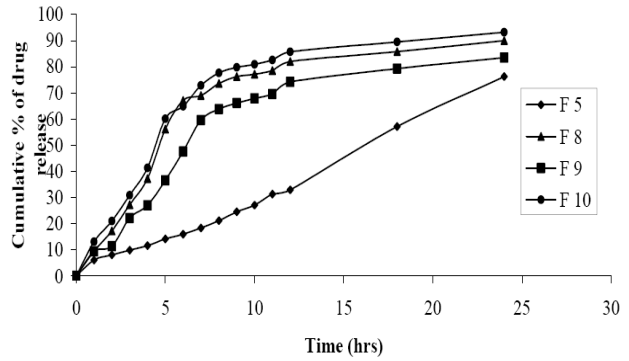


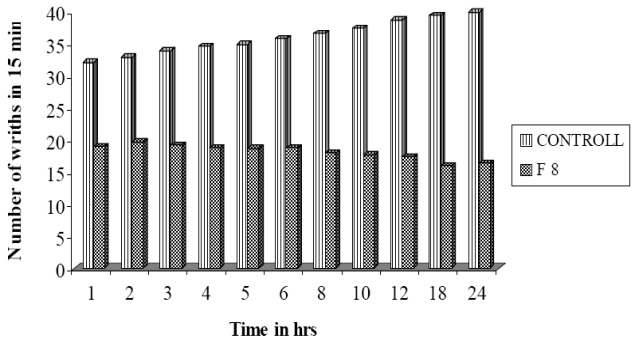
Fig.2: In-vitro release profiles of monolithic systems of F5, F8, F9 and F10



The *in vitro* drug release profiles of all monolithic systems were fairly linear with their correlation coefficients of 0.9301 to 0.9950. 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 0.8937 to 0.9582. Hence the results showed that, the mechanism of drug release from all the monolithic systems was diffusion mediated.

Among all the formulations F1 to F9, the F8 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 F8 formulation showed 61.42% reduction of formalin induced paw oedema at the end of 12 h. The data was analyzed by using P-STAT package where F8 showed a significant anti-inflammatory activity at $p < 0.05$. Same formulation F8 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 $p<0.05$. A bar graph of number of wriths 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.

Fig 3. Analgesic activity of the selected Formulation (F8)



CONCLUSION

The fabricated polymeric films containing NIM had shown uniform thickness, folding endurance, weight variation and drug content. *In vitro* skin permeation profile of the films was fairly uniform. The PVP as co-polymer enhanced drug permeation due to its hygroscopic and ampiphilic nature. 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 NIM for effective management of chronic pain, inflammation associated with rheumatoid and osteoarthritis conditions.

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