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Original Research Article

In Vitro and *In Vivo* Evaluation of Diclofenac Sodium Gel Prepared with Cellulose Ether and Carbopol 934P

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Abstract

Purpose: *To develop diclofenac sodium gel using high molecular weight hydroxypropyl methylcellulose (HPMC) and Carbopol 934P for topical and systemic delivery.*
Methods: *Diclofenac sodium gel was prepared with HPMC K100M and Carbopol 934P as gelling agents. The formulations were examined for pH, spreadability, consistency, viscosity, homogeneity, drug content and stability. In vitro drug release was evaluated using Franz diffusion cell. Carrageenan-induced rat paw oedema model was used for the evaluation of the anti-inflammatory activity of the gels. A commercial diclofenac sodium gel product was used as the reference drug.*
Results: *Formulations containing glycerin as permeation enhancer gave drug release patterns comparable to that of the reference product. The drug content of F_2, F_5 and F_9 was 99.81, 99.75 and 99.96 %, respectively. Accelerated stability results showed no significant variation in the appearance and drug release after storage for 3 months.*
Conclusion: *Diclofenac sodium gel containing HPMC K100M and Carbopol 934P exhibited pronounced anti-inflammatory activity and could be further developed for topical and systemic delivery..*

Keywords: *Diclofenac sodium, Anti-inflammatory, Hydroxypropyl methylcellulose, Carbopol, Drug release, Glycerin*

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INTRODUCTION

Drug delivery through the skin has been a promising concept for a long time because the skin is easy to access, has a large surface area with vast exposure to the circulatory and lymphatic networks and the route is non-invasive [1]. Transdermal delivery is of great importance for drugs that may cause systemic side effects, e.g., non-steroidal anti-inflammatory drugs (NSAIDs) [2].

Diclofenac sodium is an effective NSAID often used in the treatment of acute and chronic arthritic conditions. It is prescribed for long-term treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis [3]. There is great interest to develop non-oral dosage forms of diclofenac sodium to minimize its gastric side effects and to provide relatively consistent drug levels at the application site for prolonged periods [2]. Topical delivery of diclofenac sodium using various formulations has been described in the literature [4,5]. However, effective permeation of the drug through skin is difficult to achieve due to its intrinsically poor permeability, though this is relatively good compared to other commonly used NSAIDs [6]. Skin permeation enhancers can improve drug skin penetration [7].

HPMC K100M and Carbopol 934P have been used as hydrophilic polymers in the formulation of gels for transdermal drug delivery. One of their most important characteristics is high swellability, which has a significant effect on the release kinetics of the incorporated drug [8]. Cellulose ethers, especially HPMC K100M, are frequently used as the basis for sustained release hydrophilic matrix tablets [9]. The overall drug release mechanism of HPMC K100M-based pharmaceutical devices strongly depend on the design (composition and geometry) of the particular delivery system. On imbibing water, HPMC K100M swells, resulting in dramatic changes in the viscous nature of the polymer, drug concentration and increased dimension of the dosage system. Upon contact with water, the incorporated drug dissolves and diffuses out of the device. Depending on the chain length and degree of substitution of the HPMC type used, the polymer itself dissolves more or less rapidly. Diffusion, water uptake and erosion are the most important rate-controlling mechanisms of commercially available controlled release products [8]. A series of grades based on the molecular fraction of these polymers are used at concentrations between 1 and 5 % in topical gel formulation.

Carrageenan-induced paw oedema in mice has been widely used for the evaluation of anti-inflammatory activity of drugs [10].
The aim of this study was to develop suitable topical formulations of diclofenac sodium using HPMC K100M and Carbopol 934P as gelling agents and glycerin as permeation enhancers.

EXPERIMENTAL

Materials

Diclofenac sodium was purchased from Yarrow Chem. Products, Mumbai, India. HPMC K100M was obtained as a gift from Colorcon, Mumbai, India. Carbopol 934P was purchased from Genuine Chemicals, Mumbai, India. All organic solvent used were of analytical grade.

Solubility analysis

The solubility of diclofenac sodium was determined using various solvents of different polarities including methanol, 95 % ethanol, water, glacial acetic acid, ether, chloroform and phosphate buffer (pH 6.8). The vials containing the solvent and excess diclofenac sodium were kept in a shaker for 24 h. It was filtered through Whatmann filter paper. The filtrate was analyzed by UV spectrophotometry to determe the amount of diclofenac sodium.

Fourier transform-infrared spectroscopy (FT-IR) study

FT-IR study was used to check compatibility and interaction between the drug and the polymers. The spectra of pure diclofenac sodium and the physical mixture of diclofenac sodium and polymer, embedded in KBr discs,.were recorded in the range of 4000 cm^{-1} and 400 cm^{-1} using IR spectroscopy (Shimadzu, IR Affinity- 1).

Preparation of diclofenac sodium gel

For formulations F_1, F_2 and F_3, 1 g of diclofenac sodium was dissolved in 15 ml of glycerin with the aid of mild heat (solution A). Weighed quantity of HPMC K100M was added to 75 ml of distilled water and stirred until dissolved (solution B). Solutions A and B were mixed thoroughly and the final weight made up to 100 g. For formulations F_4, F_5 and F_6, 1 g of diclofenac sodium was dissolved in 15 ml of glycerin with the aid of mild heat (solution A). Weighed quantity of Carbapol 934P was added to 75 ml of distilled water, stirred until dissolved and then neutralized with 10 % NaOH (solution B). Solutions A and B were mixed thoroughly and the final weight made up to 100 g. For formulations F_7, F_8 and F_9, 1 g of diclofenac sodium was dissolved in 15 ml of glycerin with the aid of mild heat, and methyl paraben and propyl paraben added (solution A). Weighed quantity of sodium alginate was added to 75 ml of distilled water and stirred until dissolved (solution B). Solutions A and B were mixed thoroughly and the final weight made up to 100 g. The composition of the formulations is outlined in Table 1.

The three different hydrophilic polymers were used for study and in each formulation code the combination of two polymers were used by keeping one of the polymers constant. The different nine formulation of diclofenac sodium gel (F_1-F_9) with their code are listed in table 1. The gel were kept in plastic well closed container and stored at room temperature until the time of analysis.

In vitro evaluation of diclofenac gels [11]

The diclofenac sodium gels were subjected to evaluation for the following parameters - pH, spreadability, consistency, viscosity, homogeneisity [12], drug content, *in vitro* drug release studies, *in vivo* anti-inflammatory activity by carrageenan induced paw oedema and accelerated stability studies.

Table 1: Composition of diclofenac sodium gel

Formulation code	Drug (g)	HPMC K100M (g)	Carbopol 934P (g)	10% NaOH (ml)	Glycerin (ml)	Propyl paraben (g)	Distilled water to (g)
F_1	1	2.0	0.25	q. s.	15	-	100
F_2	1	2.0	0.50	q. s.	15	-	100
F_3	1	2.0	0.75	q. s.	15	-	100
F_4	1	-	0.50	q. s.	15	0.1	100
F_5	1	-	0.50	q. s.	15	0.1	100
F_6	1	-	0.50	q. s.	15	0.1	100
F_7	1	1	-	q. s.	15	0.1	100
F_8	1	1.5	-	q. s.	15	0.1	100
F_9	1	2.0	-	q. s.	15	0.1	100

Determination of pH of gel formulations

The pH of the gel formulations was measured with a pH meter (Eutech, Cyberscan) using 1 % aqueous solutions of the gels at room temperature.

This parameter was determined with a wooden block and glass slide apparatus.The gel (approx. 20 g) was added to the pan and the time for the upper slide (movable) to separate completely from the fixed was noted. Spreadability was calculated formulas in Eq 1.

$S = W \times L/T$ …… (1)

where S = spreadability, W = weight tide to upper slide, L = length of glass slide, and T = time taken to separate the slide completely from each other

Consistency [12]

Measurement of consistency of the gels was carried out by dropping a cone attached to a holding rod from a fix distance of 10 cm in such way that it falls in the centre of a glass cup filled with the gel. The penetration by the cone was measured from the surface of the gel to the tip of the cone inside the gel. The distance traveled by the cone after 10 s was noted.

Viscosity

The viscosity of the formulations was determined using a Brookfield digital viscometer (model DV-II, USA) equipped with spindle S27.. The gel sample (5 g) was placed in the sample holder of the viscometer and allowed to settle for 5 min and the viscosity measured a rotating speed of 50 rpm at room temperature (25 - 27 ^oC).

Homogeneity

All the gels formed were tested for homogeneity by visual inspection after the gels have been allowed to set in a container. They were tested for their appearance and presence of any aggregates.

Drug content

A quantity (100 mg) of the gel was dissolved in 100 ml of phosphate buffer of pH 6.8. The volumetric flask containing gel solution was shaken for 2 h on a mechanical shaker to allow the drug to dissolve completely. The solution was filtered and drug content determined spectrophotometrically at 276 nm using phosphate buffer (pH 6.8) as blank.

***In vitro* evaluation of diclofenac sodium release**

Pretreated skin of albino mice was used in the Franz diffusion cell experiment. The receptor compartment contained 100 ml of phosphate buffer pH 6.8. One gram of the test formulation or reference was applied to the skin over an area of 1.131 cm^2 and placed across the donor compartment. The donor cell was exposed to ambient temperature and covered with parafilm to prevent evaporation. The temperature of the diffusion medium was maintained at 37 ± 1 ^oC while the buffer solution was stirred continuously with a Teflon-coated magnetic bar at 500 rpm. Samples (1 ml each) were withdrawn from the release medium at 30, 60, 90 and 120 min and replaced with an equal volume of fresh buffer solution to maintain sink conditions. The samples were analyzed spectrophotometrically at 276 nm against their respective blank.

In vivo evaluation of anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced rat paw oedema method. Male albino rats of Wister strain (150 - 200 g) were used after due approval of the Institutional Animal Ethical Committee (IAEC) of Institute of Pharmaceutical Education, Boradi, India (Reg. no. 1301/a/08/CPCSEA). The animals were kept in plastic cages with soft bedding (6 per cage) under standard condition of light and dark cycles and had free access to food (standard pellet diet) and tap water. The rats were allowed to acclimatize for one week prior to the experiment. Food was withdrawn 12 h before and during the experiment.

The animals were divided into four groups with three animals in each group. The first and second groups served as controls receiving normal saline and gel base without drug, respectively. The third group received diclofenac sodium ethosomal gel and the fourth group received plain diclofenac gel (commercial). Carrageenan solution (1 %w/v in normal saline) was used to induce inflammation. The animals were placed singly in observation chambers for 10 minute to minimize any stress-related behavioral changes. The thickness (mm) of the paw was measured at 0, 1, 2, 3, and 4 h after carrageenan administration, using a digital vernier caliper (Alex) with a sensitivity of 0.01 mm.

Accelerated stability studies

All the selected formulations were subjected to accelerated stability test over a period of three months as per ICH guidelines at a temperature of 40 ± 2 ^{o}C/ and 75 % relative humidity (RH). All the formulations were analyzed for changes in appearance, pH and drug content as described above.

Statistical analysis

The results were expressed as mean ± standard deviation. The data were analyzed by one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison. A level of significance of $p < 0.05$ was set to determine any significance.

RESULTS

Drug-excipient compatibility

There were no significant changes in the peak pattern of the IR spectra of pure diclofenac sodium and of the combined drug/polymer mixture (fig 1), which implies that there was no interaction between the drug and the polymers.

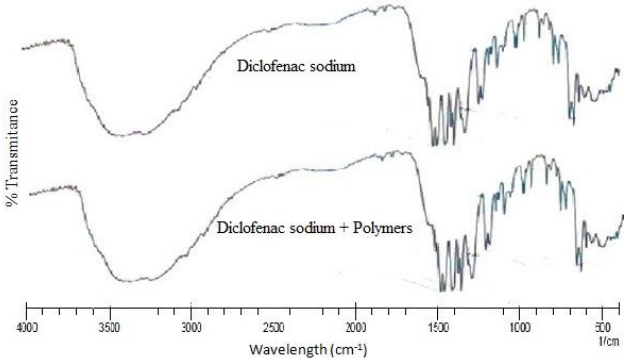

Fig 1: FT-IR spectra of pure diclofenac sodium and diclofenac sodium/polymer mixtures

Physical properties of gel

Precipitation occurred in some of the gel batches (F_1, F_3, F_4, F_6, F_7 and F_9) of polymer based gel containing diclofenac sodium which was probably due to incompatibility. Hence, these batches were discarded and remaining batches (F_2, F_5 and F_8) were used in further studies.

The results for pH, spreadability, viscosity, consistency, homogeneity and drug content are shown in Table 2. Spreadability data indicate that the gel is easily spreadable by a small amount of shear. Consistency reflects the capacity of the gel to get ejected in uniform and desired quantity when the tube is squeezed. Consistency in terms of distance travelled by the cone was 6 mm. The gel formulations were homogeneous in texture and fell within a pH range of 6.8 to 7.4 which is within the normal skin pH in healthy people. The results show that there was no significant difference between the viscosities of the gel formulations and that of the reference.

Table 2: Properties of some diclofenac sodium gel formulations

Batch	pH	Spreadability (g.cm/s)	Viscosity (dyn·s/cm)	Consistency (mm)	Homogeneity	Drug Content (%)
F_2	7.4	5.6	0.94×10^{-3}	6	Very good	99.81
F_5	6.8	3.8	1.60×10^{-3}	6	Good	99.75
F_9	7.1	3.9	1.70×10^{-3}	6	Good	99.96

In vitro diclofenac sodium release

Diclofenac sodium release from the F_2 formulation was comparable with that of the reference standard (commercial formulation), as Table 3 shows. The pH of F_2 and the commercial gel was was 6.8.

Table 3: *In vitro* release characteristics of diclofenac sodium gel

Time (min)	Drug release (%)	
	F_2	Ref*
30	38.54	39.64
60	64.78	65.86
90	81.23	82.92
120	96.87	97.34

**Reference standard (commercial formulation*

In vivo anti-inflammatory activity of diclofenac gel

The results of the anti-inflammatory test (Fig 2) indicate there was no significant difference between the anti-inflammatory activities of the test formulations and the reference.

Stability of the gels

The results of the accelerated stability studies are shown in Table 4. The appearance of the gels remained clear and no significant variation in pH was observed after subjecting the formulations to stability stress for 3 months. Drug content was 96.91, 95.33 and 95.65 % formulations F_2, F_5 and F_9, respectively, after the 3-month period.

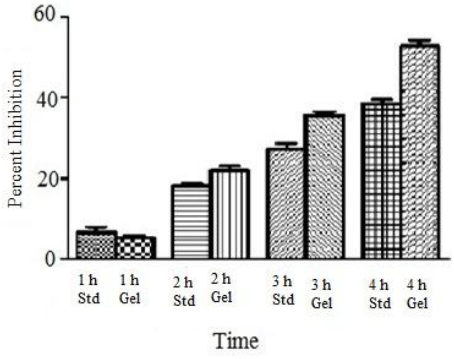

Fig 2: Inhibition of rat paw edema by reference standard (Std) and test (Gel) formulations

DISCUSSION

The aim of this study was to develop suitable topical gel formulations of diclofenac sodium gel using HPMC K100M and Carbopol 934P as a gelling agents and glycerin as permeation enhancer.

The apparent viscosity of the test formulations was comparable to that of the reference standard. Due to the viscous and hydrophilic nature of HPMC K100M and Carbopol 934P, the complex might have expanded more in water than the individual polymers would in water and hence the increase in solution viscosity. The use of glycerin as permeation enhancers significantly increased drug release rate. It is generally agreed that *in vitro* drug release data for topical formulation cannot be used to accurately predict permeation across the skin, due to the barrier properties of stratum corneum which can be

Table 4: Accelerated stability data for diclofenac sodium gel

Formulation	Month	Appearance	pH	Drug content (%)
F_2	0	Clear	7.4	99.81
	1	Clear	7.4	98.27
	2	Clear	7.3	97.54
	3	Clear	7.3	96.91
F_5	0	Clear	6.8	99.75
	1	Clear	6.8	98.87
	2	Clear	6.7	96.05
	3	Clear	6.6	95.33
F_9	0	Clear	7.1	99.96
	1	Clear	7.1	97.12
	2	Clear	7.1	96.43
	3	Clear	7.0	95.65
Commercial gel	0	Clear	6.8	99.90
	1	Clear	6.7	98.70
	2	Clear	6.6	97.20
	3	Clear	6.5	95.90

altered by the presence of various permeation enhancers in the formulation.

The difference between the anti-inflammatory activities of the reference standard and test gels after 1 and 2 h was not significant, but after 3 and 4 h, the test gel exhibited significantly higher activity than that of standard formulation.

CONCLUSION

Diclofenac sodium gels prepared using HPMC K100M and Carbopol 934P produced better spreadability and consistency as compared to carbopol 934P and sodium alginate gel (F_5) and sodium alginate and HPMC K100M gel (F_9) formulations and hence should be further developed for scale-up to industrial production.

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