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Research Article

FORMULATION AND *IN-VITRO*/*EX-VIVO* CHARACTERIZATIONS OF MICROEMULSION-BASED HYDROGEL FORMULATION OF ACECLOFENAC FOR TOPICAL APPLICATION

ABDUL BAQUEE AHMED[1]*, ROWSHAN AHMED[1], RUPA SENGUPTA[2]

[1]Department of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati - 781 017, Assam, India.
[2]Department of Pharmacognosy, Rofel Shri G.M.Bilakhia College of Pharmacy, Namdha Road, Vapi - 396 191, Gujarat, India.
Email: baquee1977@gmail.com

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ABSTRACT

Objectives: In this study, microemulsion-based hydrogel (MBH) formulation of aceclofenac was prepared for topical administration in the management of pain and inflammation to overcome the gastrointestinal toxicity of the drug occurs with oral administration.

Methods: The MBH formulation was prepared by two-step methods. In the first step, the drug loaded o/w microemulsion was prepared first by titration method and in the second step; xanthan gum was added slowly to the microemulsion under homogenization to produce clear MBH. The developed MBH formulations were characterized by *in-vitro* evaluations, stability studies, and skin irritancy test. The *ex-vivo* permeation across rat epidermis using modified Keshary-Chien diffusion cell and anti-inflammatory activity of the selected MBH formulation was also evaluated in rat hind paw edema model.

Results: The developed MBH formulations showed good stability and acceptable physicochemical properties. The selected formulation (MBH2) showed the highest skin permeation rate (transdermal flux, 193.59±5.01 μg/cm^2/h; lag time, 0.80±0.01 h) and a maximum of 70.96% inhibition of the hind paw edema was measured after 8 h of the study.

Conclusion: Thus, the results obtained in this study suggest the feasibility of the MBH formulation of aceclofenac for topical application for the treatment of pain and inflammation.

Keywords: Aceclofenac, Microemulsion-based hydrogel, Transdermal flux, *Ex-vivo* permeation.

INTRODUCTION

A microemulsion is a single optically isotropic and thermodynamically stable liquid solution consists of oil phase, surfactant, cosurfactant, and an aqueous phase. The microemulsion is either an o/w or w/o emulsion producing a transparent product that has a droplet size <0.15 μm and does not have the tendency to coalesce [1]. Microemulsions have several advantages over conventional formulations such as good thermodynamic stability, high drug solubilizing capacity, the high permeation rate of drug across stratum corneum (SC) from microemulsion [2]. The surfactant and cosurfactant in the microemulsion may reduce the diffusion barrier of the SC by acting as permeation enhancers [1]. In the last decades, much research has been focused on the topical delivery of drugs using microemulsion such as estradiol [3], meloxicam [4], triptolide [5], and lidocaine [6].

Recently, different hydrogel matrices such as carbomer 940, xanthan gum, and carrageenan have been used to increase the viscosity of microemulsion for topical application [3,7-10]. The addition of hydrogel matrix into the microemulsion resulted in the formation of the microemulsion-based hydrogel (MBH), which is more suitable for topical application when compared with microemulsion [11].

Aceclofenac (2-[(2, 6-dichlorophenyl) amine] phenylacetoxyacetic acid) is an orally effective non-steroidal anti-inflammatory drug of the phenylacetic acid group, which possesses remarkable anti-inflammatory, analgesic, and antipyretic properties [12]. It is used in the treatment of osteoarthritis and inflammatory disease of the joints [13,14]. The oral administration of aceclofenac causes gastrointestinal ulcers and bleeding, causes anemia with chronic use [14,15]. To avoid the systemic toxicity of the drug arise with oral administration, one of the promising methods is to administer the drug through transdermal route for better therapeutics effect [16].

Aceclofenac is a highly lipophilic drug having log (P) value about 1.705, molecular weight of 354.2 Da, pKa 4-5 and melting point in the range of 151-152°C can be considered ideal for transport of the drug through the skin [12].

Therefore, in this study, an attempt has been made to develop an MBH formulation of aceclofenac for topical application. The developed MBH formulations were characterized by *in-vitro* evaluations, stability studies, and skin irritancy test. The *ex-vivo* permeation across rat epidermis using modified Keshary-Chien diffusion cell and anti-inflammatory activity of the selected MBH formulation was also evaluated in rat hind paw edema model.

METHODS

Materials

Aceclofenac was a gift sample from IPCA Pharmaceuticals Pvt. Ltd. (Sikkim, India). Castor oil, polyoxyethylene sorbitan monooleate (Tween 80), and ethanol were purchased from Merck Chemicals (Mumbai, India). Xanthan gum and methylparaben (Himedia Laboratories Pvt. Ltd., Mumbai, India); sodium bromide (Loba Chemie Pvt., Ltd., Mumbai, India) and chloroform (Thomas Baker Chemicals Pvt., Ltd., Mumbai, India) were procured and used in this investigation. All other chemicals and solvents used in the studies were of analytical grade.

Methods

Preparation of MBH formulations

The details composition of MBH formulations were shown in Table 1. Aceclofenac loaded MBH formulations were prepared by two-step methods. First, aceclofenac was added to the oil, surfactant and cosurfactant mixture (10% v/v castor oil, 25% v/v tween 80/ethanol at 4:1 ratio) and after that, the required quantity of water was added

Table 1: Composition of MBH formulations

Formulations	Aceclofenac (% w/v)	Castor oil (% v/v)	Tween 80/ethanol (% v/v)	Water (% v/v)	Xanthan gum (% w/w)
ME	2	10	25	65	-
MBH1	2	10	25	65	1
MBH2	2	10	25	65	1.5
MBH3	2	10	25	65	2

MBH: Microemulsion-based hydrogel, ME: Microemulsion

to the mixture drop by drop under constant stirring at 25°C to obtain the o/w microemulsion. Second, xanthan gum was added slowly to the microemulsion under homogenization to produce clear MBH. The developed formulations were left overnight at room temperature (25-27°C) for complete dissolution.

Characterization of MBH formulations

Measurement of droplet size and conductivity of microemulsion

The average droplet size and polydispersity index of the microemulsion were determined by photon correlation spectroscopy (Zetasizer Nano S90, Malvern, UK) at 25±0.5°C using He-Ne laser at 632 nm. To characterize the conductivity of the microemulsion, a CM 180 conductometer (Elico, India) was used [11].

Physical examination

The prepared MBH formulations were inspected visually for their color, appearance, and consistency [17].

Spreadability

The spreadability of the MBH formulations was determined at 24 h after dissolution. 1 g of MBH formulations were placed between two horizontal plates (20 cm × 20 cm), and the upper plate was tied with 125 g of standardized weight. The spreading diameter was measured after 1 min [18].

Determination of pH

The pH of the formulations was determined using a pH meter (TOSHNIWAL, Model CL 54, India) at 25±1°C.

Rheological study

The viscosity of MBH formulations was measured using a Brookfield rotational viscometer with spindle no. 64 (LV2, Brookfield Inc., USA). The formulation whose viscosity was to be determined was placed in the sample holder which was connected to a thermostatically controlled circulating water bath maintained at 25°C and allows the spindle to move and rotate freely in the MBH at a speed of 10 rpm and the reading was noted [19,20].

Drug content

For assay of the drug in MBH formulation, 1 g of MBH formulations were extracted with 20 ml of methanol for 30 minutes. The resultant mixture was filtered through membrane filter (pore size 0.45 mm). The absorbance of the sample was determined spectrophotometrically at 273 nm (Shimadzu, UV-1800 spectrophotometer, Japan) after appropriate dilution with phosphate buffer pH 7.4.

Stability of MBH formulations

In stability studies, the MBH2 formulation was packed in aluminum collapsible tube (5 g) and stored at 25°C/60% relative humidity (RH) and 40°C/75% RH for a period of 3-month in a stability chamber (REMI, Environmental Test Chamber, India). Samples were withdrawn in duplicate at 0 and after 1, 2, and 3 months, and their physical and chemical stabilities were assessed. Physical stability was evaluated by visual inspection for physical appearance, pH, and rheological properties. Chemical stability was expressed as aceclofenac content, which was determined spectrophotometrically [20,21].

Skin irritation study

The skin irritation study was performed as per the procedure described by Barakat NS, 2010. [22]. Briefly, the Wistar albino male rats weighing 200-250 g were used for skin irritation test. The animals were kept under standard laboratory conditions (25±1°C and 55±5% relative humidity) with free access to standard laboratory diet and water *ad libitum*. The abdominal hairs of the rats were shaved carefully avoiding peripheral damage on the previous day of the experiment [23]. The rats were divided into three groups with four animals each. Group 1 was treated as control (without any treatment), Group 2 was treated with MBH2 formulation, and Group 3 was treated with standard irritant (0.8% v/v aqueous solution of formalin) once daily for 3 days. Finally, animals were observed for any sign of flare and wheal for a period of 7-day [24].

***In-vitro* skin permeation study**

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 [25]. The epidermis was thoroughly washed with water, dried at 25% RH, wrapped in aluminum foil and stored in freeze until further use. All experiments with animals were approved by the Institutional Animal Ethics Committee of Girijananda Chowdhury Institute of Pharmaceutical Science, India (GIPS/IAEC/M.ph/2014/3).

For *in-vitro* permeation studies, skins were allowed to hydrate for 1 h before being mounted on the Keshary-Chien diffusion cell with the SC with effective surface area 1.54 cm^2 facing the donor compartment. The receptor compartment was filled with 19.5 ml of phosphate buffer pH 7.4 and its temperature was maintained at 37±0.5°C. On the SC side of the donor compartment, 1 g of the formulation was applied and covered with aluminum foil to prevent drying out. 1 ml of receptor medium was withdrawn at an interval of 1 h and at the each time of withdrawal, 1 ml of fresh corresponding receptor medium was replaced in the acceptor compartment to maintain the sink condition. The content of the drug permeated was determined spectrophotometrically at 273 nm using a Shimadzu UV-1800 spectrophotometer.

***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 [17,26]. 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 (applied topically), Group 2 received ME formulation, and the Group 3 received MBH2. Immediately after the 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 the control group.

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

Where, D is the change in paw volume in the treated group and C is the change in paw volume in the control group.

Data and statistical analysis

The steady state flux (J, µg/cm^2/h) was calculated from the slope of the linear plot of the cumulative amount permeated per unit area (µg/cm^2) 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, cm^2/S) was calculated from the flux and donor drug concentration. Data are represented as mean ± standard deviation (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

Measurement of droplet size and conductivity

The droplet size and polydispersity index of the microemulsion were found to be 101.2±2.15 to 108.6±1.25 and 0.230±0.02 to 0.343±0.08, respectively, indicated the narrow size distribution of the oil globules in the microemulsion. The conductivity of the formulations was found to be 132.5±0.04 to 136.5±0.03 µS/cm, revealed the o/w structure of the microemulsion.

Physicochemical parameters

The results of the physicochemical study are presented in Table 2. The study revealed the formation of clear and homogeneous gel with good spreadability. The spreading diameter of ME formulation after 1 min study was found to be 123±3.12 mm which was observed to be highest as compared with the other MBH formulation. These studies suggest that addition of xanthan gum in formulations increased the viscosity which may be the cause of decrease in spreadability of the MBH formulations. The pH of the MBH formulations was found to be in the range of 6.2±0.16 to 6.5±0.22, which lies within the range of normal skin pH and would not produce and skin irritation. The drug content of the MBH formulation was found to be 87±0.93 to 94±0.81%, showing content uniformity.

Viscosity

The viscosity of the formulations generally reflects its consistency. The viscosity of MBH was increased with the increase of the concentration of xanthan gum in the formulations (MBH3 > MBH2 > MBH1). The consistency of MBH containing 1% xanthan gum (MBH1) led to a low viscosity of 11.69±0.3 Pa S. However, consistency of MBH containing 2% xanthan gum (MBH3) resulted in a too high viscosity of 35.40±0.45 Pa S. The MBH containing 1.5% xanthan gum (MBH2) had a suitable viscosity of 22.88±0.38 Pa S for topical application.

Stability study

The results of stability studies revealed that the MBH2 formulation was stable at both the storage conditions in terms of physical and chemical properties. No significant change was observed for physical appearances, pH, and viscosity. The drug content of the formulation before stability and after stability study was found to be 89.03±1.02% and 88.11±0.09%, respectively, revealed no significant chemical change in the formulation after 3 months of stability study.

Skin irritancy test

The skin irritation studies were carried out to evaluate the tolerability of the MBH after application. The skin irritancy test of the selected MBH formulation (MBH2) showed that the mean value of a skin irritation score (erythema and edema) of <2. Earlier studies reported that a value between 0 and 9 indicates that the applied formulation probably would not be irritant to human skin [27,28]. Thus, the selected microemulsion is considered to be safe for the use of transdermal drug delivery.

***In-vitro* skin permeation study**

The permeation ability of MBH formulations across rat epidermis was evaluated using *in-vitro* permeation experiment, and the permeation profiles are presented in Fig. 1. The cumulative amount of aceclofenac permeated through excised rat skins was plotted as a function of time. The slope and intercept of the linear portion of the plot were derived by regression. The permeation rate at steady state (J_s, µg/cm^2/h) was calculated as the slope divided by the skin surface area. The intercept on the X-axis was taken as the lag time (T_L, h). The permeation parameters calculated from the profiles are presented in Table 3.

The permeation profile of MBH formulations followed a linear relationship (R^2>0.9 [0.92-0.98]) was obtained between amount released and the square root of time as proposed by Higuchi's theory [29] indicating diffusion controlled mechanism of drug release kinetics. The highest transdermal flux of aceclofenac from the microemulsion formulation (ME) was found to be 243.97±3.08 µg/cm^2/h, and a lag time 0.64±0.01 hrs. In another hand, the transdermal flux of MBH formulations containing various concentrations of xanthan gum MBH1 (1%), MBH2 (1.5%) and MBH3 (2%) was found to be 218.35±9.04, 193.59±5.01 and 143.62±11.66 µg/cm^2/h, respectively. The result showed an inverse relationship between permeation rate and viscosity of the formulations. As mentioned earlier that with increased concentration of xanthan gum leads to increase the viscosity of the formulation which may causes increased diffusion path length of the drug thereby reduced the permeation rate. This phenomenon is in accorded with the earlier results reported by Chen *et al*., 2006 [11]; Das and Ahmed, 2007 [17]. Statistical comparison of the transdermal flux of ME formulation showed a significant difference ($p<0.05$) with MBH3 formulation. However, the differences of the transdermal flux of MBH1 and MBH2 was not significant statistically ($p>0.05$). Thus, formulation MBH2 showed suitable viscosity for topical application

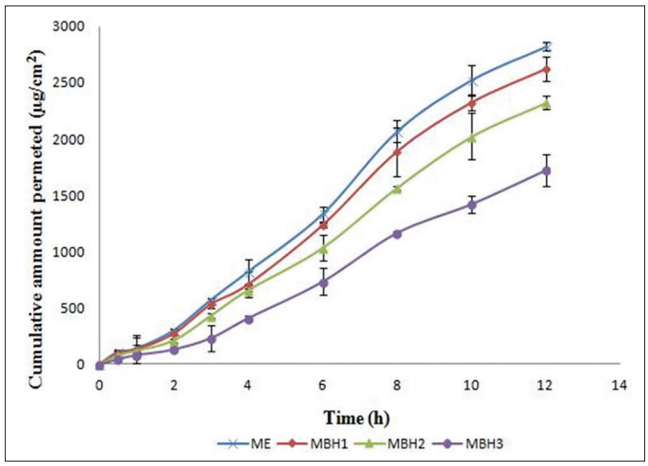

Fig. 1: *In-vitro* skin permeation profile of aceclofenac from microemulsion (ME) and microemulsion based hydrogel (MBH) formulations

Table 2: Physicochemical parameters of various MBH formulations

Formulations	pH*	Droplet size of microemulsion* (nm)	Polydispersity Index*	Drug content* (%)	Spreading diameter after 1 min* (mm)	Conductivity (µS/cm)	Viscosity (Pa S)
ME	6.2±0.16	105.3±1.05	0.310±0.05	93±1.15	123±3.12	136.5±0.03	5.81±0.38
MBH1	6.3±0.05	101.2±2.15	0.255±0.03	94±0.81	67±2.11	133.5±0.05	11.69±0.30
MBH2	6.5±0.22	108.6±1.25	0.343±0.08	89±1.02	61±1.04	132.5±0.04	22.88±0.38
MBH3	6.4±0.06	111.7±1.03	0.230±0.02	87±0.93	56±3.05	134.5±0.02	35.40±0.45

*Mean±SD, n=3, MBH: Microemulsion-based hydrogel, SD: Standard deviation, ME: Microemulsion

Table 3: Permeation parameters of aceclofenac from various formulations across rat epidermis

F code	(Mean±SD, n=4)			K_p ($cm^2/S\times10^{-6}$)	Best fit regression equation for permeation plot	R^2
	Amount permeated at 12 h (µg/cm^2)	Flux, J (µg/cm^2/h)	Lag time, T_L (h)			
ME	2819.72±37.03	243.97±3.08	0.64±0.01	3.39±0.004	Q=251.15t−98.33	0.9909
MBH1	2620.22±108.53	218.35±9.04	0.75±0.02	3.03±0.013	Q=232.26t−98.48	0.9909
MBH2	2323.15±60.02	193.59±5.01	0.80±0.01	2.69±0.007	Q=202.86t−97.78	0.9921
MBH3	1723.55±140.02	143.62±11.66	1.02±0.06	1.99±0.016	Q=149.88t−101.83	0.9855

SD: Standard deviation, ME: Microemulsion, MBH: Microemulsion-based hydrogel

Table 4: Edema volume of different treated groups of animals of anti-inflammatory activity

Treated group	Paw volume before treatment (ml) (mean±SD, n=4)	Edema volume (mean±SD, n=4) (h)					
		1	2	3	4	6	8
Control (placebo gel)	0.55±0.03	0.81±0.02	0.83±0.06	0.85±0.01	0.87±0.07	0.87±0.02	0.86±0.04
ME	0.53±0.06	0.74±0.02	0.71±0.05	0.69±0.03	0.66±0.02	0.65±0.04	0.65±0.07
MBH2	0.54±0.08	0.77±0.01	0.75±0.04	0.71±0.02	0.68±0.06	0.64±0.03	0.63±0.08

SD: Standard deviation, ME: Microemulsion, MBH: Microemulsion-based hydrogel

Table 5: Percent inhibitions of hind paw edema

Formulations	Percentage inhibition (h)					
	1	2	3	4	6	8
ME	19.23	35.71	46.66	59.37	62.50	61.29
MBH2	11.50	25.00	43.33	56.26	68.75	70.96

ME: Microemulsion, MBH: Microemulsion-based hydrogel

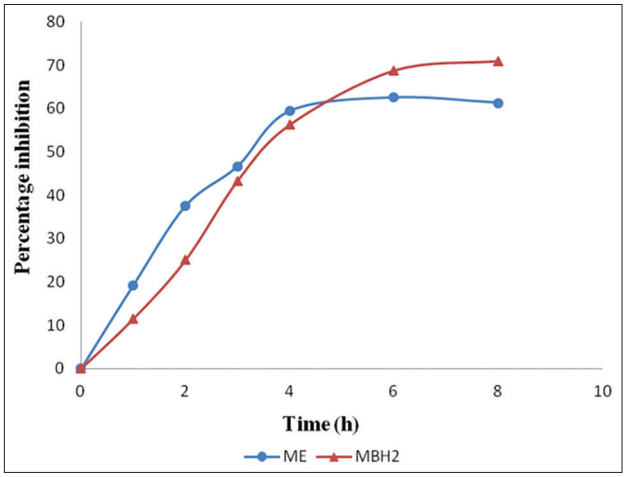

Fig. 2: Percent inhibitions of hind paw edema after oral administration of aceclofenac microemulsion (ME) and application of microemulsion based hydrogel formulation (MBH2)

with good permeation rate of 193.59±5.01 µg/cm^2/h was considered as best formulation.

***Ex-vivo* anti-inflammatory activity**

The carrageenan-induced rat hind paw edema model was used to compare the oral and topical anti-inflammatory effect of aceclofenac. The measured changes in edema volume of different treated group of animals are presented in Table 4. The result revealed that both orally administered ME (inhibited the paw edema by 62.50% at 6 h) and topically applied MBH2 (inhibited the paw edema 70.96% at 8 h) formulation significantly inhibited ($p<0.05$) the edema formation (Table 5 and Fig. 2) when compared with the control (Placebo gel). It was observed that hydrogel based formulation, MBH2, showed an initial slower permeation of drug (initial 11.5% inhibition and extended to maximum 70.96% at 8 h) with compare to microemulsion formulation, ME (initial 19.23% and extended to maximum 62.50% at 6 h). On the other hand, the difference of percent inhibition values between the ME and MBH2 formulation was not statistically significant ($p>0.05$). Hence, incorporation of aceclofenac in MBH formulation does not significantly altered the transdermal permeation of the drug but, significantly inhibited the inflammatory response induced by carrageenan. Therefore, MBH formulation may be a promising vehicle for topical delivery of aceclofenac.

CONCLUSION

In this study, MBH formulation of aceclofenac was prepared using castor oil as the oil phase of the microemulsion for good solubilizing capacity of the drug and xanthan gum as a gel matrix. The MBH2 formulation showed the highest skin permeation rate (transdermal flux, 193.59±5.01 µg/cm^2/h; lag time, 0.80±0.01 h) and inhibition of the hind paw edema (70.96% at 8 h). The results of physicochemical property, stability study and skin irritancy tests showed that the selected MBH formulation of aceclofenac was a promising vehicle for transdermal application which is expected to overcome the gastrointestinal toxicity of the drug when administered orally.

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