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Fabrication and Evaluation of Hydrogel Thickened Microemulsion of Ibuprofen for Topical Delivery

M. C. Gohel and S. A. Nagori*

Department of Pharmaceutics and Pharmaceutical Technology, L. M. College of Pharmacy, Ahmedabad 380 009, India.

Author for Correspondence: stavanpharma2000@gmail.com

Abstract

*The objective of present study was to formulate hydrogel thickened ibuprofen transdermal formulation. Eutectic mixture of camphor and menthol was chosen as oily phase, solvent for ibuprofen and powerful penetration enhancer. Tween 80, ethanol (90% v/v) and carbopol 940 were selected as surfactant, co-surfactant and hydrogel thickening agent respectively. Ternary phase diagrams were constructed to obtain the concentration range of eutectic mixture, surfactant and co-surfactant for microemulsion formulation. Hydrogel thickened microemulsions were characterized for pH, viscosity, spreadability, irritation study and in vitro drug transport study with excised rat skins. The average drug transport rate of optimized hydrogel thickened microemulsion containing 1% w/w ibuprofen, 31.84% w/w eutectic mixture of camphor and menthol, 27.21% w/w tween 80, 13.61% w/w ethanol, 23.84% w/w water, 1.5% w/w carbopol 940 and 1% w/w triethanolamine through rat skin was 1.94 µg/ml*h*cm^2. The percentage in vitro drug transport of optimized hydrogel thickened microemulsion through rat skin was 0.12 times higher than that obtained in human cadaver skin at the end of 12 h. Short term stability study of the optimized hydrogel thickened microemulsion demonstrated no considerable difference in performance characteristics.*

Key words *– Hydrogel thickened microemulsion, Ibuprofen, Eutectic mixture, Carbopol 940, Short term stability study*

INTRODUCTION

Transdermal delivery offer number of advantages over conventional systems. However, the major problem with transdermal delivery is skin which behaves as a natural barrier making difficult for most of drugs to be delivered into and through it.[1] In the last decade various attempts are made to deliver the drugs topically via microemulsions because of low skin irritation, powerful permeation ability and high drug loading capacity.[2] Microemulsion is a dispersion of oil, surfactant, co-surfactant and aqueous phase. Microemulsion is optically isotropic and thermodynamically stable liquid solution.[3] There are several permeation enhancement mechanisms of microemulsion such as an increased concentration gradient and thermodynamic activity toward skin and the permeation enhancement activity of the components of microemulsion.[4]

Even though microemulsion offers several advantages for topical delivery it is difficult to stabilize. Additionally, the low viscosity of microemulsion restrains its clinical application due to inconvenient use.[5] The problem of poor patient compliance, clinical application and stability can be overcomed by formulating hydrogel thickened microemulsion using carrageenan, carbopol, hydroxypropyl methylcellulose and xanthan gum as a hydrogel thickening agent.

Ibuprofen, a non-steroidal anti-inflammatory drug (NSAID) is very effective for the systemic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Ibuprofen topical preparations may be beneficial to the patients since it reduces the adverse side effects and avoid the hepatic first-pass metabolism. But by topical delivery, it is difficult to maintain effective concentrations, since ibuprofen posses poor skin permeation ability.[6] In order to enhance the permeation of ibuprofen, supersaturated solutions, mucoadhesive patches and vehicle containing non-ionic surfactants or fatty acid have been explored.[7-9]

The aim of the present research work was to formulate hydrogel thickened microemulsion with good stability,

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powerful permeation ability and suitable viscosity for the topical delivery of ibuprofen using eutectic mixture of camphor and menthol as oily phase, solvent for the ibuprofen, power penetration enhancer and imparts cooling effect to the skin. Carbopol 940 was used as a hydrogel thickening agent.

MATERIALS AND METHODS

Materials

Ibuprofen IP and carbopol 940 were received as gift from Zydus Cadila (Ahmedabad, India) and Maruti Chemicals Limited (Ahmedabad, India) respectively. Camphor and menthol were purchased from Gem Corporation (Ahmedabad, India) and Shreeji Pharma International (Ahmedabad, India) respectively. Ethanol IP (90% v/v) was procured from Baroda Chemicals Industries Ltd. (Baroda, India). Tween 80 and triethanolamine (TEA) was purchased from S. D. Fine Chemicals Limited (Ahmedabad, India) and Zion Chemicals Private Limited (Ahmedabad, India) respectively. The other chemicals and reagents were of analytical grade.

Determination of solubility of ibuprofen in eutectic mixture

The solubility of ibuprofen was determined in the eutectic mixture consisting of equal parts of camphor and menthol. An excess amount of ibuprofen was added to 35 ml of eutectic mixture and stirred at 100 rpm on a magnetic stirrer (Remi Electronics, India) for 30 m at 35±2° in a closed vessel. The mixture was filtered through a 0.22 µm millipore filter. The weight of undissolved solid was recorded.

Plotting of ternary phase diagrams

Ternary phase diagrams were constructed to obtain the components and their concentration ranges that can result in large existence area of microemulsion without the drug or containing 1% ibuprofen.[10] Eutectic mixture consisting of equal parts of camphor and menthol was selected as the oily phase. Tween 80 was selected as the surfactant in the study as it was readily miscible with the eutectic mixture. When co-surfactant (ethanol) was used, the ratio of surfactant to co-surfactant was 1:1, 1:2 and 2:1. The ternary phase diagrams (Figure 1) were constructed using water titration method at ambient temperature.[11] For each phase diagram, the ratio of oil to surfactant or mixture of surfactant and co-surfactant was varied from 1:9 to 9:1. Water was added drop by drop, under gentle agitation, to each oily mixture until mixture become turbid. Transparent to translucent fluid systems were characterized as microemulsion. The results are displayed in Figure 1.

Formulation of microemulsion

Ibuprofen (1% w/w) was dissolved in eutectic mixture consisting of equal amount of camphor and menthol. The ibuprofen solution was then mixed with mixture of surfactant and co-surfactant. Finally, an appropriate amount of water was added to the ibuprofen solution mixture drop by drop to get microemulsion. The composition of the different formulated microemulsion (batches F1-F9) is shown in Table 1. The selected microemulsion formulation (batch F1) was subjected to globule size measurement.

Formulation of hydrogel thickened microemulsion

Carbopol 940 was hydrated in fixed amount of water for at least 4 h and then previously formulated microemulsion was gradually added with continuous stirring till clear viscous solution was obtained. Finally, fixed amount of triethanolamine was added to get different hydrogel thickened microemulsions (batches FH1-FH9, Table 2). The hydrogels thickened microemulsions were characterized for pH, viscosity, spreadability, irritation study and *in vitro* drug transport. The results of pH and viscosity are displayed in Table 3.

Evaluations

The selected microemulsion of batch F1 was subjected to photon correlation spectroscopy (Nano ZS90, Malvern Instruments, U.K.) for globule size measurement. The measurement was performed using clear disposable zeta cell at 25°, 210.6 kcps count rate speed for 70 s with 4 attenuator. The pH of hydrogel thickened microemulsion formulations was measured using Electroquip digital pH meter at 25±1°. The viscosity of hydrogel thickened microemulsion formulations was measured at 25±2° using Brookfield viscometer (digital viscometer model DV-II+, Stoughton, MA, USA) employing spindle S96 rotated at 10 rpm. The sprayability and irritation study of the placebo hydrogel thickened microemulsion formulations was carried out onto three healthy, adult human volunteers of 23-25 years age. The volunteers were asked to apply each formulation onto the hand.

The *in vitro* drug transport study of hydrogel thickened microemulsions was carried out in Franz diffusion cell

using rat skin as a membrane. The surface area available for drug release was 4.9 cm^2. The hydrogel thickened microemulsion formulation (1 g) was placed in the donor compartment, while the receptor compartment contained 88 ml of phosphate buffer solution (pH 7.4) containing 1% w/v sodium lauryl sulphate. Sodium lauryl sulphate was used to provide sink condition. Aliquots of 10 ml samples were withdrawn at different time interval from the receptor compartment and ibuprofen was assayed spectrophotometrically at 221 nm. Equal amount of fresh dissolution medium was replaced after each withdrawal. The use of rat skin was approved for the pharmacology students by the ethical committee. Figure 2 shows the percentage drug transported at different time. The UV spectrum of ibuprofen was observed for drug-excipient interaction. The *in vitro* drug transport of selected hydrogel thickened microemulsion of batch FH1 was also determined using human cadaver skin as a membrane in Franz diffusion cell. The human cadaver skin was procured from the post mortem department of local hospital (V. S. hospital, Ahmedabad, India). The departmental review board approved the study using human cadaver skin.

The method of Bamba *et al*. was adopted to ascertain kinetics of drug transport.[12] *In vitro* drug transport data of optimized batch FH1 were analyzed by different kinetic models to evaluate the transport mechanism of ibuprofen.[13] A FORTRAN software, developed in-house, was used. The least value of sum of square of residuals (SSR) and Fisher's ratio (F) were used to select the most appropriate kinetic model (Table 4).

The similarity factor (f_2) was calculated by comparing test and reference drug transport profile using equation 1.[14]

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} * 100\right\}$$

where n is the number of pull points, R_t and T_t are percentage drug released from reference and test products respectively at time't'.

Short- term stability study

The hydrogel thickened microemulsion of batch FH1 was stored in a well closed container for two months at 25±2° and 75% RH. At the end of two months, the formulation was characterized for pH, viscosity and *in vitro* drug transport study. The centrifuge test (15 m, 10,000 rpm) was also carried out to assess the physical stability.

RESULT AND DISCUSSION

A eutectic mixture is a mixture of two or more phases at a composition that has the lowest melting point, and where the phase simultaneously crystallizes from molten solution at a particular temperature. Many substances such as chloral hydrate, betanapthol, lidocaine and prilocaine forms eutectic mixtures. Camphor and menthol forms eutectic mixture. Camphor is readily absorbed through the skin and produces a feeling of cooling similar to that of menthol and acts as mild local anesthetic and antimicrobial substance. Menthol is widely used in pharmaceuticals, confectionery, and toiletry products as a flavoring agent or odor enhancer. The aim of the present study was to develop a hydrogel thickened microemulsion using eutectic mixture of menthol and camphor which is novel, industrial acceptable, functional yet technologically difficult to copy creating a high barrier to reverse engineering by counter feiters. The eutectic mixture worked as an oily phase and solvent mixtures for the solubilization of the ibuprofen. The solubility of ibuprofen in the eutectic mixture containing equal parts of menthol and camphor was >160 mg/ml. Camphor and menthol are well known dermal penetration enhancers.[15-21] Both camphor and menthol causes leaching of the lipids present in the skin resulting into subsequent pore formation.[22] In addition, camphor and menthol posses antifungal activity of its own.[23-24] Therefore, the eutectic blend can be considered as a multifunctional excipient.

Ternary phase diagrams

The construction of pseudo-phase diagram makes it easy to find out the concentration range of components for the existence range of microemulsions. The transparent to translucent microemulsion region is presented in phase diagrams (Figure 1). No distinct phase inversion of microemulsions was observed. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. Figure 1 shows that the incorporation of co-surfactant (ethanol) increased the maximum amount of incorporated water in the oil-surfactant system with the microemulsion zone being increased in all cases compared to the co-surfactant free system. As the ratio of surfactant to co-surfactant increases, the existence area of microemulsion becomes

enlarged, reaching maximum at 2:1. Addition of surfactant co-surfactant mixture in 2:1 ratio increased the water incorporation to a maximum of 90% compared to 57% in the co-surfactant free system. Based on these results, microemulsions containing 1% ibuprofen were prepared at surfactant to co-surfactant ratio of 2:1.

Microemulsions

The microemulsions containing ibuprofen resulted in similar phase diagrams as for the microemulsions without the drug. In these formulations, the content of oil phase (eutectic mixture) was varied as 19, 29 and 39%, while the content of surfactant co-surfactant mixture was varied as 30, 40 and 50%. The detailed composition of nine different microemulsions is shown in Table 1. All these formulations existed inside the area of the microemulsion formation, thus, forming clear microemulsion at the additive concentrations examined. The droplet size of selected microemulsion (batch F1) ranged from 50-250 nm with average droplet size of 99 nm and polydispersity index (PDI) value of 0.097 indicating narrow size distribution.

Hydrogel thickened microemulsions

Hydrogel thickened microemulsions were formulated using carbopol 940 (Table 2). Table 3 shows that the pH and viscosity of the hydrogel thickened microemulsion of batches FH1-FH9 ranged from 7.1-8.04 and 51020-83998 cPs respectively. The pH and viscosity of hydrogel thickened microemulsion gradually increased with decrease in concentration of eutectic mixture and surfactant co-surfactant mixture in the formulation. None of the placebo hydrogel thickened microemulsion formulations resulted in irritation, rashes and itching in any of the volunteers. Water washability and spreadability of the hydrogel thickened microemulsions (batches FH1-FH9) was good.

The formulation excipient did not show absorbance at 221 nm. The UV spectrum remained unchanged during *in vitro* drug transport study, indicating stability of ibuprofen during the analytical procedure. Figure 2 shows that as concentration of eutectic mixture increases, the percentage drug transport also increases at each time point. The viscosity and the powerful penetration enhancer effect of the camphor and menthol are believed to affect the stated parameter. Camphor and menthol are well known dermal penetration enhancers.[15-21] Both camphor and menthol causes leaching of the lipids present in the skin resulting into subsequent pore formation.[22] Complete drug transportation was seen in hydrogel thickened microemulsion of batches FH1-FH3 formulated using highest concentration of eutectic mixture. When the content of oil (eutectic mixture) was fixed to 15.51, 23.67 and 31.84% in the hydrogel thickened microemulsion, the drug transport rate depends on the concentration of surfactant co-surfactant mixture and viscosity of the formulation. As the content of surfactant co-surfactant mixture was decreased in the hydrogel thickened microemulsion from 40.82 to 24.49%, the drug transportation rate decreases 0.008 to 0.096 times. Probable reason for it could be penetration enhancement effect of ethanol.[25] The average drug transport rate from hydrogel thickened microemulsions of batches FH1-FH9 ($\mu g/ml*h*cm^2$) were 1.94, 1.90, 1.92, 1.85, 1.80, 1.77, 1.69, 1.62 and 1.53 respectively. Fastest drug transport was observed in batch FH1 containing 31.84% eutectic mixture and 40.82% mixture of surfactant and co-surfactant in the total hydrogel thickened microemulsion formulation. Hence, considering the results of *in vitro* drug transport, hydrogel thickened microemulsion of batch FH1 was ranked as optimized batch and taken for further study.

The *in vitro* drug transport data of hydrogel thickened microemulsion of batch FH1 were analyzed for establishing kinetics of drug transport. Model fitting was done using an in-house program developed by the authors. Zero-order, first-order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas and Weibull models were tested. Table 4 depicts that the best fit was shown by Higuchi model with least sum of square of residuals (SSR) and Fischer's (F) ratio. The mechanism of transport was by anamolous diffusion (diffusion co-efficient, n=0.58).

The *in vitro* drug transport study of optimized hydrogel thickened microemulsion of batch FH1 was also studied using human cadaver skin in Franz diffusion cell. Statistically the transport profiles through rat skin (reference profile) and human cadaver skin (test profile) were dissimilar (f_2=48). The percentage *in vitro* drug transport of optimized batch through rat skin at the end of 12 h was 0.12 times higher than that obtained in human cadaver skin indicating rat skin is more permeable than

human cadaver skin.

Short term stability study

Short term stability study of the hydrogel thickened microemulsion of batch FH1 was carried out for two months at 25±2° and 75% RH. The pH and viscosity of the hydrogel thickened microemulsion at the end of stability study was 6.25 and 51185 cPs respectively. No change of phase separation was observed during 2 months. The centrifuge test showed that hydrogel thickened microemulsion had a good physical stability. *In vitro* drug transport profile of hydrogel thickened microemulsion of batch F1 before (reference profile) and at the end of stability study (test profile) was similar (f_2 = 87). The amount of drug transported at the end of 12 h from hydrogel thickened microemulsion of batch F1 was 98.9%.

CONCLUSION

A hydrogel thickened ibuprofen microemulsion was formulated into the laboratory for transdermal application using eutectic mixture of camphor and menthol as oily phase and carbopol 940 as hydrogel thickening agent. The components and their concentration ranges for the formation of microemulsion were obtained using the construction of ternary phase diagram. Their concentrations were optimized after the evaluation of *in vitro* drug transport. The presence of eutectic mixture of camphor and menthol and ethanol in the hydrogel thickened microemulsion increased the transportation rate of ibuprofen.

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Table No. 1 - Composition of different formulated microemulsion

Batch code	Amount of each ingredient (% w/w)			
	Ibuprofen	Eutectic	S/C *	Water
F1	1	39	50	10
F2	1	39	40	20
F3	1	39	30	30
F4	1	29	50	20
F5	1	29	40	30
F6	1	29	30	40
F7	1	19	50	30
F8	1	19	40	40
F9	1	19	30	50

*S/C * is the mixture of surfactant to co-surfactant (2:1)*

Table No. 2 - Composition of different formulated hydrogel thickened microemulsion

Batch code	Ingredients			
	Carbopol 940	Water	Microemulsion	Triethanolamine
FH1	1.5 g	20 g	100 g of batch F1	1 g
FH2	1.5 g	20 g	100 g of batch F2	1 g
FH3	1.5 g	20 g	100 g of batch F3	1 g
FH4	1.5 g	20 g	100 g of batch F4	1 g
FH5	1.5 g	20 g	100 g of batch F5	1 g
FH6	1.5 g	20 g	100 g of batch F6	1 g
FH7	1.5 g	20 g	100 g of batch F7	1 g
FH8	1.5 g	20 g	100 g of batch F8	1 g
FH9	1.5 g	20 g	100 g of batch F9	1 g

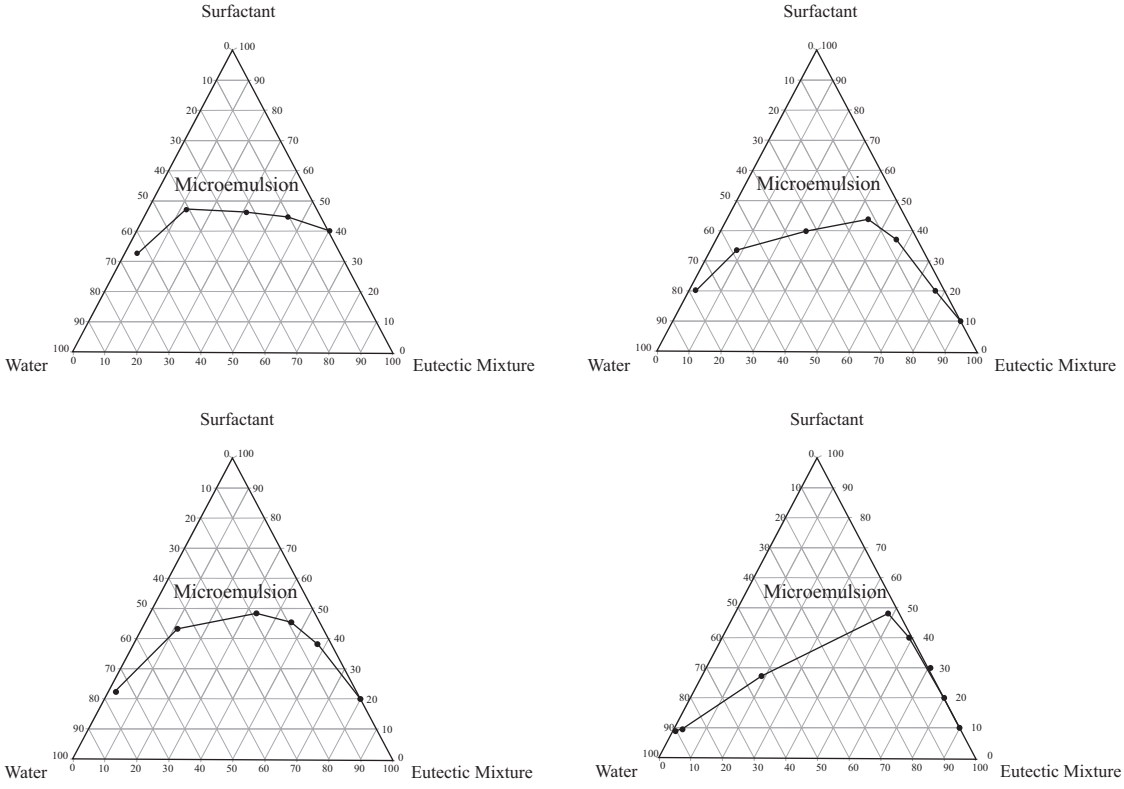
Table No. 3 - Results of pH and viscosity of the various hydrogel thickened microemulsion formulations

Test	Batch code								
	FH1	FH2	FH3	FH4	FH5	FH6	FH7	FH8	FH9
pH (a±0.05)	7.1	7.23	7.5	7.59	7.68	7.74	7.9	7.98	8.04
Viscosity (b±250 cPs)	51020	52551	55080	68756	69112	70121	82324	830127	83998

Table No. 4 - Results of model fitting of batch FH1

Parameter	Models					
	Zero order	First order	Higuchi	Hixon Crowell	Korsmeyer-Peppas	Weibull
F *	134.46	5186.52	14.23	23.64	23.24	50.55
SSR^{+}	806.76	31119.16	85.42	141.84	116.21	252.78

Fig. 1. The ternary phase diagrams of eutectic mixture, tween 80 and water in presence and absence of co-surfactant (ethanol).



The ternary phase diagrams of eutectic mixture, tween 80 and water in presence and absence of co-surfactant (ethanol); (a) co-surfactant free system,(b) 1:1 ratio of tween 80 and ethanol, (c) 1:2 ratio of tween 80 and ethanol, (d) 2:1 ratio of tween 80 and ethanol.

Fig. 2. Percentage drug transport from different formulations (FH1-FH9).

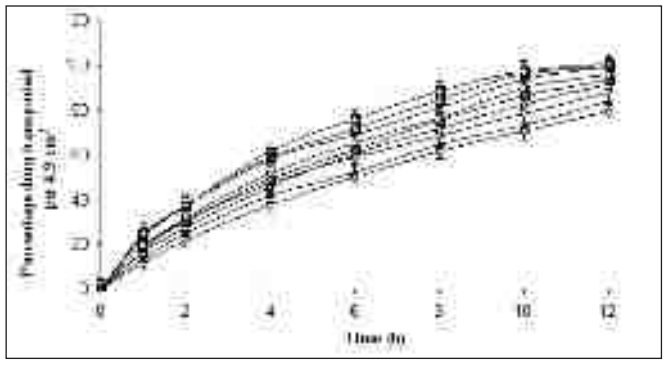


Fig. 2. Percentage drug transport from different formulations (FH1-FH9); FH1(–■–)
FH 2 (–▣–), FH 3(–●–), FH 4(–□–), FH 5(–▩–), FH 6(–△–), FH 7(–○–)
FH 8 (–▲–),FH 9 (–◊–)

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