Research Article

In Vitro Sonicated Transdermal Transport Across Hairless Rat Skin Using Optimized Batch of Ketorolac Tromethamine Gel

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ABSTRACT

The aim of present work is to develop sonophoretic transdermal drug delivery system of Ketorolac tromethamine (KT) and evaluation of it. In vitro dissolution study was carried out for simple gel, proniosomal gel and marketed gel in phosphate buffer pH 7.4. The high release rate formulation is optimized using response surface methodology by face centered central composite design (FCCCD). In the present study, various batches of gel of KT were prepared along with carbopol 940 and PEG 400 in different combinations as per the design expert (8.0.5). The prepared gels were evaluated for clarity, homogeneity, viscosity, drug content, pH, spreadability, extrudability, *in vitro* permeation studies. The optimized batch was further subjected to kinetic modeling studies and sonophoretic treatment in both continuous and pulsed modes across hairless rat skin. It was concluded from the data that the sonophoretic treatment of formulation had more *in vitro* drug permeation as compared to the permeation without sonophoresis.

Keywords: Carbopol 940, Ketorolac tromethamine, PEG 400, Sonophoresis, Transdermal.

INTRODUCTION

The delivery of drugs transdermally (through the skin) provides several important advantages over traditional oral and intravenous delivery routes. Transdermally delivered drugs avoid the risk and inconvenience of intravenous therapy, usually provide less chance of an overdose or under dose, allow easy termination, and permit both local and systemic treatment effects. Transdermal drug delivery offers controlled release of the drug into the patient, it enables a steady blood level profile, and resulting in reduced systemic side effects.[1] Systemic as well as topical delivery of drugs via the transdermal route is limited by the low skin permeability which is attributed to the stratum corneum (SC), the outermost layer of the skin. The SC consists of disk-like dead cells (keratinocytes) containing keratin fibers and water, surrounded by densely-packed lipid bilayers. The ordered structure of the lipid bilayers confers a highly impermeable character to the SC.[2,3] Different approaches including chemical enhancers, iontophoresis and sonophoresis have been investigated to increase skin permeability.[3, 4]

Ultrasound at frequencies between 20 kHz and 16 MHz has been shown to transiently enhance the skin permeability in a process referred to as sonophoresis.[2] The ultrasound probably enhances drug transport by cavitation, microstreaming, and heating.[5,6] Ultrasound mediated transdermal delivery of key compounds was first reported in 1954 by Fellinger and Schmid through successful treatment of digital polyarthritis using hydrocortisone ointment in combination with ultrasound.[5, 7, 8] Ultrasound frequencies in the range of 1-3 MHz and applied intensity levels between 1 and 3 W/cm^2 were used because of the availability of commercial equipment.[8] Sonophoresis by ultrasound has several advantages. It has a low risk of burning the skin, is not necessary to ionize the drugs, and its permeability is approximately 5 cm and its treatment time is short. The biological changes made by the mechanical and thermal effects of ultrasound includes the promotion of blood circulation, an increase in the tissues' regenerative power, an increase in the membrane permeability, an improvement in tissue circulation, change of peripheral nerve conduction velocity, and muscle relaxation, reduction of pain. Because NSAIDs are administered for an extended period, the current resurgence of interest in the transdermal administration as a route for systemic drug delivery using sonophoresis is a logical approach for increasing the rate of drug permeation across the epithelium.[9]

Ketorolac tromethamine (KT) is a non-steroidal agent with potent analgesic and moderate anti- inflammatory activity. KT works by competitive blocking of the enzyme cyclooxygenase (COX) which is involved in the production of various chemicals in the body, some of which are known as prostaglandins. Ketorolac (as tromethamine salt) is administered intramuscularly and orally in divided multiple doses for short-term management of post operative pain (30 mg q. i. d. by IM injection and 10 mg q. i. d. as oral tablets). This frequent dosing, which results in unacceptable patient compliance, is required due to the short half-life of the drug (4-6 h). Although oral bioavailability of KT was reported to be 90% with a very low first-pass metabolism, its short biological half-life and many adverse effects, such as upper abdominal pain and gastrointestinal ulceration, restrict its oral use. To avoid invasive drug therapy such as injections and to eliminate

frequent dosing regimen with oral administration, a transdermal drug delivery system has been studied as an alternative dosage form. Its high analgesic activity and low molecular weight make KT a good candidate for transdermal delivery.[10]

The current study aims at developing and optimizing the gel formulation of KT using Carbopol 940 and PEG 400 and optimizes the formulation using RSM. Use of response surface methodology has been proved to be useful tool on the development and optimization. Different steps involved in RSM include experimental design; regression analysis, constraint optimization and validation.[11] The effect of continuous and pulsed ultrasound modes were studied in vitro on the permeation of optimized formulation of gel across hairless rat skin.

MATERIALS AND METHODS

Materials

Ketorolac tromethamine was obtained as a gift sample from Ranbaxy Laboratories Ltd., Gurgaon. Carbopol 940 was purchased from Macleod Pharmaceuticals, Baddi. PEG 400, ethanol, triethanolamine were purchased from S.D. Fine-Chem Limited, Mumbai. Propylene glycol was obtained from Central Drug House Ltd., New Delhi. Cholesterol, lecithin and span 60 were obtained from Hi Media Lab. Pvt. Ltd., Mumbai. All other chemicals used were of analytical grade. Freshly prepared distilled water used throughout the study.

Methods

Preparation of various gels of Ketorolac tromethamine

Simple gel

Table 1: Composition of the KT gel

Composition	KT gel (g)
KT	0.1
Carbopol 940	1.0
Propylene glycol	20.0
Polyethylene glycol 400	5.0
Glycerin	10.0
Peppermint oil	2.0
Ethanol	10.0
Triethanolamine	1.5
Distil. Water	50.4
Total	100

Procedure: About 0.1g of KT was weighed and dissolved in 10g of ethanol. To this solution, specified quantity of glycerin and propylene glycol was added and dissolved (solution A). Weighed quantity of carbopol 940 was added to the distilled water, added PEG 400 and stirred to dissolve the same. The solution was then neutralized and made viscous by addition of triethanolamine (solution B). Solution B was then added drop wise in Solution A with constant stirring and the final weight was made up to 100g.[12, 13]

Proniosomal gel

Proniosomal gel preparation involved mixing of surfactant (24.4g), cholesterol (2.7g), lecithin (24.4g) and the drug (0.1g) with 26.7g of ethanol. After mixing all the ingredients, it was covered with a lid to prevent the loss of solvent and warmed on a water bath at 60° - 70°C until the surfactant dissolved completely. To it added 21.7g of aqueous phase (phosphate buffer pH 7.4 solution). It was warmed again to form a clear solution, which on storage for overnight under dark converted into proniosomal gel.[14, 15]

Characterization method for Ketorolac tromethamine gel

The prepared simple gel, proniosomal gel and drug dispersed in marketed gel were characterized by *in-vitro* dissolution studies (Release rate).

In vitro dissolution studies

Dissolution study was carried out by using USP apparatus-II, paddle type for 8 hr. The stirring rate was 50 rpm. Phosphate buffer pH 7.4 was used as medium (900 ml) and was maintained at 37 ± 5^0C. Samples (5ml) were collected at regular interval of time (0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hr) and assayed for dissolution spectroscopically at 322 nm. Each dissolution rate test was repeated thrice and average values were reported.

Experimental design for formulations of carbopol gel

Two independent variables, the amount of Carbopol 940 (X_1) and PEG 400 (X_2) were studied at 3 levels each. The central point (0, 0) was studied at quintuplicate. All other formulation and processing variables were kept invariant throughout the study. Table 2 summarized an account of the 13 experimental runs studied, their factor combinations, and the translation of the coded levels to the experimental units employed during the study as per the software design expert (8.0.5). Cumulative % drug permeation (% CDP) was taken as the response variables.

Table 2: Formula used for the formulation of KT simple gels as per design expert

Formulation code	Drug (mg)	Carbopol 940 (g)	PEG 400 (g)
KT_1	100	0.5	2.5
KT_2	100	2	2.5
KT_3	100	0.5	10
KT_4	100	2	10
KT_5	100	0.5	6.25
KT_6	100	2	6.25
KT_7	100	1.25	2.5
KT_8	100	1.25	10
KT_9	100	1.25	6.25
KT_{10}	100	1.25	6.25
KT_{11}	100	1.25	6.25
KT_{12}	100	1.25	6.25
KT_{13}	100	1.25	6.25

Procedure for preparation of KT Simple gels

Gels were prepared by the method as described above.

Evaluation of gels

Physical appearance and Homogeneity

The physical appearance and homogeneity of the prepared gels were tested by visual observations after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.[13, 16-17]

Clarity

The clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows; turbid: +, clear: ++, very clear (glassy): +++.[18]

Measurement of pH

The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were calculated.[13, 16, 18-19]

Viscosity study

The measurement of viscosity of the prepared gel was done with the Brookfield Viscometer.[16, 18-19]

Spreadability

The spreadability of the gel was determined using the following technique: 0.5g gel was placed within a circle of 1 cm diameter premarked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5min. The increase in the diameter due to spreading of the gels was noted.[17]

Extrudability

The formulations were filled into collapsible aluminium tubes. The tubes were pressed to extrude the 0.5 cm ribbon of the gel in 10 second and the extrudability of formulations was checked.[18]

Drug content

A specific quantity (100mg) of gel was taken and dissolved in 100ml of phosphate buffer of pH 7.4. The volumetric flask containing gel solution was shaken for 2hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometrically at 322 nm using phosphate buffer (pH 7.4) as blank.[13, 16, 19]

In vitro permeability studies

Franz diffusion cell is one of the most widely used systems for *in vitro* skin permeation studies. The cell consists of a small donor and receptor compartment which is stirred by a teflon coated magnetic bead. The drug delivery is by the vertical movement of drug from donor phase through the skin into the receptor phase. Albino rat skin was mounted with the stratum corneum side facing the donor compartment. The available diffusion area between cells was 6.21 cm^2. In the donor compartment, the formulation of gel was placed in intimate contact with the skin. The receptor compartment was filled with phosphate buffer pH 7.4, kept at constant temperature of 37±0.5^{0}C and stirred by a magnetic stirrer. The top of the donor compartment was covered with aluminium foil. At appropriate intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 24 hr), 5 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. Samples were analyzed spectrophotometrically at 322 nm.

Preparation of rat skin

The *in vitro* experiments using rat skin were carried out after obtaining approval of the Institutional Animal Ethics Committee of Kurukshetra University, Kurukshetra, India and their guidelines were strictly followed. The skin of male albino rats (150-200 gm) was used as the main barrier for permeation studies of KT. Hairless rats were killed by cervical dislocation. Skin was excised from the abdominal region and washed with purified water. The subcutaneous tissues adhering to the skin were separated with the help of scalpels and dermis side was wiped with isopropyl alcohol to remove the residual adhering fat. Following pretreatment, the skin was cut and trimmed to appropriate size and was used immediately.[20]

Sonophoretic treatment

Optimized batch of the gel was further considered for the sonophoretic treatment. Ultrasound was applied using ultrasound generator operating at a frequency of 1 MHz. A 1 MHz frequency was used since a variable frequency generator was not available. The diameter of ultrasound probe was 1 cm. The ultrasound was applied in 2 different ways- firstly, *concurrent ultrasound studies* (in this, sonication was undertaken immediately after topical deposition of the medicated gel) and secondly, *pretreatment ultrasound studies* (in this, the unmedicated marketed gel was applied on the skin. Then, ultrasonic treatment was given to the skin and after that the gel was wiped out and now, the medicated gel was applied on the skin). Ultrasound was applied both for continuous and pulsed mode (1:1).

In the present study, the ultrasound transducer was placed in the donor compartment, having KT gel, of franz diffusion cell. The receptor compartment was filled with phosphate buffer pH 7.4 and stirred with a magnetic stirrer. The available diffusion area between cells was 6.21 cm^2. The treatment was given using intensity 1 W/cm^2 for 30 min in continuous and pulsed mode. The transducer was kept stationary. At appropriate intervals (1, 2, 4, 6, 8, 10, 24 hr), 5 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. Samples were analyzed spectrophotometrically at 322 nm.

Mathematical kinetic assessment for drug release mechanism

Release kinetics is an integral part for the development of a dosage form because if the kinetics of drug release is known, one can also established *in vivo in vitro* (IVIVC) correlation. Mathematical approach is one of scientific methods to optimize and evaluate the error in terms of deviation in the release profiles of formulated products during the formulation development stage. Mathematical model approach important in research and development because of its simplicity and their interrelationships may minimize the number of trials in final optimization, thereby improving the formulation development process. The permeation profile of the optimized batch was fitted to the different kinetic models.[21]

In vitro drug release data were fitted to kinetic models

Qt versus t (zero order)

Log (Q_0-Q_t) versus t (first order)

Qt versus square root of t (Higuchi)

log %Qt versus log %t (Korsmeymer-Peppas)

Where Qt is the amount of drug released at time t. The criteria for selecting the most appropriate model are lowest sum of square of residuals (SSR) and highest R^2 value. Lowest sum of square of residuals (SSR) indicate the minimum variance between the predicted and observed dissolution data. Highest R^2 value indicates linearity of dissolution data.

RESULTS AND DISCUSSION

In- vitro dissolution study

In vitro dissolution study was carried out for simple gel, proniosomal gel and marketed gel in phosphate buffer pH 7.4. The % cumulative drug release from the formulations was given in figure 1.

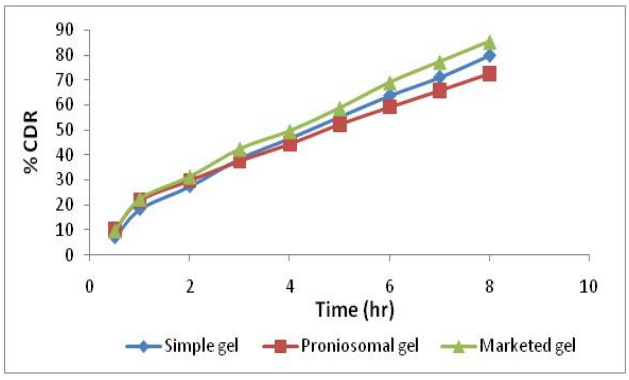

Figure 1: Dissolution profile of simple gel, proniosomal gel and marketed gel

The release rate of simple gel was higher than the proniosomal gel in comparison to the marketed preparation. So, according to the data obtained from the release pattern, simple gel was used for further optimization studies.

Preparation and evaluation of simple gels

Gels were successfully prepared. Formulated gels were subjected to various characterization parameters for their evaluation.

Evaluation of gels

All studies were carried out in triplicate and average values were recorded (table 3).

Clarity

All gels were found to be transparent and were free from presence of particles.

Homogeneity

All the gel formulations showed good homogeneity with absence of lumps.

pH

The pH of the gel formulations was in the range of 6.3 to 6.9, which lies in the normal pH range of the skin and would not produce any skin irritation.

Viscosity measurement

Viscosity of various formulated gels was found in the range of 8,675 to 13,817 centipoises.

Spreadability

Spreadability diameter for different formulations showed good spreadability i.e. gel is easily spreadable.

Extrudability

The extrudability of formulations was found to be good.

Drug Content analysis

The drug content of the gel formulations was in the range of 97.24 to 101.46, showing content uniformity.

In vitro permeability studies

In vitro permeation studies of the formulations by franz diffusion cell were performed using pH 7.4 phosphate buffer as medium and measuring drug concentration spectrophotometrically at 322 nm. The cumulative percent of drug permeates at different time intervals were shown in figure 2.

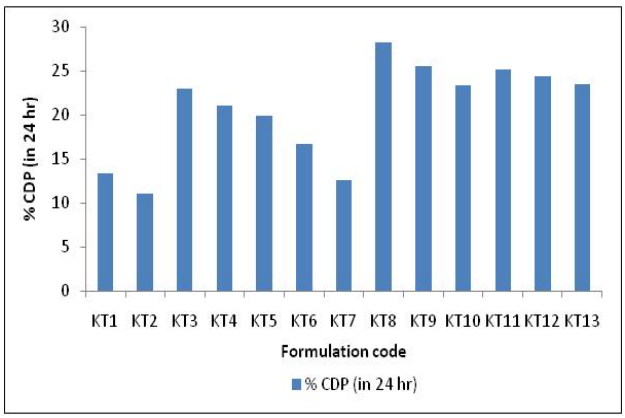

Figure 2: % CDP for formulation KT_1 to KT_{13}

Table 3: Evaluation of the prepared formulations of gel

Formulation Code	Clarity	Homogeneity	pH	Viscosity measurement (cps)	Spreadability (cm)	Drug Content analysis	Extrudability
KT_1	++	Homogenous	6.9	8,675	4.1	98.92	+
KT_2	++	Homogenous	6.2	12,889	2.7	98.53	+
KT_3	+++	Homogenous	6.5	8,953	3.7	101.30	+
KT_4	++	Homogenous	6.3	13,817	2.6	99.95	+
KT_5	+++	Homogenous	6.5	8,851	3.8	98.82	+
KT_6	++	Homogenous	6.6	13,098	2.7	99.02	+
KT_7	++	Homogenous	6.5	9,832	2.8	98.92	+
KT_8	++	Homogenous	6.3	10,928	3.1	101.46	++
KT_9	+++	Homogenous	6.9	10,219	2.9	98.74	++
KT_{10}	++	Homogenous	6.4	10,676	2.8	99.13	+
KT_{11}	+++	Homogenous	6.3	10,704	2.9	97.24	++
KT_{12}	++	Homogenous	6.7	10,346	3.0	98.65	++
KT_{13}	++	Homogenous	6.6	10,482	2.8	99.48	++

NOTE: + Satisfactory, ++ Good, +++ Excellent

Table 4: ANOVA for response surface quadratic model

Response factor	Model F-value	p-value Prob>F	Lack of fit	
			F-value	p-value
% CDP	47.84	< 0.0001	2.90	0.165

An increase in carbopol content was associated with a corresponding decrease in the drug-permeation rate. This could be due to extensive swelling of the polymer which created a thick gel barrier for drug diffusion. The drug permeation was increased linearly with the increasing concentration of PEG 400 (hydrophilic polymer). This is due to the fact that dissolution of aqueous soluble fraction of the polymer matrix leads to the formation of gelaneous pores. The formation of such pores leads to decrease the mean diffusion path length of drug molecules to release into the diffusion medium and hence, to cause higher release rate.

Formulation KT_8 showed maximum drug permeation in 24 hr which contained optimum concentration of carbopol (1.25 g) and maximum concentration of PEG 400 (10 g). All other evaluation parameters like clarity, pH, viscosity, drug content, spreadability and extrudability are suggestive of good characteristic properties of optimized formulation.

Optimization of formulations using face centered central composite design (FCCCD)

Response surface methodology (RSM) for simple gel

Response surface methodology allows understanding of the behavior of the system by demonstrating the contribution of the independent variables. An experimental design organizes the experiments in such a manner that the required information is obtained as efficiently and precisely as possible. Runs or trials are the experiments conducted according to the selected experimental design.

ANOVA- Analysis of variance

Analysis of variance of the responses indicated that response surface model developed for % cumulative drug permeate (24 hr) was significant and adequate, without significant lack of fit (table 4).

Mathematical Modeling

Mathematical relationship generated using multiple linear regression analysis for the studied response variables are expressed as equation given below:

$$\% CDP = + 24.05 - 1.21 X_1 + 5.9X_2 + 0.12 X_1 X_2 \ldots\ldots (1)$$

The polynomial equation comprised the coefficient for intercept, first-order main effect, interaction term and higher order effect. The sign and magnitude of the main effect signified the relative influence of each factor on the response.

Cumulative drug permeation (%CDP)

The polynomial equation (1) for % CDP denoted that the coefficient X_1 bear a negative sign and X_2 bear a positive sign. Therefore, increasing the concentration of Carbopol 940 was expected to decrease the % CDP and increasing the concentration of PEG 400 was expected to increase the % CDP.

Mathematical modeling to study the *in-vitro* permeation kinetics of optimized batch

The model for best fit was predicted from the value of R^2. For an ideal fit, value of R^2 was 1. Hence, the model which gives the R^2 value nearest to 1 describes the order of drug permeation. From the results of data fitting to various models, it was found that the optimized batch KT_8 showed Higuchi model of drug release , i.e. mechanism followed for the drug release from the KT gel was diffusion controlled and was square root of time dependent.

Sonophoretic treatment of optimized batch (KT_8)

Sonophoresis significantly increased the permeability of KT across rat skin in comparison to control. Based on *in vitro* skin permeation studies by franz diffusion cell, significant enhancement was achieved with *pretreatment ultrasound studies* for both in continuous and pulsed mode (P_C, P_P) as compared to *concurrent ultrasound treatment* for both in continuous and pulsed mode (C_C, C_P) shown in figure 3.

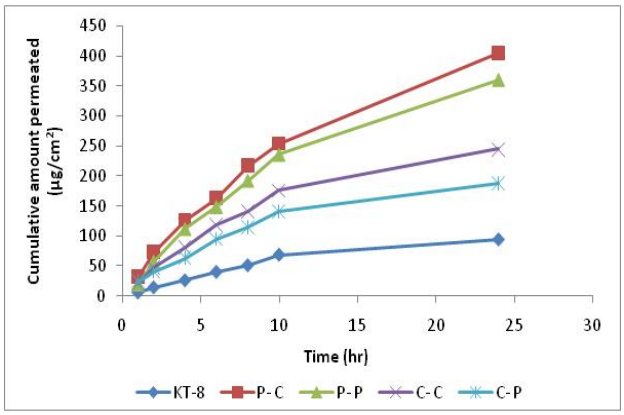

Figure 3: Effect of ultrasound parameters on KT transport across rat skin

Table 5: Permeability Flux and Enhancement factor for different protocols

Different protocols	Flux (µg/cm²/h)	Enhancement Factor
Passive (KT_8)	3.73	-
P_C	15.58	4.17
P_P	14.2	3.8
C_C	9.3	2.49
C_P	6.9	1.85

Moreover, the continuous mode had greater enhancement on KT permeation than pulsed mode of ultrasound application for both the protocols. The permeation rate followed the order:

$$P_C > P_P > C_C > C_P > KT_8$$

After the sonophoretic treatment, the cumulative amount permeation of KT increased from 93.79 to 404.98µg/cm^2. For further illustration, the flux and the enhancement factor were calculated from the slopes of the graph and listed in table 5. The highest flux value was observed with the pretreatment studies with continuous mode (15.58µg/cm^2/h) as compared to the other protocols.

Therefore, of all the experimental combinations of the sonophoresis, pretreatment for continuous mode (P_C) gave the most promising results i.e. it enhanced the passive permeation of drug by 4.17 fold.

CONCLUSION

It was concluded from the data obtained on the basis of various types of characterization parameters that the sonophoretic treatment of formulation increased *in vitro* drug permeation as compared to the passive permeation due to modification of the barrier properties of skin. From the results of data fitting to various models, it was found that the optimized batch showed Higuchi model of drug release.

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