

FORMULATION, EVALUATION AND OPTIMIZATION OF TRANSDERMAL GEL OF KETOROLAC TROMETHAMINE USING FACE CENTERED CENTRAL COMPOSITE DESIGN

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ABSTRACT

Objective: The present research has been undertaken with the aim to develop a transdermal gel formulation of Ketorolac tromethamine (KT), which would attenuate the gastrointestinal related toxicities associated with oral administration.

Methods: In vitro dissolution study was carried out for carbopol 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). DSC and FTIR were used to characterize the gels. 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.

Results: No chemical interaction was found between KT and polymers. KT₈ was found to be optimized formulation according to the FCCCD. The optimized formulation was further subjected to kinetic modeling studies which showed Higuchi model of drug release.

Conclusion: It was concluded from the results that the optimized formulation showed maximum *in-vitro* drug permeation. Stability studies under accelerated condition showed satisfactory results for the optimized formulation.

Keywords: transdermal; ketorolac tromethamine; carbopol 940; PEG 400; FTIR

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 underdose, 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, resulting in reduced systemic side effects [1].

The U.S.P. defines gels as semisolids, either suspension of small inorganic particles or large organic molecules interpenetrated with liquid. Gels are transparent or translucent semisolid formulations containing a high ratio of solvent/gelling agent. When dispersed in an appropriate solvent, gelling agents merge or entangle to form a three-dimensional colloidal network structure, which limits fluid flow by entrapment and immobilization of the solvent molecules. The network structure is also responsible for gel resistance to deformation and hence, its viscoelastic properties [2-3].

Ketorolac tromethamine (KT) is a nonsteroidal 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 [4].

The current study aims at developing and optimizing the gel formulation of KT using Carbopol 940 and PEG 400 and optimizes the formulation using response surface methodology (RSM). Use of RSM 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 [5]. The prepared gels were evaluated for clarity, homogeneity, viscosity, drug content, pH, spreadability, extrudability, *in-vitro* permeation studies.

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. All other chemicals used were of analytical grade. Freshly prepared distilled water used throughout the study.

Methods

FTIR studies

The FTIR spectra of the pure drug (ketorolac tromethamine) and its physical mixture (drug + carbopol 940 + PEG 400) were recorded with FTIR spectrophotometer. The samples were prepared by using potassium bromide and scanned for the absorbance at 4000-400/cm, Perkin Elmer.

Differential Scanning Calorimetry (DSC)

The DSC thermograms of pure drug (ketorolac tromethamine) and its physical mixture (drug + carbopol 940 + PEG 400) were carried out to investigate any possible interaction between the drug and the

utilized polymers. DSC analyses were performed using a Mettler TA 4000 apparatus, equipped with a DSC 25 cell. Weighed samples (1-5mg, Mettler MX5 microbalance) were scanned in Al pans pierced with a perforated lid at $10^{\circ}\text{Cmin}^{-1}$ in the 25-350°C temperature range, under static air.

Preparation of various gels of Ketorolac tromethamine

➤ Carbopol 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 dropwise in Solution A with constant stirring and the final weight was made up to 100g to get the final gel preparation [6-7].

➤ Proniosomal gel

Procedure: Proniosomal gel preparation involved mixing of span 60, cholesterol, lecithin and the drug with 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 (span 60) dissolved completely. To that an aqueous phase (phosphate buffer pH 7.4 solution) was added. It was warmed again

to form a clear solution, which on storage for overnight converted into proniosomal gel [8-9].

Table 2: Composition of the KT proniosomal gel

Composition	KT Proniosomal gel (g)
KT	0.1
Span 60	24.4
Lecithin	24.4
Cholesterol	2.7
Ethanol	26.7
Aqueous solution (Phosphate buffer 7.4 pH)	21.7

Characterization method for Ketorolac tromethamine gel

The prepared carbopol 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 across egg membranes 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 $37 \pm 5^{\circ}\text{C}$. Samples (5ml) were collected periodically at 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hr and assayed for dissolution spectroscopically at 322 nm. Each sample was replaced with equal volume of fresh dissolution medium, and 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 3 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 variable.

Table 3: Formula used for the formulation of carbopol gels as per design expert

S. No.	Formulation code	Drug (mg)	Carbopol 940 (g)	PEG 400 (g)
1	KT ₁	100	0.5	2.5
2	KT ₂	100	2	2.5
3	KT ₃	100	0.5	10
4	KT ₄	100	2	10
5	KT ₅	100	0.5	6.25
6	KT ₆	100	2	6.25
7	KT ₇	100	1.25	2.5
8	KT ₈	100	1.25	10
9	KT ₉	100	1.25	6.25
10	KT ₁₀	100	1.25	6.25
11	KT ₁₁	100	1.25	6.25
12	KT ₁₂	100	1.25	6.25
13	KT ₁₃	100	1.25	6.25

Procedure for preparation of Ketorolac tromethamine carbopol gels

Carbopol gels were prepared by the method as described above.

Evaluation of carbopol gels

a) 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 [7, 10-11].

b) 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): +++ [11].

c) 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 [7, 10-12].

d) Viscosity study

The measurement of viscosity of the prepared gel was done with Brookfield Viscometer [10-12].

e) 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 [13].

f) Extrudability

The extrusion of the gel from the tube is an important during its application and in patient acceptance. This study is useful in explaining whether the gel is removing from the collapsible tube during application in proper manner or not. Gels with high consistency may not extrude from the tube whereas, low viscous gels may flow quickly, and hence suitable consistency is required in order to extrude the gel from the tube. The formulations were filled into collapsible aluminum 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 [12].

g) 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 [7, 10-11].

h) 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 Teflon coated magnetic bead (figure 1). 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 cm². 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°C and stirred by a magnetic stirrer. The top of the donor compartment was covered with aluminum 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.



Fig. 1: Experimental Set up for *in vitro* permeation studies

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 [14].

Mathematical kinetic assessment for drug release mechanisms

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 formulation was fitted to the different kinetic models [15].

In vitro drug release data were fitted to kinetic models

Qt versus t (zero order)

Log (Q₀-Q_t) versus t (first order)

Qt versus square root of t (Higuchi)

log %Qt versus log %t (Korsmeyer-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 R² value. Lowest sum of square of residuals (SSR) indicate the minimum variance between the predicted and observed dissolution data. Highest R² value indicates linearity of dissolution data.

Accelerated stability studies of optimized formulation

The stability studies were conducted according to ICH guidelines by storing the formulation at 40 ± 2°C / 75% RH in stability chamber for three months. The formulation was analyzed for the change in appearance, pH or drug content by procedure stated earlier [7, 10-11].

RESULTS AND DISCUSSION

FTIR

The principal peaks of Ketorolac tromethamine were observed at 3350 cm⁻¹ [NH stretch], 1373 cm⁻¹ [C-N vibrations], 1655 cm⁻¹ [C=O stretch (Diaryl ketone)], 1701 cm⁻¹ [C=O stretch (acid)], 1049 cm⁻¹ [OH bending (alcoholic group)], 1466 cm⁻¹ [C=C (aromatic stretch)], 725, 779 cm⁻¹ [C-H bending (aromatic)]. FTIR of physical mixture showed that there is no interaction between drug and the polymers employed in formulation. Fig. 2 and 3 showed the spectra of pure drug and physical mixture respectively.



Fig. 2: FTIR of pure drug

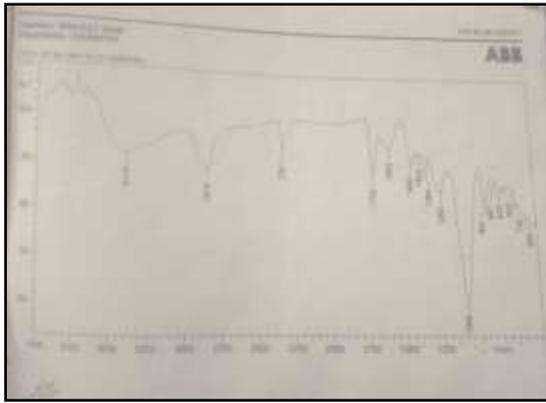


Fig. 3: FTIR of physical mixture

DSC study

The DSC of KT showed the long and sharp characteristic endothermic peak at 161.39°C associated with the melting of KT. DSC scans of pure drug, physical mixture were presented in fig. 4 and 5 respectively. DSC of physical mixture showed endothermic peak at 159.37°C, the slight change in characteristic peak may be due to fusion of excipients present in the physical mixture. The results indicated that there was no interaction between drug and the polymer.

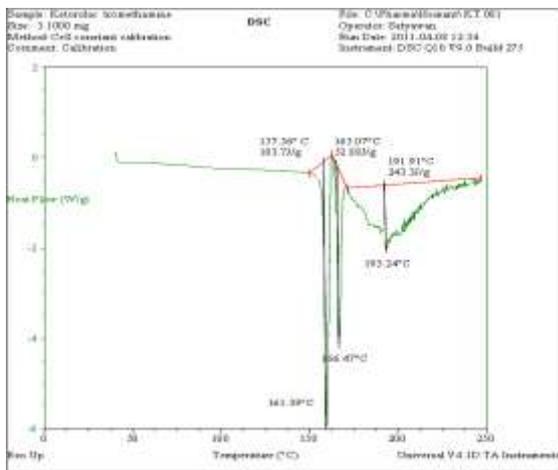


Fig. 4: DSC of pure drug

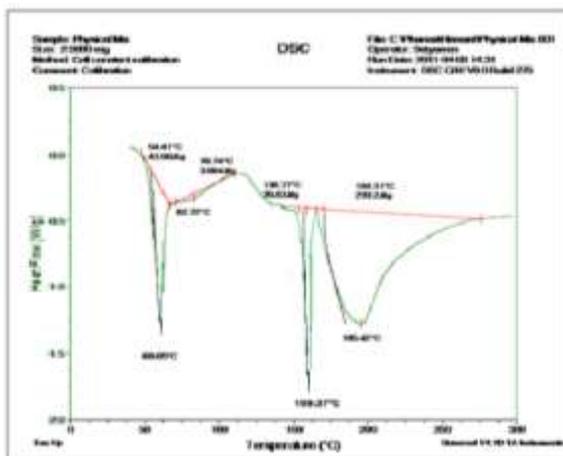


Fig. 5: DSC of physical mixture

In- vitro dissolution study

In vitro dissolution studies were carried out for carbopol gel, proniosomal gel and marketed gel in phosphate buffer pH 7.4. The % cumulative drug release from the formulations was given in fig. 6.

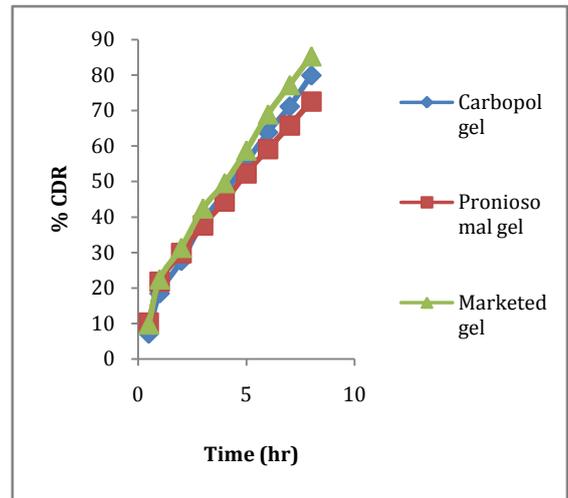


Fig. 6: Dissolution profile of carbopol gel, proniosomal gel and marketed gel

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

Preparation and evaluation of carbopol 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 4).

a) Clarity

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

b) Homogeneity

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

c) 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.

d) Viscosity measurement

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

e) Spreadability

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

f) Extrudability

The extrudability of formulations was found to be good.

g) Drug Content analysis

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

Table 4: Evaluation of the prepared formulations of carbopol 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

h) *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 7.

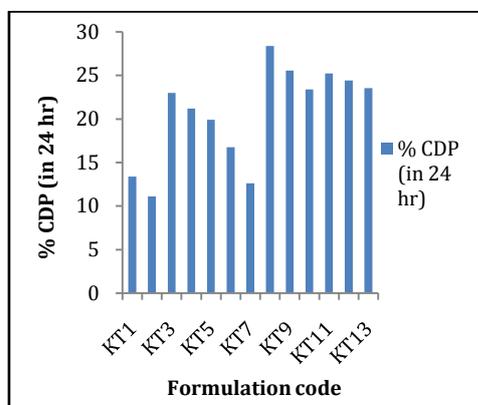


Fig. 7: % CDP for formulation KT₁ to KT₁₃

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 KT₈ 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 carbopol 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 5 predicts the data for the analysis of variance.

Mathematical Modeling

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

$$\% \text{ CDP} = + 24.05 - 1.21 X_1 + 5.9X_2 + 0.12 X_1 X_2 \dots\dots (1)$$

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.

Response surface analysis

The 3-dimensional response surface plot and the corresponding contour plot for the studied response parameter, % CDP (24 hr) revealed the effect of selected independent variables on the response.

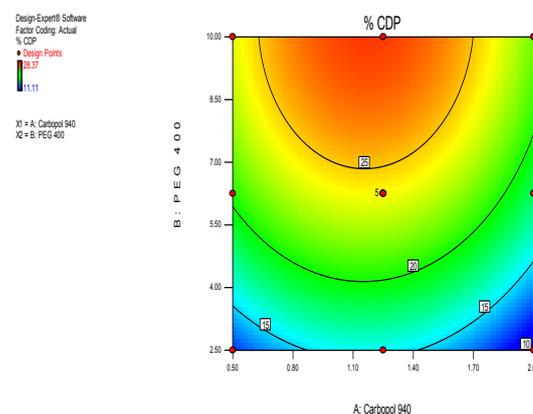


Fig. 8: Contour plot showing the influence of two factors on % CDP

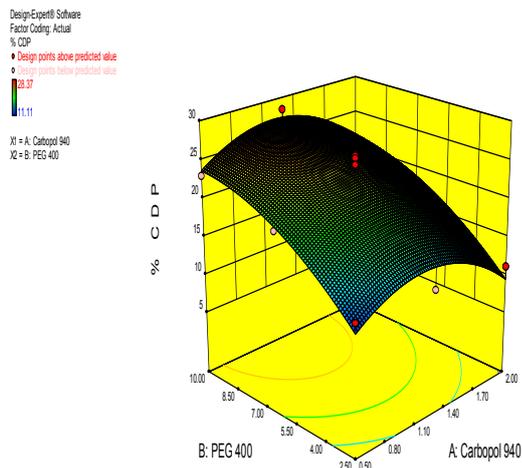


Fig. 9: 3D response surface plot showing the relationship between various levels of two factors on % CDP

Table 5: 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

Table 6: Value of R² obtained from different kinetic models

Formulation Code	R ²			
	Zero order	First order	Higuchi model	Korsmeyer Peppas model
KT _B	0.772	0.8	0.955	0.934

Table 7: Stability study of optimized batch of gel

Formulation	Months	Appearance	pH	Drug content
KT _B	0	Clear	6.3	101.46
	1	Clear	6.3	99.89
	2	Clear	6.2	98.54
	3	Clear	6.2	97.67

CONCLUSION

It was concluded from the results that the optimized formulation showed maximum *in-vitro* drug permeation 28.37%. All other evaluation parameters like clarity, homogeneity, viscosity, drug content, pH, spreadability, extrudability were suggestive of good characteristic properties of optimized formulation.

The optimized formulation was further subjected to kinetic modeling studies. From the results of data fitting to various models, it was found that the optimized formulation showed Higuchi model of drug release. The stability study of the optimized formulation showed satisfactory characteristics without being drastically influenced.

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Cumulative drug permeation (%CDP)

The polynomial equation (1) for % CDP denoted that the coefficient X₁ bear a negative sign and X₂ 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. This was further revealed by the response surface plots (figure 8 and 9).

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

The model for best fit was predicted from the value of R². For an ideal fit, value of R² was 1. Hence, the model which gives the R² value nearest to 1 describes the order of drug permeation (table 6). From the results of data fitting to various models, it was found that the optimized formulation KT_B 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.

Accelerated stability studies of optimized formulation KT_B

During the accelerated stability studies the appearance was clear and no significant variation in pH was observed and drug content is 97.67% after 3 months (table 7).

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