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Application of Simplex Lattice Design for the Development of Transdermal Gels of Diclofenac Sodium

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The present study deals with the preparation and evaluation of diclofenac sodium gels using an indigenously available synthetic gelling agent, Acrypol 940. A Simplex lattice design was employed for preparation of the gel possessing optimized characteristics. The amount of ethanol (X_1), propylene glycol (X_2) and polyethylene glycol 400 (X_3) were chosen as the independent variables to study the combined effect of cosolvents. The % of drug permeated in 1, 3, 5 and 7 h (Y_7) through the rat skin in phosphate buffer (pH 7.2) were selected as dependent variables. Full and refined models were derived for the prediction of the response variable Y_7. Based on the results of multiple linear regression analysis and *F*- statistics, it is concluded that higher % of drug release can be obtained when X_1, X_2 and X_3 are kept in an equal transformed proportion. The batch containing 33 % w/w of ethanol, 8.4 % w/w of propylene glycol and 8.4 % w/w of PEG 400 showed maximum drug release over 7 h in the *in vitro* dissolution test. The probable reasons for improved drug dissolution are permeation enhancing effect, altered drug affinity for the solvent and controlled viscosity of the gel. A contour plot is also presented to visualize the effect of the selected independent variables on Y_7. The drug release data was well described by Korsemeyer and Peppas model. Considering the % of diclofenac sodium permeated in 1, 3, 5 and 7 h, an equation is also derived for the expression of dissolution profile. The application of the equation is demonstrated for predicting % drug permeated for an extra-design checkpoint. A permeation study of the best batch was also conducted on human cadaver skin. The release profiles obtained with rat skin and human cadaver skin were found to be comparable.

Diclofenac sodium, a non-steroidal antiinflammatory agent, is frequently prescribed for the long term treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Gastrointestinal side effects such as bleeding, ulceration or perforation of the intestinal wall are commonly seen when the drug is administered orally[1]. To avoid these adverse effects, researchers have used alternate routes of administration[2,3]. Delivery of diclofenac sodium via skin offers the potential advantage of bypassing hepato-gastrointestinal first pass metabolism associated with the oral administration. It is prescribed at a dose of 75-150 mg daily in divided doses by peroral oral route. The dosing frequency can be reduced if the patients are advised to use topical products along with the conventional tablets.

It has been reported that mineral oil, a common ingredient in cream based products, induces arthritis in rats[4]. Therefore, an aqueous based product was selected in the present investigation. Natural and synthetic polymers have been tried by researchers for the preparation of gels, among them Carbomer® has interesting characteristics. Acrypol 940, is an indigenously available, synthetic high molecular weight, crosslinked polymer of acrylic acid which conforms to USP/NF specifications as Carbomer. The gelation mechanism depends on the neutralization of carboxylic acid moiety to form a soluble

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salt. It is hydrophilic, has excellent thickening efficiency at high viscosity and produces sparkling clear gels in aqueous or hydroalcoholic solutions.

Stratum corneum provides the principal resistance to percutaneous penetration because of its relatively low permeability. The commonly used approaches to promote drug penetration through skin are, use of surfactants, organic solvents and appropriate solvent blends.[5,6]

Diclofenac sodium is not easily absorbed on transdermal administration. Nishihata and co-workers[7] used hydrogenated soya lecithin for improving its percutaneous transport from formulated topical products. Rana and others prepared primary amine and pyrrolidone ion-pairs to enhance *in vitro* percutaneous penetration of diclofenac sodium[8].

Co-solvents have been widely used both as a vehicle and a penetration enhancer in transdermal formulations[9,10]. In addition to regulating the ionization of the drugs, co-solvents may alter the barrier properties of the skin, which in turn modify the drug transport profile. The objectives of the present study were to systematically investigate the impact of different co-solvents on release characteristics and percutaneous absorption of diclofenac sodium. The applications of Simplex experimental design are well documented in formulation design[11,12]. The amount of ethanol, propylene glycol and PEG 400 were chosen as independent variables and % drug released in 7 h (Y_7) was selected as a dependent variable.

EXPERIMENTAL

Diclofenac sodium and Acrypol 940 were received as gift samples from Sharda drugs, India and Corel Pharma-Chem, India respectively. Ethanol (Alembic chemical works, India), propylene glycol and polyethylene glycol 400 (PEG 400, Ases Chemical works, India) were used as received. All the other chemicals were of analytical grade.

Preparation of diclofenac sodium gels:

Ten gel formulations of diclofenac sodium were prepared using a mixture of deionized water and the co-solvents (ethanol, propylene glycol and PEG 400) as the vehicle. Acrypol 940 was used as a gelling agent at a concentration of 1 % w/w. Alcoholic solution of diclofenac sodium was added to the aqueous dispersion of Acrypol 940. Propylene glycol and PEG 400 were added with stirring. The mixture was gelled by the addition of triethanolamine and the final weight of the gel was adjusted to 100 g with deionized water. The entrapped air bubbles were removed by keeping the gel in a vacuum oven for 2 h. The gels were stored in wide mouthed glass bottles. The design layout is shown in Table 1.

The pH of the gels was determined using a pH meter (Systronics, India) after dispersing 0.5 g gel in 50 ml of deionized water. The viscosity of the gels was measured using a Brookfield viscometer (Brookfield Engineering Labs., Inc., USA).

Preparation of rat skin:

Male Wistar rat skin was used in the study. Dorsal fur was removed with a mechanical hair clipper and depilatory cream was applied to remove small hair. The skin was wiped clean with distilled water. The animal was sacrificed after 24 h by cervical dislocation at the neck and dorsal sections of the skin were excised. The adhering tissues to the skin and other visceral debris were scrapped off by a blunt knife. The skin was then delipidized by placing it in the mixture of chloroform:methanol:0.1 M potassium chloride in the ratio of 1:2:0.8 for 20 min. The delipidized skin was washed with physiological saline before mounting on the diffusion cell.

In vitro penetration study:

Zuber *et al.*[13] developed a simple technique for studying the dissolution rate of ointments through a membrane by employing a dissolution cell. In the present investigation, the *in vitro* penetration study was carried out in a modified USP basket assembly (Fig. 1). The cylindrical wire mesh (40 #) was completely covered with plastic adhesive tape in order to prevent access of dissolution medium from the sides. The study was carried out using synthetic membrane (Millipore, 0.45 µm) for all the preliminary batches (Table 2) and rat skin for the batches prepared in the experimental design (Table 1). One gram of the gel sample was placed on the membrane or skin and the basket was partially dipped into the dissolution medium (100 ml phosphate buffer; pH 7.2, 37± 0.5°). The fluid was agitated using a magnetic stirrer (100 rpm). Samples (5 ml) were withdrawn at regular intervals and filtered through 0.45 µm membrane filter. The drug content in the filtrate was determined after appropriate dilution with the dissolution medium. The absorbance was measured on a Hitachi double beam UV/VIS spectrophotometer at 276 nm[14]. An equation was derived by fitting weighted linear regression model to the data obtained

TABLE 1: DESIGN LAYOUT FOR SIMPLEX LATTICE DESIGN

Batch No.	Variable Level in Coded Form X_1	X_2	X_3	Y_7 (%)
1	1	0	0	20.1
2	0	1	0	11.2
3	0	0	1	14.1
4	0.5	0.5	0	13.2
5	0	0.5	0.5	16.2
6	0.5	0	0.5	15.8
7	0.33	0.33	0.33	23.1
8	0.17	0.17	0.66	19.1
9	0.17	0.66	0.17	16.1
10	0.66	0.17	0.17	20.1
11*	0.3	0.35	0.35	20.1
Independent Variables			Real Values	
			0	1
X_1 = Amount of Ethanol (g)			25	50
X_2 = Amount of propylene glycol (g)			0	25
X_3 = Amount of PEG 400 (g)			0	25

Note : Each batch contained 1 % w/w of diclofenac sodium and 50 % w/w water, Y_7 = Percentage drug released in 7 h (n = 3), *represents the extra design Check-point

in triplicate (Absorbance = 0.0297*Concentration + 0.03592).

Preparation of human cadaver skin:

The human skin obtained from the chest region of the cadaver was utilized for the study. The subcutaneous fat and hair was removed. The epidermis was separated by heat separation method. It was then utilized for *in vitro* penetration study for batch 7[15].

RESULTS AND DISCUSSION

Preliminary trials were conducted using the co-solvents separately and also in different combinations. A control batch was prepared in which none of the co-solvents was added. *In vitro* dissolution study of the preliminary batches was carried out using a synthetic membrane (Millipore, 0.45 µm). The control batch showed good

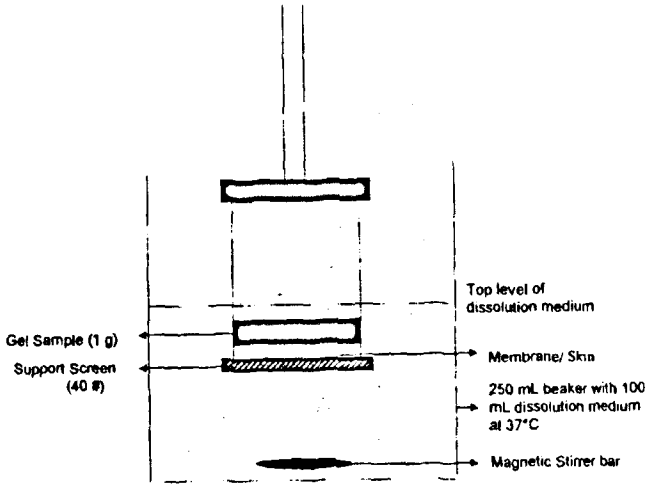

Fig. 1 : Modified Diffusion Cell for *in vitro* dissolution study of transdermal gels

clarity but the viscosity of the gel was comparatively higher than the batches containing co-solvent and the drug release was found to be very slow (6.2% in 7 h). The probable reason for the slower drug release is slow diffusion through the viscous gel.

Ethanol is an efficient penetration enhancer. Moreover, it is a good solvent for diclofenac sodium[16]. A minimum of 25 % w/w of ethanol was required to get an acceptable consistency. The data shown in Table 2 reveals that the % drug release can be modulated by changing the amount of ethanol. A linear relationship was observed between % of ethanol and Y_7 (r = 0.9805). The amount of ethanol was not increased beyond 50 % w/w as it yielded a hazy product. The affinity of the polymer for water is probably reduced when higher amount of alcohol used and thus it results in conformational changes in the polymer making the gel hazy. Hence, it was decided to use ethanol in the range of 25-50 % w/w in the experimental design.

Propylene glycol may act as a synergistic vehicle in the transdermal formulations. To study its effect on the drug release, a part of water was replaced by propylene glycol (10-25 % w/w, batches P_6-P_8). The concentration of ethanol was kept constant at 25 % w/w in these batches. The combination of both the solvents showed improved drug diffusion. A linear correlation was also observed between % w/w propylene glycol and Y_7 for the batches P_2, P_6, P_7 and P_8 (r = 0.9712). Propylene glycol was not used beyond 25% w/w in the experimental design as the gels containing higher percentage of propylene glycol showed poor spreadability. Hence, it was decided to use propylene glycol in the range of 0-25% w/w in the experimental design.

PEG 400 was tried in the range of 10 to 25 % w/w (batches P_9-P_{11}). The concentration of ethanol was kept constant at 25 % w/w in these batches. A linear relationship was also observed between % w/w of PEG 400 and Y_7 for the batches P_2, P_9, P_{10} and P_{11} (r = 0.9846). The % of PEG 400 was not increased beyond 25% w/w in the experimental design. It has been reported that flux of the drug was decreased when PEG 400 was used beyond 40 % w/w due to an increase in the drug affinity for the vehicle[17]. Compared to propylene glycol, the effect of PEG 400 on the drug diffusion was found to be much better at all the three levels of the cosolvent. It can be concluded that by replacing a part of water with other co-solvents such as ethanol, propylene glycol and PEG 400 one can improve the physical characteristic and performance of the gels. The pH of all the preliminary batches was found to be in between 6 and 7 and viscosity of all the batches ranged from 3000 to 5000 cPs.

To investigate the combined effect of the three co-solvents i.e. ethanol, propylene glycol and PEG 400, batches P_{12} and P_{13} were prepared. The presence of all the three cosolvents in the formulation helped to improve the drug release from 6.2 to 16.2 % (Table 2).

Simplex Lattice Design:

The results of the preliminary trials were used for constructing the Simplex lattice design (Table 1). All the formulations, prepared within the factor space, yielded transparent gels. A first-order linear interactive model Eq (1) was derived. The coefficients were calculated using the procedure outlined by Bolton[18].

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{123}X_1X_2X_3 \quad (1)$$

where Y is the response parameter and b_i is the estimated coefficient for the factor X_i. The main effects (X_1, X_2 and X_3) represent the average results of changing one factor at a time from its low to high value. The interactions (X_1X_2, X_2X_3, X_1X_3 and $X_1X_2X_3$) show how the response changes when two or more factors are simultaneously changed. The fitted equation relating the percent drug released in 7 h (Y_7) to the transformed factors is shown in Eq (2).

$$Y_7 = 20.17X_1 + 10.95X_2 + 14.15X_3 - 10.30X_1X_2 + 13.66X_2X_3 - 5.19X_1X_3 + 211.21X_1X_2X_3 \quad (2)$$

The Y_7 values for all the ten batches show a wide variation i.e. the response ranged from a minimum of 11.2 % to a maximum of 23.1 %. The data clearly indicate that the Y_7 is strongly dependent on the independent factors selected in the study. The significance level of the coefficients of the factors X_1X_2 (p=0.097) and X_1X_3 (p=0.314) were greater in magnitude than 0.05 and hence they were omitted from the full model for evolving a refined model Eq (3).

$$Y_7 = 19X_1 + 10.11X_2 + 13.88X_3 + 15.93X_2X_3 + 175.42X_1X_2X_3 \quad (3)$$

This argument is further investigated by testing the model in portions [19]. The computation steps for calculating the value of F are shown in Table 3. The calculated value of F was 3.181. The critical value of F for α = 0.05, v1=2, and v2= 3 is 9.55. Since the calculated value of F is lower in magnitude than the critical value, we can conclude that the polynomial terms (X_1X_2 and X_1X_3) do not

TABLE 2 : PERCENTAGE DRUG RELEASED FROM THE PRELIMINARY BATCHES

Batch No.	Alcohol (% w/w)	Propylene glycol (% w/w)	PEG 400 (% w/w)	% drug released in 7 h*
P_1	-	-	-	6.2
P_2	25.0	-	-	8.4
P_3	30.0	-	-	13.4
P_4	40.0	-	-	17.2
P_5	50.0	-	-	21.4
P_6	25.0	10.0	-	9.1
P_7	25.0	15.0	-	10.4
P_8	25.0	25.0	-	12.3
P_9	25.0	-	10.0	10.0
P_{10}	25.0	-	15.0	12.1
P_{11}	25.0	-	25.0	15.2
P_{12}	25.0	10.0	10.0	14.3
P_{13}	25.0	15.0	15.0	16.2

Water was used as solvent in batch P_1, * n=3

significantly contribute to the prediction of Y_7. The results of the full and refined models are depicted in Table 3. The equation 3 can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative). In the mixture model, the coefficient for the higher order term (b_{123}) is often numerically very large. This does not mean that it is significant, either statistically or physically.

The joint effect of the variables can be best interpreted by the help of a contour plot (Fig 2), which is drawn using the refined model. Since each contour represents the mixture that gives the same response value, the best mixture can be chosen according to cost, characteristics, performance, etc. Fig 2 depicts that as we move towards the interior of the triangle, improvement in drug release can be obtained. The results indicate that the presence of all the three solvents is required in the formulation to get improved drug release. Batch 7 may be ranked as the best batch in the factor space as it showed maximum drug release and hence it was upheld for further studies. The improved drug release may be attributed to improved permeation and/or altered drug solubility in the cosolvent blend.

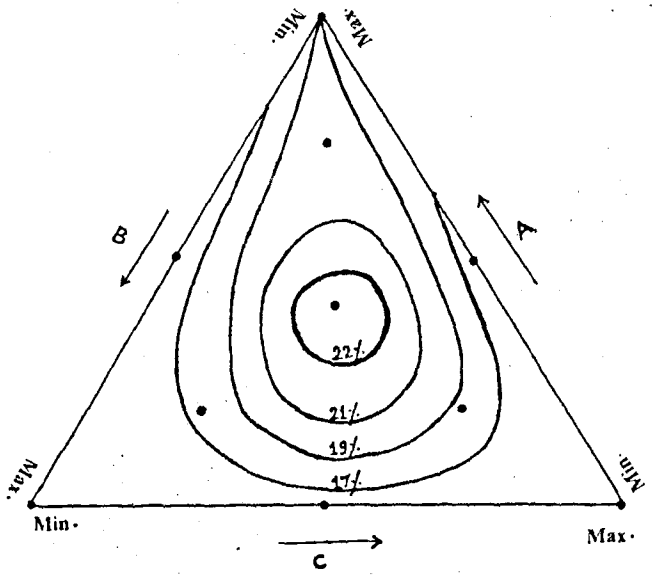

Fig. 2 : Contour Plot Lines for the effect of ethanol (A), propylene glycol (B) and PEG 400 (C) on the % drug released in 7 h. (Y_7)

The goodness of fit test proposed by Bamba and coworkers[20] was used to determine the kinetics of drug release. The release profile fitted best to Korsemeyer and Peppas equation (log time vs. log fraction of drug released,

TABLE 3 : SUMMARY OF RESULTS OF REGRESSION ANALYSIS AND ANOVA FOR MEASURED RESPONSE (Y_7)

Response		b_1	b_2	b_3	b_{12}	b_{23}	b_{13}	b_{123}
Full Model (FM)		20.17	10.95	14.15	-10.13	13.66	-5.199	211.21
Refined model (RM)		19.00	10.11	13.88	-	15.93	-	6 175.42
Sample calculation for testing the model in portions :								
		DF	SS	MS	F	R^2	*SSE*1 - *SSE*2 = 7.35-2.36 = 4.99	
Regression	FM	7	117.43	16.78	2.36	0.980	No. of parameters omitted = 2	
	RM	5	112.43	22.49	15.29	0.939	(b_{12} and b_{13}) MS of Error (full model) = 0.785	
Error	FM	3	2.36	0.79			F = (4.99/2)/0.785 = 3.18	
	RM	5	7.35	1.47			Critical F = 9.55 (v_1 = 2, v_2 = 3)	

F=0.2043) showing the least residual sum of square as compared with Weibull equation (log-log plot of time vs-ln (1-m), where m= fraction dissolved at time t, F=0.2147) or Higuchi equation (square root of time Vs percentage drug released, F=1.147). This superiority is however statistically insignificant as shown by the F-ratio test. The values of correlation coefficients were found to be 0.9971,0.9972 and 0.9915 for Korsemeyer and Peppas, Weibull and Higuchi models respectively. The values of slope and intercept were found to be 0.6696 and -2.3987 for the Korsemeyer and Peppas model respectively. From the value of the slope (n), it may be concluded that the drug is released by diffusion of anomalous type.

Peck and co-workers[21] derived mathematical relationship for the expression of dissolution profile from matrix tablets. An effort is made in the present investigation to derive a similar type of relationship. A linear interactive model was generated using the data of percentage drug released at 60, 180, 300 and 420 min from all the ten batches of simplex design. The Korsemeyer and Peppas model fitted well to the data set and hence log of time was chosen as an additional independent variable. The multiple linear regression analysis (Microsoft EXCEL® - Ver 5.0) was performed using the actual values. The derived equation describing the dissolution pattern is shown below:

$$Y = -0.38X_1 - 0.32X_2 - 0.23X_3 - 0.01X_1X_2 + 0.21X_2X_3 - 0.01X_1X_3 + 0.001X_1X_2X_3 + 14.01 * \log time \quad (4)$$

where Y is the percentage drug dissolved at time 't'. The R^2 was found to be 0.9263, indicating a good fit. The *F*-test was found to be significant at $p < 0.05$. The derived equation may be used for calculating the percent drug release from different batches within the factor space.

The objective of this study was to maximize drug release and percutaneous absorption. The batches 1, 7 and 10 (Table 1) showed superior drug permeation as compared to the other batches in the Simplex design. The final selection was done considering the amount of ethanol used in the selected batches. Batch 7 contained minimum amount of ethanol (33 % w/w) as compared to the batches 1 and 10 containing 50 % w/w and 41.5 % w/w of ethanol respectively. Moreover, the % of drug release was found to be highest from batch 7. The viscosity and the pH of batch 7 were found to be 3600 cPs and 6.2 respectively. The appearance of gel and the drug release profile remained unchanged when batch 7 was stored for one month at 45° and 60% RH.

A check- point was selected close to the setting of batch 7 (X_1 = 0.3, X_2 = 0.35 and X_3 = 0.35) to validate the derived equation. The predicted and observed dissolution profile for the check-point is depicted in Fig 3. The experimental release data (Y_7 = 20.1%) compare quite well to the release profile predicted from the mathematical model (Y_7 = 19.1%). The drug release profile of batch 7 was compared with the release profile of a commercial product (Voveran-Emulgel® containing 1.16% w/w of diclofenac diethylammonium equivalent to 1% w/w of diclofenac sodium) (Fig. 4). The performance of both the preparations was found to be almost identical.

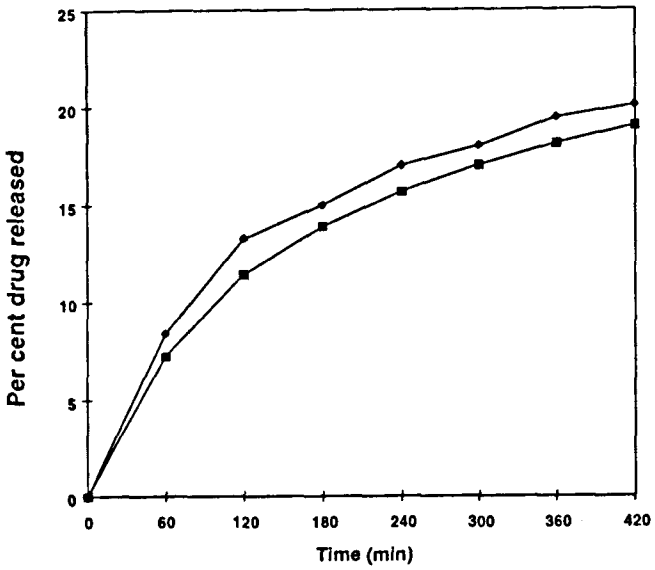

Fig. 3 : Comparative dissolution profiles of check point. Observed (◆———◆) and predicted (▮———▮)

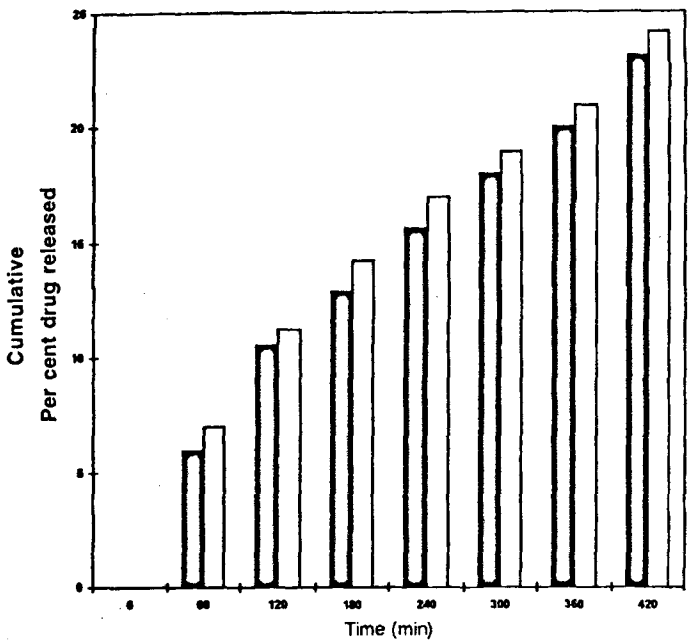

Fig. 4 : Comparative dissolution profiles. Batch 7 (□) and market preparation (▮)

The release rates of batch 7 from rat skin and human cadaver skin were compared using the Students *t*-test. Statistically insignificant difference was observed with rat and human cadaver skin (t_{cal} = 0.7973, $t_{critical}$ = 1.7613).

It may be summarized that the % drug release from the gels can be modulated by choosing an optimum solvent blend. The probable reasons for improved drug release are penetration enhancing effect and decreased drug affinity for the solvents. The cost of production of the optimized formulation will be less as compared to the preparations containing state of the art permeation enhancers. The systematic formulation approach helped us in evolving an optimal formulae.

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