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Formulation development and evaluation of Indomethacin emulgel

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ABSTRACT

The purpose of the present study was to develop and optimize the emulgel system for IND (Indomethacin), using 2 types of gelling agents: Carbopol 934 and Xanthan Gum. The prepared emulgels were evaluated in terms of appearance, pH, spreadability, viscosity, drug content and in-vitro drug release. In-vitro release study demonstrated diffusion controlled release of IND from formulation up to 12 hours. The drug release profile exhibited zero order kinetics. All the prepared emulgels showed acceptable physical properties concerning colour, homogeneity, consistency, spreadability, pH value, and with higher drug release than conventional gel as per USP. The emulgel was optimized using a two factor, two-level factorial design. Influence of type of gelling agent was also investigated. Mathematical equations and response surface plots were used to relate the dependent and independent variables. Each formulation was optimized from carbopol 934 based & from xanthan gum based formulations using contour plot and response surface plot. The optimized formulations were found to be C3 and G3 containing lower concentration of light liquid paraffin and higher concentration of emulsifiers. The optimized formulae were evaluated for Anti-inflammatory activity, skin permeation and stability for 3 months. In case of all evaluation parameters Xanthan gum based formulation showed better properties so, As a general conclusion, it was suggested that the IND emulgel formulation prepared with Xanthan Gum having the oil phase concentration in its low level and emulsifying agent concentration in its high level was the formula of choice.

Key words: Indomethacin, Carbopol 934, Xanthan Gum, optimization, Anti-inflammatory activity.

INTRODUCTION

Indomethacin (IND) is a potent nonsteroidal anti-inflammatory drug with analgesic and antipyretic properties. Like other NSAIDs, the most common side effect of IND in oral dosage forms is gastrointestinal irritation. Long term use of NSAIDs is associated with severe gastropathy [1]. Thus, alternative routes of administration for these drugs are being currently investigated. Recently, more attention has been focused on emulgels for topical drug delivery [2-5].

When gels and emulsions are used in a combined form the dosage forms are referred to as emulgels [6]. Both oil-in-water and water-in-oil emulsions are extensively used for their therapeutic properties and as vehicles to deliver various drugs to the skin [7]. Emulsions possess a certain degree of elegance and are easily washed off whenever desired. They also have a high ability to penetrate the skin.

In recent years, there has been great interest in the use of novel polymers with complex functions as emulsifiers and thickeners because the gelling capacity of these compounds allows the formulation of stable emulsions and creams by decreasing surface and interfacial tension and at the same time increasing the viscosity of the aqueous phase [8]. Natural polymers like Xanthan Gum have many advantages over synthetic gelling agent like Carbopol 934 [9]. The presence of a gelling agent in the water phase converts a classical emulsion into an emulgel. Emulgels for dermatological use have several favourable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining and transparent with long shelf life & pleasing appearance [10].

In the development of emulgel dosage form, an important issue is to design an optimized formulation with an appropriate drug diffusion rate in a short period of time and minimum number of trials. For this purpose, a computer based optimization technique with a 2-level factorial design utilizing a polynomial equation has been widely used. This technique requires minimum experimentation and time, thus is far more effective and cost-effective than the conventional methods of formulating emulgel dosage forms [11].

The aim of this work was to develop and optimize emulgel formulation of IND with 2 types of gelling agent Carbopol 934 and Xanthan Gum separately, using 2^2 factorial design. Optimized formulations evaluated for anti-inflammatory activity & ex vivo skin permeation study. The influence of the type of the gelling agent was also investigated.

MATERIALS AND METHODS

Materials

Indomethacin was received as a gift sample from Micro Labs Ltd, Bangalore (India). Carbopol 934 was purchased from Manish Pharmaceuticals, Mumbai (India). Xanthan Gum was received as a gift sample from CP Kelco, Mumbai (India). Light liquid paraffin, Span-80, Tween-80, Methyl paraben and Propyl paraben were purchased from Loba Chemie, Mumbai (India). All other chemicals and reagents used were of analytical grade. Deionised distilled water was used throughout the study.

White hairless male albino rats weighing between (170and 200 gm) were selected for evaluation of the anti-inflammatory activity by measurement of oedema size resulting from carrageenan injection in the right hind paw region of the body and skin irritation test. Animals were housed six per cage in the animal facility of the Appasaheb Birnale college of Pharmacy, Sangli (MAH). Animals were kept under constant temperature (25 ± 1^0c) and a 12 hr light dark cycle. Each animal was allowed free access to standard food pellets and water. All the animals were acclimatized in the animal facility for at least 2 weeks prior the experiments[12]. All animal study experiments were conducted in accordance with the approval of the Animal Ethical committee, Appasaheb Birnale college of Pharmacy, Sangli (IAEC/ABCP/07/2012-13) and as per Ethical guidelines for animal use [13].

Preparation of emulgel

The composition of emulgel formulations is shown in table 1. First, the gel was prepared by dispersing Carbopol 934 in heated purified water (80 °C), and the dispersion was cooled and left overnight. The oil phase of the emulsion was prepared by dissolving Span 80 in liquid paraffin while the aqueous phase was prepared by dissolving Tween 80 in purified water. Methyl and Propyl parabens were dissolved in propylene glycol whereas indomethacin was dissolved in ethanol, and both solutions were mixed with the aqueous phase. Both the oily and aqueous phases were separately heated to 70 to 80 °C then the oily phase was added to the aqueous phase with continuous stirring until cooled to room temperature. The obtained emulsion was mixed with the gel in 1:1 ratio with gentle stirring to obtain the emulgel. Finally pH of emulgel was adjusted by using triethanolamine [14].

Same procedure was followed for Xanthan Gum as gelling agent instead of using Carbopol 934.

Table 1: Quantative Composition of Emulgel formulations

Ingredients (%w/w)	C1	C2	C3	C4	G1	G2	G3	G4
Indomethacin	1	1	1	1	1	1	1	1
Carbopol 934	1	1	1	1	-	-	-	-
Xanthan Gum	-	-	-	-	1	1	1	1
Light Liquid paraffin	5	7.5	5	7.5	5	7.5	5	7.5
Tween 80	0.6	0.6	1	1	0.6	0.6	1	1
Span 80	0.9	0.9	1.5	1.5	0.9	0.9	1.5	1.5
Propylene glycol	7	7	7	7	7	7	7	7
Ethanol	10	10	10	10	10	10	10	10
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Purified water q.s.	100	100	100	100	100	100	100	100
Triethanolamine q.s	Adjust pH 6-6.5							

Preparation of conventional Indomethacin Gel as per USP (Standard Gel):

As the marketed formulation of Indomethacin Gel is not available in Indian local market, so for the purpose of comparative study conventional Indomethacin gel was prepared as per USP. 1 gm of Indomethacin was transferred to a suitable beaker, and dissolved it in 55 mL alcohol. That solution was transferred to glass mortar, and slowly added the Carbomer 941 so that it is thoroughly distributed. Any white lumps were pressed out until a smooth gel

was formed. The purified water was slowly added with mixing. A sufficient quantity of alcohol was added to make up the final volume up to 100 mL and mix. The gel was transferred to wide mouth container [15].

Experimental Design

A 2^2 factorial design was conducted to study the effect of independent variables (i) Concentration of Light liquid paraffin (X1) and (ii) Concentration of emulsifying agent (X2) on dependent variables % cumulative drug release at 12 hours (Y_1) and spreading coefficient (Y_2). Actual and coded values for independent variables are listed in table 2 while all the batches ware prepared according to the experimental design.

Two type of gelling agents Carbopol 934 as synthetic gelling agent & Xanthan Gum as natural gelling agent were taken. Same experimental design was applied for both gelling agents.

Table 2: Factors and Levels for 2^2 Factorial Design

Coded values	Actual Values	
	X_1	**X_2**
-1	5 %w/w	1.5 %w/w
+1	7.5%w/w	2.5% w/w

X1: Conc. Of Light liquid paraffin; X2: Conc. of emulsifying agent

Characterization of Emulgel

Physical Appearance and pH Determination

The IND emulgels were inspected visually for their color, homogeneity, consistency, and the pH values of 1% aqueous solutions of the emulgels were measured by a digital pH meter.

Spreading Coefficient

Spreading coefficient (Spreadability) was determined by apparatus suggested by Lalit Kumar *et.al.* 2010 [16]. It consists of a wooden block, which is attached to a pulley at one end. Spreading coefficient was measured on the basis of 'Slip' and 'Drag' characteristics of emulgels. A ground glass slide was fixed on the wooden block. An excess of emulgel (about 2 g) under study was placed on this ground slide. The emulgel preparation was then sandwiched between this slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. Weight of 500 mg was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the emulgel between the two slides. Measured quantity of weight was placed in the pan attached to the pulley with the help of hook. Time in seconds taken by two slides to slip off from emulgel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the following formula-

$S = M. L / T$

Where, M = wt. tied to upper slide; L = length of glass slides ; T = time taken to separate the slides.

Rheological Study

The viscosity of the developed emulgel formulations was determined by using a cone and plate type of Brookfield viscometer (Brookfield viscometer RVT) with spindle No.7. The maximum shear rate was 100 RPM while minimum shear rate was 10 RPM.

Drug content determination

IND content in emulgel was measured by dissolving known quantity of emulgel in solvent (ethanol) by Sonication. Filtration of resulting solution was done by using whatman filter paper no.41. Absorbance was measured after suitable dilution at 319 nm using UV/VIS spectrophotometer (JASCO, V-550, Japan).

In Vitro Drug Release Studies

The *in vitro* drug release studies were carried out using a modified vertical Franz diffusion cell (with effective diffusion area 1.44 cm^2 and 15.5 ml cell volume).The formulation was applied on Nylon membrane 0.45 µm (which was previously soaked in Phosphate buffer pH 7.4 for 24 hours); which was sandwiched between donor and receptor compartment of the franz diffusion cell. Phosphate buffer pH 7.4 + ethanol (80:20) was used as a dissolution media. The temperature of the cell was maintained at 37±0.2 ^{0}C by kept it in water bath. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead at 50 rpm. The samples (1.0 ml aliquots) were withdrawn at suitable time interval and analyzed for drug content by UV visible spectrophotometer at 321 nm after appropriate dilutions.

Data analysis
The drug release data were evaluated by the curve fitting method using PCP-Disso software. In the present study the release profile follows the Peppas model. The Peppas model shows the drug release mechanism deviates from Fick's laws and shows anomalous transport. This is demonstrated by following equation:

$M_t / M_\infty = k.t^n$

Where $\mathbf{M_t}$ is the drug released at time **t**, $\mathbf{M_\infty}$ is the quantity of drug released at infinite time, **k** is the kinetic constant and ***n*** is the release exponent.

Optimization of Emulgel Formulations
Response surface methodology (RSM) is a widely practiced approach in the development and optimization of drug delivery systems. Based on the principle of design of experiments (DoE), the methodology encompasses the use of various types of experimental designs, generation of polynomial equations, and mapping of the response over the experimental domain to determine the optimum formulation(s). The technique requires minimum experimentation and time, thus proving to be far more effective and cost-effective than the conventional methods of formulating dosage forms. Various computations for the current optimization study were performed using Design Expert software (Design Expert trial version 8.0.7.1; State-Ease Inc., Minneapolis, MN, USA). The 3-D response surface graphs and the 2-D contour plots were also generated by the Design Expert software. These plots are very useful to see interaction effects of the factors on responses.

Characterization of Optimized Formulations
Globule size and its distribution in emulgels:
This study was performed for the optimized batches each from carbopol 934 based and xanthan gum based gels. Globule size and distribution was determined by Malvern zetasizer. A 1.0 gm sample was dissolved in purified water and agitated to get homogeneous dispersion. Sample was injected to photocell of zetasizer. Mean globule diameter and distribution was obtained [17, 18].

Photomicrography
Morphology of emulsion was studied under light microscope. Optimized batches of the emulgel were viewed under light microscope to study their shape. The emulgel was suitably diluted, mounted on glass slide and viewed by light microscope under magnification of 40 X [19].

Animal Study Experiments
●Treatment:
The animals were divided into four groups, each consisting of six animals.
Group A: was treated with normal saline, Control Group
Group B: was treated with optimized gel from carbopol based gels (C_3).
Group C: was treated with optimized gel from xanthan gum based gels (G_3).
Group D: was treated with standard gel prepared as per USP. (1% Indomethacin gel USP)

●Statistical Analysis for the Results:
The statistical analysis for the results was carried out on results of the mid of experiment using Graph Pad Instat software to determine significance of the obtained results between the prepared medicated emulgel and the plain one.

Skin irritation test
Various preparations, when applied dermally, might elicit skin irritation. Therefore, to access the skin sensitizing potential, Indomethacin emulgel was applied to dorsal skin of albino rats. The animals were housed in propylene cages, with free access to standard laboratory diet and water. Animals were acclimatized for at least seven days before experimentation. The formulations were applied and the site of application was occluded with gauze and covered with non sensitizing micro porous tape. The development of Erythema and Edema was monitored for 3 days [20].

Skin Permeation study
Ex vivo diffusion study was carried out by using rat skin, and procedure was similar to that of *in vitro* diffusion study. Only nylon membrane was replaced with rat skin membrane. Cumulative corrections were made to obtain the total amount of drug diffused at each time interval and *ex vivo* parameters were calculated [21, 22].

The average cumulative amount of drug permeated per unit surface area of the skin was plotted versus time. The slope of the linear portion of the plot was calculated as flux *J*ss (μg/cm^2/h), and the permeability coefficient was calculated using the following formula:

$K_p = J_{ss} / C_v$

Where K_p : permeability coefficient, C_v : Total amount of drug

Edema size induced by Carrageenan injection (Anti-inflammatory study)
Certain amount of gel (100 mg) was applied topically to the right hind paw of the rats. The area of application was occluded with bandages and it was left in place for two hours. The dressings were then removed and the gel remaining on the surface of the skin was wiped off with a piece of cotton. The animals were then injected with 0.1 ml of 1% freshly prepared carrageenan solution in saline in plantar region of right hind paw. The right paw thickness was measured from form ventral to dorsal surface, with a cotton thread before and 1, 2, 3, & 4 hrs after sub-plantar injection. The size of edema was expressed as the increase in paw thickness (in mm) after carrageenan injection [23-25].

% Inhibition of oedema = [($D_{control} - D_{treated}$)/ $D_{Control}$]*100

where $D_{control}$ = mean diameter of rats paw in controlled group, $D_{treated}$ = mean diameter of rats paw in test group.

Stability Studies
The optimized emulgel formulations were prepared; packed in aluminium collapsible tubes and subjected to stability studies at 40 ^{0}C/75 % RH for a period of 3 months as per ICH Guidelines. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance, pH, rheological properties, drug content and drug release.

RESULTS

The present work was aimed to increases stability of emulsion and to increase the penetration through skin by formulating emulgels with Carbopol 934 and Xanthan gum as well as to compare natural gelling agent to synthetic gelling agent. The prepared formulations were characterized for physical appearance, pH, spreadability, viscosity, drug content, in- vitro drug release. Optimized formulations evaluated for animal study experiments and stability studies.

Physical appearance
All formulation batches were found to be homogenous yellowish milky emulsions previously while emulgels were found to be yellowish white viscous creamy preparation. The pH values of all prepared formulation ranged from 6 – 6.5 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Spreadability
The spreadability of Carbopol based emulgel formulations & and of Xanthan gum based formulation is depicted in table 3. From the combined graph of all formulation it was concluded that all the developed formulation showed acceptable spreadability (Fig. 1). Xanthan gum based formulations showed better spreadability than Carbopol based formulations.

Table 3: Spreadability of emulgel formulations (mean ± S.D,n= 3).

Formulation	C1	C2	C3	C4	G1	G2	G3	G4
Spreadability (gm.cm/sec.)	18.6±0.2	15.8±0.3	19.7±0.1	17.3±0.3	23.5±0.2	20.5±0.1	25.3±0.2	22.4±0.4

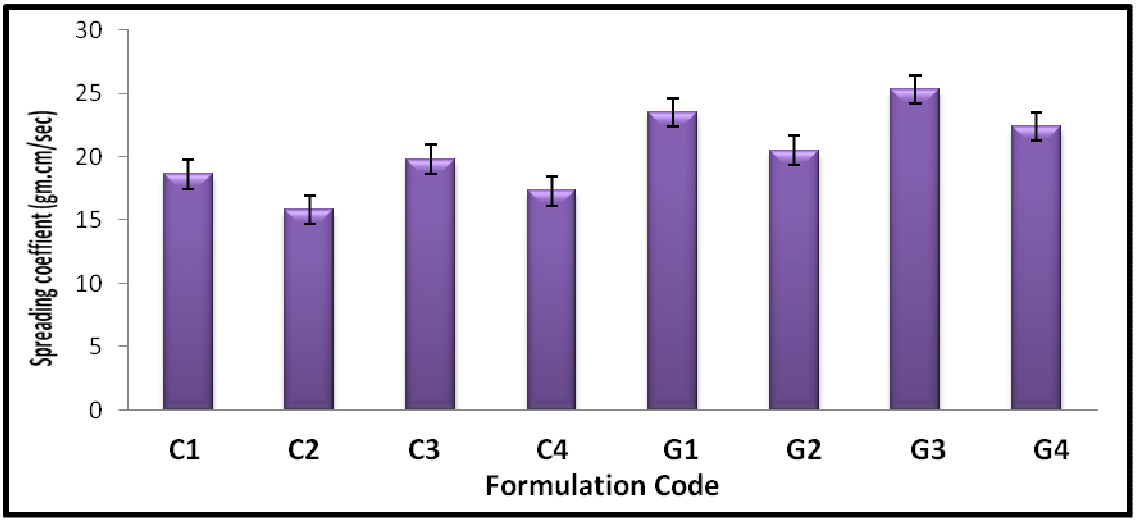


Fig. 1: Spreadability of emulgel formulations (mean ± SD; n=3)

Rheological Study

In case of carbopol 934 based formulations the highest viscosity was found in formulation C2. It may be due to high level of the liquid paraffin concentration and low level of emulsifying agent concentration. The lowest viscosity was found in formulation C3 having high level of emulsifying agent conc. Same in case of Xanthan gum based emulgel formulations G2 with Highest viscosity and G3 with lowest viscosity (Table 4). As compared to all carbopol 934 based formulations xanthan gum based formulations show considerably low viscosity which is more beneficial for maximum amount of drug release. (Fig. 2)

Table 4: Rheological study emulgel formulation (mean± SD, n =3)

Viscosity (mPas)								
RPM	C1	C2	C3	C4	G1	G2	G3	G4
10	4237±0.43	4792±0.58	3877±0.98	3549±0.54	3549±0.54	3971±0.98	3371±0.65	3684±0.75
100	1252±0.11	1314±0.21	1029±0.32	955±0.13	955±0.13	1023±0.25	899±0.31	986±0.38

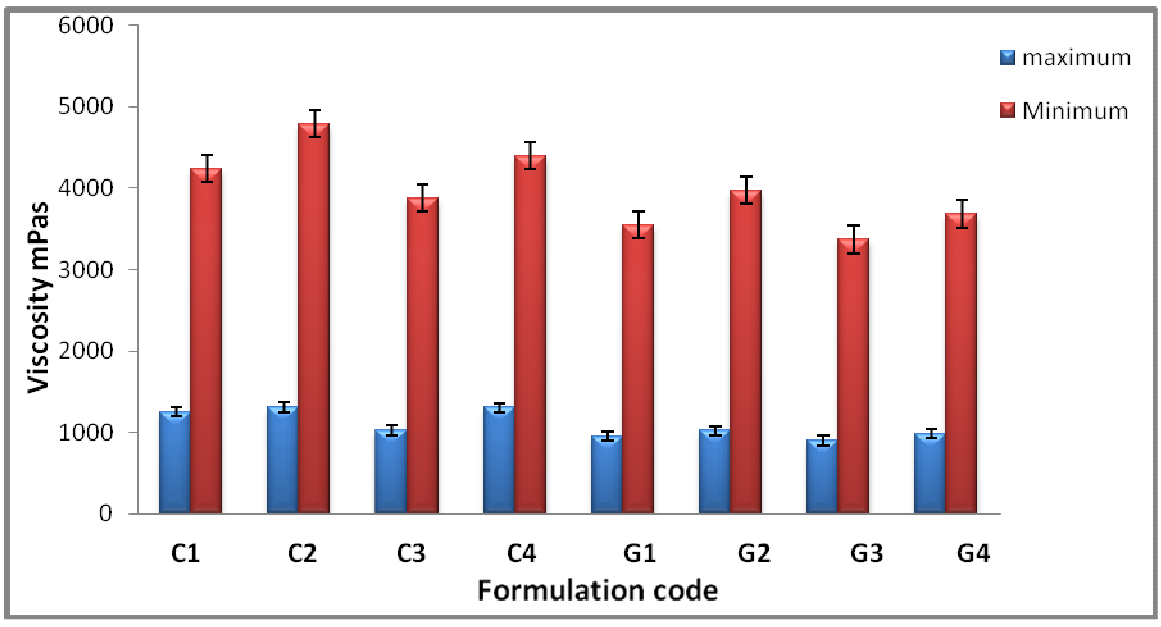


Fig. 2: Viscosity of emulgel formulations (mean ± SD;n=3)

Drug Content Determination

Drug content was calculated using the following equation, which was obtained by linear regression analysis of calibration curve. The drug content of all emulgel formulation is found within range 97 %-102%.

Y = 0.0199 x + 0.0093
R^2 = 0.9998

***In vitro* Drug Release**

The *in vitro* release profile of IND from its various emulgel formulations is being depicted in Fig.3 .It was observed that all emulgel formulations showed better drug release as compared to standard gel prepared as per U.S.P which had 55.67% drug release at 12 hrs. In case of carbopol 934 based formulations the release of the drug can be ranked in the following descending order: C3 > C1 > C4 > C2, Where the amounts of the drug release after 12 hrs were 78.91%, 74.09%, 68.37%, 64.23% respectively while in case of xanthan gum based formulation the release of the drug can be ranked in the following descending order: G3 > G1 > G4 > G2, Where the amounts of the drug release after 12 hrs were 90.12%, 83.58%, 79.50%, 71.98% respectively. From these results it can be concluded that Xanthan gum based formulations show higher drug release in comparison with corresponding carbopol 934 based formulations.

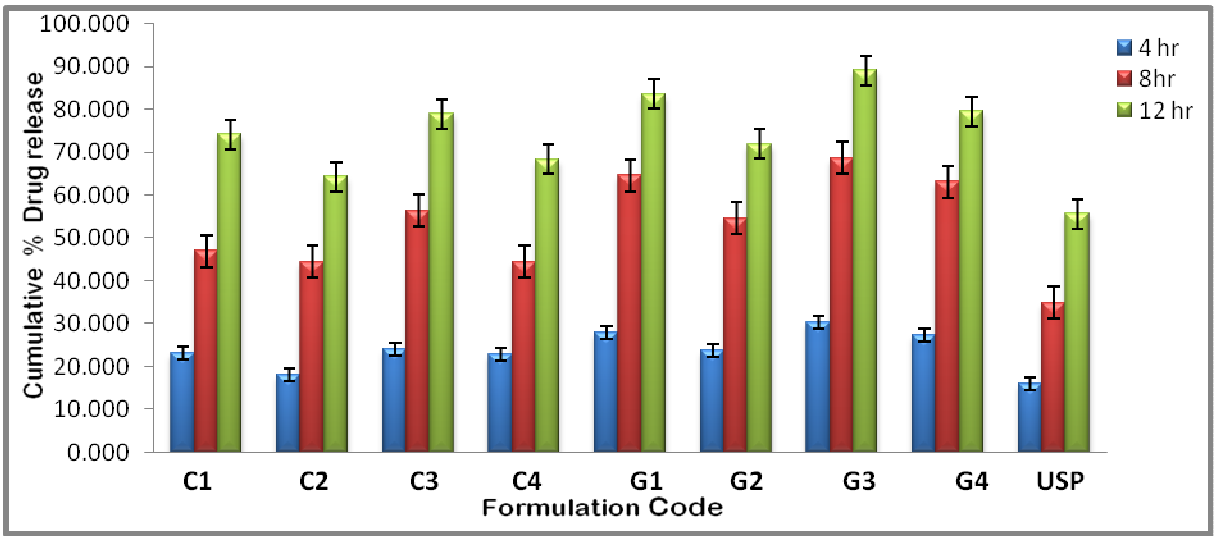


Fig.3: *In-vitro* drug release profile from emulgel formulations (mean ± SD;n=3)

Kinetic Study and Mechanism of drug release:

In order to better characterize the drug release behaviour, the release kinetic parameters were calculated in Table 5, and data was fitted to the Korsmeyer–Peppas equation.

$M_t / M_\infty = k.t^n$

Where $\mathbf{M_t}$ is the drug released at time **t**, $\mathbf{M_\infty}$ is the amount of drug loaded in gel , **k** is the kinetic constant and ***n*** is the release exponent characterizing the release mechanism. The calculated exponent, n, gives an indication of the release mechanism.

When n=1, the release is zero-order kinetic, which controlled is by time dependence (case II). When 0.5<n<1, the release is called “anomalous” and both swelling and diffusion play an important role. When the drug diffusion rate is slower than the relaxation rate of the polymeric chains, the diffusion is Fickian; whereas when the relaxation process is slow compared to diffusion, zero-order release kinetics occurs. When the drug diffusion rate and the polymeric relaxation rate are of the same order of magnitude, anomalous diffusion is observed and the value of n falls between 0.5 and 1.0[26]. As can be seen from the data listed in Table 5 the best fit model for all formulation is IND release the Korsmeyer– Peppas model and values of n for all formulations found to be in range 0.98-1.00 presented zero-order release kinetics. This means that the relaxation process of Carbopol 934 and xanthan gum is slow compared to IND diffusion; the IND releases from emulgel principally through a diffusion-controlled mechanism. In this condition, the amount of released drug corresponds to the concentration of IND in the emulgel.

Table 5: Kinetic study parameters of emulgel formulations

Model		Carbopol 934 based batches				Xanthan Gum based batches			
		C1	**C2**	**C3**	**C4**	**G1**	**G2**	**G3**	**G4**
Korsmeyers – peppas	K	6.1741	4.1844	5.4204	5.4236	8.0626	5.9303	9.2510	7.3097
	R	0.9965	0.9985	0.9976	0.9995	0.9959	0.9978	0.9956	0.9951
First order	K	-0.0927	-0.0779	-0.1142	-0.0843	-0.1323	-0.0999	-0.1571	-0.1206
	R	0.9553	0.9742	0.9659	0.9807	0.9704	0.9829	0.9568	0.9739
Higuchi matrix	K	16.9384	15.2473	19.2291	16.2689	21.0316	18.1563	22.6603	20.0875
	R	0.9098	0.9036	0.9115	0.9269	0.9299	0.9262	0.9353	0.9248
Hixson Crowel	K	-0.0264	-0.0228	-0.0316	-0.0245	-0.0356	-0.0284	-0.0405	-0.0331
	R	0.9745	0.9838	0.9816	0.9913	0.9867	0.9911	0.9833	0.9860
Release Exponent "n"		0.9773	0.9912	0.9956	0.9840	0.9581	0.9978	0.9287	0.9826
Best fit model		Peppas	Peppas	Peppas	Peppas	Peppas	Peppas	Peppas	Peppas

Optimization by experimental design

Based on previous experimental work the light liquid paraffin concentration that could form emulsion was found to be 5-7.5 % w/w and was selected as oil phase concentration to identify the optimum proportion of light liquid paraffin. The conc. of emulsifying agents that could form stable emulsion was selected as variable and that was found to be 1.5-2.5% w/w. The mathematical equations given by software are depicted in **equation no1- equation no 4.**

The responses percent drug diffusion (Y_1) and spreadability (Y_2) in C1-C4 emulgel batches were found to be Y_1, 78.91-64.24%; & Y_2, 20.42-15.51 gm.cm/sec. While the responses percent drug diffusion (Y_1) and spreadability (Y_2) in G1-G4 emulgel batches were found to be higher as compared to C1-C4 formulations (Y_1, 89.10-71.99%; & Y_2, 25.38-20.46 gm.cm/sec). The maximum % drug release and spreadability was observed in case of C3 and G3 batches having low level (5%w/w) of light liquid paraffin conc. and High level (2.5%w/w) of emulsifying agent conc. As compared to C3; G3 formulation shows higher % drug release and spreadability. (Fig. 4 & Fig. 5).

Final Equations in Terms of Actual Factors: (For C1-C4 batches).

Y_1 (percent drug diffusion) Eq.No.1

$= +87.94 - 5.10\ X_1 + 2.24\ X_2$

Y_2 (Spreadability) Eq. No.2

$= +22.85 - 1.31\ X_1 + 1.63\ X$

Final Equation in Terms of Actual Factors: (For G1-G4 batches)

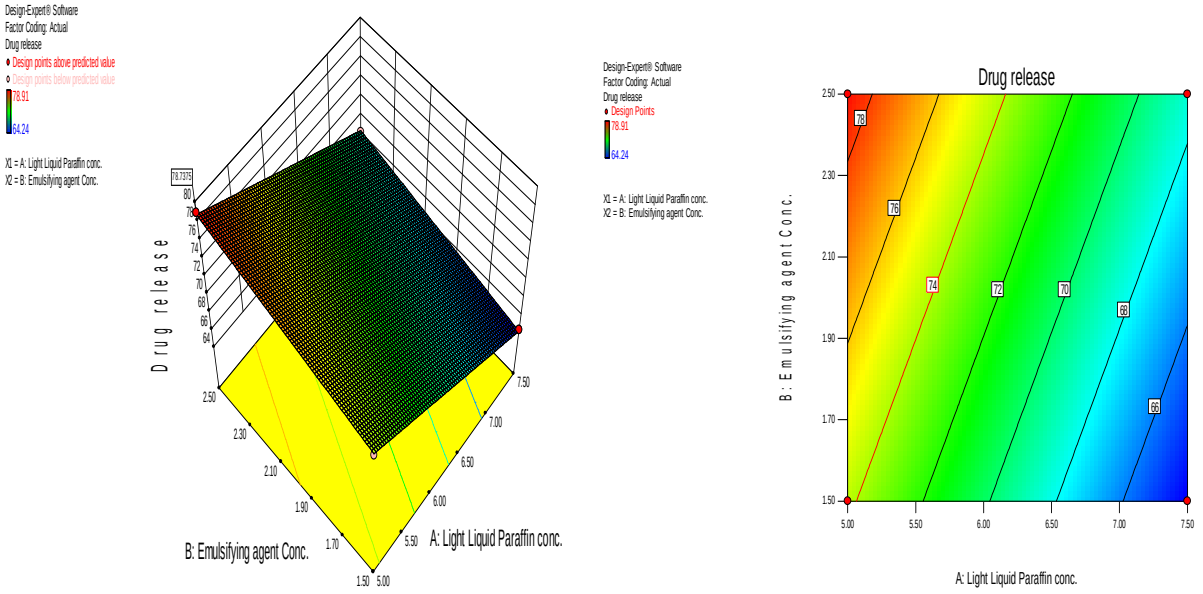
Y_1 (percent drug diffusion) Eq. No. 3

$= +95.01 - 4.44\ X_1 + 7.03X_2$

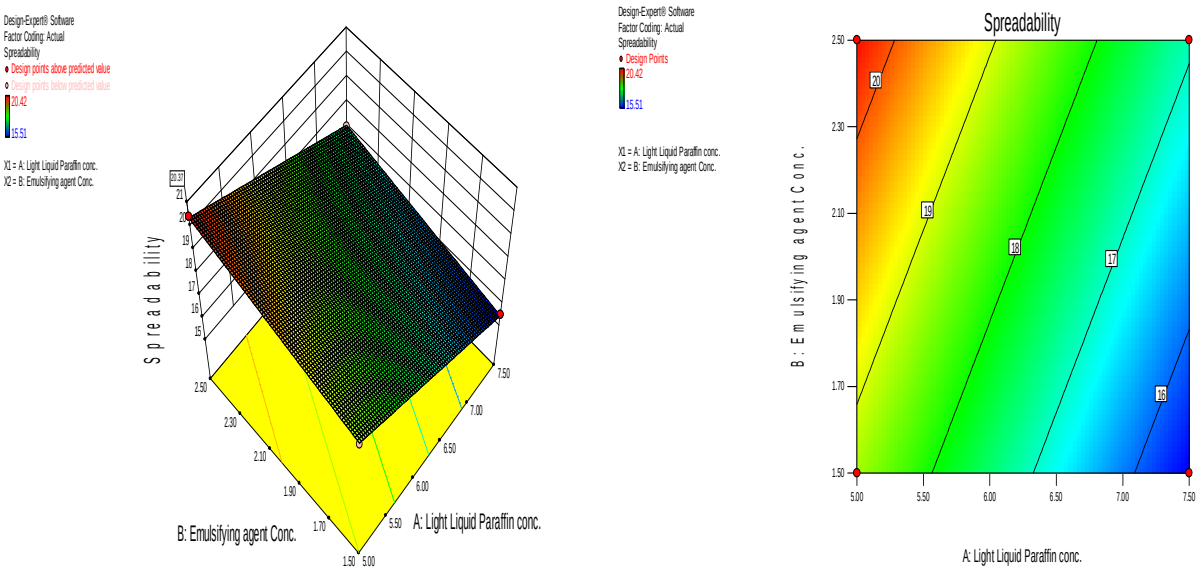
Y_2 (Spreadability) Eq. No. 4

$= +26.39 - 1.17X_1 + 1.98\ X_2$

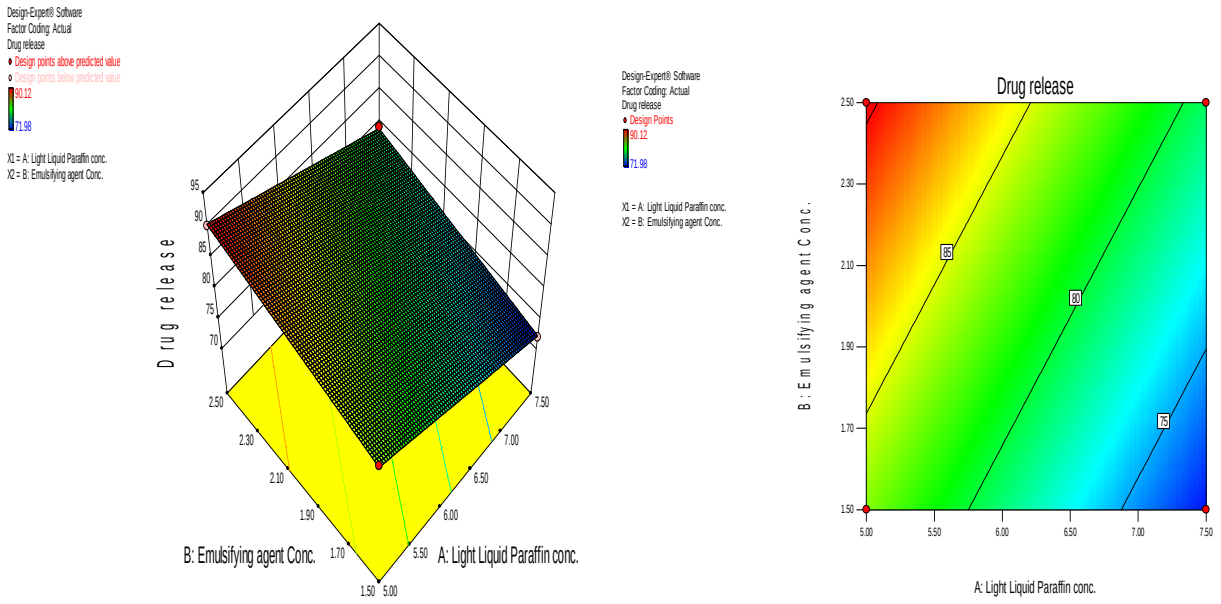
In both cases a negative value represents an effect that favours the optimization, while a positive value indicates an inverse relationship between factor and response. It is evident that the independent variable X_1 (%w/w Conc. of light liquid paraffin) was found to have negative effect on responses: percent drug diffusion (Y_1) and Spreadability (Y_2). The Independent variable X_2 was found to have positive effect on the percent drug diffusion (Y_1) and Spreadability (Y_2).



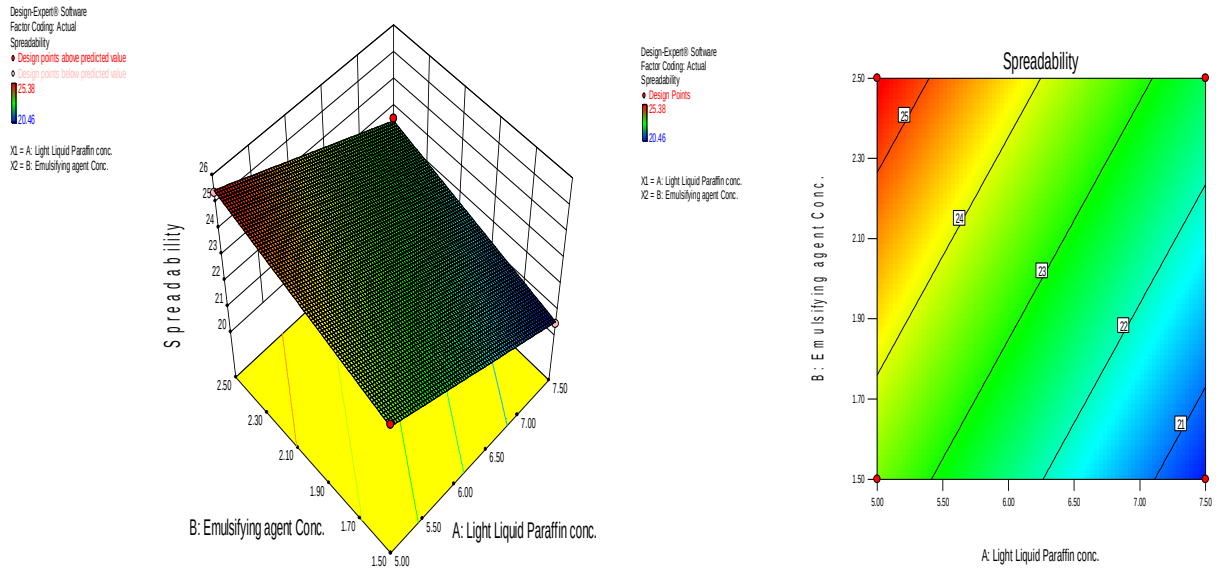
For Response Y1 : % Drug release



For Response Y2: Spreadability



For Response Y1: % Drug release



For Response Y2: Spreadability

Fig. 5: Surface response and Contour plots for Formulations G1- G4

Data Analysis

Formulations C3 and G3 had higher % drug diffusion & spreadability. Details of analysis of variance study were depicted in Table 6.

Table 6: ANOVA for dependent variables for batches C1-C4 & G1-G4

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	P value Prob > F
For Y1 = % Drug release at 12 hr (C1-C4)					
Model	123.96	2	61.98	520.75	**0.0310**
Residual	0.12	1	0.12	-	-
Cor total	124.08	3	-	-	-
For Y2 = Spreading Coefficient (C1-C4)					
Model	13.42	2	6.71	670.76	**0.0273**
Residual	0.010	1	0.01	-	-
Cor total	13.43	3	-	-	-
For Y1 = % Drug release at 12 hr (G1-G4)					
Model	172.85	2	86.43	359.96	**0.0372**
Residual	0.24	1	0.24	-	-
Cor total	173.09	3	-	-	-
For Y2 = Spreading Coefficient (G1-G4)					
Model	12.56	2	6.28	670.76	**0.0479**
Residual	0.029	1	0.029	-	-
Cor total	12.59	3	-	-	-

In both cases Values of "Prob > F" less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. For both responses in case of each type of gelling agent the best fit model is 2FI. For Carbopol 934 based batches the value of correlation coefficient was found to be 0.9990 indicates good fit and the "Pred R squared" of 0.9847 is in reasonable agreement with the "Adj R Squared" of 0.9971 in case of %drug release while the value of correlation coefficient was found to be 0.9993 and the "Pred R squared" of 0.9881 is in reasonable agreement with the "Adj R Squared" of 0.9978 in case of Spreadability. For Xanthan Gum based batches the value of correlation coefficient was found to be 0.9986 indicates good fit and the "Pred R squared" of 0.9778 is in reasonable agreement with the "Adj R Squared" of 0.9958 in case of %drug release while the value of correlation coefficient was found to be 0.9977 and the "Pred R squared" of 0.9633 is in reasonable agreement with the "Adj R Squared" of 0.9931 in case of Spreadability. These results clearly indicate that the % drug diffusion and spreadability both are strongly affected by the variables selected for study.

Table 7: Summary of Regression analysis for Responses Y1 and Y2

2 FI Model	For (C1-C4) Formulations			For (G1-G4) formulations		
	R^2	Adjusted R^2	Predicted R^2	R^2	Adjusted R^2	Predicted R^2
Response(Y1)[a]	0.9990	0.9971	0.9847	0.9986	0.9958	0.9778
Response(Y2)[b]	0.9993	0.9978	0.9881	0.9977	0.9931	0.9633

a: % drug release at 12 hr; b: Spreadability

Validation of Response Surface Methodology (RSM)

Two optimized formulations were obtained from the RSM, the composition, and predicted responses which are listed in Table 8. To confirm the validity of the calculated optimal parameters and predicted responses, the optimum formulations were prepared according to the above values of the factors and subjected to *ex vivo* permeation studies. From the results presented in Table 7, the predicted error was below 5%, indicating that the observed responses were very close to the predicted values. Percentage prediction error is helpful in establishing the validity of generated equations and to describe the domain of applicability of RSM model.

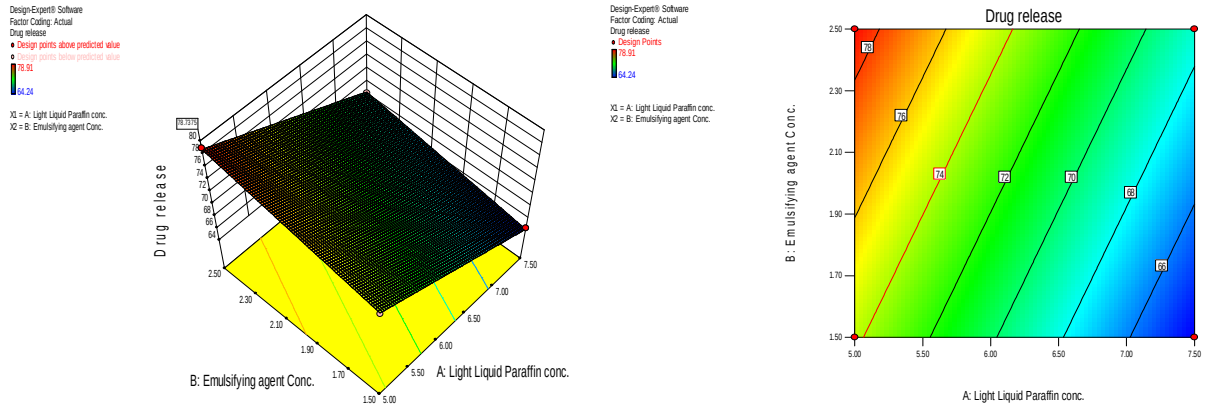
Table 8: Composition of predicted & experimental value with % error

Formulation code	Response	Predicted value	Experimental Value	% Error
C3	Release(%)	78.74	78.91	+0.21
	Spreadability	20.37	20.42	+0.24
G3	Release(%)	90.37	90.12	-0.27
	Spreadability	25.47	25.38	-0.35

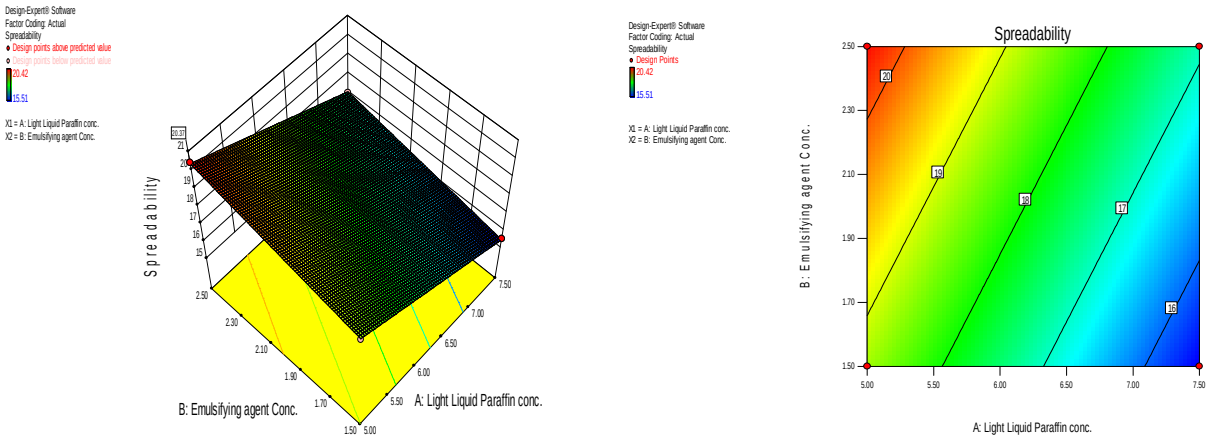
Characterization of Optimized Formulations

Globule size and its distribution in emulgel

Mean globule size in formulation C3 and G3 was found to be 479.4 nm & 287.7 nm respectively. The poly dispersity index (PDI) of these formulations was found to be 0.228 and 0.140 respectively.



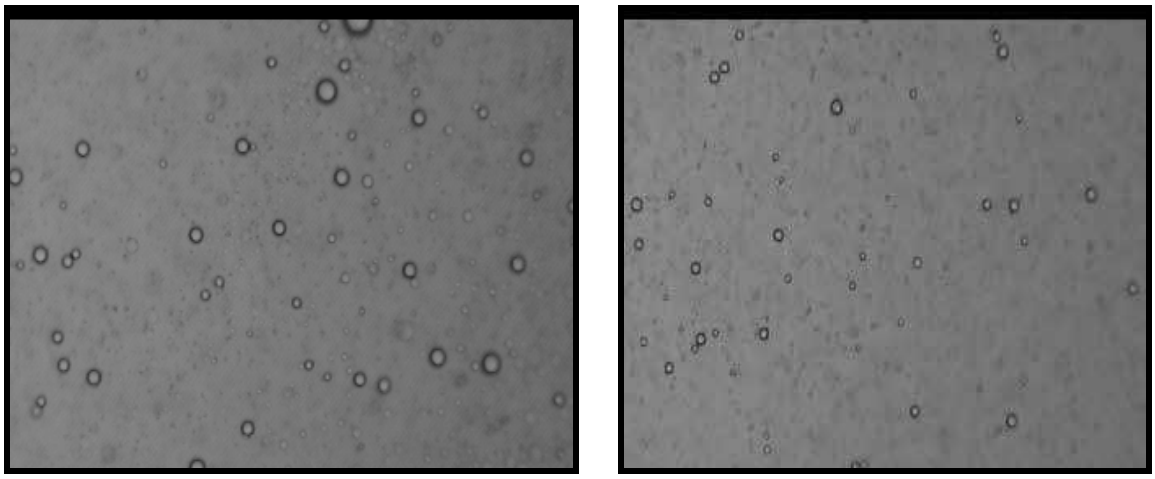
For Response Y1 : % Drug release



For Response Y2: Spreadability

Photomicrography

The suitably diluted emulsions of optimized batches (C3 and G3) were observed under light microscope at 40X (Fig.7). From the photomicrograph, nearly spherical globules of emulsion were observed. Though this study does not give any exact estimate of size however it gives a general idea about formation of emulsion and success of the method used.



(a) **(b)**

Fig. 7: Photomicrographs of Formulation C3 (a) and G3 (b)

Animal Study Experiments

Skin irritation Test

The skin irritation test was carried out to evaluate the tolerability of emulgel formulation after application. It was observed that emulgels were very well tolerated by rat, and no any allergic symptoms like erythema and/ or edema were seen even after 3days.

Skin permeation study

The ex vivo release study of Optimized batches C3 and G3 having low level of light liquid paraffin (5%w/w) and high level of Emulsifying agent conc.(2.5%w/w) compared with the standard gel prepared as per USP (1 % Indomethacin Gel USP). The amount of drug permeated through skin in 12 hours was 71.21% and 81.75 % respectively. In case of standard gel formulation, skin permeation was found to be 36.08%. Both emulgel formulations exhibited higher flux and permeation coefficient as compared to 1% Indomethacin USP gel. The results showed (Table 9) that C3 has the steady state flux (J_{ss}) 282.66(µg/cm^2/h) and apparent permeation coefficient (K_p) 28.26 (cm/h)*10^{-3}, While that G3 has the steady state flux (J_{ss}) 360.35(µg/cm^2/h) and apparent permeation coefficient (K_p) 36.035(cm/h).It can be concluded that drug permeation is enhanced in the emulgels.(Fig.7)

Table 9: Comparison of diffusion parameters of optimized formulations with 1% Indomethacin gel USP. (mean± SD, n= 3)

Formulation	J_{ss} (µg/cm^2/h)	K_p (cm/h)*10^{-3}
C3	282.660±1.45	28.266±1.76
G3	360.350±1.98	36.035±1.06
1 % IND Gel USP	122.270±1.14	12.227±0.98

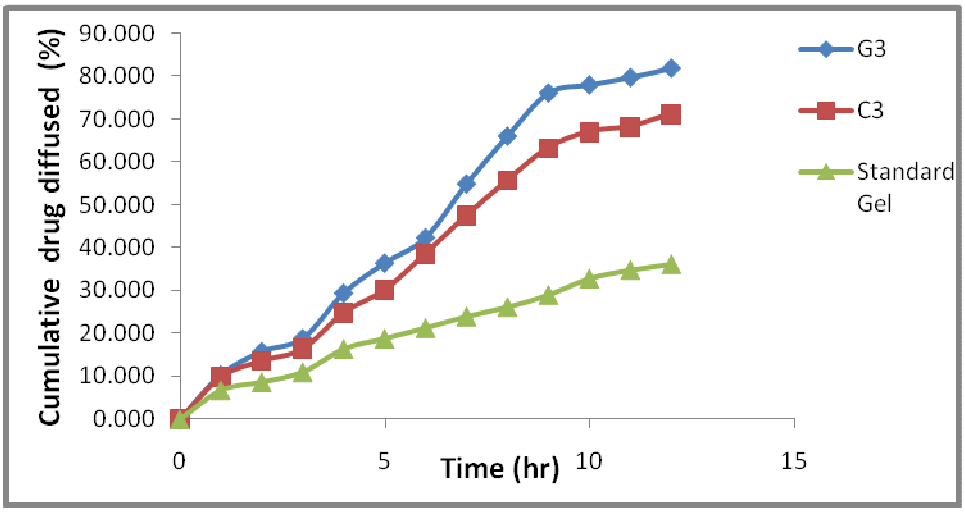


Fig. 7: Ex- vivo diffusion study of optimized emulgels through rat skin

Anti-inflammatory activity

This study was conducted by applying C3 and G3 emulgels topically at site of inflammation and also at a site away from inflammation (transdermal application) because emulgels were exhibiting high *in-vitro* release in comparison to normal gel formulation whereas skin retention was found to be negligible in emulgels. The anti inflammatory action of formulation C3 and G3 was calculated and it was compared with conventional gel prepared as per USP. The % inhibition of conventional gel and both emulgel formulations are given in Table 10. The statistical analysis of results shows that there was significant ($P<0.05$) difference in the inhibition of inflammation in between the gel C3, G3 and conventional gel. So the prepared emulgel formulations are more effective than conventional gel formulation.

Table 10: Anti inflammatory activity of optimized emulgel formulation in comparison with marketed formulation (mean ± SD, n= 6).

Groups	0 hr	4 hr	% Increase in Volume	% Inhibition
Group A	2.89± 0.04	3.48±0.03	20.41±1.12	0
Group B	2.91±0.02	3.04±0.01	4.46±1.59	78.1
Group C	3.08±0.05	3.15±0.04	2.27±1.44	88.8
Group D	2.87±0.05	3.11±0.03	8.39±0.96	59.0

Stability studies

Accelerated Stability studies at 40 ^{0}C/75 % RH indicated that the physical appearance, rheological properties, drug release in the prepared emulgel remained unchanged upon storage for 3 month. The pH observed of prepared emulgels through 3 months of storage was in between 6 to 6.5. Rheological properties and spreadability were obtained uniformly. Emulgel formulation was maintaining drug level after 3 months of accelerated stability. No any significant change was observed in case of drug release of prepared C3 and G3 emulgel formulations.

DISCUSSION

The drug Indomethacin is potent NSAID with common side effects like gastrointestinal irritation. All topical emulgel preparations had better physical properties in concern with consistency, Homogeneity, pH, Spreadability and Viscosity. Spreadability is a term expressed to denote the extent of area on which the gel readily spreads on application to the skin. The therapeutic efficacy of a formulation depends upon spreadability of formulation. Since all emulgel formulations have good spreadability, they have better therapeutic efficacy. Rheological behaviour of the emulgels indicated that the systems were shear thinning in nature showing decrease in viscosity at the increasing shear rates. As the shear stress is increased, the normally disarranged molecules of the gelling material are caused to align their long axes in the direction of flow. Such orientation reduces the internal resistance of the material and hence decreases the viscosity. The data depicted in table 4 shows that no particular trend was evident, though all formulations exhibited shear thinning properties. As xanthan gum based formulations (G1-G4) have better spreadability and low viscosity as compared to carbopol 934 based formulations (C1-C4); they have better therapeutic efficacy. The drug content in case of all emulgel formulation is within acceptance limit.

The higher drug release observed with formulations C3 and G3. This finding may be due to presence of liquid paraffin in its low level and the emulsifying agent in its high level which lead to an increase in the hydrophilicity of the emulgel, which, in turn, facilitates penetration of the release medium into the emulgel and diffusion of the drug from the emulgel. It was observed that all the formulation become liquefied and diluted at the end of the experiments, indicating water diffusion through the membrane. All Xanthan gum based formulations showed higher drug release as compared to carbopol based formulations. The drug release profile exhibited zero order kinetics having diffusion controlled mechanism of drug release.

On the basis of response surface methodology two formulations; each from xanthan gum based & from carbopol 934 based formulations were optimized. Observed poly dispersity index for C3 & G3 prove the homogeneity of emulgel formulations. The small globule size of xanthan gum based emulgel G3 results in better drug release as compared to carbopol based emulgel C3. Photomicrography study ensures formation of stable emulsion in gel & used method was good.

No any allergic signs observed in skin irritation test revealed patient compliance with emulgel formulations. As compared to conventional gel formulation as per USP, emulgel formulations showed better skin permeation. In case of anti-inflammatory study, optimized emulgels have better activity than conventional gel.

Stability study data indicates that emulgel formulations were stable at accelerated stability study conditions.

CONCLUSION

It can be concluded from the above results and discussion that Indomethacin emulgel formulations prepared with Carbopol 934 and Xanthan gum showed acceptable physical properties, drug content and drug release. The optimized batch of emulgel with the liquid paraffin in its low level and the emulsifying agent in its high level proved to be the formula of choice, since it showed the highest drug release in both type of gelling agent. As compared to carbopol 934 based formulations; Xanthan Gum based formulations showed more promising results so natural gelling agent Xanthan gum is better gelling agent than Synthetic gelling agent Carbopol 934.

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