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FORMULATION AND EVALUATION OF RECTAL *IN SITU GEL* FOR AN ANTI-INFLAMMATORY DRUG

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ABSTRACT

The rectum has been an accepted site of drug delivery. Its principal applications have been for local therapy. Some populations are less willing to accept this route as a standard method for drug delivery, but it is the most easily accessible area of the lower GI tract.[1,3] Diclofenac sodium is the most popular NSAIDs have a relatively high therapeutic index in comparison to other NSAIDs. Its half-life is 2 hours. It is a non-steroidal anti- inflammatory drug (NSAID) with analgesic and antipyretic properties. Diclofenac is used to treat pain, dysmenorrhea, ocular inflammation, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and actinic keratosis. In the present study, various *in situ* gelling rectal formulations using Poloxamer 407 with and without microemulsions were prepared. Different formulations of Diclofenac sodium using Poloxamer 407 in concentration of 15-20% w/w (B1-B10) were prepared with and without microemulsion addition. Further, different formulations of poloxamer 407 (18%w/w) were also prepared by combining with HEC in concentration of 0.5 %w/w (B13) and HPMC E50 LV in concentration of 0.5 %w/w (B14). All these rectal formulations were evaluated for appearance, clarity, pH, gelling ability, viscosity and percent drug release. The formulations showing transparent and clear appearance, quick and stable gelation, shear thinning properties, excellent sustained drug release up to 82- 98% after 8hrs were selected for further characterization. The selected formulations were then subjected for studying the stability to refrigerated condition (5°±3). The optimized formulations, B4 (18%w/w poloxamer with drug microemulsion), B13 (18%w/w poloxamer, 0.5 %w/w HEC and drug microemulsion) and B14 (18%w/w poloxamer, 0.5 %w/w HPMC E50 LV and drug microemulsion) were found to be very clear, transparent, forming quick and stable gels with shear thinning behavior. The optimized formulations B4, B13 and B14 showed excellent mucoadhesion and sustained drug release.

KEYWORDS: Diclofenac sodium, Poloxamer 407, Captex 200, Carbopol 974, Ethyl oleate etc.

INTRODUCTION

Ideally, rectal dosage forms should remain long enough in the rectum without being ejected for ensuring maximal absorption of the drug. For this purpose, a possible strategy consists in imparting bio adhesive properties to the formulation, either to improve a local effect or to enhance drug absorption. Such a strategy has already been successfully attempted for improving rectal absorption of drugs. However, it should be kept in mind that in addition to the immobilization of the dosage form, drug release has to be modulated for occurring and being completed during the immobilization period, making the formulation of such systems quite complicated. The rectal route has been used to deliver many drugs and offers similar advantages over oral delivery to those by the nasal route. The rectal route offers many advantages such as

1. A very weak enzymatic degradation of drugs because of the very limited presence of proteolytic enzymes and other enzymes in the rectum.
2. A partial avoidance of the first hepatic passage.
3. Transport by the lymphatic system of a large quantity of drug.
4. A constant and static environment.
5. A perfect availability for the patients who have problems of vomiting and nauseas and
6. Finally, it is a way of avoiding.

MATERIALS AND METHOD

Materials

Diclofenac sodium was obtained as a gift sample from Amoli Organics Ltd, Mumbai, India. Poloxamer 407 was received as a gift from BASF, Mumbai, India. Carbopol 974 was a gift sample from Noveon, Mumbai. HPMC

E50 LV was purchased from Colorcon, Asia Pvt. Ltd. Verna-Goa. HEC (Natrosol 250) was received as a gift from Research Lab. Fine Chemicals, Mumbai. Capmul MCM was received as a gift from Abitec Corporation, US.

Method

Preparation of Microemulsion

An accurately weighed oil, surfactant, and co-surfactant were added in a glass vial and mixed by vortexing. Diclofenac sodium was added to above formulation and sonication was done until the drug was completely dissolved. To above solution, an accurately weighed quantity of water was added & mixed by vortexing till a transparent clear microemulsion was obtained. The mixture was stored at room temperature until further use. Table no.1 indicates different microemulsion formulation using Captex 200 or Capmul MCM EP as oil phase.

Table 1: Composition of microemulsion formulations prepared with S:Cos ratio,1:8.

Formulation code	Name of Formulation	Composition (%w/w) of different components in Microemulsion with S:Cos ratio 1:1*		
		% Oil	% S mix	% water (% w/w)
A	(Capmul MCM+Tween 80+PEG 400)	1.50%	12%	84%
B	(Captex 200P+Tween 80+PEG400)	1.50%	12%	84%

*Each formulation contains 2.5% w/w of Diclofenac sodium

Evaluation of Microemulsion

pH:[4,7]

The pH of formulation was determined by using pH meter. The pH meter was calibrated before each use with standard pH 4.7 and 9.2 buffer solutions. The formulation temperature was maintained at 25±3°C. The pH meter electrode was immersed in formulation and the pH was recorded.

Conductance[7]

The conductance of microemulsion formulations was determined by using conductometer. The conductometer was calibrated before each use with standard KCl solution. The formulation temperature was maintained at 25±3°C. The conductometer electrode was immersed in formulation and the conductance was recorded.

Determination of viscosity[7,9]

The viscosity of the solution was determined using programmable viscometer (Brookfield LVDV-II) with small sample adopter and it was operated under following conditions. 7ml of prepared Microemulsion solution was transferred in sample cell which was placed carefully within the adopter. The guard leg was placed around the adopter and the volume of sample was stirred slowly using a motor driven stirring element. The viscosity values were recorded from the display window at 20 rpm.

Fig. 1: Brookfield viscometer.

Thermodynamic stability studies[11,12]

These studies included exposure of formulation to thermal (both low & high) as well as mechanical stress & observing the effects on the phase separation, clarity of the microemulsion formulation. The test was carried out in two parts;

a) Alternate heating (40°C) / cooling (4°C) Cycle

It includes storage of formulations at each of these temperatures viz. 4°c and 40°c alternately for not less than 48hr for three cycles. The Microemulsion formulations were observed for any kind of instability by evaluating them for any change in self- emulsification time and optical clarity and only stable formulations were selected for subsequent test of centrifugation.

b) Centrifugation

It included centrifugation of Microemulsion formulation for 30 min at 3500rpm & formulations were observed for instability by evaluating them for change in phase separation, and optical clarity.

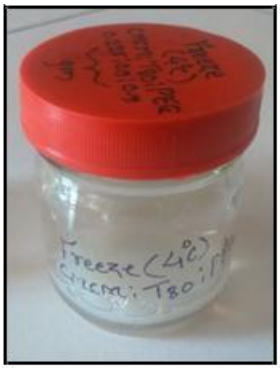

Fig. 2: Microemulsion kept at 4°C for cooling cycle.

3. Globule size analysis

Droplet size distribution of Microemulsion diluted with water was determined using Nanophox (NX0088, Symptec GmbH, Germany). 1 ml of Microemulsion Sample was diluted to 10 ml using purified water. All the measurements were carried out in triplicates at a temperature of 24.99°C and at a fixed angle of 90° to the incident laser beam. Data was analyzed by software (Version 5.7.1.0) and values of mean particle size and particle size distribution were recorded.

3. *In vitro* diffusion study[12,13]

The study was performed using Franz diffusion cells with available diffusion area of 1.77cm^2 and with 36 ml volume. Approximately, 1 ml of Diclofenac sodium loaded microemulsion was placed in the donor compartment and the receptor compartment was filled with mixture of phosphate buffer solution (pH 7.4) & 10% PEG 400, maintained at 37±1°C and stirred by using magnetic stirring bars (300rpm).For *in vitro* release studies, artificial dialysis membrane was soaked in the same buffer solution for 24 hrs before mounting on a diffusion cells. At 0.5,1,2,3,4,5,6,7,8 hrs, all the receptor liquid was withdrawn and for ensuring "sink condition", volume of the liquid was replaced immediately. Then the samples were filtered and Diclofenac sodium concentrations were assayed by UV spectrophotometric method at 277 nm.

Fig. 3: Franz diffusion assembly for microemulsion formulation.

Formulation of *in situ* gelling rectal solution by using different gelling agents[10,11,12]

The *in situ* gelling rectal solutions of Diclofenac sodium were prepared by using different gelling agents.

The formulation were prepared by following ways-

1) Development of rectal *in situ* gels of Diclofenac sodium with and without microemulsions

A. Using individual polymer (Table 2). I] Poloxamer 407

B. Using combination polymers with microemulsions (Table 3).

I] Poloxamer 407 with carbopol 974

II] Poloxamer 407 with polycarbophil III] Poloxamer 407 with HEC

IV] Poloxamer 407 with HPMC E50 LV

Table 2: Composition of *in situ* gels of Diclofenac sodium using poloxamer 407 with drug.

Formulation code	ME(formulation B)	% Poloxamer 407
B1	+	15
B2	+	16
B3	+	17
B4	+	18
B5	+	20
B6	-	15
B7	-	16
B8	-	17
B9	-	18
B10	-	20

Rectal gels containing 2.5 % Diclofenac sodium

(+) indicates formulations with ME

(-) indicates formulations without ME

Table 3: Composition of *in situ* gels of Diclofenac sodium using combination of polymers.

Formulation code	Poloxamer 407 (%w/w)	Microemulsion (B)	Carbopol 974 (%w/w)	Polycarbophil (%w/w)	HEC (%w/w)	HPMC E50 LV (%w/w)
B11	18	+	0.5	-	-	-
B12	18	+	-	0.5	-	-
B13	18	+	-	-	0.5	-
B14	18	+	-	-	-	0.5

All above formulations contained 2.5 % w/w of Diclofenac sodium dissolved in Microemulsion containing 1.5%w/w Captex 200P, 6% Tween 80, 6% Polyethylene glycol and distilled water to make 15ml of solution.

The following steps were involved in the preparation of rectal solutions of Diclofenac sodium using poloxamer and other co-polymers.

Evaluation of *in situ* gelling rectal formulations of all batches prepared

The rectal formulations were evaluated for various characteristics as follows

1. Test for appearance and clarity[12,13]

The rectal formulations were observed carefully for colour, odour and presence of suspended particulate matter if any. The clarity of solutions was further assessed by observing them against a dark and white background.

Formulations were graded as follows:

Turbid -

Slightly turbid +

Clear solution ++ Clear and transparent +++

2. Determination of pH values of rectal formulations

The pH of rectal formulation was determined by using pH meter. The pH meter was calibrated before each use with standard pH 4, 7 and 9.2 buffer solutions. The formulations temperature maintained at 25 ± 3^0C. The pH meter electrode was immersed in formulation and the pH was recorded.

3. Test for gelling ability

The individual rectal formulations (100µl) were added into glass vials. The transition of solution to viscous gel was observed visually and numerical scores were assigned depending on

- Quickness of gel formation
- Time taken for collapse of gel structure on shaking the vials

The formulations were graded as shown in table 4

Table 4: Gradation of gelling ability.

Sr. No.	Grade	Observation
1	-	No phase transition
2	+	Formulation of gel after 60sec and gel collapsed within 1hr
3	++	Formulation of gel after 60sec and gel collapsed within 3hr
4	+++	Formulation of gel within 60sec and gel remained stable for more than 6-7hr

4. Spreadability Test

For spreadability measurement of *in situ* gel, slanting type of apparatus were used which are given below in fig.5 one drop of formulation was kept on 0 marking of the scale of apparatus and measure the distance travelling from 0 marking of the scale of apparatus in cm.

Fig. 5: Spreadability apparatus.

5. Determination of viscosity of rectal formulations

The viscosity of the gel was determined using programmable viscometer (Brookfield LVDV-II) with T-bar spindle code S95 and it was operated under following conditions.

The spindle was attached to the lower shaft of the viscometer. The motor was turned on and spindle was rotated within the container containing 20ml of performed gel. The helipath movement was controlled to avoid touching of the spindle to any part of the sample holder especially the bottom. A typical run involved changing the angular velocity from 0.5 to 100rpm at a controlled speed which was changed after every 10sec (0.5… 100rpm). The viscosity values at each rpm were noted from the display window.

6. Mucoadhesive strength study by texture analyzer[23]

For mucoadhesive strength measurement, Brookfield texture analyzer apparatus was used. The force required to detach the gels from goat rectal mucosa was measured

as mucoadhesive strength.

7. Test for stability of selected *in situ* gelling rectal formulations

Test for stability of selected *in situ* gelling rectal formulations at refrigerator conditions in depicted in table 5.

Table 5: Details of stability test at refrigerator conditions.

Sr. No.	Specifications	Standard values
1	Duration of study	30 days
2	Temperature condition	5±3°C
3	Frequency of testing	Day 0, day 15, day 30

To assess the shelf life of optimized gel formulations, the stability tests were conducted (5±3°C) for the period of 30 days in refrigerator condition. The formulations were filled in glass containers and were stored at refrigerator conditions (5±3^{0}C). The samples were tested initially and then at 15 days and 30 days intervals for the following parameter.

I] Appearance/Clarity II] $_p$H
III] Gelling ability IV] Viscosity
V] Drug content by UV spectrophotometric method
VI] *In vitro* drug release of rectal formulations using dialysis membrane.

RESULT AND DISCUSSION

Formulation and evaluation of Microemulsions of Diclofenac sodium:-

1. Solubility Studies

The microemulsion, consisting of oil, surfactant, cosurfactant, drug and water should be clear and monophasic liquid at ambient temperature. Solubility studies were aimed at identifying suitable oil, surfactant and cosolvent system for the development of Diclofenac sodium microemulsion. Identifying the suitable oil, surfactant, and cosurfactant having maximum solubilizing potential for the drug investigation is very important to achieve optimum drug loading.[4] The result of Diclofenac sodium solubility in different oils is shown in table no. 6 and fig no.6 as well as solubility in surfactant and co-surfactant is shown in table no. 7 and fig no 7

Among the various oils, Captex 200 showed the highest solubility of Diclofenac sodium (125.00±1.20mg/ml) followed by Capmul MCM (120.00±2.56mg/ml), Caprol PGE (110.00±1.92 mg/ml), Captex 500 (109.46±0.232mg/ml), Oleic acid (108.00±1.09 mg/ml) Cremophor (81.87±1.53 mg/ml), Ethyl oleate (80.01±0.232 mg/ml), Transcutol P (28.58±2.067 mg/ml). Solubility of Diclofenac sodium in surfactant was found to be 54.89±0.856 mg/ml in Tween 20 and 56.50±1.09 mg/ml in Tween 80. Solubility of Diclofenac sodium in co-solvent was determined and found to be 62.80±3.09 mg/ml in propylene glycol and 102.10±2.98 mg/ml in polyethylene glycol 400. Captex 200 P and Capmul MCM were selected for further study, considering high solubility of Diclofenac sodium in these oils.

Table 6: Solubility of Diclofenac sodium in Various Oils.

Sr. No.	Name of Oil	Solubility of Diclofenac sodium at 25±1^{0}C (mg/ml)*
1	Capmul MCM	120.00±2.56
2	Oleic acid	108.00±1.09
3	Caprol PGE860	110.00±1.92
4	Ethyl oleate	80.01±0.232
5	Captex 500	109.46±0.232
6	Captex 200P	125.00±1.20
7	Captex 350	112.00±0.90
8	Cremophor	81.87±1.53

*Results are expressed as Mean ±SD (n=3)

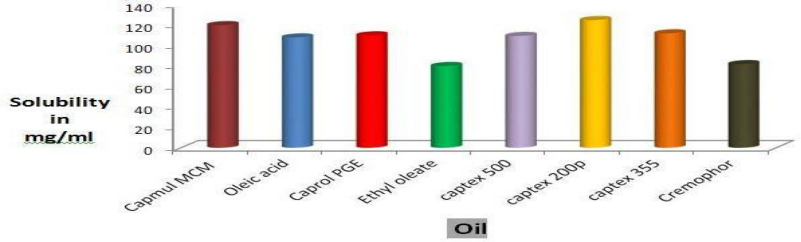

Figure 6: Solubility of Diclofenac sodium in various oils.

Table 7: Data for solubility of Diclofenac sodium in various surfactants / cosurfactants.

Sr. No.	Name of surfactants and cosurfactants	Solubility of Diclofenac sodium at 25±10C (mg/ml)*
1	Tween 80	56.50±1.09
2	Tween 20	54.89±0.856
3	Transcutol p	28.58±2.067
4	Polyethylene Glycol 400	102.10±2.98
5	Propylene glycol	62.80±3.09

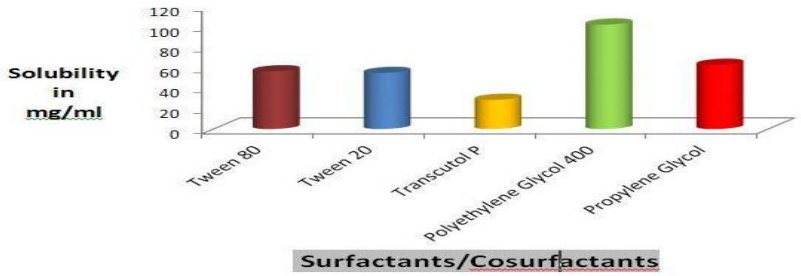

Figure 7: Solubility of Diclofenac sodium in various surfactants/cosurfactants.

Preliminary screening of surfactants

Nonionic surfactants are generally considered less toxic than ionic surfactants. They are usually accepted for oral ingestion. Various surfactants were compared for their emulsification efficiencies using oil phase. The amount of oil required to produce turbidity in various surfactant solutions are demonstrated in Table 8. From the results, it was inferred that the oily phase exhibited good emulsification efficiency with Tween 80. Thus, Tween 80 was selected as a surfactant for further study considering its good emulsification efficiency for both oils, Capmul MCM & Captex 200 P.

Table 8: Data for Emulsification efficiency of surfactants.

Sr. No.	Surfactants	The amount of oil (Capmul MCM) required to produce turbidity in 2.5 ml of 15% surfactant solution	The amount of oil (Captex 200P) required to produce turbidity in 2.5 ml of 15% surfactant solution
1	Tween 80	0.08ml	0.1 ml
2	Tween 20	0.05 ml	0.07 ml

Diclofenac sodium showed maximum solubility in Polyethylene glycol 400 (102.10 mg/ml) hence it was selected as cosolvent for further study. Solubility of Diclofenac sodium was also determined in the blends of selected oils, surfactants & cosurfactants & the results are shown in table 9 & fig.8 In both the blends , drug was found to be highly soluble which indicates the possibility of sufficient drug loading in microemulsion system using above selected oils, surfactants & cosurfactants.

Table 9: Data for solubility of Diclofenac sodium in various Blends.

Sr. No.	Mixture of Oil & Smix(1:1)in ratio 9:1	Solubility of Diclofenac sodium at 25±1°C(mg/ml)*
1	A(Capmul MCM+Tween 80+PEG 400)	157.65±0.0897
2	B(Captex 200P+Tween 80+PEG400)	196.84±0.0899

*Represents mean ±S.D (n=3)

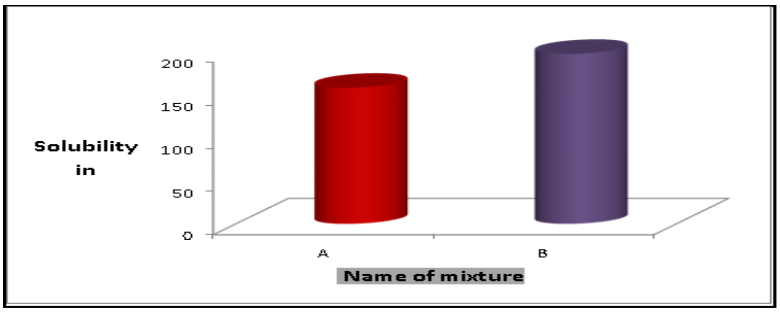

Figure 8: Solubility of Diclofenac sodium in various Blends.

Thermodynamic stability studies[8,9]

In order to evaluate the possibility of metastable formulations, stress testing is required. Thus, the selected formulations were subjected to different thermodynamic

stability test by using heating cooling cycle and centrifugation. The results of thermodynamic stability studies are expressed in Table 11. The results of thermodynamic stability studies revealed that there was no significant change in the Microemulsion formulations. No phase separation, turbidity and creaming or cracking were observed. Thus, all the formulations were found to be thermodynamically stable.

Table 11: Data for Thermodyanamic stability test of different formulations.

Formulation code	Appearance & clarity before thermodynamic stability study	Appearance or clarity after thermodynamic stability studies		Inference
		H/C*	Cent*	
A	++	++	++	**Stable**
B	++	++	++	**Stable**

Globule size analysis

Droplet size of microemulsion formulation is a critical parameter in the adapted strategy of enhancing the drug bioavailability. The smaller the droplet size, the larger interfacial surface area will be provided for drug absorption. The mean droplet sizes of the selected microemulsion formulations are reported in Table 12. The average droplet size of the formulation B was found to be less than 100 nm (33.72, X_{50}). Size distribution curve of formulation B is shown in Figure no 15.

Table 12: Data for Globule size analysis

Formulation code	Particle size(nm)		
	X_{10}	**X_{50}**	**X_{90}**
B	27.96	33.72	40.71

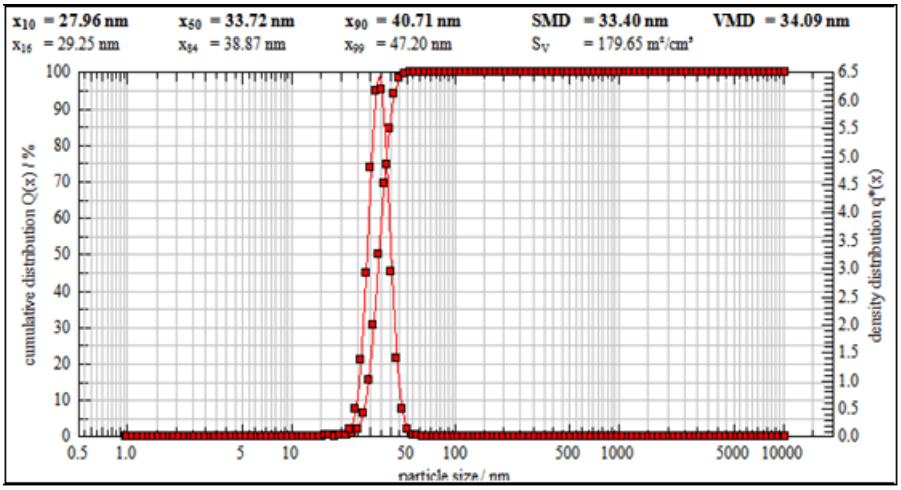

Figure 15: Droplet size distributions of microemulsion formulation B.

***In vitro* diffusion study using dialysis membrane**

The results of *in vitro* diffusion study of microemulsion formulations through dialysis membrane using Franz diffusion cell are given in table 13 & figure 15.

Table 13: Average*(±SD) cumulative percentage drug diffused of Diclofenac sodium from Capmul MCM(A) & Captex 200(B) loaded microemulsion formulations (S:Cos ratio 1:1) through dialysis membrane.

Time (hr)	Average*(±SD) Cumulative percentage drug diffused	
	A	**B**
0	0	0
0.5	1.57±0.06	1.941±0.12
1	4.698±0.14	5.370±0.86
2	10.778±0.35	9.625±0.04
3	16.879±1.34	12.185±0.16
4	19.115±0.07	14.991±0.18
5	24.915±1.08	21.058±0.36
6	29.505±0.87	28.503±0.40
7	32.046±0.36	36.973±0.13
8	35.938±0.17	56.556±2.84

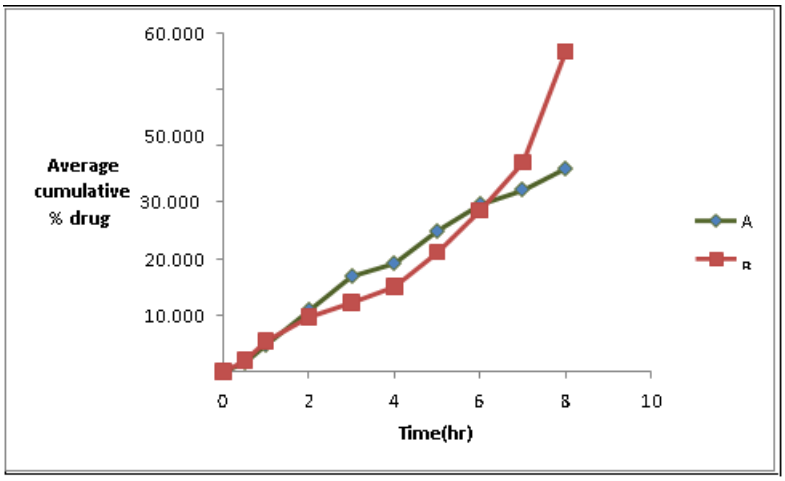

Figure 16: In vitro drug diffusion profile of Capmul MCM(A) & Captex 200(B) loaded microemulsion system formulations (S:Cos ratio,1:1) through dialysis membrane.

Table 13 & figure 16. represents the cumulative % drug diffused from the formulations A and B, in 8 hrs through dialysis membrane. For formulations A & B ,% drug diffused in 8 hrs varied from 35.938±0.17 to 56.556±2.84,which shows the drug diffusion greatly depends upon type of oil used in microemulsion formulation.

Formulation and evaluation of in situ gelling of rectal formulations:

In order to overcome the drawback of conventional rectal formulations, an *in situ* gelling system of Diclofenac sodium was formulated.[1,3] The polymers undergoing solution to gel phase transitions, as a result of physical or chemical change were selected for the study. Poloxamer 407 is a block copolymer consisting of PEO and PPO units in ratio 7:3 and undergoes temperature dependent sol to gel phase transition. At the concentration of (15% w/v) or higher in aqueous solution, it is transferred from low viscosity solution to a gel under ambient temperature, while concentration higher than 20% led to difficulty in preparation and application of formulation (remains gel at room temperature). Hence, *in situ* gelling formulation of Poloxamer 407 in concentration range of 15% w/v to 20% w/v were prepared and evaluated so as to obtain an optimum formulation, which resulted into quick and firm gel at body temperature. One major problem with Poloxamer 407 is its weak gel strength, resulting into lower residence time on mucosal surface. In an attempt to formulate a retentive mucoadhesive *in situ* gelling formulation different polymers like HPMC E50 LV, Carbopol 974, HEC (Natrosol 250) and Polycarbophil were combined with Poloxamer 407,Liturature review suggested the use of these mucoadhesive polymers even at lower concentrations of 0.5 % w/v. Thus, 0.5 % w/v of these polymers were added to Poloxamer 407. All the formulations were evaluated further for appearance and clarity, gelling ability, gelation temperature, pH, viscosity, drug content, *in vitro* drug release and mucoadhesive strength.

Evaluation of in situ gelling formulations with drug

Table 14: Data for evaluation of in situ gel formulation with drug.

Formulations	Formulation code	Appearance & Clarity	pH	Gelling ability	Gelation temperature	spreadability test(cm)	% drug Content
With Microemulsion(B)	B1	Clear	7.4	+++	40	5	97.00±1.1
	B2	Clear	7.6	+++	20.35	3.7	98.15±0.0
	B3	Clear	7.7	+++	18.5	3.5	98.12±1.4
	B4	Clear	7.8	+++	16	3.1	99.48±0.5
	B5	Clear	7.9	+++	15	2.9	98.96±0.05
Without Microemulsion	B6	Clear	7.4	+++	38	5.5	96.7±0.10
	B7	Clear	7.6	+++	36.5	4	98.09±0.08
	B8	Clear	7.7	+++	34	3.7	98.11±0.67
	B9	Clear	7.8	+++	33	3.3	99.23±2.3
	B10	Clear	7.8	+++	30	3.1	96.05±2.15

Table 15: Physical characteristics of in situ gelling rectal formulation of poloxamer 407 with mucoadhesive polymers + Diclofenac sodium.

Formulation code	Appearance and Clarity	pH	Gelling ability	Spreadability Test (cm)	% drug content
B11	+	7.5	+	1	95.89±0.09
B12	++	7.7	+	1.1	98.00±1.21
B13	++	7.4	+++	1.3	99.45±1.35
B14	++	7.3	+++	1.4	99.28±0.78

Appearance and clarity

The formulations of Poloxamer 407 alone and with HEC, HPMC E50 LV & Polycarbophil were found to be very clear without any precipitation. The formulations containing carbopol 974 were found to be turbid, which might be because of precipitation as a result of the incompatibility of Diclofenac sodium with these polymers. Hence these formulations were not considered for further study.

pH of rectal formulations

The pH of rectal formulations formulated using Poloxamer 407 was found to be in the range 7-8. These pH values were considered to be acceptable since the rectal pH ranges between 7-8. Hence no discomfort in rectum might occur on instillation.

***In situ* gelling ability of formulations**

Percent drug content

The Percent drug content of *in situ* gelling rectal formulations is given in table no.14 & 15 and found to be in the range 95.89% to 99.48 %.

Viscosity of rectal formulations

There are no specifications as such for the viscosity of *in situ* gelling systems. These systems are expected to undergo shear thinning (decreasing in viscosity at increasing shear rate) in gel state due to the thixotropic behavior of the gels formed.

The viscosities of rectal formulation were determined using Brookfield viscometer and shown in table no. 16,17,18,19,20 and fig no.17,18,19,20,21,22,23,24.

Construction of Pseudo ternary phase diagrams

For the present study, Capmul MCM & Captex 500 were selected as oils and Tween 80 as surfactant. The Polyethylene glycol 400 was selected as cosolvent for both the systems as Diclofenac sodium showed very good solubility in Polyethylene glycol 400 (102.10 mg/ml).Ternary phase diagram using selected oils, Smix (in different ratio of S:Cos) & water were plotted & one phase region were identified.

From o/w microemulsion regions, various compositions for microemulsion system were selected. Figure no.9,10,11,12,13,14 indicates pseudoternary phase diagrams of system using Capmul MCM EP/Captex 500,Tween 80 & Polyethylene glycol 400(in different ratios like1:1,1:2,2:1) and water.

An ideal *in situ* gelling system should be a free flowing liquid with low viscosity at non-physiological conditions (pH 4.0-25^{0}C) to allow reproducible administration into the rectum. It should also undergo *in situ* phase transition to form strong gel capable of withstanding shear forces in the rectum and sustain drug release at physiological condition in PBS (pH 7.4 and 37^{0}C).

Gelation Temperature

Gelation temperature of the formulations was found to be decreased with increased in the Poloxamer concentration. When microemulsion was added to the gels, gelation temperature was found to be further decreased. Formulations, in which mucoadhesive polymers were added, were found to be gel even at refrigerated temperature. Hence, these formulations cannot be used as *in situ* gelling system but can be used as gel formulations.

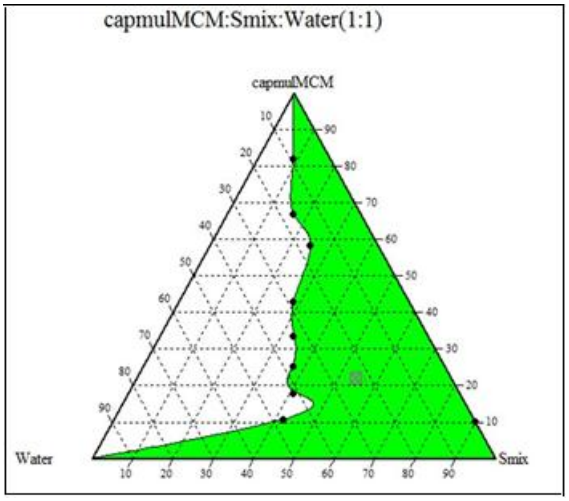

Figure 9: Pseudoternary phase diagram of system with following components: Capmul MCM (oil), Tween 80:Polyethylene glycol 400 (1:1) (S mix) and water.

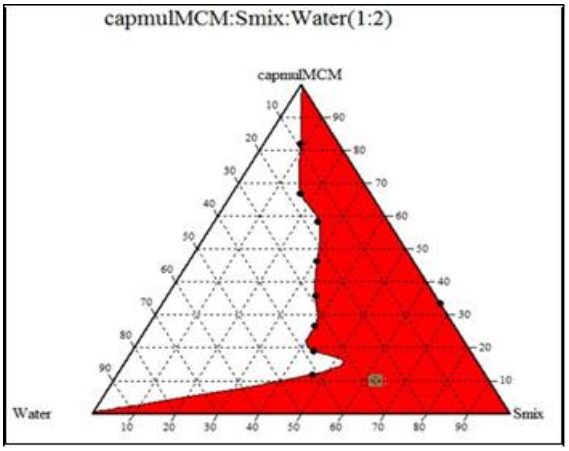

Figure 10: Pseudoternary phase diagram of system with following components: Capmul MCM (oil),Tween 80:Polyethylene glycol 400 (1:2) (S mix) and water.

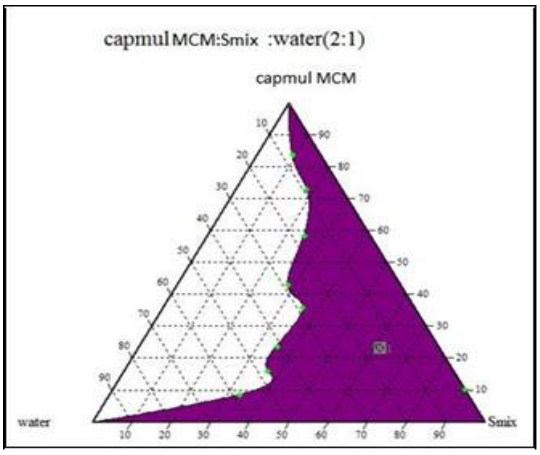

Figure 11: Pseudoternary phase diagram of system with following components: Capmul MCM (oil),Tween 80:Polyethylene glycol 400(2:1) (Smix) and water.

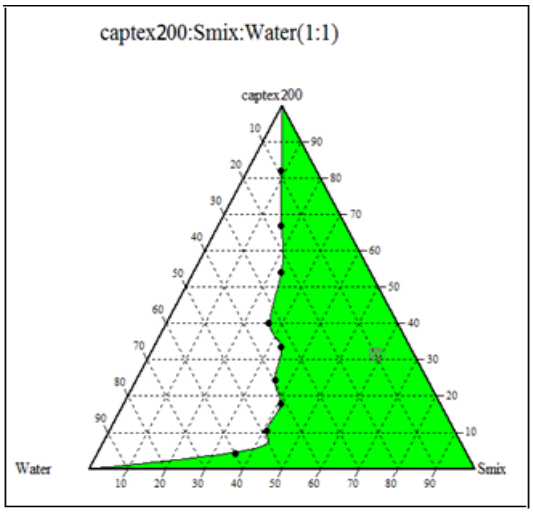

Figure 12: Pseudoternary phase diagram of system with following components: Captex 200(oil),Tween 80:Polyethylene glycol 400(1:1)(Smix) and water.

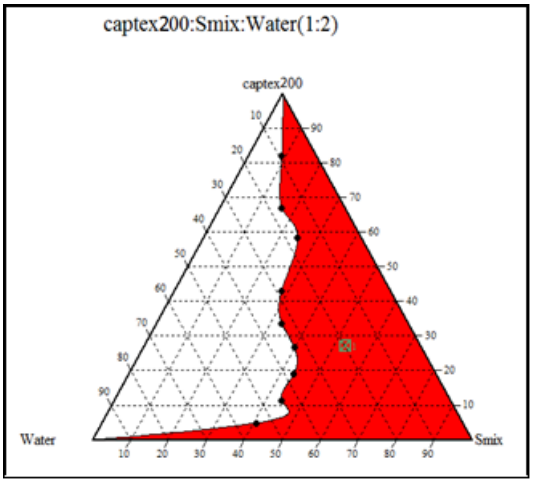

Figure 13: Pseudoternary phase diagram of system with following components: Captex 200(oil),Tween 80:Polyethylene glycol 400(1:2) (Smix) and water.

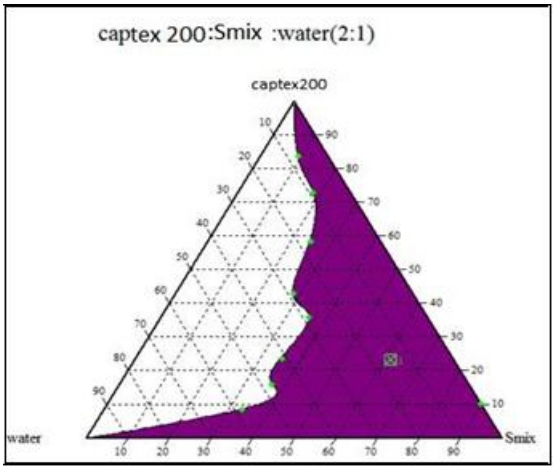

Figure 14: Pseudoternary phase diagram of system with following components: Captex 200(oil),Tween 80:Polyethylene glycol 400(2:1) (Smix) and water.

From pseudoternary phase diagram, Fig no.9,10,11,12,13 and 14. it is evident that for the system of Capmul MCM, Tween 80 & Polyethylene glycol 400, the O/W microemulsion region was found to be decreased as compared to the system of Captex 200, Tween 80 & Polyethylene glycol 400 for different ratio (1:1,1:2,2:1).

Formulation of Diclofenac sodium microemulsions

On the basis of preliminary studies, two liquid microemulsion formulations of Diclofenac sodium, with oils, Capmul MCM (formulation A) and Captex 200P (formulation B), Tween 80 (surfactant) and PEG 400 (cosolvent) were selected. These microemulsion composed of 1.5% w/w of oil, 6% w/w of surfactant, 6 % w/w of cosurfactant and 84% w/w of water.

Evaluation of Microemulsion Formulations

All the prepared Microemulsion formulations were evaluated for optical clarity, percent drug content, thermodynamic stability studies, *in vitro* drug permeation study and globule size analysis (Table. 10).

Appearance & clarity

The prepared microemulsion formulations were optically clear & transparent.

They were marked as Turbid (-), slightly turbid (+) and transparent (++).

pH:[4,8]

The pH of microemulsions were found to be in the range 7.5-8.0.These pH values were considered to be acceptable since the rectal pH ranges between 7-8.

Conductance

The conductance of microemulsion formulations was determined by using conductometer. The conductance of microemulsion formulations was found in the range of 7-12(microsiemens/cm).

Percent Drug Content

Estimation of Diclofenac sodium content in microemulsion formulations was carried out by UV spectrophotometric method at 277nm.The content of Diclofenac sodium in various microemulsion formulations was found to be in range 95-100% w/w.

Viscosity of microemulsion formulations

The viscosity of microemulsion formulations was carried out by Brookfield viscometer. The viscosity of microemulsion formulations (A and B) was found to be 240cps and 350cps respectively.

Percent Transmitance

% Transmittance in microemulsion formulation was carried out by UV spectrophotometric method at 283.5 nm. The percentage transmittance of Diclofenac sodium microemulsion formulations was found to be 100 % and 99.8 % respectively.

Table 10: Data for evaluation of different microemulsion formulation.

Sr. No.	Formulati o n code	Appearance & clarity	pH	Conductance (ms/cm)	% Drug content	Viscosity in cps (20 rpm)	% Transmissio n
1	A	++	7.6	9.45	95.36	240	100
2	B	++	8	12	97.29	350	99.8

Table 11: Viscosity of B1 & B6 in situ gel formulation at varying rpm.

Formulation code	B1				B6			
	Before Dilution		After Dilution		Before Dilution		After Dilution	
RPM	A	D	A	D	A	D	A	D
0.5	1400	1200	800	650	1400	1000	600	540
1	1100	1000	600	520	900	800	400	250
2	950	750	550	400	750	700	300	200
2.5	720	640	300	240	680	640	240	160
4	575	450	250	200	500	475	200	150
5	420	328	200	160	440	360	160	120
10	260	170	140	80	250	220	80	60
20	130	100	100	70	115	100	65	54
50	46	34	68	24	80	76	45	32
100	30	26	14	10	62	56	32	10

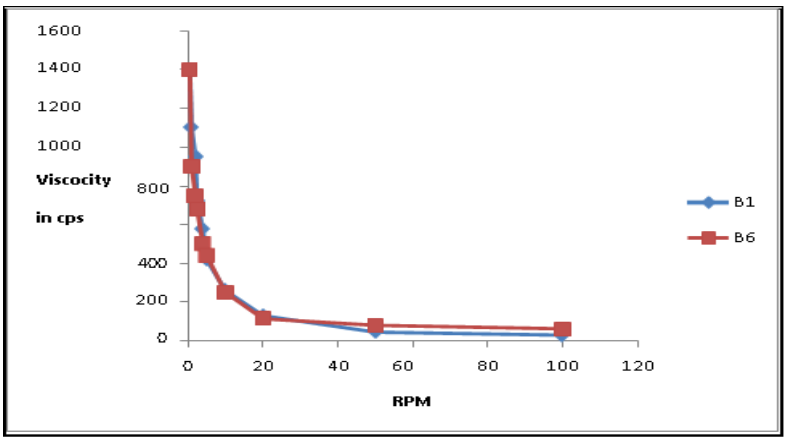

Figure 15: Viscosity of B1 & B6 in situ gel formulation at varying rpm.

Table 12: Viscosity of B2 & B7 in situ gel formulation at varying rpm.

Formulation code	B2				B7			
	Before Dilution		After Dilution		Before Dilution		After Dilution	
RPM	A	D	A	D	A	D	A	D
0.5	8240	7820	8400	7668	12400	11800	1560	840
1	7500	6995	7560	5600	9600	8900	1400	800
2	6750	5890	6800	5100	6100	4750	500	400
2.5	4360	3480	6000	4450	4650	3750	480	320
4	3345	2950	5654	3120	3875	2400	200	150
5	2390	1900	4820	2200	3260	2020	160	120
10	2050	1548	2160	1744	1820	1160	100	80
20	1905	1345	1864	1080	1125	730	80	50
50	887	714	1244	560	434	388	45	36
100	410	334	772	442	197	195	22	18

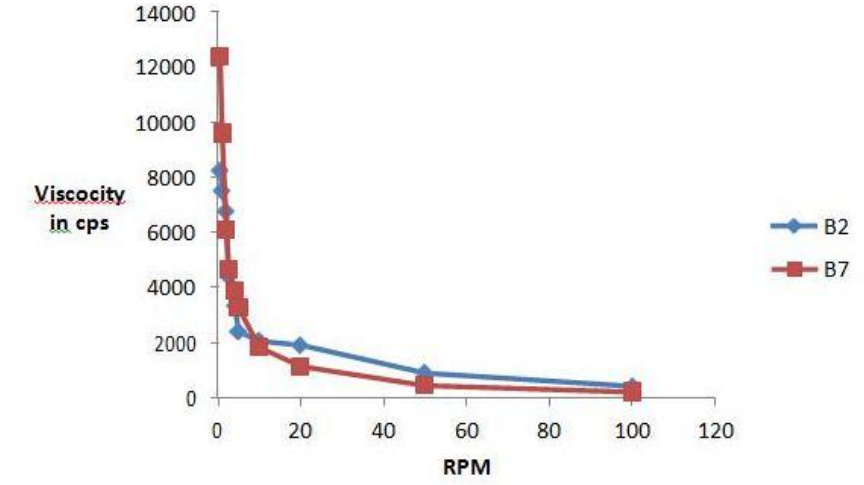

Figure 16: Viscosity of B2 & B7 *in situ* gel formulation at varying rpm.

Table 13: Viscosity of B3 & B8 in situ gel formulation at varying rpm.

Formulation Code	B3				B8			
	Before Dilution		After Dilution		Before Dilution		After Dilution	
RPM	A	D	A	D	A	D	A	D
0.5	45700	34650	28000	17600	18400	12655	1150	1200
1	28450	25600	20500	11500	10450	8425	1000	800
2	18720	12650	6700	5500	3246	3110	800	550
2.5	15760	10884	4520	3620	2400	2100	480	320
4	8300	5889	3100	2950	1750	1680	250	200
5	7140	5020	2800	2600	1675	1464	120	100
10	3420	2340	1950	1480	1000	985	100	80
20	1945	1156	1300	840	940	825	90	78
50	756	682	980	720	850	725	55	32
100	495	230	502	306	680	475	22	20

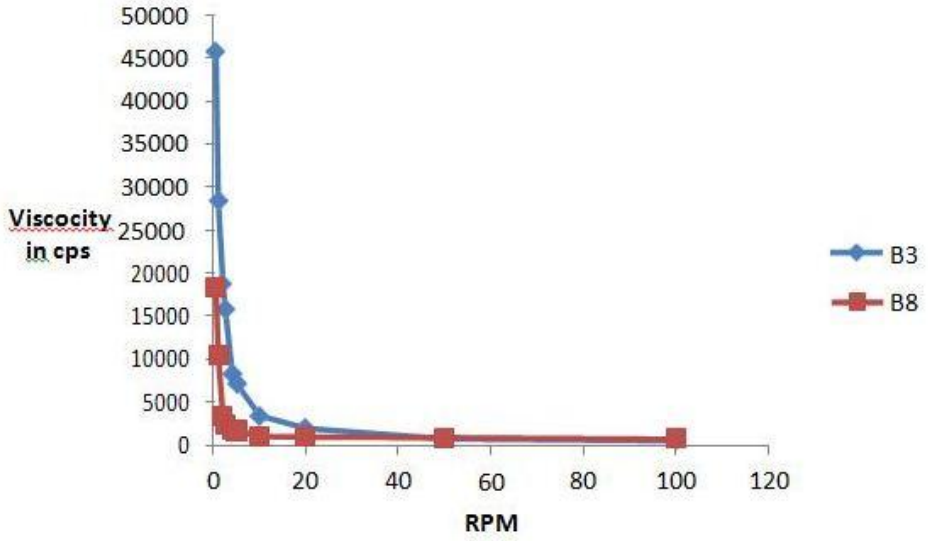

Figure 16: Viscosity of B3 & B8 in situ gel formulation at varying rpm:

Table 13: Viscosity of B4 & B9 in situ gel formulation at varying rpm.

Formulation code	B4				B9			
	Before Dilution		After Dilution		Before Dilution		After Dilution	
RPM	A	D	A	D	A	D	A	D
0.5	60,100	47496	57,118	32650	35950	21600	1400	1200
1	52,630	31560	50,112	25800	23500	12400	1200	600
2	48,265	23950	45,267	18900	15420	7000	500	400
2.5	39,260	18450	36,385	17780	9750	4860	400	320
4	30,459	15779	27,576	15450	3997	2900	350	300
5	20,690	10049	18,429	12860	1890	1725	240	200
10	12,000	7049	10,380	9130	960	850	220	160
20	6,100	4165	5,807	4200	495	316	180	150
50	1,120	813	1,000	780	316	268	100	72
100	380	292	359	324	146	96	62	22

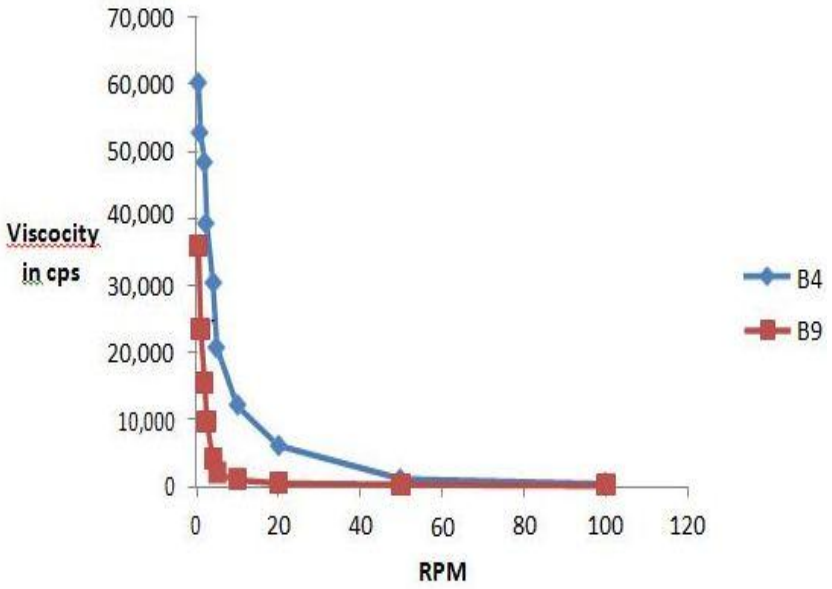

Figure 17: Viscosity of B4 & B9 *in situ* gel formulation at varying rpm.

Table 14: Viscosity of B5 & B10 *in situ* gel formulation at varying rpm.

Formulation code	B5				B10			
	Before Dilution		After Dilution		Before Dilution		After Dilution	
RPM	A	D	A	D	A	D	A	D
0.5	60400	46475	55355	42656	53000	46650	14900	14400
1	53000	36400	47000	30007	27850	25200	7500	6100
2	34400	24225	29656	22250	13800	12500	5400	3500
2.5	18450	9250	15450	7850	6450	6171	1450	1350
4	15600	8650	10490	6560	2550	2660	1050	950
5	10900	7950	7220	4390	1310	1440	980	810
10	5740	3400	3350	3000	790	698	656	569
20	2450	1640	1500	1050	465	427	407	356
50	920	574	550	350	354	339	350	269
100	618	314	342	210	300	294	250	234

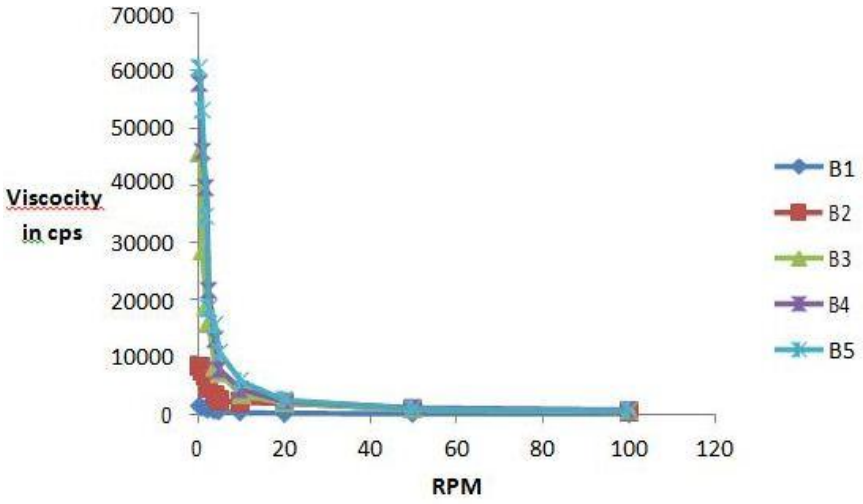

Figure 18: Viscosity of microemulsion loaded *in situ* gel formulation with microemulsion at varying rpm.

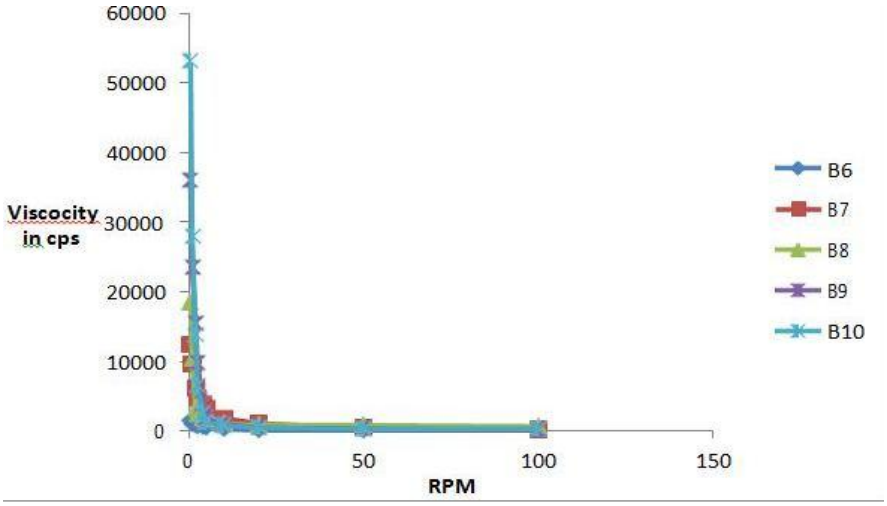

Figure 19: Viscosity of microemulsion loaded *in situ* gel formulation without microemulsion at varying rpm: Viscosities of microemulsion based formulations containing poloxamer 407 with mucoadhesive polymers

1) B11

Table 15: Viscosity of Carbopol loaded rectal *in situ gel* with microemulsion.

RPM	Before dilution	After dilution
0.5	62800	59575
1	52400	48783
2	35250	31890

2.5	21750	18780
4	13100	10950
5	11720	8146
10	6560	3396
20	2450	1010
50	1200	730
100	523	346

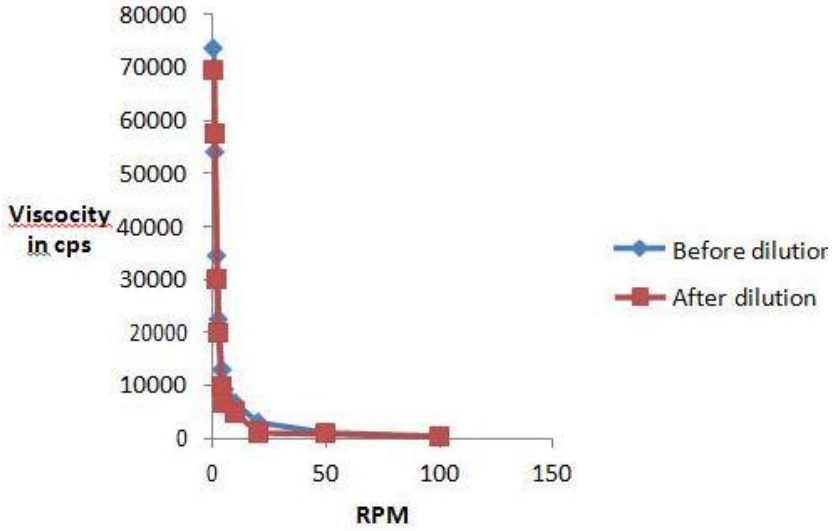

Figure 20: Viscosity of Carbopol loaded rectal *in situ* gel with microemulsion.

2) B12

Table 16: Viscosity of Polycarbophil loaded rectal *in situ gel* with microemulsion.

RPM	Before dilution	After dilution
0.5	73600	69360
1	54000	57405
2	34500	29950
2.5	22420	19995
4	12810	9740
5	9120	6543
10	6540	4820
20	2970	1016
50	1052	840
100	378	266

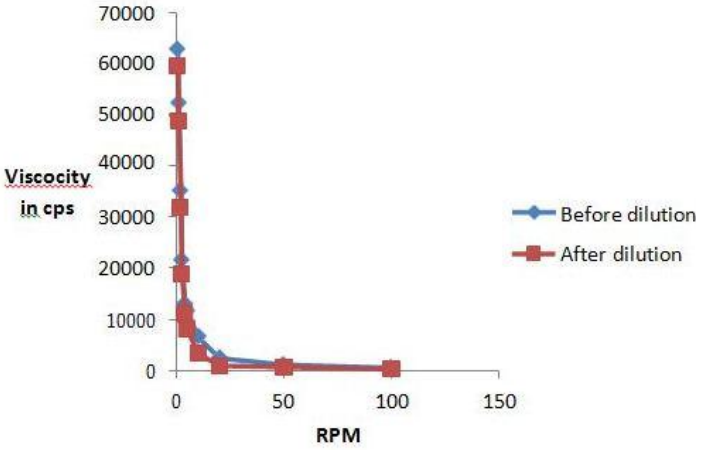

Figure 21: Viscosity of Polycarbophil loaded rectal in situ gel with microemulsion

3) B13

Table 17: Viscosity of HEC loaded rectal in situ gel with microemulsion.

RPM	Before dilution	After dilution
0.5	96450	93480
1	72620	59000
2	55320	47745
2.5	32770	29500
4	17100	13110
5	12420	10060
10	7820	4350
20	3130	2060
50	1182	790
100	782	350

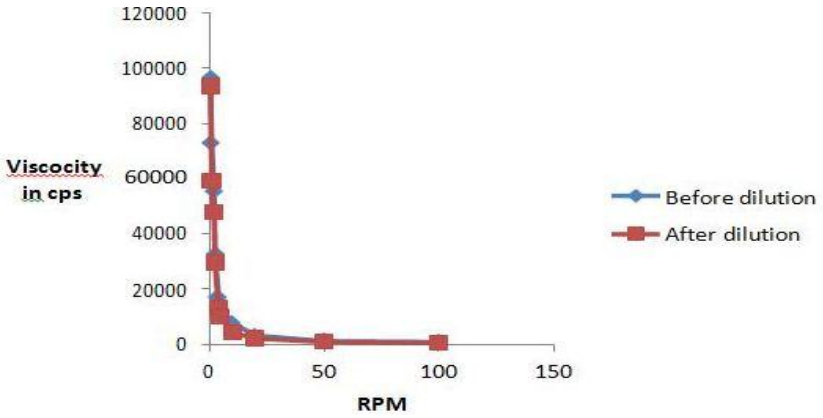

Figure 22: Viscosity of HEC loaded rectal in situ gel with microemulsion.

4) B14

Table 18: Viscosity of HPMC LV50 loaded rectal *in situ* gel with microemulsion

RPM	Before dilution	After Dilution
0.5	92000	89750
1	75600	73206
2	52360	49200
2.5	47520	42345
4	34890	30390
5	27950	26000
10	12052	10390
20	5360	3450
50	1010	895
100	657	507

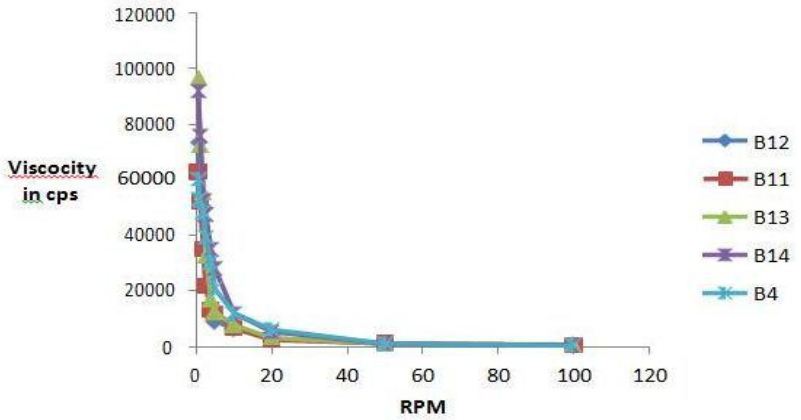

Figure 24: Rheogram of microemulsion loaded rectal in situ gel with microemulsion.

From the above rheograms, it was observed that as the polymer concentration was increased to 20% w/w, the

effect of dilution on viscosity was not significant. Addition of microemulsion increased the viscosities of the formulations before and after the dilution. When the mucoadhesive polymers were added to the Poloxamer formulations, there was increase in the viscosities of the resulting gels. Viscosity of formulation with HEC was found to be highest as compared to other polymers. All gel formulations showed thixotropic behavior at high shear.

As the viscosity of microemulsion gels with 20% w/w of Poloxamer was very high and the resulting gel was difficult to handle. Hence, this formulation was not studied further.

Release of drug from rectal formulations

Table 19: Average (±SD) cumulative percentage release of Diclofenac sodium from microemulsion based rectal *in situ* formulations prepared with Poloxamer 407.

Time (hrs)	B2	B3	B4
0	**0**	**0**	**0**
0.5	9.134±0.18	8.182±0.24	13.493±0.60
1	19.282±0.24	16.233±0.53	24.021±1.75
2	33.571±0.18	26.135±0.35	29.988±0.86
3	37.944±0.77	42.417±0.12	47.041±0.93
4	45.471±0.30	47.601±0.65	57.856±2.62
5	56.730±0.12	66.705±0.66	71.575±0.13
6	66.331±0.06	70.521±0.35	83.091±0.67
7	77.957±0.12	82.717±1.72	96.710±0.31

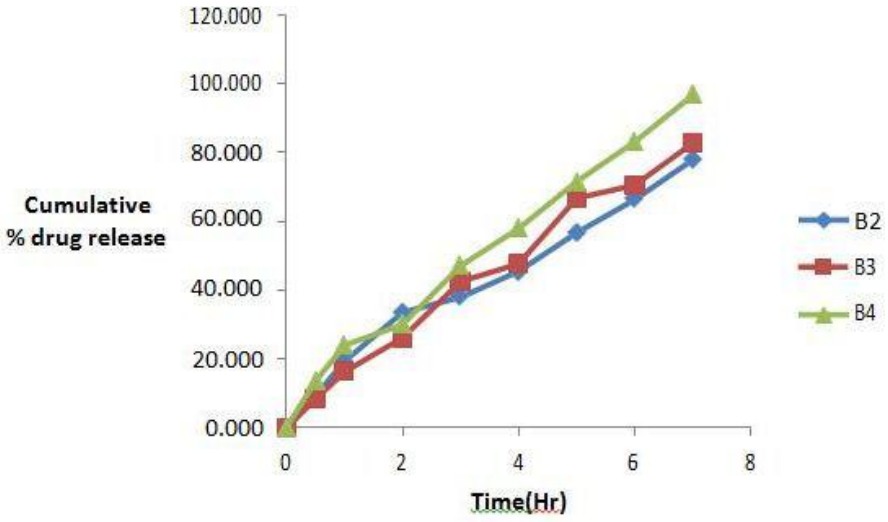

Figure 25: Average (±SD) cumulative percentage release of Diclofenac sodium from microemulsion based rectal in situ formulations prepared with Poloxamer 407.

Table 20: Average (±SD) cumulative percentage release of Diclofenac sodium from rectal formulations prepared with Poloxamer 407 without microemulsion.

Time (hrs)	B7	B8	B9	B10
0	0	0	0	0
0.5	7.478±1.18	5.372±0.69	4.940±0.65	2.456±1.68
1	16.207±1.56	21.702±0.95	16.801±1.55	14.503±0.52
2	23.170±0.57	24.512±1.33	29.879±1.82	16.989±0.65
3	34.018±0.18	44.601±0.60	44.735±1.32	29.299±1.56
4	44.518±1.31	51.921±2.00	52.639±2.05	37.920±1.57
5	50.699±0.60	58.746±0.74	72.888±1.87	43.332±1.95
6	59.432±1.89	72.964±0.79	79.371±1.11	62.285±1.34
7	70.212±0.42	78.184±0.91	89.803±0.41	71.940±1.13

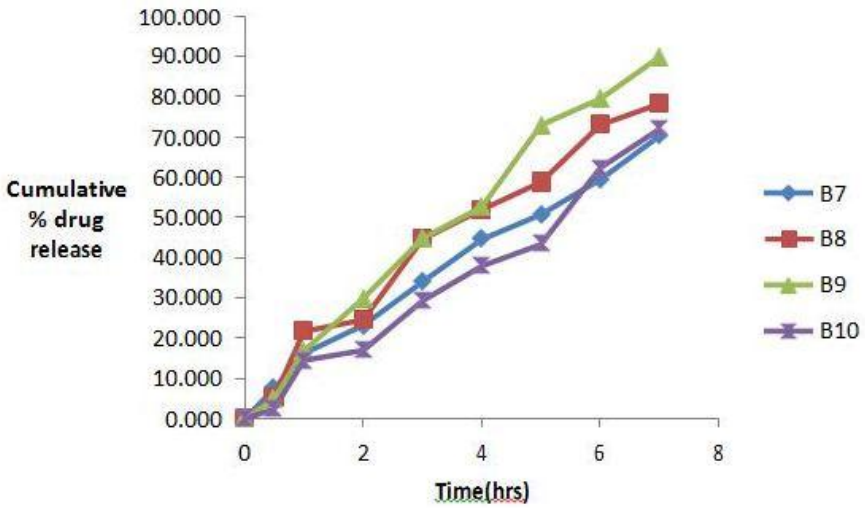

Fig. 26: Average (±SD) cumulative percentage release of Diclofenac sodium from rectal formulations prepared with Poloxamer 407 without microemulsion.

When the concentration of Poloxamer 407 was increased, present drug release was decreased which is contradictory to the available literature. This might be attributed to the surface active properties of Poloxamer. Increased Poloxamer concentration increases the micellization and increased solubilization of this Diclofenac sodium in micelles, which is otherwise insoluble. Thus increase in Poloxamer concentration till 18 % increased drug release. Further increase in Poloxamer concentration to 20 % w/w, decreased the release due to drastic increased in the viscosity of gel. This viscosity was not sufficiently decreased even after dilution.

Table 21: Average (±SD) cumulative percentage release of Diclofenac sodium from rectal formulations prepared with Poloxamer 407 with mucoadhesive polymers.

Time (hrs)	B11	B12	B13	B14
0	0	0	0	0
0.5	9.260±0.12	7.714±0.59	13.973±0.06	15.040±0.06
1	15.568±0.06	15.042±0.05	24.894±0.24	25.307±0.06
2	27.818±0.06	24.663±0.53	30.386±0.06	32.874±0.07
3	38.560±0.19	42.169±0.06	47.384±0.06	50.947±0.25
4	45.863±0.63	55.679±0.18	59.178±0.06	63.823±0.13
5	67.890±0.32	70.780±1.13	71.086±0.06	76.416±0.19
6	80.362±0.39	82.023±0.22	82.687±0.06	89.084±0.45
7	96.996±1.86	97.103±0.78	98.258±0.47	98.048±6.57

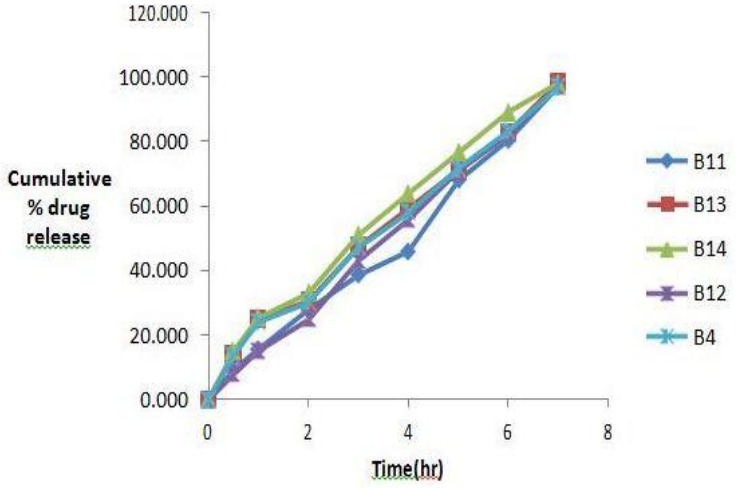

Figure 27: Average (±SD) cumulative percentage release of Diclofenac sodium from rectal formulations prepared with Poloxamer 407 with mucoadhesive polymers.

Fig. 27 indicates dissolution of microemulsion gel with mucoadhesive polymers. It was observed that there was

not much significant difference in the release profiles from different gels. Poloxamer is with weak mechanical strength, presence of water caused erosion to this gel structure due to which drug is released at a faster rate.

In the presence of microemulsion mucoadhesive polymers couldn't decrease the release of drug, although there was increase in the viscosity of the gels. This is because in presence of water, these gels tend to erode fast.

Kinetics of drug release from rectal formulations

The cumulative amount of drug released from selected rectal formulations at different time intervals was fitted to zero order, first order, higuchi matrix and Korsemayer Peppas model to find out the mechanism of drug release. The coefficient between the time and cumulative amount of drug release was also calculated to find the fit to appropriate kinetics. The result are depicted in table 22.

Table 22: Release kinetics of rectal formulations.

Code	Zero order	First order	Matrix		Korsmeyer peppas	
	R^2	R^2	R^2	K	R^2	N
B2	0.9998	0.9609	0.9304	67.038	0.999	1.01
B3	0.9994	0.9555	0.9301	69.941	0.9996	1.0117
B4	0.9992	0.863	0.9211	74.297	0.9996	1.0367
B7	0.999	0.9754	0.9255	60.008	0.9992	1.0632
B8	0.9992	0.9676	0.9378	65.366	0.9983	0.9803
B9	0.9993	0.9355	0.9354	76.095	0.998	0.9787
B10	0.9986	0.9748	0.934	61.56	0.9916	0.8592
B11	0.9763	0.8628	0.8928	3.023	0.9717	0.7908
B12	0.9972	0.9655	0.9416	3.1735	0.9969	0.8591
B13	0.9884	0.8605	0.9632	4.072	0.9908	0.7157
B14	0.9845	0.9154	0.9692	4.2914	0.9936	0.7132

Value of Korsemeyer- Peppas model was found to be between 0.85 to 1.0 for formulations B2 to B10 which indicates zero order kinetics, representing drug release at controlled rate. When mucoadhesive polymers were added, n value of Korsemeyer Peppas was found to be between 0.7 to 0.8 indicating anomalous behavior.

Mucoadhesive strength of rectal formulations

Rectal mucoadhesion relies on the interaction of a polymer and the mucin coat covering the surface of the rectum. Structurally, mucin consists of a protein or polypeptide core with carbohydrate side chains branching off the core. The polymer with many hydrophilic functional groups (eg. Carboxyl group, hydroxyl group and sulphate) can establish electrostatic interactions and hydrogen bond with the underlying surface of these non-covalent forces, hydrogen bonding appears to be the most important.[23]

The mucoadhesive potential of all *in situ* gel formulations was evaluated by using goat mucosal membrane. Weight (gm) required to detach gel formulation from the excised mucosa was measured and force was calculated.

Force of adhesion (N) = $\frac{\text{Bioadhesive strength(g)} \times \text{Gravitational force(cm/s)}}{\text{Area of mucosal surface exposed(cm}^2\text{)}}$

The mucoadhesive strength of various *in situ* gel formulations is shown in table 23

Table 23. Mucoadhesive force of rectal formulations.

Formulation	Mucoadhesive strength (gms)	Adhesiveness(gm.cm)
B14	9.17	0.9
B13	14.27	1.4
B4	8.66	0.85

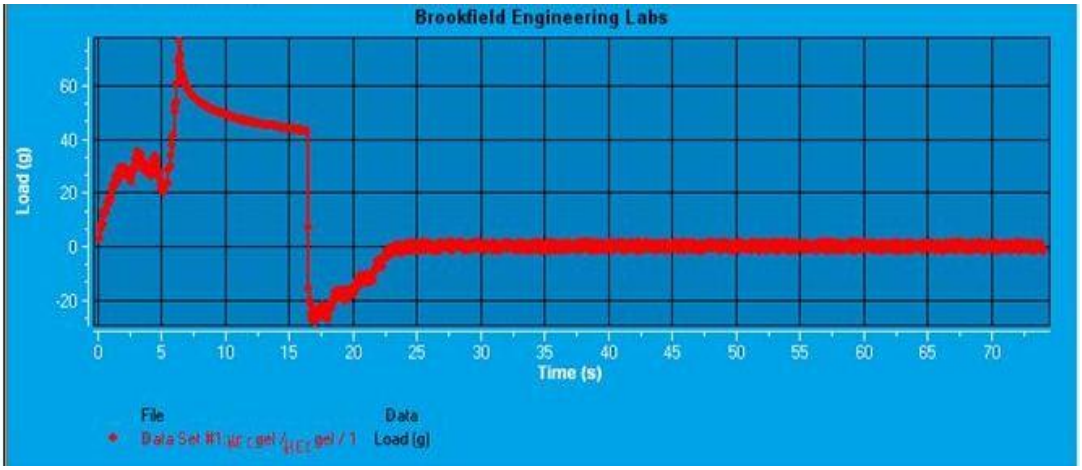

Figure 7.33: Mucoadhesion strength measurement graph of B13 rectal gel.

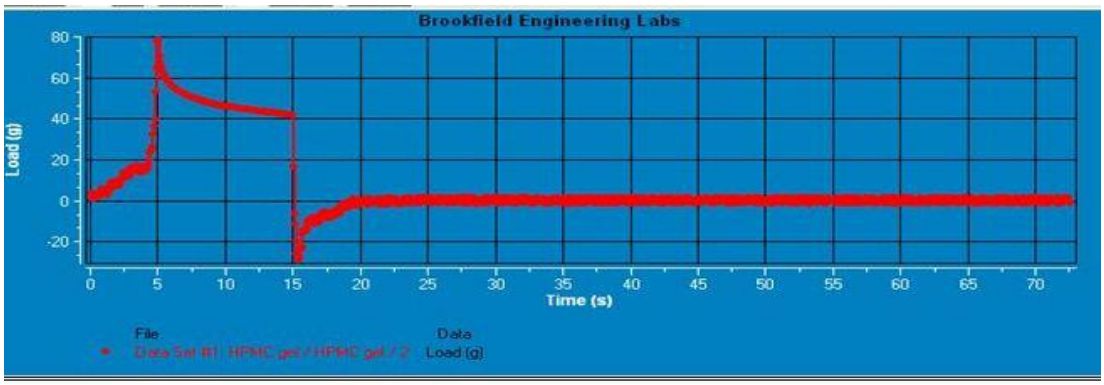

Figure 7.34: Mucoadhesion strength measurement graph of B14 rectal gel.

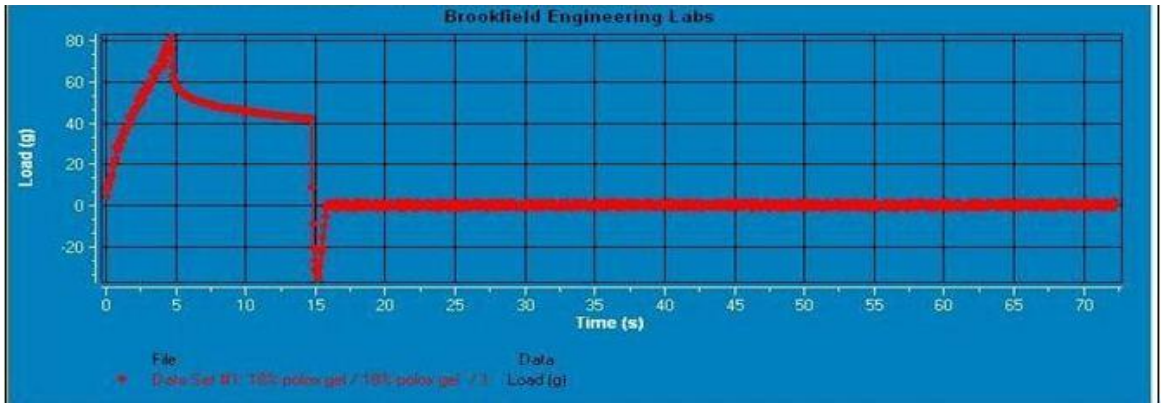

Figure 7.35: Mucoadhesion strength measurement graph of B4 rectal gel.

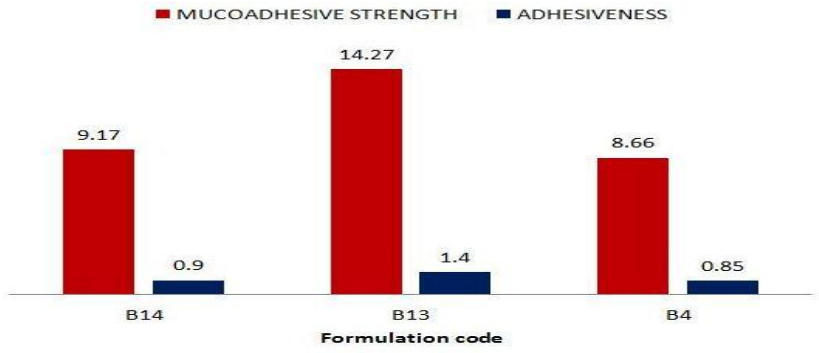

Figure 35: Rheogram of Mucoadhesive force and force of adhesion of selected rectal formulations.

Mucoadhesion was found to be increased with addition of mucoadhesive polymers to the Poloxamer.

Mucoadhesion of formulation containing HEC was found to be highest as compared to other gels (adhesiveness, 1.4 g.cm).

Test for stability of selected in situ gelling rectal formulations of Diclofenac sodium

The optimized rectal formulation (B4) was stored for 30 days at refrigerated environmental conditions (5±3°C). Effect of temperature on their stability during storage was assessed by evaluating their appearance, gelling ability, pH, viscosity, uniformity in drug content and *in vitro* drug release characteristics. The results are shown in Table below:

Table 24: Evaluation of selected rectal formulations of Diclofenac sodium before and after stability.

Evaluation parameter	B4	
	Before (Initial)	After (Final)
Appearance/Clarity	++	++
pH	7.87	7.6
Gelling Ability	+++	+++
Viscosity at 10 rpm	12,000	11,580
Percent drug content	99.48±0.5	98.5±0.5

Table 25. represent the dissolution profiles of selected rectal formulation stored at refrigerated conditions (5±3°C).

Time (hr)	B4	
	Before	After
0	0	0
0.5	13.493±0.60	12.123±0.12
1	24.021±1.75	22.873±1.46
2	29.988±0.86	28.110±1.21
3	47.041±0.93	45.980±0.38
4	57.856±2.62	55.952±0.09
5	71.575±0.13	70.114±0.07
6	83.091±0.67	81.021±2.80
7	96.710±0.31	94.491±2.56

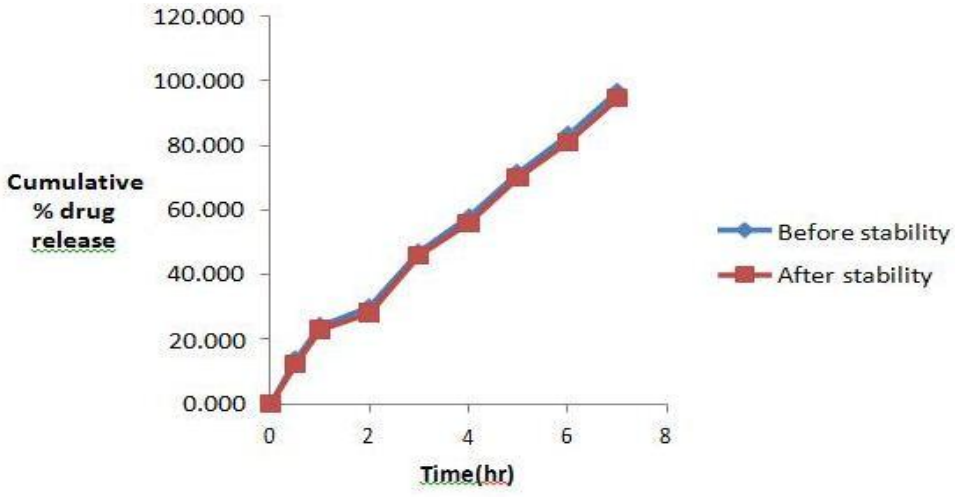

Figure 36: represent the dissolution profiles of selected rectal formulation stored at refrigerated conditions (5±3°C) before and after stability.

Formulation B4 did not show any change in appearance, clarity and color at the storage condition. It was also observed that there was no change in the gelation properties of the prepared *in situ* gelling formulations. There was no change in drug content of the formulations. The viscosity of the formulations found to be decreased with time and the change in viscosity was very negligible.

CONCLUSION

In situ gelling formulations of Diclofenac sodium were successfully developed using Poloxamer as temperature sensitive polymer. The resulting gels were found to be very clear, transparent, forming quick and stable gels

with shear thinning behavior, good mucoadhesion and sustained drug delivery.

The microemulsion of Diclofenac sodium was formulated and evaluated for appearance and clarity, pH, percent drug content, percent transmittance, conductance, *in-vitro* diffusion studies, thermodynamic stability studies and particle size analysis. Microemulsions prepared with Captex 200 were found to possess 33 nm globule size, excellent thermodynamic stability and desired diffusion characteristics.

Microemulsion based Poloxamer gels of Diclofenac sodium were prepared and found to possess high viscosity, sustained release characteristics, increased.

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