International Journal of Drug Development & Research
| July-September 2012 | Vol. 4 | Issue 3 | ISSN 0975-9344 |
Available online http://www.ijddr.in
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SJR Impact Value 0.03 & H index 2

Development and Characterization of Clarithromycin Emulgel for topical delivery

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Abstract

Topical drug delivery has been used for centuries for the treatment of local skin disorders. Emulgel have emerged as one of the most interesting topical delivery system as it has dual control release system i.e. gel and emulsion. One side the topical applications of the drug offers the potential advantages of delivering the drug directly to the site of action and secondly delivering the drug for extended period of time at the effected site. The major objective behind this formulation is enhancing the topical delivery of hydrophobic drug (Clarithromycin) by formulating Clarithromycin Emulgel using high molecular weight water soluble polymer of Hydroxypropyl methylcellulose (HPMC K4M), Carbopol 940, Carbopol 934. That possesses very high viscosity, transparency, film forming properties at low concentration and reported to be useful in formation of gel with an objective to increase transparency and spreadability. Oleic acid is used as permeation enhancer. The prepared Emulgel were evaluated for their physical appearance, pH determination, viscosity, spreadability, extrudability, *in vitro* drug release, *ex vivo* drug release, anti microbial activity and stability. All the prepared Emulgel showed acceptable physical properties, homogeneity, consistency, spreadability, viscosity and pH value. The best formulation F1 and F4 showed comparable antimicrobial activity when they compared with marketed Azithromycin gel. The *in vitro* release rate of Emulgel was evaluated using Diffusion cell containing dialysis membrane with phosphate buffer pH 5.5 as the receptor medium. The release rate of the F1 Formulation was found to follow Higuchi model. The Emulgel were found to be stable with respect to physical appearance, pH, rheological properties and drug content at all temperature and conditions for three month.

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Key words:
Emulgel, Clarithromycin, Extrudability, Spreadability, Rheological properties.

***How to Cite this Paper*:**
Joshi Baibhav*, Singh Gurpreet, Rana AC, Saini Seema "Development and Characterization of Clarithromycin Emulgel for topical delivery" Int. J. Drug Dev. & Res., July-September 2012, 4(3): 310-323

Article History:----------
Date of Submission: 28-07-2012
Date of Acceptance: 05-08-2012
Conflict of Interest: NIL
Source of Support: NONE

INTRODUCTION

Clarithromycin is a novel macrolide antibiotic with a methoxy group (-OCH_3) attached to the C-6 position of erythromycin, which makes it more acid stable than erythromycin.[1] Clarithromycin is a broad spectrum antibiotics it is active against both gram positive and gram negative microbacteria like

staphylococcus aureus, E.coli, Klebsiella, Proteus [2,3] Clarithromycin is used to treat soft tissue and skin infections, Clarithromycin is also used to treat both upper and lower respiratory tract infection, *Helicobacter pylori* infections.[4,5] Clarithromycin acts by binding to the 50S bacterial ribosomal subunits, causing inhibition of RNA dependent protein synthesis.[6] Acne vulgaris (acne) is a chronic inflammatory disorder of the sebaceous gland with the formation of comedones, papules, pustules, nodules, or cysts as a result of obstruction and inflammation of pilosebaceous units (hair follicles and their accompanying sebaceous gland) It is the most common disorder treated by dermatologists. [7] The term acne is derived from Greek word "acme" which means "prime of life". Although generally considered to be a benign, self limiting condition, acne may cause severe psychological problems or disfiguring scars that can persist for a lifetime. [8] Four factors are responsible the development of acne:

1. Plugging of the hair follicle with abnormally cohesive desquamated cells.
2. Sebaceous gland hyperactivity.
3. Proliferation of bacteria (especially *Propionibacterium acnes, staphylococcus aureus* [9]) within sebum.
4. Inflammation.

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. 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. Emulsions possess a certain degree of elegance and are easily washed off whenever desired. They also have a high ability to penetrate the skin. [10] In addition; the formulator can control the viscosity, appearance and degree of greasiness of cosmetic or dermatological emulsions. Oil-in-water emulsions are most useful as water washable drug bases and for general cosmetic purposes, while water-in-oil emulsions are employed more widely for the treatment of dry skin and emollient applications. [11] Gels for dermatological use have several favourable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, nonstaining, compatible with several excipients and water-soluble or miscible.[12] Emulgels are emulsions, either of the oil-in-water or water in-oil type, which are gelled by mixing with a gelling agent. They have a high patient acceptability since they possess the previously mentioned advantages of both emulsions and gels.[13,14] Therefore, they have been recently used as vehicles to deliver various drugs to the skin. They are also called as creamed gel, quassi emulsion, gelled emulsion. [15-19] In the present study, topical Emulgel formulations of Clarithromycin were prepared using carbopol 934, carbopol 940 and HPMC K4M as gelling agent of varying concentrations and oleic acid is used as penetration enhancer. The influence of the concentration of gelling agents on drug release was investigated. After *in vitro* evaluation of Emulgel formulations, *ex vivo* permeation was evaluated across rat epidermis and anti-microbial activity of formulations were also evaluated with marketed formulation.

MATERIALS

Clarithromycin was procured from Ind Swift Pvt. Ltd., Carbopol 934 and Carbopol 940 was obtained from Loba chemicals Mumbai. HPMCK4M was obtained as gift sample from Colorcon Asia Pvt. Ltd. Goa. Dialysis membrane was procured from Hi media, Mumbai, *Staphylococcus aureus* strain was obtained from IVRI Palampur (HP). All other chemicals were used of analytical grade and without any further chemical modification.

PREPARATION OF EMULGEL

Different formulations were prepared using varying amount of gelling agent. The method only differed in process of making gel in different formulation. The preparation of emulsion was same in all the formulations. The gel bases were prepared by dispersing Carbopol 934 and Carbopol 940 in distilled water separately with constant stirring at a moderate speed using mechanical shaker. Formulations F1, F2 and F3 were prepared by Carbopol 934 and F4, F5 and F6 by Carbopol 940 as gelling agent. In formulations F7, F8 and F9 the gel were prepared by dispersing HPMC in heated distilled water (75°C) and the dispersion was cooled and left overnight. The pH of all the formulations was adjusted to 5.5 to 6.5 using tri ethanol amine (TEA).

The oil phase of the emulsion was prepared by dissolving Span 80 in light liquid paraffin while the aqueous phase was prepared by dissolving Tween 80 in purified water. Methyl and propyl paraben were dissolved in propylene glycol and mixed with aqueous phase Clarithromycin, being hydrophobic was dissolved in oil phase. Oleic acid was also mixed in oil 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 it got cooled to room temperature. The obtained emulsion was mixed with the gel in 1:1 ratio with gentle stirring to obtain the Emulgel [10, 20]. The composition of different formulations has been discussed in Table 1.

Table 1: Composition of Clarithromycin Emulgel Formulations (%w/w)

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9
Clarithromycin	1	1	1	1	1	1	1	1	1
Oleic acid	2	2	2	2	2	2	2	2	2
Light liquid paraffin	4	4	4	4	4	4	4	4	4
Acetone	2	2	2	2	2	2	2	2	2
Spans 80	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylene Glycol	5	5	5	5	5	5	5	5	5
Tweens 80	1	1	1	1	1	1	1	1	1
Methyl Paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl Paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Carbopol 934	1	1.2	1.3	-	-		-	-	-
Carbopol 940	-	-	-	1	1.2	1.3	-	-	-
HPMCK4M	-	-	-	-	-	-	1	1.2	1.3
Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

CHARACTERIZATION OF EMULGEL

PHYSICAL APPEARANCE

The prepared Emulgel formulations were inspected visually for their pH, colour, homogeneity, consistency, grittiness and phase separation. [21]

MEASUREMENT OF pH

The pH of Emulgel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml of distilled water and it was placed for two hours. The measurement of pH of each formulation was done in triplicate and average values were calculated. [22]

FTIR SPECTRA

The IR absorption spectrum of the pure drug was taken in the range of 4000-400 cm^{-1} using KBr pellet method. The major peaks were reported for evaluation of purity. Observed peaks are similar to

reported peaks of Clarithromycin and there is no interaction of Clarithromycin with the excipients as shown in given FTIR spectra because the major peaks of Clarithromycin are retained with excipients .

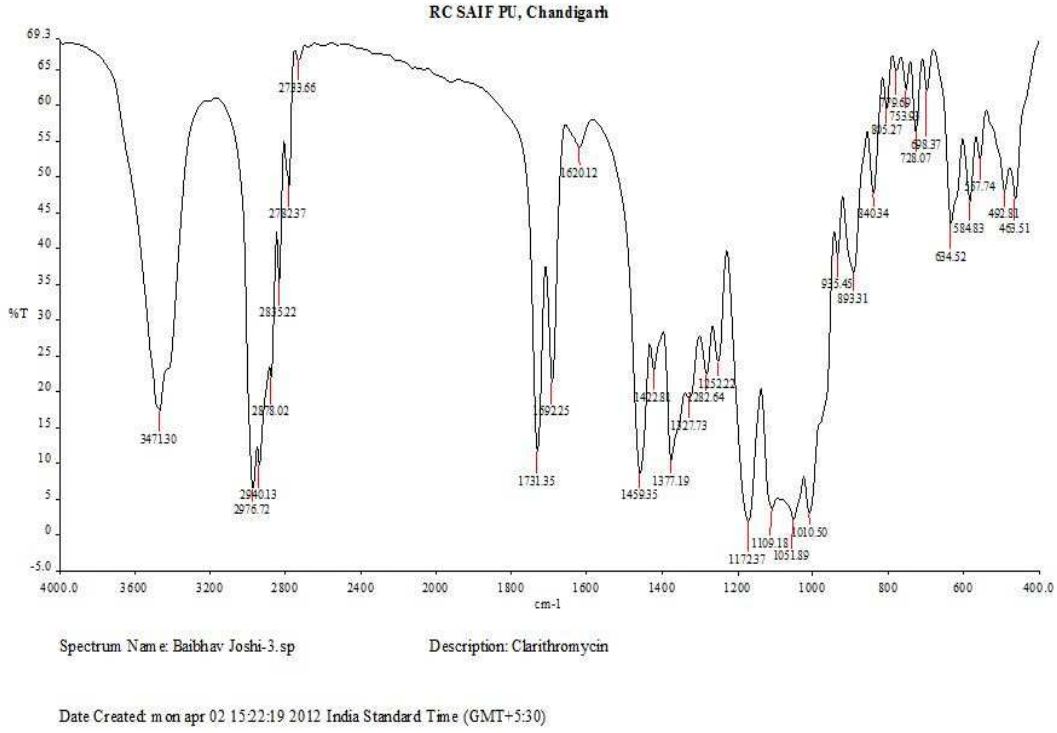


Fig 1: FTIR of Clarithromycin.

Table 2: Showing the major peaks of functional groups obtained during FTIR

Energy (Wave number)	Assignment
1692	C=O (Ketone carbonyl)
1731	Lactone carbonyl
1422	N-CH_3
2780-3000	Alkane stretching peaks
3450	Hydrogen bonds between OH groups
1000-1200	-C-O-C-Stretch
1340-1400	CH_2 Group

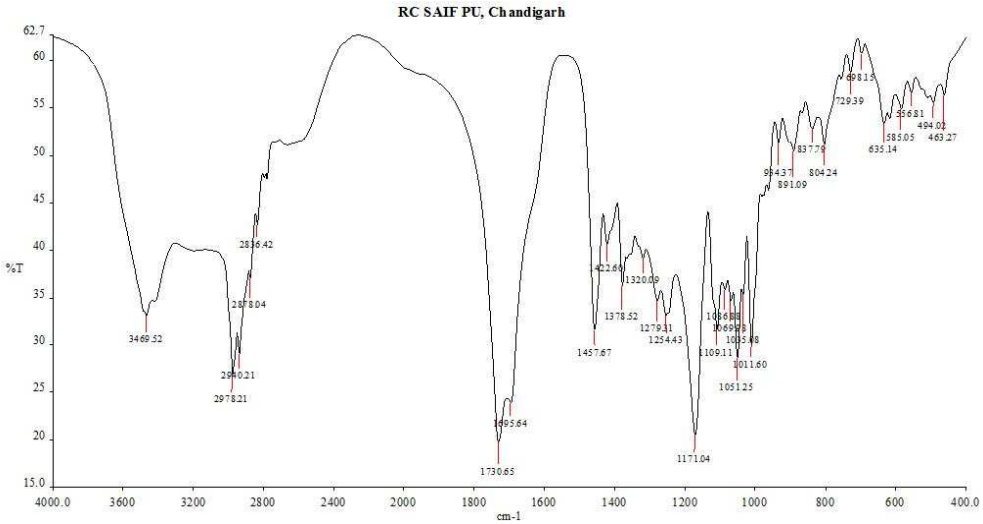


Fig 2: FTIR of Clarithromycin and carbopol 934

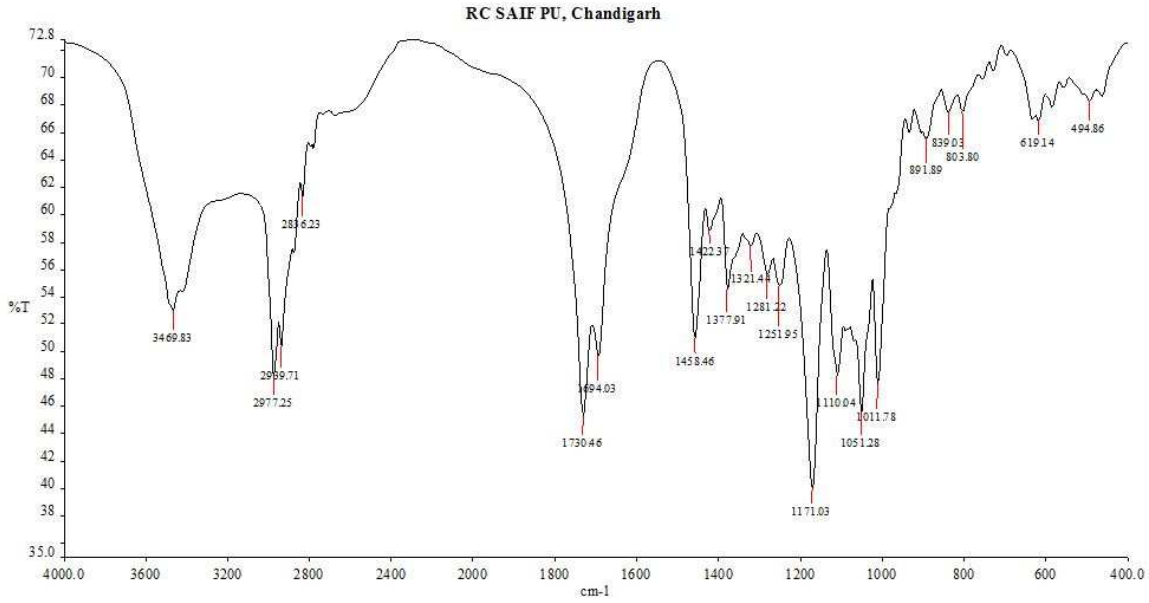


Fig 3: FTIR of Clarithromycin and carbopol 940

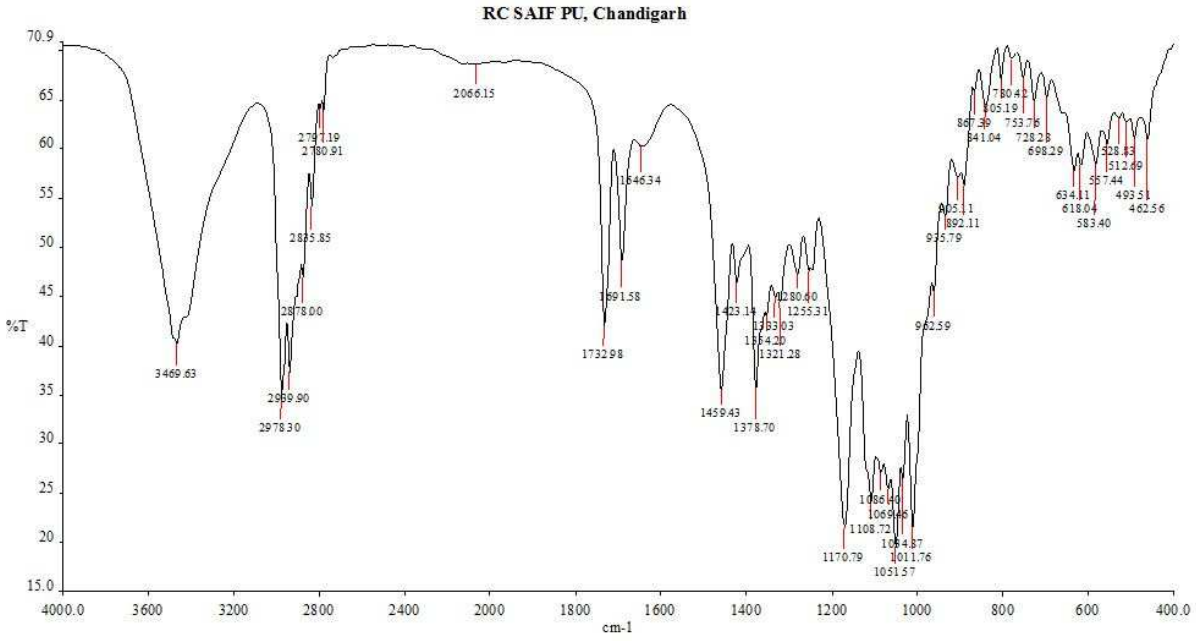


Fig 4: FTIR of Clarithromycin and HPMCK4M

SPREADING COEFFICIENT

Spreading coefficient was determined by apparatus suggested by Mutimer et al (1956). 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 Emulgel. [23] A ground glass slide was fixed on the wooden block. [24] An excess of Emulgel (about 2 gm) under study was placed on this ground slide. 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 one g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of Emulgel between the two slides. [25] Measured quantity of weight was placed in the pan attached to the pulley with the help of hook. The time (in sec) required by the top slide to separate from ground slide was noted.[26, 23] A shorter interval indicates better Spreading coefficient.

It is calculated by using the formula: **S = M. L / T**

Where M = weight tied to upper slide

L = length of glass slides

T = time taken to separate the slides.

RHEOLOGICAL STUDY

The viscosity of the formulated batches was determined using a Brookfield Viscometer (RVDV-I Prime, Brookfield Engineering Laboratories, USA) with spindle 07. The formulation whose viscosity was to be determined was added to the beaker and was allowed to settle down for 30 min. at the assay temperature (25±1°C) before the measurement was taken. Spindle was lowered perpendicular in to the centre of Emulgel taking care that spindle does not touch bottom of the jar and rotated at a speed of 50 rpm for 10 minutes. The viscosity reading was noted down. The average of three readings were taken in 10 minutes was noted as the viscosity of Emulgel. [27]

EXTRUDABILITY TEST (TUBE TEST)

Extrudability test is based upon the determination of weight required to extrude 0.5 cm ribbon of Emulgel in 10 sec from lacquered collapsible aluminum tube. The test was performed in triplicate and the average values were calculated. The extrudability was then calculated by using the following formula. [28]

Extrudability = Weight applied to extrude Emulgel from tube (in gm) / Area (in cm²)

DRUG CONTENT DETERMINATION

Weigh accurately 1 gm of Emulgel and it was dissolved in 100 ml of Methanol. The volumetric flask was kept for 2 hours and shaken well in a shaker to mix it properly. The solution was passed through the filter paper and filtered. The absorbance was measured spectrophotometrically at 288 nm after appropriate dilution against corresponding Emulgel concentration as blank. The drug content was determined using following formula. [29, 30]

Drug Content = (Concentration × Dilution Factor × Volume taken) × Conversion Factor.

***In Vitro* DRUG RELEASE STUDY**

The *in vitro* drug release studies of the Emulgel were carried out in modified Diffusion cell using Dialysis membrane (Himedia laboratories Pvt Ltd: dry, unwashed, open ended; flat width: 28.46 mm; inflated diameter: 17.5 mm; Length: 1 m). The membrane was soaked in phosphate buffer pH 5.5 for 9-12 h was clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter; 4-16 cm² area). Then Emulgel was spread uniformly on the dialysis membrane. 50 ml of phosphate buffer was taken in a beaker, which was used as receptor compartment. The donor compartment was kept in contact with receptor compartment. This whole assembly was kept on a magnetic stirrer and the solution on the receptor side was stirred continuously using a magnetic bead and temperature of the cell was maintained at 37°C. A similar blank set was run simultaneously as a control. Sample (5 ml) was withdrawn at suitable time intervals and replaced with equal amounts of fresh dissolution media. The Samples were analyzed spectrophotometrically at 288 nm and the cumulative percent drug release was calculated. [31] The difference between the readings of drug release and control was used as the actual reading in each case. [32]

***Ex vivo* DRUG RELEASE STUDY**

The *ex vivo* drug release study of selected formulations (F1 and F4) was carried out in a modified Diffusion cell, using wistar male rat skin. A section of skin was cut and clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter; 4-16 cm² area) keeping the dorsal side upward. Then Emulgel was spread uniformly on the membrane. Phosphate buffer pH 5.5 was used as dissolution media. The donor compartment was kept in contact with receptor compartment. This whole assembly was kept on a magnetic stirrer and the

solution on the receptor side was stirred continuously using a magnetic bead and temperature of the cell was maintained at 37°C. [33, 34] Sample (5 ml) was withdrawn at suitable time intervals and replaced with equal amounts of fresh dissolution media. The Samples were analyzed spectrophotometrically at 288 nm and the cumulative percent drug release was calculated.

MICROBIOLOGICAL ASSAY

Clarithromycin stable topical prepration is used for topical prepration in acne. [35] The main microbe that cause acne is found to be staphylococcus aureus. [36] The microbiological assay is done to determine the activity of prepared formulation with the strain staphylococcus aureus and the Sabouraud agar medium ditch plate technique was used, this technique is used for evaluation of bacteriostatic or fungistatic activity of a compound. It is mainly applied for semisolid formulations. Previously prepared Sabouraud's agar dried plates were used. One gram of emulgel are placed in a ditch cut in the plate. After incubation for 18 to 24 hours at 37 ˚C and then the percent inhibition was measured as follows: [10]

$$\% \text{ Inhibiton} = \frac{L2}{L1} \times 100$$

where

L1- Total length of the streaked culture.

L2- Length of inhibiton.

RELEASE KINETICS OF SELECTED FORMULATION (F1 AND F4)

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing.

- Zero order (cumulative % drug release v/s. time).
- First order (log cumulative % drug retained v/s. time).
- Higuchi model (cumulative % drug retained v/s. Square root of time).
- Peppas model (log cumulative % drug release v/s. log time). [37, 38, 39]

STABILITY STUDY

Stability study was performed on F1 and F4 formulations. The preparations were packed in collapsible aluminum tubes (5 g) and subjected to stability studies at 25°C/ 60 % RH & 30°C/65 % RH, for a period of 3 months. Samples were withdrawn at interval of 45-days and were evaluated for physical appearance, rheological properties, and drug content. All the test results were found to be in limits. Hence the formulations were stable under stated storage condition. [40]

RESULTS AND DISCUSSION

Physical Appearance

Emulgel formulations were white viscous creamy preparation with a smooth homogeneous texture and glossy appearance. Results have been discussed in Table 3.

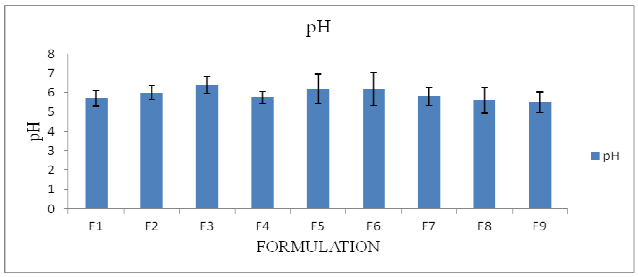
Table 3: Physicochemical characteristics of Clarithromycin Emulgel formulations

S. No	Formulation code	Colour	Phase separation	Grittiness	Homogeneity	Consistency
1	F1	White	None	-	Excellent	+++
2	F2	White	None	-	Excellent	+++
3	F3	White	None	-	Excellent	+++
4	F4	White	None	-	Excellent	+++
5	F5	White	None	-	Excellent	+++
6	F6	White	None	-	Excellent	+++
7	F7	Transparent	None	-	Fair	+
8	F8	Transparent	None	-	Good	++
9	F9	Transparent	None	-	Good	++

Excellent +++, Good ++, Satisfactory +

MEASUREMENT OF pH

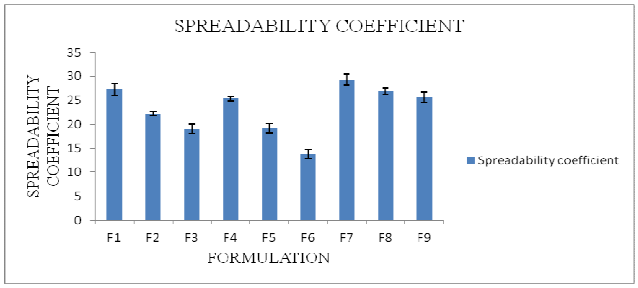
The pH of the Emulgel formulations was in the range of 5.5 ± 0.54 to 6.4 ± 0.43, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values as a function of time for all formulations. The data is shown below in Graph 1.



Graph 1: pH of Different Formulations F1- F9

SPREADABILITY TEST

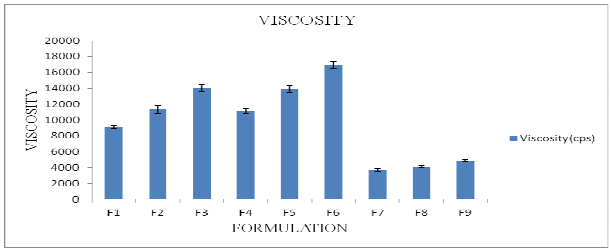
Spreadability test was carried out for all the formulations. The spreadability indicates that the Emulgel is easily spreadable by small amount of shear. Spreadability of the Emulgel decreases with the increase in the concentration of the polymer. The spreadability is very much important as it shows the behaviour of Emulgel when it comes out from the tube. Given in graph 2.



Graph 2: Spreading Coefficient of Different Formulations F1-F9

RHEOLOGICAL STUDY

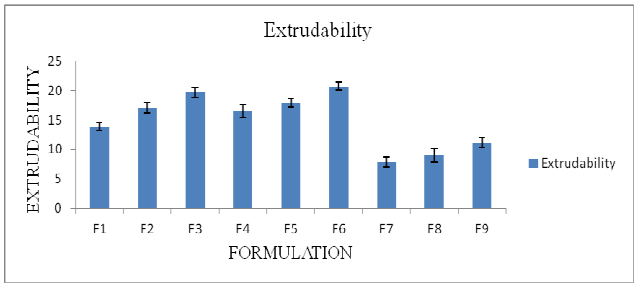
The Emulgel was rotated at 50 rpm for 10 min with spindle 07. The corresponding reading was noted. The viscosity of the Emulgel was obtained (Graph 3). The viscosity of the formulations increases as concentration of polymer increases.



Graph 3: Viscosities of Different Formulations F1-F9

EXTRUDABILITY

The gels were filled into collapsible tubes after formulating them. The extrudability of the formulation has been checked as shown below in graph 4.



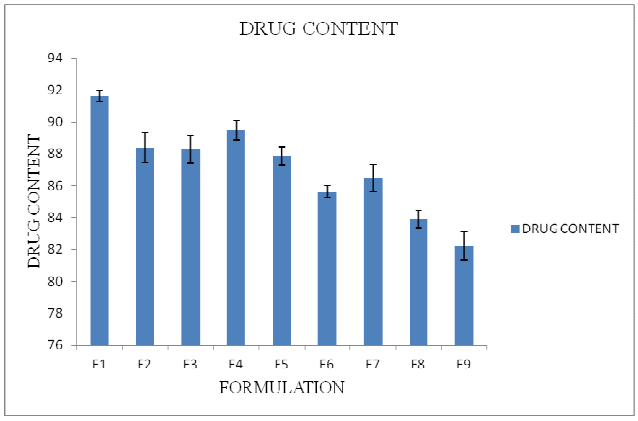
Graph 4: Extrudability of Formulations F1 – F9

DRUG CONTENT

The drug content of the formulated Emulgel was estimated spectrophotometrically at λmax 288. The results were in the limits as shown in Table 4 and Graph 5.

Table 4: Drug content of all formulations

FORMULATION	DRUG CONTENT
F1	91.60 ± 0.37
F2	88.38 ± 0.92
F3	88.26 ± 0.87
F4	89.48 ± 0.61
F5	87.83 ± 0.56
F6	85.63 ± 0.38
F7	86.47 ± 0.85
F8	83.91 ± 0.55
F9	82.24 ± 0.88



Graph 5: Drug Content of Formulations F1 – F9

In Vitro DRUG RELEASE

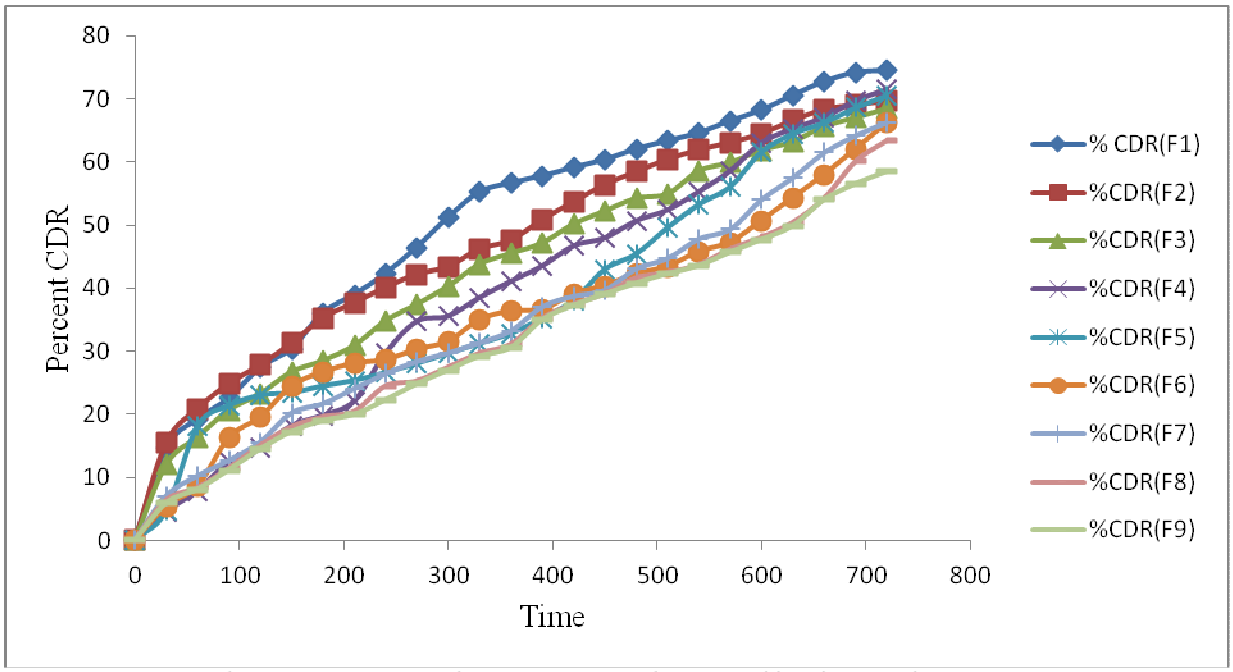
The release of Clarithromycin from the Emulgel was varied according to concentration of polymer. The release of the drugs from its emulsified gel formulation can be ranked in the following descending order: F1 > F4> F5 > F2 >F3 > F6 > F7>F8>F9 Where the amounts of the drug released after 8 hours were 74.47%, 71.52%, 70.5%, 69.65%, 68.43%, 66.13%, 66.32%, 63.46%, 58.43% respectively. The progressive increase in the amount of drug diffusion through membrane from formulation attributed to gradual decrease in the concentration of polymer. It has been concluded that, if we increase the concentration of polymer, the diffusion of drug through the membrane also decreases. The cumulative % drug release profile of all the formulation batches has been shown in Table 5(a) and 5(b) and graph is plotted between cumulative % drug releases versus time as shown in graph 6.

Table 5(a): *In-vitro* drug release study of clarithromycin emulgel formulations (F1-F5)

Time (hours)	F1	F2	F3	F4	F5
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.5	14.68±0.015	15.44±1.25	11.98±1.77	4.53±0.35	4.66±1.19
1.0	19.19±0.005	20.85±0.31	16.36±1.51	7.883±0.56	18.11±2.42
1.5	22.53±0.02	24.83±0.05	20.61±1.96	12.38±1.13	21.35±1.07
2.0	27.39±0.003	27.81±1.46	23.22±0.55	14.67±2.72	22.99±2.41
2.5	30.11±0.36	31.25±2.24	26.68±0.56	18.17±0.11	23.41±1.93
3.0	36.04±0.23	35.23±0.57	28.46±2.43	19.81±0.96	24.47±0.81
3.5	38.93±0.47	37.66±1.98	30.88±2.02	22.08±2.64	25.34±0.76
4.0	42.27±0.15	40.08±1.11	34.77±2.67	29.69±1.49	26.59±0.93
4.5	46.45±0.89	42.17±2.87	37.42±1.90	34.71±0.13	28.02±1.03
5.0	51.19±0.20	43.39±0.91	40.27±0.32	35.55±2.50	29.66±1.47
5.5	55.21±0.115	46.19±1.32	43.76±1.86	38.49±1.56	31.2±0.57
6.0	56.64±0.65	47.56±0.65	45.48±1.61	41.06±1.84	32.79±1.98
6.5	57.74±0.11	50.85±1.81	47.14±0.34	43.5±2.47	35.24±0.41
7.0	59.09±1.18	53.65±1.90	50.12±0.56	46.69±1.73	38.02±1.61
7.5	60.32±0.32	56.39±2.49	52.20±1.43	47.95±0.15	42.95±0.69
8.0	62.02±1.04	58.43±1.09	54.21±0.33	50.68±2.69	45.42±0.57
8.5	63.32±2.09	60.41±0.26	54.92±0.79	52.39±2.31	49.56±1.77
9.0	64.56±0.42	61.9±2.65	58.56±0.53	55.29±0.69	53.26±0.71
9.5	66.48±0.01	63.06±0.32	59.88±0.7	58.61±0.57	56.08±0.63
10.0	68.18±2.46	64.48±0.51	61.84±0.83	63.06±1.39	61.58±2.85
10.5	70.54±2.06	66.55±2.54	63.2±2.22	65.27±0.21	64.45±0.53
11.0	72.7±0.69	68.33±1.86	65.6±1.48	67.11±1.47	66.21±2.78
11.5	74.14±2.13	69.04±0.74	67.0±1.27	69.69±0.41	68.57±0.58
12.0	74.47±1.27	69.65±0.65	68.43±1.16	71.52±1.54	70.5±2.63

Table 5(b): *In-vitro* drug release study of clarithromycin emulgel formulations (F6-F9)

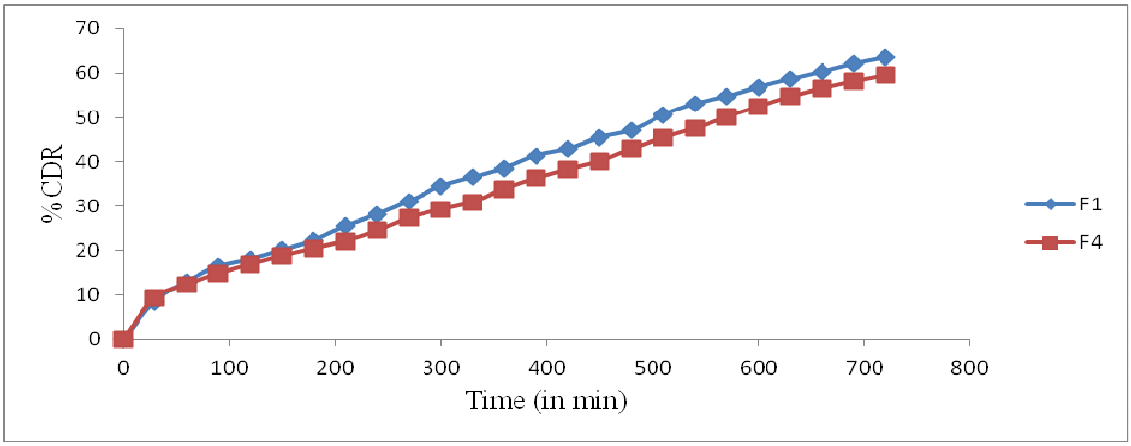
Time (hours)	F6	F7	F8	F9
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.5	5.428±2.2	7.01±0.09	6.395 ±1.19	5.911±1.17
1.0	8.59±0.97	10.204±2.49	8.346±0.67	8.0911±2.69
1.5	16.24±1.28	12.662±0.86	11.44±2.87	11.15±0.53
2.0	19.53±0.11	15.577±2.63	15.05±0.05	14.479±2.74
2.5	24.41±0.58	20.1966±1.15	18.01±2.23	17.199±0.44
3.0	26.67±1.42	21.6675±0.33	19.58±0.38	18.960±0.31
3.5	28.03±2.29	24.050±0.91	20.479±0.83	19.941±0.76
4.0	28.62±1.13	26.4191±1.96	24.492±2.50	22.234±2.24
4.5	30.26±2.23	28.360±0.04	25.30±1.16	24.803±0.19
5.0	31.53±0.94	29.707±2.55	27.48±1.06	26.972±2.36
5.5	34.95±1.14	31.3433±0.48	29.70±1.41	29.168±0.21
6.0	36.32±1.83	33.22±2.69	31.14±2.61	30.541±0.29
6.5	36.60±2.26	37.05±0.62	35.27±1.11	34.941±1.11
7.0	39.02±0.19	38.732±2.50	37.46±1.08	37.23±0.15
7.5	40.34±2.24	39.69±1.13	39.16±0.09	39.008±1.33
8.0	42.24±1.98	43.09±0.27	41.66±0.74	40.756±1.68
8.5	43.39±1.37	44.734±1.71	42.52±1.91	42.22±1.70
9.0	45.77±2.49	47.835±1.91	43.76±1.46	43.532±1.14
9.5	47.24±1.19	49.37±2.42	46.22±0.99	45.653±2.31
10.0	50.68±1.73	54.05±2.48	48.11±1.97	47.648±1.67
10.5	54.32±0.63	57.552±0.05	50.49±0.71	49.83±0.89
11.0	57.97±1.59	61.515±1.99	54.30±0.70	53.96±2.52
11.5	62.04±1.83	64.229±2.08	59.84±2.74	56.50±2.49
12.0	66.13±0.75	66.32±2.65	63.46±0.59	58.43±1.83



Graph 6: *In Vitro* Cumulative % Drug Release Profile of Formulations F_1 – F_9

***Ex vivo* Release Study**

This study was carried out only on two best optimized formulations. The study showed the release of the drugs from its emulsified gel formulation F1 and F4 were 63.43% and 59.55% respectively in 8 hours. The results are show in Graph 7.



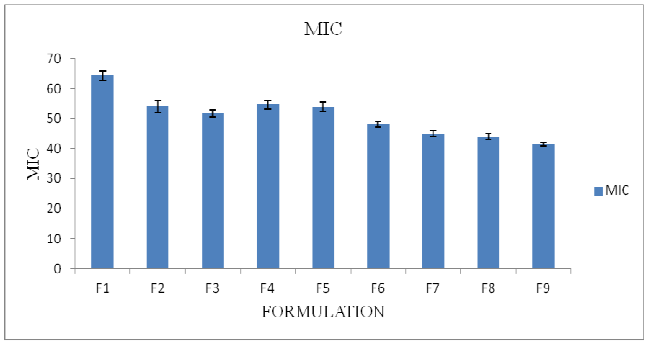
Graph 7: *Ex Vivo* Cumulative % Drug Permeation Profile of Formulations F1 and F4

MICROBIOLOGICAL ASSAY:

The use of control plates allowed that the plain emulgel bases were microbiologically inert toward the *staphylococcus aureus* strain. The antimicrobial activity of clarithromycin in its different emulgel formulations as well as with commercially available gel of Azithromycin is shown below. Percentage inhibition was taken as a measure of the drug antimicrobial activity.The highest activity was observed with F1 and F4 where the percentage inhibition found to be 64.3 ± 1.52% and 54.6 ± 1.5%, while the lowest activity found with F9 where the percentage of inhibition is 41.6 ± 0.57% and the percentage inhibition of of marketed Azithromycin gel was found to be 51.03 ± 1.23 which is less then best(F1) formulation.

Table 6: Percentage inhibition

Formulation	MIC(%) ± S.D
F1	64.3 ± 1.52
F2	54 ± 2.08
F3	51.6 ± 1.08
F4	54.6 ± 1.5
F5	53.8 ± 1.5
F6	48 ± 1
F7	45 ± 1
F8	44 ± 1
F9	41.6 ± 0.57



Graph 8: Percent inhibition of all formulation.

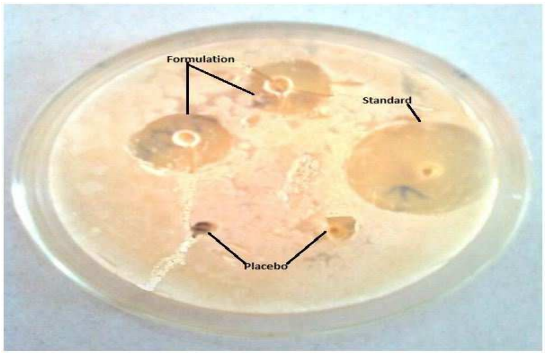


Fig 5: MIC obtained in culture media with formulation(F1).

Fig 6: Showing % inhibition of marketed formulation

Table 7: Comparison between marketed formulation and test formulation

Test Formulation (F1)	Marketed Formulation
64.3 ± 1.52	51.03 ± 1.23

STATISTICAL ANALYSIS

From ANOVA all the result was expressed as Mean ± Standard Error (SEM). The data was analyzed by using one way Analysis of Variance (ANOVA) followed by Tukey and Dunnett tests by using Graph pad prism software. The value of $p<0.05$ was considered to be statistical significant.

KINETICS OF DRUG RELEASE

The results obtained in *in vitro* release studies were plotted in different kinetic models. Regression co-efficient (R^2) values of different kinetic models are shown in Table 8. This indicated that the release data of best formulation (F1) follows Higuchi model kinetics and F4 follows zero order kinetics because the value of R^2 is greater in this model. The mechanism of drug release is determined by Korsmeyer Peppas where 'n' is the release exponent hence the mechanism of drug release is non- fickian diffusion for both F1 and F4 formulations given in table.

Formulation	Zero order		First order		Higuchi		Korsemeyer Peppas	
	N	R^2	N	R^2	n	R^2	n	R^2
F1	5.424	0.928	-0.046	0.987	22.73	0.991	0.548	0.989
F4	5.890	0.991	-0.044	0.985	23.37	0.959	0.880	0.995

Table 8: Regression analysis (r^2) of release data based on best curve-fitting method for different formulations of emulgel of clarithromycin (n=3)

STABILITY STUDY

All the prepared Emulgel formulations were found to be stable upon storage for 3 months, no change was observed in their physical appearance, pH, rheological properties and drug content.

CONCLUSION

From the above results we can conclude that Clarithromycin Emulgel formulations prepared with either Carbopol 934, Carbopol 940, HPMC K4M showed acceptable physical properties, drug release, and antimicrobial activity, which remained unchanged upon storage for 3 months. However, the Carbopol 934 based Emulgel in its low concentration with the formulation code F1 proved to be the formula of choice, since it showed the highest drug release and very good antimicrobial activity when compared to the marketed Azithromycin gel. So, Clarithromycin Emulgel can be used as an anti microbial broad spectrum medication for topical drug delivery.

ACKNOWLEDGEMENT:

Authors are thankful to Colorcon Asia private limited, Goa for providing free gift sample of HPMC K4M and also thankful to Rayat Institute of Pharmacy, Railmajra S.B.S Nagar (Punjab) for providing facilities for work in the research article.

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