Research Article

Formulation Development and *Invitro* Evaluation of Clarithromycin Topical gel

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ABSTRACT

Utility of gel based drug delivery systems are being employed in recent past for therapeutic effectiveness of topical applied drugs. Clarithromycin is a novel macrolide antibiotic. Topical route for Clarithromycin was selected up to avoid GIT irritation and to maximize the drug concentration at the site of action. In the present study an attempts were made to formulate and evaluate topical gels of Clarithromycin. Estimation of Clarithromycin was done in pH 5.5 phosphate buffer spectrophotometrically at 205nm showed good correlation coefficient. All the prepared gels were evaluated for their appearance, pH, drug content, homogeneity, spreadability, SEM, *in vitro* release. Visually, formulated Hydroxy Propyl Cellulose gels were sparkling and transparent. The pH range of formulated Hydroxy Propyl Cellulose gels was found to be suitable for topical application. The drug content of formulated gel was found in the range of 99.61. The viscosity measurement was done for selected gels using Brookfield viscometer at room temperature and found good consistency. The spreadability of formulated Hydroxy Propyl Cellulose gels was found to be good. The *in vitro* release was carried out using dialysis membrane in pH 5.5 phosphate buffer. The *in vitro* drug release was plotted according to release kinetics, to precisely know the mechanism involved in the drug release. Among all gel formulations the drug release was greater in formulation F4 -99.65±0.86.

Keywords: Clarithromycin, Topical gel, Hydroxy Propyl Cellulose, in-vitro, viscosity.

INTRODUCTION

Topical dosage forms are responsible to deliver drugs across the skin and can be applied directly on external surface of the body by rubbing, installation, spreading, spraying. The topical drug delivery produce local effect and avoid the GIT irritation, prevent the first pass metabolism and also helpful to improving the Bioavailability of drug and the drug which is going to be directly act on the site of action. Gels are transparent opaque semi solids. It contains gelling agents to form three-dimensional colloidal network structure. Gel based formulations are dermatological use have many favorable properties such as easily spreadable, grease less, easily removable, thixotropic, water soluble or miscible, non-staining and emollient. When gels are compare to ointments, creams these are non-sticky, it requires low energy during formulation and have aesthetic values.[1]

Clarithromycin drug is a macrolide antibiotic. It fights with bacteria in body. It is used to treat different types of bacterial infection affecting the skin and respiratory system and also used together with other medicine to treat stomach ulcers caused by helicobacter pylori, haemophilus influenza, *staphylococcus aureus*, E.coli.[2]

Clarithromycin is stable at acid pH so it is well absorbed from GIT. It has poor bioavailability [50%] in presence of intestinal metabolic enzyme cytochrome P450 (cyp3A).

The main criteria of the present work is formulation development of Clarithromycin topical gel by using four types of gelling agents Na CMC, Hydroxy propyl cellulose, Guar gum, Poloxamer 407 and study the gelling agents affecting on the release of drug.[3,4]

MATERIALS AND METHODS

MATERIALS

Clarithromycin, pure drug was a gift sample from Aurobindo pharma Ltd, Hyderabad. Na CMC, Hydroxy propyl cellulose, Guar gum, Poloxamer 407 procured from S.D. fine chemicals Pvt, Ltd. All other chemicals were used of analytical grade and without any chemical modification.

METHODS

Preparation of Clarithromycin topical gel

- Clarithromycin gel formulations were prepared using Sodium Carboxy Methyl Cellulose (Na CMC), Hydroxy Propyl Cellulose (HPC), Guar Gum and Polaxomer 407 as gelling agents.
- Gelling agent (0.5%, 1% and 1.5%) was dispersed in a calculated amount of water with constant stirring using magnetic stirrer at a moderate speed overnight to ensure complete hydration.
- Clarithromycin was dissolved in methanol (95%) and added the octyl alcohol (1.25%) to the dispersion. A few ml of water was taken and heated up to 75^{o}Cand then the weighed quantities of Methyl Paraben (0.015%) was added and stirred until to get clear solution. Then the above solution was allowed to cool up to 45^{o}C.

- In gels adjust the pH of the gel compatible with the normal pH of the skin by using TEA (triethanolamine) (0.1%) until the desired pH value was approximately reached (5.5 -7). During pH adjustment, the mixture was stirred gently with spatula until homogenous Clarithromycin gel was formed**.**
- The final weight of the gel was adjusted to 100 mg . Entrapped air bubbles were removed by keeping the gels in vacuum desiccators.
- The prepared Clarithromycin gels were filled in lacquered aluminium collapsible tubes and stored in dark and cool place.[5]

Table 1: Formulation of Clarithromycin gels using different types of gelling agents (%w/w)

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Clarithromycin(mg)	100	100	100	100	100	100	100	100	100	100	100	100
Sodium Carboxy Methyl cellulose (mg)	0.05	0.10	0.15	-	-	-	-	-	-	-	-	-
Hydroxy Propyl Cellulose (mg)	-	-	-	0.05	0.10	0.15	-	-	-	-	-	-
Guar gum (mg)	-	-	-	-	-	-	0.05	0.10	0.15	-	-	-
Polaxomer 407	-	-	-	-	-	-	-	-	-	0.05	0.10	0.15
Octyl Alcohol (ml)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Methylparaben(mg)	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Water(ml)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

*All ingredients in milli grams

Physicochemical Evaluation of clarithromycin topical gel

Gels are evaluated for their homogeneity, pH, viscosity, spreadability, drug content, stability studies, release kinetic studies, in-vitro release studies and FTIR studies.

Measurement of pH

The pH of various clarithromycin gel formulations was determined by using digital pH meter, which was calibrated before each use with standard buffer solutions. The electrode was inserted into the sample solution prior to taking the reading at room temperature. Each measurement was carried out in triplicate and the average pH was calculated.[6,7]

Homogeneity

All developed Clarithromycin gels were tested for homogeneity by visual inspection after the gels have been set in the container. They weretested for their color, appearance and presence of any aggregates or lumps.[8]

Viscosity

The viscosity of the different Clarithromycin gel formula was determined at 25$^{\circ C}$ using Brookfield digital viscometer. The gels were rotated at 20 and 30rpm with spindle no. 64. At each speed, the corresponding dial reading was noted. Evaluation was conducted in triplicate.[9]

Spreadability test

A sample of 0.5 g of each developed Clarithromycin gels formula was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading of gel was expected. Diameters of spreaded circles were measured in centimetre and were taken as relative values for spreadability. Evaluation was conducted in triplicate and the average spreadability values were calculated.[1]

Drug content

100mg of gel from each formulation were weighed and it was dissolved in 100ml phosphate buffer ofpH 5.5.the conical flask containing gel was shaken for 2hrs on mechanical shaker in order to get complete solubility of clarithromyin. The resulting solution is filtered through whatman filter paper, the clarithromycin content was analysespectrophotometrically at 205nm using an UV spectrophotometer (Elico, india). Each measurement was carried out in triplicate and the average clarithromycin content in the topical gel was calculated.[11]

***In-vitro* release study**

Franz diffusion cell (with effective diffusion area 3.14 cm ^{2} and 24.5 ml cell volume) was used for the studies. Prepared Clarithromycin gel (500 mg) was applied uniformly on the surface of dialysis membrane (spectrum laboratories dry, unwashed, open ended; flat width: 28.46 mm; inflated diameter: 17.5 mm; Length: 1 m).

The membrane was clamped between the donor and the receptor compartment of diffusion cell. The receptor chamber was filled with freshly prepared PBS (pH 5.5) solution to solubilise the Clarithromycin. The receptor chamber was stirred by magnetic stirrer at 50 RPM; the temperature was maintained at 37 ± 0.50$^{\circ c}$.

The samples (5.0 ml aliquots) were collected at suitable time interval. Samples were analyzed for Clarithromycin content by UV visible spectrophotometer after appropriate dilutions. Cumulative corrections were made to obtain the total amount of Clarithromycin permeation at each time interval.[12]

Release kinetics study

The mechanism of drug release were find out from the Clarithromycin topical gel by interpreated the in*vitro* release data with different kinetic models.They named as zero order, first order, Higuchi, Korsemeyer-Peppas. A criterion for selecting the most appropriate model was based on goodness of fit, high regression coefficient value.

FTIR Spectra

Drug-excipients compatibility studies were carried out using FT-IR infrared spectrum of pure drug Clarithromycin was taken in between 400 to 4000cm^{-1} by using KBr pellet method. The study was carried out on individual pure drug Clarithromycin and optimized formulation F4.[13]

SEM (Scanning Electron Microscope) Studies

The surface morphology of the layered sample was examined by using SEM (JEOL Ltd.,Japan). The small amount of gel was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to an aluminum stubs were coated with a thin layer (30^{0}A) of gold by employing POLARON - E 3000 sputter coater. The samples were examined by SEM with direct data capture of the images onto a computer.[14]

RESULTS AND DISCUSSION

Characterization of formulations

Homogeneity

The prepared Clarithromycin gel formulae were inspected visually for their colour and syneresis. All developed gel formulae showed good homogeneity with absence of lumps and syneresis.

pH determination

The pH values of all developed Clarithromycin formulae was in range 6-7 which is considered acceptable to avoid the risk of irritation upon application to the skin. There was no significant change in pH values as a function of time for all Clarithromycin topical gel formulations.

Viscosity

Viscosity is an important physical property of Clarithromycin topical gel formulations, which affects the rate of drug release. In general, an increase of the viscosity vehicles would cause high degree of cross linking with a consequent decrease of the rate of Clarithromycin release. Viscosity increased (from 2819.42 to 6122.49cPs,) as polymer concentration increased in all gel formulations. Viscosity of gel formulation with Guar gum was high as compared to that of HPC, Poloxamer 407, and Na CMC.

Drug content

After various formulation of Clarithromycin gel the drug content of the formulated gel was estimated by Elico spectrophotometer at λmax 205 nm in phosphate buffer of pH 5.5. The results were in the official limits shows in Table No.2.

Spreadability

The spreadability is very much important as it shows the behavior of Clarithromycin gel comes out from the tube. The spreadability indicates that the Clarithromycin topical gel is easily spreadable by small amount of shear. The diameter of spreaded circles ranged from 2-5 cm seen with Na CMC and 2-4 cm seen with HPC and 2-3 cm seen with Guar gum and 3-4 cm seen with Poloxamer 407 Spreadability Data revealed that Spreadability of the Clarithromycin topical gel decreases with the increase in the concentration of the gelling agents as expressed by the lower diameter of the spreaded circle.

***In vitro* release studies**

The in-vitro release profile of Clarithromycin topical gel formulae it was represented in Fig 1.1-1.3 It was observed that the release of the Clarithromycin drug fromits different formulae can be ranked in the followin g descending order F4>F11>F5>F12>F6>F10>F3>F7>F2> F8>F1>F9 order where the amounts of the drug released after 12hrs were 99.65%, 98.14%, 96.10%, 96.02%, 95.74%, 94.23%,85.91 %,78.92 %,78.51 %, 72.3%,74.26 %, and 62.8 % respectively. These results suggested that F4 is effective for topical application as highest percentage of drug released after 12hrs (99.65%). It was observed that the most influenced factor in the Clarithromycin release is polymer type followed by the concentration of the polymer.

Drug Release Kinetic Study:

The release data analysis was carried out using the different kinetic models. The Regression coefficient (R^2) values of different kinetic models are tabulated in table. This indicated that the release data of best formulation [F4] showed best fitting to Higuchi model kinetics.

Table 2: Characteristic properties of gel formulations [F1-F12]

Formulation code	pH	Viscosity (cps)	Drug content (%)	Spreadability (cm)	Color	Phase separation	Clarity
F2	6.74±0.02	2952.36±2.36	97.89	3.5	Brownish	NO	++
F3	6.58±0.02	3561.85±1.58	97.95	2.1	Brownish	NO	+++
F4	6.89±0.015	2468.93±2.42	99.61	3.9	White	NO	+++
F5	6.36±0.01	3429.70±3.57	96.29	3.8	White	NO	+
F6	6.91±0.01	3989.88±1.66	97.56	2.4	White	NO	+++
F7	6.98±0.03	4276.47±1.85	97.89	2.9	White	NO	+++
F8	6.23±0.025	5633.84±1.98	98.55	2.7	White	NO	++
F9	6.57±0.01	6122.49±1.33	98.97	2.5	White	NO	+
F10	6.75±0.03	3245.66±1.27	98.63	4.2	Transparent	NO	++
F11	6.98±0.015	4557.31±2.47	96.44	3.6	Transparent	NO	+++
F12	6.94±0.02	4889.32±2.41	99.13	3.1	Transparent	NO	+

Excellent +++, Good ++, Satisfactory +

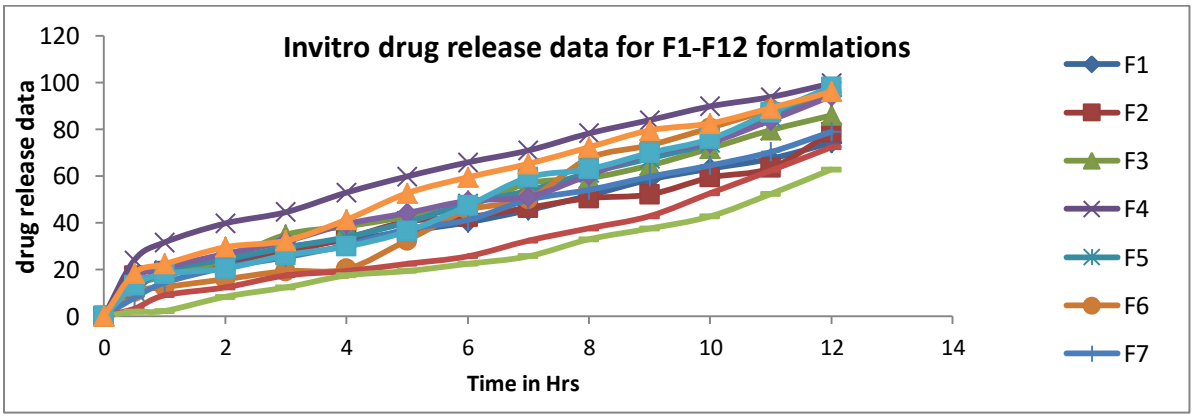

Figure 1: *In vitro* Drug release study for F1 to F12 formulations

The mechanism of Clarithromycin release is fickian diffusion for F4 formulations given in table no.3.

Drug-Excipients Compatibility Studies:

Drug-Excipient compatibility can be determined by FT-IR analysis. FT-IR study revealed that there was no major change in the position of peak values obtained in the Clarithromycin alone and in formulation of Clarithromycin topical gel with Excipients, which shows that there was no interaction between Clarithromycin and Excipients. Results are shown in figures.

SEM of Clarithromycin Gel of optimized formulation

From the SEM monograph the gel morphology was studied there by it shows irregular surface along with smear layer and dentinal tubules was present.

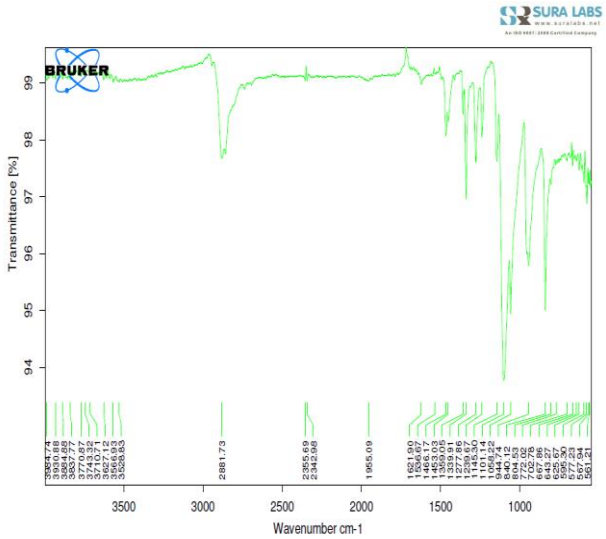

Figure 2: FT-IR of pure drug Clarithromycin

Table 3: Release Rate Kinetics to Dissolution Data for optimised formulation

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG (%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0	0	0	2.000	48.26	0.0512	-0.885	100	4.642	4.642	0.000
23.93	**0.5**	0.707	1.379	0.301	1.881	47.860	0.0418	-0.621	76.07	4.642	4.237	0.404
31.68	**1**	1.000	1.501	0.000	1.835	31.680	0.0316	-0.499	68.32	4.642	4.088	0.554
39.77	**2**	1.414	1.600	0.301	1.780	19.885	0.0251	-0.400	60.23	4.642	3.920	0.722
44.51	**3**	1.732	1.648	0.477	1.744	14.837	0.0225	-0.352	55.49	4.642	3.814	0.827
52.97	**4**	2.000	1.724	0.602	1.672	13.243	0.0189	-0.276	47.03	4.642	3.610	1.032
59.84	**5**	2.236	1.777	0.699	1.604	11.968	0.0167	-0.223	40.16	4.642	3.425	1.217
65.81	**6**	2.449	1.818	0.778	1.534	10.968	0.0152	-0.182	34.19	4.642	3.246	1.396
70.91	**7**	2.646	1.851	0.845	1.464	10.130	0.0141	-0.149	29.09	4.642	3.075	1.566
78.29	**8**	2.828	1.894	0.903	1.337	9.786	0.0128	-0.106	21.71	4.642	2.790	1.852
83.94	**9**	3.000	1.924	0.954	1.206	9.327	0.0119	-0.076	16.06	4.642	2.523	2.119
89.88	**10**	3.162	1.954	1.000	1.005	8.988	0.0111	-0.046	10.12	4.642	2.163	2.479
93.82	**11**	3.317	1.972	1.041	0.791	8.529	0.0107	-0.028	6.18	4.642	1.835	2.806
99.65	**12**	3.464	1.998	1.000	0	8.304	0.0100	-0.002	0.35	4.642	0.705	3.937

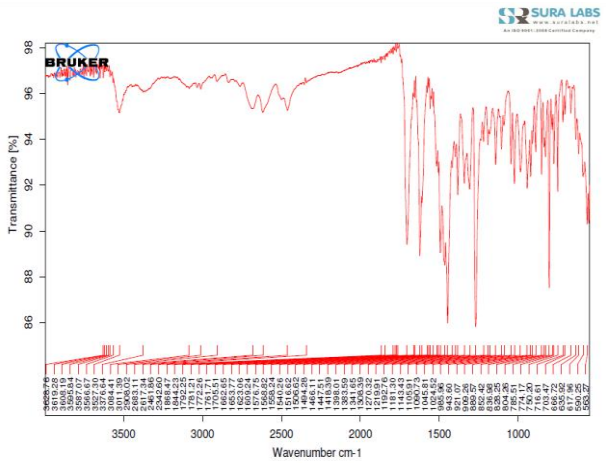

Figure 3: FT-IR of optimized formulation (F4)

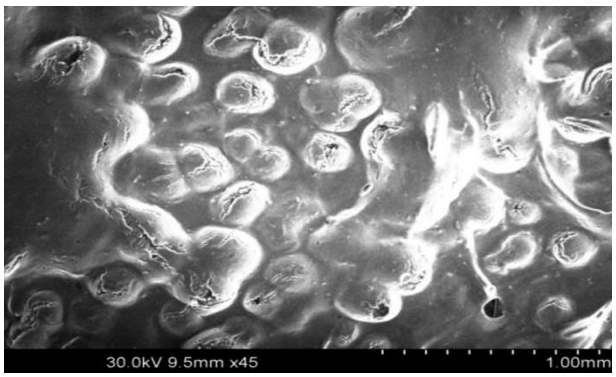

Figure 4: SEM of Clarithromycin Gel of optimized formulation

CONCLUSION

From the above results we can conclude that Clarithromycin gel was formulated with different types of gelling agents Na CMC, HPC, Guar gum and Poloxamer 407 showed favorable and acceptable physical properties concerning color, pH, homogeneity, spreadability, drug content, consistency of drug release study. Among all the formulation F4 proved to be the formula of choice since HPC gels shows superior drug release after that Na CMC, Guar gum, Poloxamer 407 these gelling agent were showed decreasing order of Clarithromycin release. In HPC gel formulation the Clarithromycin release was decrease with increase in HPC concentration because polymer concentration increase, viscosity increase so finally HPC contain Clarithromycin topical gel is used to treatment of skin infections. However, further preclinical and clinical studies are recommended to support its efficacy in humans.

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