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FORMULATION AND CHARACTERIZATION OF MUPIROCIN GEL AND EVALUATION OF IN VITRO ANTIMICROBIAL ACTIVITY AGAINST STAPHYLOCOCCUS AUREUS

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Abstract:

Mupirocin is a broad-spectrum antimicrobial agent available as ointment, and creamin the market.
However, the present investigation aims to formulate Mupirocin gel for topical application for convenient drug delivery. Gels are non-greasy and sustained release formulations possessing better adherence properties. Carbopol 934P was taken as gelling agent for the formulation of gel. The prepared gel was evaluated for its physical appearance, texture, pH, viscosity, spreadabilityand in-vitro performance.A stability study of prepared gel was performed.The formulated gel was compared with marketed Mupirocin ointment Bactroban (brand name)and was found to be effective than marketed preparation.

Keywords: Mupirocin, Gel, Spreadability, Zone of inhibition.

Introduction:

A novel antibacterial agent with peculiar chemical structure, Mupirocin(9-[(E)-4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-[[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2 yl] methyl] oxan-2-yl]-3-methylbut-2-enoyl]oxynonanoic acid) (Fig.1) was isolated from Pseudomonas fluorescens in 1971.It has a distinct mode of action than the available antibacterial agents at that time.(1,2) Mupirocin is effective against a wide variety of gram-positive and gram- negative bacteria like Staphylococcus aureus, Staphylococcus epidermidi Streptococcusspyogens, Haemophilusinfluenzae, Neisseria gonorrhea,Branhamellacatarrhalis,and Pasteurellamultocida.(1)

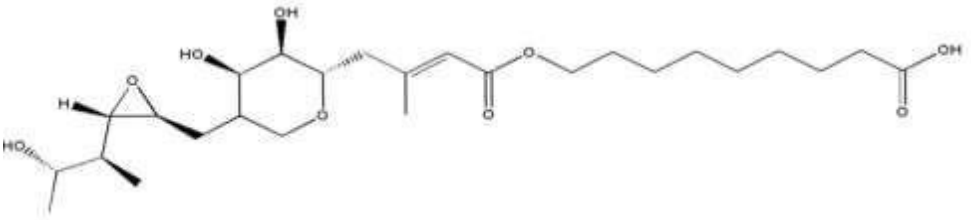

Figure 1. Structure of Mupirocin

It acts by inhibiting the synthesis of bacterial RNA and protein without causing substantial toxicity to the human cell as it shows very less interference with mammalian isoleucyl-transfer-RNA synthetase.(1,3,4).Market existence of Mupirocin is limited totopical formulations as 2% Mupirocin cream and ointmentfor the treatment of skin ailments(5)due to its metabolism to inactive form monic acid A (6).Attempt has been made for developing alternatenanosized carriers loaded with Mupirocin like Polymeric nanocapsule(7), Liposomes for parenteral use(2),Liposomal hydrogel for burn(8)and Nanostructured lipid carriers for intravenous route(9). In the present study, the goal was to develop a topical gel delivery of Mupirocin for improving its transport across the skin. Gel isa semisolid dosage form with a three-dimensional matrix of natural or synthetic polymers interpenetrated with liquid. Their biocompatibility, network structure, and molecular stability of the incorporated actives make it suitable vehicle (10). Gels possess better stability and superior application properties in comparison to creams and ointments. Gel systems are less greasy and can facilitate better absorption characteristics.They can provide sustained release with improved patient compliance in comparison to ointment and cream, due to its intermediate behavior between solid and liquid states of matter (11).

Material and method:

Mupirocin IP(> 99%) was obtained as a gift sample from KawmanPharma,Cuddalore Tamil Nadu, India. Carbopol 934P, Triethanolamine, Propylparaben, Methylparaben and Propylene glycol were purchased from Qualigens Fine Chemicals, A Division of Galaxo India Limited, Mumbai.S. aureus strain was obtained from the Department of Biotechnology,ITS Institute of Health and allied sciences,Muradnagar,Ghaziabad,U.P,India.

Preformulation study of Drug:

Infrared (IR) Spectrum:

IR Spectrum of Mupirocin was obtained using FTIR (Jasco FTIR -4100, Japan). One mg of the drug was mixed with 100 mg of potassium bromide (KBr) in a mortar by triturating and the mixture was pressed to 10 ton/cm^2 into a pellet. The sample was then scanned at 4000– 400 cm^{-1} (Figure 2).

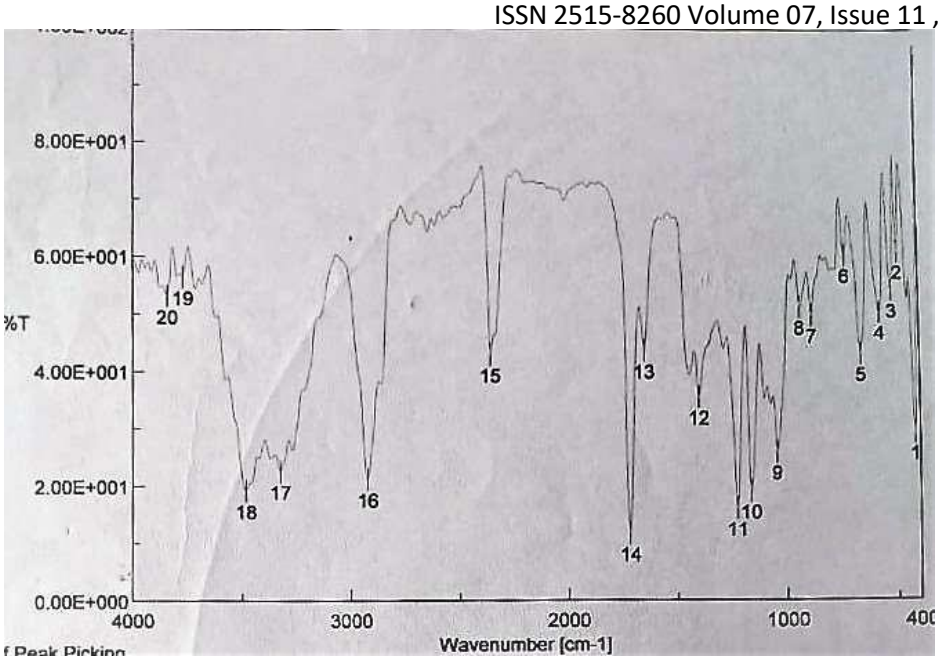

Figure 2.IR Spectra of Mupirocin

Melting Point:

The melting point of Mupirocin was determined using the capillary melt method.The appropriate amount of drug was filled through the open end of a glass capillary (sealed at one end), by gentle tapping on the soft surface of a table.The filled capillary was placed in the Melting point apparatus (PERFIT,INDIA) and the temperature at which melting occurs was noted.(12)

Formulation of the Gel loaded with Mupirocin:

The gel-type drug reservoir was prepared by dissolving Carbopol 934 P in distilled water in three different concentrations(0.5%,0.75 % and 1 %)(Table 1).Carbopol 934P was weighed and was kept in deionized water (quantity sufficient to make 100 gram) ina cool and dark place for 24 hrs. A 0.02% w/w mixture of methyl and propylparaben (in a ratio 2:1) was dissolved in propylene glycol followed by the addition of Mupirocin (2% w/w) with continuousmagnetic stirring at 50 rpm for 2 minutes (13). The prepared gel was neutralized by the addition of 0.1 ml triethanolamine and stirred again to get clear transparent gel.

Table 1: Composition of Mupirocin Gel

Formulation	Ingredients			
	Carbopol 934P	Mupirocin	Parabens (Methyl and Propyl in a ratio of 2:1)	Triethanolamine
F1	0.5% w/w	2% w/w	0.02% w/w	1.0 ml
F2	0.75% w/w	2% w/w	0.02% w/w	1.0 ml
F3	1.0% w/w	2% w/w	0.02% w/w	1.0 ml

Characterization of Mupirocin loaded Gel

Physical observation:

All the gels were visually observed for physical appearance and texture.

pH Determination:

the pH of all three gel formulations was determined by using a digital pH meter (HI-2213 bench pH Meter; Hanna Instruments). For pH determination, 0.5 gm of formulation was taken and dispersedin 20 ml of distilled water by stirring on a magnetic stirrer for 20 minutes at room temperature and measurement was done by immersingthe pH sensor probe in dispersion. All readings were taken in triplicates.(14)

Viscosity Study:

The viscosity measurement of Mupirocin gel was performed with Brookfield Viscometer with T bar mounted on helipath drive motor with special coupling (Brookfield LVDV-II+Pro). 100 gm gel was taken for viscosity measurement at room temperature and normal atmospheric pressure. T bar spindle with helipath was rotated for 1 minute at 4 rpm moving up and down during rotation to determine the percentage torque at multiple points. Viscosity was calculated in centipoises (cp) and average value was calculated (Table 2)(15)

Spreadability:

The spreadability of the formulations was determinedby measuring the spreading diameter of 1 g of gel samplebetween two horizontal glass plates (10 cm× 20 cm) after oneminute. The standard weight applied to the upper plate was25 g. Each formulation was tested thrice (13).

In-vitro drug release

The *in-vitro* drug release studies of all three gel formulations of Mupirocin were carried out using Franz diffusion cell. Dialysis membrane (Hi-Media, Mumbai, India) having molecular weight cut-off between 12,000–14,000 was soaked in double-distilled water for 12 h before mounting in Franz diffusion cell. Gel was placed in the donor compartment and phosphate buffer (pH 7.4)was kept in the acceptor compartment.The temperature of the receptor compartment was maintained at 37±0.5 °C with the help of a circulating water bath.Buffer in the acceptor compartment was stirred continuously (100 rpm) using amagnetic bead.The sample (1.0 ml) was withdrawn at 0, 0.5, 1, 12 hrsandreplaced with an equal amount of fresh media to maintain the sink condition. The samples were analyzed spectrophotometrically (UV-1800, Shimadzu, Japan) at 220 nm. Calculations for cumulative%drug releasewas performed.(16)

Release kinetic study:

Release data of Mupirocin from gels were fitted to differentmathematical models (17) like zero-order kinetics (concentration-independent kinetics), first-order (concentration-dependent kinetics), and Higuchi model.

Stability study:

Stability study of gel formulation F2 was carried out at 25^0C with 60%RH and 40^0C with 75% RH for 90 days, sampling was done at 0,30,60 and 90 days for any change in physical appearance, texture,pH change, viscosity, and spreadability (18).

Antimicrobial activity of Mupirocin gel:

A zone of inhibition test was carried out against clinical Staphylococcus aureus strain by using the agar diffusion method. The zone of inhibition of formulation F2 was compared with blank gel (without drug) as control and a marketed Mupirocin ointment preparation(Bactroban). A sterile cotton swab was dipped into the bacterial suspension and swabbed over the surface of the agar plates.Three cups were bored ineach Petri dish with the help of a sterile steel borer of 6mm and an amount equivalent to 2% Mupirocin gel,Bactroban ointment and blank gel were placed in the cup and incubated for 48 hrs at 37^0C.The zone of inhibition of all three were calculated as diameter (in millimeter).(19)

Results and Discussion:

IR spectra of Mupirocin (Figure 2) revealed peaks corresponding to the major functional group in the range of 1721.3 cm^{-1} which may be due to carbonyl stretching (C=O) of the ester group present in the molecule. The peak corresponding to 2922.33 cm^{-1} indicates the possible acidic hydroxyl group (R-COOH).Peak at 3770.02 cm^{-1}may be indicative of free hydroxyl (R-OH) group present terminally in the molecule (Figure 1). Hence, the molecule is interpreted as Mupirocin due to the presence of the above-stated peaks.The melting of the drug was found to be 78^0C, which is similarto the reported one (77^0C to 78^0C).(20)

Three gel formulations were prepared which contains 2% w/w Mupirocin as API, 0.02% w/w mixture of methyl and propylparaben (in a ratio of2:1) as preservative with three different concentration(0.5%,0.75% and1.0% w/w) of Carbopol 934P(Table 1). The method of preparation was mixing the ingredients witha magnetic stirrer at 50 rpm to avoid air bubble entrapment.All the three gels were transparent and smooth.Table 2 displays the observations for physical appearance and texture. The pH is a very important parameter for any formulation as it affects the stability of API as well as patient compliance.The formulation with a neutral pH range is necessary to avoid any skin irritation after application (21). The pH of three gel formulations was found in a neutral range for formulation.The values are mentioned in Table 2. Since all the pH belongs to the neutral range,the gel may supposed to be nonirritant to the skin. It may be because of the same concentration of triethanolamine used in all three formulations.The slight difference in pH of all three formulations may be due to different concentrations of carbopol 934P.

Viscosity is an important criterion for topical gel preparation. The value of viscosity was found to be80465 ± 97.34 cp,92054 ± 89.73 cp, 105400 ± 94.19cp for F1, F2 & F3 respectively.(Table 2,Figure 3)It was observed during the study that the concentration of Carbopol 934P hasa major impact on viscosity. As the concentration of Carbopol was increased from 0.5 % to 1.5 % the viscosity increases. The possible reason forthis increase may be high network crosslink density at higher concentrations. The swelling of carbopol depends on the concentration and solvent used, which ultimately governs the viscosity(15).

Table 2: Properties of Gel formulations

Formulation	Physical appearance	Texture	pH	Viscosity(cp)	Spreadability (mm)
F1	Transparent	Smooth	7.6 ± 0.33	80465 ± 97.34	25 ± 5.3

F2	Transparent	Smooth	7.4 ± 0.28	92054 ± 89.73	23 ± 3.4
F3	Transparent	Smooth	7.3 ± 0.31	105400 ± 94.19	18 ± 1.9

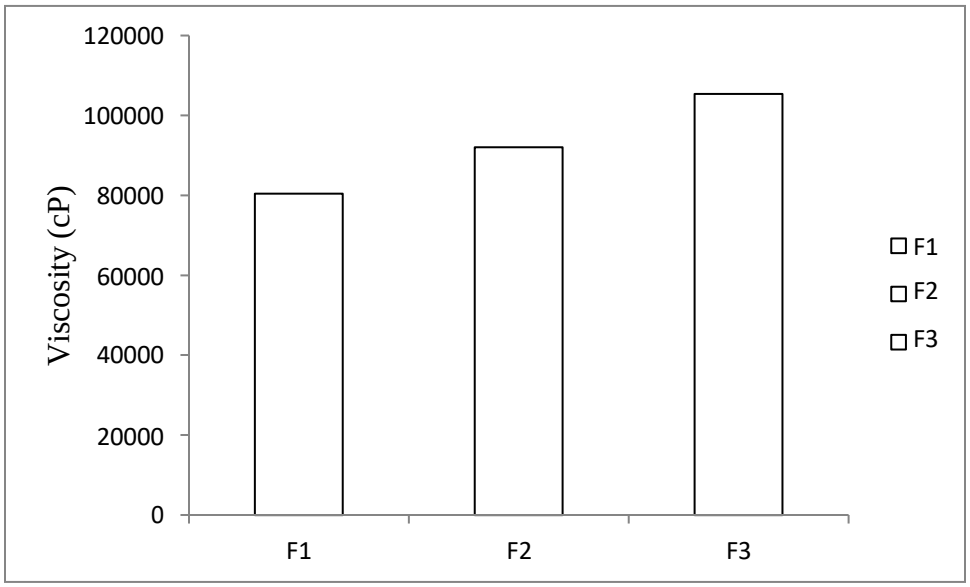

Figure 3: Viscosity of gel formulations

Spreadability of gel is the extent of the area to which it spreads on application to the site and playsan important role in the overall performance of gel formulation. It ensures an even layer of gel to deliver a standard dose to the site of application.Spreadability of prepared gels were 25 ± 5.3 mm, 23 ± 3.4 mm and 18 ± 1.9 mm for F1,F2 and F3 respectively(Table2,Figure 4). A decrease in spreadabilitywas found on increasing carbopol concentration. It may be due to increase in crosslinking and consequently increase in repulsion between the chains of carbopol 934P (22).

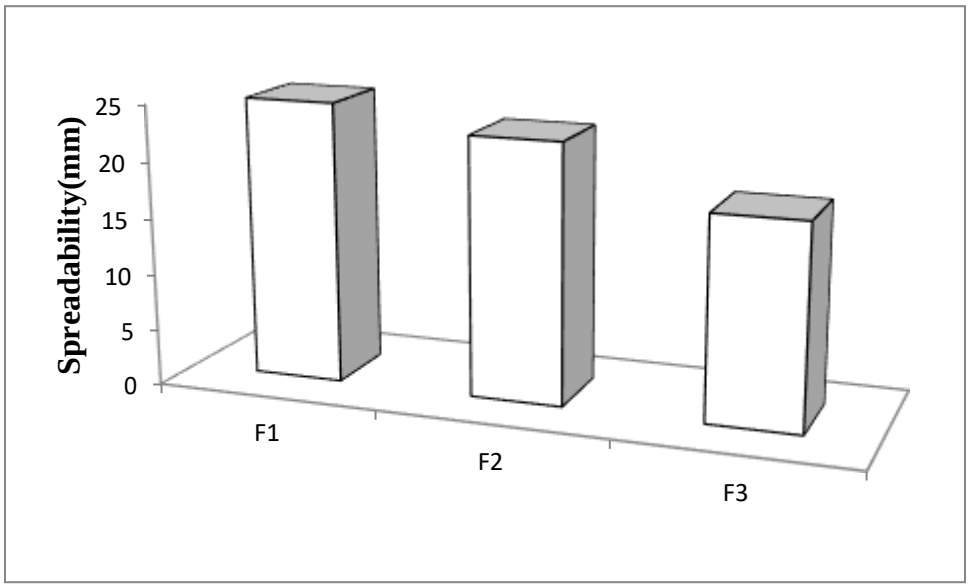

Figure 4: Spreadability of gel formulations

In-vitro release of formulation is important forpredicting the in vivo fate of formulation.An in-vitro release study was performed for all the three gel formulations in phosphate buffer pH 7.4.All the formulations released more than 98 % of the drug in 12 hrs. F1 showed 98.1±2.29 %, F2 showed 99.2±3.41, while 99.1 ± 3.22 % drug release was found for F3 in 12 hrs.No significant difference in the release of all three formulations was observed (Figure 5). Amongst three formulations GelF2 was selected because of the high percentageof drug release.For release kinetics of gel F2,the models of release were fitted.The value of correlation (r^2) for zero-order kinetics was found to be 0.872 followed by 0.739 for the Higuchi model and 0.724 for the first-order kinetics (Table3).The value of correlation(r^2) was highest for zero-order (0.872) but not equal to one and it reveals that the release profile is close to zero-order than first order.

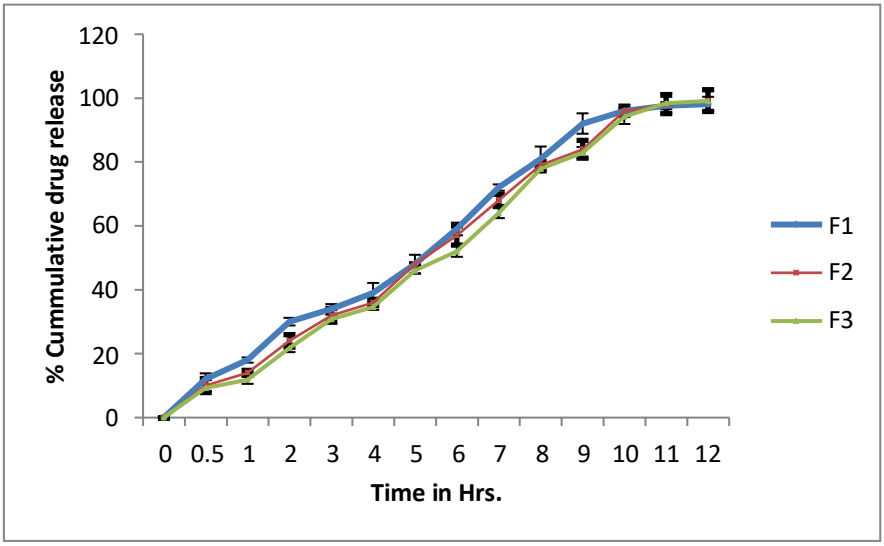

Figure 5. In-vitro release profile of Gel F1,F2, and F3

Table 3: Release kinetic study of Gel F2

Formulation	Release kinetic models		
Gel F2	Zero-order	First-order	Higuchi model
	r^2	r^2	r^2
	0.872	0.739	0.724

The formulation gelF2 was evaluated for stability in terms of physical appearance,texture,pH, viscosity and spreadability for 90 days(Table 4). This showsa very slight change in the above parameters which is not significant and hence the gel F2 was found to be stable for 90 days in terms of the above-listed parameters.

Table 4: Stability studies of Gel F2

Storage condition	**Days of sampling**	**Parameters evaluated**				
		Physical appearance,	**Texture**	**pH**	**Viscosity(cP)**	**Spreadability(mm)**
25^0C/60%RH	0	Transparent	Smooth	7.4 ± 0.28	92054 ± 9.73	23 ± 3.4

	30	Transparent	Smooth	7.38 ± 0.80	91840 ± 10.31	23.9 ± 2.1
	60	Transparent	Smooth	7.39 ± 0.47	91875 ± 3.38	23.6 ± 5.2
	90	Transparent	Smooth	7.3 ± 0.24	92009 ± 11.79	22.8 ± 5.9
40^0C/75%RH	0	Transparent	Smooth	7.4 ± 0.28	92054 ± 9.73	23 ± 3.4
	30	Transparent	Smooth	7.31 ± 0.39	91989 ± 9.98	23.4 ± 1.1
	60	Transparent	Smooth	7.52 ± 0.12	92435 ± 11.90	22.6 ± 6.1
	90	Transparent	Smooth	7.8 ± 0.81	92289 ± 9.73	22.9 ± 7.3

In-vitroantimicrobial activity of gel F2 was performed by using bacterial strain of Staphylococcus aureus and compared with blank gel (without Mupirocin) and marketed Bactroban ointment.To determine the zone of inhibition of all three formulation experiments was performed by agar diffusion method. Zone of inhibition was measured as diameter in millimeter (mm) where no bacterial growth was observed.In the present study,the zone of inhibition of blank was found to 5 mm, for Bactroban it was 18 mm and 20 mm for gel F2 (Table 5,Figure 6&7).

Table 5: Zone of inhibition of prepared gel formulation, Marketed formulation and Blank against Staphylococcus aureus

Formulation	Zone of inhibition(mm)
Blank Gel	5
Marketed ointment	18
Gel F2	20

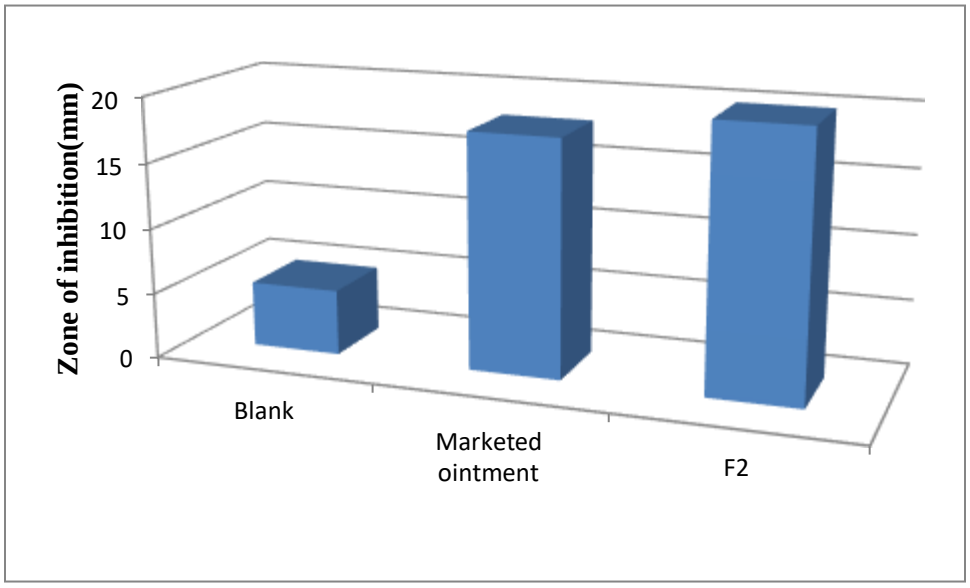

Figure 6: In-vitro antimicrobial activity

Figure 7: Zone of inhibition

The zone of inhibition reveals that the prepared gel has good antibacterial activity than marketed preparation. It might be due to the incorporation of Mupirocin in gel form which consequently enablesthe release of the drug in a sustained manner and was able to create an optimum concentration for a longer duration of time hence having good antimicrobial activity than conventional ointment.

Conclusion:

Mupirocin gel was successfully prepared by using Carbopol 934P as gelling agent. All three gel formulations have a similar physical appearance and smooth independent ofthe concentration of gelling agent. the pH of all the three gel formulations lies in the neutral range. Viscosity and spreadability were found to be affected by the concentration of gelling agent. Gelling agent affects the release of Mupirocin from gel and it was highest for gel F2, which showed a release pattern close to zero-order kinetics. Gel F2 was stable upto 90 days and hada larger zone of inhibition than marketed ointment Bactroban.In view of the above findings, drug delivery carrier gel was found to be a better alternative for the delivery of mupirocin in comparison to available market formulation.

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