WOUND- HEALING ACTIVITY OF A PREPARED LONG ACTING GEL LOADED WITH CIPROFLOXACILIIN HCL MICROSPHERES IN ALBINO RATS

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Summary

The drug-loaded microspheres were prepared by solvent evaporation technique[1, 2] and loaded into 1%, 2% and 3% carbopol gel formulations. The gel formulations were evaluated for drug content and viscosity .The *in vitro* diffusion studies revealed that the 3% carbopol gel loaded with microspheres exhibited sustained release upto 12 hrs and drug release was through diffusion process. Wound healing activity was determined on Wister rats using excision wound models .The animals were divided into three groups of six animals each. Group I animals treated with only the gel base , group II received the gel formulation with the pure drug ciprofloxacillin Hcl(Fg4) and group III (Fg3) treated with the gel formulation loaded with microspheres. Healing was assessed by the rate of wound contraction which was also supported by the histopathological studies. Increased wound contraction was seen with the formulations containing gel loaded with the microsheres.Stability studies showed no significant changes in the drug content and physical appearance.

Key words: Topical gels, Ciprofloxacin HCl, Wound healing

Introduction

Wound is a physical trauma where the skin is torn, cut or punctured. Exposed to air there are chances of microorganism entering the wound which leads to wound contamination and finally development of infection.The most cutaneous infections are caused by *S.aureus* and *S.pyrogenes*. Topical antimicrobial agent helps in preventing entry of microorganism into wound, which leads to fast healing of wound.Quinolones belongs to synthetic class of antimicrobial agent with potent antimicrobial activity. Ciprofloxacin hydrochloride is a fluroquinolone derivative with potent antibacterial activity against most gram+ve and gram-ve bacterial[3].

As the drug is known to possess superior antibacterial activity against a wide range of microorganisms, a topical drug delivery system localizing the drug at the skin will be much effective for the treatment of skin infections[4]. Hence in the present work topical controlled release gels[5] are prepared by incorporating the prepared microspheres to control the release rate of the drug over a period of time, so that the frequency of application of the formulation can be reduced, which also enhances patient compliance with better wound healing activity.

Materials and Method

Carbopol 934 (SD Fine chemicals, Mumbai); Ciprofloxacin hydrochloride (gift sample from Dr Reddy's laboratories), Hydrabad. Methyl paraben and Propyl paraben (Loba Chemicals, Mumbai), other chemicals and reagents used were of analytical grade.

Preparation of carbopol 934[6] gel:

1%, 2%, and 3% concentration of Carbopol gel were prepared by adding appropriate amount of Carbopol 934 in distilled water and was stirred gently, allowed to soak for 2 hrs. Required quantity of triethanolamine was added to form gel.

Preparation of Carbopol 934[7] gel containing sodium cmc microspheres:

Optimized sodium cmc microspheres equivalent to 350mg of drug (Ciprofloxacin HCl) was taken and incorporated during the preparation of gels of 1%, 2%, and 3% concentrations respectively. Finally triethanolamine was added in required quantity to adjust the pH and to obtain the gel.

Preparation of Carbopol 934 gel containing pure drug Ciprofloxacin HCl:

100g of 3% concentration of Carbopol gel was prepared, into which 350mg of pure drug, ciprofloxacin HCl was incorporated. Triethanolamine was added in required quantity to adjust the pH and to obtain the gel.

Table 1: Formulation chart of Gels

Ingredients (% w/v)	**Fg1**	**Fg2**	**Fg3**	**Fg4**
Ciprofloxacin HCl	Loaded M/S*	Loaded M/S	Loaded M/S	0.35g
Carbopol 934	1	2	3	3
Triethanolamine	Q.S	Q.S	Q.S	Q.S
Methyl paraben	0.1	0.1	0.1	0.1
Propyl paraben	0.5	0.5	0.5	0.5
Distilled water	Q.S	Q.S	Q.S	Q.S

Determination of drug content

1 gram of formulation was taken in 100 ml of 6.8pH buffer. Aliquots of different concentration were prepared by suitable dilution. The absorbance was measured at 271 nm using UV-spectrophotometer.

Determination of viscosity

The viscosity of all the formulations was determined by using Brookfield digital viscometer using spindle No.T 91 at 50 rpm, which was maintained at $37^{o} \pm 0.5^{0}$C.

***in Vitro* Release Studies [8]:**

A modified open diffusion cell was used for drug release from the gel formulations. Commercial semi permeable membrane, which was, soaked overnight in 6.8pH buffer, was stretched over the open end of glass tube. 1g of gel was placed carefully between the donor and receptor compartment. The receptor compartment contained 100ml buffer of pH 6.8 maintained at 37°C ± 5°C and stirred on a magnetic stirrer at 200 rpm. Samples of 5 ml were withdrawn from receptor compartment every hour and replaced with equal volumes of fresh receptor medium and were analysed for Ciprofloxacin HCl by UV (1601 A, Shimadzu Co, Japan) Spectrophotometer at 271nm.

Wound contraction studies [9, 10, 11]

The study protocol was approved by institutional Animal Ethics committee. Excision open wound model was selected for the wound healing activity. Albino rats of Wister strain of either sex weighing between 160-180g were selected and were divided into three groups of six animals each. Rats were anesthetized with anaesthetic ether and depilated at the predetermined site before wounding. An excision wound was inflicted by cutting away approximately 2sq cm full thickness of the predetermined area on the anterior-dorsal side of each rat. Each rat was kept in a separate cage with food and water. The control group (group I) received only the gel base. In one of the experiment group (group II) received the gel formulation Fg4 containing the pure drug ciprofloxacillin Hcl and the group III received the gel formulation loaded with microspheres. All the test formulations were applied twice daily for 16 days starting from the day of wounding. Wound healing property was evaluated by wound contraction percentage and wound closure time. Wound area was measured by tracing the wound margin using a transparent paper in each 2 days interval and healed area was calculated by subtracting from the original wound area. The wound contractions were measured as the percentage of wound reduction in the wound area for every two days. The percentage wound contraction was determined using the following formula:

$$\text{Percent wound contraction} = \frac{\text{Healed area}}{\text{Total wound area}} \times 100$$

Stability studies

The formulation weight equivalent to 100 mg of drug were packed individually in suitable screw tight container and subjected to following protocol. The optimized formulation was subjected to stability studies by storing at 25ºC/60% RH, 30 ºC/65% RH and 40ºC/75% RH for 90 days. These samples were checked for changes in physical appearance and drug content.

Results and Discussions

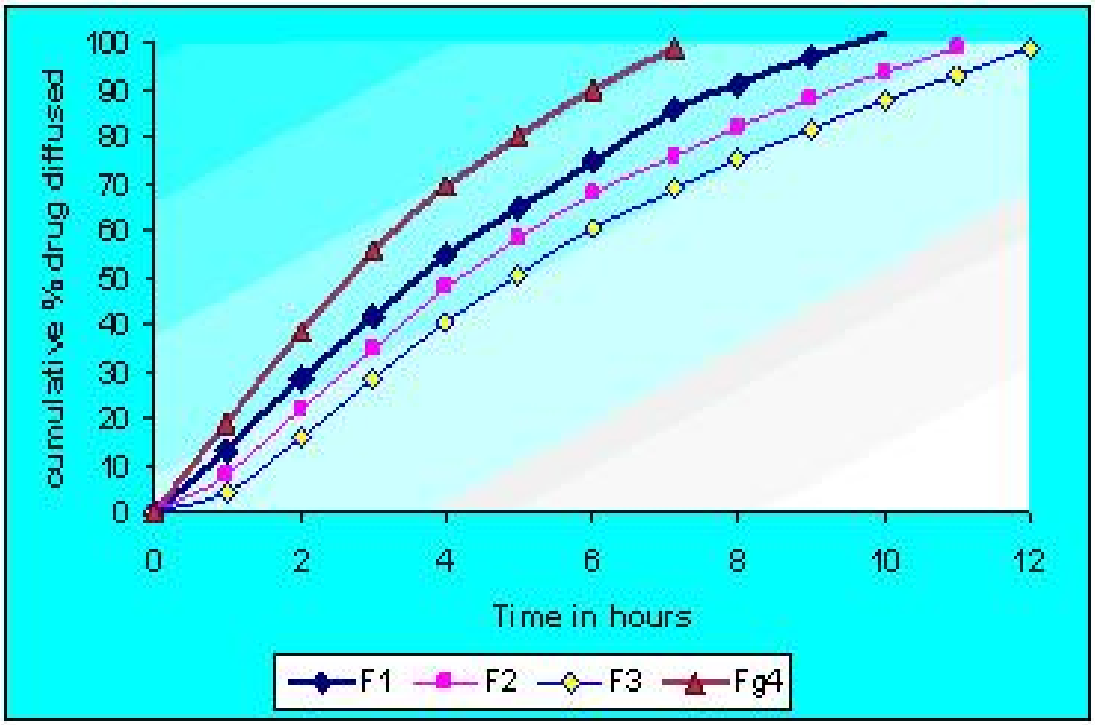
In-vitro drug release Studies

From the *in vitro* release data, it was observed that the concentration of the polymer affected the drug release from the gels, which were loaded with microspheres. Carbopol 934 is a hydrophilic polyacrylic acid polymer and its carboxyl groups become highly ionized after neutralization, forming a gel due to electrostatic repulsion among charged polymer chains[12].

The ionization of carboxyl groups in the Carbopol molecule at pH 7 results in uncoiling of the polymer chains and forms a rigid gel, thus affecting diffusion of drugs in the polymeric matrix. Viscosity of the gels reduces at lower pH, which could be attributed due to incomplete uncoiling of the polymer chains, resulting in an increased amount of free water and thus expanding the aqueous channels in the gel.

The diffusion study results showed that with increase in concentration of Carbopol 934, the rate of drug release[13] decreases. The reason for this can be based on the viscosity and swellability of the polymers.

It was observed that with increase in concentration of Carbopol 934, the viscosity was also increased. The diffusion study results (figure 1) showed that with increase in concentration of Carbopol 934, the rate of drug release decreases. The drug release from the gels was linear and was more controlled with the formulations containing 3%w/v of Carbopol 934 compared to formulations containing 1%w/v and 2%w/v of Carbopol 934. The formulation F1 showed a release of 97.3 % at the end of 8hours, Formulation F2 showed a release of 98% at the end of 10hours and F3 of 99 % at the end of 12 hours. Where as, the gel formulation (F4) containing pure drug gave a release of 99% at the end of 6hours only.



Wound contraction studies: Wound healing is essential to the human body. The wounded body is disposed to threats, such as bleeding, microbes, and dehydration. The healing process is principally similar in distinct connective tissues and epithelia. The healing of the wound requires orchestrated collaboration between different tissues and cell lineages. Wounds may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissue. Wound healing is a process that is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and other extra cellular macromolecules that are later remodelled to form scar. Inflammation is often associated with pain and fever.

Several factors delay or reduce wound healing, including bacterial infection, necrotic tissue, interference with blood supply, lymphatic blockage and etc. Generally if the above factors could be inhibited /controlled by any agent, increasing healing rate could be achieved. Ciprofloxaciliin Hcl is an antibacterial agent[14] which was found to be an effective agent in the form of a long acting gel. Exogenous application of certain antibacterial agents help in keeping the wound area sterile prevents the microbial growth hence preventing any further infection, which augment the healing process.

The reports of wound contraction studies in rats are reported in Table 2.The results indicated that both the formulations enhance the wound healing in excision open wounds. The control group showed little contraction(64.8%) whereas the rate of wound contraction was maximum (99%) on the 12th day in the Fg3 treated groups and 85% in the Fg4 treated groups respectively. On day 16, complete wound closure was seen in Fg3 treated group. The wound closure for Fg4 and control groups was 18 days and 21 days respectively. Wound contraction studies depicted that the gel formulations containing loaded microspheres had a promising wound contraction property when compared to the gel formulation with pure drug.

Table 2: Excision wound studies

Post wounding days	% Of wound contraction (Mean ± SD)*		
	Group I (Control)	Group II (pure drug Fg4)	GroupIII (microsphere Fg3)
0	0	0	0
2	6.6 ± 1.03	16.2 ± 2.01	33.2 ± 1.24
4	14.8 ± 096	24.6 ± 1.01	38.3 ± 1.04
6	19.8 ± 0.81	49.0 ± 0.97	66.5 ± 0.94
8	24.7 ± 1.04	52.5 ± 1.20	78.1± 0.99
10	39.8 ± 0.93	78.7 ± 0.98	83.5 ±0 .78
12	64.8 ± 1.00	85.8 ± 0.97	99.1 ± 0.99

Standard Deviation, n=3

Stability studies

These samples were analyzed and checked for changes in physical appearance and drug content at regular intervals. The obtained data is presented in Table 3. From the table, it is clear that the formulation did not undergo any chemical changes/interaction during the study period.

Table 3: Stability study for drug content of formulation Fg3

Stability condition	Sampling (days)	Drug content (mg) Mean ± SD*
25 ºC/60% RH	0	98.61± 0.38
	15	98.60 ± 1.10
	45	98.60 ± 1.12
	90	98.59 ± 0.86
30 ºC/65% RH	15	98.58 ± 0.98
	45	98.57 ± 1.18
	90	98.56 ± 1.06
40 ºC/75% RH	15	98.55 ± 0.82
	45	98.52 ± 1.46
	90	97.49 ± 1.56

- **Standard Deviation, n=3**

Conclusion

The prepared delivery system was found to be suitable for topical applications on wounds, which would limit the indiscriminate use of systemic administration of broad spectrum of antibiotics. Fg3 formulation showed 12 hrs of sustained drug release. The *in vitro* drug release studies showed that, the release of drug was found to be diffusion controlled. Results of wound contraction studies revealed the good wound healing activity of the formulations. Stability studies showed that there were no significant changes in the drug content and physical appearance.

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