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Research Article

IN VITRO ANTIBACTERIAL ACTIVITIES STUDY OF POLYMERIC CIPROFLOXACIN SUSPENSIONS

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ABSTRACT
To study the *in vitro* antibacterial activities of mucoadhesive suspensions containing Ciprofloxacin, three different formulations were prepared by using three polymers, such as Hydroxypropyl methylcellulose (HPMC) (S_1), Carbapol934 (S_2) and Carbapol940 (S_3), along with some common ingredients (bases). For the investigation, agar well diffusion method was performed taking *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* and *Escherichia coli* (ATCC 25922). Apart from *S. aureus*, S_1 and Ciprofloxacin in distilled water (S_4) produced more or less similar zones of inhibition against all the strains used. S_2 was more potent than S_4 against *S. aureus*, while S_3 was not more effective than S_4 as far as their antibacterial activities were concerned. Moreover, S_4 was not inferior to Ciprofloxacin in citrate buffer, marketed product containing Ciprofloxacin and Ciprofloxacin disc. The negative controls of the study, i.e., the different bases, distilled water and citrate buffer did not show any antibacterial activity. Considering the overall antibacterial activity pattern of different formulations, it may be concluded that S_1 was the most potent, while S_2 was more effective than S_3. In addition, S_1 and S_4 were almost equally potent against all the strains.
KEYWORDS: Ciprofloxacin, HPMC, C934, C940, Antibacterial activity

INTRODUCTION

Now-a-days mucoadhesive suspensions are being prepared for several purposes [1, 2]. In some of these suspensions, antibacterial substances are also incorporated. To study their antibacterial activities very few *in vitro* methods are available [3]. Such investigations are essential to know the availability/release of the drug from a base (containing a polymer with other substances). Sometimes drug release from the base is reduced, as a result of which that formulation may not be considered as a suitable preparation to control bacterial infections effectively.

Considering the importance of the availability of the drug from the suspensions, three different formulations of Ciprofloxacin (Cipro) were prepared in the present study. Their *in vitro* antibacterial activities were compared with those of the pure drug both in water and in citrate buffer, marketed suspension of Ciprofloxacin, disc containing Ciprofloxacin, different bases and distilled water against *Staphylococcus (S.) aureus* (ATCC 25923), *Bacillus (B.) subtilis* and *Escherichia (E.) coli* (ATCC 25922). Since such study reports are scanty, it is not possible to compare our results with those of others.

For the above-mentioned study, mucoadhesive suspensions of an antibacterial agent, Ciprofloxacin, were prepared using bases containing three different polymers. Hydroxypropyl methylcellulose (HPMC) and two grades of carbopol polymer, having different crosslinking agents such as C934 and C940, were selected for our investigation. HPMC is propylene glycol ether of methyl-cellulose having high swellability upon contact with water [4]. It is one of the most commonly used hydrophilic biodegradable polymers for developing mucoadhesive formulations, because it works as a pH-independent gelling agent [5-9]. On the other hand, carbopol polymers form hydrogel that change their swelling behaviour upon exposure to an external stimulus, such as change in pH [10, 11], temperature [12], light, or electric field, and are known as "environmentally responsive polymers" or "smart gels" [13,14]. Carbopol polymers have recently attracted considerable interest in the field of drug delivery as the means of providing an on-off release by shrinking and swelling in response to the change in pH [15-18].

MATERIALS AND METHODS

i) Materials

The following materials were used for the study: Ciprofloxacin was obtained from Dr. Reddy's Lab, Hyderabad, India, as a gift sample. Hydroxypropyl methylcellulose (HPMC E15 LV Premium) was supplied by Loba Chemie Pvt. Ltd., India. It was having methoxy group (23.8%) and hydroxypropoxy group (8.3%). Pluronic F 68 and Soya lecithin were purchased from Himedia Laboratories Pvt. Ltd., India. C934, C940, Glycerol, Citric acid, Sodium citrate, Methyl praraben sodium, Propyl paraben sodium, Sorbitol solution I.P. and Sucrose were supplied by Cosmo Chem. Laboratory, Pune, India. Tri-sodium citrate dehydrate purified was obtained from Merck Specialities Private Limited, Mumbai, India. Ultra pure water was obtained from a Millipore Milli-Q UV water filtration system. For the antibacterial activity study, different media and Ciprofloxacin Susceptibility test discs were obtained from Himedia Laboratories Pvt. Ltd., India.

ii) Samples Used

a) Formula for the Preparation of Mucoadhesive Suspensions-

(Percentage with respect to Ciprofloxacin)

Polymer ($S_1/S_2/S_3$)*	5%
Pluronic F 68	5%
Soya lecithin	1%
Sorbitol Solution (80%)	7.2%
Glycerin	0.8%
Simple Syrup IP	40%
Distilled water q.s. up to	100ml

Concentration of Ciprofloxacin used in the formulation - 1.25gm/25ml of distilled water

The pH was adjusted to 5.5.

*S_1- HPMC; S_2 – C934; S_3 – C940.

b) Other Samples-

S_{1b}-S_1 without Ciprofloxacin;

S_{2b}- S_2 without Ciprofloxacin;

S_{3b}- S_3 without Ciprofloxacin;
S_4 - Pure Ciprofloxacin (1.25gm) was mixed with 25ml of distilled water;
S_5 - Pure Ciprofloxacin (1.25gm) was mixed with 25ml of Citrate buffer of pH 5.5;
S_6 - Marketed product – CIPROLAR Suspension (Lark Laboratotries (India) Ltd, New Delhi):
Ciprofloxacin suspension each 10ml contain 250 mg Ciprofloxacin;
S_7 - Disc concentration - 10 µg Ciprofloxacin /disc;
S_8 – Distilled water;
S_9 – Citrate buffer of pH 5.5.

iii) Methods

Preparation of Formulations-

Praparation of Bulk A

In a beaker, 6 ml of distilled water was heated up to 80° C. Sucrose (10 gm) was added under continuous stirring. The temperature was monitored in such a way so that it should not fall below 70° C, till the sucrose was completely dissolved. The prepared syrup was cooled properly at room temperature and kept overnight. Syrup was filtered using 120 mesh nylon cloth.

Praparation of Bulk B

Five millilitre of distilled water was taken in a beaker to which 1.8 ml of sorbitol solution and 0.2 ml glycerin were added. The mixture was stirred properly. To this solution, pluronic F 68 (5%), soya lecithin (1%) and HPMC/C934/C940 (5%) in w/w of drug were added with continuous stirring.

Preparation of Mucoadhesive Suspension and Ultrasonication

Five millilitre of distilled water was taken in another beaker to which 1.25gm of Cipro was added. To the drug suspension, the bulk B and bulk A were added with continuous stirring. The volume was made up to 25 ml by Ultra pure water. The pH was adjusted to 5.5 by citrate buffer. Homogenization was carried out for at least 20 min by ULTRASONIC HOMOZENIZER LABSONIC^R M (SARTORIUS), having operating frequency 30 KHZ and line voltage 230 V/50 HZ, using the probe made up of Titanium of diameter 7 mm and length 80 mm. The setting knob "cycle" was adjusted to 0.8, indicating sound was emitted for 0.8 s and paused for 0.2 s. In this manner, we could expose our sample with 100% amplitude, while reducing the heating effect to 80%. This LABSONIC^{R}M generates longitudinal mechanical vibrations with a frequency of 30,000 oscillations / s (30 KHZ). The probes bolted to the sound transducer were made of high-strength Titanium alloys, built as λ /2 oscillators. It amplified the vertical oscillation, and transferred the ultrasonic energy via its front surface with extremely high power density into the sample that was to be subjected to ultrasonic waves. In our study, stress applied was sound wave and in addition, mild rise in temperature of the sample occurred during ultrasonication which helped in the homogenization of the suspension.

Method of Antibacterial Activity Study-

The nutrient agar well diffusion method as described by Schillenger and Luke (1989) was performed for our study. Sterile nutrient agar medium was inoculated with 0.1ml of fresh overnight nutrient broth culture of each bacterium (approx.10^7CFU/ml) and poured into sterile petriplates [19]. In each plate, wells of 6mm in diameter were punched using a sterile borer and the plates were allowed to dry for 5min [19, 20]. For the present study, mucoadhesive suspensions of Ciprofloxacin with HPMC containing base (S_1), Ciprofloxacin with C934 containing base (S_2) and Ciprofloxacin with C940 containing base (S_3), pure Ciprofloxacin in distilled water (S_4), pure Ciprofloxacin in citrate buffer (S_5), marketed suspension of Ciprofloxacin (S_6), disc containing Ciprofloxacin (S_7), bases (S_{1b}, S_{2b} and S_{3b}) (at different concentrations as mentioned earlier), distilled water (S_8) and citrate buffer (S_9) were used against *S. aureus* (ATCC 25923), *B. subtilis* and *E. coli* (ATCC 25922). 50 µl of each sample was dispensed into different wells using sterile micropipettes. For our study, S_{1b}, S_{2b}, S_{3b}, S_8 and S_9 were used as negative controls. After holding the plates at room temperature for 2 hours to allow diffusion of the samples and controls into the nutrient agar medium, the plates were incubated at 37 ^{0}C for 24hrs. The plates were examined for inhibition of the bacterial growth around the wells after the incubation period. The diameters of the zones of inhibition in each case were measured [19].

RESULTS

Against *S. aureus*, S_1 and S_2 were more active than S_4, while S_3 and S_4 were equally potent. Moreover, S_2 and S_3 were inferior to S_4 in case of *B. subtilis*. Against *E. coli*, all the samples (S_1, S_2, S_3 and S_4) showed more or less similar zones of inhibition. Considering the overall pattern of antibacterial activities, S_4 was not inferior to S_5, S_6 and S_7. Marketed product containing Ciprofloxacin and Cipro disc were not very effective against the strains used in this study. The negative controls of the study, i.e., the bases (S_{1b}, S_{2b} and S_{3b}), distilled water (S_8) and citrate buffer (S_9) did not show any antibacterial activity (Table 1). The zones of inhibition of different samples against the three bacterial strains have been shown in Figure 1.

DISCUSSION

Apart from *S. aureus*, S_1 was the most potent mucoadhesive suspension against the strains used. Between S_2 and S_3, S_2 was more effective than S_3. Only S_1 and S_4 were almost equally potent against all the strains. S_2 was better than S_4 only against *S. aureus*, while S_3 was not more potent than S_4 as far as their antibacterial activities were concerned. Moreover, S_4 was more potent than S_2 and S_3 only against *B. subtilis*.

Since the bases did not produce any zone of inhibition against the strains, some formulations showing more zones of inhibition than those of S_4 against *S. aureus* indicated that the bases had got potentiating effect on the antibacterial property of Cipro. On the other hand, S_4 was more or equally potent against some strains as compared with S_2 and S_3. The difference in antibacterial activities between different suspensions may be due to either the effect of bacterial metabolites which may influence the rate of release or the interaction between the drug and the base.

At least those formulations were not inferior to pure drug in buffer, marketed suspension containing Ciprofloxacin and disc containing Ciprofloxacin. This indicates that all the formulations should be studied further.

CONCLUSION

Considering the antibacterial activity pattern, it could be concluded that, instead of using S_4, S_1 may also be considered as a potent mucoadhesive suspension (as they are equipotent). S_1 could be preferred over S_4 therapeutically, if the results of other relevant studies (e.g., pharmaceutical / pharmacokinetic evaluations) of S_1 would be better than those of S_4. S_2 may also be considered as a better option than S_4, only against *S. aureus*, when the results of the above-mentioned investigations of S_2 would be satisfactory. In addition, S_3 should not be ignored. So, all the formulations,

particularly S_1 and S_2, are potentially promising mucoadhesive suspensions.

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Table 1. ANTIBACTERIAL ACTIVITIES OF DIFFERENT SUSPENSIONS, PURE DRUG, MARKETED SUSPENSION AND NEGATIVE CONTROLS

Micro-organisms	Average Zone of Inhibition (mm)											
	S_1	S_{1b}	S_2	S_{2b}	S_3	S_{3b}	S_4	S_5	S_6	S_7	S_8	S_9
S. aureus	54.8	0	57.7	0	50.8	0	50.5	47.8	38.8	28.3	0	0
B. subtilis	45.4	0	40.8	0	35.5	0	47.3	38.3	38.7	27.0	0	0
E. coli	32.6	0	32.5	0	32.3	0	32.4	32.3	11.7	12	0	0

S_1 – Ciprofloxacin with HPMC containing base; S_{1b} - HPMC containing base; S_2 - Ciprofloxacin with C934 containing base; S_{2b} - C934 containing base; S_3 - Ciprofloxacin with C940 containing base; S_{3b} - C940 containing base; S_4 – Ciprofloxacin in distilled water; S_5 – Ciprofloxacin in citrate buffer; S_6 –Marketed Ciprofloxacin suspension; S_7 – Ciprofloxacin containing disc; S_8 – Distilled water; S_9 – Citrate buffer

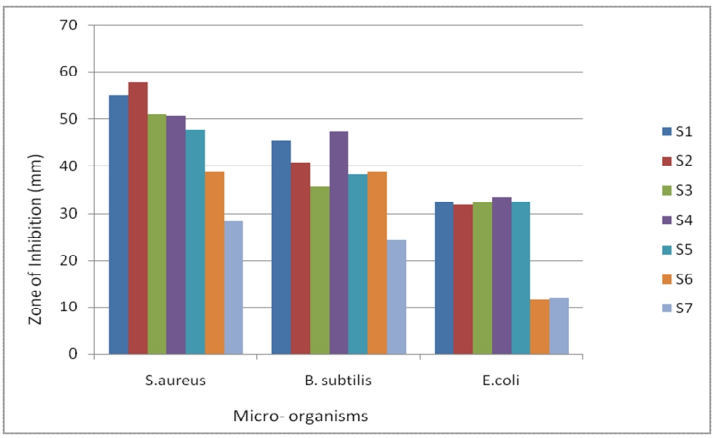

Figure 1. Zones of inhibition of different samples against the three bacterial strains

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