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Research Article

In vitro antibacterial activities study of norfloxacin containing mucoadhesive suspensions

Subhashree Sahoo*[1], Chandra Kanti Chakraborti[1], Pradipta Kumar Behera[2]

[1]Department of Pharmaceutics, Kanak Manjari Institute of Pharmaceutical Sciences, Rourkela-769015, Orissa, India

[2]School of Chemistry, Sambalpur University, Jyoti Bihar-768019, Orissa, India

ABSTRACT

To study the *in vitro* antibacterial properties of mucoadhesive suspensions containing Norfloxacin, 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 antibacterial activities study of the suspensions agar well diffusion method was performed taking *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* and *Escherichia coli* (ATCC 25922). Considering the overall antibacterial activity pattern of different suspensions, it may be concluded that S_1 was the most potent, and S_2 and S_3 were more or less equally effective formulations. Antibacterial properties of all the suspensions were either more or similar to those of pure drug in water (S_4). At least those formulations were not inferior to S_4, Marketed suspension containing Norfloxacin and Disc containing Norfloxacin. The negative controls of the study, i.e., the different bases and distilled water did not show any antibacterial activity.

Keywords: Antibacterial Activity; Norfloxacin; HPMC; C934; C940

INTRODUCTION

Now-a-days mucoadhesive suspensions are being prepared for several purposes (Jain *et al,* 2011; Sahoo *et al*, 2011a). In some of these suspensions, antibacterial substances are also incorporated. To study their antibacterial activities very few *in vitro* methods are available (Sahoo *et al*, 2011b). 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 Norfloxacin (Norflox) were prepared in the present study. Their *in vitro* antibacterial activities were compared with those of the pure drug in water, marketed suspension of Norfloxacin, disc containing Norfloxacin, different bases and distilled water against *Staphylococcus (S.) aureus* (ATCC 25923), *Bacillus (B.) subtilis* and *Escherichia (E.) coli* (ATCC 25922).

* Corresponding Author
Email: subha77t@yahoo.co.in
Contact: +91-9861376820; Fax +91-661-2480752
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For the above-mentioned study, mucoadhesive suspensions of an antibacterial agent, Norfloxacin, 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 (Fatimi *et al*, 2008). It is one of the most commonly used hydrophilic biodegradable polymers for developing mucoadhesive formulations, because it works as a pH-independent gelling agent (Phaechamud, 2008; Siepmann and Peppas, 2001; Gao *et at*, 1996; Talukdar, 1996; Katiknani, 1995). On the other hand, Carbopol polymers form hydrogel that change their swelling behaviour upon exposure to an external stimulus, such as change in pH (Qiu and Park, 2001; Bettini *et al*, 1995), temperature (Bromberg and Ron, 1998), light, or electric field, and are known as "environmentally responsive polymers" or "smart gels" (Galaev and Mattiasso, 1999). 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 (Yoshida et al, 1998; Jeong and Gutowska, 2001; Gupta et al, 2002).

MATERIALS AND METHODS

Materials

The following materials were used for the study: Norfloxacin was obtained from Dr. Reddy's Lab, Hydera-

bad, 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, 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 antibacterial activity study different media and Norfloxacin Susceptibility test discs were obtained from Himedia Laboratories Pvt. Ltd., India.

Samples Used

a) Formula for the Preparation of Mucoadhesive Suspensions

(Percentage with respect to Norfloxacin)

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 Norfloxacin used in the formulation - 500mg/25ml of distilled water

*S_1- HPMC; S_2 – C934; S_3 – C940.

b) Other Samples

S_{1b}-S_1 without Norfloxacin;

S_{2b}- S_2 without Norfloxacin;

S_{3b}- S_3 without Norfloxacin;

S_4 - Pure Norfloxacin (500mg) was mixed with 25ml of distilled water;

S_5 - Marketed product – BACIGYL Suspension (ARISTO Pharmaceutical Pvt. Ltd. Mumbai): Norfloxacin suspension each 5ml contain 100 mg Norfloxacin;

S_6 - Disc concentration 10 µg Norfloxacin/disc;

S_7 – Distilled water

Preparation of Formulations

Preparation 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 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 500 mg of Norflox 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. 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 (Bayoub *et al*, 2010). In each plate, wells of 6mm in diameter were punched using a sterile borer and the plates were allowed to dry for 5min (Bayoub *et al*, 2010; Ganjewala *et al*, 2009). For the present study, mucoadhesive suspensions of Norfloxacin with HPMC containing base (S_1), Norfloxacin with C934 containing base (S_2) and Norfloxacin with C940 containing base (S_3), pure Norfloxacin in distilled water (S_4), marketed suspension of Norfloxacin (S_5), disc containing Norfloxacin (S_6), bases (S_{1b}, S_{2b} and S_{3b}) (at different concentrations as mentioned earlier) and distilled water (S_7) 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

Table 1: Antibacterial activities of different suspensions, pure drug, marketed suspension and negative controls

Name of Micro-organisms	Average Zone of Inhibition (mm)									
	S_1^a	S_{1b}^b	S_2^c	S_{2b}^d	S_3^e	S_{3b}^f	S_4^g	S_5^h	S_6^i	S_7^j
S.aureus	40	0	34.3	0	27.3	0	24.7	24	20	0
B.subtilis	30.3	0	28	0	29.3	0	28	23	18	0
E. coli	24	0	21.3	0	23.3	0	18.3	23	12	0

a – Norfloxacin with HPMC containing base; b - HPMC containing base; c - Norfloxacin with C934 containing base; d - C934 containing base; e - Norfloxacin with C940 containing base; f - C940 containing base; g – Norfloxacin in distilled water; h – Marketed Norfloxacin suspension; i – Norfloxacin containing disc; j – Distilled water

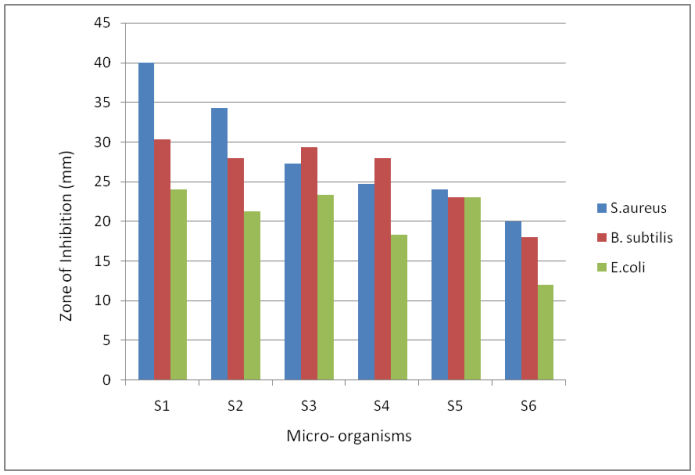

Figure 1: Zones of inhibition of different samples against the three bacterial strains

sterile micropipettes. For our study S_{1b}, S_{2b}, S_{3b} and S_7 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. After the incubation period, the plates were examined for inhibition of the bacterial growth around the wells. The diameters of the zones of inhibition in each case were measured (Bayoub *et al*, 2010).

RESULTS

Against *S. aureus*, both S_1 and S_2 were more effective than S_4, while S_3 was more or less similar to S_4. Between S_1 and S_2, it was observed that S_1 was more potent than S_2 against that strain. In case of S_1, the zone of inhibition was more than S_4 against *B. subtilis*, on the other hand, it was more or less similar in S_2, S_3 and S_4. All the suspensions (S_1, S_2, S_3) were more potent than S_4 against *E. coli*. The zones of inhibition produced by Norflox containing marketed product (S_5) and Norflox Disc (S_6) were smaller than those of S_4 against all the strains. The negative controls of the study, i.e., the bases (S_{1b}, S_{2b} and S_{3b}) and distilled water 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

Mucoadhesive suspension of Norfloxacin with HPMC containing base (S_1) was the most effective suspension against all the strains used. It was more potent than S_4 as far as its antibacterial properties were concerned. Against *S. aureus* and *E. coli*, S_2 and S_3 showed more zones of inhibition than those of S_4, while S_2, S_3 and S_4 were equally effective in case of *B. subtilis*. Since the bases did not produce any zone of inhibition against the strains, almost all the formulations showing more zones of inhibition than those of S_4 indicated that the bases had got potentiating effect on the antibacterial property of Norflox. Moreover, 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.

Considering the overall antibacterial pattern, it may be mentioned that S_1 was the most potent formulation. In addition, S_2 and S_3 were more or less equally effective suspensions. At least, the antibacterial activities of pure drug (S_4) were retained in all the formulations (such as S_1, S_2 and S_3). Moreover, all the suspensions were not inferior, if not better, to the pure drug (S_4), Marketed suspension (S_5) and Norfloxacin susceptible disc (S_6).

Since such study reports are scanty, it is not possible to compare our results with those of others.

CONCLUSION

Considering the overall antibacterial activity pattern of different suspensions, it may be concluded that Nor-

floxacin with HPMC containing base was the most potent, and Norfloxacin with C934 containing base and Norfloxacin with C940 containing base were more or less equally effective formulations. Antibacterial properties of all the suspensions were either more or similar to those of pure drug in water. At least those formulations were not inferior to pure drug in water, Marketed suspension containing Norfloxacin and Disc containing Norfloxacin. So, these formulations could be considered as better options for therapeutic use, if their results of other relevant studies (e.g., pharmaceutical / pharmacokinetic evaluations) would be satisfactory.

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