RESEARCH PAPER

Received 3 September 1996
Accepted 15 February 1997
Indian J. Pharm. Sci., 1997, 59(2) pp.61-67

Preparation and evaluation of Alginate Microspheres containing Norfloxacin and Ciprofloxacin

K.S. AITHAL* AND N. UDUPA
College of Pharmaceutical Sciences, Kasturba Medical College, Manipal - 576 119 (D K) Karnataka.

Microspheres containing norfloxacin and ciprofloxacin using Sodium and calcium alginates were prepared and the possible drug- excipient chemical interaction was evaluated by various spectral methods. There was no chemical interaction between these drugs and the alginates. The dissolution and diffusion of these drugs were delayed and the stability at various accelerated stability conditions was improved on entrappment of these drugs in alginates.

NORFLOXACIN (NOR) and ciprofloxacin (CIP) are fluroquinolone antibacterial drugs used in urinary tract infection, gonorrhoea, and vaginitis. They are readily soluble in the gastric pH due to the formation of salts. They were reported to have poor stability at elevated temperatures and exposure to sunlight due to the ring opening and formation of ethylene diamine derivative and formamide derivative[1]. Sodium alginate (SA) is the sodium salt of alginic acid which is soluble in water forming a viscous colloidal solution and insoluble in ethanol, ether, chloroform and aqueous acids below pH 3.0. It is a linear polymer of beta - D- (1->4)-linked mannuronic acid units(M) and alpha-L-(1->4)- linked guluronic acid units(G) residues[2,3]. The physicochemical properties including solubility dissolution of certain drugs viz., dextran, theophylline, diazepam[4-6] are reported to be affected by incorporating them into SA and CA beads or microspheres leading to the formation of sustained release formulations. These alginates were reported to give acid resistance property to drugs like Sulphamethoxazole which dissolve quickly in gastric juice. It was reported that an increase in the amount of alginates decreased the release rate of sulphamethoxazole[7]. The release kinetics of sodium salicylate and theophylline from the controlled release matrix containing calcium alginate(CA) and SA system is reported in the literature[8]. Pilocarpine incorporated into CA matrix is commercially available for opthalmic implant.

In the present work, NOR and CIP were dispersed in the alginate films and entrapped into microspheres of SA and CA separately and their effect on the physicochemical characteristics of these drugs was evaluated. The preformulation parameters evaluated in this study may be used in the preparation of dental, buccal, opthalmic, rectal, intradermal implants for local delivery of these drugs.

MATERIALS AND METHODS

Norfloxacin (Dr. Reddy's Laboratory, Hyderabad, India), Ciprofloxacin (Cipla, Bangalore, India), Sodium Alginate (viscosity of 2% w/v solution, 250 cps, Sigma chemicals, UK) were the chemicals used as received. UV spectrophotometer (UV 240, Shimadzu, Tokyo, Japan), IR spectrophotometer (IR 408 Shimadzu, Tokyo, Japan), X-ray diffractometer (PW 1140/90, Philips Ltd. The Netherlands), dissolution apparatus (Thermonik, Campbel Electronics,

* For correspondence

Bombay, India), diffusion apparatus (locally fabricated) were the instruments used.

Preparation of various formulations containing NOR and CIP

Entrapment of NOR in sodium alginate

The entrapment was found to be maximum when the drug to the SA or CA was in the ratio of 1:15 w/w. Hence this ratio was chosen for the study[9]. About 1500 mg of SA was triturated in a mortar using distilled water (30 ml) at 40° to get a pasty consistency. It was further triturated by adding water (20 ml) and the trituration was continued for about 15 min to get a uniform consistency. The contents were transferred quantitatively into a beaker and 100 mg of NOR was uniformly dispersed with constant stirring for about 15 min. using a magnetic stirrer (100 RPM) to get a uniform dispersion of the drug. Ethanol (95% v/v, 200 ml) was added dropwise to precipitate SA along with the drug. Formation of the aggregate masses was avoided by constant and gentle stirring of the contents while adding ethanol. Addition of ethanol was continued until the precipitation was complete. The residue was thoroughly washed with water to remove the unentrapped drug. The residue was dried in vacuum and used for further analysis.

Entrapment of NOR in calcium alginate

NOR (100 mg) was uniformly dispersed in a mucilage prepared by triturating 1500 mg of SA in 50 ml of water by gentle stirring. This was added to 200 to 250 ml of 1% w/v calcium chloride solution dropwise with constant and gentle stirring to precipitate the calcium alginate simultaneously trapping the drug dispersed in it. The amount of calcium chloride chosen was sufficient to precipitate the calcium alginate completely. It was filtered and washed with distilled water thrice. The residue was dried in vacuum and used for further analysis.

Dispersion of NOR in sodium alginate

The drug and SA in the ratio 1:15 w/w were dispersed in distilled water (20 ml) and triturated for about one hour for proper mixing. It was poured into a tray and the water was evaporated at 25°. The flakes formed were air dried, powdered and sieved through mesh 44 and the formulation was named as SA film. As calcium alginate was insoluble in water, NOR-CA film was not prepared.

The physical mixture (phy. mix) of NOR with SA and NOR with CA in the ratio 1:15 w/w were prepared by triturating the ingredients for about one hour.

CIP was used instead of NOR and the formulations were prepared on similar lines as stated above.

Evaluation of the drug excipient interaction

The standard plots for NOR in 0.01N HCl at 275 nm and for CIP in 0.1N NaOH at 272 nm in the concentration range 0 to 10mcg/ml were prepared. The effect of SA and CA on the λmax and absorptivity was evaluated. For X-ray diffractometric analysis, the experimental settings were: Ni-filter Cu K α radiation (λ=1.541836 A), tube setting 30 KV, 20 mA, angular speed (2θ) $2^{\circ}min^{-1}$. The IR spectrum was recorded using KBr disc method at a pressure of 400 Kg cm^{-2}.

Stability studies were carried out using temperature controlled incubators maintained at 25±1°, 37±1° and 45±1° and humidity chamber maintained at 82.0±2% RH. Known amount of the samples were also exposed to direct sunlight and the photodegradation was evaluated by estimating the drug remaining spectrophotometrically. These studies were carried out for a period of three months. From the graph of log percentage drug remaining versus time, the degradation rate constate (k) was evaluated, IR spectrum of the samples were also taken before and after exposure to these accelerated conditions. A thin layer chromatographic procedure was

performed using silica gel (G_{254} grade). The solvent used to dissolve the formulation was chloroform: dichloromethan (4:1) and the solvent system used was chloroform: glacial acetic acid: methanol: water (20:10:3:3). The lower organic layer was used as the mobile phase. The chromatogram was run using plain drug as the reference sample and compared with those of the formulations. Any new spots obtained were identified and their R_f value was calculated.

Dissolution rate studies were carried out using USP XXI dissolution rate test apparatus in 100 ml of buffer solutions (pH 7.2, KH_2PO_4/NaOH; pH 1.2 HCl/KCl) at 37±1°, 100 RPM. The plain drug, phy.mix, and the formulations were subjected to this study. Diffusion studies were carried out using indigeneously fabricated diffusion apparatus attached with a sigma dialysis membrane (Sigma chemicals Ltd., USA, cut off mol. wt. 12000). The diffusion medium used was buffer solution of pH 1.2 (100ml) at 37±1°, 100 RPM. The plain drug, phy. mix. and the formulations were subjected to this study.

RESULTS AND DISCUSSION

Entrapment study

The percentage of NOR entrapped in SA and CA was 46.7 ± 3.7, 74.3 ± 4.9 respectively when the drug : excipient was in the ratio 1:15. Similarly the percentage of CIP entraped in SA and CA was 49.9 ± 3.5, 78.8 ± 3.5 respectively at the same ratio (9).

Interaction studies

The intensity of absorption and the λmax remained the same for plain drug and the preparations containing equivalent amount of the drug indicated that the UV absorption characteristics of these drugs were not affected by the excipients.

Though the plain drug had a well resolved XRD pattern, neither the preparations nor pure SA/CA gave a well resolved XRD pattern. This could be due to the low drug content in the preparations. Hence XRD also could not provide definite evidence for the interactions.

The IR spectrum of NOR-SA and CIP-BCD phy.mix. showed all the signals of the individual compounds with expected decrease in intensity for those of NOR. The IR peaks of SA or CA in the preparations were not shifted, indicating non involvement of any functional groups of SA or CA with the drug in the formulations. Thus no chemical interaction involving the functional groups of the two ingredients in these formulations could be predicted.

Stability study of NOR and CIP with alginates

The new IR signals appeared in the case of plain drug were absent in the formulations except the phy. mix. indicated the restriction offered by the alginates on the ring opening of the drug leading to the formation of degraded products. Similarly the new TLC spots obtained corresponding to the partial degradation of the plain drugs were not found in the case of formulations prepared excepting the phy. mix. indicated the absence of the degraded products in the formulations. Thus, both these techniques helped in the qualitative evaluation of the stability studies and revealed the absence of the degraded products in the formulations.

The degradation rate constant (k) value for NOR-SA microspheres at 25° was lower than that of both the plain drug and the phy. mix. All the other samples gave a higher value. The k value for NOR-SA microspheres and in NOR-SA phy.mix. at 37° was lower than that of plain drug. whereas, other samples gave a higher k value. Similar observations were made at 45°. The k values for all the NOR-alginate preparations at 82% RH were higher than that of the plain drug indicating their lesser stability. On exposure to direct sunlight, the k value for all the samples were considerably reduced compared to the plain drug and it was least for SA and CA microspheres indicating that the stability of the drug was improved when it was incorporated with

Table - 1
Degradation rate constant of norfloxacin and ciprofloxacin with alginates

Samples	Rate constant values at various stability conditions (k) x 10^{-4} $days^{-1}$				
	25°	37°	45°	82% RH	Under sunlight
Plain drug	1.791 (1.534)	3.071 (2.303)	3.838 (3.838)	2.303 (2.303)	20.471 (17.912)
Drug+SA Phy.mix.	2.303 (1.791)	2.814 (2.558)	3.326 (3.838)	3.966 (5.629)	10.235 (3.838)
Drug in SA film	2.452 (2.303)	3.302 (2.303)	3.826 (2.558)	5.162 (11.515)	7.676 (5.630)
Drug in SA microspheres	1.152 (1.279)	1.279 (1.407)	1.791 (1.867)	3.327 (3.455)	5.885 (2.814)
Drug+CA Phy.mix.	2.415 (1.152)	3.071 (1.535)	3.905 (2.303)	4.891 (1.791)	8.444 (23.030)
Drug in CA microspheres	5.117 (2.315)	5.630 (2.558)	5.629 (5.118)	7.421 (2.315)	6.141 (8.956)

Values in paranthesis corresponds to ciprofloxacin

alginates. Thus it can be inferred that SA microspheres could provide better stability to NOR almost at all accelerated stability conditions studied, compared to CA microspheres (Table- 1).

The k values were found to be least for CIP-SA microspheres and slightly more for CA microspheres at elevated temperatures indicated that SA microspheres offered protection against degradation of CIP at elevated temperatures and the SA film could do so only at 45° compared to CA microspheres. The k values for all the samples at 82% RH were more than the plain drug except for the CA phy.mix. indicating that on incorporating CIP with alginates the degradation of the drug was pronounced at higher moisture conditions. However, when the samples were exposed to sunlight, the degradation of the drug was considerably reduced in all the cases except the CA phy.mix., as could be seen from the lower value of k. Thus both SA and CA alginates provide excellent protection to degradation of CIP under sunlight. It could be concluded that SA is superior to CA as a carrier to provide better stability to CIP in the form of microspheres (Table-1).

Dissolution data of NOR with alginates

The decrease in the dissolution of NOR in a buffer solution of pH 1.2 when incorporated with various alginates was in the order of CA microspheres < SA microspheres < SA phy. mix. < SA film < CA phy.mix < plain NOR (Table-2). The decrease in the dissolution of NOR from the phy.mix. and from the film is probably due to the swelling of the polymer and leaching of the drug through the pores. When the drug was entrapped in the SA microspheres, the dissolution might have been further affected by this process. The dissolution of the drug from its phy.mix. with CA was not significantly delayed compared to the plain drug. This was due to poor solubility of CA compared to SA at pH 1.2.

Table - 2
Dissolution and Diffusion data of NOR and CIP-alginate system

Samples	Dissolution				Diffusion	
	pH 1.2		pH 7.2		pH 1.2	
	$t_{50\%}$ (min)	$t_{80\%}$ (min)	$t_{50\%}$ (min)	$t_{80\%}$ (min)	$t_{50\%}$ (min)	$t_{80\%}$ (min)
Plain drug	1.0 (6.7)	1.7 (13.5)	8.8 (22.0)	48.0 (52.0)	38.0 (40.0)	141.0 (103.0)
Drug + SA Phy.mix.	25.0 (22.5)	135.0 (68.0)	48.0 (38.5)	114.0 (63.5)	109.0 (125.0)	>180.0 (>180.0)
Drug in SA film	13.0 (23.5)	100.0 (94.5)	45.0 (50.0)	103.0 (98.0)	126.0 (134.0)	>180.0 (>180)
Drug in SA microspheres	104.0 (144.0)	>180.0 (>180.0)	64.0 (53.0)	162.0 (>180.0)	>180.0 (>180)	>180.0 (>180)
Drug + CA Phy.mix.	8.5 (10.5)	13.0 (22.0)	12.0 (22.0)	51.0 (57.5)	41.0 (48.0)	180.0 (132.0)
Drug in CA microspheres	>180.0 (172.0)	>180.0 (>180.0)	132.0 (104.0)	>180.0 (>180.0)	>180.0 (>180.0)	>180.0 (>180.0)

Values in paranthesis corresponds to ciprofloxacin

However it was considerably delayed on incorporation of NOR into CA microspheres which was reflected in the $t_{50\%}$ and $t_{80\%}$ values. This may be attributed to the resistance offered by the polymer membrane (Table-2). Similar decrease in the dissolution of sulphamethoxazole, salicylic acid and theophylline[8] was reported when they were incorporated with SA and CA.

The dissolution of NOR in the buffer solution of pH 7.2 was also decreased in the order CA microsphres < SA microsphres < SA phy.mix. < SA film < CA phy.mix < plain NOR (Table-2). Though the plain drug registered a decrease in its dissolution when the pH was increased from 1.2 to 7.2, its dissolution from the microspheres was increased with increase in pH. This could be due to increased solubility of the polymers at pH 7.2 compared to pH 1.2.

When dextran was incorporated into SA and CA beads, the dissolution of it was very less in pH 1.2 solution but it was considerably increased when the pH was changed from 1.2 to 6.8. The increased solubility of dextran in pH 6.8 was predicted to be due to increased solubility of the polymer at this pH[4].

Dissolution of CIP with alginates

The dissolution of CIP from the SA preparations were decreased in buffer solution of pH 1.2 (Table-2). From the SA phy.mix., the drug dissolution was decreased probably due to the leaching of drug through the swollen polymer matrix. The dissolution from the SA film was further decreased giving rise to $t_{50\%}$ and $t_{80\%}$ values of 23.5 and 94.5 min respectively probably due to the time lag required for the swelling of the film. However, from the SA microspheres, CIP dissolved very slowly compared to the plain

drug or phy.mix. as indicated by the increased values of $t_{50\%}$ and $t_{80\%}$. Incorporation of CIP into CA microspheres further decreased the dissolution of CIP as shown by the $t_{50\%}$ and $t_{80\%}$ values viz., 172 min and < 180 min respectively.

Similar controlled release of theophylline containing alginates in the aqueous release of theophylline containing alginate microspheres in acidic pH[5] and diazepam from CA microcapsules[6] was also reported.

The dissolution of CIP in buffer solution of pH 7.2 was also considerably decreased when incorporated with alginates. The dissolution of CIP from the SA film and SA microspheres was less compared to that of the plain drug. Its dissolution from the CA microsphere was very slow compared to other formulations evaluated. The $t_{50\%}$ and $t_{80\%}$ values for various drug- alginate preparations indicated that both SA and CA could be effectively used to control the release of the drug (Table-2). The dissolution of CIP from the microspheres was more effectively controlled in a buffer of pH 1.2 compared to that of pH 7.2. This information may be useful in the preparation of controlled release formulations of CIP to release the desired amount of drug at various sites in the oral route of administration.

Diffusion data of NOR and CIP with alginates

The diffusion of NOR in buffer solution of pH 1.2 was considerably decreased by incorporating with alginates. This decrease is in the order CA microspheres < SA microspheres < SA film < SA phy.mix. < CA phy.mix < plain NOR (Table-2). This could be due to the swelling of the polymer in the diffusion medium that creates additional barrier for the diffusion of the drug across the membrane. Julian et al., (1988) reported the decreased permeation of acetaminophen in hydrated CA film.[10]

The phy.mix of CIP and SA could decrease the diffusion of CIP in pH 1.2. This is supported by increase in the diffusion $t_{50\%}$ and $t_{80\%}$ values from 40 to 125 min and 103 to >180 min respectively for the SA phy.mix. compared to the plain drug. When it was incorporated into SA film, the diffusion was further decreased. The diffusion of CIP from the SA microspheres was very less as indicated by the higher values of $t_{50\%}$ and $t_{80\%}$ (table-2). Similarly its diffusion from the CA microspheres was also effectively controlled. The diffusion of CIP from CA microspheres was less compared to SA microspheres. Hence these two polymers could effectively control both the dissolution and diffusion of CIP in buffer solution of pH 1.2.

In summary, norfloxacin and cirprofloxacin could be incorporated with sodium alginate in the form of film and microspheres. The dissolution of these drugs was considerably delayed both in buffer solutions of pH 1.2 and 7.2. The microspheres was found to be more effective than the film in controlling the drug release. These drugs could also be entrapped into calcium alginate microspheres which resulted in the delayed dissolution of these drugs. Calcium alginate is found be more effective than sodium alginate in delaying the drug release especially in pH 1.2. The stability of these drugs under various accelerated stability conditions was found to be better when they were incorporated with alginates wherein SA is found to be better than CA.

Sodium alginate is superior to calcium alginate in imparting better stability to both norfloxacin and ciprofloxacin at elevated temperatures and under exposure to direct sun light. The drug release was considerably delayed when these drugs were entrapped into the microspheres and calcium alginate was found better than sodium alginate. Thus these materials can be successfully used in the preparation of sustained release dosage forms containing norfloxacin and ciprofloxacin. This information may find its use as preformulation parameters in the preparation of sustained release dosage forms containing these drugs.

REFERENCES

1. Claude, M. Analytical profile of drug substances, 2nd Ed., Academic Press Inc., London., 1991, 557.

2. Reynolds, J.E.E., Martindal's The Extra pharmacopoeia, 28th Ed., The pharmaceutical press, London, 1982, 984.

3. Whistler, E.L. and BEMiller, J.N. Industrial gums, Polysaccharides and their derivatives, 2nd Ed. Academic press Inc., London, 1973, 619.

4. Chong, K.K. and Lee, K.J., **Int. J. Pharm.**, 1992, 79, 11.

5. Zatz, J.L. and Woodford, L., **Drug Develop. Ind. Pharm.**, 1987, 13, 2159.

6. Chowdhary K.P.R. and Babu, K.V., **Indian J. Pharm. Sci.**, 1988, 50, 173.

7. Badwan A.A., Abumalooh A., Sallam, K., Abukalaf. V and Jawan, O., **Drug Develop Ind. Pharm.**, 1985, 11, 239.

8. Forni, F. Iannucelli, V. Coppi, G and Bernabei, M.T., **Boll. Chim. Farm.**, 1990, 129, 155.

9. Aithal, K.S., Physicochemical study of drugs with various excipients in solid dosage forms. Ph.D. Thesis, University of Mangalore, 1996, 131.

10. Julian, T.N., Radebaugh, G.W. and Wisniewski, S.J., **J. Controlled Release.**, 1988, 7, 165.