IJPPR (2021), Vol. 13, Issue 3 *Research Article*

INTERNATIONAL JOURNAL OF BIOPHARMACEUTICAL & TOXICOLOGICAL RESEARCH

FORMULATION AND EVALUATION OF RELEASE BEHAVIOR OF MICROSPHERES FROM CHITOSAN ENCAPSULATING PEFlOXACIN

Yashpal Sangwan*1, Sumit Sigroha2, Ravi Kumar2, Tanuj Hooda3, Harsh Kumar3

1) Nav Chetna Institute for Pharmacy Professionals, Jind
2) Fitlife health care, Gurgaon
3) Faculty of Pharmacy, Vaish Institute of Pharmaceutical Education and Research, Rohtak, Haryana

Keywords:
Microspheres, pefloxacin, scanning electron microscopy, N-methylated chitosan.

Corresponding Author-
Yashpal sangwan
H.NO. 450 Urban Estate Near Jaat Dharamshal Jind-126102, Haryana, India
E-mail:
sangwan.yashpal@gmail.com
Contact No: +91-9812384235

ABSTRACT:
Poor solubility of chitosan at neutral pH and higher limits its application as an adsorption enhancer in the basic environment of the intestinal mucosa. The chitosan only can be dissolved in the acidic condition. To overcome these disadvantages of classical chitosan solution, it is essential to prepare chitosan derivatives which are water soluble around neutral pH. Microspheres were prepared from N-methylated chitosan by cross-linked with glutaraldehyde as the shell. The CS samples having different molecular weight were prepared by hydrolysis with 30 ml of 3 M HCl (30 ml/1 g of CS) at 60 °C. Structure and morphology was characterized with IR spectroscopy and scanning electron microscopy. Pefloxacin encapsulated in the microspheres was rapidly released into phosphate buffer solution (pH 7.4), whereas it was only slowly released in 0.1 M HCl (pH 1.2). The degree of swelling of microspheres at pH 7.4 was higher than that at pH 1.2. The release mechanism of the microsphere was proposed to be non-fickan diffusion through the swollen microspheres, and to be controlled by the Mw and the cross-linking density of shell. The current study explains about the release behavior of encapsulated drug.

Introduction:

Pefloxacin is a synthetic chemotherapeutic agent used to treat life threatening bacterial infections. Pefloxacin was developed in 1979 (German Patent Roger Bellon /Dainippon). It was approved in France for human use in 1985. Pefloxacin is commonly referred to as a fluoroquinolone (or quinolone) drug and is a member of the fluoroquinolone class of antibacterial. It is an analog of norfloxacin. It is a synthetic fluoroquinolone, belonging to the 3rd generation of quinolones. Pefloxacin is extensively prescribed in France [1]. Recently, microspheres have attracted great attention because of a variety of applications such as delivery vesicles for drugs, DNA, antigens, and protection proteins and enzymes, especially for controlled drug-delivering systems employing bio-polymers as raw material [2-8]. Usually, drugs are encapsulated to mask taste and odour, to stabilize the quality of the drug, to improve glutaraldehyde gastrointestinal (GI) tolerance and to provide

sustained release after oral administration. Chitin, obtained from lobster, shrimp and crab shell waste, is the second most abundant polysaccharide found in nature. In the 21st century, chitin and its derivative (chitosan) face new opportunities to contribute functional materials and environmentally friendly materials to meet the diverse needs of today's society because of their nontoxic, biodegradable, biocompatible, antibacterial, etc. Therefore, chitosan has been extensively used in medical and pharmaceutical areas, as it would be advantageous to use them as drug formulations [9-10].

Experimental Methods

Reagents and chemicals

Pefloxacin (Pelox, i.v. Injection, 400 mg/100 ml 5% dextrose solution) were purchased from Glenmark (Majesta) Pharmaceuticals and Wockhardt Pharmaceuticals, India respectively. Fluoresceine isothiocyanate (FITC) was procured from Himedia, Mumbai, India. ELISA kit (Specific anti-rat IgG) and Freund's incomplete adjuvant were purchased from Banglore Genni, India. Collagenasewas obtained as a gift sample from Central Leather Research Institute, Chennai, India. All other chemicals used were of analytical grade. Chitosan (case sensitive with chitosan) having molecular weight of 13.44 • 104 and 93% degree of deacetylation was purchased from purex laboratories (i) pvt. Ltd. - Exporter, Manufacturer & Supplier of Chitosan based in Bangalore, India.

Preparation of Microsphere

Chitin is isolated from shells of crustaceans (for example shrimp, crab and lobster) by treating shells with 2.5 NaOH at 75°C and with 1.7 HCl at room temperature for 6 hours11. Deacetylation can be done by alkaline treatment or by enzymatic reaction. The alkaline deacetylation is carried out by treating chitin with NaOH at high temperature. The degree of deacetylation increases with increasing temperature or NaOH concentration. Water soluble half N-acetylated chitosan samples were obtained by N-acetylation with acetic anhydride. The water-soluble half N-acetylated chitosan and chitooligomer had no significant antimicrobial activity. Moreover, water-soluble chitosan and chitooligomer promoted the growth of C. albicans. In contrast water-insoluble chitosan in acidic medium exhibited inhibitory effect against these microorganisms. The water-insoluble chitosan with molecular weight around 5×104 were the optimum for antimicrobial action in these tested samples. The antimicrobial mechanism of dissolved water-insoluble chitosan was hypothesized as forming an impervious layer around the cell. The results suggest that optimum chitosan as food preservative should be water-insoluble chitosan from mild depolymerization of native chitosan [12].

The CS samples having different molecular weight were prepared by hydrolysis with 30 ml of 3 M HCl (30 ml/1 g of CS) at 60 °C. By controlling the hydrolysis time to 0, 4, 8, and 12 h, four samples (coded as 0–3) were obtained. The hydrolysed samples were dialyzed against running water for 4 days, and then centrifuged 14.47g for 20 min. The resultant precipitates were washed several times with distilled water and acetone, and then vacuum- dried at 40 °C. N-Methylated chitosan (NMC) was prepared according to above method [13-14]. The portion of NMC was dissolved in distilled water and cyclohexane was added as the oil phase and 15% pefloxacine acetic acid solution was added after the addition of aqueous glutaraldehyde. The resultant mixture was stirred at 57 g and 40 °C for 1 h to form oil-in-water (O/W) emulsion. After addition of 27 % w/w aqueous glutaraldehyde aqueous (0.6 ml), the system was stirred at 12 g for 30 min to yield the emulsion droplets covered with cross linked NMC and pefloxacine. The resulting products were precipitated by coagulating with acetone. The precipitated products prepared from NMC 0–3 were coded as NMGF 0–3, respectively.

Microsphere Characterization

Surface morphology

Microsphere shape and appearance were evaluated by scanning electron microscopy (SEM) (Scanning Electron Microscope Jeol JX 840-A JEOL Ltd, Tokyo, Japan). Samples for SEM analysis were prepared by gold-sputtering the microspheres in an argon atmosphere.

Size distribution

Particle size analysis was performed by the light diffraction method with a Coulter apparatus, model LS230 (Coulter Corp, Hialeah, FL); this instrument works on laser diffraction optics and on another system based on polarized light of 3 wavelengths termed PIDS. The size range of the LS230 version is from 0.04 μm to 2000 μm. The samples of

microspheres were suspended in filtered water, sonicated for 30 seconds, and subsequently analyzed. Three analyses were performed for each sample of microspheres.

Swelling Test

The dry microspheres encapsulated with pefloxacine (25mg) were immersed in phosphate buffer solution and the mixture was diluted to 1000 ml (pH 7.4) for 48 h at room temperature until equilibrium of swelling had been reached. The swollen samples were collected by centrifugation, and then blotted with filter paper to remove the water on the surface, and immediately weighed and the degree of swelling (SW) was calculated using the following equation:

$$Sw\ (Wt\ \%) = \{(W-W0)/W0\} \times 100\%$$

Where the W and W0 are the weight of the microsphere at the equilibrium swelling state and at the dry state respectively.

Drug Release Study

Currently, the most common methods used to study drug release from microspheres are the sample and separate and the dialysis methods. The more conventional method is the sample and separate method, often referred to as the tube method, in which drug-loaded microspheres are introduced into a sealed tube or vial or a stoppered Erlenmeyer flask containing buffer, and release is followed over a specified time [15-20].

The release results were analyzed by using an empirical equation as follows

$$Mt/M = ktn$$

Where Mt/M is the amount of the release pefloxacin (%) at time (h).

Release Behavior of Drug

In vitro drug release

In vitro release tests were carried out by a dialysis method: 30 mg of microspheres were placed into dialysis tubs (cutoff 12 000-14 000 D). They were then were poured in 5 ml of water in bottles closed by screw stoppers at 37°C and shaken twice a day. At fixed time intervals, 1 ml of release medium was removed and replaced with 1 ml of fresh water to maintain sink conditions [21-22].

In vitro release behavior of Pefloxacin from the microspheres with different Mw at pH 7.4 and 1.2 media was done. At pH 7.4, pefloxacine releases more slowly from the microspheres having high Mw of NMC than from that of low Mw for the same release time period. Interestingly, pefloxacin releases more rapidly at pH 7.4 than at pH 1.2, the release half times t50 for NMGF0, NMGF2, and NMGF3 are 2.8, 1.8, and 1.7 h at pH 7.4, and 6.0, 5.0 and 4.4 h at pH 1.2, respectively. It was observed that pefloxacin in therapeutic dose-dosage regimen (Pefloxacin 6.66 mg.kg−1 (BID, i.m.) 7 days Following medication (rats were medicated for 7 days and then immunized) did not reduce the anti-BSA antibody titre ($P>0.05$) in rats i.e. following medication (FM), during medication (DM) and after seven days of medication (ASDM) in comparison of control group (CONTR; GM 6). Hence, the data manifest that pefloxacin does not modulate the humoral immune response in rats elicited by BSA-loaded GM (GM6). Pefloxacin does not affect the humoral immune response triggered by EV vaccine fraction 1 antigen. Moreover, pefloxacin does not augment the IL-2 level. Therefore, pefloxacin does not interfere with the generation of instructive immune response [23].

Result and Discussion

From the SEM images of the microspheres of NMGF0 (Figure1), before releasing, exhibit smooth surfaces, most of the microspheres become smaller. The size of the sample microspheres in pH 1.2 medium is larger than that of its counterpart in pH 7.4; this suggests the cross-linked shell is more stabilized at pH 1.2 than in pH 7.4 media.

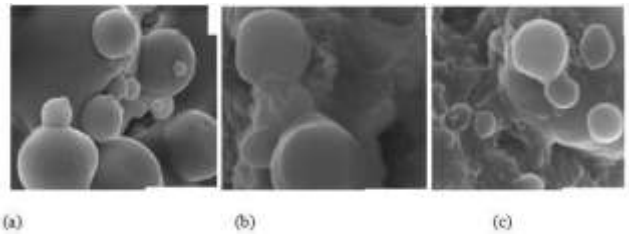

Figure 1. SEM images of the NMGF0 microspheres, before releasing (a), after releasing by pH 1.2 medium (b) and by pH 7.4 medium.

As deduced from the SEM images of the microspheres of NMGF0 (Fig. 2) the NMGF0 microspheres, before releasing, exhibit smooth surfaces; this indicates that the cross-linked NMC has formed a tight exterior shell because the microspheres are formed by the surface

fabrication, and ofloxacin has been encapsulated inside the cross-linked shell of the microspheres, as shown in Figure 1a. The size of the microspheres lies in the range from 2 to 14 lm. After releasing, the microspheres remain spherical shapes, and have smooth surfaces. However, most of the microspheres become smaller, as shown in Figures. 1b and c.

Swelling ratio

Dependence of the degree of swelling on the Mw of NMC samples and pH of the medium for the microsphere is shown in the figure 2. The microspheres in pH 1.2 have a lower percentage swelling degree than those in phosphate buffer pH 7.4. Therefore, the swelling ability of the microspheres is weakened in an acid environment. This can be explained in terms of the electrostatic interaction between the cross-linked NMC and the –COOH groups of pefloxacine, and the Schiff‟s base of the cross-linked network, as a result of the reaction between the –CHO groups and the –NHCH3 groups of NMC samples, is easily destroyed in pH 7.4 medium. With an increase of the Mw of NMC sample concentration, the swelling degree of the microspheres decreases, because the microspheres with high Mw have a higher density of shell, leading to a decrease of solvent resistance.

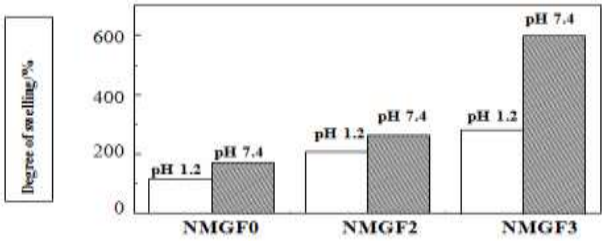

Figure 2. Degree of swelling for microspheres with different Mw of NMC samples at pH 7.4 and 1.2 conditions.

Conclusion

The encapsulated pefloxacin was quickly released in a –HCl buffer (pH 7.2), whereas a small amount of pefloxacin was released under acid conditions (pH 1.2) because of the strong electrostatic interaction between -NH2 groups of NM-chitosan and -COOH groups of pefloxacin. A series of microspheres encapsulated with pefloxacine were prepared from NMC having different Mw. The degree of swelling of microspheres in phosphate buffer solution, pH 7.4, was much higher than that in 0.1 M HCl (pH 1.2), and decreased with an increase of Mw of the NMC ofloxacin in the microspheres was more speedily released at pH 7.4 than at pH 1.2. In addition, release speed of pefloxacine in the microspheres having high degree of quaternization of NMC was higher than that with low degree of quaternization. The pefloxacin release mechanism was a kind of non-fickian diffusion through the swollen macrostructure of microspheres and was controlled by the Mw. At pH 7.4, a large amount of pefloxacin was released from the microspheres in a short time because of the rapid swelling of the microspheres. However, the release only depended on the diffusion of pefloxacin at relatively low pHs, this resulted in a relatively low release.

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