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Research Article

FORMULATION AND *IN VITRO* EVALUATION OF SODIUM ALGINATE NANOSPHERES CONTAINING OFLOXACIN

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ABSTRACT

The aim of present work was to formulate sodium alginate nanospheres of Ofloxacin by controlled gellification method and to evaluate its *invitro* release characteristics. The particle size analysis was carried out by scanning electron microscopy and the size range was found to be 656.6 ± 0.28 nm. The carrier capacity of sodium alginate was evaluated in terms of drug to polymer ratio and the maximum drug loading was 33.2% for the batch 20mcg/ml. And where there was an increase in drug concentration the drug loading capacity was reduced.

In vitro release studies were carried out on all drug loaded batches through dialysis method and release kinetics was also determined. The study revealed that the release of drug from the nanospheres followed Fickian diffusion with acceptable release**.** The formulated Ofloxacin nanospheres could be a possible approach to treat bacterial infection.

Keywords: Nanospheres, Sodium Alginate, Ofloxacin, Controlled Gellification, *In Vitro* Release.

INTRODUCTION

Nanotechnology by manipulation of characteristics of materials such as polymers and fabrication of nanostructures is able to provide superior drug delivery systems for better management and treatment of diseases. The nanostructures employed as drug delivery systems have multiple advantages which make them superior to conventional delivery systems [1, 2].

For a large majority of pharmaceutical formulations in contemporary use, their 'specificity' towards appropriate sites of a disease is not based on their ability to accumulate selectively in the target organ or tissue. Usually, they are more or less evenly distributed within the body. Moreover, to reach the target area, the drug has to cross-different biological barriers such as organs, cells, even intracellular compartments, where it can cause undesirable side effects, or be partially inactivated. The best solution to this issue is drug targeting [3].

The drug Ofloxacin selected for our study is a synthetic chemotherapeutic second generation fluoroquinolone antibiotic used to treat pneumonia and bronchitis caused by *Haemophilus influenzae* and *Streptococcus pneumoniae*. It is also used in treating skin infections caused by *staphylococcus aureus,* and *streptococcus pyogenes* bacteria and also used to treat sexually transmitted diseases, such as gonorrhea and chlamydia, but is not effective against syphilis. It is often used to treat urinary infections and prostate infections caused by E. coli. Some strains of *streptococcus*, *enterococcus*, and anaerobic bacteria are resistant to Ofloxacin.

Even though the drug having wider application it is associated with substantial number of serious adverse drug reactions, such as tendon damage (including spontaneous tendon ruptures) and peripheral neuropathy (which may be irreversible); such reactions may manifest long after therapy had been completed, and, in severe cases, may result in life-long disabilities. Ofloxacin has also been associated with severe psychiatric adverse reactions. Hepatotoxicity has also been reported with the use of Ofloxacin[6]. To reduce the above mentioned adverse effects incorporation of the selected drug in to the polymer in the form of nanoparticles could be a possible approach. So we have selected a natural bio-polymer for our study, as the use of natural biopolymers specifically polysaccharides in drug delivery has a particular interest due to their desirable biocompatible, biodegradable, hydrophilic and protective properties. Entrapment in biopolymers of therapeutic agents including: peptides, proteins and polynucleotides have been shown to maintain their structure and activity and protect them from enzymatic degradation. Moreover, many of these polymers, particularly hydrogels, are naturally hydrophilic, which is advantageous since this property is thought to contribute to longer *invivo* circulation times and allows encapsulation of water-soluble biomolecules [4]. Alginate is an anionic polysaccharide consisting of linear copolymers of α-L-guluronate and β-D-mannuronate residues. Alginates which are a group of hemo-compatible polymers have not been found to accumulate in any major organs and have shown evidence of *in vivo* degradation[7]**.** In the presence of Calcium ions, ionic interactions between the divalent Calcium ions and the glucuronic acid residues cause Alginates to form gels. The properties of Calcium-Alginate nanospheres make them one of the most widely used carriers for controlled release systems. Coating of these nanospheres with other polymers has been shown to improve their stability during (shelf-life) storage and their half-life in biological fluids [5]. Based upon all the above discussions we have planned to formulate sodium alginate nanospheres containing Ofloxacin and evaluate its physiochemical properties, exploring alternative routes of administration like nanoparticle to develop a targeted drug delivery system and to act locally on the organ of infection with enriched therapeutic efficacy and abridged side effects thereby providing patient compliance.

MATERIALS AND METHODS

Materials

Ofloxacin was received as a gift sample from Tanmed Pharmaceutical, Chennai. Sodium alginate was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Poly-L-lysine and calcium chloride were purchased from S.D. Fine-Chem. Ltd., Mumbai, India. All other ingredients were of laboratory grade.

Method of preparation

Sodium alginate nanospheres containing Ofloxacin were prepared by controlled gellification method reported by Rajaonarivony et al [7]. 2ml of calcium chloride (18mM) was added to 38ml of sodium alginate solution (0.1%w/v) to induce gellification. Then 16ml of poly-l-lysine (0.1%w/v) was added to form a polyelectrolyte complex [8]. The nanospheres suspension obtained was stirred for 2 h and kept overnight for stabilization. The nanospheres were separated by High Speed Lab Centrifuge (ACMAS TECHNOCRACY PVT LTD) at 20000 rpm for 45 minutes and dried under vacuum to

form a flaky mass, which on re-dispersion in sterile water for injection produced discrete particles[9, 10].

Determination of particle size

The nanospheres were spread over a glass slide and dried under vacuum at room temperature (25ºC). The sample was shadowed in a cathodic evaporator with a gold layer 20nm thick. The diameters of all the spheres in each field were calculated using a JSM-6400 scanning electron microscope (Tokyo, Japan).[11]

Study on drug to polymer ratio

To determine the drug: polymer ratio[12], five batches of nanospheres containing various concentrations of drug were prepared. In each batch the concentration of drug was varied, while other processing variables were kept constant. Ofloxacin equivalent to 10µg/ml was dissolved in 38ml of sodium alginate solution (0.1%). Then 2 ml of calcium chloride (18mM) and 16ml of poly-l-lysine (0.1%) was added, stirred for 2 h and kept overnight. The resulting nanospheres were separated at 20000 rpm by ultracentrifugation. The nanospheres were dried under vacuum. This batch coded OFA-I. Similarly OFA-II, OFA-III, OFA-IV and OFA-V were prepared, containing 20, 30, 40 and 50µg/ml of Ofloxacin, respectively. The batches were subjected to particle size analysis by SEM [13, 14].

Determinations of drug content

An ultracentrifugation technique was used to separate the free drug from nanospheres and to estimate the drug loading of the nanospheres. A quantity of drug loaded nanospheres from each batch equivalent to 1mg was added to 50ml of normal saline and stirred continuously for 2 hrs and then the final colloidal suspensions were ultra- centrifuged at 10000 rpm at 22 ± 2 ° C for 0.5 h. The supernatant was analyzed for drug content by measuring the absorbance at 291 nm using UV spectrophotometer[15].

In vitro release studies

The drug release assessment was carried out following the procedure reported earlier, which employed a diffusion cell. A Sigma dialysis membrane was fixed to one end of a permeation cell [16]. The nanospheres were placed in the cell donor compartment and the cell was immersed in a beaker containing 50ml of phosphate buffer (pH 7.4) which serve as a receptor compartment. The cell was immersed to a depth of 1cm below the surface of the receptor solvent. The medium in the receptor compartment was agitated using a magnetic stirrer and temperature of 37 ± 10C. 5ml of the sample from the receptor compartment was taken at various intervals of time over a period of 24 h and each time replaced with fresh buffer. The samples withdrawn were estimated spectrophotometrically at 291nm [17].

RESULTS

Preparation of nanospheres by controlled gellification involves formation of calcium alginate complexes. The interaction between the calcium ions and the alginate polymer transpired at the level of the oligopolyglucuronic sequences. Furthermore, calcium ions induced a parallel packing of the oligopolyglucuronic sequences to give egg-box structures and the addition of poly-l-lysine allowed only strengthening of this system to obtain small and well defined particles. The obtained nanospheres were spherical and discrete with an average particle size of 656.6 ± 0.28 nm. (Fig-1)

Drug-loading capacity[18, 19]

The drug carrier capacity of sodium alginate with respect to Ofloxacin was determined assessing the drug: polymer ratio. As Table 1 shows, the drug loading capacity was 33.2% at 20mcg/ml and reduces further on increase with drug concentration. Thus, it can be said that the saturation capacity of the polymer with respect to the selected drug occurred at a relatively lower concentration and at a faster rate. (Table-1)

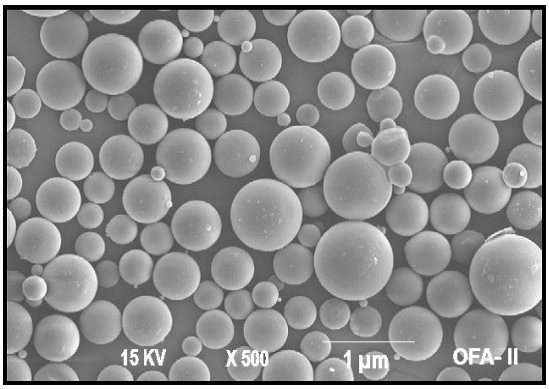

Fig. 1: Scanning Electron Micrograph of Sodium Alginate Nanospheres Containing Ofloxacin

Table 1: Ofloxacin loading efficiency of sodium alginate nanospheres

S. No.	Formulation code	Drug concentration (mcg/ml)	Drug loading (%)
1.	OFA I	10	13.4 ± 0.2
2.	OFA II	20	33.2 ± 0.6
3.	OFA III	30	28.3 ± 0.8
4.	OFA IV	40	26.1 ± 0.4
5.	OFA V	50	25.6 ± 0.7

Release kinetic analysis[20]

The *in vitro* study of all the five batches showed an initial burst release of 15 to 19% for all the batches within 30 min and 78 to 83% of the drug was released in a slow manner over 24 h. The batch OFA II showed about 96% of release at the end of 24hrs, which was found to be more when compared to other batches. All the batches showed a good sustained release of drug.

In order to predict and correlate the release behavior of the drug from the hydrophilic matrix it is necessary to fit it to a suitable model. Hence, the release data were fitted according to the well-known exponential equation often used to describe the drug release behavior from polymeric system.

$$m_t / m_\infty = kt^n \quad \text{----------(1)}$$

Where m_t/m_∞ is the fractional release of the drug 't' is the release time, *'k'* is a constant which indicates the properties of the macromolecular polymeric system and n is the release exponent indicative of the mechanism of release. The 'n' values used for analysis of the drug release mechanism from the Ofloxacin nanospheres were determined from log (m_t/m_∞) vs log (t) plots. To calculate the release constant, k, the logarithm of the remaining Ofloxacin in nanospheres plotted versus time. The release of drug from nanospheres followed first order kinetics over a 24 h period. Drug loading is another factor that influenced the drug release rate from the nanospheres. Generally, the drug loading is 33.2% at 20mcg/ml and reduces on increase in concentration of the drug. The values of 'k', 'n' and 'r' for five different batches are reported in Table, and the 'n' value was within 0.5132 to 0.5583. The results of kinetic analysis reveal that the release of Ofloxacin B from sodium alginate nanospheres followed Fickian diffusion. (Table-2)(Fig-2)

Table 2: *In vitro* release kinetics of Ofloxacin- loaded nanospheres

S. No.	Formulation code	Drug concentration (mcg/ml)	First order plot		Peppa's	
			$k \times 10^{-3}$	r	n	r
1.	OFA I	10	2.6357	0.9951	0.5199	0.9447
2.	OFA II	20	2.2236	0.9957	0.5583	0.9404
3.	OFA III	30	2.8735	0.9708	0.5223	0.9131
4.	OFA IV	40	3.5046	0.9926	0.5437	0.9322
5.	OFA V	50	2.8368	0.9956	0.5132	0.9265

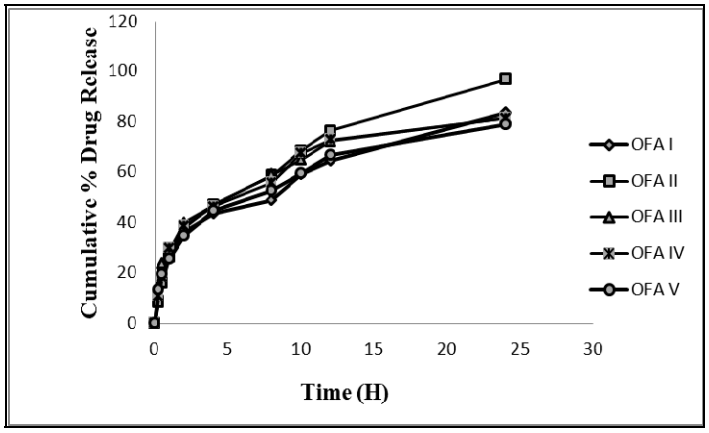

Fig 2: *In vitro* release profiles of Ofloxacin from different batches of drug loaded sodiumalginate nanospheres. (♦)OFA I-10µg/ml, (■) OFA II-20µg/ml, (▲) OFA III-30µg/ml, (×) OFA IV- 40µg/ml and (●) OFA- V-50µg/ml

DISCUSSION

Sodium alginate nanospheres containing Ofloxacin were successfully formulated by controlled gellification method. The formulated sodium alginate nanospheres of Ofloxacin were found to be an effective natural carrier in terms of discrete particle size, optimum drug loading capacity, satisfactory *in vitro* release characteristics. It could also be an ultimate route of administration in case of bacterial infection. Further *in vivo* studies can prove to have enriched drug release and abridge side effects, and also its ability to act as a passive targeting.

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