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Research Article

DEVELOPMENT OF CROSS-LINKED CHITOSAN MICROSPHERES FOR PROLONGED DELIVERY OF MACROMOLECULAR DRUGS

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ABSTRACT

The present investigation was designed to prepare stable chitosan microsphere capable of releasing macromolecular drugs for periods ranging from days to months. The prepared chitosan microsheres were evaluated for the shape, particle size, and size distribution. The chitosan microspheres were formulated by glutraldehyde cross linking of an aqueous acetic acid dispersion of chitosan in a non-aqueous dispersion medium consisting of tolune and span 80 used as emulsifying agent. The procedure for preparing chitosan microspheres was standardized by adjusting various parameters like concentration of chitosan gel, volume and concentration of cross linking agent, cross linking time and stirrer speed. The chitosan microspheres were smooth and spherical without aggregation. The particle size range was 1- 20µm. The concentration of the chitosan polymer was found to influence the size of the chitosan microspheres. The glutraldehyde cross-linked chitosan microsphere can be an alternative for the prolonged delivery of macromolecular drugs.

INTRODUCTION

The Macromolecular drugs include polypeptides, hormones, polysaccharides, antigens, antibodies and similar compounds. In controlled release technology, biodegradable polymeric carriers offer potential advantages for the sustained release of macromolecular drugs[1-3]. A number of biodegradable polymeric carriers have been investigated for the controlled release technologies. The biodegradable polymers include poly(lactic acid), poly(E-caprolactone), poly(anhydrides), poly(lactic acid-co-glycolic acid) and poly(ortho esters). Some of the natural polymers such as proteins and polysaccharides have also been investigated as potential drug carriers[4-6]. Sustained release of macromolecular drugs from polymeric matrices has received increasing attention in recent years. Macromolecular drugs like vaccines are required to be given in multiple divided doses. For a vaccine to be effective, it requires two or three booster doses after primary immunization. The efficiency of vaccine is reduced if only one dose is given without boosting. In developing countries like India, the drop–out rate of individuals receiving the first dose but not the successive doses is high. Therefore the conversion of multiple dose vaccines into single dose vaccines is more effective and more appropriate[7]. When WHO commenced the children's vaccine initiative in 1990, one of the goals was to support the development of vaccine for single administration based on biodegradable microspheres of poly(lactic- co-glycolic acid)[8-9]. In the present study we have tried to develop a stable chitosan microspheres by optimising various parameters such as concentration and volume of chitosan gel and cross linking agent, stirrer speed and crosslinking time. Chitosan is a biodegradable

non toxic and cheap polymer[10-11]. Microspheres have good spherical geometry and smooth surface and prepared by crosslinking chitosan from a acetic acid dispersion. Preliminary evaluation of the particle size distribution and spherical geometry, sterilisation and swelling properties of microspheres have been carried out.

MATERIALS AND METHODS

Chitosan was obtained from Central Institute of Fisheries and Technology, Cochin, India and used without further purification. sodium chloride, acetic acid, toluene, acetone, Span-80, hydrochloric acid, all analytical grade were obtained E-MERK, Glutraldehyde 50% (biological grade), from Sigma, Chemical Company, St. Louis, USA.

The chitosan microspheres were prepared by emulsion crosslinking method. The microsphere preparation were standardised by optimising various parameters like concentration and volume of chitosan gel and cross linking agent, stirrer speed, crosslinking time.

The chitosan microspheres were prepared following the techniques reported earlier with minor modifications[12-15]. The optimised procedure is as follows. Briefly, 1% (w/v) aqueous solution of chitosan was prepared in 3% (w/v) acetic acid containing 2% (w/v) sodium chloride. The resulting solution was stirred for one hour at 3000 rpm to form a gel and was kept overnight for stabilization. Glutaraldehyde Saturated Toluene (GST) was prepared by adding equal volume of glutaraldehyde and toluene and then stirred at 2000 rpm and kept overnight for stabilization. One ml of 1% chitosan gel is dissolved in equal volume of 0.01N HCL and to this added 50 ml of toluene containing 5 ml of span 80 and stirred at 2000 rpm for one hour. To the above emulsion 10 ml of GST is added dropwise and stirred for 4 hours. The product is centrifuged and washed thrice with toluene and acetone. The microsphere is dried at 37^{0}C and collected as free flowing powder.The procedure is repeated in similar manner for 2% (w/v) chitosan gel.

Sterilization of Chitosan microspheres

The chitosan microspheres prepared are subjected to sterilization, if it is required to be given by injection. The microspheres were sterilized by dry heat at 80^{o}C for 16 hours as reported by Jameela and co-workers.

Swelling Properties

The 100 mg of the chitosan microspheres were dispersed in 100 ml of phosphate buffer of p^H 7.4 in a conical flask and incubated at 37^{o}C for 24 hours. The microspheres were then centrifuged and separated. The collected microspheres were dried and analyzed under SEM for the change in surface characteristic.

RESULTS AND DISCUSSIONS

The chitosan microspheres were prepared by emulsion cross-linking method. The preformulation studies was performed by adjusting various parameters like, stirrer speed, cross linking time, volume and concentration of cross linking agent and cross linking time etc.(Table 1 to 5)

The Microspheres were highly spherical in appearance as seen in SEM (Figure 1). The surface of chitosan microspheres was smooth without aggregation. All the microspheres were below 20 µm in diameter. The average particle size of chitosan microshere was found to be 19 µm as seen in the particle size distribution analysis (Figure 2). The chitosan microspheres were found to be free flowing and could dispersed in water without aggregation.

The viscosity of chitosan gel was found to influence the size of chitosan microspheres. The average particle size of chitosan microsphere prepared from 1% chitosan gel was found to be 17 µm and that of the 2% chitosan gel was found to be 19 µm. This may be due to the increased viscosity of chitosan gel which contributes for the increased size. The chitosan polymer after sterilization did not show any changes in the surface morphology (Figure 3). The chitosan microspheres swell rapidly in aqueous dispersion (Figure 4). After swelling it showed increased pore size and can be used for incorporating macro molecular drugs for prolonged release.

The Chitosan polymer is a biocompatible and naturally occurring polysaccharide, which is biodegradable by the action of lysozyme. The major drawback with chitosan polymer is its insolubility in water and in common organic solvent. This can be advantageous that the water soluble impurities can be excluded. Although chitosan of commercial grade often comes with different degree of deacetylation, it can be acetylated or deacetylated to the desired degree[16]. The chitosan is susceptible to cross linking by dialdehydes such as

glutaraldehyde and such cross linking effectively controls drug diffusion from the matrix.

Table 1: Effect of polymer concentration on microsphere formation

S. No.	Polymer concentration	Average particle size
1	1%	17µm
2	2%	19µm
3	0.50%	no stable microspheres are formed

Table 2: Effect of volume of emulsifying agent

S. No.	Percentage of emulsifying agent	Result
1	8%	no stable microspheres
2	10%	stable microspheres without aggregation
3	12%	aggregation of microspheres is seen

Table 3: Effect of stirring rate

S. No.	Stirring Rate	Result
1	1500	no stable microspheres formed
2	2000	stable microspheres without aggregation
3	2500	aggregation of microspheres

Table 4: Effect of cross- linking time on microspheres formation

S. No.	Cross- linking time in hours	Result
1	3.5	no stable microspheres formed
2	4	stable microsheres without aggregation
3	4.5	aggregation of microspheres

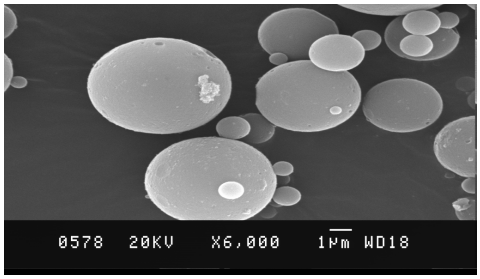
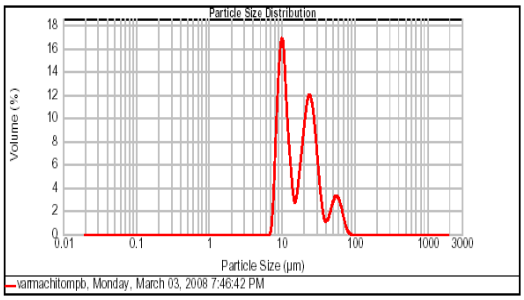
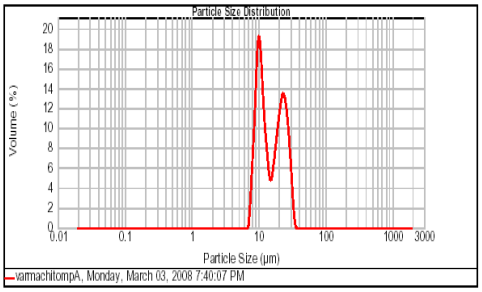


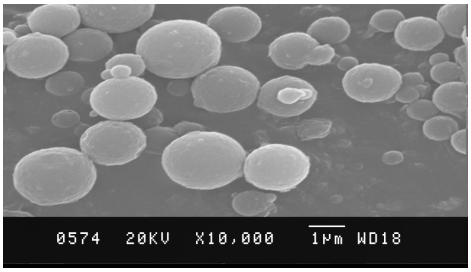
Fig. 1: Smooth spherical chitosan microspheres seen under SEM



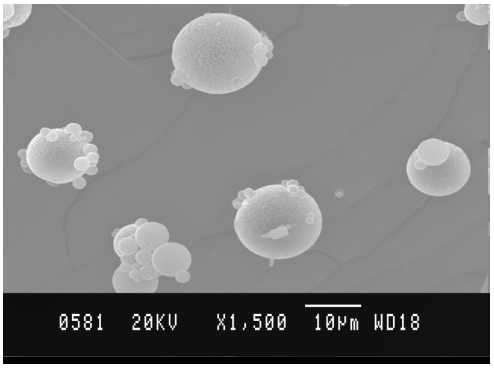
A. Particle size distribution analysis of microspheres with 1% gel



B. Particle size distribution analysis of microspheres with 2% gel (Fig. 2)



Sterilised chitosan microspheres showing no change in surface characteristic seen under SEM (Fig.3)



Swelling of chitosan microspheres showing increased pore size seen under SEM (Fig.4)

Table 5: Effect of concentration and volume of cross linking agent

Concentration of cross linking agent	Volume of cross- linking agent			
	20ml	15ml	10ml	5ml
100%	aggregation of microspheres	aggregation	aggregation	no stable microspheres formed
50%	aggregation	aggregation	stable microsphere without aggregation	no stable microspheres formed
25%	no stable microspheres formed	no stable microspheres formed	no stable microspheres formed	no stable microspheres formed

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