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FORMULATION AND EVALUATION OF CROSS LINKED CHITOSAN MICROSPHERES CONTAINING MITOMYCIN-C

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ABSTRACT

The purpose of this research was to formulate and systematically evaluate in vitro performances of chitosan microspheres containing Mitomycin-C. Chitosan microspheres containing Mitomycin-C were prepared by simple emulsification phase separation technique using glutaraldehyde as a cross-linking agent. Results of preliminary trials indicate that volume of cross-linking agent, time for cross-linking, polymer-to-drug ratio, and speed of rotation affected characteristics of microspheres. Microspheres were discrete, spherical, and free flowing. The microspheres exhibited good in vitro wash-off test and also showed high percentage drug entrapment efficiency. The best batch exhibited a high drug entrapment efficiency of 75% and a swelling index is 1.42. The drug release was also sustained for more than 12 hours. The polymer-to-drug ratio had a more significant effect on the dependent variables. The effect of chitosan concentration, cross linking agents and conditions were evaluated with respect to entrapment efficiency, particle size, surface characteristics and in vitro release behaviours.

KEY WORDS

Microspheres, Drug delivery, Targeting, Drug release, Chitosan.

INTRODUCTION

Oral controlled release (CR) dosage forms (DFs) have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. Microspheres carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems[1-3]. They have varied applications and are prepared using assorted polymers[4]. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes[5-8]. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site[9-12]. Chitosan (obtained by deacetylation of chitin) is a cationic polymer that has been proposed for use in microsphere systems by a number of authors[13-17]. Chitosan was selected as a polymer in the preparation of mucoadhesive microspheres because of its good muco adhesive and biodegradable properties. Hence, there is a need to develop an oral drug delivery system that is convenient for patients. Various synthetic and natural polymers like alginate, chitosan and polyesters have been used to develop drug delivery systems for entrapping and delivering drugs orally[18]. The objective of the present investigation was to develop an extended and controlled release composition and formulation of Mitomycin C using chitosan polymer which otherwise demands prolonged chemotherapy and to identify the modulation of drug release from the formulated matrix devices and demonstrate its utility in pharmaceutical drug carrier systems.

MATERIALS

Chitosan was obtained from Shreeji laboratories, Mumbai. Mitomycin-C was obtained from Glenmark (Mumbai, India). Glutaraldehyde was obtained from Orchid Health care pvt Ltd. All other reagents used were analytical grade.

METHODS

Preparation of microspheres

Chitosan solutions of varying concentrations of 0.5%, 1%, 1.5%, 2% solutions were prepared by dissolving them in dilute acetic acid (1% v/v). The core material Mitomycin-C (10mg) dispersed with the aqueous phase (chitosan solution) in a homogenizer at 5000 rpm for 20min. This solution was added to the liquid paraffin to form water in oil (W/O) emulsion. The dispersion was stirred at 710 rpm for 30 minutes after the addition of glutaraldehyde solution. After allowing cross linking for varying time, microspheres were washed with distilled water repeatedly and vacuum dried for 12 h. Four different formulations are prepared and they are coded as F1, F2 and F3, F4.

***Particle size measurement*[19]**
The size of the prepared microspheres was measured by the optical microscopy method using a calibrated stage micrometer for randomly selected samples of all the formulations.

Surface morphology of microspheres
Scanning electron microscopy of the chitosan microspheres was carried out to examine the surface morphology. The microspheres were mounted on metal stubs and then coated with a 150 Å layer of gold. Photographs were taken using Jeol Scanning Electron Microscope (Jeol. JSM-5610LV SEM).

In vitro drug release
Drug release from the microspheres was determined using phosphate buffer pH 7.4 as the release medium. Microspheres were suspended in 50 ml of the dissolution medium in a 100 ml glass vials and stirred on a magnetic stirrer at 100 rpm in a thermo stated bath at 37^{o}C. 2 ml samples were withdrawn at appropriate time intervals and centrifuged at 5000 rpm. Supernatants were diluted suitably and absorbance of the resulting solution was measured at 365 nm using the dissolution medium as blank. The residue was re dispersed in 2 ml of the fresh dissolution medium and replaced back into the vial.

Determination of drug content
Practical drug content was determined by taking a weighed quantity of chitosan microspheres (approximately 100 mg) in a 100ml volumetric flask. Sufficient quantity of water was added to make the volume 100 ml. The suspension was shaken vigorously and then left for 24 hours at room temperature with intermittent shaking. Supernatant was collected by centrifugation and drug content in supernatant was determined by UV spectrophotometry at suitable wavelength (365 nm)using a shimadzu UV visible spectrophotometer (SHIMADZU, Spectrascan-2200, Japan).

***Determination of percentage drug entrapment (PDE)*[19]**
Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula:

$$PDE = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Theoretical drug content was determined by calculation assuming that the entire drug present in the chitosan solution used, gets entrapped in microspheres and no loss occurs at any stage of preparation of microspheres.

RESULT AND DISCUSSION

The present study was taken to formulate and evaluate sustained release microspheres of Mitomycin C by simple emulsification and phase separation technique. Various batches were made and these formulations are shown in table 1. Cross-linking with glutaraldehyde is reported to produce greater number and more stable cross-links. This may be the reason of the smaller particle size obtained for the glutaraldehyde cross-linked microspheres. This is also reflected in the in-vitro drug release study which shows significantly slower drug release rates from microspheres cross-linked with glutaraldehyde. Four batches prepared with different polymer ratios were evaluated for physical properties like SEM, particle size, percentage drug content, in vitro dissolution of mitomycin c microspheres were tabulated in table 2&3 and figures are shown in figure 1,2&3.

Table no.1

Formulation of chitosan microspheres containing Mitomycin c

Content	F1	F2	F3	F4
Mitomycin C	10	10	10	10
Chitosan	0.5%	1.0%	1.5%	2.0%
Acetic acid	1%	1%	1%	1%
Glutaraldehyde	30ml	30ml	30ml	30ml

Table no.2

Particle size, Drug content, Entrapment efficiency of Mitomycin C microspheres

Parameter	F1	F2	F3	F4
Particle size	98±4.8	96±4.2	95±5	110±5.2
Drug content(%)	57	61	68	79
Entrapment efficiency(%)	57	57	65	79

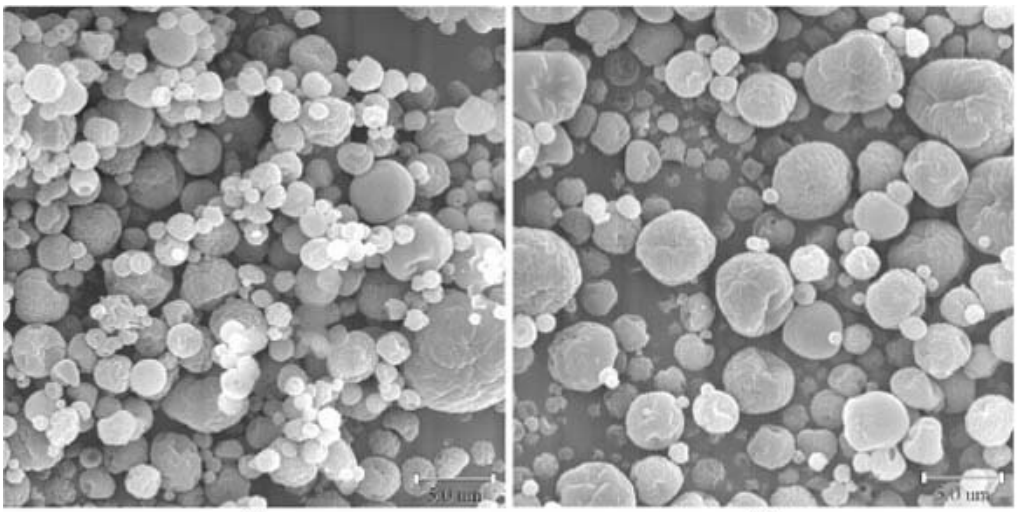
Table no.3

In vitro release studies of cross linked Chitosan Microspheres in buffer pH 7.4

Time in hours	F1(0.5% chitosan)	F2 (1% chitosan)	F3 (1.5%chitosan)	F4 (2% chitosan)
1	29.58	33.52	21.15	33.71
4	33.36	41.41	24.22	38.10
8	66.22	66.28	34.35	41.14
12	95.03	70.10	65.20	50.54

Figure1

SEM microphotographs of Chitosan microspheres obtained by spray drying from aqueous solutions with: a) 0.5%w/w chitosan; b) 2%w/w chitosan



(a) (b)

Fig 2
In vitro release studies of cross linked Chitosan Microspheres in buffer pH 7.4

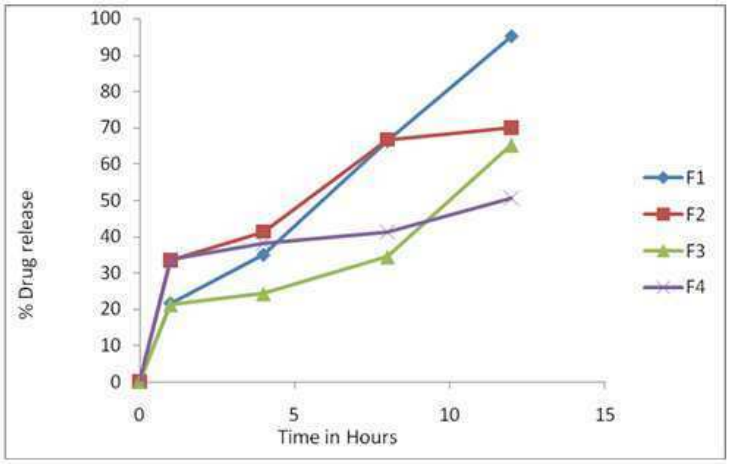
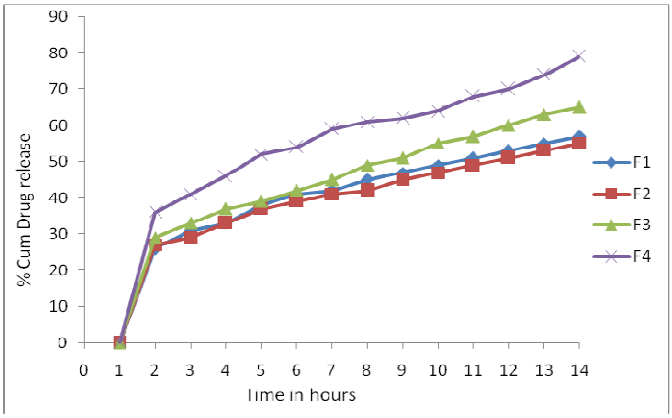


Fig 3
Drug entrapment efficiency of Chitosan microspheres



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