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Preparation and evaluation of cross-linked chitosan microspheres containing furosemide

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Abstract

Chitosan microspheres containing furosemide were prepared from a w/o emulsion system using liquid paraffin as the external phase and a solution of chitosan in acetic acid as the disperse phase. Discrete spherical furosemide microspheres having a 350–690 μm diameter range were produced. Microsphere properties were affected by the preparation variables such as the type and concentration of chitosan, drug concentration, cross-linking process, the viscosity of oil and stirring rate during the preparation. The results were examined kinetically. Dissolution data indicated that the release followed the Higuchi matrix model.

Keywords: Chitosan; Microsphere; Cross-linking; Furosemide; Release mechanism

1. Introduction

Chitosan is a natural polymer which possesses valuable properties as a biomaterial. Recently, it has been reported to have useful pharmaceutical applications in different drug delivery systems (Miyazaki et al., 1988; Nishioka et al., 1990). On the other hand, very few studies on the preparation of chitosan microcapsules and microspheres have been published thus far (Kawashima et al., 1985; Nishioka et al., 1990; Li et al., 1991; Lin and Lin, 1992; Thanoo et al., 1992). However, it is difficult to use a common method such as the solvent evaporation technique to prepare chitosan microspheres due to the only acidic solubility of this polymer. Thanoo et al. (1992) prepared cross-linked chitosan microspheres. The aim of this study was to prepare cross-linked chitosan microspheres containing furosemide and to discuss the effect of several factors on microsphere properties.

2. Materials and methods

2.1. Materials

The following materials were obtained from the indicated sources: chitosan (Sea Cure 243; 20–200 mPa s viscosity grade; Sea Cure 340, 560 mPa s viscosity grade (Pronova A/S, Norway); Sea Cure 210; chitosonium malate, 20 mPa s

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viscosity grade (Pronova A/S, Norway); furosemide (Hoechst, Turkey); glutaraldehyde (25% aqueous solution), chloroform, acetic acid (E. Merck, Germany); liquid paraffin (Atabay, (Turkey); Merkez; (Turkey); soybean oil (Turkey); mineral oil (Sigma, U.S.A.). The viscosity grades were 170.8, 28.5, 61.5 and 27 cps, respectively.

2.2. Preparation of cross-linked chitosan microspheres

A 4% solution of chitosan was prepared in aqueous acetic acid. Furosemide was dispersed in this solution and mixed well. This solution was added to the liquid paraffin to form a water-in-oil (w/o) emulsion. The dispersion was stirred at 710 rpm (Ika-Werk Janke Kunkel, Germany) for 30 min after the addition of glutaraldehyde solution. The product was filtered and washed with chloroform several times and finally with water and dried at 50°C. All batches were prepared at least three times. A number of variables such as drug concentration, type and concentration of chitosan, the type of oil, cross-linking process and stirring rate were investigated to optimize the microsphere properties (Table 1).

2.3. Physical characterization of microspheres

Scanning electron microscopy (SEM) (Joel JVA 840A, Japan) was used to evaluate the shape and surface characteristics of the microspheres. Size and size distributions were measured by sieve analysis (Retsch Haan, Germany).

2.4. Determination of drug content

A weighed quantity of microspheres was extracted with ethanol for 24 h, then the drug content was spectrophotometrically (Shimadzu 2100 S, Japan) assayed at 271.6 nm. Each determination was carried out in triplicate.

2.5. In vitro release studies

A weighed amount of microspheres was suspended in phosphate buffer (pH 7.4 USP, 50 ml) contained in a 100 ml glass bottle. This dissolu-

Table 1
Codes of cross-linked microspheres containing furosemide

Variables	Values	Abbreviations
Drug concentration (mg/ml)	14.3	A.1
	35.7	A.2
	71.4	A.3
Chitosan concentration (%)	4	B.1
	7.5	B.2
	10	B.3
Type of chitosan	Sea Cure 243	C.1
	340	C.2
	210	C.3
Type of oil	mineral oil (Sigma)	D_1
	soybean oil	D_2
	liquid paraffin (Atabay)	D_3
	liquid paraffin (Merkez)	D_4
Concentration of gluteraldehyde (%)	25	E_1
	20	E_2
	15	E_3
	12.5	E_4
Amount of glutaraldehyde (ml)	1	F_1
	3	F_2
	5	F_3
Stirring rate (rpm)	400	G1
	650	G2
	1000	G3

tion medium was stirred at 100 rpm in a horizontal laboratory shaker and maintained at constant temperature (37 ± 0.1°C) in a water bath. Samples were periodically removed and analyzed spectrophotometrically at 276.2 nm. The means of three determinations were given. No absorption due to chitosan was observed.

3. Results and discussion

Fig. 1 shows scanning electron micrographs of chitosan microspheres with and without drug. Incorporation of drug into the microspheres altered the surface morphology of furosemide microspheres to a significant extent. The scanning electron micrographs revealed that addition of drug imparts a high degree of surface roughness to the spheres.

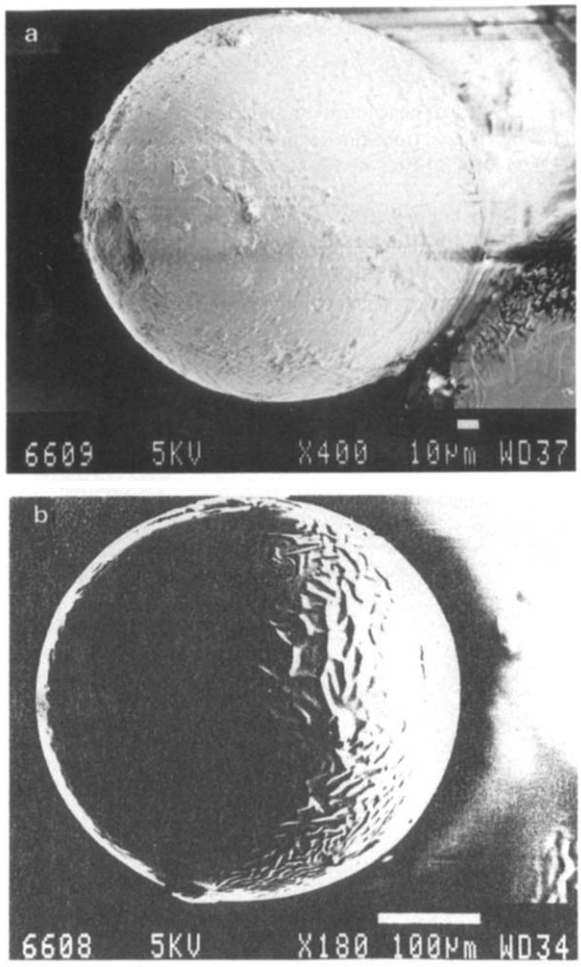

Fig. 1. Scanning electron micrographs of cross-linked chitosan microspheres with (a) and without (b) drug.

Table 2
Incorporation efficiency and mean particle size of furosemide-loaded chitosan microspheres

	Mean particle size (µm) (±SD)	Incorporation efficiency (%)
A.1	668.7 ± 63.4	20.03
A.2	634.3 ± 10.7	21.87
A.3	582.2 ± 60.6	25.35
B.1	668.7 ± 63.4	20.03
B.2	677.6 ± 73.1	35.29
B.3	675.6 ± 121.1	37.51
C.1	668.9 ± 63.4	20.03
C.2	612.8 ± 16.1	6.89
C.3	603.3 ± 44.8	15.16
D.1	548.8 ± 114.8	28.19
D.2	471.7 ± 137.3	23.57
D.3	633.1 ± 70.2	21.96
D.4	668.7 ± 63.4	20.03
E.1	668.7 ± 63.4	20.03
E.2	298.2 ± 114.9	20.84
E.3	550.0 ± 99.6	31.17
E.4	541.5 ± 67.2	33.03
F.1	668.7 ± 63.4	20.03
F.2	518.2 ± 145.7	10.49
F.3	599.0 ± 48.9	15.62
G.1	597.8 ± 127.6	29.45
G.2	459.0 ± 195.6	24.80
G.3	589.4 ± 123.19	27.88

Drug content and micromeritic properties of furosemide loaded chitosan microspheres are given in Table 2. On the other hand, one of the important factors related to microsphere formation, as reported earlier (Li et al., 1991), is the viscosity of the chitosan-acetic acid solution. Microspheres did not form at low concentration of chitosan-acetic acid solution. A highly concentrated solution made the dropping process difficult and microspheres could not readily be formed. Therefore, the concentration of chitosan was chosen as 4% w/v. In contrast to the findings of Lin and Lin (1992), acetic acid concentration has no importance in microsphere formation and properties. This may be due to the difference in origin of the chitosan samples and to the preparation technique. Fig. 2 represents the effect of

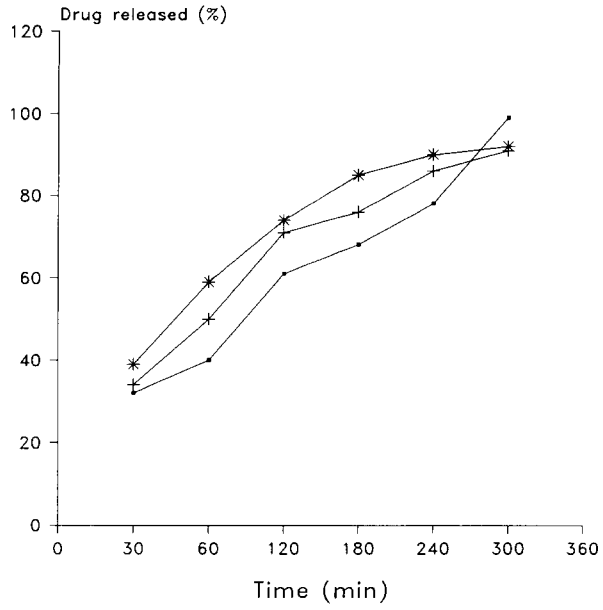

Fig. 2. Effect of drug concentration on drug release from furosemide microspheres. (□) A1, (+) A2, (*) A3.

drug concentration on drug release properties from chitosan microspheres. As the amount of drug incorporated increased, furosemide release

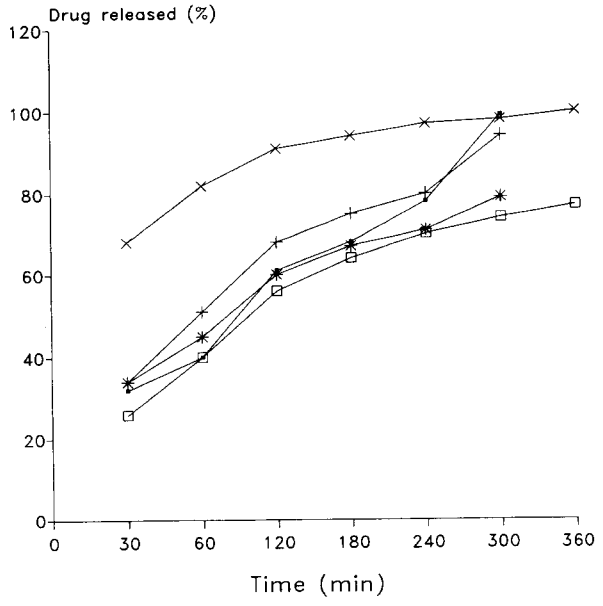

Fig. 3. Effect of the type and concentration of chitosan on drug release from microspheres. (□) B1, (+) B2, (*) B3, (□) C2, (×) C3.

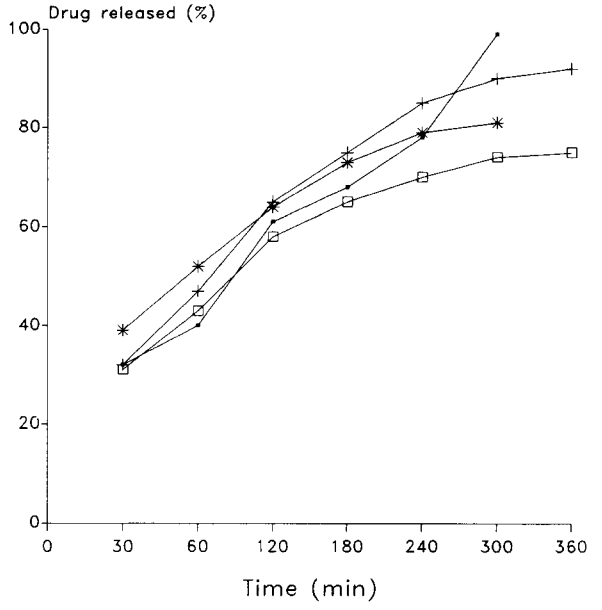

Fig. 4. Effect of concentration and amount of glutaraldehyde on drug release from furosemide microspheres. (□) E2, (+) E3, (*) E4, (□) F1, (×) F2, (◇) F3.

was also increased. Moreover, the type of chitosan has a considerable effect on the drug release characteristics of microspheres (Fig. 3). In

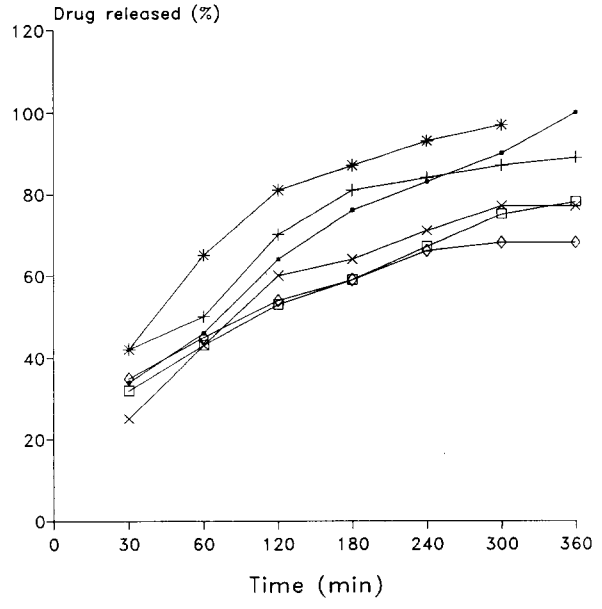

Fig. 5. Effect of oil properties on drug release from furosemide microspheres. (□) A1, (+) D1, (*) D2, (□) D3.

Table 3
Kinetic data of furosemide-loaded chitosan microspheres

Kinetics		A1	B2	B3	G1	G2	G3
Zero-order	k	0.135	0.155	0.198	0.214	0.148	0.154
	r	0.927	0.960	0.964	0.961	0.913	0.895
First-order	k	1.43×10^{-3}	-1.71×10^{-3}	-3.30×10^{-3}	-2.34×10^{-3}	3.41×10^{-3}	-3.50×10^{-3}
	r	0.997	0.987	0.957	0.987	0.956	0.975
Higuchi	k	5.300	3.661	4.667	5.076	4.614	46.501
		0.985	0.988	0.987	0.989	0.988	0.986
Baker and Lonsdale	k	4.57×10^{-4}	5.56×10^{-4}	1.00×10^{-3}	6.00×10^{-4}	9.79×10^{-4}	1.04×10^{-3}
		0.997	0.993	0.967	0.957	0.976	0.963

k, release rate constant; r, coefficient of correlation.

contrast to the report of Nishioka et al. (1990), no significant effect of chitosan concentration on drug release was observed. Several factors can affect the drug release process from chitosan microspheres (Lin and Lin, 1992; Thanoo et al., 1992). Among these factors, the degree of cross-linking may play an important role. Thanoo et al., (1992) reported that the least cross-linked microspheres released the drug at a faster rate; however, a significant difference was not observed in the release properties of microspheres cross-linked with different amounts of glutaraldehyde aqueous solution (Fig. 4). However, the concentration of glutaraldehyde solution also affected the release of furosemide. As the concentration of glutaraldehyde increased, the extent of cross-linking process increased.

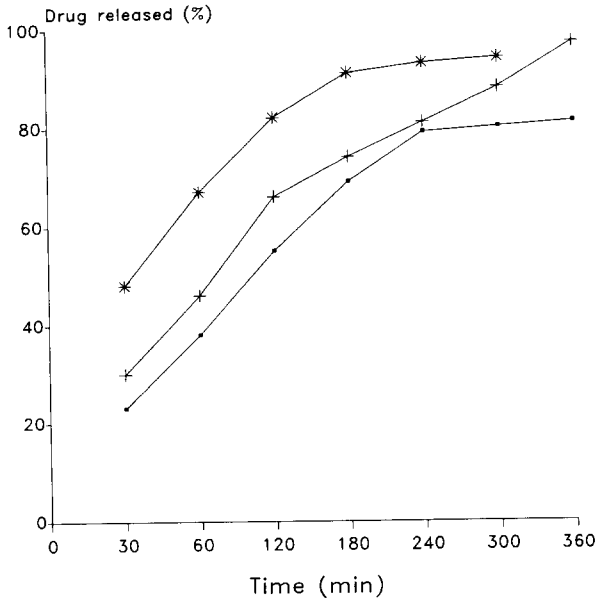

Fig. 6. Effect of stirring rate on drug release from furosemide microspheres. (□) 400 rpm, (+) 650 rpm, (*) 1000 rpm.

As observed in Fig. 5, the viscosity of oil used in microsphere preparation may change the drug release properties of the microspheres. Also, process variables such as stirring rate affected furosemide release from chitosan microspheres (Fig. 6).

In order to investigate the release mechanism, the release data were fitted to models representing zero-order, first-order, Higuchi's (1963) square-root of time and Baker and Lonsdale (1974) equation, indicative of release mechanisms related solely to time, drug diffusion or its dissolution rate, respectively. The following equation was used for the Baker and Lonsdale model: $(3/2)[1-(1-F)^{2/3}]-F=KT$ where F is the fraction of drug released, K denotes a constant and T is time.

The linear regression analyses for some trials are summarized in Table 3. As seen in Table 3, the coefficient of determination (r^2) was about 0.915–0.986 in each case, indicating that the data corresponds to diffusion control.

Table 4
Coefficients and exponents of furosemide release functions according to $Q(t) = at^n$ for chitosan microspheres

	A1	B2	B3	G1	G2	G3
a	0.983	1.006	0.939	0.566	0.858	1.292
n	0.358	0.361	0.416	0.556	0.443	0.286
r^2	0.990	0.988	0.978	0.984	0.980	0.951

r^2, coefficient of determination; n, release exponent; a, coefficient in above equation.

A more stringent test was used to distinguish between the mechanisms of drug release. Release data were analyzed according to the empirical equation (Higuchi, 1963; Cardinal, 1984):

$$Q(t) = at^n$$

where $Q(t)$ is the fraction of drug released after time t and a denotes a coefficient.

Different values of n (release exponent) indicate different release mechanisms. Values for the coefficient a and the exponent n are listed in Table 4. The values of n were in the range 0.286–0.556, and thus the release process is diffusion-controlled.

In conclusion, chitosan microspheres containing furosemide were prepared from a w/o emulsion system, using liquid paraffin as the external phase and a solution of chitosan in acetic acid as the disperse phase. Microsphere properties are affected by preparation variables such as the type and concentration of chitosan, glutaraldehyde concentration, drug concentration, the viscosity of oil and stirring rate.

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