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CROSS-LINKED CHITOSAN MICROSPHERES OF PENTAZOCINE FOR INTRANASAL ADMINISTRATION

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Cross-linked chitosan microspheres of pentazocine for intranasal systemic delivery were prepared using glutaraldehyde as the cross-linking agent. Microspheres were evaluated for physical characteristics like; Particle size, incorporation efficiency, swelling ability, invitro bioadhesion and drug release characteristics in pH 6.6 phosphate buffer. The invitro release profiles were examined kinetically. The data indicated that the release followed Higuchi's matrix model. The extent of cross-linking did not affect the rate and extent of drug release significantly.

Keywords: *Pentazocine; Chitosan; Microspheres; Intranasal; Cross-linking*

Introduction

Recently, extensive pharmaceutical research has been devoted to find alternative routes of systemic delivery, and intranasal administration has attracted much interest. This route holds good potential for administration of various drugs with the avoidance of the first metabolism, attainment of better bioavailability and better therapeutic profiles (1). Chitosans are linear polysaccharides with low toxicity that are biodegradable and biocompatible and have been considered good . for various biomedical and pharmaceutical applications (2).

Pentazocine (PZ) is used as a potent analgesic in chronic pains as in cancer traumatic and post operative pains. As per the guidelines of WHO for cancer management, analgesics like PZ are the drugs of choice (3). PZ is highly pass metabolic drug. By per oral route, only 20-25% of PZ enters into systemic circulation. But nasal route avoids first pass metabolism and bioadhesive microspheres provide longer retention time, thereby improving absorption (4). So, PZ seemed to be a good candidate to develop as a bioadhesive dosage form for intranasal systemic delivery.

Thus, in the present work cross linked chitosan microspheres (CLCM) were prepared and the effect of cross linking on particle size, incorporation efficiency, swelling ability, *invitro* bioadhesion and drug release were evaluated. Kinetics of drug release mechanisms were also studied.

Materials

Pentazocine (Ranbaxy, Dewas), chitosan (purified, viscosity grade 50) (CIFT, Cochin), glutaraldehyde and liquid paraffin (S.D. Fine Chem, Mumbai), Span 80 and Tween 80 (Wilson Lab, Mumbai), acetone (Qualiges Fine Chem, Mumbai), dihydrogen potassium orthophosphate (Glaxo, Mumbai), sodium hydroxide (E.Merc,

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Mumbai) (all Indian companies) and double distilled water were used. All chemicals used were of analytical reagent grade.

Methods

Preparation of CLCM: The formula for CLCM are shown in Table 1 and were prepared by emulsion-cross linking method (5). A 4% w/v solution of chitosan was prepared in 5% v/v aqueous acetic acid solution. PZ (40 mg) was dispersed in this solution and mixed well. This solution was added to light liquid paraffin containing 5% w/v Span 80 to form a water in oil (w/o) emulsion. The dispersion was stirred at 1200 rpm after the addition of 2 ml glutaraldehyde saturated toluene (6) initially followed by addition of aqueous glutaraldehyde solution at the end of 20 mins and the reaction was carried out for a total time of 0.5 to 2 hr. The product was filtered and washed several times with acetone and finally with water and dried at room temperature. Formulation variables such as conc. of cross-linking agent, amount (volume) of cross-linking agent and cross-linking reaction time were investigated to optimize the microsphere properties.

Table 1. Formula for different batches of crosslinked chitosan microspheres (CLCM)

Crosslinked chitosan microspheres (CLCM)	Concentration of aqueous glutaraldehyde (%)	Amount of aqueous glutaraldehyde (ml)	Crosslinking time (hr)
A_1	25	X* + 1	1
C_1	20	X* + 1	1
C_2	15	X* + 1	1
D_1	25	X* + 0.5	1
D_2	25	X* + 2	1
E_1	25	X* + 1	0.5
E_2	25	X* + 1	2

x* = 2 ml of glutaraldehyde saturated toluene. All the batches of microspheres were prepared using 40 mg drug, 4% w/v chitosan, stirring speed 1200 rpm and light liquid paraffin as oil phase.

Evaluation of CLCM Size and shape of microspheres: All the microspheres were evaluated to determine the particle size and shape using a microscope fitted with ocular micrometer.

Incorporation efficiency: To determine this, 25 mg of PZ loaded CLCM were washed with 10 ml of phosphate buffer (pH 6.6) containing 0.1% w/v Tween 80, to remove surface associated drug. The microspheres were then digested in 10 ml of 10% v/v glacial acetic acid for 12 hr at room temperature (25 ± 2^{0}C) to release entrapped drug. Drug contents were analysed spectrophotometrically (Jasco 9800, Tokyo, Japan) at 278 nm and the percent of incorporation efficiency was calculated as follows: Surface associated drug % or Entrapped drug % (w/w) = (Actual drug content/Theoretical drug content) x 100. Total incorporation efficiency (%) = Surface associated drug % (w/w) + Entrapped drug % (w/w).

Swelling ability: All the CLCM were also evaluated for their swelling ability (in triplicate) by allowing them to swell to equilibrium in phosphate buffer (pH 6.6) and by estimating their equilibrium fluid content using a reported method (7).

In vitro bioadhesion test: All the CLCM were further studied for *in vitro* bioadhesion using the method described in the literature (8). 50 mg of microspheres were placed in albino rabbit small intestine. The intestine with microspheres was placed in a dessicator maintained at 80% R.H. and room temperafure (25 ± 2 ^{0}C) to allow hydration of microspheres for 20 min. The mucosal lumen was thoroughly washed with phosphate buffer (pH 6.6). The washings were dried at 70^{0}C in hot air oven. The ratio of applied and adhered microspheres were computed as percent bioadhesion.

In vitro release study: In vitro evaluation of all the developed CLCM (in triplicate) were done by following a reported method (4). 20 mg of microspheres were suspended in 400 ml of

phosphate buffer (pH 6.6) contained in a beaker and kept at 37 ± 0.2°C under continuous stirring (100 rpm). At selected time intervals, 5 ml samples were withdawn through a hypodermic syringe fitted with a 0.4 μm Millipore filter, and replaced with the same volume of prewarmed fresh buffer solution to maintain the constant volume of the receptor compartment. The samples were analysed UV spectrophotometer at 278 nm (9). Released drug contents were computed from the calibration curve of PZ.

Results and Discussion

The results of physical characteristics of CLCM are shown in Table 2. When observed microscopically, CLCM were found to be discrete and spherical in shape. It was observed that the particle size of microspheres were not influenced by the concentration (batches C_1, C_2) and the amount (batches D_1, D_2) of the crosslinking agent as compared to batch A_1. However, size was significantly increased when cross linking reaction time was increased to 2 hr (batch E_2 70 μm) as compared to all other microspheres.

Incorporation efficiency: Data shown in Table 2 indicated that the drug loading efficiency.of the microspheres were significantly ($P < 0.05$) affected by the concentration & amount of cross linking agent and also by the cross linking time.

Swelling ability: The swelling ability of CLCM expressed as equilibrium fluid content in Table 2 indicated that this ability was influenced significantly ($P<0.05$) by the time of cross linking induced. The concentration and the amount of cross linking agent also affected the swelling ability of microspheres.

In vitro bioadhesion: Results shown in Table 2 indicated that concentration, amount and time of cross linking altered the bioadhesive properties of microspheres to marginal extent. All the microspheres exhibited good bioadhesive property.

In vitro release study: The in vitro release profiles of PZ from CLCM are shown in Figs 1-3. The drug release profiles observed from all the microspheres exhibited a biphasic pattern of drug release. An initial burst effect due to immediate release of surface associated drug followed by slow and controlled release phase resulted from the controlled diffusion of entrapped drug. Results indicated that

Table 2. Physical characteristics of prepared crosslinked chitosan microspheres

S. No	Batch No	Mean particle size (μm + S.D.)	Incorporation efficiency			Equilibrium fluid content (%) (Mean ± S.D.) (n=3)	%Mean bioadhesion ± SEM (n=3)
			Surface associated drug %w/w	Entrapped drug %w/w	Total incorporation efficiency %w/w		
1	A_1	50 ± 5.87	25.55	12.01	37.56	60.5 ± 0.8	90.3 ± 1.12
2	C_1	49.5 ± 5.27	25.15	16.82	41.97	67.3 ± 0.6	96.1 ± 1.53
3	C_2	52.5 ±6.18	25.69	25.55	51.24	71.4 ± 0.3	97.4 ± 0.92
4	D_1	49.5 ± 6.32	33.16	24.69	57.85	64.5 ± 0.7	93.1 ± 0.89
5	D_2	50 ± 4.95	11.52	16.07	27.59	52.3 ±0.7	84.5 ± 1.56
6	E_1	46.5 ± 5.31	25.32	18.85	44.17	56.4 ± 0.5	86.1 ± 0.98
7	E_2	70 ± 4.82	26.79	10.54	37.33	67.5 ± 0.7	95.3 ± 1.42

the rate and extent of drug release were not influenced significantly ($P > 0.01$) by both concentration and the amount of glutaraldehyde (Figs 1, 2). The different times of cross linking also did not show significant effect on drug release profiles except for batch E_2 which exhibited lower rate and extent of drug release as compared to batches A_1 and E_1 and was attributed to the fact that glutaraldehyde had sufficient time (2 hrs) to crosslink chitosan linkages in case of E_2 as compared to lower time of cross linking in A_1 (1 hr) and E_1 (0.5 hr). Thus, in our opinion increasing the cross linking reaction time may lead to a further reduction in the drug release which could prove to be a better alternative (10, 11) for not using higher concentrations of glutaraldehyde as it is not safe at high concentrations.

To examine the release mechanism, the release data were fitted to models representing zero order, first order and Higuchi's square root of time. The linear regression analysis are summarized in Table 3. The observation of coefficient of determination (r^2) values indicated that drug release from CLCM followed diffusion control mechanism.

Table 3. Kinetics of *invitro* pentazocine release from chitosan microspheres

S. No	Batch No	Zero-order		First-order		Higuchi	
		K	r	K	r	K	r
1	A_1	0.0914	0.8862	-1.117×10^{-4}	0.9774	0.3083	0.9832
2	C_1	0.0951	0.8827	-1.411×10^{-4}	0.9784	0.3220	0.9736
3	C_2	0.0971	0.8815	-1.858×10^{-4}	0.9426	0.3289	0.9727
4	D_1	0.0923	0.8830	-1.172×10^{-4}	0.9773	0.3120	0.9721
5	D_2	0.0915	0.8889	-1.083×10^{-4}	0.9751	0.3077	0.9741
6	E_1	0.0978	0.8911	-1.518×10^{-4}	0.9830	0.3291	0.9774
7	E_2	0.0937	0.9339	-9.68×10^{-5}	0.9926	0.3058	0.9934

K = Release rate constant

r = Coefficient of correlation

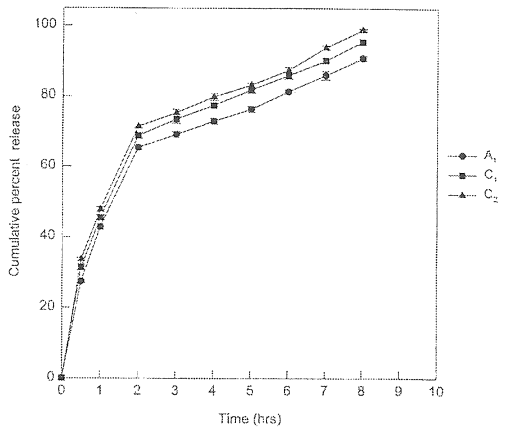

Fig. 1. *In vitro* release profiles of pentazocine from different cross linked chitosan micro-spreheres in phosphate buffer (pH 6.6)

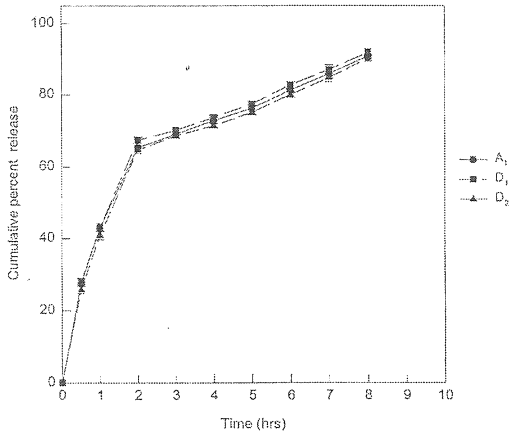

Fig. 2. *In vitro* release profiles of pentazocine from different cross linked chitosan micro-spheres in phosphate buffer (pH 6.6)

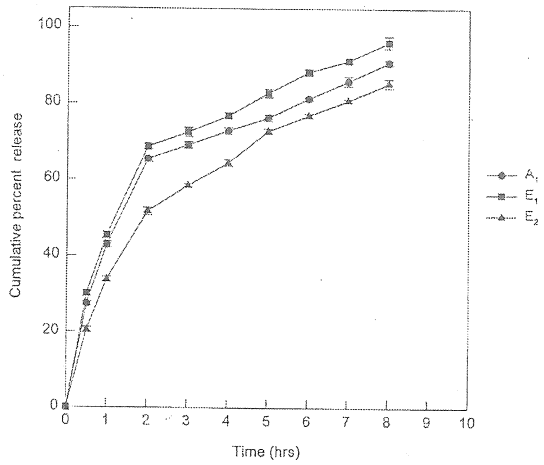

Fig. 3. *In vitro* release profiles of pentazocine from different cross linked chitosan microspheres in phosphate buffer (pH 6.6)

To examine further the release of PZ from CLCM, the results were analysed according to $Q(t) = at^n$ equation (12,13) where Q(t) is the fraction of drug released after time 't' and 'a' denotes a coefficient. Values for the coefficient 'a' and the release exponent 'n' are listed in Table 4. The values of n were in the range of 0.3234 - 0.4758 which is further indicative of drug release as diffusion controlled.

Table 4. Coefficient and exponent of pentazocine release according to $Q(t) = at^n$ for chitosan microspheres

Batch No	Equation coefficent (a)	Release exponent (n)	Coefficient of determination (r^2)
A_1	0.4136	0.4003	0.9725
C_1	0.4521	0.3790	0.9782
C_2	0.4758	0.3649	0.9794
D_1	0.4219	0.3975	0.9709
D_2	0.3972	0.4177	0.9700
E_1	0.4433	0.3960	0.9788
E_2	0.3234	0.4747	0.9888

In conclusion, the cross linked chitosan microspheres exhibited significant bioadhesive property and could potentially be used as bioadhesive microspheres for controlled and sustained intranasal systemic delivery of pentazocine. Further, its potential to improve bioavailability of pentazocine could be established by invivo evaluation in animals and/or humans.

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