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Chitosan based pentazocine microspheres for intranasal systemic delivery: development and biopharmaceutical evaluation

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Bioadhesive chitosan microspheres (Ms) of pentazocine (Pz) for intranasal systemic delivery were prepared with the aim of avoiding the first pass effect, and thus improving the bioavailability and achieving sustained and controlled blood level profiles, as an alternative therapy to injection and to obtain improved therapeutic efficacy in the treatment of chronic pain such as cancer, trauma and post-operative pain, etc. The formulation variables were drug loading, polymer concentration, stirring rate during crosslinking and oils. The microspheres (Ms) were subjected to evaluation for physical characteristics, such as particle size, incorporation efficiency, swelling ability, *in vitro* bioadhesion, *in vitro* drug release characteristics and *in vivo* performance in rabbits. Application of *in vitro* data to various kinetic equations indicated matrix diffusion controlled drug delivery from chitosan Ms. Drug loading, polymer concentration and stirring speed influenced the drug release profiles significantly while oils had negligible effect. *In vivo* studies indicated significantly improved bioavailability of Pz from Ms with sustained and controlled blood level profiles as compared to i.v., oral and nasal administration of drug solution. Good correlation was observed between *in vitro* and *in vivo* data.

1. Introduction

Pentazocine (Pz) having both agonistic and weak opioid antagonistic activity, is used as a potent analgesic for chronic pain such as cancer, trauma and postoperative pain. According to the guidelines of the World Health Organization for cancer pain management, analgesics like Pz are the drug of choice [1]. Pz is highly first-pass metabolised and when given by the oral route only 20–25% of Pz enters into the systemic circulation [2]. At the same time, the parenteral route is associated with pain and discomfort and needs the assistance of medical personnel. In an attempt to circumvent these problems, alternative routes of drug administration for systemic delivery, are being investigated. Nasal administration of drugs along with bioadhesive polymers in combination with a non-toxic enhancer seem to offer potential as an alternative therapy to injection [3–5]. Chitosan is a cationic, hydrophilic, biodegradable and biocompatible polymer with low toxicity and it has been found that chitosan has no effect on nasal mucociliary clearance nor on cilia beat frequency [6, 7].

These observations have given an impetus to develop chitosan microspheres (Ms) as a promising formulation of Pz for administration by the intranasal route, as an alternative therapy to injections, for the better treatment of chronic pain.

2. Investigations, results and discussion

The shape and surface morphology of the various batches of Ms prepared were determined by scanning electron microscopy. The Ms were found to be discrete and spherical in shape. The incorporation of drug increased the roughness of the Ms as compared to the placebo spheres.

The results (Table 1) indicated that as the amount of polymer (batches B_1, A_1, B_2) and drug (batches A_2, A_1, A_3) in the Ms were increased, the particle size also increased [8]. This could be attributed to an increase in the relative viscosity of the medium which might cause an increase in the interfacial tension, resulting in the formation of larger particles during emulsification. The particle size seemed to decrease progressively with an increase in the rate of stirring (batches F_1, A_1, F_2).

The drug incorporation efficiency (Table 1) was found to be directly proportional to the polymer concentration and drug loading, but inversely proportional to the rate of stirring. The increase in drug incorporations with an increase in drug loading may be due to an increase in the concentration of the drug that is available for incorporation as the drug dissolves completely in the formulation environment (acidic). Similar behaviour with an increase in the polymer concentration is attributed to an increased viscosity, which results in the formation of bigger microspheres, thus increasing the incorporation efficiency. Though there

Table 1: Physical characteristics of prepared bioadhesive 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	A_2	40 ± 5.59	13.98	11.52	25.50	65.4 ± 0.3	92.8 ± 0.98
3	A_3	60 ± 7.20	32.58	16.90	49.48	56.7 ± 0.7	88.2 ± 1.25
4	B_1	40 ± 5.10	16.28	15.55	31.83	52.8 ± 0.7	85.2 ± 1.38
5	B_2	75 ± 6.85	30.84	14.52	45.36	64.7 ± 0.3	93.8 ± 1.27
6	F_1	75 ± 8.14	25.41	26.13	51.54	65.2 ± 0.8	94.5 ± 0.93
7	F_2	40 ± 3.95	23.99	10.99	34.98	57.4 ± 0.6	86.9 ± 1.17
8	G_1	48.5 ± 5.86	32.50	23.36	55.86	60.7 ± 0.7	90.8 ± 1.32
9	G_2	42.5 ± 6.38	27.91	35.61	63.52	56.8 ± 0.7	85.4 ± 2.11

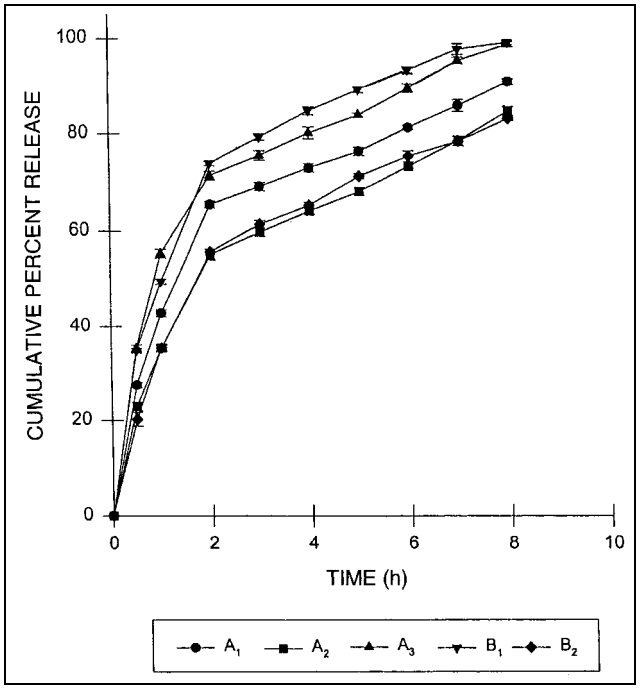

Fig. 1: *In vitro* release profiles of pentazocine from different chitosan microspheres in phosphate buffer (pH 6.6)

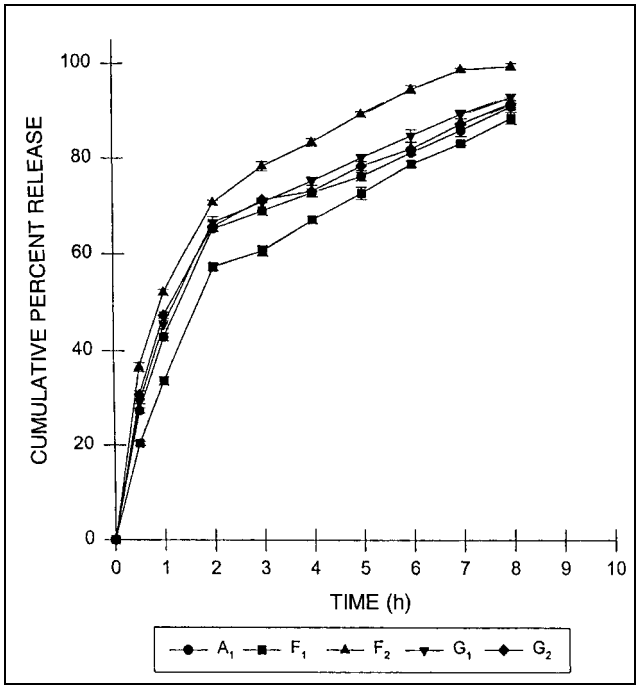

Fig. 2.: *In vitro* release profiles of pentazocine from different chitosan microspheres in phosphate buffer (pH 6.6)

is a comparative increase in the drug incorporation efficiency with an increase in the drug/polymer ratio, the overall incorporation efficiency in all batches ranged from 25.50% (batch A_2) to 63.52% (batch G_2). The low overall incorporation efficiency is due to the fact that drug molecules preferentially stayed at the surface and a substantial proportion may be lost during successive washings. The decrease in the incorporation efficiency with an increase in the rate of stirring may be due to a reduction in the mean diameter of the Ms.

The swelling ability is shown in terms of equilibrium fluid content [16] in Table 1. The prepared Ms in phosphate buffer showed equilibrium fluid contents ranging from 52.8% (Batch B_1) to 65.4% (Batch A_2).

The study of *in vitro* bioadhesion revealed that all the batches of Ms showed good mucoadhesivity (Table 1) ranging from 85.2% (batch B_1) to 94.5% (batch F_1). Chitosan, being a cationic polyelectrolyte, binds strongly with mucin, which is an anionic polyelectrolyte, at around neutral pH [9, 10].

The effects of drug concentration on release characteristics are shown in Fig. 1. The rate and extent of drug release from A_3 (60 mg/ml) was higher than from A_1 (40 mg/ml) and A_2 (20 mg/ml) which is attributed to the presence of a higher amount of drug in A_3 than in A_1 and A_2. All the release profiles followed a biphasic release pattern, an initial burst effect being followed by slow and controlled drug release. The burst effect is due to the immediate release of surface associated drug followed by slow and controlled release from the entrapped drug in the Ms.

The release profiles from the three batches 2% (B_1), 4% (A_1) and 6% w/v (B_2) of chitosan are shown in Fig. 1. A significant ($p < 0.001$) decrease in the extent of drug release was observed with the increase in the polymer concentration and is attributed to the density of the polymer matrix and also to increase in the diffusional path length which the drug molecules have to traverse. Profiles still followed a biphasic pattern.

The results shown in Fig. 2 indicated that the rate and extent of drug release was significantly ($p < 0.01$) increased with increase in the stirring rate during crosslinking of the chitosan polymer which is attributed to reduction in the mean diameter of the Ms which in turn causes an increase in the surface area of the Ms.

The release profiles shown in Fig. 2 indicate that the change of oil had no significant effect on the rate and extent of drug release.

Table 2: Kinetics of *in vitro* pentazocine release from chitosan microspheres

S.No.	Batch No	Zero-order		First-order		Higuchi	
		K (mg/h)	r	K (h^{-1})	r	K ($mg/h^{1/2}$)	r
1	A_1	0.0914	0.8862	-1.117×10^{-4}	0.9774	0.3083	0.9832
2	A_2	0.0875	0.9167	-8.81×10^{-5}	0.9804	0.2890	0.9880
3	A_3	0.0951	0.8713	-1.917×10^{-4}	0.9552	0.3244	0.9680
4	B_1	0.1005	0.8733	-2.17×10^{-4}	0.9852	0.3427	0.9703
5	B_2	0.0887	0.9089	-8.78×10^{-5}	0.9791	0.2951	0.9831
6	F_1	0.0959	0.9251	-1.058×10^{-4}	0.9889	0.3147	0.9880
7	F_2	0.1005	0.8808	-2.566×10^{-4}	0.9682	0.3413	0.9750
8	G_1	0.0941	0.8869	-1.276×10^{-4}	0.9850	0.3178	0.9756
9	G_2	0.0896	0.8762	-1.145×10^{-4}	0.9770	0.3046	0.9706

K = release rate constant
r = coefficient of correlation

Table 3: Coefficient and exponent of pentazocine release according to $Q(t) = a\ t^n$ for chitosan microspheres

Batch No.	Equation coefficent (a)	Release exponent (n)	Coefficient of determination (r^2)
A_1	0.4136	0.4003	0.9725
A_2	0.3461	0.4417	0.9832
A_3	0.5056	0.3351	0.9751
B_1	0.4955	0.3683	0.9786
B_2	0.3325	0.4764	0.9739
F_1	0.3308	0.5020	0.9796
F_2	0.5082	0.3516	0.9879
G_1	0.4357	0.3870	0.9769
G_2	0.4480	0.3603	0.9753

In order to investigate 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 analyses are summarized in Table 2. The examination of coefficient of determination (r^2) values indicates that drug release followed the diffusion control mechanism from the Ms.

A more stringent test was used to distinguish between the mechanisms of drug release. Release data were analyzed according to the empirical equation [11, 12] $Q(t) = a\ t^n$, where Q (t) is the fraction of drug released after time 't' and 'a' is a coefficient. Values for the coefficient 'a' and the release exponent 'n' are listed in Table 3. The values of n were in the range of 0.3351–0.5020 which was further indicative of the drug release following a diffusion control mechanism.

Batches containing three different drug loadings were selected for the *in vivo* study. The blood concentration-time profiles for the Ms, nasal solution, oral solution and Pz I.V. are shown in Fig. 3. The bioavailability parameters such as C_{max}, T_{max}, AUC (0–24 h) and absolute bioavailability for different formulations are shown in Table 4. Though the C_{max} for the nasal solution was much higher and was attained much more rapidly than that for microspheres, the decline in drug concentration was equally rapid, suggesting frequent administration of nasal solution would be required which might result in pulse type profiles, while the blood-time profiles for the microspheres were more sustained and controlled. The AUC for the Ms were found to be closer to AUC values for i.v. administration and were much higher than the AUC values for nasal and oral solutions. Such a low AUC for oral solution is attributed to the first pass effect of pentazocine. The absolute bioavailability data indicated that microspheres A_1, A_2 and A_3 were 96.5%, 81.8% and 89.9% bioavailable, respectively, as compared to nasal solution 70.8% and oral solution 15.8%. The results thus indicate that all the microspheres were not only able to improve the bioavailability of the drug by the intranasal route due to avoidance of first pass effect, but also to provide sustained and controlled blood level profiles of Pz as compared to nasal and oral solutions and the I.V. route of administration. Further, correlation of *in vitro* data at 0.5 h, 1 h and 8 h with C_{max} values [13] for all three Ms exhibited good correlation.

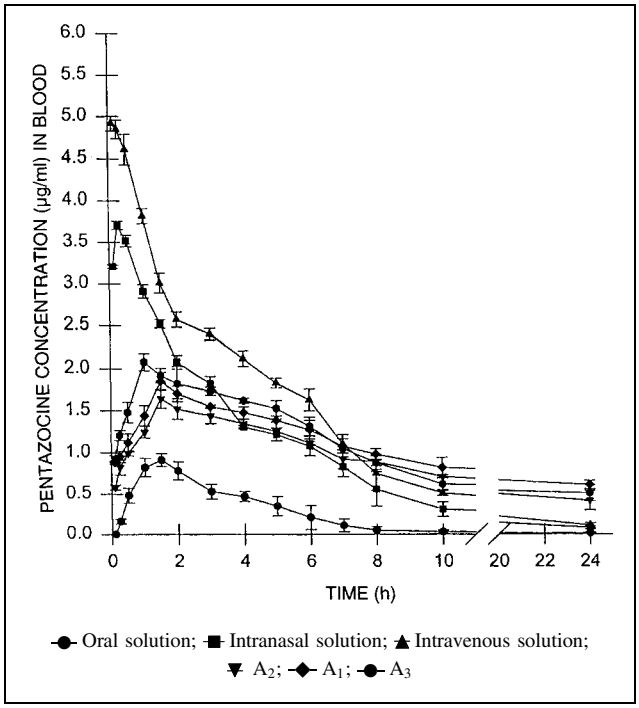

Fig. 3: Profiles of average blood concentrations following administration of pentazocine (5 mg/kg) to rabbits through different formulations

Based on the findings it was concluded that the chitosan Ms could potentially be used for intranasal delivery of drugs like Pz which undergo extensive first pass metabolism. However, studies on a larger group will have to be conducted to strengthen our findings in rabbits. The effect on ciliary movement and toxicity need to be well established.

3. Experimental

3.1. Preparation of chitosan microspheres

The formulae for the various batches of Ms are shown in Table 5. Ms were prepared using the following technique [14]. A 4% w/v solution of chitosan was prepared in 5% v/v aqueous acetic acid solution and Pz was homogeneously dispersed into this. This dispersion (26 g) was mixed with 150 ml of (light/heavy) liquid paraffin containing 5% v/v span 80 to form a water in oil (w/o) emulsion. This dispersion was stirred at 1200 rpm after addition of 2 ml glutaraldehyde saturated toluene [15] and the stirring continued at room temperature (25 °C). More aqueous glutaraldehyde solution

Table 4: Bioavailability data for pentazocine after administration of aqueous drug solution by different routes and of intranasal bioadhesive chitosan microspheres in rabbits

Formulation	C_{max} (µg/ml) (Mean* ± SEM)	T_{max} (h) (Mean* ± SEM)	AUC (0–24 h) (µg h/ml) (Mean* ± SEM)	Absolute bioavailability (%) (Mean* ± SEM)
Intravenous	4.92 ± 0.09	–	23.47 ± 2.82	100.00
Nasal solution	3.71 ± 0.05	0.25 ± 0.02	16.64 ± 1.97	70.80 ± 5.10
Oral solution	0.91 ± 0.08	1.50 ± 0.05	3.71 ± 1.29	15.80 ± 1.50
A_1	1.85 ± 0.11	1.5 ± 0.03	22.66 ± 2.41	96.50 ± 8.40
A_2	1.64 ± 0.11	1.5 ± 0.02	19.21 ± 2.08	81.80 ± 6.20
A_3	2.08 ± 0.10	1.0 ± 0.04	21.10 ± 1.89	89.90 ± 6.71

*n = 6

Table 5: Formulae for different batches of chitosan microspheres

Formulation variables	Batch								
	A_1	A_2	A_3	B_1	B_2	F_1	F_2	G_1	G_2
Drug loading (mg/ml)	40	20	60	40	40	40	40	40	40
Chitosan concentration (% w/v)	4	4	4	2	6	4	4	4	4
Stirring rate (rpm)	1200	1200	1200	1200	1200	1000	1400	1200	1200
Type of oil	Light liquid paraffin	Light liquid paraffin	Light liquid paraffin	Light liquid paraffin	Light liquid paraffin	Light liquid paraffin	Light liquid paraffin	Light: Heavy liquid paraffin 1 : 1	Heavy liquid paraffin

was added at 20 min intervals for preparing Ms of different crosslinking densities and the reaction was continued for a total of 3 h. The product was filtered, and washed several times with acetone and finally with water and dried at room temperature. Formulation variables such as drug concentration, polymer concentration, stirring rate and the type of oil were investigated to optimize the Ms properties.

3.2. Evaluation of chitosan microspheres

3.2.1. Surface characteristics

The shape and surface morphology of various batches of Ms were determined by scanning electron microscopy.

3.2.2. Particle size

Particle size was determined by observing 100 Ms per batch under microscope fitted with an ocular micrometer.

3.2.3. Incorporation efficiency

25 mg of pentazocine loaded chitosan Ms were washed with 10 ml of phosphate buffer of pH 6.6 containing 0.1% w/v Tween 80, to remove surface associated drug. Then the Ms were digested in 10 ml of 10% v/v aqueous glacial acetic acid solution for 12 h at room temperature to release entrapped drug, and the actual drug content was measured by analysing the solution UV spectrophotometrically (Shimadzu-1601, Tokyo, Japan) at 278 nm. Incorporation efficiency was then calculated by using the following formula:
Surface associated drug % (w/w) or entrapped drug % (w/w) = (actual drug content/theoretical drug content) × 100. Total incorporation efficiency (%) = surface associated drug % (w/w) + Entrapped drug % (w/w)

3.2.4. Swelling ability

The swelling ability (in triplicate) of the prepared chitosan Ms was determined by allowing them to swell to their equilibrium in phosphate buffer of pH 6.6 followed by estimation of equilibrium fluid content [16].

3.2.5. In vitro bioadhesion

In vitro bioadhesion (in triplicate) was determined using a previously reported method [17]. 50 mg of Ms were placed on albino rabbit small intestine. The intestine samples with Ms were placed in a dessicator maintained at 80% R.H. and room temperature (25 ± 2 °C) to allow hydration of Ms for 20 min. The mucosal lumen was thoroughly washed with phosphate buffer (pH 6.6). The washings were dried at 70 °C in a hot air oven. The ratio of adhering to applied microspheres was calculated as percent bioadhesion.

3.2.6. In vitro release

The *in vitro* drug release from different batches of Ms was evaluated (in triplicate) using phosphate buffer of pH 6.6 as the elution medium. 10 mg drug equivalent of Ms were suspended in 400 ml of elution medium contained in a beaker, maintained at 37 ± 0.2 °C under continual stirring (100 rpm). Samples were withdrawn at regular time intervals through a hypodermic syringe fitted with a 0.4 μm Millipore filter, the withdrawn volume was replaced by the same volume of fresh medium, and the samples were analysed spectrophotometrically at 278 nm.

3.2.7. In vivo studies

Three promising batches of chitosan Ms were evaluated *in vivo* in rabbits. Six healthy albino rabbits of either sex weighing 2.0 ± 0.2 kg with normal diet were used for the study. Food was withdrawn 12 h prior to the study with water ad libitum. A washout period of one week was allowed.

Drug (5 mg/kg in each case) administration to rabbits was done as (a) an oral gastric tube, (b) an intravenous bolus injection of sterile drug solution into the ear vein, (c) an intranasal instillation of 50 μl nasal drug solution in each nostril using a Hamilton microlitre syringe attached to 5 cm of polyethylene tube inserted 1.7 cm inside the nostril and (d) bioadhesive chitosan Ms (batches A_1, A_2, A_3) sprayed in the nostril by a previously reported method [18] in a cross over fashion on different occasions. All nasal preparations were administered to conscious rabbits in a supine position and the rabbits were kept in this position for 1 min after drug administration.
Blood samples (0.7 ml) were withdrawn at predetermined time intervals of 0, 0.10, 0.25, 0.5, 1, 1.5, 2.3, 4, 5, 6, 8, 10 and 24 h from the middle ear vein of the rabbit using a 26 gauge needle and syringe and were collected in heparinized vials and stored at −10 °C until assayed. All blood samples were analysed by a spectrophotofluorometer (Jasco FP-777, Tokyo, Japan) following a previously reported method [19, 20]. Bioavailability parameters (C_{max}, T_{max}, AUC_0^{24} and absolute bioavailability) were calculated for various formulations and results from the microspheres were compared with the conventional formulations.

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