Drug Delivery, 10:21–28, 2003

1071-7544/03 $12.00 + .00
DOI: 10.1080/10717540390169539

Taylor & Francis
healthsciences

Sodium Alginate Microspheres of Metoprolol Tartrate for Intranasal Systemic Delivery: Development and Evaluation

P. S. Rajinikanth, C. Sankar, and B. Mishra
Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi, India

Bioadhesive sodium alginate microspheres of Metoprolol tartrate (MT) for intranasal systemic delivery were prepared to avoid the first-pass effect, as an alternative therapy to injection, and to obtain improved therapeutic efficacy in the treatment of hypertension and angina pectoris. The microspheres (Ms) were prepared using emulsification–cross-linking method. The formulation variables were drug loading, polymer concentration, cross-linking agent concentration, and cross-linking time. The Ms were evaluated for characteristics, like particle size, incorporation efficiency, swelling ability, in vitro bioadhesion, in vitro drug release, and in vivo pharmacodynamic performance in rabbits against isoprenaline-induced tachycardia. Treatment of in vitro data to different kinetic equations indicated matrix-diffusion controlled drug delivery from sodium alginate Ms. Polymer concentration, cross-linking agent concentration, and cross-linking time influenced the drug release profiles significantly. In vivo studies indicated significantly improved therapeutic efficacy of MT from Ms with sustained and controlled inhibition of isoprenaline-induced tachycardia as compared with oral and nasal administration of drug solution.

Keywords Metoprolol Tartrate, Microspheres, Nasal Delivery, Sodium Alginate

Hypertension is one of the major risks associated with heart diseases. Metoprolol tartrate (MT) is a widely used β_1-blocker for hypertension (Reynolds 1993). Due to its high first-pass metabolism, only 38% of this drug enters systemic circulation via oral route (Lennard et al. 1982). Its plasma half-life is less than 4 hr causing frequent administration (Lennard et al. 1983). Also frequent doses of parenterals are uncomfortable to patients and needs the assistance of medical personnel. Alternative routes of administration are of much interest: Nasal administration of drugs along with bioadhesive polymers in combination with a nontoxic enhancer seem to offer potential as an alternative therapy to injection and to avoid first-pass metabolism (Nagai and Machida 1990; Popovici and Szasz 1992; Dondeti et al. 1996). Sodium alginate is a hydrophilic, biodegradable, biocompatible, mucoadhesive, nontoxic, and inexpensive polymer and could be used for controlled delivery of drugs (Stockwell et al. 1986; Nakano and Ogata 1984; Bowersock et al. 1996).

These observations have given us impetus to develop sodium alginate microspheres (Ms) as a promising formulation of MT for intranasal administration for an alternative route to injections, and for better management of hypertension.

Received 22 April 2002; accepted 1 September 2002.

Address correspondence to B. Mishra, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221005, India. E-mail: bmishra@banaras.ernet.in

MATERIALS AND METHODS

Metoprolol tartrate (Astra Zeneca Ltd., Bangalore), sodium alginate (purified medium viscosity grade, Central Drug House, Delhi), calcium chloride (S.D. Fine Chemicals Ltd., Mumbai), hydroxypropylmethylcellulose (Qualigens Fine Chemicals, Mumbai), Span 80 (Wilson Labs, Mumbai), heparin injection (Biological E. Ltd, Hyderabad), pentobarbitone sodium (Ranbaxy Labs, New Delhi) (all Indian companies), and isoprenaline (National Lab, Italy) were used. All other chemicals used were of analytical grade.

Preparation of Sodium Alginate Microspheres

The formula, for the various batches of Ms are shown in Table 1. Ms were prepared using reported method of Wan et al. (1992) with little modification. MT was dispersed in an aqueous solution containing 5% w/v sodium alginate and HPMC in (9:1) ratio. The solution was dispersed in a hexane solution containing 2% v/v Span 80 using a mechanical stirrer (Remi stirrer, Mumbai, India) at 1500 rpm. The resultant emulsion was stirred for 30 min. A calcium chloride solution (7.5% w/v) was added and the dispersion was stirred for another 10 min. Ms were collected by filtration, washed three times with isopropyl alcohol, and finally dried at room temperature. To optimize the preparation of Ms, the formulation variables like drug concentration, polymer concentration, concentration of cross-linking agent, and cross-linking time were used.

TABLE 1
Formula for different batches of MT-loaded sodium alginate microspheres

Formulation variables	Batch code of microspheres										
	A	A1	A2	A3	B1	B2	B3	C1	C2	D1	D2
Drug loading (mg/ml)	20	30	40	50	20	20	20	20	20	20	20
Sodium alginate concentration (% w/v)	2	2	2	2	4	5	6	2	2	2	2
Calciumchloride concentration (% w/v)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	5	7.5	2.5	2.5
Cross-linking time (min)	5	5	5	5	5	5	5	5	5	10	25

Morphology and Particle Size Analysis

The shape and surface morphology of various batches of Ms prepared were determined by scanning electron microscopy (SEM-JEOL Model 8404, Japan, at magnification 500×). Particle size was determined using an optical microscope (Olympus, New Delhi, India) fitted with a stage and an ocular micrometer.

Incorporation Efficiency

The incorporation efficiency was determined by the method of Jeffery et al. (1993). First, 25 mg of MT-loaded Ms were washed with 10 ml phosphate buffer of pH 6.6 to remove surface associated drug. Then the Ms were digested in 10 ml of 5N sodium hydroxide for 2.5 hr at room temperature ($25 \pm 2^{\circ}$C) to release any entrapped drug. The samples were centrifuged at 560 ×g for 10 min to eliminate the nonsoluble residue and the supernatant layer was removed to measure the actual drug content by analyzing the solution by ultraviolet spectrophotometry (Shimadzu-7800, Tokyo, Japan) at 272 nm. Incorporation efficiency was calculated using the following formula:

- Surface associated drug % (w/w), or
- Entrapped drug % (w/w) = (Actual drug content/total drug content) × 100.
- Total incorporation efficiency % (w/w) = Surface associated drug % (w/w) + entrapped drug % (w/w).

Swelling Ability

The swelling ability (in triplicate) of the 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 (Thanoo and Jayakrishnan 1990).

In Vitro Bioadhesion

In vitro bioadhesion (in triplicate) was determined by following a previously reported method (Ranga Rao and Buri 1989). Microspheres of 50 mg were placed on albino rabbit small intestine. The intestine with Ms was placed in a humidity temperature control cabinet (Narang Scientific Works Pvt. Ltd, New Delhi) at 80% relative room humidity and temperature of $25 \pm 0.5^{\circ}$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 adhered and applied microspheres were computed as percent bioadhesion.

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 elution medium. First, 10 mg drug equivalent of Ms was suspended in 400 ml of elution medium placed in a beaker, maintained at $37 \pm 0.2^{\circ}$C with continuous stirring of 100 rpm (Illum et al. 1987). 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 analyzed spectrophotometrically at 272 nm.

Pharmacodynamic Studies

Three promising batches of Ms were evaluated in vivo by measuring the isoprenaline-induced tachycardia in rabbits. The study was designed with a slight modification of the Kemken et al. (1991) method. Six healthy albino rabbits of either sex, weighing 2.4 ± 0.2 kg, and fed with Hind Lever diet, were used for the study. Food was withdrawn 12 hr prior to the study with water ad libitum. A washout period of 1 week was allowed. Rabbits were anesthetized by intraperitoneal injection of 35 mg/kg pentobarbitone sodium in sterile normal saline. The anesthesia was maintained by hourly administration of intraperitoneal pentobarbitone sodium at doses of 6 mg/kg.

Electrocardiograph electrodes (stainless steel needles) were set subcutaneously (one each in right and left arms, and right and left legs). Lead I or Lead II was used for recording ECG on a student physiograph. The chart speed was kept at 5 mm/sec. Heart rate was determined by counting the "R-waves" of the ECG.

A catheter (scalp vein needle gauge 24) was placed in the marginal ear vein. Heparinized saline (50 IU/ml) was filled in the catheter immediately after each injection to keep the catheter patent and to overcome its dead volume. Body temperature of the rabbit was maintained by electrical light source.

Normal heart rate of the rabbit was recorded before administration of isoprenaline. One intravenous slow infusion (for 30 sec) of a standard dose of isoprenaline (0.25 μg/kg) in saline

solution was given at intervals of 30 min and heart rate was recorded. Drug (2 mg/kg in each case except intravenous) administration to rabbits was through four routes:

1. Oral gastric tubing.
2. Intravenous bolus injection of sterile drug solution into ear vein (dose 0.2 mg/kg).
3. Intranasal instillation of 50 μl aqueous drug solution containing 0.5% w/v carboxymethylcellulose in each nostril using Hamilton microlitre syringe attached with 5-cm polyethylene tube inserted 1.7 cm inside the nostril.
4. Bioadhesive Ms (batches A, B_1, and B_2) sprayed (Maitani et al. 1989) in the nostril, in a crossover fashion on different occasions.

All nasal preparations were administered to rabbits in a supine position and the rabbits were kept in this position for 1 min after drug administration. Isoprenaline doses (0.25 μg/kg) were administered at 5, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min after MT dosing. Heart rate (beats/min) was recorded before and after every dose of isoprenaline administration.

The percent inhibition of isoprenaline-induced tachycardia was calculated by

$$\text{Percent inhibition} = (HR_0 - HR/HR_0) \times 100$$

where HR_0 is the number of heart beats induced by isoprenaline before drug administration and HR the number of heart beats shown by isoprenaline after MT administration through various routes/formulation.

Pharmacodynamic parameters** E_{max}, T_{max}, AUC and absolute bioavailability were calculated for various formulations and results from microspheres were compared with the conventional formulations.

RESULTS AND DISCUSSION

Morphology and Size of Microspheres

Morphology of the various batches of Ms prepared were found to be discrete and spherical in shape. A microphotograph of one batch of Ms is shown in Figure 1. The results (Table 2) indicated that as the amount of polymer (batches A, B_1, B_2, and B_3) in the Ms were increased, the particle size also proportionally increased. Decreasing the alginate concentration to 2% w/v resulted in clumping of microspheres, whereas high sodium alginate concentration (at 6% w/v) resulted in formation of discerte micropheres with size 84 μm. This could be attributed to an increase in the relative viscosity at higher concentration of polymer and formation of larger particles during emulsification.

The mean diameter of the prepared Ms was marginally increased with an increase in drug loading (batches A, A_1, A_2, and A_3). Similar increases in the mean diameter of microspheres also were observed with increase in calcium chloride concentration as well as cross-linking time (batches C_1, C_2, D_1, and D_2). The addition of higher amount of Ca^{2+} that is available for cross-linking the guluronic acid unit of sodium alginate results in formation of relatively more cross-linked guluronic acid. This result further increases the viscosity of the formulation medium, thereby leading to formation of larger microspheres.

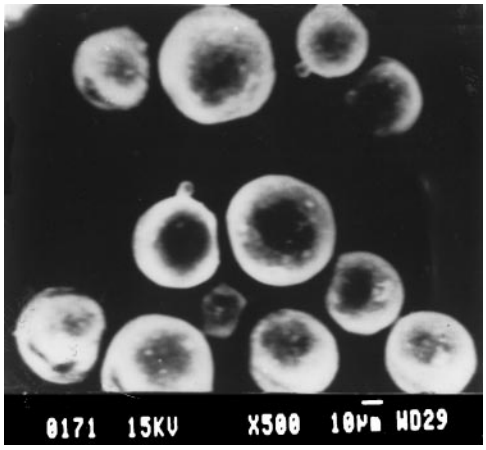

FIG. 1. Scanning electron micrograph of MT-loaded microspheres.

Incorporation Efficiency of Microspheres

The incorporation efficiencies were generally higher for batches cross-linked with 2.5% w/v calcium chloride (batch A, A_1, A_2, A_3, B_1, B_2, and B_3) for 5 min. The incorporation efficiency, however, showed, an inverse relationship with increasing calcium chloride concentration and cross-linking time (Table 2).

The incorporation efficiency increased progressively with increasing drug concentration until 40 mg/ml. An increase in Metoprolol-loading from 40 to 50 mg/ml caused a marginal decrease in the incorporation efficiency suggesting that quantity of sodium alginate becomes insufficient to entrap the drug (Lemoine et al. 1998). The incorporation efficiency also was found to be proportional to sodium alginate concentration. The method adopted for the preparation of microspheres could be responsible for the observed higher incorporation efficiency. An aqueous solution of sodium alginate–containing drug was dispersed in an organic phase to form a w/o type of emulsion. The added calcium chloride solution was merged with internal aqueous phase of alginate-containing drug resulting in formation of gel instantaneously and entrapping drug in the resultant three dimensional lattice of the ionically cross-linked alginate.

Increase in the alginate concentration resulted in the formation of larger microspheres entrapping greater amounts of the drug. The presence of more alginate overcame the problem of insufficient polymer concentration that was responsible for a decrease in entrapment efficiency when Metoprolol concentration was increased from 40 to 50 mg/ml, as discussed earlier. Other investigators have reported higher incorporation efficiencies with high molecular weight drugs in contrast to

**[Maximum % inhibition effect of isoprenaline induced heart rate (E_{max}), maximum time to achieve E_{max} (T_{max}), area under % inhibition of isoprenaline induced heart rate-time (AUC) curve].

TABLE 2
Physical characteristics of prepared MT-loaded sodium alginate microspheres

Batch code	Mean particle size (μm) (mean* ± SD)	Incorporation efficiency			Equilibrium fluid content in phosphate buffer (pH 6.6) (%) (mean* ± SD)	Bioadhesion (%) (mean* ± SEM)
		Surface associated drug (% w/w)	Entrapped drug (% w/w)	Total incorporation efficiency (% w/w)		
A	55.30 ± 18.92	23.60	53.70	77.30	64.04 ± 0.392	76.24 ± 1.178
A1	62.11 ± 17.82	25.74	60.10	80.81	63.84 ± 0.516	72.87 ± 1.873
A2	65.57 ± 18.72	26.35	55.85	82.21	64.28 ± 1.159	75.56 ± 1.890
A3	68.72 ± 21.87	28.92	42.33	71.25	65.01 ± 0.279	74.24 ± 0.992
B1	72.74 ± 21.74	15.80	68.98	86.78	61.01 ± 0.732	69.50 ± 1.270
B2	79.81 ± 16.74	19.10	69.00	88.11	57.91 ± 1.540	67.56 ± 1.876
B3	84.47 ± 20.78	24.78	63.20	91.80	50.91 ± 1.112	71.24 ± 1.890
C1	60.57 ± 20.12	19.24	34.48	53.72	61.54 ± 1.074	60.75 ± 1.779
C2	65.72 ± 19.75	24.35	23.56	47.80	58.16 ± 0.961	55.37 ± 1.654
D1	70.98 ± 21.47	21.95	35.80	57.78	64.41 ± 0.523	65.30 ± 1.720
D2	74.50 ± 20.27	25.40	24.50	49.90	60.72 ± 0.657	57.80 ± 1.092

*$n = 3$.

lower molecular weight drugs like indomethacin (Shiraishi et al. 1993) and nitrofurantoin (Hari et al. 1996). MT with a molecular weight of 684.82 can be considered a drug with high molecular weight.

Interestingly, the least concentration of calcium chloride used in the present study (2.5% w/v) was sufficient to decrease the porosity of alginate matrix as evidenced by higher entrapment efficiency of MT, which is a water soluble drug.

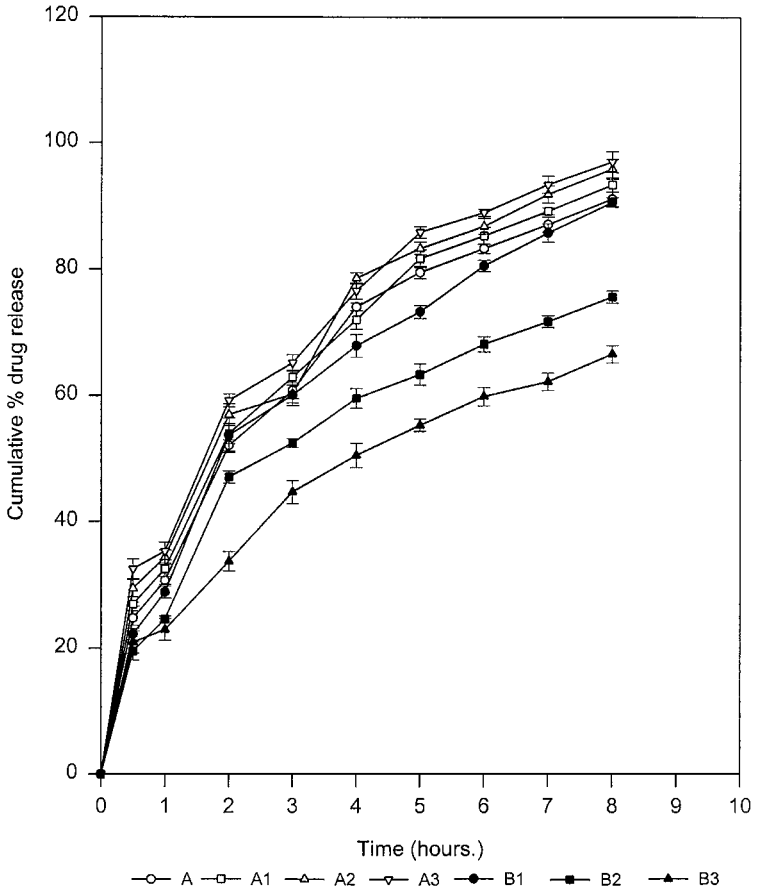

FIG. 2. In vitro release profiles of MT show the effect of drug loading and polymer concentration on drug release from microspheres (bars represent SD).

The decrease in incorporation efficiency with increase in cross-linking time and concentration of calcium chloride could be attributed to incomplete emulsification as a result of higher viscosity of the internal aqueous phase.

The swelling ability is shown in terms of equilibrium fluid content in Table 2. The prepared Ms in phosphate buffer showed equilibrium fluid contents ranging from 50.91% to 65.01%.

The study of in vitro bioadhesion revealed that the all the batches of prepared Ms had good bioadhesive property ranging from 76.24% (batch A) to 55.37% (batch C_2). The increase in the concentration of cross-linking agent ($CaCl_2$) and cross-linking time significantly altered the bioadhesive property of microspheres (batch A, C_1, C_2, D_1, and D_2).

In Vitro Release

To study the effect of drug loading on MT release, the MT was used at four different concentrations: 20 (A), 30 (A_1), 40 (A_2), and 50 (A_3) mg/ml. The release profiles for these batches are shown in Figure 2. The results indicated no significant difference ($p > .01$) between the rate and extent of drug release from the four batches and all the batches of Ms delivered around 90% of drug in 8 hr.

The effect of polymer (sodium alginate) concentrations, 2% (A), 4% (B_1), 5% (B_2), and 6% w/v (B_3), on MT release from different batches of Ms are also shown in Figure 2. A significant ($p < .01$) decrease in the rate and extent of drug release was observed with the increase in polymer concentration in Ms and is attributed to an increase in the density of the polymer matrix and in the diffusional path length that the drug molecules have to traverse.

The release of MT from these batches was characterized by an initial phase of high release (burst effect) followed by a second phase of moderate release. This biphasic pattern of release is a characteristic feature of matrix diffusion kinetics (Lemoine et al. 1998). The initial burst effect was considerably reduced with an increase in polymer concentration. The fact that an increase in sodium alginate concentration resulted in better incorporation efficiency could be the reason for the observed decrease in burst effect because the amount of surface-associated drug decreases with an increase in incorporation efficiency.

The cross-linking/ionotropic gellation of sodium alginate matrix with calcium chloride is well established. Sodium alginate is a linear copolymer consisting of $\beta(1 \rightarrow 4)$ mannuronic acid (M) and $\alpha(1 \rightarrow 4)$ L guluronic acid (G) residues. These uronic acid residues are arranged in homopolymeric block of type MM, GG,

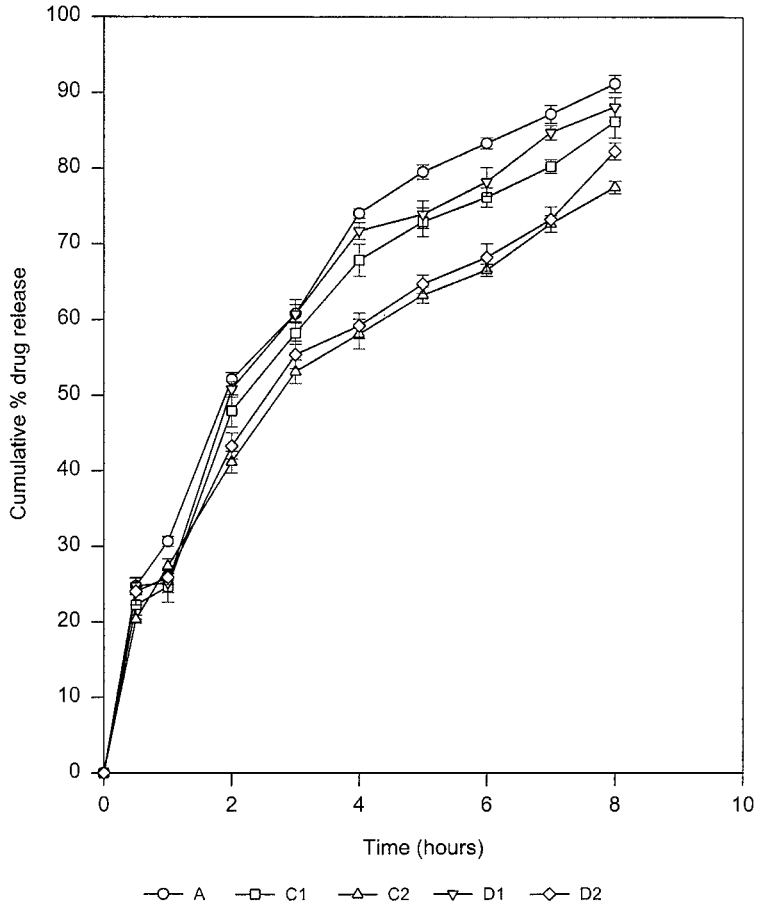

FIG. 3. In vitro release profiles of MT show the effect of cross-linking agent concentration and cross-linking time on drug release from microspheres (bars represent SD).

or heteropolymeric block of MG. The principle of cross-linking or gelation of sodium alginate with calcium chloride is based on the formation of tight junction between the GG residues.

In the present study, the effect of a cross-linking agent (calcium chloride) at three different concentrations, 2.5 (A), 5 (C_1), and 7.5% w/v (C_2), and at three different cross-linking times, 5 (A), 10 (D_1), and 25 min (D_2), on drug release from Ms also were studied.

The results shown in Figure 3 indicated that rate and extent of drug release was significantly ($p < .05$) decreased with the increase in the concentration of calcium chloride and cross-linking time that is attribute to formation of tight junction between the MM/GG residues of sodium alginate with calcium ion.

The results indicated that there was not much difference between the profiles of the batches A and D_1 with cross-linking times of 5 and 10 min, respectively. However, a more pronounced reduction in drug release was observed with batch (D_2) with 25 min cross-linking time (Figure 3).

In all the Ms other than D_1 and D_2, a cross-linking time of 5 min was allowed. The observed results indicated that the cross-linking time of 5 min might not have been sufficient for complete cross-linking to occur. Although the cross-linking of sodium alginate with calcium chloride is considered to occur instantaneously, some time should be given for the calcium chloride solution to penetrate the outer calcium alginate residue that is formed instantaneously to expeditiously cross-link with the inner sodium alginate matrix.

To investigate the drug 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 3. The examination of coefficient of determination (r^2) values indicated 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 following empirical equation (Higuchi 1963; Cardinal 1984)

$$Q(t) = a\,t^n$$

where Q(t) is a fraction of drug released after time t and a is a coefficient and n is release exponent. The values for coefficient a and the release exponent n are listed in Table 4. The values n were in the range of 0.4060–0.5120, further indicative of the drug release following a diffusion control mechanism.

TABLE 4
Coefficient and exponent of MT release according to $Q(t) = a\,t^n$ for the prepared microspheres

Batch code	Equation coefficient (a)	Release exponent (n)	Coefficient of determination (r^2)
A	0.6481	0.4995	0.9858
A1	0.6455	0.5037	0.9940
A2	0.6969	0.4864	0.9885
A3	0.6635	0.5026	0.9900
B1	0.6384	0.4974	0.9876
B2	0.7838	0.4192	0.9812
B3	0.7492	0.4060	0.9840
C1	0.6896	0.4807	0.9850
C2	0.5556	0.5120	0.9888
D1	0.7440	0.4467	0.9709
D2	0.6990	0.4700	0.9954

In Vivo Pharmacodynamic Studies

Ms containing three different concentrations of sodium alginate were selected for in vivo study. The pharmacodynamic profiles for the prepared microspheres (batch A, B_1, and B_2), nasal solution, oral solution, and intravenous (I.V.) solution are

TABLE 3
In vitro release kinetic parameters of MT-loaded sodium alginate microspheres

		Zero-order		First-order		Higuchi	
S. no.	Batch code	K (mg/h)	r	K (h^{-1})	r	K ($mg/h^{1/2}$)	r
1	A	0.6562	0.9960	−7.7012	−0.9846	0.1870	0.9923
2	A1	0.6039	0.9640	−5.9720	−0.9096	0.1725	0.9930
3	A2	0.1003	0.9549	−6.0527	−0.9841	0.0288	0.9863
4	A3	0.0978	0.9637	−4.9411	−0.9851	0.0297	0.9918
5	B1	0.1078	0.9640	−8.5650	−0.9661	0.0311	0.9873
6	B2	0.1263	0.9595	−12.5413	−0.9938	0.0361	0.9893
7	B3	0.1254	0.9568	−14.6495	−0.9733	0.0324	0.9701
8	C1	0.1069	0.9524	−8.6065	−0.9955	0.0307	0.9864
9	C2	0.1166	0.9666	−8.9510	−0.9510	0.0331	0.9897
10	D1	0.1095	0.9836	−7.7879	−0.9907	0.0308	0.9972
11	D2	0.1053	0.9669	−5.7014	−0.9528	0.0296	0.9968

K = the release rate constant; r = coefficient of correlation.

TABLE 5
Pharmacodynamic parameters for MT following administration of intranasal bioadhesive sodium alginate microspheres in rabbits ($n = 6$) in comparison to aqueous drug solution administered by different routes

Formulations	Dose (mg/kg)	E_{max} (%) (mean ± SEM)	T_{max} (min) (mean ± SEM)	AUC (0–8 h) (mean ± SEM)	Absolute bioavailability (%) (mean ± SEM)
Intravenous	0.20	87.87 ± 7.42	5.0 ± 0.0	97.24 ± 6.24	100.00
Nasal solution	2.00	65.84 ± 4.74	10.0 ± 0.0	129.48 ± 7.84	14.57 ± 1.92
Oral solution	2.00	50.23 ± 3.12	15.0 ± 0.0	82.57 ± 3.94	8.54 ± 1.72
Microspheres					
A	2.00	49.37 ± 5.12	240.0 ± 0.0	304.92 ± 19.25	35.21 ± 1.97
B_1	2.00	40.57 ± 3.25	180.0 ± 0.0	282.28 ± 12.54	32.07 ± 2.34
B_2	2.00	41.30 ± 4.07	300.0 ± 0.0	256.74 ± 17.72	28.74 ± 1.73

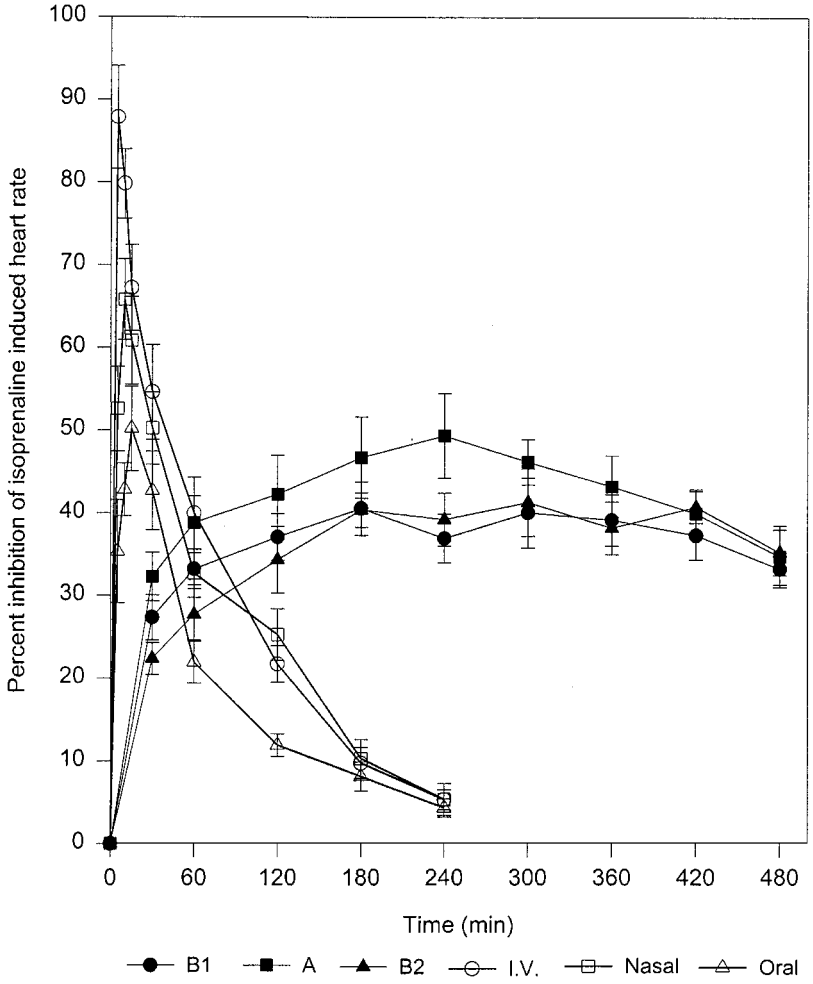

FIG. 4. Percent inhibition of isoprenaline-induced heart rate following administration of MT microspheres in rabbits in comparison to aqueous drug solution administered through different routes (bars represent SEM).

shown in Figure 4. The prepared Ms batches (A, B_1, and B_2) showed a gradual increase in percent inhibition of heart rate, followed by a maintenance of percent inhibition effect of MT on heart rate for 8 hr. This result showed a controlled and sustained effect of the drug when administered intranasally as Ms (Figure 4). Batches A, B_1, and B_2 exhibited maximum inhibitory effect of 49.37 ± 5.12, 40.57 ± 3.25, and $41.30\% \pm 4.07$ at 4, 3 and 5 hr, respectively. On the other hand, aqueous drug solutions administered through I.V., nasal, and oral routes though inhibited heart rates that varied significantly initially after administration, an inhibitory effect on the heart rate declined rapidly thereafter.

The pharmacodynamic parameters such as E_{max}, T_{max}, $AUC_{(0-8\ h)}$, and absolute bioavailability for different formulations are shown in Table 5. Although the E_{max} for nasal solution was much higher and was attained more rapidly than that for microspheres, the decline in inhibition effect on heart rate was equally rapid. This effect suggests that frequent administration of nasal solution would be required that might result in pulse type of profile, whereas the inhibitory effect in isoprenaline-induced heart rate for microsphere were more sustained and controlled. The AUC for the MS was found to be higher than that of I.V., oral, and nasal administrations. The AUC value for oral solution was much lower than AUC of I.V. and nasal administrations and is attributed to first-pass effect of MT. The absolute bioavailability of Ms batches (A, B_1, and B_2) were approximately 35.21 ± 1.97, 32.07 ± 2.37, and $28.74 \pm 1.24\%$ for batches A, B_1, and B_2 respectively, as compared with $14.57 \pm 1.92\%$ and $10.54 \pm 1.79\%$ for drug solution administered through nasal and oral route, indicating significantly ($p < .001$) high bioavailability for Ms. The results thus indicate that all the microspheres were not only able to improve the bioavailability of drug by the intranasal route due to avoidance of first-pass effect, but also were able to provide sustained and controlled delivery of MT as compared with nasal, oral, and I.V. routes.

CONCLUSION

The results of our present study clearly indicated promising potentials of sodium alginate microspheres for delivering MT intranasally and could be viewed as a potential alternative to conventional dosage forms. However, extensive pharmacokinetics and phamacodynamic studies are required to establish a correlation, if any, before establishing intranasal MT delivery as an alternative. Because sodium alginate is a biocompatable polymer, we would expect it not to cause any deleterious effect or toxic response in the nasal mucosal cavity even if used for prolonged periods. To evaluate the biocompatibility of the polymer with the nasal mucosa, appropriate histopathological studies are required.

REFERENCES

Bowersock, T. L., HogenEsch, H., Sucknow, M., Porter, R. E., Jackson, R., and Park, K. 1996. Oral vaccination with alginate microspheres system. *J. Control. Rel*. 39:209–220.

Cardinal, C. R. 1984. Drug release from matrix devices. In *Recent Advances in Drug Delivery Systems*, ed. J. M. Anderson and S. W. Kim, 229–248. New York: Plenum.

Dondeti, P., Zia, H., and Needham, T. E. 1995. In vivo evaluation of spray formulation of human insulin for nasal delivery. *Int. J. Pharm*. 122:91–105.

Hari, P. C., Chandy, T., and Sharma, C. P. 1996. Chitosan/calcium alginate microscapsules for intestinal delivery of nitrofurantoin. *J. Microencapsul*. 13:319–329.

Higuchi, T. 1963. Mechanism of sustained action medication. *J. Pharm. Sci*. 52:1145–1149.

Illum, L., Jorensen, H., Bisgaard, H., Krogsgaard, O., and Rossing, N. 1987. Bioadhesive microspheres as a potential nasal drug delivery system. *Int. J. Pharm*. 39:189–199.

Jeffery, H., Davis, S. S., and O'Hagan, D. T. 1993. The preparation and characterization of poly(lactide-co-glycolide) microparticle. II. The entrapment of model protein using a (water-in oil)-in water emulsion solvent evaporation technique. *Pharm. Res*. 10:362–367.

Kemken, J., Zieuler, A., and Muller, B. W. 1991. Investigations into the pharmacodynamic effects of dermally administered microencapsulation containing β-blockers. *J. Pharm. Pharmacol*. 43:679–684.

Lemoine, D., Wauters, F., Bouchend, S., and Preat, V. 1998. Preparation and characterization of alginate microspheres containing a model antigen. *J. Pharm. Sci*. 176:9–19.

Lennard, M. S., Silas, J. H., Freestone, S., Ramsay, L. E., Tucker, G. T., and Woods, H. F. 1983. Oxidative phenotype a major determinant of metoprolol metabolism and response. *N. Engl. J. Med*. 307:1558–1560.

Lennard, M. S., Silas, J. H., Freestone, S., Ramsay, L. E., Tucker, G. T., and Woods, H. F. 1983. Differential stereoselective of metoprolol. *Biochem. Pharmacol. Ther*. 307:732–737.

Maitani, Y., Machida, Y., and Nagai, T. 1989. Influence of molecular weight and charge on nasal absorption of dextran and DEAE-dextran in rabbits. *Int. J. Pharm*. 49:23–27.

Nagai, T., and Machida, Y. 1990. Bioadhesive dosage forms for nasal administration. In *Bioadhesive Drug Delivery System*, ed. V. Lenaerts and R. Gurny, 169–178. Boca Ration, FL: CRS Press.

Nakano, M., and Ogata, A. 1984. Examination of natural gums as matrices for sustained release of theophylline. *Chem. Pharm. Bull*. 32:782–785.

Popovici, A., and Szasz, I. 1992. Mucoadhesive gel with polyuranides for endonasal administration. In *Minutes European Symposium Buccal and Nasal Administration as an Alternative to Parenteral Administration*, ed. D. Duchene, 292–296. Paris: Editions de Sante.

Ranga Rao, K. V., and Buri, P. 1989. A novel *in situ* method to test polymers and coated microparticles for bioadhesion. *Int. J. Pharm*. 52:265–270.

Reynolds, J. P. 1993. β-Blockers. In *Martindale, The Extra Pharmacopoeia*, 5th ed., 635–636. London: Pharmaceutical Press.

Shiraishi, S., Imai, T., and Otagiri, M. 1993. Controlled release preparation of indomethacin using calcium alginate gel. *Biol. Pharm. Bull*. 16:1164–1168.

Stockwell, A. F., Davis, S. S., and Walker, S. E. 1986. In vitro evaluation of alginate gel system as sustained release drug delivery system. *J. Control. Rel*. 3:167–176.

Thanoo, B. C., and Jayakrishnan, A. 1990. Preparation of hydrogel beads by alkaline hydrolysis of crosslinked poly(methyl methacrylate) microspheres. *J. Appl. Polym. Sci*. 39:1153–1161.

Wan, L. S. C., Heng, P. W. S., and Chan, L. W. 1992. Drug encapsulation in alginate microsphere by emulsification. *J. Microencapsul*. 9:309–316.