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Development and *in vitro* evaluations of gelatin A microspheres of ketorolac tromethamine for intranasal administration

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Gelatin A microspheres (MS) of ketorolac tromethamine (KT) for intranasal systemic delivery were developed with the aim to avoid gastro-intestinal complications, to improve patient compliance, to use as an alternative therapy to conventional dosage forms, to achieve controlled blood level profiles, and to obtain improved therapeutic efficacy in the treatment of postoperative pain and migraine. Gelatin A microspheres were prepared using the emulsification-crosslinking technique. The drug was dispersed in polymer gelatin and formulated into a w/o emulsion with liquid paraffin, using glutaraldehyde as a crosslinking agent. The formulation variables were drug loading and the concentrations of polymer (gelatin), copolymer (chitosan) and the crosslinking agent. All the prepared microspheres were evaluated for physical characteristics, such as particle size, incorporation efficiency, swelling ability, *in vitro* bioadhesion on rabbit small intestine and *in vitro* drug release characteristics in pH 6.6 phosphate buffer. All the microspheres showed good bioadhesive properties. Gelatin A and chitosan concentrations, percentage of the crosslinking agent and also the drug loading affected significantly the rate and extent of drug release. The data indicated that the KT release followed Higuchi's matrix model.

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Ketorolac tromethamine (KT) is a potent non-narcotic analgesic with moderate anti-inflammatory activity (1). Clinical studies indicate that KT has a single dose efficacy, higher than morphine for postoperative pain and excellent applicability in the emergency treatment of pain (breakthrough cancer pain) and in the treatment of migraine headache (2). However, it suffers from incomplete oral absorption and exposure of the stomach to large amount of drug at a time. When administered as the conventional formulation, it causes gastrointestinal complications including ulceration and bleeding. Hence,

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several approaches have been tried to develop non-oral formulations in addition to injections (3, 4). Among the non-invasive routes, nasal administration has a promising potential as a viable alternative for systemic medication of drugs (5). Nasal deliveries of KT with drug solution and powder forms have already been reported but rapid nasal mucociliary clearance limits its sustained bioavailability (6, 7). KT, having elimination half-life of 5 h needs sustained medication. Bioadhesive microspheres give more residence time to facilitate absorption through nasal mucosa against nasal mucociliary clearance (8). Gelatin A is an acid hydrolytic product of collagen, bioadhesive and biodegradable polymer that can be used in controlled drug delivery (9). These observations prompted us to develop microspheres based on gelatin A as a promising formulation of KT for intranasal systemic administration, as an alternative route to injections and for better management of all types of pain.

EXPERIMENTAL

Materials

Ketorolac tromethamine and chitosan (purified, viscosity grade 50) were gift samples obtained from Ranbaxy (India) and CIFT (India), respectively. Gelatin A, glutaraldehyde and liquid paraffin (S.D. Fine Chem, India), Span 80 and Tween 80 (Wilson Lab, India), acetone and isopropanol (Qualigens Fine Chem, India), dihydrogen potassium orthophosphate (Glaxo, India), sodium hydroxide (E. Merck, India) were purchased. Doubly distilled water was used. All chemicals used were of analytical reagent grade.

Phosphate buffer pH (6.6) was prepared by mixing 250 mL of potassium dihydrogen orthophosphate (0.2 mol L^{-1}) (Glaxo, India) with 82 mL of sodium hydroxide (0.2 mol L^{-1}) (E. Merck, India) and making up the total volume to 1000 mL with distilled water. The final pH was adjusted to 6.6 ± 0.05 using a digital pH meter (Toshniwal Pvt. Ltd, India).

Methods

Preparation of microspheres. – The formulae for MS are shown in Table I. They were prepared by the emulsion-crosslinking method (10). The drug (KT) was dispersed in an aqueous gelatin A solution (10%, *m*/*V*, preheated at 40 °C) and the dispersion was added dropwise to liquid paraffin while stirring the mixture at 1500 rpm at 40 °C for 10 min. This gave water in oil (w/o) emulsion. Stirring was continued for a further 10 min at 15 °C and the microspheres were washed three times with acetone and isopropanol, respectively, air-dried, and then dispersed in 5 mL of aqueous glutaraldehyde-saturated toluene solution (25%,*V*/*V*) at room temperature for 3 h to allow crosslinking. The resulting microspheres were finally washed three times with water by centrifugation and freeze-dried. The chitosan-gelatin based microspheres were prepared by exactly the same method as mentioned above except that the chitosan solution prepared in 1% (*V*/*V*) glacial acetic acid was first mixed with the gelatin solution, the drug was dispersed into it and then emulsification was followed as above (10). Percentage composition of chitosan and gelatin is given in Table I. Formulation variables, such as the amount of the drug

Table I. Formulae for different batches of gelatin A microspheres of ketorolac tromethamine

MS	Gelatin A (%, *m/V*)	Chitosan (%, *m/V*)	Drug (mg mL^{-1})	Glutaraldehyde (%, *V/V*)
A1	10	–	20	25
A2	7.5	–	20	25
A3	12.5	–	20	25
B1	10	–	10	25
B2	10	–	30	25
C1	10	–	20	20
C2	10	–	20	15
C3	10	–	20	–
D1	8	2	20	25
D2	6	4	20	25
D3	4	6	20	25

and different concentrations of gelatin, chitosan and crosslinking agent were assayed to obtain the microspheres of optimum properties and characteristics.

Size and shape of microspheres. – All the MS were evaluated with respect to their size and shape using a microscope (Olympus, NWF 10x, India) fitted with an ocular micrometer and a stage micrometer.

Incorporation efficiency. – To determine the incorporation efficiency, 25 mg of KT loaded MS were washed with 10 mL of phosphate buffer (pH 6.6) containing 0.1% (*V/V*) Tween 80 to remove the surface associated drug. The MS were then digested in 10 mL of 0.1 mol L^{-1} HCl for 12 h at room temperature (25 ± 2 °C) to release the entrapped drug. Drug content was analyzed spectrometrically (Jasco 9800, Japan) at 322 nm (11). Percent of total incorporation efficiency was calculated as follows:

total incorporation efficiency (%, *m/m*) =
= surface associated drug (%, *m/m*) + entrapped drug (%, *m/m*)

Swelling ability. – MS were also evaluated with regard to their swelling ability (in triplicate) by allowing them to swell to their equilibrium in phosphate buffer (pH 6.6) and by estimating their equilibrium fluid content using a reported method (12).

In vitro *bioadhesion test.* – MS were further studied for *in vitro* bioadhesion using the method described in the literature (13). Unfasted albino rabbits (2–2.5 kg) (Zoological Emporium, BHU, India) were anaesthetized with pentobarbital (gift sample from Biologicals, E. Ltd, India) (75 mg kg^{-1} body mass *i.p.*).The intestine was dissected and washed with phosphate buffer (pH 6.6) at room temperature (25 ± 2 °C). The tissue was used within 24 h after dissection. All the procedures were carried out under the guidance of the Ethical Committee, BHU, India. MS (50 mg) were placed on albino rabbit small intestines. The intestines with MS were placed in a dessicator maintained at 80% relative humidity and room temperature (25 ± 2 °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 °C in a hot air oven. The ratio of applied and adhered microspheres was computed as percent bioadhesion.

In vitro *release study. – In vitro* evaluation of MS (in triplicate) was done using our earlier reported standard method (14). MS (20 mg) were dispersed in 400 mL of phosphate buffer (pH 6.6) in a beaker and maintained at 37 ± 0.2 °C under continuous stirring (100 rpm). At selected time intervals, 5-mL samples were withdrawn 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 a constant volume of the receptor compartment. The samples were analyzed spectrometrically at 322 nm. The released drug content was computed from the calibration curve of KT.

RESULTS AND DISCUSSION

Physical characteristics of MS are shown in Table II. When observed microscopically, MS were found to be discrete and spherical in shape. It was observed that as the amount of polymer and drug increased in the microspheres, the particle size also increased proportionally. However, the concentration of the crosslinking agent did not significantly affect the particle size. The increase in the particle size observed with an increase in the polymer and/or drug concentrations could be attributed to an increase in the relative viscosity of the medium, which may have caused and increase in the interfacial tension (14). This resulted in the formation of larger particles during emulsification. However, at 12.5% *(m/V)* of gelatin A, a large variation in the particle size (40.0 ± 12.25 μm)

Table II. Physical characteristics of prepared microspheres of ketorolac tromethamine

MS	Particle size (μm)[a,b]	Incorporation efficiency			Equilibrium fluid content (%)[b,c]	Bioadhesion (%)[a,c]
		Surface drug (%, *m/m*)	Entrapped drug (%, *m/m*)	Total incorporation efficiency (%, *m/m*)		
A_1	32.5 ± 6.9	25.6	12.0	37.6	70.8 ± 0.5	87.4 ± 1.5
A_2	20.0 ± 3.5	14.0	11.5	25.5	68.2 ± 0.6	84.3 ± 1.6
A_3	40.0 ± 12.3	28.6	10.9	39.5	71.5 ± 2.8	86.2 ± 1.8
B_1	28.0 ± 5.0	18.8	8.5	27.4	72.1 ± 0.5	88.8 ± 2.2
B_2	36.5 ± 7.0	23.4	20.1	43.5	70.2 ± 0.8	86.5 ± 3.3
C_1	30.5 ± 7.0	24.0	11.0	35.0	80.4 ± 0.6	89.9 ± 2.0
C_2	31.5 ± 8.3	19.5	9.4	28.9	84.7 ± 0.7	90.8 ± 2.3
C_3	28.0 ± 10.0	20.3	8.1	28.4	88.0 ± 2.0	86.0 ± 0.9
D_1	34.0 ± 8.3	13.2	26.0	39.3	76.5 ± 1.2	94.8 ± 0.6
D_2	44.0 ± 6.0	12.1	28.2	40.3	74.2 ± 0.8	96.4 ± 1.3
D_3	50.0 ± 5.0	15.9	30.4	46.3	74.2 ± 0.4	97.5 ± 1.4

[a] Mean ± SD value.
[b] $n = 100$
[c] $n = 3$

was observed. Incorporation of chitosan yielded larger microspheres; this might be due to the more viscous nature of chitosan than gelatin solution.

The drug incorporation efficiency was found to be proportional to polymer concentration and drug loading. The increase in drug incorporation with an increase in drug loading may be due to an increase in the drug concentration. Similar behavior with an increase in the polymer concentration may be attributed to increased viscosity, which results in the formation of larger microspheres, thus increasing the incorporation efficiency. Incorporation of chitosan resulted in more drug entrapment than the surface associated drug. The swelling ability of MS was more affected by different concentrations of the crosslinking agent compared to the chitosan concentration and the amount of the drug (Table II).

Results shown in Table II indicate that the gelatin concentration and the crosslinking agent marginally altered the bioadhesive properties of MS. However, incorporation of chitosan increased considerably the bioadhesive property of MS. The improved bio-

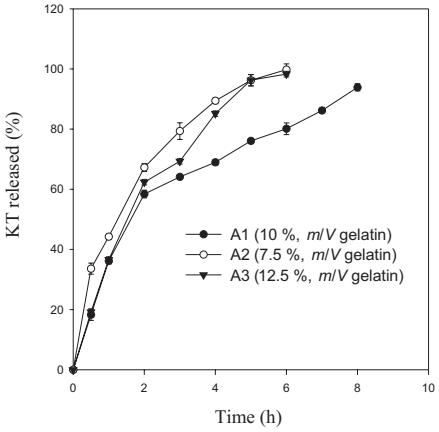

Fig. 1. *In vitro* release (mean ± SD, $n = 3$) profiles of ketorolac tromethamine in phosphate buffer (pH 6.6) from gelatin A microspheres containing different concentrations of gelatin A.

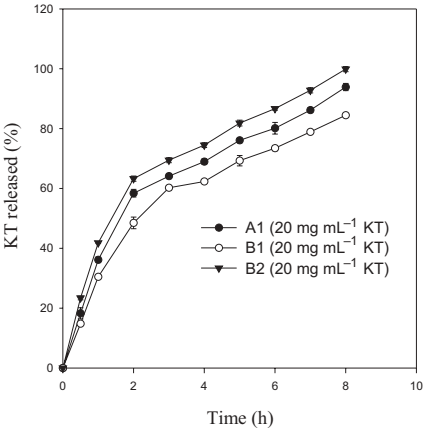

Fig. 2. *In vitro* release (mean ± SD, $n = 3$) profiles of ketorolac tromethamine in phosphate buffer (pH 6.6) from gelatin A microspheres containing different drug concentrations.

adhesive property of MS with chitosan is attributed to the cationic nature of chitosan (mucin being anionic) (15). Gelatin A significantly contributed to bioadhesion as well (16): all the microspheres exhibited good bioadhesive properties.

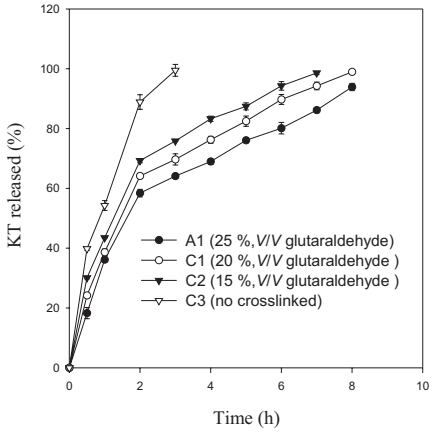

Fig. 3. *In vitro* release (mean ± SD, $n = 3$) profiles of ketorolac tromethamine in phosphate buffer (pH 6.6) from gelatin A microspheres crosslinked with different concentrations of glutaraldehyde.

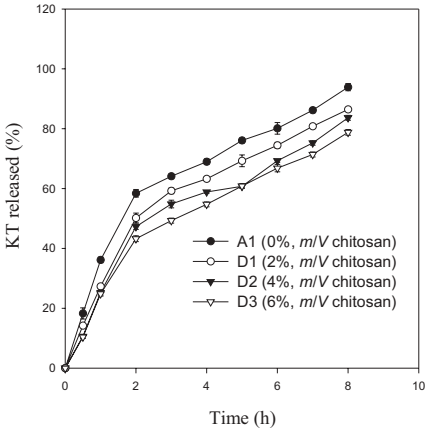

Fig. 4. *In vitro* release (mean ± SD, $n = 3$) profiles of ketorolac tromethamine in phosphate buffer (pH 6.6) from gelatin A microspheres incorporated with different concentrations of chitosan.

The *in vitro* release profiles of KT from MS are shown in Figs. 1–4. The drug release profiles observed in all the MS exhibited a biphasic pattern of drug release, an initial burst effect due to immediate release of the surface associated drug, followed by a slow and controlled release phase resulting from the controlled diffusion of entrapped drug. A significant ($p < 0.01$) decrease in the rate and extent of drug release was observed with the increase in gelatin concentration in MS (Fig. 1) and is attributed to the increase in the density of the polymer matrix and also an increase in the diffusional path length that the drug molecules have to traverse. *In vitro* drug release proportionally increased with in-

creasing the drug concentration (Fig. 2). Not crosslinked MS, C_3, released about 100% of the drug within 4 h. As expected (17), with an increase in the crosslinking agent concentration, a respective decrease in the rate and extent of drug release was observed (Fig. 3). Incorporation of chitosan in MS resulted in a lower burst effect and prolonged drug release compared to MS A_1, which does not contain chitosan (Fig. 4). This might be due to more drug entrapment within the MS (Table II) and also to the more insoluble property of chitosan-based MS than the gelatin-based MS; this might have caused the swelling-controlled drug release from the MS (18). Our observations clearly indicated that the in-

Table III. Kinetics of in vitro *ketorolac tromethamine release from gelatin A microspheres*

MS	Zero-order		First-order		Higuchi	
	k (mg h^{-1})	R	k (h^{-1})	R	k (mg $h^{-1/2}$)	R
A1	0.1029	0.9420	–7.7909	0.9713	0.0297	0.9798
A2	0.0684	0.9580	–3.5592	0.9569	0.0221	0.9902
A3	0.0789	0.9581	–3.4522	0.9735	0.0239	0.9881
B1	0.1067	0.9443	–1.0190	0.9927	0.0309	0.9841
B2	0.1015	0.9441	–5.2684	0.9406	0.0293	0.9814
C1	0.0989	0.9440	–4.3621	0.9440	0.0286	0.9827
C2	0.0990	0.9308	–3.7500	0.9623	0.0288	0.9762
C3	0.0602	0.9271	–2.8471	0.8023	0.0212	0.9710
D1	0.1067	0.9499	–9.5515	0.9893	0.0307	0.9855
D2	0.1135	0.9529	–1.0877	0.9748	0.0325	0.9820
D3	0.1177	0.9662	–12.0731	0.9848	0.0335	0.9909

k – release rate constant, R – coefficient of correlation

Table IV. Coefficient and exponent of ketorolac tromethamine release from gelatin A microsphere[a]

MS	Equation coefficient (a)	Release exponent (n)	R^2
A1	0.0361	0.5369	0.9722
A2	0.0445	0.5270	0.9748
A3	0.0435	0.5260	0.9873
B1	0.0280	0.5700	0.9758
B2	0.0463	0.5021	0.9761
C1	0.0515	0.4890	0.9833
C2	0.0676	0.4489	0.9823
C3	0.1035	0.3891	0.9396
D1	0.0303	0.5538	0.9823
D2	0.0281	0.5575	0.9765
D3	0.0234	0.5860	0.9824

R^2 – Coefficient of determination
[a] according to $Q(t) = a\ t^n$

corporation of chitosan gave a better bioadhesive property and controlled drug release. Chitosan is reported to show an absorption promoting effect in mucosal delivery (19) and, KT being a drug of incomplete oral absorption, it is expected that the developed MS will exhibit better mucosal bioavailability.

To examine the drug release kinetics and 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 III. The coefficient of determination (R^2) values (calculated from the plots of Q *vs.* t for zero-order, *log* $(Q_0 - Q)$ *vs.* t for first-order and Q *vs.* $t^{1/2}$, respectively, where Q is the amount of drug released at time t, $(Q_0 - Q)$ is the amount of the drug remaining after time t) were much closer to 1 for the Higuchi kinetics, thus indicating that the drug release from MS followed a diffusion controlled mechanism. To understand the mechanism of diffusion controlled release of KT from MS, the results were further analyzed according to equation (20):

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

where $Q(t)$ is the fraction of the drug released after time t and a denotes a coefficient, n is the release exponent. When $n \leq 0.5$, this indicates a quasi-Fickian diffusion mechanism, for $n > 0.5$, an anomalous non-Fickian solute diffusion is observed, whereas $n = 1$ indicates zero-order kinetics. Values for a and n are listed in Table IV. The values of n were in the range of 0.3891–0.5860, which is a further indication of the diffusion-controlled drug release.

CONCLUSIONS

The gelatin A microspheres exhibited a significant bioadhesive property and could potentially be used as bioadhesive microspheres for controlled and sustained intranasal systemic delivery of KT. Further, their potential to improve KT bioavailability through the nasal route could be established by *in vivo* evaluation of MS in animals and/or humans.

REFERENCES

1. W. H. Rooks, The pharmacological activity of ketorolac tromethamine, *Pharmacotherapy* **10** (1990) 30S-32S.
2. J. R. Andrade, M. Maslanka, T. Maneatis, L. Bynum and M. Burchmore, The use of ketorolac tromethamine in the management of postoperative pain, *Orthopedics* **17** (1994) 157–166.
3. S. D. Roy and E. Manoukian, Permeability of ketorolac acid and its ester analogs (prodrug) through human cadaver skin, *J. Pharm. Sci.* **83** (1994) 1548–1553.
4. K. Park, D. Verotta, S. K. Gupta and L. B. Sheiner, Passive versus electrotransport facilitated transdermal absorption of ketorolac, *Clin. Pharmacol. Ther.* **63** (1998) 303–315.
5. D. Duchene and G. Ponchel, Nasal administration: A tool for tomorrow's systemic administration of drugs, *Drug Dev. Ind. Pharm.* **19** (1993) 101–122.
6. G. Santus, R. Rivolta, G. Bottoni, B. Testa, S. Canali and S. Peano, Nasal formulations of ketorolac tromethamine: technological evaluation-bioavailability and tolerability in rabbits, *Farmaco* **48** (1993) 1709–1723.

7. M. Quadir, H. Zia and T. E. Needham, Development and evaluation of nasal formulations of ketorolac, *Drug Deliv.* **7** (2000) 223–229.
8. L. Ryden and P. Edman, Effect of polymers and microspheres on the nasal absorption of insulin in rats, *Int. J. Pharm.* **83** (1992) 1–10.
9. Y. Tabata and Y. Ikada, Protein release from gelatin matrixes, *Adv. Drug Deliv. Rev.* **31** (1998) 287–301.
10. Y. Tabata and Y. Ikada, Synthesis of gelatin microsphere containing interferon, *Pharm. Res.* **6** (1989) 422–427.
11. *United States Pharmacopoeia 24*, United States Pharmacopoeial Convention, Rockville 2001, p. 948.
12. B. C. Thanoo and A. Jayakrishnan, Preparation of hydrogel beads by alkaline hydrolysis of crosslinked poly(methylmethacrylate) microspheres, *J. Appl. Polym. Sci.* **39** (1990) 1153–1161.
13. K. V. Ranga Rao and P. Buri, A novel in situ method to test polymers and coated microparticles for bioadhesion, *Int. J. Pharm.* **52** (1989) 265–270.
14. C. Sankar, M. Rani, A. K. Srivastava and B. Mishra, Chitosan based pentazocine microspheres for intranasal systemic delivery – development and biopharmaceutical evaluation, *Pharmazie* **56** (2001) 223–226.
15. H. Park, M. Amiji and K. Park, Mucoadhesive hydrogel effective at neutral pH, *Proc. Int. Symp. Control. Rel. Bioact. Mater.* **16** (1989) 217–218.
16. K. Morimoto, H. Katsumata, T. Yabuta, K. Iwanaga, M. Kakemi, Y. Tabeta and Y. Ikada, Evaluation of gelatin microspheres for nasal and intramuscular administrations of salmon calcitonin, *Eur. J. Pharm. Sci.* **13** (2001) 179–185.
17. B. C. Thanoo, M. C. Sunny and A. Jayakrishnan, Cross-linked chitosan microspheres: preparation and evaluation as a matrix for the controlled release of pharmaceuticals, *J. Pharm. Pharmacol.* **44** (1992) 283–286.
18. N. A. Peppas and N. M. Franson, The swelling interface number as a criterion for prediction of diffusional solute release mechanisms in swellable polymers, *J. Polym. Sci.* **21** (1983) 983–997.
19. P. Artursson, M. Lindmark, S. S. Davis and L. Illum, Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (Caco-2), *Pharm. Res.* **11** (1994) 1358–1361.
20. C. R. Cardinal, *Drug Release From Matrix Devices*, in *Recent Advances in Drug Delivery Systems* (Eds. J. M. Anderson and S. W. Kim), Plenum, New York 1984, pp. 229–248.

S A Ž E T A K

Priprava i *in vitro* evaluacija mikrosfera ketorolak trometamina sa želatinom A za intranazalnu primjenu

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Pripravljene su želatinske mikrosfere ketorolak trometamina za intranazalnu primjenu kao alternativa uobičajenim ljekovitim oblicima, s ciljem da se izbjegnu gastro-intestinalne nuspojave, poboljša podnošljivost od strane pacijenta, postigne kontrolirana koncentracija ljekovite tvari u krvi te da se poboljša učinkovitost u terapiji postoperativnih bolova i migrene. Mikrosfere su pripravljene metodom emulzifikacijskog umrežavanja. Od ljekovite tvari dispergirane u želatini i tekućeg parafina pripravljena je emulzija tipa voda/ulje pomoću glutaraldehida kao sredstva za umrežavanje. U pripravcima je varirana količina ljekovite tvari, polimera (želatine), kopolimera (kitozana) i sredstva za umrežavanje. Određena je veličina čestica mikrosfera, količina ljekovite tvari i sposobnost bubrenja, *in vitro* bioadhezija na sluznici tankog crijeva zeca te *in vitro* oslobađanje pri pH 6,6 u fosfatnom puferu. Sve mikrosfere imaju dobra bioadhezivna svojstva.

Na oslobađanje ljekovite tvari značajno utječu koncentracija želatine A, kitozana, sredstva za umrežavanje i udio ljekovite tvari. Oslobađanje ljekovite tvari sljedilo je Higuchijev matriks model.

Ključne riječi: ketorolak trometamin, želatina A, kitozan, intranazalna primjena, mikrosfere

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