ORIGINAL ARTICLES

Induakaka Ipcowala College of Pharmacy[1], New Vallabh Vidyanagar, Anand; A. R.College of Pharmacy & G. H. Patel Institute of Pharmacy[2], Vallabh Vidyanagar, Anand; Arihant School of Pharmacy & Bioresearch Institute[3], Gandhinagar, Gujarat, India

Development and characterization of mucoadhesive microspheres for nasal delivery of ketorolac

C. D. Nagda[1], N. P. Chotai[2], D. C. Nagda[2], S. B. Patel[1], U. L. Patel[3]

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Dr. Chotai N. P., A.R.College of Pharmacy & G.H.Patel Institute of Pharmacy, Vallabh Vidyanagar, Anand, Gujarat, India-388120
pharmacist_chotai2002@yahoo.co.in

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The objective of the present investigation was to prepare mucoadhesive microspheres of ketorolac for nasal administration by means of a solvent evaporation technique using carbopol (CP), polycarbophil (PL) and chitosan (CS) as mucoadhesive polymers. The prepared microspheres were characterized for morphology, swelling behavior, mucoadhesion, interaction studies, drug encapsulation efficiency, *in vitro* drug release, release kinetics, and *ex vivo* nasal cilio toxicity studies. The effects of various process variables on the particle size of the microspheres were investigated. Drug encapsulation efficiency and particle size of the microspheres ranged from 52–78% w/w and 14–46 μm respectively. Interaction studies revealed that there were no drug-polymer interactions. The *in vitro* release profiles showed prolonged-release of the drug. *In vitro* release data showed a good fit with the Higuchi model, and indicated Fickian diffusion. No severe damage was found to the integrity of nasal mucosa after *ex vivo* experiments.

1. Introduction

Ketorolac tromethamine (KT), a pyrrolizine carboxylic acid derivative, is a potent anti-inflammatory drug. This non-steroidal and non-narcotic drug is administered systemically (*via* oral and parenteral routes) for the control of mild to moderate pain as well as some post-operative and cancer pain (Brocks and Jamali 1992). Administration of this non-selective COX inhibitor by the oral route causes many gastrointestinal side effects, e.g. nausea, vomiting, gastric irritation, peptic ulceration and bleeding, limiting its clinical use, when ingested as a 20 mg single daily dose or in divided doses (Wagner et al. 2007; Buckely and Brogden 1990). The management of moderate to severe pain requires the maintenance of a consistent therapeutically effective drug concentration in the body over a period of time. Sustained-release dosage forms deliver the drug at a consistent rate over an extended period of time. KT has a fairly short half life of 4–6 h (Mroszczak et al. 1987). Therefore, it is imperative to design a prolonged release dosage form to reduce frequency of dosing and adverse effects, especially since the duration of treatment is typically longer for NSAIDs (Sankar and Mishra 2003; Nagda et al. 2009; Basu et al. 2010). Studies of sustained release formulations of KT, such as sustained release tablets (Vatsaraj et al. 2002), osmotic tablets (Arora et al. 2002), liposomes (Rouzi et al. 2005), microspheres (Rokhade et al. 2006) and non-oral formulations such as transdermal (Amrish and Sharma 2009), ocular gel (Karatas and Baykara 2006), parenteral microspheres (Puri and Bansal 2004; Sinha and Trehan 2005; Mathew et al. 2007), nasal powder (Quadir et al. 2000), nasal gel (Chelladurai et al. 2008) and nasal microspheres (Sankar and Mishra 2003), have been reported by many researchers. Nasal delivery of KT with drug solution and powder forms have already been reported but rapid nasal mucociliary clearance limits its absorption and thereby affects the bioavailability.

Nasal drug delivery for systemic effects has been practised since antiquity. However, over the past two decades, the nasal route has been used as an alternative to parenteral injections (Ugwoke et al. 2005). In fact, there are an increasing number of nasally administered dosage forms for systemic application currently on the market (Ugwoke et al. 2005; Jadhav et al. 2007). The nasal route is advantageous because of the rapid absorption of drug molecules across the nasal membrane and the relative ease of administration (Ugwoke et al. 2001). Many small molecules like dihydroergotamine, metoclopramide, butorphanol tartrate, and sumatriptan succinate, as well as larger molecules such as vitamin B12, vasopressin, and calcitonin, have been successfully delivered intranasally (Ugwoke et al. 2005).

Limitations of nasal drug delivery include possible local tissue irritation, and rapid clearance of the therapeutic agent from the site of absorption that may alter the nasal bioavailability of drugs significantly. To overcome the rapid removal of the drug from site of absorption, the addition of bioadhesive materials has been investigated (Harris et al. 1989; Nagai et al. 1984). Mucoadhesive microspheres provide more residence time to facilitate absorption through the nasal mucosa against nasal mucociliary clearance (Jain et al. 2004).

The aim of the present study was to design suitable microsphere formulations that allow absorption through the nasal mucosa for polar NSAIDs, such as ketorolac tromethamine. The microspheres were prepared by solvent evaporation techniques using three different mucoadhesive polymers, carbopol, polycarbophil and chitosan. The microspheres prepared were characterized

Table 1: Effect of experimental variables on particle size

Variables		Mean particle size (μm)		
		CP microspheres	PL microspheres	CS microspheres
Drug to polymer ratio	1:3	19.84 ± 1.59	19.40 ± 1.92	20.82 ± 2.13
	1:4	23.89 ± 3.41	24.61 ± 2.01	25.45 ± 2.22
	1:5	28.05 ± 1.62	29.91 ± 2.41	30.41 ± 2.57
Surfactant concentration (%w/w)	2	28.05 ± 1.62	24.61 ± 2.01	30.41 ± 2.57
	3	22.74 ± 2.71	19.23 ± 1.51	21.38 ± 1.64
	4	15.82 ± 1.93	14.42 ± 2.97	16.52 ± 2.10
Volume of processing medium (mL)	100	42.15 ± 3.81	46.12 ± 4.01	46.12 ± 4.01
	250	28.05 ± 1.62	24.61 ± 2.01	30.41 ± 2.57
	500	20.18 ± 1.61	17.39 ± 1.40	17.39 ± 1.40
Stirring speed (rpm)	800	36.81 ± 2.49	37.90 ± 1.75	37.90 ± 1.75
	1000	28.05 ± 1.62	24.61 ± 2.01	30.41 ± 2.57
	1200	19.53 ± 1.20	22.40 ± 2.15	22.40 ± 2.15

Standard conditions: Drug-to-polymer ratio = 1:5; surfactant concentration = 2% w/w; volume of processing medium = 250 mL; and stirring speed = 1000 rpm

for their surface morphology, swelling behavior, mucoadhesion, drug release profile and nasal ciliotoxicity of the prepared formulations.

2. Investigations, results and discussion

2.1. Preparation of mucoadhesive microspheres

It is important that the size of the microspheres for nasal delivery should be in the range of 10 − 180 μm, since particles below 10 μm could be carried with the airstream down into the lungs (Pereswetoff-Morath 1998; Brime et al. 2000). Larger particles will mainly deposit in the anterior unciliated portion of the nose. Hence, particle size distribution is a vital factor in the characterization of nasal microspheres (Rathananand et al. 2007). The process and formulation parameters were varied to study the effect on mean particle size as shown in Table 1. Some parameters such as drug concentration (0.5 g) and volume of non-solvent (50 ml) were kept constant to study the influence of other parameters on particle size.

2.2. Effects of processing parameters on microsphere characteristics

2.2.1. Effect of drug-to-polymer ratio

The drug-to-polymer ratio appears to influence the particle size of microspheres, as shown in Table 1. The mean particle size of the microspheres significantly increased with increase in the polymer concentration and was in the range of 19 to 30 μm. This observation may be because of the increase in viscosity of the droplets (due to the increase in concentration of polymeric solution). This increase is high enough to make dispersion and subdivision of droplets difficult. Increase in mean particle size due to increased viscosity of the polymer solution has also been reported by Dubey et al for chitosan microspheres (Dubey and Parikh 2004).

2.2.2. Effect of emulsifier concentration

Microspheres were prepared with various concentrations of Span 80 ranging from 1 to 4%. The main function of an emulsifier in droplet stabilization is to form a thin film around the droplets to prevent their coalescence (Dinarvand et al. 2004). These droplets are stabilized by the use of Span 80 (2% w/w). At concentrations lower than 2%, the amount of stabilizer may not be sufficient to cover the entire body of droplets. The lowest concentration of Span 80 required to form the stable emulsion was found to be 2% and, as the concentration increased from 2% to 4%, the mean particle size was reduced (Table 1).

2.2.3. Effect of processing medium

It can be seen from Table 1 that the mean particle size of microspheres decreased with increase in volume of processing medium from 100 to 500 ml. As the volume of the internal phase is decreased, the shearing efficiency of the mixer probably decreases, which, in turn, produces larger microspheres, and the mean distance between the droplets decreases (Dinarvand et al. 2004). This may, in turn, increase the chances of coalescence between droplets, which fuse together and cause aggregation of the microspheres prepared (Reddy et al. 1990). Volumes of

Table 2: Effect of drug to polymer ratio on encapsulation efficiency, yield and mucoadhesion

Polymer type	Batch	Drug to polymer ratio	Practical drug content (%)*	Encapsulation efficiency* (%)	Yield (%)*	Mucoadhesion* (%)
	SEKTC1	1:3	15.12 ± 0.67 (25)	60.51 ± 2.67	52.58 ± 2.16	66 ± 3.61
CP	SEKTC2	1:4	13.91 ± 0.55 (20)	69.44 ± 2.72	62.13 ± 1.92	77 ± 4.36
	SEKTC3	1:5	12.53 ± 0.58 (16.5)	75.84 ± 3.51	67.81 ± 2.19	82 ± 3.06
	SEKTP1	1:3	14.11 ± 0.56 (25)	56.37 ± 2.25	48.20 ± 3.49	69 ± 2.65
PL	SEKTP2	1:4	13.45 ± 0.47 (20)	67.15 ± 2.37	60.31± 2.91	77 ± 4.04
	SEKTP3	1:5	8.67 ± 0.59 (16.5)	52.49 ± 3.56	56.02 ± 2.54	84 ± 4.0
	SEKTS1	1:3	15.53 ± 1.01 (25)	62.21 ± 4.04	58.01 ± 4.02	68 ± 1.73
CS	SEKTS2	1:4	14. 31 ± 0.74 (20)	71.48 ± 3.71	61.48 ± 2.54	73 ± 3.21
	SEKTS3	1:5	12.99 ± 0.47 (16.5)	78.62 ± 2.87 Ψ	69.16 ± 3.12	86 ± 2.51 Ψ

*Each observation is the mean (± SD) of three determinations. Values in parenthesis indicate theoretical drug content. Ψ $p < 0.05$, Significant difference compared with CP and PL microspheres. Standard conditions: surfactant concentration = 2% w/w; volume of processing medium = 250 mL; and stirring speed = 1000 rpm

processing medium of 100 ml and 500 ml caused spheres of irregular morphology. In these proportions, the microspheres obtained were discrete, but the high viscosity gradient of liquid paraffin had an impact on surface morphology. The spheres were shriveled due to leaching of liquid paraffin through the pores during recovery. However, a volume of processing medium of 250 ml resulted in the formation of uniform microspheres without affecting their morphology (Shanmuganathan et al. 2008).

2.2.4. *Effect of stirring speed*

Stirring speed is a parameter of primary importance in the emulsification step because it provides the energy to disperse the aqueous phase into the oil phase. When the stirring rate was increased, the mean particle size of microspheres was decreased (Table 1). Higher stirring speeds result in high shear and kinetic energy and thus prevent particle agglomeration (Vivek et al. 2007). On decreasing the stirring speed from 1200 to 800 rpm, we noticed an increase in mean particle size and agglomeration of the microspheres. Nevertheless, a stirring speed of 1000 rpm was found to be optimal, yielding microspheres of uniform size and with a narrow size range and spherical shape. The results clearly demonstrate that the mean particle size of microspheres was inversely proportional to the stirring speed, as increasing the stirring speed resulted in small sized microspheres (Shanmuganathan et al. 2008).

2.3. *Yield and encapsulation efficiency*

The yield and entrapment efficiency of drug loaded microspheres of all the batches are shown in Table 2. All the prepared batches showed good encapsulation efficiency ranging from 52 to 78%. When the microspheres yields were compared with each other, the results showed that microsphere yield increased with increasing amount of polymer (Table 2).
One-way ANOVA results indicated that CS concentration has a significant impact on encapsulation efficiency. Significant differences were obtained for each level of CS concentration for KT microspheres. These findings showed that the effect was considerable at the lowest and highest CS concentration. Microspheres based on CS revealed significant differences in encapsulation efficiency ($p < 0.05$); in particular SEKTC3 microspheres had the highest encapsulation efficiency while SEKTP3 showed the lowest (Table 2). The encapsulation efficiency of prepared microspheres increased with increasing drug to polymer ratio except for PL microspheres. Higher polymer concentration in the emulsion droplets led to enhanced efficiency of KT encapsulation, which could be due to the high viscosity of the aqueous phase tending to restrict migration of the inner aqueous/drug phase to the external oil phase and enhancing drug entrapment efficiency (Maiti et al. 2009).

2.4. *Scanning Electron Microscopy*

The polymeric composition of the microspheres has no influence on their morphology, as is illustrated in Fig. 1. SEM micrographs of drug loaded microspheres and blank microspheres showed similar morphology (data not reported). The SEM photomicrographs of the microspheres revealed that they are spherical, nonporous, and uniform with a smooth surface. The particles appeared to be aggregate in nature with no evidence of collapsed particles. Normally, microspheres obtained from natural polymers are not perfectly spherical because of variations in the molecular weight and other properties of the polymer, but we obtained microspheres with a uniformly smooth surface, with no deformed surfaces. This may be because of the low viscosity of the light liquid paraffin employed as the external phase. The microspheres formed may not have experienced much resistance from the dispersion medium due to its low viscosity (Mathew et al. 2007). Incorporation of KT in the polymeric network had no influence on the surface or morphological characteristics of microspheres prepared by the solvent evaporation method.

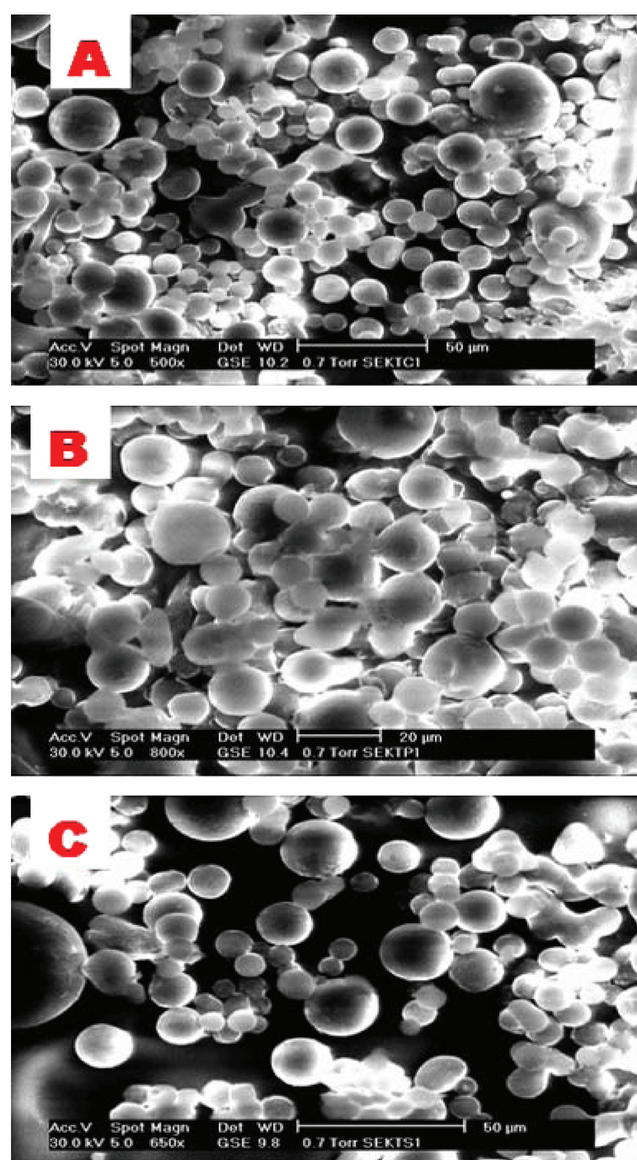

Fig. 1: Scanning electron photomicrographs of drug-loaded microspheres: (A) microspheres of CP (SEKTC1); (B) microspheres of PL (SEKTP1); (C) microspheres of CS (SEKTS1)

2.5. *Swelling index*

An important requirement for polymers is their ability to swell by absorbing water (here from the mucus layer in the nasal cavity) thereby forming a gel like layer in which interpenetration of polymers and glycoprotein chains can take place and binding can occur rapidly. The swelling index is shown in terms of fluid intake capacity and depends on polymer content. The CP and PL microspheres showed the highest degree of swelling (428% and 408% respectively) in comparison with CS microspheres (348%), as shown in Fig. 2. The swelling index of microspheres prepared with different polymers decreased as follows: CP > PL > CS microspheres. As the polymer content of the microspheres increased, so did their swelling ability. According to results of the one way ANOVA test, the swelling profiles of the

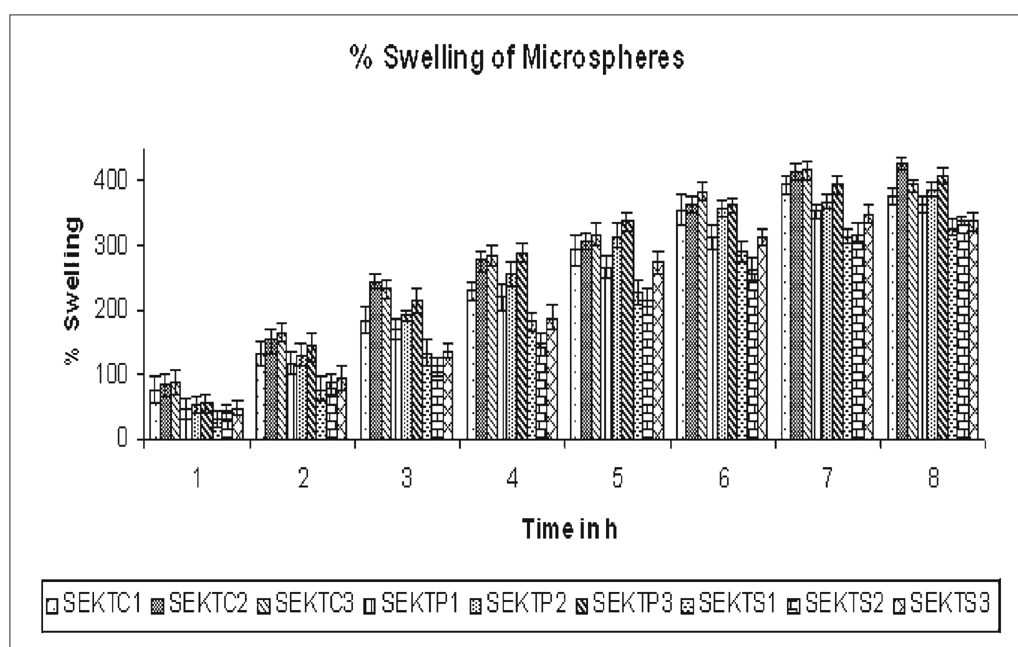

Fig. 2: Percentage swelling profiles of microspheres. Each observation is mean ± SD of three determinations

prepared mucoadhesive microspheres were found to be different ($p < 0.05$) at each time point (Fig. 2).

Water uptake into the polymer depends upon the extent of hydrodynamic free volume and the availability of hydrophilic functional groups for the water to establish hydrogen bonds. The high swelling properties of the CP and PL microspheres could be attributed to their ionized ability to uncoil the polymer into an extended structure. Their high molecular weight and large number of carboxyl groups could be possible reasons for the higher swelling ability of CP and PL microspheres compared with CS microspheres (Agarwal and Mishra 1999). The highest swelling seen with PL microspheres could be because of the chemical structure, i.e. polyacrylic acid crosslinked with divinyl glycol, which swells very well in water (Burjak et al. 2001).

2.6. *Mucoadhesion*

Mucoadhesion studies were carried out to ensure the adhesion of the microspheres to the mucosa for a prolonged period of time at the site of absorption. Mucoadhesives are generally polymers with numerous hydrophilic functional groups, capable of forming hydrogen bonds. This is due to the fact that on hydration, expansion of the polymeric surface takes place, thereby resulting in an increased area of contact between the polymer and the mucin, and permitting a greater degree of interpenetration and interdiffusion. The data in Table 2 show an increasing trend of mucoadhesive strength with increasing polymer content, consonant with the literature (Varma et al. 2004).

It can be seen from the one-way ANOVA results that the CS concentration had a significant effect on mucoadhesion ($p < 0.05$). Comparison of microspheres based on different concentrations of polymer but containing the same amount of the drug, CS microspheres vs. PL microspheres, showed that SEKTS3 had significantly higher mucoadhesion than PL microspheres ($p < 0.05$); on the other hand, SEKTS3 and SEKTC3 did not show a statistically significant differences with respect to the mucoadhesive properties of the corresponding SEKTS3 ($p > 0.05$). This indicates that mucoadhesion is influenced by the amount of polymer with respect to the drug.

The results could be interpreted by the fact that CS, a cationic polymer, forms additional interactions between its amino groups and negatively charged carboxylic and sulfuric groups of the nasal mucus membranes (He et al. 1998). This resulted in CS microspheres having the highest mucoadhesion strength in comparison with the other polymeric microspheres of CP and PL. The formation of weak hydrogen bonds with mucous molecules by the hydrophilic functional groups present in CP molecules may be responsible for its mucoadhesion. On the other hand, the mucoadhesion of PL microspheres was poor, perhaps due to its nonionic character, while the presence of drug molecules could prevent formation of the hydrogen bonds responsible for mucoadhesion (Burjak et al. 2001). In addition, the type, amount, and molecular weight of the polymer could play a significant role in mucoadhesion.

2.7. *FTIR and DSC*

The IR spectra of prepared microspheres were recorded in comparison with IR spectra of both pure KT and blank microspheres as shown in Fig. 3. The IR spectra of KT showed peaks at 3360, 1588 and 1278 nm representing–COOH stretching, –C=O stretching and –C–N stretching respectively. The peaks at 1561 nm and 730 nm showed as major peaks for the drug. All the above peaks were present in drug loaded microspheres, confirming the presence of the drug in the polymer without any interaction.

The thermal behavior of prepared microspheres was studied in comparison with thermograms of both pure KT and blank microspheres as shown in Fig. 4. The DSC-thermogram of pure KT showed an endothermic peak at 159 °C, corresponding to its melting point. KT loaded polymeric microspheres exhibited a single melting peak at 153 °C due to presence of KT in the polymeric matrix. However, there was a slight decrease in the melting point of the drug when prepared in the form of microspheres. The evaluation of the thermograms obtained from DSC revealed no interaction between the polymer and the drug in the microspheres.

2.8. *In vitro drug release study*

In vitro release profiles were investigated for the drug loaded microspheres and compared with the release profile of the pure

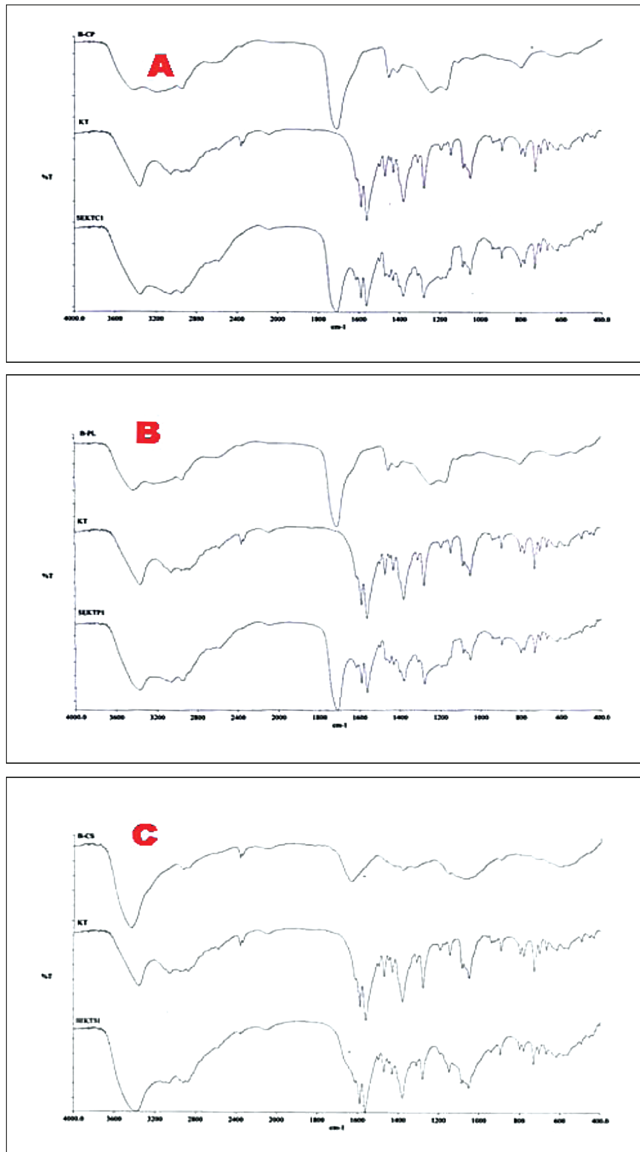

Fig. 3: Comparative IR spectra of KT, blank microspheres and drug-loaded microspheres: (A) microspheres of CP; (B) microspheres of PL; (C) microspheres of CS

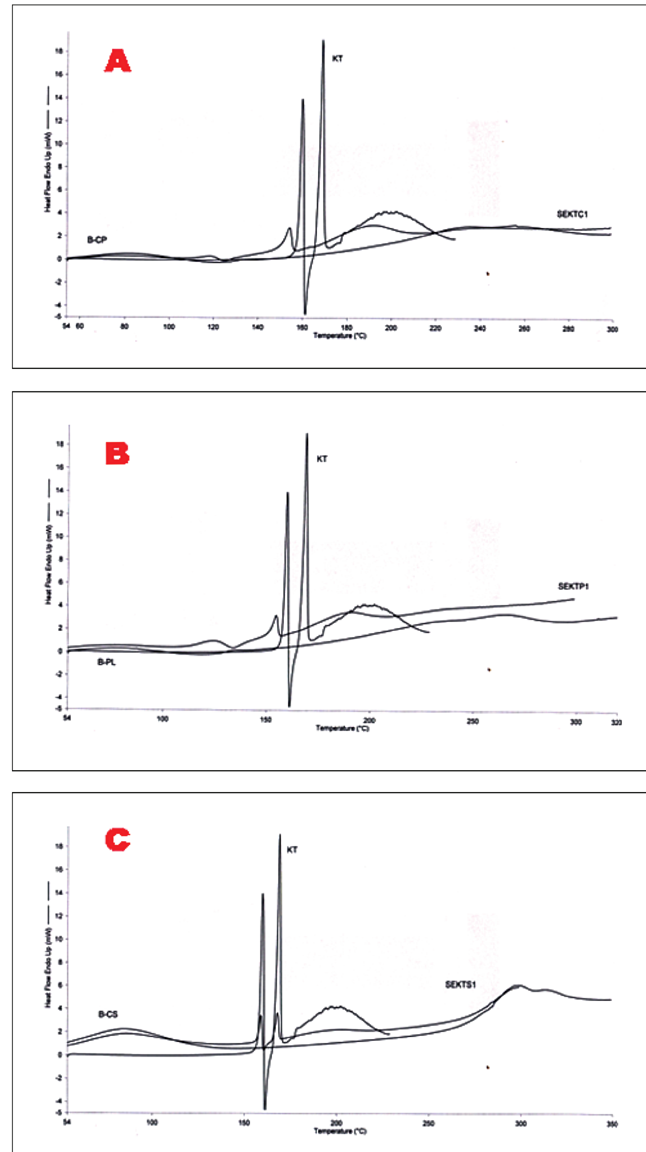

Fig. 4: Comparative DSC spectra of KT, blank microspheres and drug-loaded microspheres: (A) microspheres of CP; (B) microspheres of PL, (C) microspheres of CS

drug as shown in Fig. 5. The rate of release of KT powder was significantly faster (approximately more than 98% of the drug released in 2 h). Loading KT into a polymeric matrix led to a prolonged diffusion/release rate. The decrease in the rate of release depended on the kind of polymer used and the drug to polymer ratio. In fact, about 85–95% of drug release was achieved up to 8 h from the prepared microspheres.

The % cumulative release was slower in the case of SEKTC3, SEKTP3 and SEKTS3 than with the other formulations. This is because, as the polymer content of the polymer matrix increases, matrix swelling also increases due to the extremely hydrophilic nature of the polymers (Rokhade et al. 2006). According to the results of one-way ANOVA, the drug release was found to be significantly different at each time interval ($p < 0.001$) as well as between drug products ($p < 0.05$), implying that the release profiles were not equivalent (Fig. 5). From the results of Tukey's Multiple Range test, it was found that the percentage release from the prepared microspheres was statistically significantly different ($p < 0.05$) at the time points after 30 min (<25% drug release), 4 h (approximately 50% drug release) and 8 h (>85% drug release). It is apparent from the results of the one-way ANOVA that the release profiles had differing shapes, in terms of course of diffusion and percentage drug release.

Swelling of microspheres is an important factor affecting the release of drug incorporated in them. It has been found that drug release from highly hydrated CP and PL microspheres is faster than that from less hydrated CS microspheres. Slow cumulative drug release from microspheres may be attributed to the

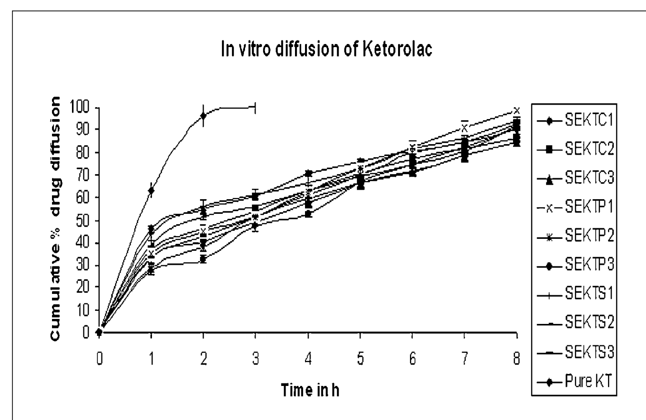

Fig. 5: Cumulative percentage diffusion of ketorolac from different mucoadhesive microspheres prepared using different polymers (n = 3)

increased density of the polymer matrix and also to an increase in the diffusional path length that the drug molecules have to traverse (Hafner and Filipovic 2007). The swollen polymeric network might act as a barrier to penetration of the medium, thereby suppressing the diffusion of KT from the swollen polymeric matrix. It may be shown that the high swelling ability of KT loaded microspheres, small cores and large contact surface between swollen microspheres lead to similar release profiles for all the microspheres (Nagda et al. 2009; Martinac et al. 2005). The batches (SEKTC3, SEKTP2 and SEKTS3) which demonstrated satisfactory encapsulation, mucoadhesion and drug release properties amongst all the prepared batches were chosen for *ex vivo* trials.

2.9. Release kinetics

The *in vitro* release data obtained were fitted to various kinetic equations. Correlations of individual batches with the different equations applied are given in Table 3. The release rates were determined from the slope of the appropriate plots. All the prepared microspheres showed a higher correlation with the Higuchi plot than with zero order, first order or Baker and Lonsdale plots. The Korsmeyer–Peppas equation was applied to the *in vitro* release data in order to clarify the diffusion mechanism. The release exponent n was determined and is given in Table 3. Microspheres prepared with CP and CS demonstrated ($n < 0.5$) a fickian diffusion mechanism, while, microspheres prepared with PL showed ($n > 0.5$) anomalous (non-fickian) diffusion.

2.10. Nasal cilia toxicity

Nasal ciliotoxicity studies were performed to evaluate the potential toxic effects of KT and the excipients used in the microspheres on the nasal mucosa. Nasal mucosa treated with phosphate buffer pH 6.6 (negative control) showed no nasociliary damage and the nasal mucosa remained intact, whereas extensive damage to the nasal mucosa coupled with loss of nasal cilia was observed with the positive control (Fig. 6). However, the application of KT to nasal mucosa showed only mild nasal mucosal damage associated with the loss of few nasal cilia. The untoward effect of KT powder may be due to its acidic structure (Kumar et al. 2009; Tas et al. 2006). Morphological changes in nasal epithelia exposed to drug loaded microspheres were milder than those exposed to KT alone and isopropyl alcohol. No apparent damage was observed in nasal mucosa treated with KT loaded microspheres, thus substantiating the safety of the excipients and drug used in the formulations. Thus, this result showed that the mucosa remained intact after exposure to microspheres exposure and retained good morphology.

2.11. Conclusion

The present investigation describes a nasal drug delivery system for ketorolac, prepared by the solvent evaporation technique using aqueous solutions of carbopol, polycarbophil and chitosan. The prepared chitosan mucoadhesive microspheres were of a suitable size for nasal administration and showed satisfactory encapsulation, swelling, mucoadhesion properties and prolonged drug release characteristics with almost negligible irritant and toxic effects to the nasal mucosa.

3. Experimental

3.1. Materials

Ketorolac tromethamine (KT) was from a a sample donated by Symed Labs Limited, Hydrabad, (India). Carbopol 974® PNF (CP) and Noveon AA-1 (Polycarbophil, PL) were donated by Lubrizol Advanced Materials Inc, Mumbai (India). Chitosan (CS, > 85% deacetylation), was kindly provided by the Central Institute of Fisheries Technology, Kochi (India). All other reagents and solvents were of analytical grade and used without further purification.

3.2. Preparation of ketorolac mucoadhesive microspheres

Mucoadhesive microspheres were prepared by the water-in-oil (W/O) emulsification solvent evaporation technique (Abd El-Hameed and Kellaway 1997). Different amounts of CP and PL were dispersed in deionized water and mixed by rapid vortexing; the pH was adjusted to 7 using dilute aqueous sodium hydroxide. Different amounts of CS were added gradually to a solution of aqueous acetic acid (1.0% w/v). KT (0.5 g) was dissolved in each polymeric solution to achieve drug-to-polymer ratios of 1:3, 1:4 and 1:5 respectively (Table 1). The solution was added dropwise into a 250 ml solution of light liquid paraffin containing 2% Span 80. The resulting solutions were stirred at 1000 rpm. The samples were heated to 60–70 °C to promote evaporation of water. Solid polymer microspheres were subsequently separated from the oil by centrifugation, washed with hexane and dried in a vacuum oven at 40 °C for 24 h. Each formulation was prepared in triplicate for further studies.
The process and formulation parameters such as emulsifier concentration (1%, 2%, 3% and 4%), volume of processing medium (100, 250 and 500 ml) and stirring speed (800, 1000 and 1200 rpm) were varied to study their effect on mean particle size. The other parameters such as drug concentration and volume of non-solvent were kept constant.

3.3. Encapsulation efficiency

Twenty five milligrams of accurately weighed drug loaded mucoadhesive microspheres were added to 100 ml of 0.1 N HCl (Mathew et al. 2007). The resulting mixture was shaken continuously on a mechanical shaker for 24 h. Subsequently, the solution was filtered and 1 ml of the filtrate was diluted with 0.1 N HCl and analyzed spectrophotometrically at 322 nm using a Shimazdu UV-1700 UV/VIS double beam spectrophotometer (Shimazdu, Kyoto, Japan). The encapsulation efficiency was calculated as the amount of KT found (UV-spectrophotometric determination) with respect to the theoretical amount of total solid in the solution (polymer and KT) using Eq (1):

$$Drug\ Encapsulation\ Efficiency\,(\%) = \left\{\frac{Practically\ Derived\ Drug\ Content}{Theoretical\ Drug\ Content}\right\} \times 100 \quad (1)$$

3.4. Particle size

The particle size of the prepared microspheres was determined using a microscopic imaging analysis technique (Filipovic et al. 1996). Particle size analysis of the microspheres was performed using an AXIOPLAN microscope (Zeiss MPM400 Germany) equipped with a computer-controlled image analysis system (Zeiss KS300 Germany). In all experiments at least 100 particles were examined.

3.5. Scanning electron microscopy (SEM)

A scanning electron microscope (ESEM TMP with EDAX, Philips, Netherlands) was used to characterize the surface topography of the microspheres. The microscope was equipped with an electron optical system (EOS) consisting of a 0.5–30 kV capacity electron gun and an electron detector. The microspheres were placed on a metallic support with thin adhesive tape and were coated with gold under vacuum. The surface was scanned and photographs of the drug loaded microspheres were taken at an accelerating voltage of 30 kV.

3.6. Swelling index

The swelling ability of the microspheres was determined by allowing the microspheres to swell to equilibrium in phosphate buffer (pH 6.6) (Hascicek et al. 2003; Jain et al. 2004). Accurately weighed amounts of microspheres (50 mg) were immersed in a slight excess of phosphate buffer (pH 6.6) and kept for 8 h. At intervals of 1 h, the samples were removed, blotted with a piece of paper towel to absorb excess buffer on the surface and reweighed. The difference between the weight initially and after swelling was studied up to 8 h. The following formula was used to calculate percentage swelling:

$$Ssw = \left(\frac{Ws - Wo}{Ws}\right) \times 100 \quad (2)$$

Table 3: Various parameters of model equations for *in vitro* release kinetics

Batch	Higuchi model		Zero order		First order		Baker & Lonsdale model		Krosemeyer Peppar model	
	r^2	K_h	r^2	K_0	r^2	K_1	r^2	K_{bl}	r^2	n
SEKTC1	0.9742	29.77	0.8327	8.98	0.9859	−0.010	0.9918	0.032	0.9916	0.351
SEKTC2	0.9934	29.60	0.8969	9.17	0.9917	−0.094	0.9865	0.029	0.9929	0.405
SEKTC3	0.9925	28.70	0.9249	9.04	0.9776	−0.088	0.9653	0.027	0.9812	0.428
SEKTP1	0.9903	34.04	0.9528	10.89	0.8124	−0.188	0.8894	0.047	0.9867	0.499
SEKTP2	0.9931	32.89	0.9571	10.53	0.9512	−0.126	0.9441	0.036	0.9915	0.540
SEKTP3	0.9774	31.19	0.9715	10.38	0.9152	−0.117	0.8992	0.033	0.9771	0.567
SEKTS1	0.9775	30.30	0.8565	9.25	0.9258	−0.121	0.9544	0.037	0.9896	0.347
SEKTS2	0.9816	29.01	0.8771	8.95	0.9288	−0.100	0.9426	0.031	0.9846	0.361
SEKTS3	0.9930	28.29	0.9090	8.83	0.9803	−0.084	0.9718	0.026	0.9851	0.404

K, release rate constant; r^2, coefficient of determination; n, release exponent

where, Ssw = percentage swelling of microspheres; Wo = initial weight of microspheres;and Ws = weight of microspheres after swelling.

3.7. Mucoadhesion

Mucoadhesion of the different microsphere systems was assessed using the method reported by Jain et al (2004). A strip of sheep nasal mucosa was mounted on a glass slide. One hundred milligrams of accurately weighed mucoadhesive microspheres in dispersion form was placed on the nasal mucosa. This glass slide was incubated for 15 min in a desiccator at 90% relative humidity to allow the polymer to interact with the membrane and finally placed in the cell attached to the outer assembly at an angle 45°. Phosphate buffer saline (pH 6.6), previously warmed to 37 ± 0.5 °C, was circulated to the cell over the microspheres and membrane at the rate of 1 ml/min. Washings were collected after 1 h and centrifuged to separate out microspheres and finally dried at 50 °C. The washed-out microspheres were weighed and the percentage mucoadhesion was calculated using eq:

$$\textit{Percent Mucoadhesion} = \left(\frac{Wo - Wt}{Wo}\right) \times 100 \qquad (3)$$

where, Wo = weight of microspheres applied; Wt = weight of microspheres washed out.

3.8. Fourier Transform Infrared Spectroscopy (FTIR)

Spectra were recorded for the pure drug, drug-loaded microspheres and blank microspheres using FTIR (Spectrum GX, Perkin-Elmer, USA). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400–4000 cm^{-1} and the resolution was 2 cm^{-1}.

3.9. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry scans of the drug, blank microspheres and drug-loaded microspheres were performed using a DSC-PYRIS-1 instrument (Perkin-Elmer, USA). The analysis was performed with a heating range of 50–300 °C and a rate of 10 °C min^{-1}.

3.10. In vitro drug release study

In vitro drug release of the pure drug (KT) and prepared microspheres was studied using a Franz diffusion cell (Hafner and Filipovic 2007; Hascicek et al. 2003). A dialysis membrane (cut-off Mw 12,000) was placed between

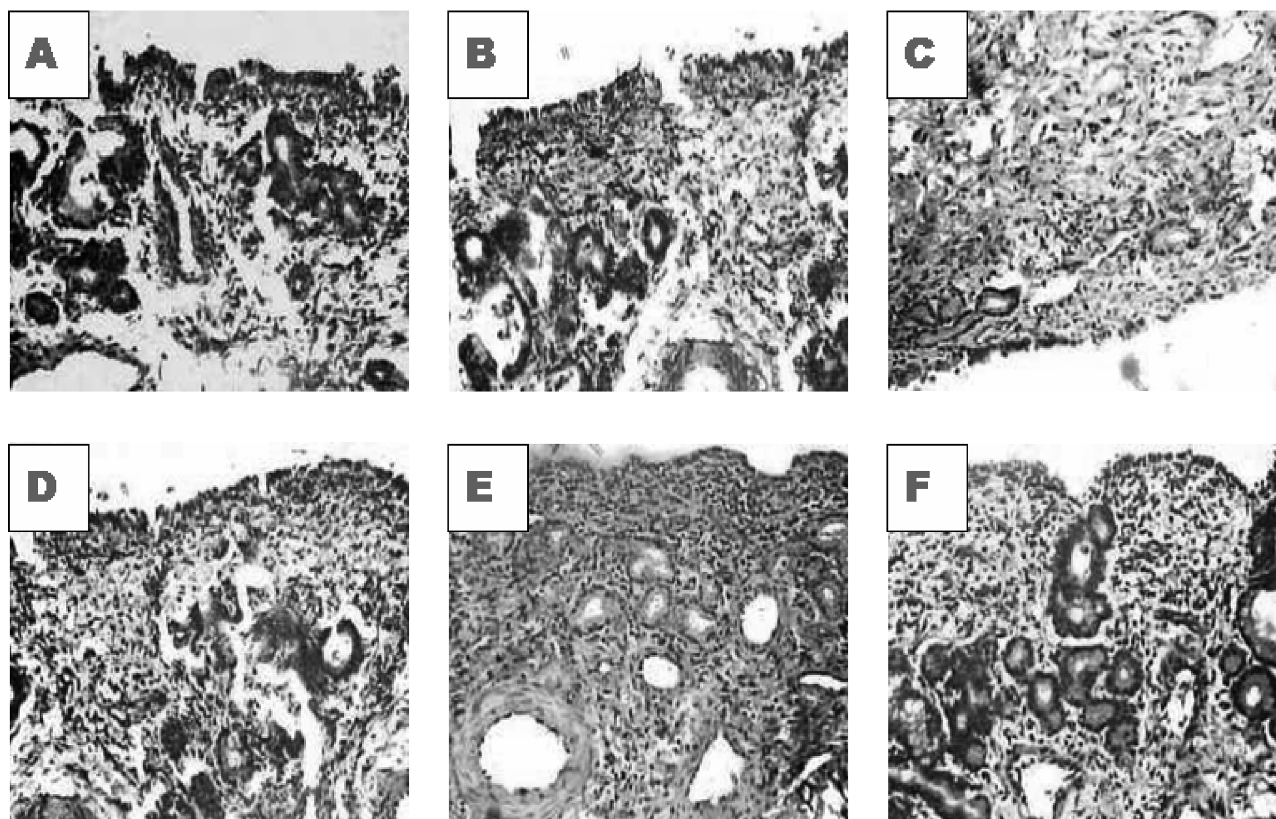

Fig. 6: Cross section of Sheep nasal epithelium treated with (A) isopropyl alcohol; (B) pure KT; (C) Negative control; (D) SEKTC3, microspheres of CP; (E) SEKTP2, microspheres of PL; (F) SEKTS3, microspheres of CS

the microsphere sample and a receptor compartment containing phosphate buffer solution (pH 6.6). KT loaded microspheres equivalent to 10 mg of KT were applied to the dialysis membrane. The volume of the receptor compartment was 20 ml (Hascicek et al. 2003). The temperature of the receptor medium was adjusted to 37 ± 1 °C. The contents of the receptor compartment were continuously stirred with a magnetic stirrer. Aliquots of 1.0 ml were withdrawn from the receptor compartment at hourly intervals for 8 h and replaced with the same amount of fresh buffer solution. The aliquot was analyzed for drug content at 322 nm after appropriate dilution against phosphate buffer saline pH 6.6 as blank. All experiments were performed in triplicate.

3.11. Release kinetics

In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study were fitted to various kinetic equations (Costa and Lobo 2001). The kinetic models used were zero-order, first-order, Higuchi matrix, and Baker and Lonsdale. To define a model which representing a better fit for the formulation, drug release data were analyzed by the Peppas equation. Regression coefficients (r^2 values) were calculated for the linear curves obtained by regression analysis of the above plots.

3.12. Ex-vivo nasal cilio toxicity of mucoadhesive microspheres

Freshly excised sheep nasal mucosa, except for the septum, was collected from the slaughter house in saline phosphate buffer pH 6.6 (Kumar et al. 2009; Tas et al. 2006). Six sheep nasal mucosa pieces (N1, N2, N3, N4, N5, N6) of uniform thickness were selected and mounted on Franz diffusion cells. N1 was treated with 0.5 ml of pH 6.6 saline phosphate buffer (negative control), N2 with 0.5 ml of isopropyl alcohol (positive control), N3 with 10 mg of KT in pH 6.6 phosphate buffer and N4 to N6 with KT loaded microspheres equivalent to free KT (10 mg), one formulation from each polymer (SEKTC3, SEKTP2 and SEKTS3), for 1 h. After 1 h, the mucosa was washed with pH 6.6 saline phosphate buffer and subjected to histological studies to evaluate the toxicity of the KT loaded polymeric microspheres (Mahajan and Gattani 2009).

After removal of the sheep nasal mucosa from the diffusion cell, the tissues were placed in 10% v/v buffered formaldehyde solution, and fixed for 72 h. For the purpose of histological study, the tissues were dehydrated in ascending concentrations of ethanol (70, 80, 90, 96, and 99% v/v) and sequentially embedded in paraffin wax blocks according to the standard procedure (Tas et al. 2006), and sectioned at 5 μm thickness. They were further deparaffined with xylol, and histologic observations were performed after staining the functional nasal tissues with hematoxylin-eosin. The slides were examined using light microscopy.

3.13. Statistical analyses

All the reported determinations were performed in triplicate. One-way analysis of variance (ANOVA) followed by Tuckey's multiple range test was performed to determine the least significant difference for the relevant evaluations. Differences were considered significant at $p < 0.05$.

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References

Abd El-Hameed M, Kellaway I (1997) Preparation and *in vitro* characterization of mucoadhesive polymeric microspheres as intra-nasal delivery systems. Eur J Pharm Biopharm 44: 53–60.

Agarwal V, Mishra B (1999) Design, development, and biopharmaceutical properties of buccoadhesive compacts of pentazocine. Drug Dev Ind Pharm 25: 701–709.

Amrish C, Sharma PK (2009) Transdermal delivery of ketorolac. Yakugaku Zasshi 129: 373–379.

Arora R, Srivastava AK, Rani M, Mishra B (2002) Investigation on osmotic matrix tablets as controlled delivery systems of ketorolac tromethamine. Acta Pharm Turcica 44: 87–96.

Basu SK, Kavitha K, Rupeshkumar M (2010) Evaluation of ketorolac tromethamine microspheres by chitosan/gelatin b complex coacervation. Sci Pharm 78: 79–92.

Brime B, Ballesteros MP, Frutos P (2000) Preparation and *in vitro* characterization of gelatin microspheres containing levodopa for nasal administration. J Microencap 17: 777–784.

Brocks DR, Jamali F (1992) Pharmacokinetics of ketorolac tromethamine. Clin Pharmacokin 23: 415–427.

Buckely M, Brogden R (1990) Ketorolac: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential. Drugs 39: 86–109.

Burjak M, Bogataj M, Velnar M, Grabnar I, Mrhar A (2001) The study of drug release from microspheres adhered on pig vesical mucosa. Int J Pharm 224: 123–130.

Chelladurai S, Mishra M, Mishra B (2008) Design and evaluation of bioadhesive *in-situ* nasal gel of ketorolac tromethamine. Chem Pharm Bull 56: 1596–1599.

Costa P, Lobo J (2001) Modeling and comparison of dissolution profiles. Eur J Pharm Sci 13: 123–133.

Dinarvand R, Mahmoodi S, Farboud E (2004) Effect of process variables on particle size of gelatin microspheres containing lactic acid. Pharm Dev Tech 9: 291–299.

Dubey RR, Parikh RH (2004) Two-stage optimization process for formulation of chitosan microspheres. AAPS PharmSciTech 5: 1–9.

Filipovic J, Becirevic M, Skalko N, Jalsenjak I (1996) Chitosan microspheres of nifedipine and nifedipine–cyclodextrin inclusion complexes. Int J Pharm 135: 183–190.

Hafner A, Filipovic-Grcic J (2007) Development and *in vitro* characterization of chitosan-based microspheres for nasal delivery of promethazine. Drug Dev Ind Pharm 33: 427–436.

Harris SA, Ohin M, Svensson S, Lethagen D, Nilsson IM (1989) Effect of viscosity on the pharmacokinetics and biological response to intranasal destiiopressin. J Pharm Sci 78: 470–471.

Hascicek C, Gonul N, Erk N (2003) Mucoadhesive microspheres containing gentamicin sulfate for nasal administration: preparation and *in vitro* characterization. Farmaco 58: 11–16.

He P, Davis SS, Illum L (1998) *In vitro* evaluation of the mucoadhesive properties of chitosan microspheres. Int J Pharm 166: 75–88.

Jadhav KR, Gambhire MN, Shaikh IM, Kadam VJ, Pisal SS (2007) Nasal drug delivery system-factors affecting and applications. Current Drug Therapy 2: 27–38.

Jain SK, Chourasia MK, Jain AK, Jain RK (2004) Development and characterization of mucoadhesive microspheres bearing salbutamol for nasal delivery. Drug Deliv 11: 113–122.

Karatas A, Baykara T (2006) Studies on release of ketorolac tromethamin and indomethacin from ophthalmic hydrogel inserts. J Fac Pharm Ankara 35: 255–268.

Kumar M, Pathak K, Misra A (2009) Formulation and characterization of nanoemulsion-based drug delivery system of risperidone. Drug Dev Ind Pharm 35: 387–395.

Mahajan HS, Gattani SG (2009) Nasal administration of ondansetron using a novel microspheres delivery system. Pharm Dev Technol 14: 226–232.

Maiti S, Dey P, Kaity S, Ray S, Maji S, Sa B (2009) Investigation on processing variables for the preparation of fluconazole-loaded ethyl cellulose microspheres by modified multiple emulsion technique. AAPS PharmSciTech 10: 703–715.

Martinac A, Filipovic-Grcic J, Perissutti B, Voinovich D, Pavelic Z (2005) Spray-dried chitosan/ethylcellulose microspheres for nasal drug delivery: Swelling study and evaluation of *in vitro* drug release properties. J Microencapsul 22: 549–561.

Mathew ST, Devi SG, Sandhya KV (2007) Formulation and evaluation of ketorolac tromethamine-loaded albumin microspheres for potential intramuscular administration. AAPS PharmSciTech 8: E1–E9.

Mroszczak J, Lee FW, Combs D, Sarnquist FH, Huang BL, Wu AT, et al. (1987) Ketorolac tromethamine absorption, distribution, metabolism, excretion, and pharmacokinetics in animals and humans. Drug Metab Dispos 15: 618–626.

Nagai T, Nishimoto Y, Nanibu N, Suzuki Y, Sekine K (1984) Powder dosage form of insulin for nasal administration. J Control Release 1: 15–22.

Nagda C, Chotai N P, Patel U, Patel S, l SoniT, Patel P, Hingorani L (2009) Preparation and characterization of spray-dried mucoadhesive microspheres of aceclofenac. Drug Dev Ind Pharm 35: 1155–1166.

Pereswetoff-Morath L (1998) Microspheres as nasal drug delivery systems. Adv Drug Del Rev 29: 185–194.

Puri V, Bansal AK (2004) *In vitro–in vivo* characterization of release modifying agents for parenteral sustained-release ketorolac formulation. Drug Dev Ind Pharm 30: 619–624.

Quadir M, Zia H, Needham TE (2000) Development and evaluation of nasal formulations of ketorolac. Drug Deliv 7: 223–229.

Rathananand M, Kumar DS, Shirwaikar A, Kumar R, Sampath Kumar D, Prasad RS (2007) Preparation of mucoadhesive microspheres for nasal delivery by spray drying. Ind J Pharm Sci 69: 651–657.

Reddy PB, Dorle AK, Krishna DR (1990) Albumin microsphere: effect of process variables on size distribution and *in-vitro* release. Drug Dev. Ind. Pharm 16: 1791–1803.

Rokhade AP, Agnihotri SA, Patil SA, Mallikarjuna NN, Kulkarni PV, Aminabhavi TM (2006) Semi-interpenetrating polymer network microspheres of gelatin and sodium carboxymethyl cellulose for controlled release of ketorolac tromethamine. Carbohyd Polym 65: 243–252.

Rouzi B, Tosi G, Forni F, Vandelli MA (2005) Ketorolac tromethamine liposomes: Encapsulation and release studies. J Liposome Res 15: 175–185.

Sankar C, Mishra B (2003) Development and *in vitro* evaluations of gelatin A microspheres of ketorolac tromethamine for intranasal administration. Acta Pharm 53: 101–110.

Shanmuganathan S, Shanumugasundaram N, Adhirajan N, Ramyaa Lakshmi TS, Babu M (2008) Preparation and characterization of chitosan microspheres for doxycycline delivery. Carbohyd Polym 73: 201–211.

Sinha VR, Trehan A (2005) Formulation, characterization, and evaluation of ketorolac tromethamine-loaded biodegradable microspheres. Drug Deliv 12: 133–139.

Tas C, Ozkan CK, Savaser A, Ozkan Y, Tasdemir U, Altunay H (2006) Nasal absorption of metoclopramide from different Carbopol 981 based formulations: *In vitro*, *ex vivo* and *in vivo* evaluation. Eur J Pharm Biopharm 64: 246–254.

Ugwoke MI, Agu RU, Verbeke N, Kinget R (2005) Nasal mucoadhesive drug delivery: Background, applications, trends and future perspectives. Adv Drug Deliv Rev 57: 1640–1665.

Ugwoke MI, Verbeke N, Kinget R (2001) The biopharmaceutical aspects of nasal mucoadhesive drug delivery. J Pharm Pharmacol 53: 3–22.

Varma M, Singla AK, Dhawan S (2004) Release of diltiazem hydrochloride from hydrophilic matrices of polyethylene oxide and carbopol. Drug Dev Ind Pharm 30: 543–553.

Vatsaraj N, Zia H, Needham T (2002) Formulation and optimization of a sustained-release tablet of ketorolac tromethamine. Drug Deliv 9: 153–159.

Vivek K, Reddy H, Murthy RSR (2007) Comparative study of some biodegradable polymers on the entrapment efficiency and release behavior of etoposide from microspheres. Pharm Dev Tech 12: 79–88.

Wagner W, Khanna P, Furst DE (2007) Nonsteroidal anti-inflammatory drugs, disease-modifying antirheumatic drugs, nonopioid analgesics and drugs used in gout. In: Katzung BG (ed.) Basic and Clinical Pharmacology, 10th ed., New York, p. 586.