Design and Evaluation of Bioadhesive *in-Situ* Nasal Gel of Ketorolac Tromethamine

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The present study was aimed at developing safe and effective bioadhesive gelling systems of ketorolac tromethamine, a potent non-narcotic analgesic with moderate anti-inflammatory activity for nasal systemic delivery. Chitosan and pectin based gelling systems were prepared with variables like polymer concentration and type. These systems were characterized in terms of their physical properties, *in vitro* bioadhesion, *in vitro* drug release and long-term stability. The anti-inflammatory activity and mucosal irritancy of selected gels were also evaluated in rats and these results were compared with per oral, intraperitoneal and nasal solution administration of ketorolac tromethamine. All the prepared formulations gelled immediately at the nasal mucosal pH and showed longer contact time. Addition of hydroxypropyl methylcellulose (HPMC) in both chitosan and pectin based gelling systems increased the viscosity and gel strength. All the formulated gels exhibited pseudoplastic rheology and diffusion-controlled drug release. The results from stability studies revealed that the prepared thermogels showed marginal decrease in viscosity but at the same time, no significant difference in drug content, and *in vitro* release characteristics were observed before and after accelerated studies. The developed gelling systems produced only mild to negligible irritant effect to nasal mucosae as compared to control group.

Key words ketorolac tromethamine; bioadhesive; gelling system; chitosan; pectin; nasal delivery

Aqueous polymer solutions that are transformed into gels by changes in environmental conditions, such as temperature and pH, thus resulting in *in-situ* hydrogel formation, have recently attracted the attention of many investigators for practical biomedical or pharmaceutical applications.[1—3] Ketorolac tromethamine (KT) is a potent non-narcotic analgesic with moderate anti-inflammatory activity and clinical studies indicate that it has a single dose efficacy, greater than morphine for postoperative pain and has excellent applicability in the emergency treatment of pain (break through cancer pain) and in the treatment of migraine headache. However, this drug is not currently available as nasal formulation. With intranasal delivery, a drug is absorbed directly into the systemic circulation, bypassing the problems that occur with oral administration, including fast onset of therapeutic effect without the discomfort and inconvenience of an injection.

Pectin is a plant-derived polysaccharide commonly used in foods and drugs. Solutions containing drug plus pectin can be stored as liquids, and form gels when applied to the nasal mucosa.[4] Chitosan is a linear polysaccharide derived from crustacean shells and is currently being investigated for many pharmaceutical applications because of its biocompatibility and biodegradability characteristics.[5] These observations made an impetus to prepare and evaluate chitosan and pectin based gelling systems of KT.

Experimental

Materials KT, chitosan (purified, viscosity grade 50) and pectin were gift samples from Ranbaxy (India), CIFT (India) and India Citrus Products Ltd. (India), respectively. Hydroxypropyl methylcellulose (HPMC K15) (Sigma-Aldrich India Ltd., India), glacial acetic acid (S.D. Fine Chem., India), hydrochloric acid (Qualigens Fine Chem., India), dihydrogen potassium orthophosphate (Glaxo, India) and sodium hydroxide (E. Merck, India) were purchased. Double distilled water was used. All other chemicals used were of analytical reagent grade. Dialysis membrane (Sigma-Aldrich), Digital pH meter (Toshniwal Pvt. Ltd., India) and Brook Field viscometer (Tokimec Co., Japan) were also used.

Methods For preparation of pectin or chitosan based gelling systems (Table 1); drug was mixed with polymer solution prepared in distilled water or 0.1 M glacial acetic acid, respectively and pH was adjusted to 4. For the preparation of HPMC K15 incorporated gels, HPMC K15 was also added while preparing pectin/chitosan solution and rest of the procedures were the same.

The pH, angular viscosity and drug content of the prepared gels were studied using digital pH meter, Brookfield viscometer and UV–VIS spectrophotometer (JASCO 7800, Tokyo) at 322 nm, respectively.

Gelation Studies Gelation studies were carried out by following a reported method[6] in different pH buffers (pH 5.0, 6.0, 6.6, 7.4) and were assessed by visual examination.

Gelation temperature and gel melting was assessed by a modified procedure[7] as follows: 2 ml aliquot of gel was transferred to test tubes, sealed with aluminium foil and immersed in a water bath. The temperature of water bath was increased in increments of 1 °C and left to equilibrate for 5 min at each new setting. The samples were then examined for gelation, which was said to have occurred when meniscus no longer move upon tilting through 90°. The gel melting temperature, a critical temperature when the gel starts flowing upon tilting 90°, was also recorded.

***In Vitro* Bioadhesion** Bioadhesiveness of gel formulations were evalu-

Table 1. Formula for Different Batches of Gelling Systems of KT

Composition (% (w/v))	Batch code											
	D1	D2	D3	D4	D5	D6	P1	P2	P3	P4	P5	P6
Chitosan	10	15	20	30	20	30	—	—	—	—	—	—
Pectin	—	—	—	—	—	—	10	20	30	40	30	40
HPMC K15	—	—	—	—	20	20	—	—	—	—	20	20
KT	25	25	25	25	25	25	25	25	25	25	25	25

— indicates not present.

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Table 2. Physical Characteristics of Prepared Gelling Systems of KT

Batch code	Drug content (%) (mean±S.D.)	Viscosity (cps)	Gelation temperature (°C) (mean±S.D.)	Gel strength	Mucoadhesion (%) (mean±S.D.)
D1	98.34±0.85	1550.0	35.0±0.4	++	85.69±2.54
D2	97.99±1.20	1625.0	35.7±0.1	++	88.12±1.32
D3	99.00±0.54	1780.0	36.0±0.2	++	92.36±2.02
D4	99.21±0.36	1850.0	35.7±0.4	+++	93.54±1.87
D5	98.88±1.00	1940.0	37.3±0.4	+++	80.12±1.25
D6	97.55±1.03	2200.0	37.4±0.3	+++	84.32±2.00
P1	99.22±0.35	340.0	35.3±0.4	++	71.35±1.35
P2	98.55±0.92	370.0	35.0±0.2	++	75.65±1.22
P3	98.78±0.38	395.0	35.9±0.4	++	77.23±1.65
P4	99.64±0.18	400.0	36.3±0.4	++	78.36±2.12
P5	99.46±0.78	615.0	36.5±0.2	+++	70.32±1.55
P6	99.64±0.65	686.0	36.8±0.2	+++	73.65±1.00

$n=3$ for each parameter, ++ gelation immediate, remains for few hours; +++ gelation immediate, remains for extended period (>12 h).

ated by following a reported agar plate method.[8]

***In Vitro* Drug Release Studies** *In vitro* drug release[9] from the gelling formulations was studied by dialysis (membrane thickness 0.025 mm) against phosphate buffer pH 6.6 as the diffusion medium. Aliquot samples were withdrawn at regular intervals and analysed by spectrophotometry at 322 nm. The release data were analysed according to the treatment proposed by Higuchi.[10]

Anti-inflammatory Activity in Rats *In vivo* anti-inflammatory studies of KT based formulations were conducted in male Wister rats by following a reported[11] method. The study protocol was approved by the Institutional Animal Ethical Committee. All the nasal formulations (batches D1, D4 and P4) were delivered to the nasal cavity *via* inserted tracheal cannula using a microsyringe attached to a blunt needle.[12] Also, for comparision, KT (2 mg/kg in each case) administration to rats was done through oral intubation, intraperitoneal injection and nasal instillation as aqueous solution.[12]

Histopathology Studies After the pharmacodynamic studies, the rats used for nasal gel (batch D4) treatment, were euthanized with an intravenous injection of lethal dose of pentobarbitone sodium. The medial part of the septum was surgically removed and immersed in fixative for 30—45 min in 6% formaldehyde solution. The specimens were decalcified in 10% (v/v) formic acid overnight, cut into 2—3 pieces and embedded in paraffin wax. The embedded tissues were sectioned, stained with haematoxylin–eosin (H&E) followed by periodic acid-Schiff staining (PAS)[13] and evaluated semi-quantitatively (single blinded study) for epithelial hyperplasia, goblet cell hyperplasia, and neutrophil infiltrations of (i) stroma, (ii) epithelium and (iii) lumen of the nasal cavity. For comparative analysis of mucous irritation potential, three different groups were taken, one group was untreated rats (no nasal administration), second was treated with nasal normal saline and third was treated with sodium taurodihydrofusidate (STDHF, 1% (w/v)) in normal saline.[14] All were euthanized as above and their respective nasal mucosal staining was done by following the above staining procedures.

Stability Studies To assess long term stability[15] of the prepared gelling systems of KT, formulations were stored at 40 °C/75% relative humidity (RH) in the stability chamber for 3 months. The samples were withdrawn at different time intervals (0, 1, 3 months) and observed for physical characteristics, drug content and *in vitro* drug release characteristics. The results were supported by statistical analysis using student '*t*' test and ANOVA (significance level $p<0.05$).

Results and Discussion

Physical characteristics of prepared gelling systems of KT are shown in Table 2. All the prepared solutions were in pH range of 3.8—4.9. pH <5 provides a better environment for mucoadhesion as well as longer retention of formulation in the nasal cavity because of hydrogen bonding. The drug content of all the prepared formulations was found within a range of 97.55 to 99.64% (Table 2).

Gelation and Gelling Capacity The gelation temperatures of chitosan (batches D1—D6) and pectin (batches P1—P6) gels were in the range of 35.0 to 37.4 °C and 35.3 to 36.8 °C, respectively (Table 2). All the prepared formulations gelled immediately and remained as gels for longer time (Table 2).

Addition of HPMC in both chitosan and pectin based gelling systems increased the viscosity and gel strength (batches D5, D6 and P5, P6). The higher gelation rate of the formulation with HPMC might have resulted from the stronger association of HPMC with other components *via* hydrogen-bonding,[16] leading to a prolonged retention of KT in the nasal cavity.[17] It was also observed that an increase in HPMC content resulted in marginal increase in gelation temperature (Table 2). This might be caused by the increased viscosity due to the additional mucoadhesive polymer.

Rheological Studies The formulations exhibited pseudoplastic rheology as evidenced by shear thinning and an increase in the shear stress with increased angular velocity (Table 2). The viscosity was directly dependent on the polymeric content of the formulations. Addition of HPMC led to increase in the viscosity of formulations and exhibited more pseudoplasticity (batches D5, D6 and P5, P6) as compared to batches prepared without HPMC (Table 2).

***In Vitro* Bioadhesion** The results indicated a reverse relationship between the force of adhesion of the gels and their movement on the agar (Table 2). The higher mucoadhesivity shown by the batches of chitosan containing gels (batches D1—D6) is in accordance with findings reported by Park *et al.*[18] and chitosan being a cationic polyelectrolyte binds strongly with mucin which is an anionic polyelectrolyte at neutral pH.[19] Pectin, being an anionic polymer, it also showed a better bioadhesiveness ranged from 70.32% (batch P5) to 78.36% (batch P4) (Table 2). However, incorporation of neutral polymer HPMC, increased the viscosity but failed to enhance the bioadhesivity significantly. This might be due to availability of low amount of water for swelling of HPMC and chitosan combination in the agar plate.[8]

***In Vitro* Drug Release** The release profiles of KT observed from all the gelling formulations exhibited a biphasic pattern of drug release (Figs. 1, 2). The results (Fig. 1) of the *in vitro* studies indicated that the duration of drug release was significantly prolonged ($p<0.01$, student '*t*' test; $p<0.05$, one way ANOVA), with an increase in the chitosan (Fig. 1a) or pectin (Fig. 1b) concentration from 10 to 15% (w/v) and then to 30% (w/v), which may be attributed to increase in the polymer matrix densities as well as viscosity

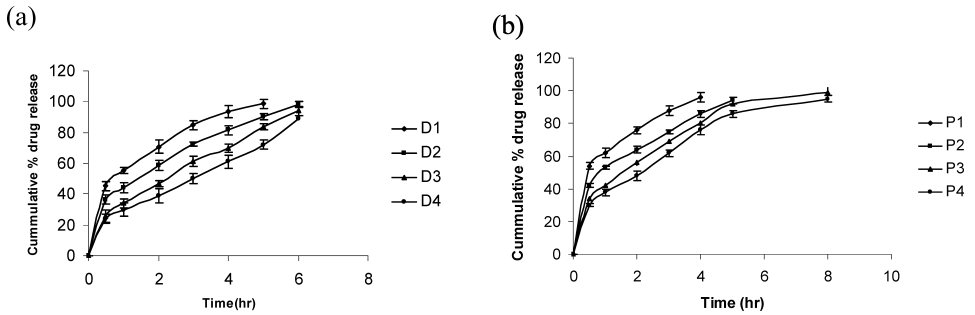

Fig. 1. Effect of Different Concentrations of (a) Chitosan and (b) Pectin on *in Vitro* Release Profiles of KT from Gelling Systems

Bars represent ±S.D. ($n=3$).

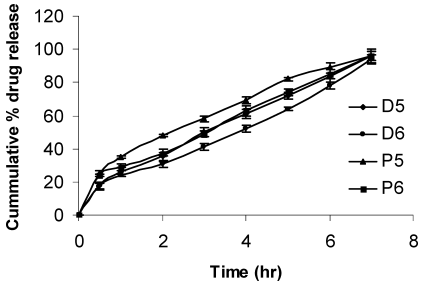

Fig. 2. Effect of Different Concentrations of HPMC on *in Vitro* Release Profiles of KT from Gelling Systems

Bars represent ±S.D. ($n=3$).

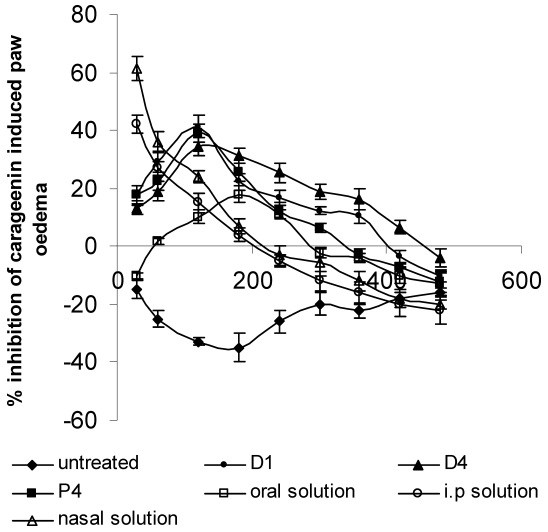

Fig. 3. Profiles Showing Inhibition Effect of Nasal Bioadhesive Gels and Other Formulations of KT (2 mg/kg) on Carageenin Induced Paw Oedema in Rats

Mean±S.E.M. ($n=5$).

(Table 2). However, increasing pectin concentration (Fig. 1b) from 30% (batch P3) to 40% (w/v) (batch P4) did not show much difference in prolonging the drug release. The release profiles also showed (Fig. 1b) that pectin 10% (batch P1) released total amount of drug within 4 h when compared to chitosan 10% (w/v) based gelling formulation (batch D1) which released 98.67% drug in 5 h of study. The amount of drug released at the end of 6 h from HPMC incorporated batches (Fig. 2) were 84% and 76% for D5 and D6, respectively. These values were much lower when compared to batches without HPMC (94%, 89% for D3, D4, respectively). This indicated that the incorporation of co-polymer HPMC could be an added advantage for prolonging the drug release from gelling systems. The same types of profiles were also seen with pectin based gelling formulation. Incorporation of HPMC in the pectin based gelling formulations (batches P5, P6) also showed significantly prolonged drug release when compared to batches without HPMC (batches P3, P4). To understand the mechanism of drug release from gelling systems, the results were further analyzed according to equation described by Peppas and Buri.[20] All the formulated gels exhibited diffusion-controlled drug release ($n=0.3498$—0.5119 for batches D1—D4 and 0.2816—0.5404 for batches P1—P4).

Stability Studies The chitosan and pectin based gels (batches D1, D4 and P4) exhibited no significant difference ($p>0.05$) in the drug content and *in vitro* release characteristics before and after storage for 1 and 3 months (40 °C/75% RH, results not shown). However, there was a marginal decrease in viscosity with increase in temperature. This is in agreement with earlier report.[21]

Anti-inflammatory Activities in Rats All three gelling systems (batches D1, D4 and P4) inhibited rat paw oedema significantly in comparison to nasal and oral drug solution and intraperitoneal injection of KT. On increasing chitosan concentration from 10% (batch D1) to 30% w/v (batch D4), significantly ($p<0.001$) increased magnitude and duration of anti-inflammatory activity (Fig. 3) was observed. When compared with pectin based gelling systems (batch P4), chitosan based gelling systems (batch D4) prolonged the anti-inflammatory activity for 7 h as compared to 5 h from P4. This might be due to stronger bioadhesion[22] between mucus (anionic) and chitosan (a cationic polymer) as compared to mucus and pectin (an anionic polymer) interaction.

The relative bioavailability of nasal gelling systems D1, D4 and P4 were 365, 424 and 301% respectively in reference to per oral solution and were 151, 175 and 124% respectively in reference to intraperitoneal injection of drug.

Histopathological Studies The results shown in Fig. 4 and Table 3 indicated that the medial region of nasal septum of untreated rats was covered with typical respiratory epithelium (Fig. 4a). The epithelia of the STDHF treated groups exhibited epithelium disruption and complete loss of some parts of the epithelium (Fig. 4b). In contrast, only slight changes were observed in the epithelium of the groups exposed to nasal gel (batch D4) (Fig. 4c). Nasal toxicity scores presented in Table 3 also showed that bioadhesive gelling

Table 3. Nasal Toxicity Scores with Nasal Formulations of KT in Rat Nasal Mucosa (n=5)

S. No	Batch	Histopathological (cytological) parameters				
		Epithelial hyperplacia	Goblet cell hyperplacia	Neutrophil infiltration to		
				Stroma	Epithelium	Lumen
1	Untreated rats	0	+	+	+	+
2	Intranasal normal saline	+	+	++	+	++
3	STDHF treated	+++	+++	+++	++	+++
4	Batch D4	+	+	++	+	++

Absence 0; mild +; moderate ++; severe +++; STDHF, (sodium taurodihydrofusidate).

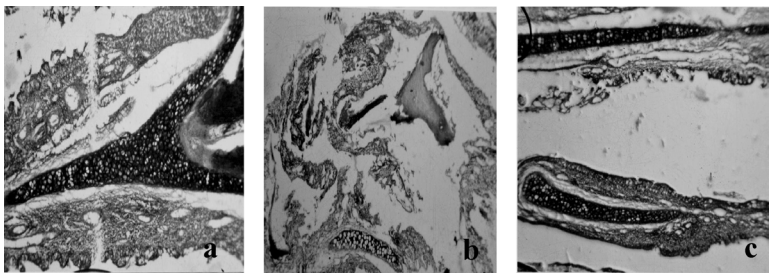

Fig. 4. Histopathological Investigation of Nasal Epithelium Showing (a) Untreated, (b) STDHF Treated and (c) Chitosan Gelling Systems of KT (Batch D4) Treated Rats

Magnification ×80.

system (batch D4) produced only mild irritation to nasal mucosae which could be reversible when compared to control groups. But, STDHF showed a severe damage to nasal mucosa.

It was concluded that the developed chitosan and pectin based bioadhesive gelling systems of KT were able to provide better deposition, distribution and residency properties of formulation in the nasal cavity and also exhibited prolonged drug release characteristics with added advantages of providing controlled anti-inflammatory effect with almost negligible irritant and toxic effects to nasal mucosae. Thus, bioadhesive gelling systems can be considered as a viable alternative for systemic medication of drugs through nasal route.

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