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Development and evaluation of nasal mucoadhesive nanoparticles of an analgesic drug.

Mohammed S. Khan[1]*, Rohitash K[1], Vijaykumar M[1], Suresh C Pandey[1], Gowda D. Vishakante[1], Faruqui M. Ahmed[2], Aquil R Sidiqui[3]

[1]Dept. of Pharmaceutics, JSS College of Pharmacy, JSS University, Sri Shivarathreeshwara Nagara, Mysore-570015, India.
[2]Royal College of Pharmaceutical Education & Research, Malegaon, India
[3]Bhagwan Pharmacy College, Aurangabad, India

ABSTRACT

Aim of the present study was to develop and evaluate mucoadhesive nanoparticles of chitosan using Tramadol HCl. Spray drying method was employed for producing nanoparticles using different drug to polymer ratio. Nanoparticles were evaluated for variables like yield, drug loading, entrapment efficiency, swelling, in-vitro mucoadhesion, particle size, polydispersity index & zeta potential, scanning electron microscopy, transmission electron microscopy, X-ray diffraction study and drug polymer compatibility by Differential scanning calorimetry & Fourier Transform Infrared Radiation studies. From the above studies it is considered that Tramadol HCl loaded chitosan nanoparticles is a promising delivery through nasal route for relief of pain.

Keywords: spray drying, mucoadhesive, nanoparticles, nasal route.

INTRODUCTION

Oral routes are the most preferable route for administration of active ingredients. However certain limitation barred the oral administration especially in case of hydrophilic drugs due to low bioavailability, insufficient intestinal transit time and reliance on paracellular transport (due to tight junction that limits the passage of hydrophilic drugs) [1, 2].

Nasal route had been considerably focused by current researchers as a alternative to target drug molecules directly to brain with help of olfactory neurons providing a loop to enter drug molecules to enter the central nervous system [3]. The only factor that limits the nasal delivery is nasal mucociliary clearance. Drug residence time is drastically affected by this factor by not allowing the drug molecule to get effectively absorbed which completely eradicates the sustainability of drug molecules in nasal drug administration. However this limitation can be overcome by using bioadhesive polymers which will increase the nasal residence time which in turns allows drug to get effectively absorbed by providing a longer contact of drug with nasal mucosa leading to enhanced drug absorption.

Drug can penetrate nasal mucosa via passive transport, carrier mediated transport as well as through transcytosis, It has been already reported that dry powder dosage forms such as microspheres prepared with mucoadhesive

polymers will swell in coming in contact with nasal mucosa and will form a gel like structure which control drug ciliary clearance from nasal cavity. Presence of polymeric microspheres will dehydrate (due to moisture uptake) the nasal mucosa resulting in reversible shrinkage of the mucosalcells, thus making a physical separation of the tight (intercellular) junction finally resulting in increased drug absorption [4].

Chitosan is a linear hydrophilic polysaccharide polymer of d-glucosamine. Being non toxic, chitosan has been widely researched for its potential use as a pharmaceutical ingredient in various dosage forms. Chitosan is used as a mucoadhesive polymer because of its enhanced permeation property which assist the opening of tight junctions of epithelilal cells [5-7].

Chitosan had been widely used as base material for targeting of many drugs to brain [8-10].

Tramadol HCl (TRM) is used a model drug in the present study. TRM belongs to opioid analgesic used for treatment of pain (post-surgical pain, obstetric pain, cancer pain, and chronic pain of mechanical and neurogesic origin). Half life of drug is reported to be around 5.5 hr with a usual oral dosage regimen of 50 to 100 mg every 4 to 6 hr with a maximum dosage of 400 mg/day [11-12]. Chitosan nanoparticles had been already reported for ocular drug delivery of acyclovir which proved to be promising for effective management of ocular viral infections [13].

The present study focus on developing and characterizing mucoadhesive nanoparticles of tramadol HCl in order to increase the drug bioavailabilty by avoiding first pass metabolism, achieving rapid onset of action for patient associated with severe pain.

MATERIALS AND METHODS

Tramadol HCl was received as a gift sample from Lupin Ltd, Pune, India. Chitosan (deacetylation degree 95.0%, molecular weight 50,000) was provided by Sigma Aldrich, Mumbai. All other reagents were of analytical grade or the highest grade commercially available.

Preparation of TRM loaded Chitosan nanoparticles

TRM loaded NPs were prepared using chitosan and TRM in 5 different drug to polymer ratios as shown in Table 1. Chitosan solution in aqueous acetic acid (1% w/v) was prepared by continuous magnetic stirring for 24 hrs in order to ensure complete solubilization of chitosan in the aqueous solution. To the prepared chitosan solution, TRM was added under stirring to get the final spray drying solution. 3% Lutrol F68 was added to the above solution as stabilizer. The above solution was kept for homogenization at 25000 rpm for 2 hrs (Polytron PT 1600E). Final resulting solution was spray dried by Buchi 290 spray dryer operating in the closed mode, using the Buchi 295 inert loop and nitrogen as the drying gas with other standard operating conditions (inlet temperature: 110-115 C: Outlet temperature: 80-90 C; Aspirator rate: 45-55% & Feed inlet rate: 0.5 ml/min) .Each formulation was carried out in triplicate ,n=3.

Characterization parameters evaluated for prepared NPs

Production yield [14] of NPs prepared was calculated using the weight of final product obtained after spray drying to the initial weight of drug and polymer used for preparation and % yield by using the formulae:

$$\text{Production yield} = \frac{\text{Practical Mass (NPs)}}{\text{Theoretical Mass (poymer+drug)}} \times 100 \qquad \textbf{Eq. 1}$$

Drug loading and Entrapment efficiency

Drug content: Weighed amount of NPs were taken from every batches and digested overnight in 1 % w/v acetic acid aqueous solution and is quantified for drug content spectrophometrically at 272 nm (UV-1801, Shimadzu, Japan).

Drug loading [15] and entrapment efficiency [16] was determined using the following equation:

$$\text{Drug Loading (\%)} = \frac{M_{actual}}{\text{Weighed quantity of NPs}} \times 100 \qquad \textbf{Eq. 2}$$

$$\text{Entrapment Efficiency (EE) (\%)} = \frac{\text{amount of TRM in NPs}}{\text{amount of TRM added}} \times 100 \qquad \textbf{Eq.3}$$

where M_{actual} is the actual tramadol HCl content in weighed quantity of NPs.

Measurement of particle size and zeta potential of prepared nanoparticles.
Size and zeta potential of TRM loaded NPs were measured by photon correlation spectroscopy (PCS) using Malvern Zetasizer. The particle size analysis was performed at a scattering angle of 90°C at room temperature. The concentration of the particles was adjusted to an appropriate value by pure water filtered through a 0.22 µm membrane. The diameter was averaged from three parallel measurements and expressed as mean±standard deviation.

Fourier Transform Infrared Radiation Measurements (FT-IR): FT-IR analysis was carried out for pure drug and for formuation using KBr pellet method on FTIR spectrophotometer type Shimadzu model 8033, USA in order to ascertain compatibility between drug and polymer used.

Differential scanning calorimetry (DSC):
All dynamic DSC studies were carried out on DuPont thermal analyzer with 2010 DSC module. The instrument was calibrated using high purity indium metal as standard. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10ºC/min heating rate of 10ºC/min.

Scanning electron microscopic (SEM) study
SEM photographs were taken with a scanning electron microscope Model Joel- LV-5600, USA, at the required magnification at room temperature. The photographs were observed to visualize the surface morphology of the TRM loaded NPs.

X-Ray Powder Diffraction
X-ray powder diffraction patterns were recorded at room temperature with a D8 Advance wide-angle diffractometer in the range of 5−40° of 2*θ*. Tablets for IR analysis were made with KBr and analyzed with an IR Perkin-Elmer model 1420, in the range from 4000 to 600 cm^{-1}.

Swelling Property
Swelling studies were done as procedure adopted by Juan et al & Jain et al with slight modification [17-18]. Accurately weighed NPs were allowed to swell to their equilibrium in nasal simulated fluid. Weighed amount of NPs was immersed in phosphate buffer (pH 6.6, for 5 min).

Degree of swelling was calculated using formulae:

$$\alpha = W_1 - W_2 / W_1$$

where α denotes degree of swelling, W_1= Initial weight of NPs, W_2= Weight of the NPs after swelling.

***In-vitro* mucoadhesion studies**
Mucoadhesion studies were done in accordance with procedure followed by Sofia P et al [19] with slight modifications. Drug loaded CS NPs were immersed in a 50 mL glass bicker at 37 °C containing a phosphate buffer solution (pH 6.8) for 5 min in such a way that the solution just covered the nanoparticles. Fresh nasal mucosa was obtained from local abattoir which was cut opened and was placed on nanoparticles surface so as to cover all the nanoparticles. Nasal mucosa was removed after 5 min of interval.

Mucosa with the attached nanoparticles was removed and the remaining nanoparticles on the glass beaker were dried at 60 °C till constant weight. The percent of adhered nanoparticles (AN) was estimated using the following equation:

$$AN\ (\%) = \frac{Wo - Wr}{Wo} \times 100$$

Where, Wo is the initial weight of nanoparticles and Wr the remained unattached weight of nanoparticles.

***In-vitro* drug release study**

In vitro releases studies of TRM loaded NPs were carried out in Static Franz diffusion cell. A cellulose acetate membrane (Dialysis membrane with molecular weight cut off value of 12,000-14,000, porous membrane tubing, Sigma Aldrich, Mumbai) was adapted to the end portion of the cylindrical donor compartment. NPs (100 mg) were placed in the donor compartment above the membrane. Receptor compartment contain buffer solution of pH 6.6 that was within the pH range of nasal cavity, maintained at 37°C under mild agitation using a magnetic stirrer. Samples were withdrawn periodically and were replaced with the same amount of fresh buffer medium.

Amount of drug released was assessed by measuring the absorbance at 272 nm using a single beam UV spectrophotometer (Shimadzu UV, 1801).

RESULTS AND DISCUSSION

TRM loaded chitosan NPs were successfully prepared by spray drying method. All the evaluated parameters were found to be satisfactory.

Characterization of TRM loaded NPs batches

Production yield loaded NPs prepared ranges in between 31.35±1.12 to 60.18±1.29% for TRM1 to TRM5 batches respectively as shown in Table 1. Increase in production yield of NPs was attributed to the increase in amount of polymer used in TRM1 to TRM5 batches as shown in Table 1. However complete yield was not possible as there is high risk of material adhering on the walls of the spray drier while spraying the solution. Risk of adhering can be minimized to certain extent by controlling the feed rate & other operating condition and nature of spray material used.

Table 1: Various parameters evaluated for TRM loaded chitosan NPs.

Formulation Code	Drug to Polymer Ratio	Yield of NPs (%, ±SD)	Drug Loading(%, ±SD)	Entrapment Efficiency(%, ±SD)	Degree of Swelling(*α*, ±SD)	Adhered NPs (%, ±SD)	Polydispersity Index	Average Size (nm)	Zeta Potential (mV)
TRM1	1:1	31.35±1.12	41.26±1.13	63.53±1.05	0.31±0.02	58.21±0.21	0.368 ± 0.003	121 ± 1.3	+29.9 ± 0.8
TRM2	1:2	40.15±2.14	33.11±2.23	67.53±1.17	0.36±0.33	63.21±1.13	0.377 ± 0.013	126 ± 0.3	+28.6 ± 1.2
TRM3	1:3	46.21±2.47	24.18±2.18	71.53±2.13	0.47±0.15	68.21±1.11	0.413 ± 0.022	139 ± 1.7	+30.3 ± 1.5
TRM4	1:4	53.13±2.17	17.03±1.07	76.53±1.36	0.56±0.22	73.21±1.03	0.458 ± 0.011	147 ± 2.5	+31.8 ± 2.3
TRM5	1:5	60.18±1.29	13.54±1.03	78.53±1.13	0.65±0.17	80.21±0.23	0.483 ± 0.035	153± 1.7	+33.1 ± 0.7

Drug loading of prepared NPs batches ranged in between 41.26± 1.13 to 13.54±1.03. There was a increase in drug entrapment from TRM1 to TRM5 (63.53±1.05 to 78.53±1.13) which is clearly due to the increased amount of polymer used in respective batches as shown in Table 1.

Prepared NPs formulation was subjected for swelling studies in nasal simulated fluid (pH 6.6). It was shown that all NPs batches show a considerable amount of swelling. However the rate of swelling increases from TRM 1 to TRM5 batches (0.31±0.02 to 0.65±0.17%) shown in Table 1, with maximum swelling shown by the TRM5. It is due to the fact of increased amount of polymeric content present in respective batches of NPs prepared. However all the prepared batches show satisfactory swelling properties.

Prepared TRM loaded batches of NPs were evaluated for particle size, polydispersity index & zeta potential. Particle size ranges in between 121 ± 1.3 nm to 153± 1.7 nm for TRM1 to TRM5 formulations respectively as shown in Table 1. A slight increase in particle size was observed that is due to the increased amount of polymer used in respective batches.

Mucoadhesion studies had been done in order to asses the adhesion of chitosan NPs on the nasal mucosal layer. All batches of TRM loaded NPs shows satisfactory adhesion property which is due to the mucoadhesive polymer used. It was found that there in increase in mucoadhesion from TRM1 to TRM5 NPs batches shown in Table 1 (58.21±0.21 to 80.21±0.23%) which is attributed to the increased amount of polymer that have been used in respective batches of formulations as mucoadhesive property of chitosan are mostly dependent on the ionic interaction of positively charged amine group of chitosan with negatively charged ions present within mucus (sulfonic acid & sialic acid residues) so an increase in chitosan concentration will lead to more mucoadhesive properties which is clearly proven by mucoadhesion studies.

The DSC thermogram obtained by studies for the pure drug Tramadol hydrochloride showed sharp endotherm at 181.32°C which correspond to its melting, and thermogram of the formulation showed the endotherm at 179.9°C as shown in **Figure 1.** As melting point of Tramadol hydrochloride and that of the formulation are nearer it reveals that there is no interaction between the drug and excipients used in study and drug is molecularly dispersed in chitosan.

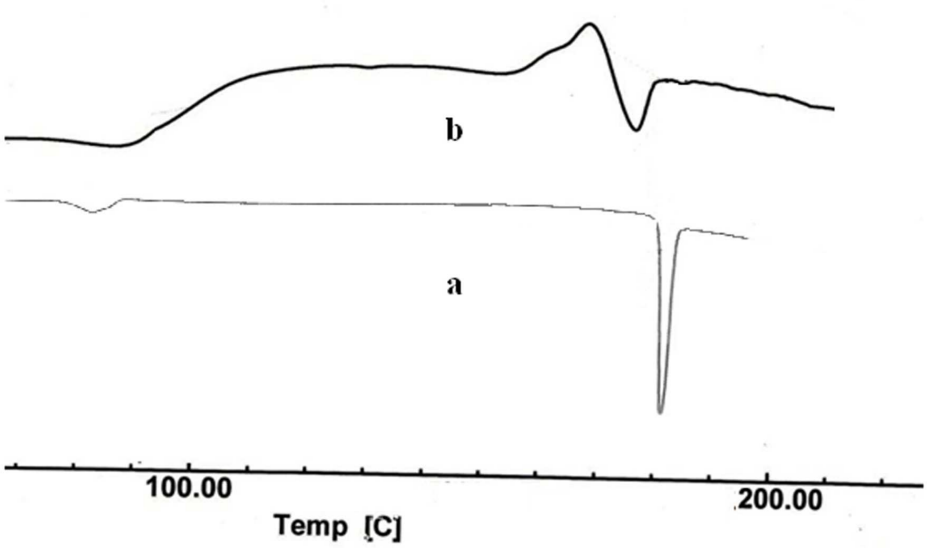

Figure 1: DSC thermogram of pure Tramadol HCl (a) and formulation TRM1 (b).

From the FT-IR peaks it can be concluded that the peaks of pure drug and formulations were found to be similar indicating that there was no significant interaction between drug and polymer used shown in **Figure 2**. Tramadol HCl has exhibited IR spectrum a broad band around 3311.77 cm^{-1} for hydroxyl, which is shown at 3317.37 cm^{-1} in formulation , methoxy at 2934.24 cm^{-1} in pure drug , 2929.67 cm^{-1} in formulation & amino group at 1051 cm^{-1} in pure drug , 1057 cm^{-1} in formulation thus ruling out any interactions between drug and polymer used. FTIR studies completely show the drug stability during the spray drying technique.

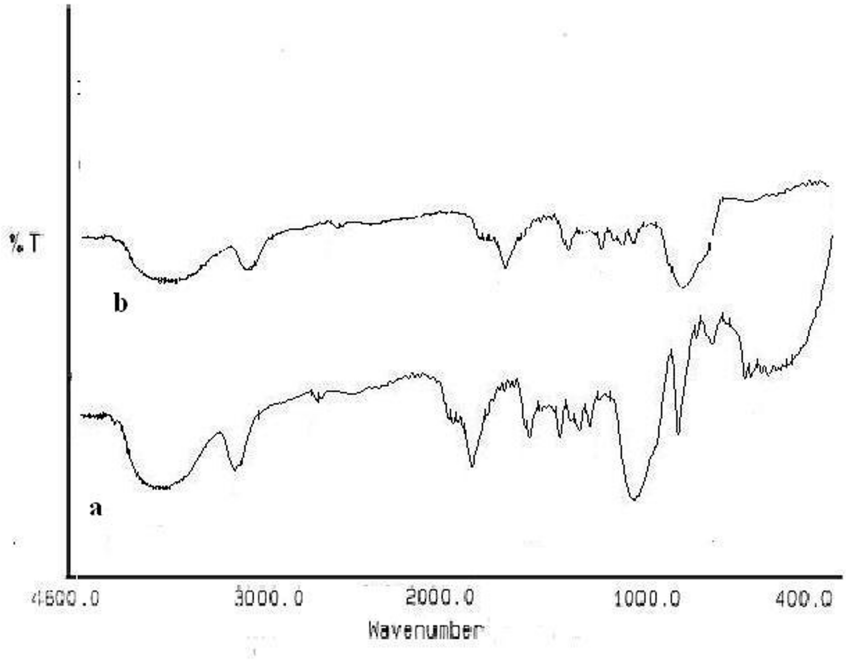

Figure 2: FTIR spectra of pure Tramadol HCl (a) and formulation TRM1 (b).

Nanoparticle surface morphology and shape were investigated by using SEM analysis. SEM figures completely revealed that the drug loaded nanoparticles were found to be distinct, spherical having a smooth surface as shown in **Figure 3**.

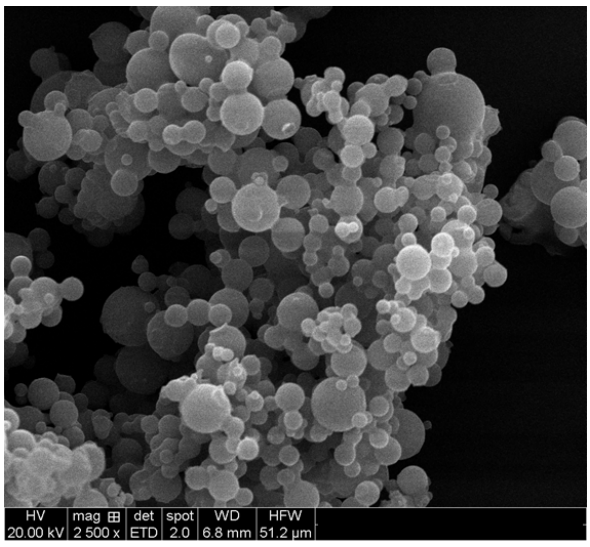

Figure 3: SEM (a) & TEM (b) images of TRM1 loaded chitosan NPs.

Pure drug & formulation were subjected for XRD studies. XRD patterns of pure drug & formulation (TRM1) were shown in Figure 4. Pure XRD pattern of Tramadol HCl shows sharp peaks indicating crystalline nature of drug. Sharp distinctive peaks of pure drug were observed 2θ angles of 25.8, 27.7, 32.5. On contrary, formulation shows broader peaks with low intensities clearly indicates that drug is present in amorphous form & drug got molecularly dispersed in the polymer.

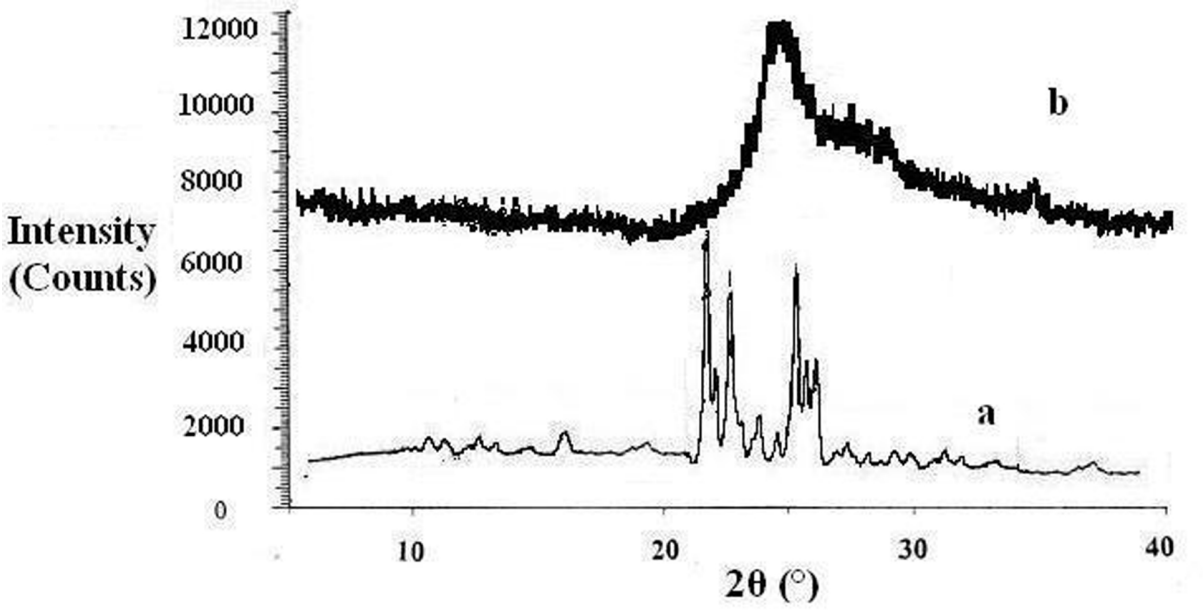

Figure 4: XRD analysis of pure Tramadol HCl (a) & formulation TRM1 (b).

In-vitro drug release studies of TRM loaded NPs was carried out in diffusion cells. Release profile of TRM loaded NPs batches were shown in Figure 5. It was clearly shown from the drug release data that an increase in amount of polymer concentration will affect the drug release. TRM1 releases 63.57±2.23% of the drug in less than 1 hr. However there is decrease in release rate of the drug in other batches due to increase in polymer concentration. TRM5 release around 30% of the drug in 1 hr. Based on the *in-vitro* release study TRM1 was chosen as optimized formulation because it shows faster drug release compared to other batches of formulations prepared indicating rapid onset of action.

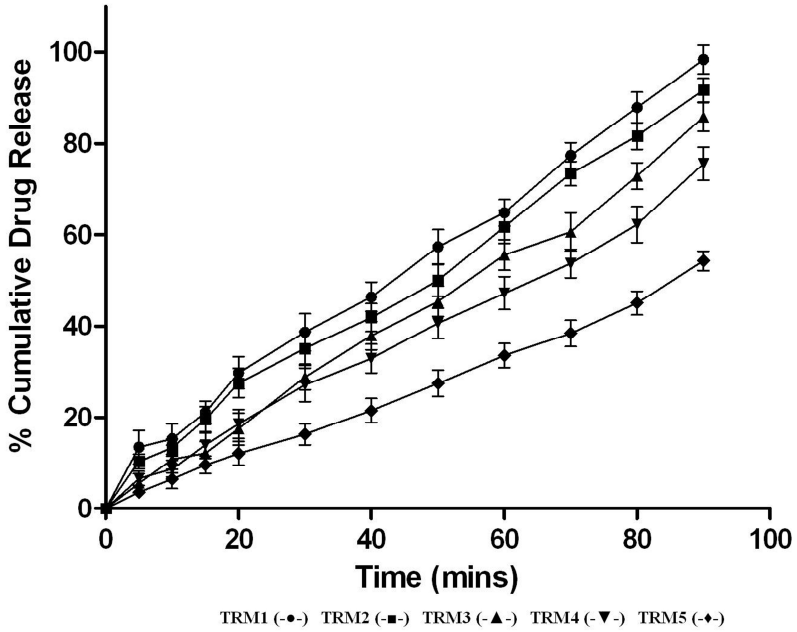

Figure 5: *In-vitro* drug release profile of various batches of NPs batches.

CONCLUSION

The above study conclude that spray drying is a appropriate method, quite reproducible to prepare mucoadhesive chitosan nanoparticles loaded with Tramadol HCl for relief of pain thorough nasal route improving the therapeutic efficacy. Adequate mucoadhesion had been achieved by the nanoparticles causing no damage to nasal mucosa. Prepared formulation shows no sign of damage to the nasal mucosa as assessed by histological studies. So prepared chitosan nanoparticles will be a promising dosage form to deliver the Tramadol HCl through nasal route.

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