International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.2, pp 01-13, 2017

Development and Evaluation of Lamotrigine loaded N-Trimethyl Chitosan Microspheres for Intranasal Administration

Jayshree B Taksande*, Kamlesh J Wadher,Rashmi V Trivedi, Milind J Umekar

Department of Pharmaceutics, ShrimatiKishoritaiBhoyar College of Pharmacy, New Kamptee, Nagpur (M.S.), India-441 002

Abstract : In the present study, we have developed and characterized the trimethyl chitosan(TMC) mucoadhesive microspheres of antiepileptic drug, Lamotrigine for intranasal administration. Firstly, TMC was synthesized by reductive methylation of chitosan and characterized by FTIR, DSC and XRD analysis. The lamotrigine loaded TMC microspheres were prepared by ionic gelation method and critically analyzed for appropriate morphological features (scanning electron microscopy), particle size, polydispersity index (PDI), zeta potential, drug entrapment efficiency, thermal behavior (differential scanning calorimetry), in vitro drug release, mucoadhesive nature and biocompatibility studies in excised sheep nasal mucosa and further evaluated for ex vivo permeation of drug through nasal mucosa.Results demonstrated that the microspheres were smooth and sphericalin shape with particle size in the range of 36.85±1.78 to 16.8±0.96µm suitable for nasal administration, with PDI 0.256 and zeta potential 29.8 mV respectively. Prepared microspheres showed high encapsulation efficiency up to 92.34±0.73%, strong bioadhesion potential and devoid of any signs of morphological toxicity in excised sheep nasal mucosa. The permeation of lamotrigine from TMC microspheres of batch TL-8 and TL-9 was 88.80% and 83.24% respectively. Thus the formulation of lamotrigine loaded TMC mucoadhesive microspheres offers promising advantages over conventional dosage forms.

Keywords : N-trimethyl Chitosan, Lamotrigine, Mucoadhesive microsphere, Intranasal administration, Epilepsy.

Introduction

The intra-nasal route offers several advantages in drug formulation development due to escape of gastrointestinal tract limitations, avoidance of first pass metabolism and highly vascularized nasal epithelium. Recently mucoadhesive system has received much attention to deliver various drugs via the nasal route as it significantly decrease the nasal mucociliary clearance rate and increase the residence time of the formulation in the nasal cavity for better absorption.[1,2] Although several bioadhesive materials have been used for nasal delivery of drugs, chitosan, a copolymer of glucosamine and N-acetylglucosamine has gain particular attention. Chitosan has been largely studied as a biomaterial and as a pharmaceutical excipient for drug delivery, because of its favorable biological properties.[3-5]It facilitates transport of drugs across the mucosal barriers,[4,6-8]is biodegradable and devoid of any potential toxicity.[9] However, Chitosan showed limited solubility in aqueous solution at physiological pH. This limitation of chitosan can be overcome by the quaternization of its amino groups resulting in N-trimethylchitosan (TMC). The positively charged TMC

showed improved solubility in neutral and basic aqueous media due to the substitution of the primary amine with methyl groups and retains excellent mucoadhesive properties.[10]Interactions developed between the negatively charged sialic acid groups of mucus substrate and the positively charged N-trimethyl groups of TMC are mainly responsible for attaining bioadhesiveness.[11] Because of this latter property, TMC has been chiefly used as absorption enhancer to improve nasal delivery of macromolecules.[12-16]

Present study investigates the relevance of TMC as mucoadhesive polymer for the preparation of microspheres of antiepileptic drug lamotrigine to provide more contact time and better absorption to attain sufficient therapeutic concentration. Lamotrigine is commonly used to treat partial seizures and tonic-clonic convulsions through oral route of administration.[17] In view of potential pharmacological application of lamotrigine in the treatment of epilepsy, the present work was designed to formulate its mucoadhesive microspheres using ionotropic gelation method and carry out its pharmaceutical evaluation. In the present study, TMC microspheres were prepared by ionotropic gelation method. The influence of various process and formulation parameters, like TMC concentration, stirring rate, volume of crosslinking agent (glutaraldehyde), on particle size and entrapment efficiency of microspheres was investigated.

Materials and Methods

Drugs and chemicals

Lamotrigine was acquired as a gift sample from Glenmark, Mumbai. Chitosan (Mw: >310kDa, DD: 75-85%) was obtained from FineChem Industries Mumbai, while glutaraldehyde was purchased from Rankem, Nagpur. All the chemicals used in experiments were of analytical grade.

Synthesis ofN-Trimethyl Chitosan

The synthesis of TMC was carried out according to previously reported procedure by with slight modifications. Chitosan by reductive methylation using methyl iodide (CH3I) as methylating agent in the presence of a strong base (NaOH) at 60◦C gives the formation of TMC. Accurately weighed 2.0 g of chitosan and 4.8 g of sodium iodide (NaI) were dissolved in 80.0 mL of N-methyl-2-pyrrolidinone in a 2-necked flask mounted on constant temperature water bath at 60◦C. Mixture was stirred constantly to dissolve chitosan completely. The flask was then connected to a condensation column, followed by the addition of 11.0 ml of 15% (w/v) aqueous NaOH solution and 11.5 ml of methyl iodide, under magnetic stirring. Further200.0 mL of ethanol was added to obtain precipitated product, separated by centrifugation at 300 rpm. The product then washed twice with acetone on a sintered glass filter and dried under reduced pressure. It was further dissolved in 80.0 ml of N-methyl-2-pyrrolidinone and heated to 60°C. After that, 4.8 g of sodium iodide, 11.0 ml of NaOH solution (15%, w/v) and 7.0 ml of methyl iodide were added with rapid stirring. The resulting mixture was then heated for 30 min. Again 2.0 ml of methyl iodide and 0.6 g pellets of NaOH were added with continuous stirring for 1 h. The obtained mixture (N,N,N-trimethyl chitosan iodide) was precipitated by again adding 200.0 ml ethanol and separated by centrifugation at 300 rpm for 5 min and washed twice with acetone. It was then filtered and dried under reduced pressure at 40 °C for 48 h. The obtained product (N,N,N-trimethyl chitosan iodide) was dissolved in 40.0 ml of sodium chloride (NaCl, 10%, w/v) solution to substitute the iodide ions with chloride ions. Again precipitated by adding ethanol and collected by centrifugation.[18]

Characterization of synthesized TMC byFourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), and X-ray diffraction Studies

FTIR spectrum of obtained TMC was recorded using Fourier transform infrared Thermo Nicolet, Avatar 370. The sample was dried in hot air oven at 50^0C for 2 hours. The samples was prepared as KBr discs compressed under pressure of 10 Ton/nm^2. The selected wave number range was from 400 to 4000 cm^{-1}. An obtained spectrum was observed for the specific peaks for the confirmation of preparation of TMC.

The thermal profile of TMC was recorded on Differential Scanning Calorimeter (Model:DSC-Mettler Toledo DSC 822e AG analytical , Switzerland) at a heating rate 10 °Cmin^{-1} from 30 to 350 °C under nitrogen purge of 50 ml/ min.

The X-ray diffractograms of prepared TMC was recorded on an X-ray diffractometer (Brucker AXS D8 advance Karlsruhe, Germany) to evaluate its crystallinities. Diffractograms were scanned in the range from 3° to 80° (2θ) with resolution of 0.02°and scanning speed of 2.0°min^{-1}. An accelerating voltage of 40 kV was applied at the current intensity of 35 mA.[19]

Preparation of TMC microspheres

Lamotrigine loaded TMC microspheres were prepared by ionotropic gelation method using glutaraldehyde as crosslinking agent. Lamotrigine was added to the aqueous TMC solution of different concentrations. This was the added drop-wise under constant stirring to polyanionic glutaraldehyde solution. The microspheres were formed by adding resultant solution through a 21G disposable syringe into mechanically agitated (800 rpm) solution of the cross-linking agent, glutaraldehyde at the rate of 30 drops/min from 5 cm height. Due to complexation between oppositely charged species, TMC undergoes ionic gelation and forms spherical particles. Microspheres were collectedby filtration, washed and dried in oven. Blank microspheres were prepared using same procedure as stated above. To optimize the preparation of microspheres, the formulation variables like drug concentration, polymer concentration, and concentration of crosslinking agent were applied.[20]

Table 1: Composition of different batches of LT microspheres

S. no	Formulation ID	Drug mg	Polymer concentration mg	Crosslinking agent ml
1	TL1	100	60	2
2	TL2	100	80	2
3	TL3	100	100	2
4	TL4	200	80	2
5	TL5	300	80	2
6	TL6	100	80	3
7	TL7	100	80	5
8	TL8	100	80	2
9	TL9	100	80	2

Characterization of TMC microspheres

The obtained microspheres were characterized for physicochemical parameters like particle size, polydispersity index (PDI) and Zeta potentialby dynamic light scattering (DLS) using Zetasizer (Nano ZS90, Malvern Instruments Ltd., Malvern, UK). Surface morphology of microspheres was determined by scanning electron microscopy (JEOL Model JSM - 6390LV).[21]

Drug loading and Encapsulation efficiency

The drug loading and encapsulation efficiency was determined by earlier methods with slight modifications.[21]Briefly, microspheres containing 10 mg lamotrigine were dissolved in methanol and set aside overnight to extract the drug. The samples were centrifuge at 560 rpm for 10 min to eliminate the nonsoluble residue. The resultant solution was filtered and the filtrate was analyzed for the drug content by UV- Visible spectrophotometer at 305 nm (Schimadzu 7800, Tokyo Japan). Methanol was used as blank. The data was obtained by repeating the process in triplicate. Encapsulation efficiency was determined by the following equation:[22]

$$Drugcontent\ \% = \frac{Qp}{Q} \times 100$$

Where, Qp = quantity of drug encapsulated in microspheres

Q = weighed quantity of microspheres

E = Qp/ Qt ×100

Where E = percent drug encapsulation in microspheres,

Qt= quantity of the drug added and

Qp=quantity of drug encapsulated in microspheres.

Ex vivo bioadhesion studies

The ex vivo bioadhesion studies of microspheres were carried out by falling liquid film method as described previously.[23] Briefly, fresh sheep nasal mucosa was obtained from local slaughter house (Kamptee), thoroughly extracted, washed with saline solution and mounted on a glassslide. Weighed amount of (50 mg) microspheres was carefully spread on nasal mucosa. Thereafter, 100 µl of simulated nasal electrolyte solution (SNES: containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl and 0.59 mg/ml $CaCl_2$ per liter) was spread on the microspheres and incubated for 15 min in a desiccator at 90% relative humidity. Then pre-warmed phosphate buffer solution (pH 6.6) was peristaltically pumped over the sheep nasal mucosa at the rate of 5 ml/ min and perfusate was collected in a beaker. After 1 h, the concentration of lamotrigine in the collected perfusate was determined using UV-Visible spectrophotometer rat the wavelength of 305 nm. The amount of microspheres equivalent to the amount of drug in the perfusate was determined. The mucoadhesion potential was determined by the following equation:[24]

Mucoadhesion potential (%) = [Concentration of adhered MS]/ [Concentration of applied MS] ×100.

Ex vivo biocompatibility studies

The mucosal toxicity studies were performed to confirm the biocompatibility of microspheres. Again sheep nasal mucosa was used because of its sensitivity than other tissue. The freshly excised nasal mucosa of sheep was obtained and washed with saline solution. After application of predefined amounts of lamotrigine microspheres (100 mg), mucosal tissues were fixed in 10 % formalin solution and implanted in paraffin. Paraffin sections (7.5µm) were stained with Hematoxylin- Eosin (HE) and observed under optical microscope (Model: DM2500, Leica Microsystems Inc, ButtaloGrove,IL). The untreated mucosa incubated with phosphate buffer solution (pH 6.6) was taken as a control.[25]

Differential scanning calorimetry

The thermal profile of lamotrigine, TMC and lamotrigine loaded chitosan microspheres were recorded on differential scanning calorimeter (DSC) (Mettler Toledo DSC 822e). The thermograms were obtained by heating the microspheres at rate of 10° C min^{-1} from 30° to 300° C using nitrogen purge of 50 ml/min.

In – vitro drug release studies

The in vitro drug release study of microspheres was performed by using Franz diffusion cell (receptor capacity: 12.0 ml; permeation area 3.14 cm^2) and dialysis membrane (Mw cut-off 12000–14000) as diffusion barrier. The membrane was equilibrated with phosphate buffer solution (pH 6.6) before dispersing the microspheres into the donor compartment. Accurately weighed microspheres equivalent to 10 mg of lamotriginewere applied uniformlyover the pre-hydrated dialysis membrane. The receptor compartment was filled with 12 ml of phosphate buffer solution (pH 6.6) that was within the pH range in the nasal cavity. The donor compartment was placed in such a way that it just touched the diffusion medium in the receptor compartment. The temperature was maintained at 37°±1°C using circulating water bath and was stirred with a magnetic stirring bar. Samples were withdrawn at predetermined time points (10 min interval)from the receptor compartment, replaced with the same amount of fresh pre-warmed buffer solution and assayed using UV - visible spectrophotometer (Spectro 2060 plus, UV Spectra TM, Analytical Technologies Ltd., Gujarat, India) at the wavelength of 305 nm. The release studies were carried out in triplicate and the mean values were plotted as percentage cumulative release versus time.[26]

Ex -vivo permeation studies

Ex vivo drug permeation studies of microspheres was carried out using Franz's diffusion cell (receptor capacity: 12.0 ml; permeation area 3.14 cm^2) and sheep nasal mucosa as prototypical diffusion membrane. The nasal mucosa was placed on the diffusion chamber with mucosal and serosal surfaces directed

toward supplier and receptor compartments, respectively. Microspheres equivalent to 10 mg of lamotrigine were spread over the mucosal membrane in supplier compartment previously soaked with 3.5 ml SNES. Further experimental procedures andsample collections were performed in the same manner as in described for in vitro drug release studies.[26,27]

Results and Discussion

Physicochemical Characterization of N-Trimethyl chitosan

Prepared TMC was characterized by the melting point of 183-185°C which complies with the melting point of reported values given in reference.The zeta potential of prepared TMC was 27.8 mV as compared to that of chitosan 18.2 mV, clearly indicating the introduction of methyl groups onto the nitrogen atoms, which increases the cationic property of TMC.[28]

FTIR studies

Fig 1 confirmed the trimethylation of chitosan by the appearance of band at 1477.19 cm^{-1} in the FTIR spectra of TMC. Thiscould be due to asymmetric stretching of C−H bonds of methyl groups which was not observed on the spectrum of chitosan alone. Anotherevidence of chitosan alteration i.e. TMC formation is the absence of the band near 1500 cm^{-1}in the spectrum of TMC, which was assigned to the angular deformation of N - H bonds of the amino groups on chitosan. The absence of bands in the range 1500–1600 cm^{-1}on the TMC spectrum due to the N -H bending demonstrated that the methylation of amino groups on chitosan has been effectively taken place. The bands in the range of 2800-3500 cm^{-1}appears to be more intense and shifted towards the higher frequency also confirm the formation of TMC. The small and broad band at 2133.32 cm^{-1} on the spectrum of TMC is due to the presence of ammonium ion. The characteristic bands of primary and secondary alcohols at 1091.45 and 1028 cm^{-1}on chitosan spectrum are persisted at 1082.89 and 1030 cm^{-1}on TMC spectrum, shows that the substitution occurred only at amino groups and not at C-3 or C-6 on the chitosan.

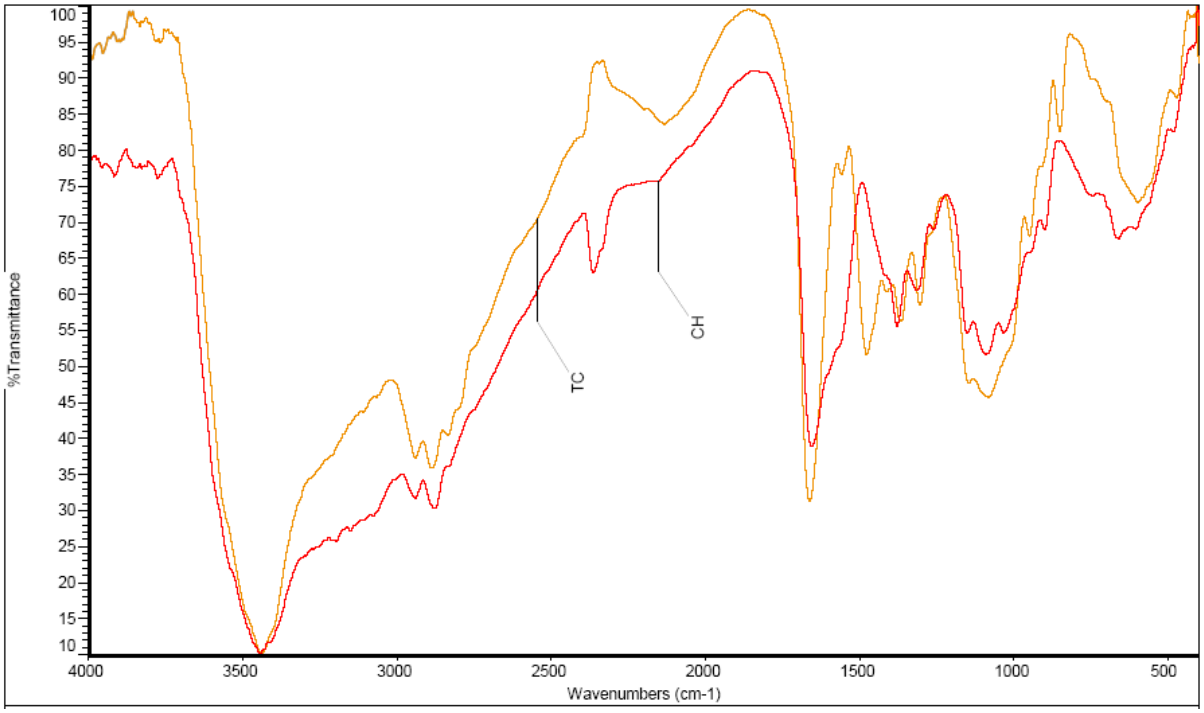

Fig. 1.FTIR spectra of Chitosan(CH) and TMC (TC)

DSC studies

As depicted in fig 2, the first thermal event occurs in the range of 43.19–133°C for chitosan and 46.07–113.64◦C for TMC, which accounts for the evaporation of water and subsequent weight loss. The weight loss as a consequence of the evaporation of water seems to depend on the presence and number of charge sites present on the polymer chains. The second thermal event occurs in the range of 282–299°C for chitosan and 253–270°C for TMC.

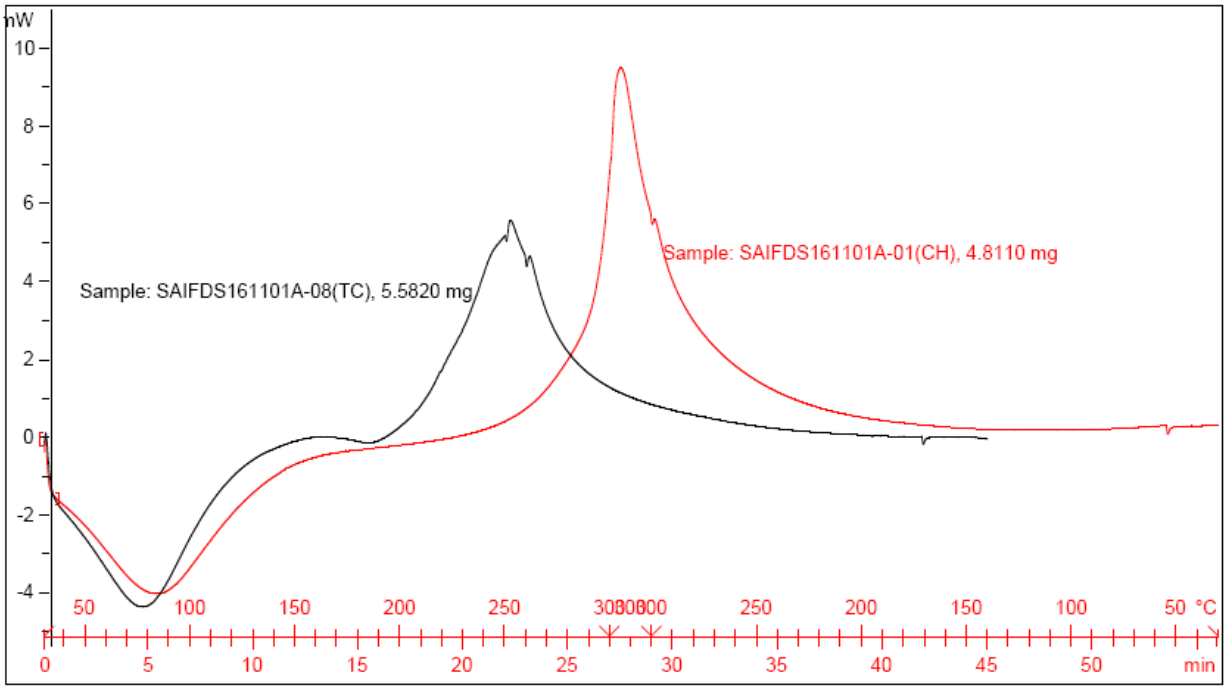

Fig.2.DSC thermograms of Chitosan (CH) andTMC (TC)

XRD studies

As depicted in fig 3, the XRD spectrum of chitosan displayed two diffraction peaks at 2θ= 9.86° and 20.38° which confirms the presence of crystalline domains on chitosan. Diffraction profiles of TMC exhibited well-defined diffraction peaks because of salt form of TMC that easily crystallizes. Intense diffraction peaks are observed in the XRD spectrum of TMC viz. at2θ = 9.302°, 20.11◦, 27.70°, 32.03◦, 45.74◦, 53.58◦, 56.70◦, 66.40◦, and 75.47◦suggesting the formation of crystalline regions with different periodic distances. The methylation of chitosan disrupts the hydrogen bonds among the amino groups leading to a disappearance of diffraction peaks in the XRD spectrum of TMC which were exhibited by bulk chitosan.[29,30]

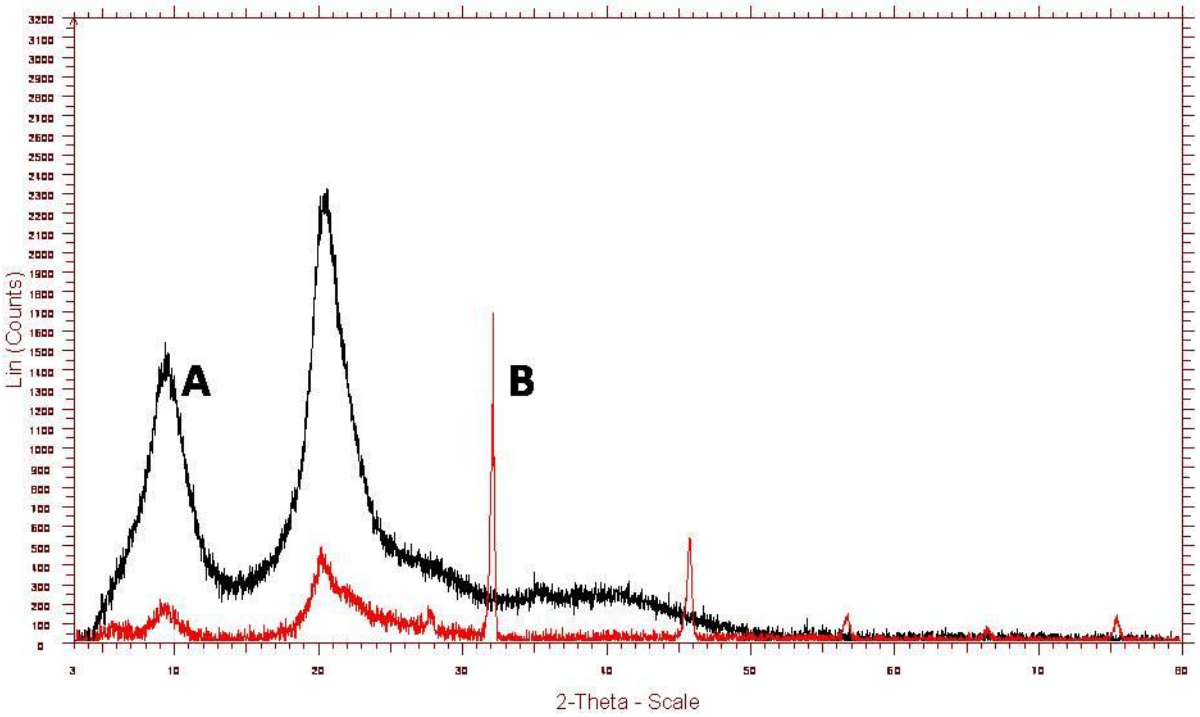

Fig. 3.X-ray diffraction patterns of Chitosan (A) and TMC (B)

Characterization of TMC microspheres

Physicochemical and morphological characterization

The percentage yield, particle size, entrapment efficiency and percent bioadhesion of different batches of microspheres are shown in Table 2. Mucoadhesive microspheres of lamotrigine were prepared by ionotropic gelation method using TMC as carrier polymer and glutaraldehyde as crosslinking agent for intranasal administration.

The particle size of microspheres varied with the concentration of TMC used. At 0.6% TMC concentration, the particle size of microspheres was 21.35±1.05 µm which was increased to 26.12±1.13 µm to34.53±1.42 µm for 0.8 % and 1% of TMC concentration respectively. This particle size range wassuitable for deposition in the nasal cavity without passing to the lower respiratory tract.[31-33]The particle size of the microsphere was directly proportional to concentration of TMC used. This may be due to increased viscosity of the TMC solution that may give larger particles. There was significant correlation between particle size and the concentration of polymer; with increasing polymer concentration particle size also increased ($p< 0.05$).PDI and zeta potential of the microspheres was found to be 0.256 and 29.8 mV respectively. PDI indicated narrow size distribution of microspheres. Percentage yield of all the batches of microspheres was found in the range of 75.03±2.85 to 98.90±0.87%. Surfacemorphology of preparedmicrospheres was determined by SEM. Lamotrigine loaded TMC microspheres showed regular shape and smooth surface(Fig. 4). SEM images indicate that lamotrigine loaded into polymeric network of TMC are spheroid particle.

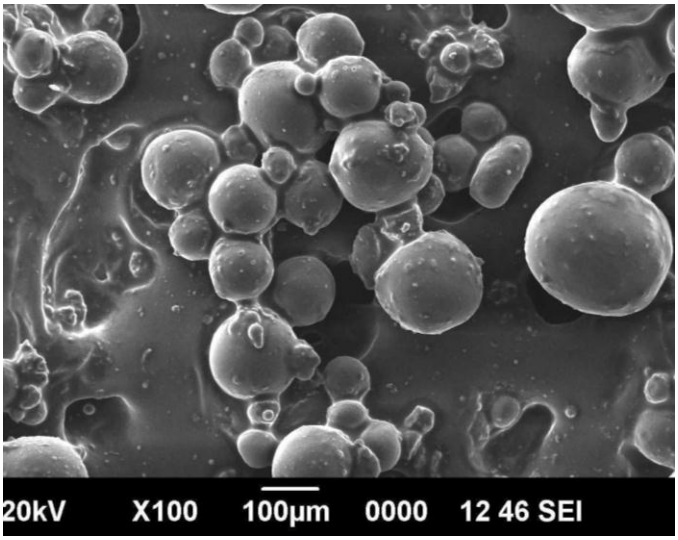

Fig 4. SEM image of lamotrigine loaded TMC microspheres

Drug Content and Encapsulation efficiency

Drug content for all the batches was from 36.63% to 41.20%. As shown in Table 2, the encapsulation efficiency increased with the increase in the concentration of TMC. It might be because of availability of more amount of polymer to entrap the drug.Batch TL1 with 0.6 % of TMC showed 74.04±1.02% entrapment of drug. This is supported by earlier studies employing TMC microspheres by ionotropic gelation technique (Ma and Liu 2010). It is noteworthy that TL2 and TL3 batches prepared at LTG: TMC ratio (1:0.8) and (1:1) showed the increase in entrapment efficiency as 85.02±0.92 and 91.64±0.64 % respectively.

Ex vivo bio-adhesion study

The in vitro bio-adhesion studies were carried out to determine the percent adhesion of formulations to nasal mucosa so as to prevent the removal of drug from the site of administration and also for enhanced permeation of drug by nasal route. The prepared microspheres showed good bio-adhesion strength ranging from 79.54±1.48 to 90.28±2.31 % as determined using excised sheep nasal mucosa. As depicted in Table 2 TMC concentration showed significant effect on bioadhesivepotential of microsphere using ionizing gelation method. As the concentration of TMC increased from 0.6 to 1 percent bioadhesion was also increased.

Table 2:Characterization of mucoadhesive microspheres

Formulation Code	Percent Yield* (%±SD)	Particle size# (µm± SD)	Drug content* (%±SD)	Encapsulation Efficiency* (%±SD)	Percent Bioadhesion* (%±SD)
TL1	91.73±1.41	21.35±1.05	39.17	74.04±1.02	82.42±2.04
TL2	93.25±1.53	26.12±1.13	39.7	85.02±0.92	86.71±1.78
TL3	98.90±0.87	34.53±1.42	40.14	91.64±0.64	90.28±2.31
TL4	89.73±0.85	32.45±2.43	39.58	85.18±1.24	85.14± 1.83
TL5	87.22±1.92	36.85±1.78	41.20	92.34±0.73	88.08±2.54
TL6	82.96±1.07	20.46±0.97	40.16	91.72±0.86	79.54±1.48
TL7	75.03±2.85	18.1±1.65	93.84	89.44±1.08	66.59±1.14
TL8	91.78±2.09	18.4±1.40	36.63	90.51±1.18	82.18±1.29
TL9	93.33±0.74	16.8±0.96	40.35	92.23±1.02	80.3±0.38

Note: * values expressed as mean ±SD; # indicates average of 100 particles ± SD

Ex -vivo biocompatibility studies

As shown in Fig.5, there were no apparent signs of any epithelial necrosis or sloughing of epithelial cells on the microsphere treated nasal mucosa.Thus, the biocompatibility of lamotrigine loaded TMC

microspheres was consistent with earlier reports that showed that TMC is the least toxic polymer owing to its high degree of deacetylation and can be applied on the nasal epithelium.[28]

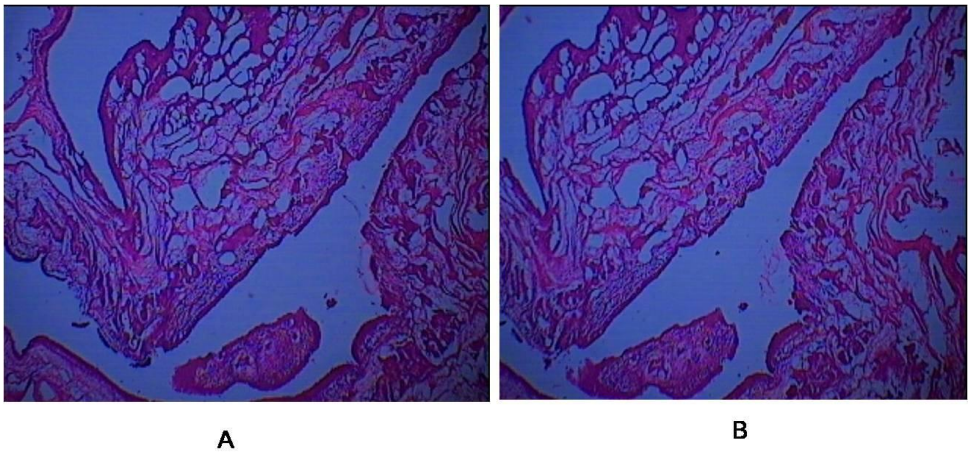

Fig. 5. Light photomicroghaph of sheep nasal mucosa, A) untreated control mucosa and (B) Lamotrigine loaded TMC microspheres- treated mucosa

Differential scanning calorimetry

DSC studies explored the thermal properties of the prepared microspheres, providing qualitative information about the physicochemical state of drug within the microspheres. DSC thermograms of lamotrigine, TMC and lamotrigine loaded TMC microspheres are shown in Fig 6. DSC thermogram of lamotrigine showed a sharp endothermic peak at 218.29° C indicating melting point of lamotrigine. By investigating the thermogram of lamotrigine loaded TMC microspheres it was found thatthe endothermic peak of drug was shifted from 218.29° to 225.25°C indicates the disappearance of melting point of lamotrigine from the thermogram.This might bedue to incorporation and molecular dispersion of lamotrigine in TMC polymer medium of the prepared microspheres formulations.

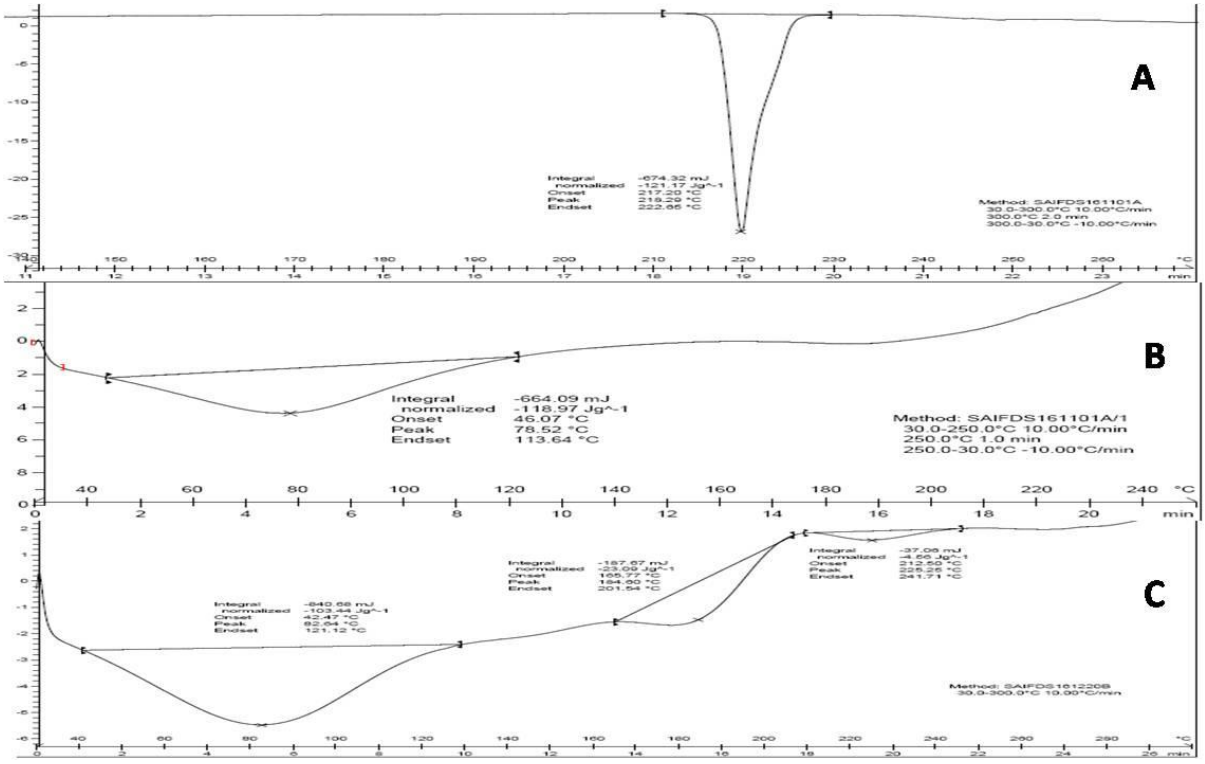

Fig 6. DCS thermograms of A) Lamotrigine, B) TMC and C) lamotrigine loaded TMC microspheres

In-vitro drug release study

The in vitro drug release studies of lamotrigine loaded TMC microspheres were performed using Franz diffusion cell.Figure 7 showed the drug release profiles of all formulationbatches of microspheres after 90 min at pH 6.6 phosphatebuffer. The release patterns of all the formulations appearedto show immediate release from the microspheres. The rate and extent of drug releasefrom microspheres were significantly retarded with anincrease in TMC concentration.Results of in vitro drug release studies suggested thatthe batch TL6, TL8 and TL9 showed faster and maximum drug release as compared to the other batches. Batch TL9 with smallest particle size and maximum entrapment efficiency released the drug at faster rate thusconsideredas an optimized formulation. The improvement of the dissolution of the drugs from the microspheres was attributed to their smaller size and entrapment efficiency that may lead to the uniformdispersion of the drug into the polymeric network of TMC.

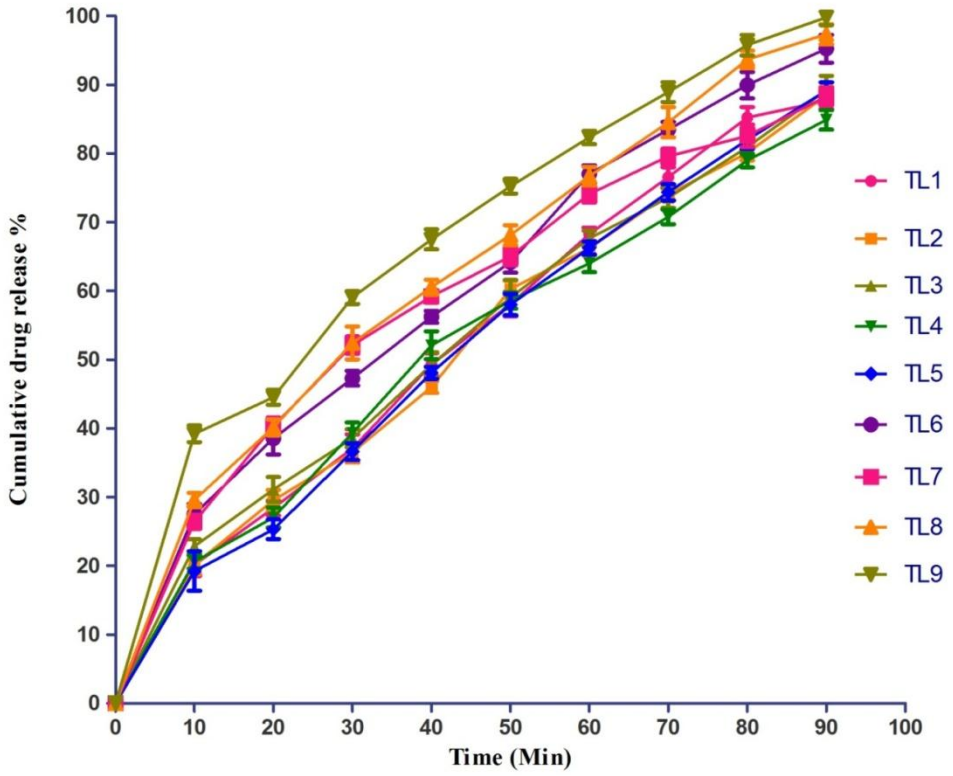

Fig. 7. In-vitro drug release profiles of lamotrigine loaded TMC microspheres

Ex -vivo permeation studies

The optimized formulations TL8 and TL9 were further subjected tothe ex-vivo permeation studies using sheep nasal mucosa.As depicted in Fig 9, the permeation of lamotrigine from TMC microspheres of batch TL8 and TL9was found to be in the range of 88.80% and 83.24% respectively. TMC polymeric material with enhanced solubility than chitosan can be used to increase the drug dissolution and absorption through mucoadhesive microspheres of poorly water soluble drugs. Smaller particle size of microspheres provides greater surface area that enhances the drug release from the formulations.

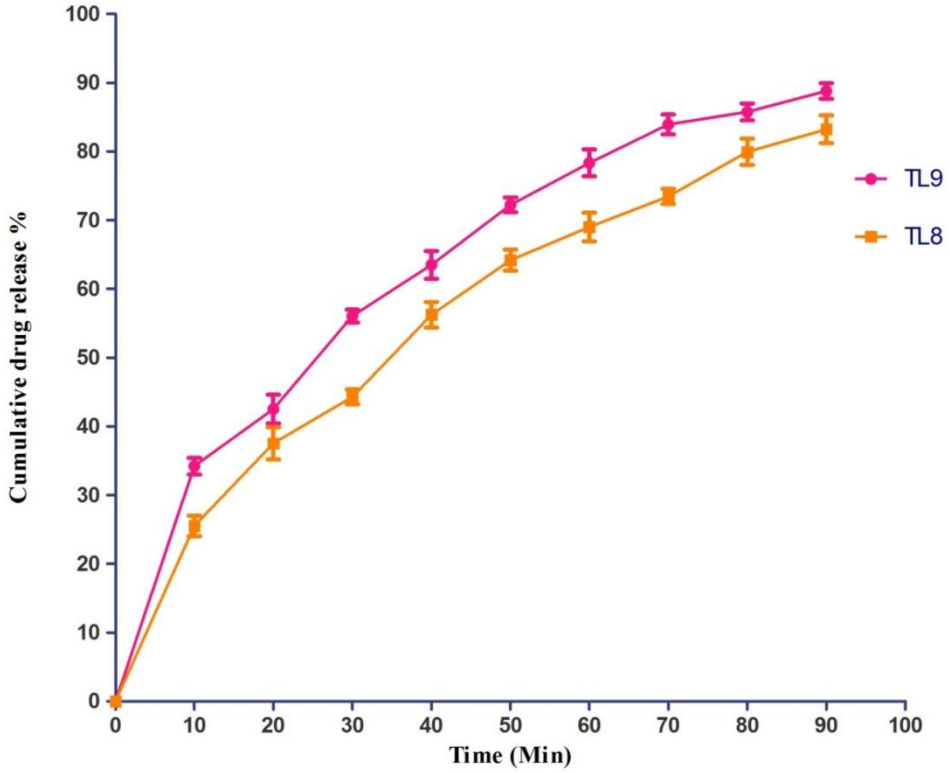

Fig. 8. Ex-vivo permeation of lamotrigine loaded TMC mucoadhesive microspheresthrough nasal mucosa

Conclusion

In the present study, N,N,Ntrimethyl chitosan was synthesized as chemically modified derivative of chitosan by reductive methylation reaction and characterized by the FTIR, DSC and XRD studies. We have prepared and optimized lamotrigine loaded TMC mucoadhesive microspheres for intra-nasal administration by ionic gelation technique. Physicochemical assessment confirmed that the lamotrigine loaded TMC microspheres showed suitable particle size for intranasal administration, more encapsulation efficiency, strong bioadhesion potential and devoid of any morphological toxicity on excised sheep nasal mucosa. In addition, permeation across excised sheep nasal mucosa exhibited good permeability of lamotrigine loaded TMC microspheres. Thus the formulation of lamotrigine loaded chitosan mucoadhesive microspheres offers promising advantagesover conventional dosage forms. It would be advantageous to carry out pharmacokinetic and pharmacodynamics studies of lamotrigine loaded TMC microspheres to ensure its therapeutic efficacy in epileptic seizures.

Funding source: The work was supported under the head of institutional research grants.

Conflict of interest: Authors declare no Conflict of interest.

References

1. Soane RJ, Ferier M, Perkins AC, Jones NS, Davis SS, Illum L., Evaluation of the clearance characteristics of bioadhesive system inhumans, Int. J. Pharm.,1999, 178,55-65.
2. Soane RJ, Hinchcliffe M, Davis SS, Illum L., Clearance characteristics of chitosan based formulation in the sheep nasal cavity, Int. J. Pharm., 2001, 217, 183-191.
3. Illum L., Chitosan and its use as a pharmaceutical excipient, Pharm. Res., 1998, 15, 1326-1331.
4. Felt O, Buri P, Gurny R., Chitosan: a unique polysaccharide for drug delivery, Drug Dev. Ind.Pharm., 1998, 24,979-993.
5. Junginger HE, Verhoef JC., Macromolecules as safe penetration enhancers for Hydrophilic Drugs-a fiction? Pharm. Sci. Technol. Today., 1998, 1, 370-376.

6. Illum L, Farraj NF, Davis SS., Chitosan as a novel nasal delivery system for peptide drugs, Pharm. Res.,1994, 11, 1186-1189.
7. Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS., Chitosan as a novel nasaldelivery system for vaccines, Adv. Drug Deliv. Rev., 2001, 51, 81–96.
8. Borchard G, Lueßen HL, De Boer AG, Verhoef JC, Lehr CM, Junginger HE., The potential of mucoadhesive polymers in enhancing intestinal peptide drug absorption: III. Effects of chitosan glutamate and carbomer on epithelial tight junctions in vitro, J. Control. Release.,1996, 39, 131-138.
9. Onishi H and Machida Y., Biodegradation and distribution of water-soluble chitosan in mice, Biomaterials., 1999, 20, 175-182.
10. Van der Lubben IM, Verhoef JC, Borchard G, Junginger, HE., Chitosan and its derivatives in mucosal drug and vaccine delivery, Eur. J. Pharm. Sci., 2001, 14, 201-207.
11. Mourya VK, Inamdar NN.,Trimethyl chitosan and itsapplication in drug delivery.,J. Mater.Sci. Mater. Med., 2009, 20, 1057-1079.
12. AmidiM ,Romejin SG, Borchard G, Junginger HE, Hennink WE, JiskootW.,Preparation and characterization ofprotein-loaded N-trimethyl chitosan nanoparticles as nasal delivery system. J. Controlled Release., 2006, 111, 107-116.
13. Du Plessis LH, Lubbe J, Straus T, Kotze AF., Enhancementof nasal and intestinal calcitonin delivery by the novel Pheroid fattyacid based delivery system, and by N-trimethyl chitosan chloride. Int. J.Pharm., 2010, 385, 181-186.
14. Jintapattanakit A, Peungvicha P, Sailasuta A, Kissel T, Junyaprasert VB. ,Nasal Absorptionand local tissue reaction of insulin nanocomplexes of trimethyl chitosan derivatives in rats, J.Pharm.Pharmacol.,2010, 62, 583-591.
15. Du Plessis LH, Kotze AF, Junginger HE., Nasal and rectaldelivery of insulin with chitosan and N-trimethyl chitosan chloride, Drug Delivery 2010, 17, 399-407.
16. 16.Nazar H, Caliceti P, Carpenter B, El-Mallah AI,Fatouros DG, Roldo M, van der Merwe SM, TsibouklisJ., A once-a-daydosage form for the delivery of insulin through the nasal route: invitroassessment and in vivo evaluation. Biomater.Sci.,2013, 1, 306-314.
17. Schiller Y and KrivoyN., Safety and efficacy of lamotrigine in older adults with epilepsyand co-morbid depressive symptoms,Clin. Med. Ther., 2009, 1, 825–833.
18. Sieval AB, Thanou M,Kotze AF, Verhoef JC, Brussee J Junginger HE. Preparation andNMR characterization of highly substituted N-trimethyl chitosan chloride, Carbohydr.Polym.,1998, 36,157-165.
19. Karavasili C, Katsamenis OL, Bouropoulos N, Nazar H, Thurner PJ, Van der MerweSM,Fatouros DG., Preparation and characterization of bioadhesivemicroparticlescomprised of low degree of quaternizationtrimethylated chitosanfor nasal administration: effect of concentration and molecularweight., Langmuir., 2014, 30, 12337−12344.
20. Pani NR, Acharyab S, Patraba S., Development and validation of RP-HPLC method forquantification of glipizide in biological macromolecules, Int. J. Bio.Macromol.,2014, 65,65-71.
21. Rajinikanth PS,SankarC, Mishra B., Sodium alginate microspheres of metoprololtartrate for intranasal systemic delivery: Development and Evaluation,Drug Delivery., 2003,10,21-28.
22. Fu-De C, Ming-Shi Y, Ben-Gang Y, Yu-Ling F, Liang W, Peng Y,HeY., Preparation ofsustained-releasenitrendipine microspheres with eudragit RS and aerosil using quasi-emulsion solvent diffusion method,Int. J. Pharm., 2003, 259, 103–113.
23. RangaRao KV and BuriP., A novel in situ method to test polymers and coated microparticlesfor bioadhesion,Int. J. Pharm., 1989, 52, 265-270.
24. SaraparnH,VimolmasL,NaruepornS, Garnpimol CR., Spray-dried mucoadhesive microspheres: preparation and transport through nasal cell monolayer, AAPS Pharm SciTech., 2006,7, E79-E88.
25. Pardeshi CV, Belgamwar VS, Tekade AR, Surana SJ.,Novel surface modified polymer–lipid hybrid nanoparticles as intranasal carriers for ropinirole hydrochloride: in vitro, ex vivo and in vivo pharmacodynamic evaluation,J. Mater. Sci., 2013, 24 ,2101-2115.
26. Pardeshi CV, Rajput PV, Belgamwar VS, Tekade AR., Formulation, optimization and evaluation of spray-dried mucoadhesive microspheres asintranasal carriers for Valsartan, J.Microencapsul., 2012, 29, 103-114.
27. Tas C, OzkanCK, Savaser A, Ozkan Y, Tasdemir U, Altunay H., Nasal absorption of metoclopramide from different Carbopol981 based formulations: In vitro, ex vivo and invivo evaluation,Eur. J. Pharm.Biopharm., 2006, 64, 246-254.

28. PardeshiCV and Belgamwar VS., Controlled synthesis of N,N,N-trimethyl chitosan for modulated bioadhesion and nasal membrane permeability,Int. J. Biol. Macromol., 2015, 82, 933-944.
29. Martins AF, Pereira AGB, Fajardo AR, Rubira AF, MunizEC., Characterization of polyelectrolytes complexes based on N,N,N-trimethyl chitosan/heparin prepared at different pH conditions, Carbohydr.Polym., 2011, 86,1266-1272.
30. Britto D and Campnana-Filho SP., A kinetic study on the thermal degradationof N,N,N-trimethylchitosan, Polym. Degrad. Stab.,2004,84, 353-361.
31. Momeni A and Mohammadi MH., Respiratory delivery of theophylline by sizetargeted starch microspheres for treatment of asthma, J. Microencapsul.,2009,26, 701-710.
32. Naikwade SR, Bajaj AN, GuravP, Gatne MM, Singh SoniP., Development of budesonide microparticles using spray-drying technology for pulmonary administration: design, characterization, in vitro evaluation, and in vivo efficacy study, AAPS Pharm. Sci. Technol., 2009, 10, 993-1012.
33. Vidgren P,Vidgren M,Arppe J, Hakuli T, Laine E, Paronen P., In vitroevaluation of spray-dried mucoadhesive microspheres for nasal administration, Drug Dev. Ind. Pharm., 1992,18, 581-597.

*****