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DEVELOPMENT AND EVALUATION OF PEPTIDE LOADED MUCOADHESIVE MICROSPHERES: IN AN EFFORT TO IMPROVE NASAL BIOAVAILABILITY OF PEPTIDE

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ABSTRACT: The nasal drug delivery system has been a promising route for delivery of proteins and peptides as it can avoid degradation in the gastrointestinal tract and metabolism by liver enzymes. However, due to the rapid mucociliary clearance, the bioavailability of proteins and peptides is still low. Hence, mucoadhesive microspheres may prolong the residence time of peptide drugs in nasal cavity and improve their absorption. In the present study chitosan microspheres loaded with a model peptide vancomycin hydrochloride were prepared. Drug-excipient compatibility studies were done to investigate and predict any possible interactions between the components in the formulation. The prime objective of the study was to check the influence of different process variables and formulation parameters on the two key parameters *viz* particle size and percentage entrapment efficiency of vancomycin loaded chitosan microspheres and to develop a formulation with desirable particle size for nasal delivery and with maximum possible entrapment efficiency. Drug-excipient compatibility study indicates that vancomycin hydrochloride is compatible with the polymer chitosan. Drug loaded chitosan microspheres were successfully prepared by the emulsification-crosslinking technique. The results indicate that the selected process variables and formulation parameters significantly affect the particle size and percentage entrapment efficiency of drug-loaded microspheres. An optimum formulation is obtained using at 4% w/v of chitosan concentration, 1:10 aqueous to oil phase ratio, 0.5 ml volume of glutaraldehyde with a cross-linking time of 1 h, with stirring speed 1000 tpm and 1% w/v concentration of Span 80 as a stabilizer. Further, optimized microspheres show controlled release for 8 h, which follows the Higuchi model.

INTRODUCTION: With the recent remarkable progress in biotechnology, a class of clinically available peptide and protein drugs has been expanding more than ever. Generally, drugs can be administered *via* various parenteral or non-parenteral routes and the choice of the route normally depends on several key factors such as clinical advantages, convenience of administration, properties of the drugs. The pharmacokinetic profile needed for therapy and cost of development [1]. The nasal mucosa is an attractive site for the non-parenteral delivery for protein therapeutics [2]. As a site for systemic absorption of drug, the nasal cavity has many advantages, which include easy access, highly vascular zed epithelial layer, relatively large surface area, porous endothelial basement membrane and avoiding the first pass metabolism [3].

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However, one of the most important limiting factors for nasal drug delivery is nasal mucociliary clearance. The typical residence time of a protein delivered to the nasal mucosa is only 15-30 min due to rapid ciliary clearance [4]. Thus, muco-adhesive microspheres have been developed to decrease the effect of mucociliary clearance.

The mucoadhesive microspheres forma gel like layer, which is cleared slowly from the nasal cavity and thus results in a prolonged residence time of the drugformulation [5]. Some bioadhesive platforms not only increase residence time at the nasal mucosa but may also increase protein solutions stability [6] and/or enhance protein uptake. These bioadhesives are typically high molecular weight polymers with flexible chains that can interact with mucin through hydrogen bonding, electrostatic, hydrophobic or van der waals interactions [7].

Various biodegradable polymers have been used as carriers for microparticulate drug delivery systems. Chitosan, a cationic polymer, is frequently used for application of macromolecules in nasal cavity due to its strong mucoadhesive property [8]. Muco-adhesion is obtained by the ionic interaction between negatively charged groups of the mucosal membrane and positively charged amine groups of the D-glucosamine unit of chitosan [9].

The permeation-enhancing property of chitosanis due to its mucoadhesive property and its ability to transiently open the tight junctions in the nasal mucosa [10]. Histological study also confirmed that chitosan does not lead to any significant histological changes in nasal mucosa [11].

In the present study, we have prepared chitosan microspheres containing a test peptide vancomycin hydrochloride; by emulsification cross-linking method for nasal delivery. Drug-excipient compatibility study is done to investigate and predict any possible physicochemical interactions between the components in a formulation and to ensure that the selected polymers are compatible with the drug.

For nasal drug delivery system particle size is one of the most important factors as it influences nasal absorption by influencing region of deposition in nostril. Further, as the therapeutic peptides are relatively expensive, so the goal was also to minimize its loss and get maximum entrapment efficiency of the peptide used. So, the prime objective of the study was to check the influence of different process variables and formulation parameters on the two key parameters *viz* particle size and percentage entrapment efficiency of vancomycin loaded chitosan microspheres and to develop a formulation with desirable particle size and maximum possible entrapment efficiency.

MATERIALS AND METHODS: Vancomycin hydrochloride was gifted by Aurobindo Pharma, Hyderabad, India. Chitosan was provided by Central Institute of Fisheries Technology, Kochi, India; as a gift sample. Light and heavy liquid paraffin and Glutaraldehyde (25% aqueous solution) were purchased from Merck Specialties Private Limited, Mumbai, India. Methanol, Acetone, n-Hexane, Tween 80 were purchased from SD Fine Chemicals, Mumbai, India. All other chemicals and reagents used were of pharmaceutical grades.

Drug-excipients Compatibility Studies: Proper design and formulation of any dosage form require consideration of the physical, chemical, and biological characteristics of drug substance(s) and all excipients to be used in the development of a product. Careful selection of the excipients used in a formulation is the key to the development of a stable and effective dosage form. The drug(s) and the excipients must be compatible with one another to produce a stable formulation. To investigate the chemical compatibility of the drug with the selected excipients, FT-IR was used.

The drug and the selected polymer were mixed physically in the ratio of 1:1, and the mixtures were placed in sealed vials for three months at room temperature. After three months, the sample was subjected to FT-IR study. Samples were finely ground with an infra-red grade of KBr then pressed into pellets, and FTIR spectra were taken in transmission using spectrum GX FT-IR spectro-meter (Perkin Elmer, USA) over the range of 4000-400 cm^{-1}. The obtained FT-IR spectrum was compared against the FT-IR spectra of pure drug and polymer alone.

Preparation of Vancomycin Hydrochloride Loaded Chitosan Microspheres: Vancomycin hydrochloride (VANCO) loaded chitosan microspheres were prepared by w/o emulsification cross-linking process [12]. Briefly, VANCO was dissolved in chitosan solution in 10 ml of aqueous acetic acid by continuous stirring using a magnetic stirrer. The prepared polymer-drug solution was then added slowly into 100 ml liquid paraffin

(heavy and light, 1:1) containing span-80 as stabilizing agent under continuous stirring. After 15 min of stirring, glutaraldehyde was added slowly under continuous stirring.

After a stipulated period of stirring time, the hardened microspheres were centrifuged, washed with hexane thrice, and finally with acetone to remove oil. Finally, the prepared microspheres were dried in a vacuum oven for 24 h at 50 °C and then stored in a desiccator. The effect of different process parameters on the two key parameters *viz.* particle size and percentage entrapment efficiency of chitosan microspheres was investigated.

Different batches **Table 1** of formulations were prepared by varying one parameter at a time and keeping the others constant.

TABLE 1: DIFFERENT BATCHES OF VANCOMYCIN LOADED CHITOSAN MICROSPHERES

Formulation code	Chitosan conc. (%w/v)	Aqueous to oil phase ratio	Volume of GA (ml)	Cross-linking time (hr)	Stirring speed (rpm)	Conc. of SPAN80
Selection of chitosan concentration						
F1	1%	1:10	0.5	1	1000	1
F2	2%	1:10	0.5	1	1000	1
F3	4%	1:10	0.5	1	1000	1
F4	6%	1:10	0.5	1	1000	1
Selection of aqueous to oil phase ratio						
F5	4%	0.5:10	0.5	1	1000	1
F3	4%	1:10	0.5	1	1000	1
F6	4%	2:10	0.5	1	1000	1
Selection of volume of glutaraldehyde (GA)						
F3	4%	1:10	0.5	1	1000	1
F7	4%	1:10	3	1	1000	1
F8	4%	1:10	5	1	1000	1
Selection of cross-linking time						
F9	4%	1:10	0.5	0.5	1000	1
F3	4%	1:10	0.5	1	1000	1
F10	4%	1:10	0.5	3	1000	1
Selection of stirring speed						
F11	4%	1:10	0.5	1	500	1
F3	4%	1:10	0.5	1	1000	1
F12	4%	1:10	0.5	1	1500	1
Selection of concentration of span 80						
F13	4%	1:10	0.5	1	1000	0.5
F3	4%	1:10	0.5	1	1000	1
F14	4%	1:10	0.5	1	1000	2

Morphological Characterization: All batches of chitosan microspheres were checked for shape and size-by optical microscopy. A light transmission microscope (Kyowa-Gertner, India) at 40X magnification was employed for morphological investigation. Further, the surface morphology of optimized VANCO loaded microspheres was examined by Scanning Electron Microscopy (SEM).

Particle Size Measurements: The particle size of the prepared microspheres was determined by using optical microscopy method [13].

Three hundred freshly prepared and dried microspheres were counted for particle size analysis by using a calibrated optical microscope at a magnification of 40X. The average diameter of 300 microspheres was considered as the mean particle size.

Determination of Percentage Entrapment Efficiency (PEE): Accurately weighed samples of VANCO-loaded chitosan microspheres were added to 100 ml of 0.1NHCl and subjected to ultrasonication. After ultrasonication samples were filtered, and the amount of VANCO was determined spectrophotometrically at 281 nm on UV spectrophotometer (Shimadzu UV-1800, Japan). The percentage of entrapment efficiency was calculated using the following equation:

% Entrapment efficiency = Actual loading / Theoretical loading × 100

***In-vitro* Drug Release Study:** The *in-vitro* drug release test of optimized VANCO loaded chitosan microspheres was performed using Franz diffusion cell with a dialysis membrane (MW cut-off 14 000, HI Media, India). The receptor compartment contained phosphate buffer solution pH 6.8 that was within the pH range in the nasal cavity and maintained at 37 °C ± 1 °C. The membrane was equilibrated before carefully dispersing the sample onto the donor side. The receptor compartment was stirred with a magnetic stirring bar. Samples were periodically withdrawn from the receptor compartment, replaced with the same volume of fresh buffer solution, and assayed by a spectrophotometer (Shimadzu-1800, Japan) at 281 nm. All the experiments were carried out in triplicate, and average values were calculated [14].

RESULTS AND DISCUSSION:

Drug-excipients Compatibility Studies: FT-IR was used to investigate and predict any possible physicochemical interactions between the components in the formulation and to ensure that the selected polymer incompatible with the drug.

As shown in **Fig. 1** pure VANCO shows its characteristic bands at 1653 cm^{-1} for C=O stretching (Amide-I), at 1505 cm^{-1} for C=C stretching, at 1230 cm^{-1} for C-O-C stretching and at 1062 $cm^{-1}$for C-N (aliphatic amine) stretching; which is in accordance with the standard FT-IR data of VANCO [15]. The pure chitosan shows a characteristic absorption band at 3441 cm^{-1}corresponding to the overlapping of its O-H stretching vibration, symmetric N-H vibration and the intermolecular hydrogen bonding of the polysaccharide moiety of CS, 2924 and 2856 cm^{-1} corresponding to C-H stretching vibration. It also shows the absorption band sat 1630 $cm^{-1}$and 1317 cm^{-1} for carbonyl stretching vibration (amide-I) and C-N stretching vibration (amide-III), respectively, while absorption at 1384 and 1416 cm^{-1} are due to symmetrical deformation of its methyl (CH_3) groups [16]. In the case of VANCO-Chitosan-mixtures, all the characteristic bands that are observed in both pure VANCO and Chitosan have again appeared, indicating that the drug-VANCO is compatible with the selected polymer chitosan.

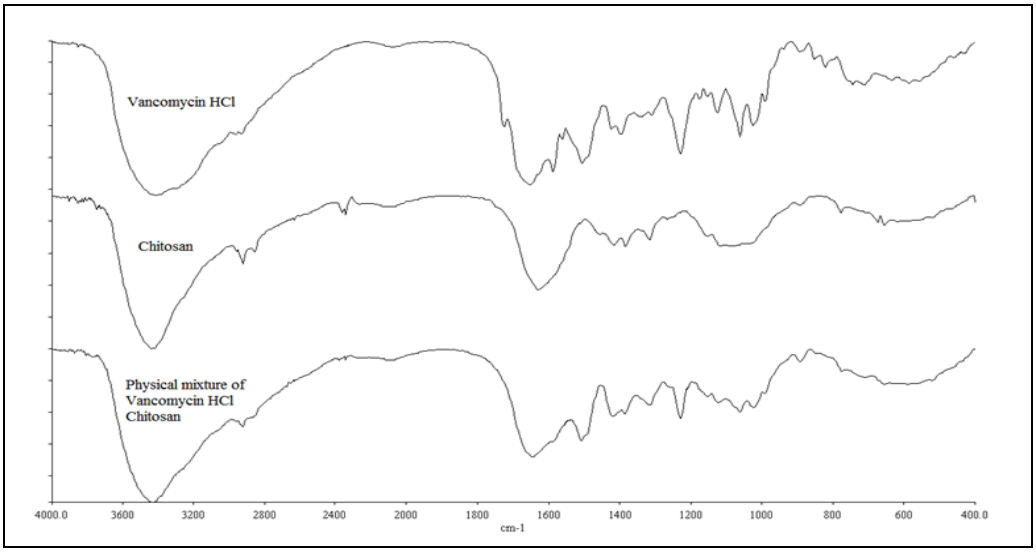

FIG. 1: FT-IR SPECTRA OF PURE DRUG-VANCO; PURE CHITOSAN; VANCO-CHITOSAN-MIXTURES

Results of Evaluation of VANCO Loaded Chitosan Microspheres: In thisstudy the VANCO loaded chitosan microspheres were prepared and evaluated for sphericity of microspheres, particle size and drug entrapment efficiency. Results of all the batches are shown in **Table 2**.

Effect of Chitosan Concentration: Microspheres were not obtained when chitosan was usedin 1% concentration (F1). Formulation F1 showed flakes type particles and not spheres. However, when chitosan is used at a concentration of 2% (F2) and 4% (F3) a well discrete, spherical microspheres with little clumping was obtained. A further, increase in chitosan concentration (at 6%), it was difficult to prepare the microspheres, due to enhanced viscosity of the polymer solution.

Effect of Aqueous to Oil Phase Ratio: Result shows that as the aqueous to oil phase ratio

increases the particle size of the prepared microspheres was also increased. When aqueous to oil phase ratio was increased from 0.5:10 (F5) to 1:10 (F3), a slight increase in particle size of the microspheres was observed (17.41 ± 1.67 µm to 18.85 ± 1.73 µm). Further, increasing the aqueous to oil phase ratio up to 2:10 (F6) results in a significant increase in the particle size (37.22 ± 1.97 µm) of the microspheres. Moreover, the prepared microspheres were aggregated. This increase in particle size and aggregation of microspheres with an increase in aqueous to oil phase ratio may be attributed to the reduced distance between droplets of the aqueous phase in an oil phase, which in turn increases the chances of coalescence between aqueous phase droplets. Hence, aqueous to oil phase ratio of 1:10 was found to be most suitable in the preparation of chitosan microspheres.

Effect of Volume of Glutaraldehyde (GA): To check the effect of cross-linking agent, different batches were taken by varying the volume of glutaraldehyde from 0.5 to 5 ml. Discrete spherical microspheres were obtained using 0.5, 3, and 5 ml of glutaraldehyde. However, the microspheres batches show a significant effect on drug entrapment efficiency. For 0.5, 3, and 5 ml of glutaraldehyde, the entrapment efficiency was found to be 85.23 ± 1.43, 78.46 ± 1.22, and 64.02 ± 1.25 respectively.

The result indicates that with an increase in the amount of glutaraldehyde the percentage entrapment efficiency of prepared microspheres decreases significantly, this may be due to a decrease in free spaces within the polymer matrix with an increase in cross-link density.

Further, during the cross-linking and hardening process, the microspheres water is exuded from the polymer matrix, which also takes away the dissolved drug and may be another contributing factor for the low incorporation efficiency [17].

TABLE 2: SPHERICITY, PARTICLE SIZE AND DRUG ENTRAPMENT EFFICIENCY OF DIFFERENT BATCHES OF VANCOMYCIN LOADED CHITOSAN MICROSPHERES

Form code	Sphericity of microspheres	Particle size (µm)	Drug entrapment efficiency
Selection of chitosan concentration			
F1	-----	-----	-----
F2	Spherical	15.39 ± 2.44	72.31 ± 2.22
F3	Spherical	18.85 ± 1.73	85.23 ± 1.43
F4	-----	-----	-----
Selection of aqueous to oil phase ratio			
F5	Spherical	17.41 ± 1.67	79.78 ± 2.76
F3	Spherical	18.85 ± 1.73	85.23 ± 1.43
F6	Aggregated	37.22 ± 1.97	85.89 ± 2.21
Selection of volume of glutaraldehyde (GA)			
F3	Spherical	18.85 ± 1.73	85.23 ± 1.43
F7	Spherical	15.45 ± 2.41	78.46 ± 1.22
F8	Spherical	10.11 ± 2.12	64.02 ± 1.25
Selection of cross-linking time			
F9	Aggregated	-----	-----
F3	Spherical	18.85 ± 1.73	85.23 ± 1.43
F10	Spherical	19.88 ± 2.32	81.03 ± 1.76
Selection of stirring speed			
F11	Spherical	38.56 ± 3.45	85.58 ± 2.02
F3	Spherical	18.85 ± 1.73	85.23 ± 1.43
F12	Spherical	07.26 ± 1.34	75.55 ± 2.19
Selection of concentration of span 80			
F13	Irregular	-----	-----
F3	Spherical	18.85 ± 1.73	85.23 ± 1.43
F14	Spherical	16.51 ± 1.64	76.45 ± 2.02

Effect of Cross-linking Time: The check the effect of cross-linking time three different batches were formulated using 0.5 h, 1 h and 3 h as cross-linking time. The lower cross-linking time *i.e* 0.5 h was found to be insufficient, as within this time, the cross-linking reaction between GA and chitosan

was not complete, and thus the resultant microspheres were aggregated and inadequately hardened. However, when the cross-linking time increases to 1 h and 3 h; microspheres with good physical properties *i.e.* discrete and smooth spherical surface were obtained and mean particle size was of 18.85 ± 1.73 µm and 19.88 ± 2.32 µm, respectively. With increasing cross-linking time, a slight increase in particle size was observed. Further entrapment efficiency decreases for a cross-linking time of 3 h (81.03 ± 1.76) when compared to 1 h (85.23 ± 1.43). Therefore, it may be concluded that an increase in curing time would increase the interaction time leading to the formation of a dense chitosan matrix, which is thought to be associated with a decrease in entrapment efficiency [18].

Effect of Stirring Speed: Results show that mean particle size of prepared chitosan microspheres decreased with increasing stirring rate. The particle size of prepared microspheres was decreased from 38.56 ± 3.45 µm to 18.85 ± 1.73 µm as stirring rate increased from 500 to 1000 rpm. Further, at a stirring rate of 500 rpm, the sphericity and smoothness of the chitosan microspheres were also affected. This may be due to the formation of larger droplet size of internal phase as a result of less efficient shearing of the chitosan solution at low stirring rate [19]. At the higher stirring rate, smooth and spherical and smooth microspheres were obtained. The mean particle size of microspheres was 18.85±1.73µm and 07.26 ± 1.34 µm for stirring rate of 1000 and 1500 rpm, respectively. As a higher stirring rate provides the required energy to the internal phase to be dispersed as fine droplets in the oil phase and, therefore, microspheres smaller in size are formed, similar findings were also reported in the related literature [20]. Further, entrapment efficiency decreases with an increase in the stirring speed from 1000 (85.23 ± 1.43) to 1500 rpm (75.55 ± 2.19).

Effect of Concentration of Span 80: Surfactants play important role in preparation of microspheres by emulsification cross-linking process as they prevent aggregation of dispersed phase. Therefore, the effects of concentration of Span 80, on the drug-loaded chitosan microspheres were studied. Irregular shape microspheres were obtained at 0.5%w/v span-80 concentration (F13). An increase in the concentration of Span-80 to 1%w/v (F3) gives discrete spherical microspheres with particle size 18.85 ± 1.73 µm and percentage entrapment efficiency 85.23 ± 1.43. However, further increase in span 80 concentration to 2.0% (F14) result in decrease in entrapment efficiency (76.45 ± 2.02), which may be due to the solubilizing effect of span 80 during the preparation of microspheres [21].

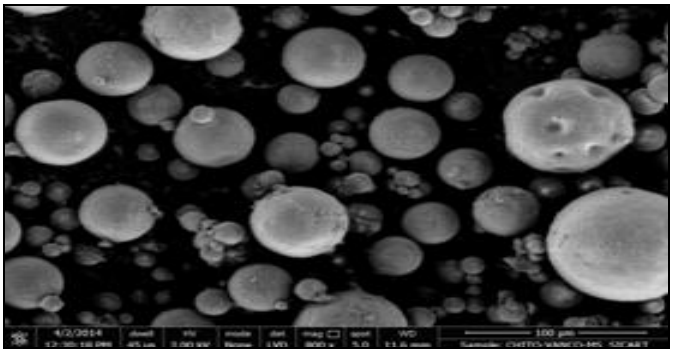

FIG. 2: SEM IMAGES OF VANCO-LOADED CHITOSAN MICROSPHERES BATCH F3

***In-vitro* Drug Release Study:** The *in-vitro* drug release behavior of VANCO from optimized chitosan microspheres Batch F3 was monitored for 8 h, and the cumulative percentage of drug release were plotted against time to obtain drug release profile **Fig. 3**. *In-vitro* drug release study revealed VANCO loaded chitosan microspheres followed a biphasic kinetic mechanism: an initial fast release mainly causes from the dissolution of drug from the surface of the microspheres, followed by a controlled release by the rate of swelling of the polymer matrix and simultaneous diffusion of VANCO from the interior of the cross-linked microspheres [22]. From the result of drug release kinetic analysis, it was found that the drug release follows Higuchi model, indicating release from chitosan microspheres was diffusion controlled.

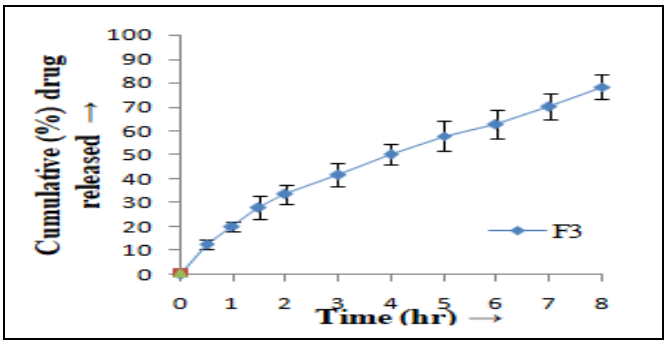

FIG. 3: *IN VITRO* DRUG RELEASE PROFILE OF VANCOMYCIN HYDROCHLORIDE FROM CHITOSAN MICROSPHERES (BATCH: F3) THE VALUES ARE MEAN ± SD (N=3)

CONCLUSION: In the present study chitosan microspheres containing test peptide vancomycin hydrochloride was prepared successfully. Polymer chitosan found to be compatible with Vanco. The emulsification cross-linking technique has proved to be useful in the preparation of Vanco-loaded chitosan microspheres for nasal delivery. The present study shows that different process variables and formulation parameters show a significant effect on two critical parameters *viz.*, particle size, and drug entrapment efficiency of drug-loaded microspheres. The optimum batch of drug-loaded chitosan microspheres can be prepared by using chitosan concentration at 4%, w/v aqueous to oil phase ratio 1:10, the volume of glutaraldehyde 0.5 ml, one-hour cross-linking time, at 1000 rpm stirring speed and 1% concentration of span 80 as a stabilizer. The optimized microspheres show a controlled release for 8 h, which follows the Higuchi model, indicating release from chitosan microspheres was diffusion controlled.

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REFERENCES:

1. Patel A, Cholkar K and Mitra AK: Recent developments in protein and peptide parenteral delivery approaches. Therapeutic Delivery 2014; 5(3): 337-65.
2. Bajracharya R, Song JG, Back SY and Han HK: Recent advancement in non-invasive formulation for protein drug delivery. Comp and structure Biot J 2019; 17: 1290-08.
3. Erdo F, Bors LA, Farkas D, Bajza A and Gizurarson S: Evaluation of intranasal delivery route of drug administration for brain targeting. Brain Research Bulletin 2018; 143: 155-70.
4. Rokade M, Tambe B and Ruparel M: In situ gel -sustained nasal drug delivery. IJPSR 2015; 6(12): 4958-66.
5. Nizic L, Potas J and Winnicka K: Development, characterization and nasal deposition of melatonin-loaded pectin/hypromellose microspheres. European Journal of Pharmaceutics Sci 2020; 141: 105115.
6. Lueben HL, Verhoef JC, Bisgaard G, Lehr CM, de-Boer AG and Junginger HE: Mucoadhesive polymers in peroral peptide drug delivery. II. Carbomer and polycarbophil are potent inhibitors of the intestinal proteolytic enzyme trypsin. Pharmaceutical Research 1995; 12: 1293-98.
7. Hanafy NA, Leporatti S and El-Kemary: Mucoadhesive hydrogel nanoparticles as smart biomedical drug delivery system. Applied Sciences 2019; 9(5): 825-36.
8. Ways TM, Lau WM and Khutoryanskiy VV: Chitosan and its derivatives for application in mucoadhesive drug delivery systems. Polymers 2018; 3(10): 267-04.
9. Cheung CFR, Ng TB, Wong HJ and Chan WY: Chitosan: an update on potential biomedical and pharmaceutical application. Marine Drugs 2015; 13: 5156-86.
10. Mohammed MA, Syeda JTM, Wasan KM and Wasan EK: An overview of chitosan nanoparticles and its application in non-parenteral drug delivery. Pharma 2017; 9: 53-79.
11. Bruinsmann FA, Pigana S and Aguirre T: Chitosan-coated nanoparticles: effect of chitosan molecular weight on nasal transmucosal delivery. Pharmaceutics 2019; 11(2): 86-05.
12. Pandey J and Tripathi PK: Formulation and evaluation of levocetirizine loaded mucoadhesive microspheres for nasal delivery. Int J of Pharma Sci and Res 2016; 7(5): 2242-51.
13. Agarwal GR, Wakte P and Shelke S: Formulation, physicochemical characterization and *in-vitro* evaluation of human insulin-loaded microspheres as potential oral carrier. Progress in Biomaterials 2017; 6(3): 125-36.
14. Kulkarni AD, Bari DB, Surana SJ and Pardeshi CV: *In-vitro*, *ex-vivo* and *in-vivo* performance of chitosan-based spray-dried nasal mucoadhesive microspheres of diltiazem hydrochloride. J of Dru Del Sci and Tec 2016; 31: 108-17.
15. Mills T, Roberson JC and Matchett CC: Instrumental data for drug analysis. CRC T and Fr Fl E 2006; 3(4): 3232-33.
16. Samanta HS and Ray SK: Controlled release of tinidazole and theophylline from chitosan based composite hydrogels. Carbohydrate Polymers 2014; 106: 109-20.
17. Muhsin MD, George G, Beagley K Ferro V, Wang H and Islam N: Effects of chemical conjugation of I-leucine to chitosan on dispersibility and controlled release of drug from nanoparticulate dry powder inhaler formulation. Molecular Pharmaceutics 2016; 13(5): 1455-66.
18. Ko JA, Park HJ and Park YS: Chitosan microparticle preparation for controlled drug release by response surface methodology. J of Microencap 2003; 20(6): 791-97.
19. Patil SB and Sawant KK: Chitosan microspheres as a delivery system for nasal insufflation. Colloids and Surfaces B Biointerfaces 2011; 84(2): 384-89.
20. Patil A, Pawar P, Gharge V, Doltade U and Doijad R: Mesalamine-loaded mucoadhesive microsphere for colon drug delivery system: effect of process variables and *in-vitro* characterization. Inter J of Pharma Inv 2018; 8:74-82.
21. Roy S, Pan Panpalia SG, Nandy BC, Rai VK, Tyagi LK, Dey S and Meena KC: Effect of method of preparation on chitosan microspheres of mefenamic acid. International J of Pharma Scien and Drug Research 2009; 1(1): 36-42.
22. Nayak UY, Gopal S and Mutalik S: Glutaraldehyde cross-linked chitosan microspheres for controlled delivery of zidovudine. J of Microencapsulation 2009; 26(3): 214-22.

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