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Mucoadhesive Microspheres of Anti-allergic Agent for Nasal Delivery

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ABSTRACT

The objective of research work was to develop and optimize mucoadhesive microspheres of anti-allergic agent (cetirizine) for nasal delivery with the aim to enhance the residence time and improve therapeutic efficacy and at the same time increase the local absorption of drug and reducing systemic side effects and also to develop unique delivery system for patients suffering from allergy and rhinitis. Mucoadhesive microspheres (Chitosan based) of cetirizine were prepared by emulsification-crosslinking method in different ratio. Glutaraldehyde was used as crosslinking agent. The mean particle size was significantly increased when high concentration of chitosan was used. Aqueous to oil phase ratio, stirring rate and dioctyl sodium sulfosuccinate (DOSS) concentration also influenced the particle size distribution of the microspheres. Microspheres were evaluated with respect to the production yield, particle size, entrapment efficiency, swelling index, FT-IR, *in vitro* mucoadhesion, cumulative percentage drug release, histological study and stability studies. Formulation Cf3 was found to be optimized. The optimized formulation Cf3 was mucoadhesive in nature which adhere onto the mucus and increase the residence time within the nasal cavity.

Key-words: Cetirizine, mucoadhesive microspheres, chitosan, nasal delivery, Emulsification crosslinking method, Anti-allergic Agent.

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Introduction

Nasal drug delivery is increasingly important as an alternative to the oral and parenteral route for systemic drug delivery. There has been increasing interest in using the nose as a route for an administration of systemically active drugs. There are number of research and review articles on nasal drug delivery. The direct drug transport into the systemic circulation, thereby avoiding hepatic first-pass metabolism and irritation of gastrointestinal membrane [1, 2]. Also nasal route is non-invasive, therefore, reduced risk of infection, ease of convenience and self-medication resulting in improved patient compliance [3].

Although nasal administration of drugs has many advantages, it is usually limited by the specific nasal morphological and physiological characteristics. One of the most important is nasal mucociliary clearance that limits the time allowed for drug absorption to occur [4, 5]

Intranasal delivery is suitable for the local and systemic delivery of diverse therapeutic compounds. Among the non-invasive routes, nasal administration offers promising potential as a viable alternative for the delivery of some drugs. Recently, microsphere technology has been applied in designing formulations for nasal drug delivery [6]. The primary rationale behind selection of microspheres is to provide a better chance for the drug to be absorbed by allowing a more intimate and prolonged contact between the drug and the mucosal membrane [7].

Cetirizine is a selective second generation histamine H1 receptor antagonist used in the treatment of allergic rhinitis and chronic urticaria [8].

Chitosan is a natural polymer that has mucoadhesive properties because of its positive charges at neutral pH, which enable an ionic interaction with the negative charges of sialic acid residues on the mucus [9, 10]. This highly mucoadhesive characteristics of chitosan provide a longer contact period for drug transport through nasal mucosa and prevents the clearance of the formulation via mucociliarly clearance mechanism [11]. Therefore, chitosan microspheres have been extensively evaluated as a drug delivery system [12,13,14,15].

The objective of the present study is to formulate and evaluate mucoadhesive microspheres of cetirizine that will increase residence time in the nasal cavity and at the same time increase the local of absorption of drug and reducing systemic side effects and also to develop unique controlled delivery system for patients suffering from allergy and rhinitis. The microspheres were prepared by emulsification cross linking method in different ratio by using mucoadhesive polymer, chitosan.

Materials and methods

Reagents and chemicals

Cetirizine was received as a kind gift from Ajenta Pharma Ltd. (Mumbai, India). Chitosan was provided by Fisher scientific, Mumbai, India. All other ingredients used were of analytical grade and were used without further purification. Spectrophotometric studies were carried out by using double-beam UV-spectrophotometer, Shimadzu, Pharma Spec 1700, Kyoto, Japan.

Preparation of mucoadhesive microspheres

Chitosan microspheres were prepared by simple w/o emulsification-cross linking process using liquid paraffin (heavy and light 1:1) as external Phase [16, 17]. Briefly, chitosan was dissolved in 2% aqueous acetic acid solution by continuously stirring until a homogeneous solution was obtained (table 1). Specified quantity of drug dispersed homogeneously by stirring in chitosan solution. This solution was added slowly to liquid paraffin (heavy and light 1:1) containing 0.2% (w/v) of DOSS as stabilizing agent under constant stirring at 1200 rpm-1375 rpm speed for 15 min using a Eurostar (IKA Labortechnik, Germany) high speed stirrer. To this w/o emulsion, Glutaraldehyde (GLA) was added slowly in definite concentration (2 ml) in different formulation and stirring was continued for 2 hrs. The hardened microspheres were separated by vacuum filtration and washed several time with hexane to remove oil. Finally, microspheres were washed with distilled water to remove unreacted GLA. The microspheres were dried for 24 hrs and then stored in vacuum desiccators until further use.

Characterization of cetirizine loaded microspheres

Particle size

The particles size of the microspheres measured by using optical microscope (OLYMPUS CH 20i).

The mean particle size was calculated by measuring more than 100 microspheres were measured randomly by optical microscope [18, 19].

Table 1 Formulation composition of cetirizine loaded mucoadhesive microspheres

Formulation & process variables		Constant parameters				
Formulations	Drug: polymer ratio	% of stabilizer used (DOSS)	Vol. of cross linking agent (Glutaraldehyde)	Aqueous to oil phase ratio	Stirring rate	Cross linking time
Cf1	1:1	0.2	2ml	10:100	1375 rpm	2 hours
Cf2	1:2					
Cf3	1:3					
Cf4	1:4					

Production yield

The production yield of microspheres of various formulation were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microspheres [20].

Determination of entrapment efficiency

Accurately weighed equivalent to 10 mg of cetirizine microspheres were crushed and dissolved in 100 ml methanol with the help of ultrasonic stirrer and kept overnight The Solution was filtered through Whatmann filter paper No.41, suitable dilution (6,8,10 mcg/ml). The samples were assayed for drug content by UV-spectrophotometer at 232.1nm. The drug entrapment efficiency was calculated using following Equations (1) [20].

$$\text{Entrapment efficiency (\%)} = \frac{\text{Mactual}}{\text{Mtheoretical}} \times 100 \qquad \text{Equ.....(1)}$$

Where M_{actual} is the actual cetirizine content in weighed quantity of powder of microspheres and $M_{theoretical}$ is the theoretical amount of cetirizine in microspheres calculated from the quantity added.

Scanning Electron Microscopy

The surface morphology of optimized formulation (Cf3) was examined by scanning electron microscopy (JSM 6390, India). The images were recorded at the 100X magnification at the acceleration voltage of 10 kv [21].

Fourier Transform Infrared Spectroscopy (FT-IR spectroscopy)

Cetirizine, Chitosan and optimized formulation (Cf3) was examined using FTIR Spectrophotometer (Shimadzu FTIR-8400S Kyoto, Japan). The measurements were made in transmittance mode in the range of 400-4000 cm^{-1} against the background spectra of pure KBr by setting resolution of 4 cm^{-1} and 50 times accumulation [22].

Swelling ability of microspheres

The swelling ability of microspheres was determined by allowing them to swell to their equilibrium in phosphate buffer of pH 6.4 [23, 24]. Swelling was determined in triplicate by using the equation 2.

$$\alpha = \frac{Ws - Wo}{Ws} \qquad \text{Equ.....2}$$

Where α is degree of swelling, Wo is initial weight of microspheres and Ws is the weight of microspheres after swelling.

Mucoadhesive Testing by in-vitro wash-off test

In Mucoadhesive properties of the microspheres were evaluated by in vitro adhesion testing method known as the wash-off method [25]. In this method freshly excised nasal mucosal membrane (3×2 cm) of goat was taken and mounted on the paddle of USP dissolution test apparatus with thread Microspheres were spread onto each wet rinsed tissue specimen, and immediately therefore the support washing onto the arm of a USP dissolution test apparatus. Operate USP dissolution test apparatus at 25 rpm of paddle in phosphate buffer 6.4 at 37°C ± 0.5°C. At the end of 30 min, 60 min, at hourly intervals up to 6 hours.

In-vitro Release Studies

The drug release study was performed using USP XXIV basket apparatus at 37°C ± 0.5°C at 50 rpm using 900 mL of phosphate buffer (pH 6.4) as a dissolution medium as per USP XXVI dissolution. Microspheres equivalent to 10 mg of cetirizine drug were used for the test. Five milliliters of sample solution was withdrawn at predetermined time intervals, filtered through a Whatmann filter paper, diluted suitably, and analyzed spectrophotometrically [26,27,28]. An equal amount of fresh dissolution medium was replaced immediately after with drawl of the test sample. Percentage drug dissolved at different time intervals was calculated at 231.0 nm.

Kinetics of Drug release

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s. t), first order [Log (Q0-Q) v/s. t], Higuchi's square root of time (Q v/s. t 1/2) and Korsemeyer Peppas double log plot (log Q v/s. log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q0-Q) is the cumulative percentage of drug remaining after time t.

In short, the results obtained from in vitro release studies were plotted in four kinetics models of data treatment as follows:-

Cumulative percentage drug release Vs. Time (zero order rate kinetics)
Cumulative percentage drug release Vs. √T (Higuchi's classical diffusion equation)
Log cumulative percentage drug release Vs. log time (Korsmeyer Peppas equation)
Log cumulative percentage drug remaining Vs. time (First order rate kinetics)

Kinetic analysis was performed and the data was evaluated after fitting to Zero order, First order, Higuchi, Peppas values observed where Regression co-efficient (R) and Diffusion exponent (n) value in case of Peppas model. Criteria for selecting most appropriate model were based on best reliability of fit indicated by 'R' value nearer to one. When drug release is concentration dependent, first order model is an indicator. Zero order model is independent of concentration of drug. Matrix model is applicable when matrix polymer is used and Peppas model is used when release mechanism is not well known Fickian diffusion exists when $n<0.5$, but at $n>0.5$ non-fickian diffusion mechanism was observed [25,29,30,31].

Histological studies

Histological studies were conducted to determine the effect of formulation on nasal mucosa. Nasal mucosa of Goat was obtained from slaughter house in saline phosphate buffer pH 6.4.The mucosa was kept in 10% formalin solution for stabilize the mucosa. Three pieces of nasal mucosa of identical size were cut and mounted on separate glass slide. One slide treated with0.5ml phosphate buffer pH 6.4(negative control) Second slide treated with 0.5 ml isopropyl alcohol (positive control) and third slide, formulation Cf3(control) and all the slide kept for 6 h. After 6 h slides were subjected to histopathology study for evaluation of nasal toxicity [32,33,34]. The specimens were visualized through Microscope at 100 x magnification at Pt. Deen Dayal Upadhaya Pashu Chikitsa Vigyan Vishwavidyalya and Gau research center Mathura, India.

Stability studies

The optimized formulation Cf3 was tested for stability studies. The formulations were divided into 3 sets of sample and stored at 4±1˚C, 25±2˚C and 60±5%RH , 37±2˚C and 65±5%RH [35,36]. After one to six month, the drug release of selected formulations was determined by the method discussed previously *in vitro* drug release studies and percentage entrapment efficiency was also carried out for the same formulation.

RESULT AND DISCUSSION

Preparation of microspheres

The microspheres of cetirizine were prepared by the emulsification crosslinking method using glutaraladehyde as crosslinking agent. The microspheres obtained under these conditions were found to be spherical and without aggregation.

Characterization of cetirizine loaded mucoadhesive microspheres

Particle Size

The mean particle sizes of the formulations were shown in the table 2. The mean particle size of microspheres ranged from 11-24 µm. The particle size mainly depends on the stirring rate and slow effect of concentration of mucoadhesive polymers, it is clear that stirring rate increases particle size decreases both at higher and lower concentration of polymers while concentration of mucoadhesive polymer had opposite effect on particle size.

Production yield

Yield of production was found in the range between 65-76.03% (see table 2). It was found that production yield of microspheres prepared by 1:3 (drug: polymer) was greater than Cf1 (1:1), Cf2 (1:2), and Cf4 (1:4). The probable reason behind this may be the high viscosity of the chitosan solution wastage of the drug-polymer solution which ultimate decreased the production yields of microspheres. Another reason for that may be agglomeration and sticking of polymer to blades of stirrer and to the wall of the beaker during microsphere formation.

Entrapment efficiency

Entrapment efficiency was high since it always exceeds 75%. It was found that with increasing the ratio of drug to polymer, the entrapment efficiency was also increased (table 2).

Table 2 Particle size of cetirizine loaded formulations.

Formulation code	Particle size (µm)	Production yield %	Encapsulation efficiency %	Mucoadhesion %	Swelling index %
Cf1	24.33±4.041	65±0.500	78.43±0.458	65±0.500	0.603±0.006
Cf2	20.66±3.055	69.53±0.451	82.03±0.153	69±0.500	0.827±0.015
Cf3	10.6±1.039	76.03±0.473	84.9±0.265	74.5±0.500	0.913±0.032
Cf4	23.66±3.215	73.9±0.794	83.4±0.436	78.5±0.500	1.04±0.051

N = mean of 3, SD±= Standard Deviation

Scanning Electron Microscopy

The optimized formulation Cf3was examined by SEM. SEM images of Cf3 in presented in figure 1. SEM analysis revealed that optimized formulation Cf3 microspheres were spherical in shape and microspheres have smooth surface.

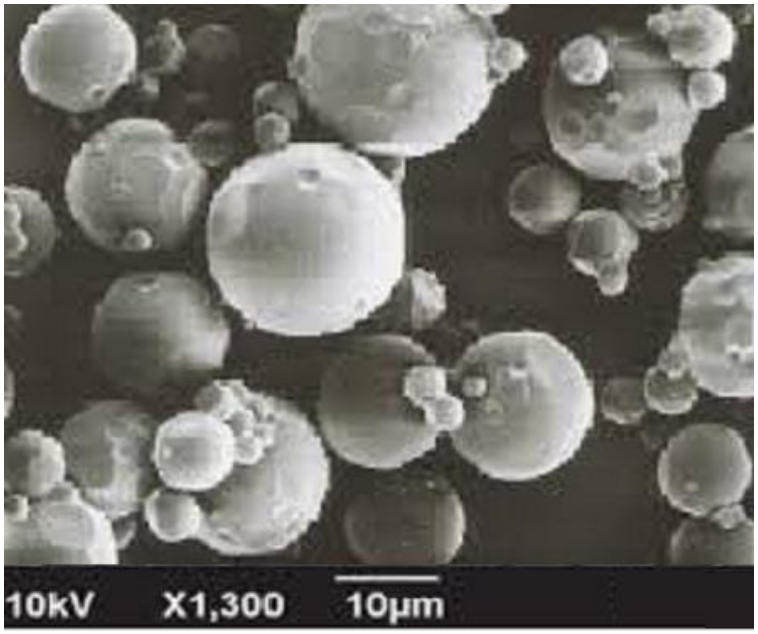

Figure 1 SEM of formulation (Cf3)

Fourier transforms infrared spectroscopy (FTIR)

FTIR spectroscopy to know any possible interaction between cetirizine, chitosan and the crosslinking agent. Cetirizine and chitosan showed characteristic peak at range of 400-4000 cm^{-1}. The FTIR spectrum of chitosan in figure 2 showed peaks corresponding to O-H stretching at 3424 cm- 1 and amine group (NH_2) stretching at 2984.1 cm-1 respectively. The spectrum of drug loaded microspheres denotes that the drug was intact in the formulation and the absence of drug-polymer interaction. Changes in the intensity of the peaks indicating no interaction between drug and polymer.

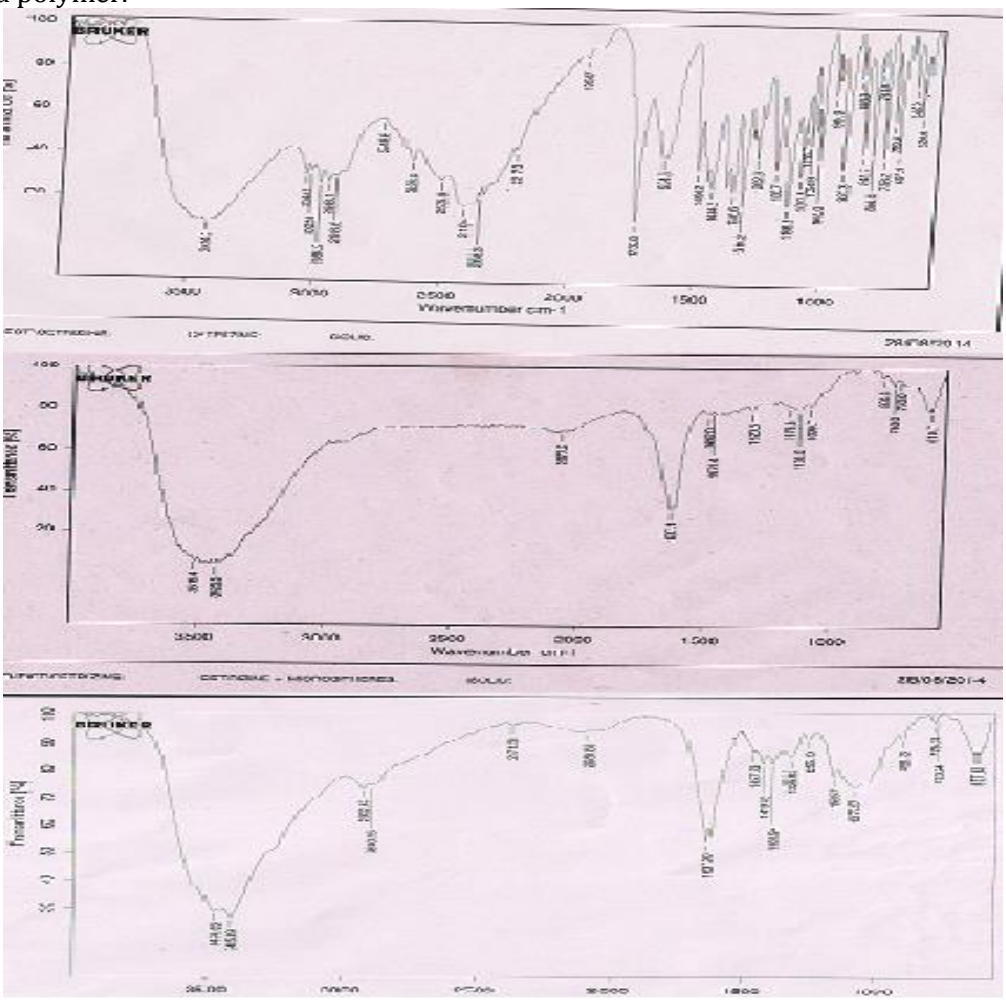

Figure 2 FTIR spectra of (a) Cetirizine, (b) Chitosan and (c) Cetirizine loaded microspheres

Swelling ability of microspheres

The swelling index of all formulation was shown in Table 2. From the table, degree of swelling for chitosan microspheres varied from 0.603±0.006 to 1.04±0.051. It is known that the degree of swelling increases marginally as the concentration of mucoadhesive polymer increases. Marginal decrease in swelling at lower level of mucoadhesive polymer may be due to the higher level of film forming polymer (chitosan) in those formulations which allows lesser penetration of water inside the polymer matrix. From this, it may be concluded that when the microspheres are in contact with mucus layer, they swell rapidly and take up liquid from the mucus layer, Hence the epithelial cells loose water and shrink which opens the epithelial tight junctions allowing drug to be absorbed.

***In Vitro* Mucoadhesion**

The mucoadhesion of cetirizine loaded nasal microspheres closely varied between 65±0.500 to 78.5±0.500 (see Table 2) and was dependent on polymer concentration. Such excellent mucoadhesion of chitosan microspheres were from the electrostatic attraction between chitosan and mucin. Moreover, the linear molecules of chitosan

expressed sufficient chain flexibility for interpenetration and entanglement. A good mucoadhesion is the high flexibility of polymer backbone structure and its polar functional groups. Such flexibility of the polymer chain is reduced if the polymer molecules are cross-linked either with each other or with cross-linking agent. The decrease in flexibility imposed upon polymer chain by cross-linking makes it more difficult for cross-linked polymer to penetrate the mucin network. Thus cross-linking effectively limits the polymer chain that can penetrate the mucus layer and could possibly decrease mucoadhesion strength.

In Vitro release studies

The *in vitro* release data of all the formulations were tabulated in Table 3. The cumulative drug release after 8hrs was found to be 79.9%, 82.53% , 84.83%, 82% respectively for the formulation Cf1 to Cf4 (table 3). The release studies of Cetirizine loaded chitosan microspheres are graphically shown in figure 3. It was clear that both the variables (stirring rate & concentration of polymer) had significant impact on drug release. As the concentration of mucoadhesive polymer increased, the drug release also increased proportionally. Stirring rate had more influence on drug release than concentration of mucoadhesive polymer. Drug release increased steeply as the stirring rate was increased from lower to higher level. This presumably is due to the smaller particle size of microspheres at higher stirring rate which leads to much larger surface area available for release and shorter path length for drug to diffuse through microspheres. The greater drug release from chitosan microspheres may be due to the higher swelling degree of chitosan which forms hydrophilic passage inside the microspheres who help drug diffuse out. The increase hydrophilic pores formed by chitosan facilitated the water penetrating into microspheres, accelerated the erosion of swelling matrix and resulted in a combination of the diffusion and erosion mechanism of drug release from microspheres. From the percent drug release graph, formulations Cf3 were showed best result.

Table 3 *In Vitro* drug release of cetirizine loaded Microspheres

Time (hrs)	Formulation Code			
	Cf1	Cf2	Cf3	Cf4
0	0	0	0	0
1	15.46± 0.321	17.46±0.451	22.8±0.265	23.5±0.500
2	23.5±0.500	25.03±0.551	30.5±0.500	31.33±0.577
3	34.03±0.551	38.5±0.500	41.93±0.603	40.9±0.656
4	40.5±0.500	43.33±0.379	51.1±0.854	49.03±1.002
5	50.5±0.500	51.83±0.289	61.56±0.404	58.2±0.231
6	60.63±0.321	62±0.500	69.93±0.379	66.03±0.451
7	69.23±0.252	70.76±0.252	74.93±0.702	72.06±1.102
8	79.9±0.361	82.56±0.503	84.83±0.764	82±1.000

N = mean of 3, SD±= Standard Deviation

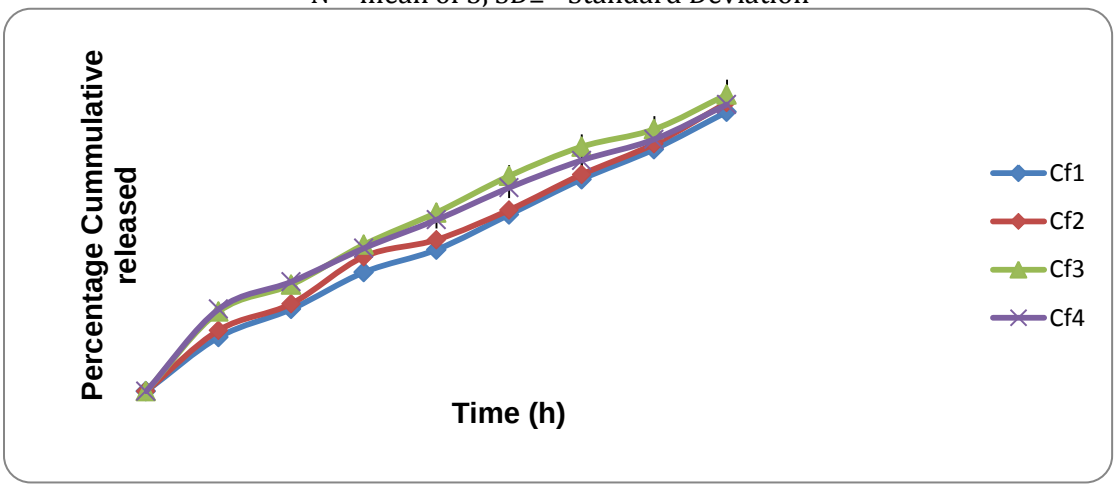

Figure 3 In vitro release of Cetirizine loaded microspheres

***In vitro* Drug release kinetics studies**

The *in vitro* drug release data of all the formulations were fit into Zero order, First order, Higuchi Equation and Korsemeyer-Peppas model. The results were shown in Table 4. The 'R' values for zero order kinetics of Cf1 to Cf4 were 0.977 to 0.983 and 'R' values for first order kinetics of Cf1 to Cf4 were 0.931 to 0.966 respectively. Among the zero order and first order equations, the Zero order Regression co-efficient (R2) value was found to be more than the First order. So all the formulations Cf1 to Cf4 followed Zero order drug release values indicate the drug release follows zero order (figure 4). To ascertain the drug release mechanism, the *in-vitro* data were also subjected to Higuchi diffusion. The 'R' values of Higuchi diffusion was 0.908 to 0.931 for formulation Cf1 to Cf4 respectively. So it confirms the drug release by Higuchi diffusion mechanism. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent (n) values of Korsemeyer-Peppas model was found to be All the formulations were subjected to Korsmeyer-Peppas plots, 'n' value ranges from 0.733 to 0.811 indicating that the drug release was by non-fickian diffusion mechanism (see table 4).

Table 4 Regression co-efficient (R) values in the analysis of release data of microspheres as per various kinetics model and Diffusion exponent (n) value of Peppas equation

Formulation Code	Zero order	First order	Higuchi matrix	Peppas plot		Best fit model
	r^2 value	r^2 value	r^2 value	r^2 value	'n' value	
Cf1	0.977	0.931	0.908	0.933	0.811	Zero order
Cf2	0.980	0.966	0.963	0.945	0.791	Zero order
Cf3	0.989	0.976	0.959	0.979	0.733	Zero order
Cf4	0.983	0.966	0.931	0.979	0.761	Zero order

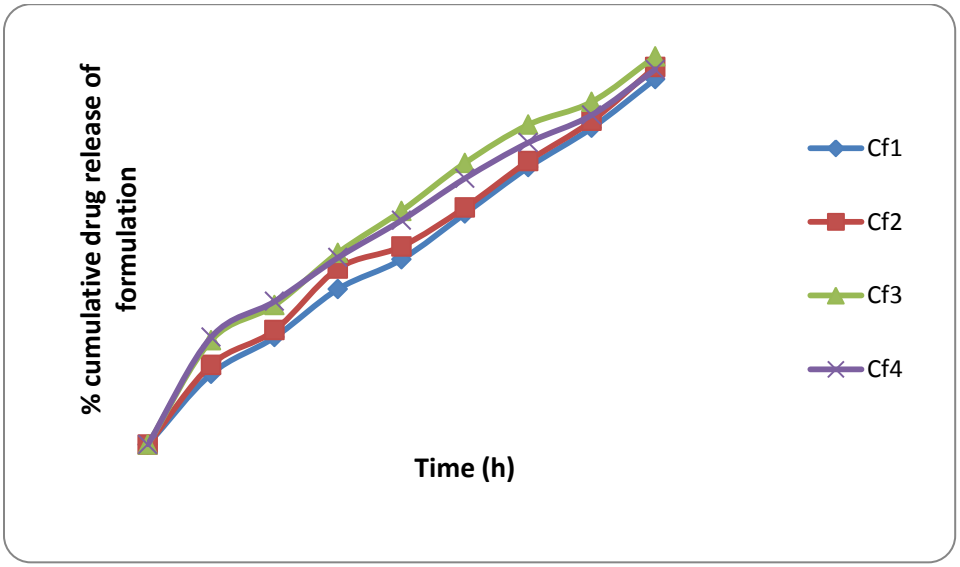

Figure 4 Zero order release kinetics of cetirizine Microspheres formulations.

Histological studies

Nasal mucosa of Goat was obtained from slaughter house in saline phosphate buffer pH 6.4.The mucosa was kept in 10% formalin solution for stabilize the mucosa. Three pieces of nasal mucosa of identical size were cut and mounted on separate glass slide. One slide treated with 0.5ml phosphate buffer pH 6.4 (negative control) Second slide treated with0 .5 ml isopropyl alcohol (positive control) and third slide, formulation Cf3 (control) and all the slide kept for 6 h. After 6 h slides were subjected to histopathology study for evaluation of nasal toxicity. The specimens were visualized through Microscope at 100 x magnification. Nasal toxicity study was performed to evaluate any toxic effect of drug and excipients were used in formulation of microspheres on nasal mucosa. In negative control treated with phosphate buffer pH 6.4 nasal mucosa appeared intact with no signs of nasal mucosa damage. While positive control with isopropyl alcohol shows extensive damage of nasal mucosa. After treating with microspheres formulations the nasal mucosa shows no sign of any damage. Hence the developed microspheres formulation can be considered as safe for nasal application (figure 5).

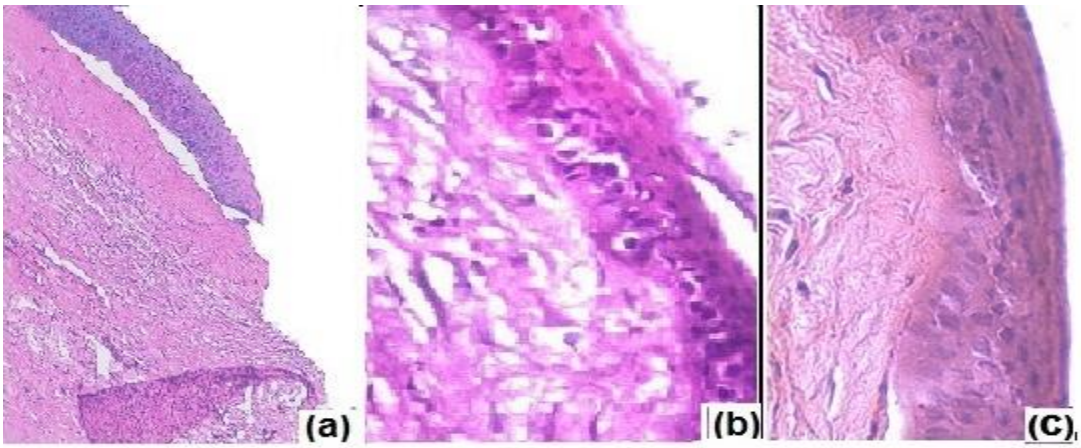

Fig.(5).Histological Study(a) Negative control (b) positive control and (c) formulation(Cf3)

Stability studies

Stability studies of the prepared cetirizine microspheres were carried out by storing the best formulation Cf3 at 4±1˚C, 25±2˚C & 60±5˚C RH and 37 ± 2˚C & 65 ± 5% RH for six month. Parameter namely percentage entrapment efficiency and cumulative percentage drug release was carried out. The result of entrapment efficiency and cumulative percentage drug release after six month of storage were shown in Table 5. These studies revealed that, there is a reduction in entrapment efficiency after storage six month at 4±1˚C, 25±2˚C & 60±5˚C RH and 37±2˚C & 65±5% RH. It was also revealed that formulations stored at 25±2˚C& 60±5˚% RH showed maximum entrapment and cumulative percentage drug release followed by the storage at 4±1˚C and 37±2˚C; 65±5% RH conditions. These results may be attributed to erosion of polymer matrix to some extent during storage (table 5).

Table 5 Stability Studies of the optimized formulations (Cf3)

Time in Month	4±1ºC		25±2ºC & 60±5% RH		37±2ºC & 65±5% RH	
	EE (%)	% CDR	EE (%)	% CDR	EE (%)	% CDR
1	84.8	84.84	84.9	84.85	84.9	84.83
2	84.8	84.8	84.8	84.84	84.6	84.79
3	84.6	84.7	84.8	84.84	83.7	84
4	84.2	84.70	84.7	84.8	83.0	84.6
5	83.2	84.6	84.6	84.7	82.9	84.6
6	83.5	84.6	84.5	84.7	82.5	84.5

Conclusion

In the present studies, it can be concluded that Cetirizine microspheres based on chitosan prepared by emulsification cross linking method may be considered a promising nasal delivery. Thus, the formulated microsphere seems to be potential candidate as intranasal controlled drug delivery system for the treatment of allergy & rhinitis.

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