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Research Article

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FORMULATION DEVELOPMENT AND CHARACTERIZATION OF CHITOSAN NANOPARTICLES OF MONTELUKAST SODIUM FOR SITE SPECIFIC DRUG DELIVERY IN MANAGEMENT OF ASTHMA

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ABSTRACT

Targeted delivery of drug molecule to lungs is being explored as an avenue for better & faster relief from pulmonary disorders. Montelukast sodium (MS), a leukotrine receptor antagonist (LTRA) used for the maintenance therapy of asthma & providing symptomatic relief from seasonal allergies, in conventional formulation undergoes major hepatic first pass metabolism, decreasing bioavailability to 64%. The objective of the present investigation was to formulate free flowing, respirable, biodegradable chitosan nanoparticles (NPs) for controlled delivery of MS to broncho alveolar tract for management of asthma. NPs loaded with MS were prepared by *Ionic gelation method*. The effect of chitosan concentration & mass ratio of chitosan: Tri poly Phosphate (TPP) was optimized on the basis of particle size, zeta potential, and % entrapment efficiency (%EE). From the results, it was observed that as the chitosan concentration increased from 0.25 to 0.85%, the particle size linearly increased with a decrease in zeta potential & %EE. However, as chitosan: TPP mass ratio was increased no significant effect on particle size & zeta potential was observed. Drug release from NPs was found to be influenced by long chain chitosan segments, chitosan: TPP mass ratio and initial burst effect, due to superficial presence of drug particles. Nanoparticulate formulation with optimized concentration of chitosan, and optimized chitosan: TPP mass ratio shows particle size of 222.5 nm, zeta potential +24.9 and %EE 61.8%. All these attributes render the optimized Montelukast loaded Chitosan Nanoparticles may be considered as promising for developed DPI of MS.

Keywords: Chitosan, Montelukast Sodium, Nanoparticles, Pulmonary delivery, TPP (Tri poly Phosphate), etc.

INTRODUCTION

The lungs are the primary organ of the respiratory system, functioning is to transport oxygen from the atmosphere into the blood, and to expel carbon dioxide from the body. The body can increase the amount of oxygen and decrease the amount of carbon dioxide in the blood stream by breathing at a faster rate or more deeply [1]. There are various diseases related to respiratory tract system like chronic obstructive pulmonary disease (COPD), asthma, lung cancer, tuberculosis, bronchitis or pneumonia. Asthma and chronic obstructive

pulmonary disease (COPD) are both common diseases, and their incidence is increasing globally, placing an increasing burden on health services in industrialized and developing countries [2]. Such diseases are characterized by airway obstruction, which is variable and reversible in asthma but is progressive and largely irreversible in COPD [3, 4]. Asthma is a respiratory condition characterized by recurrent attacks of dyspnoea (difficulty breathing) and wheezing caused by spasmodic constriction of the bronchi to variety of stimuli. During asthma attack the muscle wall contracts and the lining of the airways becomes swollen and inflamed, which cause a narrowing of the airways which is further aggravated by an increase in secretions from the mucus membrane, which block the smaller airways [5]. Corticosteroids, β-Blockers, anticholinergic, bronchodilators, mass-cell stabilizers, leukotrine antagonist, lipooxygenase inhibitors are widely used for treatment of asthma. In this article, one of the leukotriene antagonist montelukast is recommended for management of chronic asthma [6].

The Montelukast sodium (MS) is a leukotrine receptor antagonist (LTRA) used for the maintenance, treatment of asthma, chronic asthma attacks and to relive symptoms of seasonal allergies. The main drawback of conventional montelukast formulation is that it undergoes hepatic first pass metabolism. Thus, it shows plasma or biological half-life 2.5 to 5.5 hrs, thereby decreasing bioavailability upto 64%. The present work describes a delivery system, which will improve the biological half-life as well as bioavailability of Montelukast [7].

The respiratory tract has been focused as new administration route of drug molecules. Recently, a systemic application of drug compounds via the lung has created new possibilities for the pulmonary application of drug compounds. The pulmonary drug delivery presents many advantages compared to other administration routes like the amount of drug administered to patients is less, decrease systemic undesirable effects and also avoid the first pass hepatic metabolism [8, 9, 10]. Aerosols are an effective product to deliver therapeutic agents to the respiratory tract. Nebulizers, metered dose inhalers, or dry powder inhalers are commonly used for this purpose. Local delivery of medication to the lung is highly desirable, especially in patients with specific pulmonary diseases. The principal advantages of local delivery include reduce systemic side effects and also reduce higher dose levels of the applicable medication at the site of drug action. Unlike the oral route of drug administration, pulmonary inhalation is not subject to first pass metabolism. Indeed, aerosol delivery has long been viewed as a promising approach for asthma [11].

By virtue of their nanometric size range of 1-1000 nm, nanoparticles can be designed to have several advantages for controlled and targeted drug delivery, including controlled deposition, sustained release, reduced dosing frequency, as well as an appropriate size for avoiding alveolar macrophage clearance or promoting trans-epithelial transport, thus, leading to reduced macrophage uptake or clearance by reticulo-endothelial system (RES), ensuring enhanced retention and concentration at targeted site in broncho alveolar region[12].

The predominant role of polymeric nanoparticles in drug delivery system is to carry the drug molecules, to protect drugs from degradation, and to control drug release. Therapeutically used polymeric nanoparticles are

composed of biodegradable or biocompatible materials, such as poly (ε-caprolactone) (PCL), poly (lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA), alginic acid, gelatin and chitosan [12].

Chitosan is a natural polysaccharide comprising copolymers of lucosamine and Nacetyl glucosamine. It can be derived by partial deacetylation of chitin from crustacean shells. Chitosan has a positive charge when compared with many other natural polymers and is mucoadhesive. Properties such as biodegradability, low toxicity, and good biocompatibility make it suitable for use in biomedical and pharmaceutical formulations. Ionic gelation can be used to produce chitosan-TPP nanoparticles of sufficient quality for use in clinical applications [13].

The main aim of present work was to formulate free flowing, rerspirable, biodegradable chitosan nanoparticles for controlled delivery of montelukast sodium to broncho alveolar tract for management of asthma and evaluate their in vitro release profile.

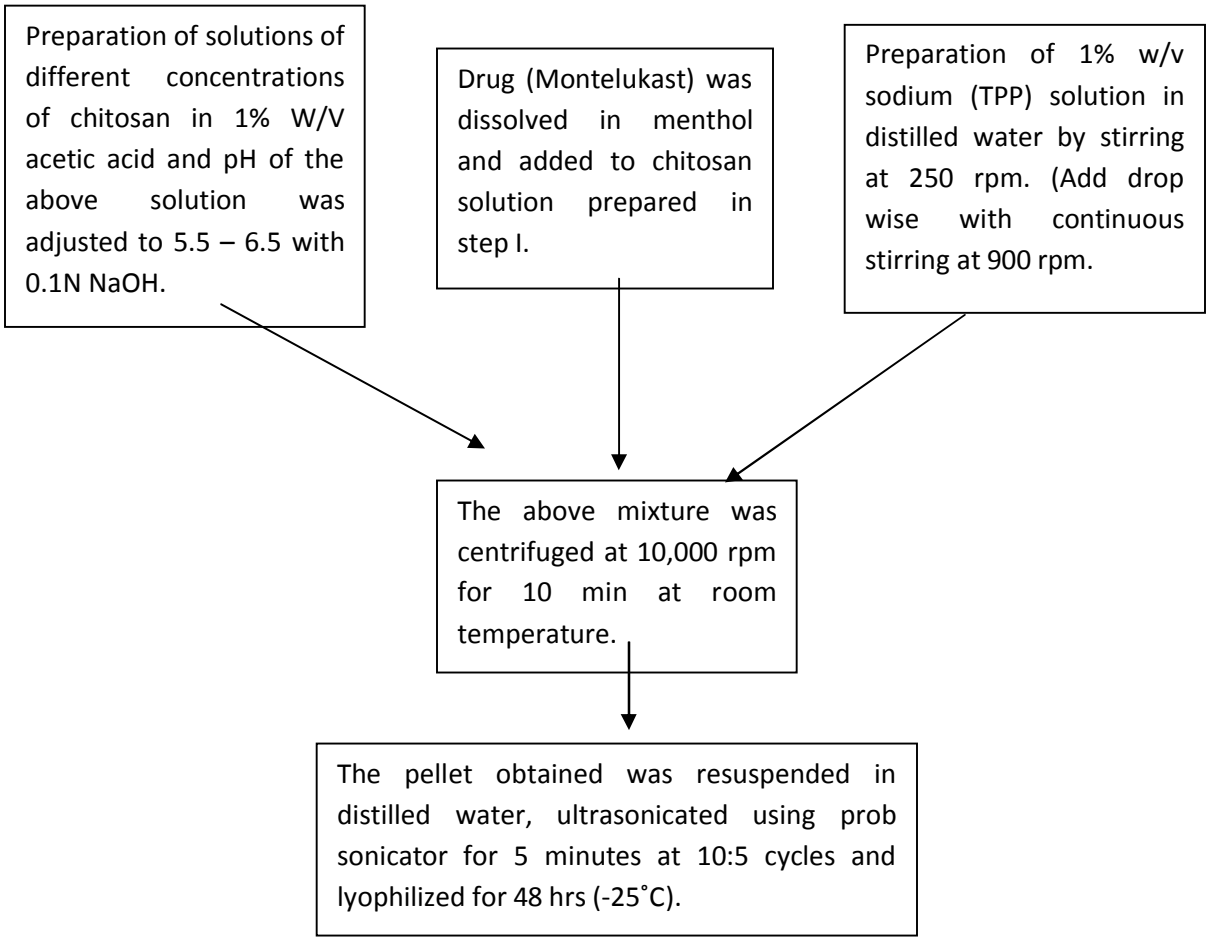
2. Materials and Methods

2.1 Materials

Montelukast Sodium was received as a gift sample from Macleods Pharma, India. Chitosan was received as a gift sample from Central Institute of Fisheries Technology, Cochin. TPP was purchased from Loba Chemie, Mumbai, India. Disodium hydrogen phosphate, Potassium dihydrogen phosphate, and Sodium chloride were received from Sisco research laboratory, Mumbai, India, and all other chemicals used were of analytical grade.

2.2 Preparation of Nanoparticles:

2.2.1 Formulation of Montelukast loaded Chitosan Nanoparticles



2.2.2 Lyophilization of Drug loaded LNPs [24]:

The Montelukast Sodium loaded chitosan pellets obtained after centrifugation at 10,000 rpm by using 0.1N HCl was redispersed in mili Q water. Trehalose was added as a cryoprotectant to Montelukast sodium loaded Nanoparticles dispersion. The above mixture was frozen at – 80˚C for 3 hrs followed by freeze drying using lyophilizer for 48 hrs at a temperature of -25˚C and a vacuum of 0.370 mbar. Characterization of lipid nanoparticles was done for particle size, Zeta Potential, % Entrapment Efficiency etc.

2.3 Factorial Design [23]:

For the optimization of the formula, design of experiment concept was used. As there are two major factors affecting the formulation, chitosan concentration and chitosan: TPP ratio, the 3^2 factorial design was used. This design analyses the effect of formulation variables such as chitosan concentration and chitosan: TPP mass ratio

(as shown in Table 1) on product characteristics such as Particle Size, Zeta Potential and % Entrapment Efficiency.

Table 1: Variables used 3^2 factorial design along with their levels indicating their coded and uncoded values

PARAMETERS	LEVELS (Depended Variables)		
	Low (-1)	Medium (0)	High (+1)
Chitosan Concentration (%) (X1)	0.10	0.25	0.40
Chitosan:TPP mass ratio (X2)	1.5	2.5	3.5

2.4 Characterization of drug loaded nanoparticles

2.4.1 Measurement of Particle Size [14-15]:

Photon Correlation spectroscopy (PCS) is a technique employed to determine the mean particle size (PCS diameter) and particle size distribution (Poly-dispersity index, PDI). It is a light scattering experiment in which the stastical intensity fluctuations in light scattered from the particles are measured. These fluctuations are due to the Random Brownian motion of the particles. Particle size of the optimized batch was determined by light scattering based on laser diffraction using Malvern zetasizer nano ZS 90 (Malvern Instruments LTd. UK) after suitable dilution. The apparatus consist of a He-Ne laser (5mw) and a sample holding cell of 5 ml capacity.

2.4.2 Zeta Potential [14-16]:

Zeta Potential is the electric potential of a particle in dispersion. It is a parameter which is very useful for the assessment of the physical stability of the colloidal dispersion. In dispersions the surfaces of the particles develop a charge due to ionization of surface groups or adsorption of ions. This charge depends on both the surface chemistry of the particles and media around these particles. The surface charge generates the potential around the particle, which is highest near the surface and decays with distance into the medium. When the particle is placed in the electric field, it will move with a characteristic velocity *v*. the velocity of the particle per unit electric field strength is called the electrophoretic mobility, which is expressed in micrometers per second per volt per centimeter (μm/s)/(V/cm). The zeta potential can be measured by determining the velocity of the particles in an electric field. In the present work, for the Zeta potential measurements a Malvern Zetasizer ZS 90 (Malvern Instruments, UK) was used.

2.4.3 Entrapment Efficiency and Drug loading [16-19]:

The %entrapment efficiency and Drug loading of Montelukast loaded Chitosan Nanoparticles was determined by centrifugation method.

10 µl of 0.1N HCl was added into drug-loaded dispersion which caused the coagulation of lipid particles. The Entire system was then centrifuged for 10 min at 10,000 rpm.

The supernatant was separated and the pellets were washed 3 times with distilled water. The free drug concentration was determined by the UV Spectrophotometric analysis of supernatant while the drug entrapped was determined by the extraction of pellets using methanol followed by vortexing for 5 minutes and then Spectrophotometric determination of the solution was done. The nanoparticulate dispersion was centrifuged at 5000 rpm for 15 min to settle down the unentrapped drug followed by dilution with methanol and drug content was analyzed spectrophotometrically at 285.5 nm. The entrapment efficiency and drug loading of Montelukast sodium (MS) was done by following equation.

$$\% EE = \left(\frac{Wa - Ws}{Wa}\right) * 100$$

$$\% DL = \left(\frac{Wa - Ws}{Wa - Ws + Wi}\right) * 100$$

Where, Wa = Amt. of drug added to formulation

W*l* = Wt. of lipid

Ws = Amt. of free drug

2.4.4 In vitro drug release [16-20]:

In vitro release of montelukast (MS) from NPs was evaluated by the dialysis bag diffusion technique. The studies of release of MS from NP were performed phosphate buffer (pH 7.4) to create a perfect sink condition. The aqueous nanoparticulate dispersion equivalent to 2 mg of Montelukast was placed in a dialysis bag (cut-off 12,000 Da; Himedia, Mumbai, India), which was previously soaked overnight in water, cleaned next morning and sealed at both ends. The dialysis bag was immersed in the receptor compartment containing 100 ml of phosphate buffer (pH 7.4), which was stirred at 100 rpm and maintained at 37 ± 2 ˚C. The receptor compartment was covered to prevent the evaporation of release medium. Samples were withdrawn at regular time intervals, and the same volume was replaced by fresh receptor medium. The samples were analyzed spectrophotometrically at 285.5 nm. All the experiments were performed in triplicate, and the average values were taken.

3. Results and Discussion:

3.1 Factorial Design:

For optimization of the formula, design of experiment concept was used. The design analyses the effect of formulation variables such as chitosan concentration and chitosan: TPP mass ratio on product characteristics such as particle size, zeta potential and % Entrapment Efficiency. For optimization of all parameters, there are three criteria to be requiring 1) particle size should be Minimum, 2) zeta potential should be Minimum and 3) % Entrapment Efficiency should be Maximum.

A) Effect of Chitosan Concentration on Nanoparticles [21-22]:

Different low molecular weight chitosan concentration were dissolved in 1% w/v acetic acid solution and prepare different concentration of chitosan (0.05 – 0.85% w/v) and mixed with TPP to formulate chitosan-TPP nanoparticles. The effect of chitosan concentration on particle size, zeta Potential, % entrapment efficiency of Montelukast sodium loaded chitosan nanoparticles are shown in Table 2.

Table 2: Effect of Chitosan Concentration on Particle Size, Zeta Potential, and % entrapment efficiency of Montelukast loaded chitosan nanoparticles

Batch	Chitosan Concentration	Particle Size (nm)	Zeta Potential (mV)	% Entrapment Efficiency
F1	0.05	Not formed		
F2	0.25	243	24	58
F3	0.45	416	22	61
F4	0.65	623	20	52
F5	0.85	786	19	53

From the table, it was observed that the particle size linearly increased with increase in chitosan concentration, whereas zeta potential and % entrapment efficiency over all decreased on increasing chitosan concentration from 0.25 to 0.85%. Decreasing the Zeta potential might be due to interaction between chitosan and drug molecule at higher concentration of polymer. At 0.25% concentration, the criteria for minimum particle size and Zeta potential is achieved and for % entrapment efficiency (58%) which is only 3% lesser than 0.45% concentration which is acceptable that is why 0.25% chitosan concentration is optimize for bulk production.

B) Effect of chitosan to TPP mass ratio on Nanoparticles [22]:

Different low molecular weight chitosan concentration were dissolved in 1% w/v acetic acid solution to make up chitosan concentration 0.05 – 0.85% w/v and mixed with TPP and nanoparticles were prepared at selected chitosan to TPP mass ratio of 0.5, 2.5, 4.5, 6.5, and 8.5 respectively. The effect of chitosan to TPP mass ratio on particle size, zeta Potential, % entrapment efficiency of Montelukast sodium loaded chitosan nanoparticles are shown in Table 3.

Batch	Chitosan to TPP mass ratio	Particle Size (nm)	Zeta Potential (mV)	% Entrapment Efficiency
F6	0.5	Not prepared		
F7	2.5	251	24	57
F8	4.5	259	25	50
F9	6.5	256	24	37
F10	8.5	261	24	23

Table 3: Effect of Chitosan to TPP ratio on Particle Size, Zeta Potential, and % entrapment efficiency of Montelukast loaded chitosan nanoparticles

It was observed that when chitosan to TPP mass ratio increased from 0.5 to 8.5, drug entrapment efficiency deceased from 57 to 23%, however, variation in chitosan: TPP ratio did not exert significant effect on particle size and zeta potential. So, 2.5 chitosan to TPP mass ratio is optimum because it fulfilled all requiring criteria for optimization like 1) particle size should be Minimum, 2) zeta potential should be Minimum and 3) % Entrapment Efficiency should be Maximum.

3.2 Optimization of formulation

From the result of above batches, it can be conclude that 0.25% chitosan concentration and 2.5 chitosan to TPP mass ratio are optimum to produce bulk mass of Drug loaded Chitosan Nanoparticles. For further optimization of formula, this data can be entering in Design-Expert®. Goals and limits of various parameters for optimization are shown in table 4. The goals that were pre determined for the formulation was to achieve the desired characteristics of nanoparticles in terms of their particle size, zeta potential and percentage drug entrapment efficiency.

Table 4: Goals and limits of various parameters for optimization

Name	Goal	Lower limit	Upper limit	Importance
A: Chitosan concentration	In Range	0.10	0.25	3
B: Chitosan: TPP mass Ratio	Minimum	1.5	2.5	3
Size	Minimum	188	331	3
Zeta potential	Minimum	+22	+29	3
%EE	Maximum	32	63	3

Based on the results of the above experimental design, formulation given in Table 5 was finalized formula from Design-Expert®.

Table 5: Final Formulation

Chitosan Concentration	Chitosan to TPP mass ratio	Particle Size (nm)	Zeta Potential (mV)	%EE
0.213	1.5	222.5	+24.9	61.8

3.3 Check point Batch Analysis:

The equations derived from Design are the good tools for the prediction of the response parameter without performing the actual experiments. To verify the result from software, it is must to perform check point batch analysis, for that any three random batches were taken for that the formulation parameters were selected from the range stated above. The response parameters by actual experiments were compared with the response parameters from the statistically derived formula showed in Table 6.

Table 6: Check point analysis

Chitosan concentration (level)	Chitosan:TPP masratio (level)	Theoretical size	Actual size± S.D	Theoretical Zeta potential	Actual ZETA potential± S.D*	Theoretical %EE	Actual % EE±S.D*
0.22	-0.22	276	271± 1.37	+24	+23±1.08	54	58±0.655
0.59	0.11	307	310±1.65	+22	+23±1.01	53	56±0.678
-0.19	0.38	252	261±2.012	+25	+24±1.09	48	51±1.062

Based on the results of the above experimental work, it can be conclude that the optimized batch showed in Design-Expert® is acceptable for further large scale production of Montelukast loaded Chitosan Nanoparticles.

3.4 Characterization and Evaluation of Optimized Batch:

3.4.1 Particle Size: The size is measured by the Photon Correlation Spectroscopy (PCS). Photon Correlation Spectroscopy is a technique used to determine the mean particle size diameter and particle size distribution expressed as Polydispersity index (PI

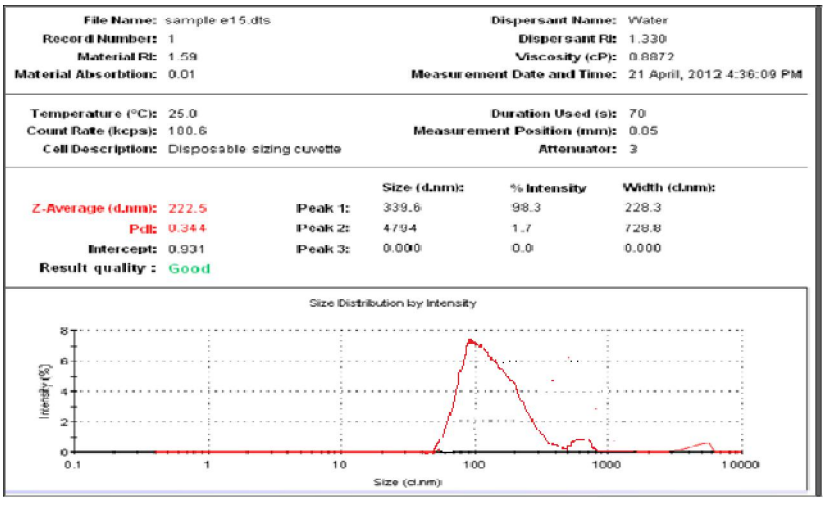


Figure 1: Results of Particle Size measurement of chitosan nanoparticles

The figure 1 shows the size measurement report of the zeta sizer. Result showed that particle size of the Nanoparticle dispersion prepared by Ionic gelation technique is 222.5 nm.

3.4.2 Zeta Potential: Zeta Potential can be measured by determining the velocity of the particles in an electric field. The zeta Potential report for the chitosan NPs is shown in figure 2.

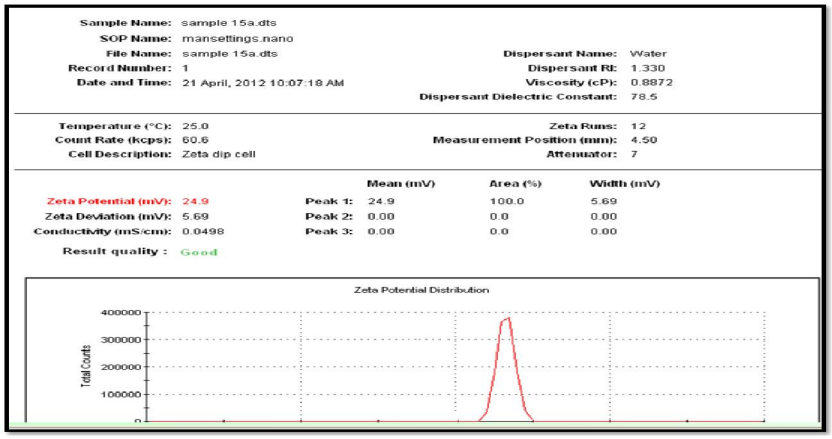


Figure 2: Results of Zeta Potential measurement

As shown in the figure 2, the zeta potential value of NPs is near to +24.9 mV. This indicates that the dispersions of NPs will remain deflocculated and would be physically stable.

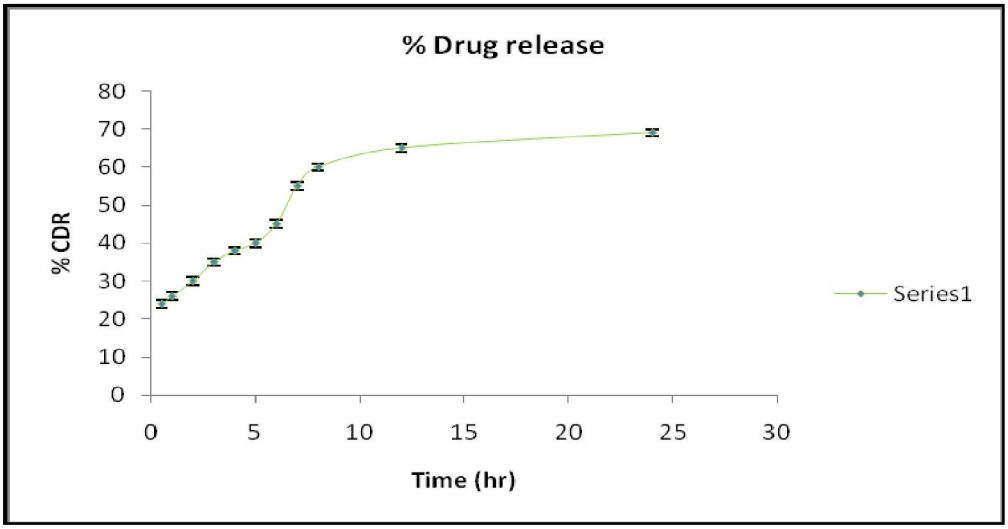
3.4.3 % Entrapment Efficiency and Drug Loading: % Entrapment Efficiency is the parameter which shows how much drug is incorporated in the polymer matrix and the drug which is in the polymer matrix will only show the controlled release property. The %EE and % Drug loading for NPs are 61% and 10.87% respectively.

3.4.4 In vitro Drug Release Study: In vitro release study indicates that sustain drug release for 24 hr after burst effect seen in the beginning. The % drug release from the MS loaded NPs is shown in the Table 5 and graphical presentation is shown in figure 3.

Table 5: In Vitro Release Study from Nanoparticles

Sr. No.	Time (hr)	% Drug Release from the Nanoparticles
1	**0**	**0**
2	**0.5**	**24.2 ± 0.2**
3	**1**	**26.2 ± 0.2**
4	**2**	**30.4 ± 0.4**
5	**3**	35.3 ± 0.3
6	**4**	38.3 ± 0.3
7	**5**	40.7 ± 0.7
8	**6**	45.5 ± 0.5
9	**7**	50.7 ± 0.7
10	**8**	60.4 ± 0.4
11	**12**	65.7 ± 0.7
12	**24**	69.8 ± 0.8

Figure 3: In vitro drug release of Montelukast Sodium from LNP



4. Conclusion:

Drug targeting to pulmonary route is effective if formulation is given in uniform quantity and appropriate particle size like nanoparticles (size 200-300 nm). The particles >10µm are removed by cough swallowing and the mucociliary process. Inhaled nanoparticles suggests that mucus clearance can be overcome by nanoparticles, possibly due to rapid displacement of particles to the airway epithelium, more stable and efficient systems with better lung delivery reaching up to targeted site than other conventional formulation. The nanoparticles were prepared by Ionic gelation method and effect of chitosan concentration and ratio of chitosan: TPP concentration was optimized for particle size, zeta potential, and % entrapment efficiency. From the results obtained for formulations F1 to F5, it was observed that the particle size linearly increased with increase in chitosan concentration, whereas zeta potential and % entrapment efficiency decreased. From the results of formulations F6 to F10, it was observed that when chitosan to TPP mass ratio increased from 0.5 to 8.5, drug entrapment efficiency deceased from 57 to 23%, however, variation in chitosan: TPP ratio did not exert significant effect on particle size and zeta potential. The drug release study clearly indicated that release of the drug was influenced by the presence of long chain segments of polymer (chitosan concentration) and chitosan to TPP mass ratio and initial burst effect was seen due to presence of drug particles on surface. Thus, on the basis of all the studies conducted, it was concluded that the batch having chitosan concentration 0.213, chitosan to TPP mass ratio 1.5, the particle size 222.5 nm, zeta potential +24.9 and entrapment efficiency 61.8% was finalized as nanoparticles formulation. Hence, these formulations hold great potential for treating disease which requires direct lung delivery with reduced drug dosage and

dosing frequency. Thus there is an improvement in patient compliance with minimized systemic side effects. All these attributes render the optimized Montelukast loaded Chitosan Nanoparticles may be considered as promising for developed DPI of MS.

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