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NANOPOLYMERIC FORMULATION DEVELOPMENT AND CHARACTERIZATION OF ANTI ASTHMATIC DRUG FOR PULMONARY DRUG DELIVERY

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ABSTRACT

Pulmonary route as a targeted drug delivery system cuts the edge over other routes of administration in terms of local, site specific, patient friendly, and needle free drug delivery. Pulmonary route minimizes the systemic toxicity of drugs administered and facilitates accomplishment of optimal drug concentrations at the site of action in asthma and allied pulmonary disorders. Montelukast sodium (MS), a leukotriene receptor antagonist (LTRA) is used for the maintenance therapy of asthma & providing symptomatic relief from seasonal allergies. The objective of the present investigation was to formulate biodegradable polymeric nanoparticles (PNP) for controlled delivery of MS to broncho alveolar tract for management of asthma. To achieve the same, we have hypothesized to prepare drug loaded biodegradable and biocompatible nanoparticles using PLGA by solvent evaporation method which leads to long term site specific controlled release of the encapsulated drug over the treatment period in management of asthma. The prepared Montelukast sodium (MS) loaded PLGA nanoparticles were evaluated on the basis of particle size, zeta potential, % entrapment efficiency (%EE), Scanning Electron Microscopy analysis, In-vitro Drug release study.

Keywords: Montelukast Sodium (MS), Polymeric Nanoparticles, Pulmonary Drug delivery, PLGA (poly lactic-co-glycolic acid).

1. 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. This may be due to diseases 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 [1]. Such diseases are characterized by airway obstruction, which is variable and reversible in asthma but is progressive and largely irreversible in COPD. 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 [2]. Corticosteroids, β-Blockers, anticholinergic, bronchodilators, mass-cell stabilizers, leukotriene antagonist, lipooxygenase inhibitors are widely used for cure of asthma. In this one of the leukotriene antagonist montelukast is recommended for management of chronic asthma [3-4].

The Montelukast sodium (MS) is a leukotriene 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 up to 64% [5]. The present work describes such delivery system, which will improve the biological half-life as well as bioavailability of Montelukast.

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. The amount of drug administered to patients is lower compared to the traditional administration routes, systemic undesirable effects decrease and the first pass hepatic and renal effects are avoided [6-8]. 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 reduced systemic side effects and 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 [5,6]. Indeed, aerosol delivery has long been viewed as a promising approach for asthma.

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 reticulum-endothelial system (RES), ensuring enhanced retention and concentration at targeted site in broncho alveolar region [8-10].

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 [11].

The main aim of present work was to formulate free flowing, respirable, biodegradable PLGA 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 METHOD

Montelukast Sodium was received as a gift sample from Macleods Pharma, India. Poloxamer was a received as a gift sample from Signet Chemicals Ltd, Mumbai. Resomer 504 (PLGA 50:50) was purchased from Evonik Industries. Acetone was purchased from SRL, Ahmedabad. Sodium hydroxide, Disodium hydrogen phosphate,Potassium dihydrogen phosphate, Sodium chloride, Sodium Laryl Sulphate were received from Finar Chemicals Ltd., Ahmedabad.

2.1 Preparation of polymeric- nanoparticles by solvent evaporation Method

In this method, aqueous phase was prepared by dissolving surfactant in distilled water & organic phase was prepared by dissolving drug MS and polymer (PLGA) in organic solvent. Then keep aqueous phase on magnetic stirrer to dissolve surfactant and then add organic phase drop wise by using syringe under continuous stirring for 4-6 hours to evaporate organic phase. The prepared Polymeric nanoparticles containing solution were

collected & evaluated.

2.2 Lyophilization of drug loaded PNP [12]:

The Trehalose as cryoprotectant was added to PNP dispersion. It was then freezed by keeping it at – 80˚C for 3 hr and then the montelukast sodium PNP sample was freeze-dried using lyophilizer (Vertis benchtop K) for 48 hr at a temperature of -25°C and a vacuum of 0.370 mbar.

2.3 Optimization of process parameters:

The following process parameters which may have impact on the various characteristics of PNPs such as particle size, PDI (Poly dispersity index), zeta potential (ZP), %EE (% Entrapment Efficiency) were evaluated. For optimization of all parameters, there are three criteria to be requiring 1) Particle size should be Minimum, 2) Zeta potential should be Minimum 3) PDI should be minimum (near to zero) and 4) % Entrapment Efficiency should be Maximum.

Effect of types of surfactant

Effect of various organic solvent

Effect of pH of aqueous phase

Effect of polymer concentration

Effect of stirring speed of magnetic stirrer

Effect of Surfactant concentration

2.4 Characterization of MS loaded PNP:

2.4.1 Measurement of Particle size and Poly dispersity Index (PDI) [13-17]:

Photon correlation spectroscopy (PCS) is a technique employed to determine the mean particle size (PCS diameter) and size distribution (poly-dispersity index, PDI). It is a light scattering experiment in which the statistical intensity fluctuations in light scattered from the particles are measured. These fluctuations are due to the random Brownian motion of the particles. The size and the PDI of the particles were measured by Malvern Zetasizer – NanoZS Model ZEN 3600 spectrometer with He-Ne laser (633nm). Measurement was carried out at a scattering angle of 173o in 10mm diameter cells, the system kept thermostated at 25oC. The apparatus consisted of a He-Ne laser (5 mw) and a sample holding cell of 5 ml capacity.

2.4.2 Zeta potential [13-15]:

Zeta potential was measured by Malvern Zetasizer – NanoZS Model ZEN 3600 spectrometer with He-Ne laser (633nm), the system kept thermostated at 25oC.Zetasizer, Malvern (Nano ZS), which measures the potential range from −120 to 120 V. This parameter is highly useful for the assessment of the physical stability of PNP.It measures the surface charge of the particles.

2.4.3 %Entrapment Efficiency [18-20]:

The %entrapment efficiency of prepared PNP were determined by measuring the concentration of free drug content in the dispersion medium. For determination take 10ml of formulation and centrifuge at ~8000 RPM for 30 min by using REMI R-24 centrifuge. Take 1 ml supernant and dilute up to 10 ml with 0.5% SLS. Measure the amount of Montelukast sodium by UV-spectroscopy. Entrapment efficiency was calculated by following equation.

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

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

Where, Wa = Amt. of drug added to formulation

W*l* = Wt. of lipid

2.4.4 Scanning Electron Microscopy analysis [21-24]:

SEM analysis was performed to study the morphological characteristics of the polymeric nanoparticles. For studying the surface morphology of Lyophilized PNP scanning electron microscopy model EVO 18 special edition (Carl Zeiss Inc, Germany) was used. Samples of PNP were placed on double sided tape, which had previously been secured to aluminium stubs. PNPs were metalized with palladium with a sputter coater and analyzed at 20kV acceleration voltage. Photomicrographs were taken at suitable magnifications.

- **In vitro drug release [24-26]:**

In vitro release of Montelukast Sodium from PNP was evaluated by the dialysis bag diffusion technique. The studies of release of Montelukast Sodium from NP were performed in PBS 7.4 buffer. The aqueous nanoparticulate dispersion equivalent to 2 mg of Montelukast Sodium was placed in a dialysis bag (cut-off 12,000 Da; fisher brand, India), which was previously soaked in warm water, then cleaned and sealed at both ends. The dialysis bag was immersed in the receptor compartment containing 250 ml of PBS

7.4 (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 282 nm. All the experiments were performed in triplicate, and the average values were taken.

3. Results and Discussion:

3.1 Effect of types of surfactant

Surfactant is used as a stabilizer to avoid PLGA NP aggregations as well as to decrease particle size. Effect of various types of surfactant on particle size, PDI, ZP and % EE of MS loaded PNPs are shown in **Table 1** and is generally depend on unique chemical structure.

Table 1: Effect of various surfactants on PNPs

Surfactants	Particle Size (nm)	PDI	Zeta Potential (mV)	%EE	Comments
Poly Vinyl Alcohol (PVA)	408.5	0.312	-25.2	69.41	Aggregation
Poloxamer-188	**184.6**	**0.213**	**-26.7**	**74.21**	**Clear**
Poloxamer-407	242.7	0.325	-22.3	71.35	Clear

Table 1 shows that formulation containing Poloxamer-188 produced PNP with minimum particle size (less than 200 nm) as well as minimum Zeta potential (-26.7) that indicates that Poloxamer-188 have a capacity to produce formulation having highest stability with minimum size of particle. The values of PDI and ZP were well in acceptable range, with maximum %EE compared to PVA and poloxamer 407. Therefore, polaxamer 188 was selected as a surfactant.

3.2 Effect of various organic solvents:

Organic solvent is used to produce interaction between PVA and PLGA NPs and solubalize all ingredients to produce Nanoparticles by precipitation. The following table shows effect of various organic solvents like dichloromethane, acetone, IPA on particle size, PDI, ZP and % EE of MS loaded PNPs.

Table 2: Effect of various organic solvents on PNPs

Organic Solvent	Particle Size (nm)	PDI	Zeta Potential (mV)	%EE
Dichloromethane	186.4	0.213	-24.2	69.42
Acetone	**194.7**	**0.197**	**-26.6**	**76.76**
IPA (Isopropyl alcohol)	165.3	0.213	-22.6	72.37

The best organic solvent has a capacity to produce least particle size with greater %EE, minimum zeta potetial and lowest PDI. The analysis shows that Acetone was quite good as compared to dichloromethane and IPA with all parameters in satisfactory range. Acetone is additional advantage like less toxic in acute or short-term studies and negative in genotoxicity studies, and can be completely evaporated from solvent evaporation process after nanoparticle formation. Hence, acetone was for further studies to optimize other process parameters.

3.3 Effect of pH of aqueous phase

Effect of pH of aqueous phase is an important parameter which affects the %EE by producing interaction between drug and polymer. The table 3 shows effect of aqueous phase pH on PNPs.

Table 3: Effect of pH of aqueous phase on PNPs

pH of aqueous phase	Particle Size (nm)	PDI	Zeta Potential (mV)	%EE
3	350.4	0.213	-29.4	60.24
7	194.7	0.197	-26.6	75.76
9	184.3	0.186	-20.4	48.32

The result of Table 3 shows that at neutral pH of aqueous phase containing formulation produced PNP with maximum %EE and acceptable Particle size. The PDI and ZP values were also in well acceptable range. So, neutral pH of aqueous phase was considered for further studies.

3.4 Effect of Polymer Concentration:

Effect of various polymer concentrations is directly affect %EE and particle size. The optimum concentration of polymer is that at which highest %EE and acceptable particle size (less than 200nm) containing NP is produced. The following table shows the effect of **polymer** (PLGA) concentration on particle size, PDI, ZP and % EE of MS loaded PNPs.

Table 4: Effect of Polymer concentration on PNPs

Polymer Concentration (PLGA) mg	Particle Size (nm)	PDI	Zeta Potential (mV)	%EE
5	147.8	0.246	-29.4	41.26
30	**196.2**	**0.223**	**-24.6**	**84.69**
50	574.6	0.328	-20.7	89.42

The table shows as concentration of polymer increase from 5 mg to 50 mg, particle size also increase. At 30mg polymer concentration, highest %EE and particle size having acceptable range (less than 200 nm) is produced. For the formulation containing 30 mg PLGA, all the values of parameters were well in acceptable range. Hence, 30mg of PLGA was considered for further evaluation.

1. Effect of surfactant concentration:

The optimum concentration is at which minimum particle size with minimum PDI because Surfactant act as stabilizer so when formulation have Minimum PDI (near to zero), can be consider as best stable Formulation. Following table shows effect of Poloxamer 188 concentrations on particle size, PDI, ZP and % EE of MS loaded PNPs.

Table 5: Effect of surfactant concentration on PNPs

Surfactant (mg)	Particle Size (nm)	PDI	Zeta Potential (mV)	%EE
25	218.6	0.192	-23.6	79.41
50	**193.4**	**0.186**	**-25.8**	**75.20**
100	203.8	0.213	-26.4	70.14
150	185.3	0.221	-27.4	60.31

The above result shows that 50mg of Poloxamer 188 produced PNP with minimum PDI and all desired values for particle size, PDI, ZP and %EE.

2. Effect of stirring speed of magnetic stirrer:

Effect of stirring speed on particle size, PDI, ZP and % EE of MS loaded PNPs are shown in **Table 6**.

Table 6: Effect of stirring speed of Magnetic stirrer on PNPs

Stirring Speed (rpm)	Particle Size (nm)	PDI	Zeta Potential (mV)	%EE
500	**173.8**	**0.176**	**-26.9**	**76.34**
1000	195.7	0.227	-25.7	72.72
1500	201.7	0.284	-27.4	71.17
2000	237.4	0.326	-26.8	69.24

The studies showed that less stirring speed give maximum %EE as well as minimum particle size. Increasing particle size and PDI with increasing stirring speed indicates that particle aggregation is produced. The other values of particle size, PDI and ZP were also in well acceptable range. Hence, 500 rpm stirring speed was considered as optimized speed for PNP formulation.

3. Final Formulation:

Final formulation was decided based on the above experimental trials. Final formulation contains following ingredients as shown in **Table 7**.

Table 7: Final Formulation

Ingredients & Process Parameters	Amount of excipients to be added in the formulation
Surfactant (Polaxamer 188)	50 mg
Polymer (PLGA)	30 mg
Organic Solvent (Acetone)	q.s
Processing Parameters	
pH	7
Stirring Speed	500 rpm

3.8 Evaluation & Characterization of Final Formulation:

When MS loaded PNPs were prepared by above mentioned ingredients & process parameters using Solvent evaporation technique, results mentioned in **Table 8** were obtained.

Table 8: Evaluation results of Final Formulation

Evaluation Parameters	Results
Particle Size	163.1 nm
PDI	0.082
Zeta Potential	-29.9
%EE	76%

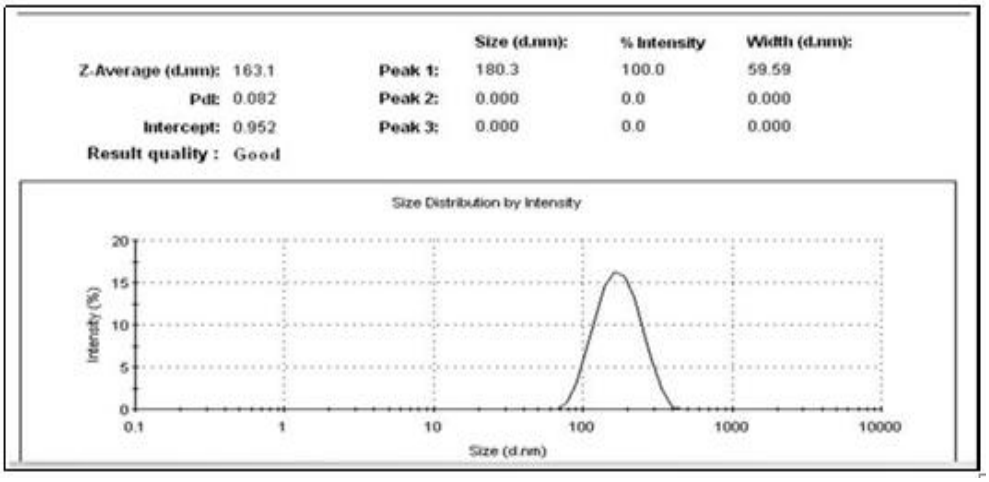

Figure 1: Results of Particle Size Measurement of PNPs

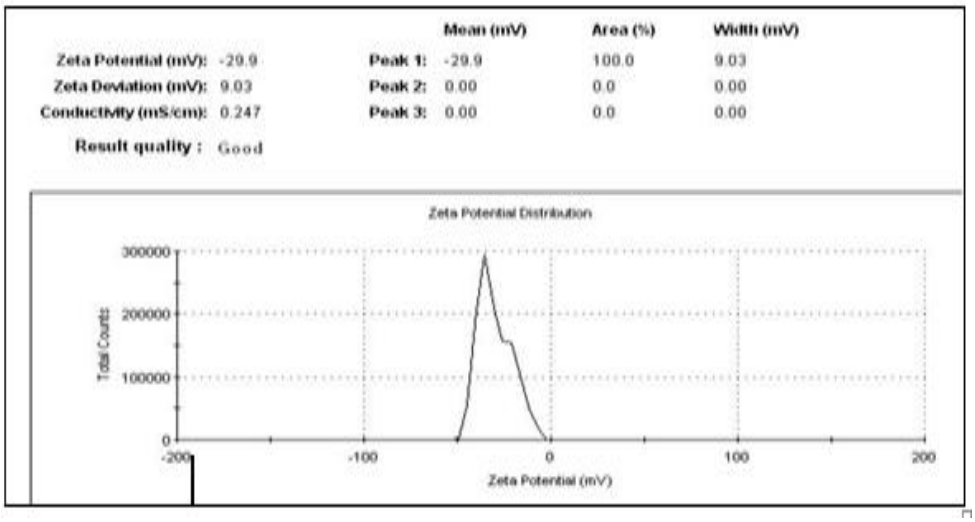

Figure 2: Results of Zeta Potential of PNPs

3.8.1 SEM:

The following figure shows the SEM image of PNP loaded with MS and results of SEM revealed that the PNP was almost uniform in size and spherical in shape.

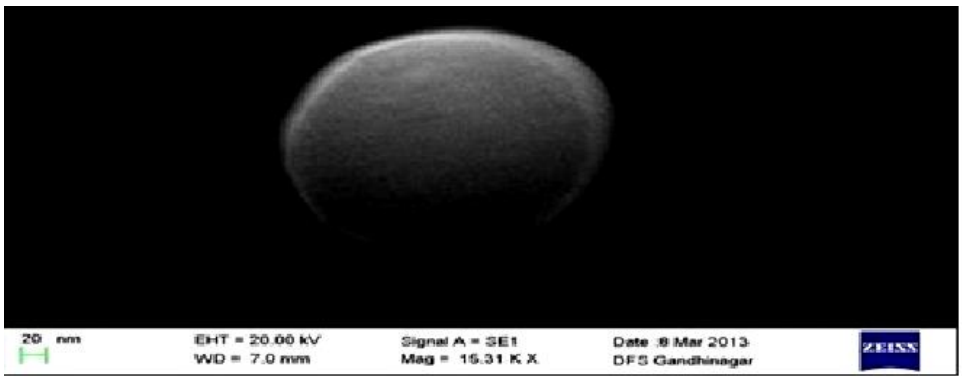

Figure 3: SEM image of MS loaded PNPs

3.8.2 **In Vitro Release Study:** In vitro release study indicates that sustain drug release for 15 hr after burst effect seen in the beginning. The % drug release from the MS loaded NPs is shown in the Table 9 and graphical presentation is shown in figure 4.

Table 9: Results of In Vitro Release Study for PNPs

Sr.No.	Time (hr)	% Drug Release from PLGA NPs
1	0	0
2	0.5	0
3	1	4.6
4	2	10.5
5	3	18.3
6	4	25.4
7	5	30.8
8	6	35.7
9	7	36.4
10	8	37.1
11	9	38.4
12	10	39.1
13	11	40.0
14	12	41.2
15	13	42.0
16	14	42.4
17	15	43.1

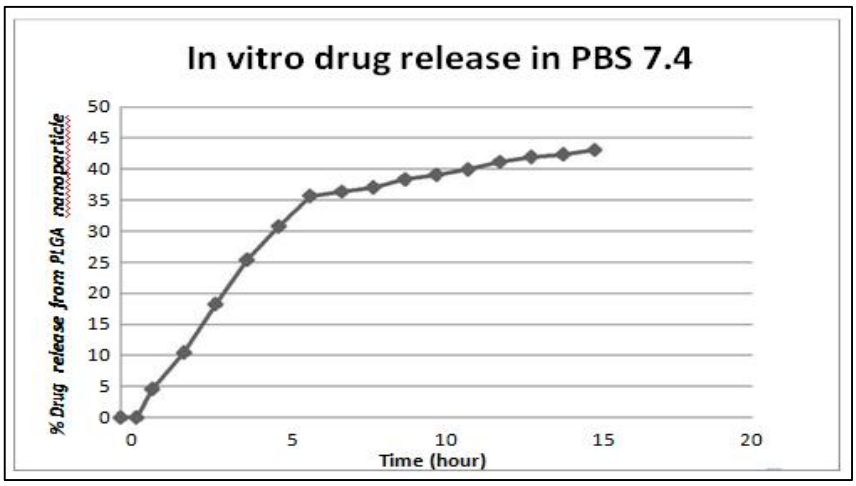

Figure 4: In Vitro Release of MS from PNPs

4. CONCLUSION

Nanoparticles delivered by pulmonary route are expected to achieve enhanced and optimal therapeutic concentrations of drug intracellularly, particularly in broncho alveolar space, and thereby enhancing drug retention and subsequently, improved drug bioavailability leading to reduced incidence of asthmatic attacks as well as drug induced adverse reactions. With this aim, various formulation and instrumental parameters like effect of types and concentration of surfactants, various polymer concentrations, various organic solvents, pH of aqueous phase, and different stirring speeds of magnetic stirrer were investigated in order to optimize formulation of polymeric nanoparticles for most critical characteristics like particle size, PDI, zeta potential and % entrapment efficiency. Prepared drug loaded biodegradable and biocompatible nanoparticles using PLGA with Montelukast sodium have all desired characteristic like Particle size, zeta potential, PDI, % Entrapment Efficiency, In-vitro drug release required for long term site specific controlled release of the encapsulated drug over the treatment period in management of asthma.

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