CHAPTER 9

PUBLICATIONS

9.1 LIST OF PUBLICATIONS

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Details of Publications</th>
</tr>
</thead>
</table>
9.2 REPRINTS OF PUBLICATIONS

FOMULATION AND EVALUATION OF GELATIN NANOPARTICLES FOR PULMONARY DRUG DELIVERY

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ABSTRACT

Pulmonary drug delivery is a non-invasive, non-systemic delivery approach for both local and systemic drugs and a method to directly target disorders of lung. The gelatin nanoparticles were prepared by double desolvation method using different concentration of gelatin as biodegradable polymer for lungs. The prepared gelatin nanoparticles were evaluated for entrapment efficiency, particle size, polydispersity index, zeta potential, transmission electron microscopy, in-vitro drug release, stability study and in-vivo study. The entrapment efficiency of all gelatin nanoparticles formulations were found to be in the range of 46.16% - 58.60%. The particle size of gelatin nanoparticle formulations (GNPs1, GNPs2, GNPs3, GNPs4) were found to be 179.9 nm, 198.4 nm, 252.4 nm and 1545 nm, respectively. The gelatin nanoparticle formulation GNPs3 was selected best formulation depending upon the particle size less than 500nm and higher entrapment efficiency as compared to GNPs1 and GNPs2. The zeta potential of gelatin nanoparticles found to exhibit stability due to positive charge on the surface. The transmission electron microscopy indicated the spherical surface of the gelatin nanoparticles. The in vitro release was found to follow higuchi plot as compared to zero order plot, first order plot and krosmeyer peppas plot. Stability studies showed that gelatin nanoparticle formulation GNPs3 was stable when tested for particle size, entrapment efficiency and in vitro drug release at refrigerated condition (5±3°C), at room temperature (25±2°C/65±5% RH) and at...
accelerated condition (40°C±2°C/75%±5% RH) according to ICH guidelines for 6 months (180 days). In-vivo study using rat model indicated the localization gelatin nanoparticles in the lungs of rat.

**KEYWORDS:** Gelatin nanoparticles, pulmonary drug delivery, terbutaline sulfate, particle size, entrapment efficiency, stability.

**INTRODUCTION**

The lung is an attractive target for drug delivery due to noninvasive means to provide high systemic bioavailability, avoidance of first-pass metabolism, more rapid onset of therapeutic action, and the availability of a huge surface area. Pulmonary drug delivery is a non-invasive, non-systemic delivery approach for both local and systemic drugs and a method to directly target disorders of lung such as asthma, chronic obstructive pulmonary diseases (COPD), emphysema, cystic fibrosis, lung cancer, tuberculosis, pulmonary hypertension and diabetes. The principal of pulmonary drug delivery system is aerosolization which means reduction in the drug particle size delivered to alveoli and bronchioles. It offers many advantages over oral, intranasal and transdermal delivery systems such as

(a) large absorptive surface area with extensive vasculature
(b) easily permeable membrane
(c) low extra cellular and intracellular enzymatic activity
(d) immense capacity for solute exchange and ultra thinness of the alveolar epithelium
(e) rapid and predictable onset of action with minimum side effects
(f) reduced costs due to reduced need for high dose exposures
(g) avoid first pass metabolism
(h) improved patience compliance.

Nanoparticles with their special characteristics such as small particle size, large surface area, and the capability of changing their surface properties have numerous advantages compared with other delivery systems. The interest in nanocarrier systems has led to increasing attention to pulmonary drug delivery. Polymeric nanoparticles are widely studied in drug delivery system for parenteral administration, and their application to the pulmonary routes is also widely recognized. The main roles of polymeric nanoparticles in drug delivery system are to carry the drug molecules, to protect drugs from degradation, and to control drug release. Nanocarrier systems can provide the advantage of sustained release in the lung tissue, resulting in reduced dosing frequency and improved patient compliance.
In the present research, gelatin was used as a biodegradable polymer to form gelatin nanoparticles. Terbutaline sulfate was a drug used to target lungs locally. Terbutaline causes bronchodilation by direct stimulation of $\beta_2$ adrenergic receptors present in bronchial smooth muscles. So, terbutaline was used to treat diseases of lungs such as asthma, chronic obstructive pulmonary diseases (COPD) etc. Terbutaline is variably absorbed from the gastrointestinal tract and about 60% of the absorbed dose undergoes first-pass metabolism by sulfate (and some glucuronide) conjugation in the liver and the gut wall.$^{[9,10]}$ So, as to avoid first pass metabolism, the drug is delivered via pulmonary drug delivery route and drug is allowed to sustain release by incorporating them in the polymeric nanoparticle formulation such as gelatin nanoparticles.

**MATERIALS AND METHODS**

Terbutaline sulfate obtained as gift sample from cipla, Baddi (H.P), gelatin obtained from the qualikem, Mumbai, acetone purchased from SD Fine chemical, chandigarh, glutaraldehyde purchased from the Qualikem, Mumbai, disodium hydrogen ortho Phosphate and Potassium dihydrogen ortho phosphate purchased from Central Drug House, New Delhi. Sodium Chloride is purchased from Qualikem, Mumbai. Hydrochloric acid and sodium hydroxide is purchased from SD Fine Chemicals, Chandigarh.

**Preparation of Gelatin Nanoparticles**$^{[11,12]}$

Gelatin nanoparticles were prepared by the double desolvation method. In this method, 20 ml of 1.25%, 2.5%, 5% and 7.5 % (w/v) gelatin aqueous solution was heated to 50 °C with continuous stirring at 600 rpm followed by the addition of 20 ml of acetone (as desolvating agent). The supernatant was discarded, and the precipitate was dissolved in 20 ml hot water. The pH value was adjusted to 2.5 with 0.1 N HCl. Before the second desolvation step, 60 mg of the terbutaline sulfate was added and then 48 ml acetone was added in the gelatin solution to desolvate the gelatin nanoparticles with continuous stirring at 600 rpm. The nanoparticles were cross-linked by 800 μl of glutaraldehyde and stirred at 1000 rpm overnight. The nanoparticles were sonicated for 5 mins. Finally, acetone was removed by vacuum drying, and the fabricated GNP were purified and resuspended in deionized water. These particles were stored at 4°C for further applications. The composition of different formulations of gelatin Nanoparticles are shown in the table 1 below.

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**Entrapment Efficiency**\(^{[13]}\)

The prepared nanoparticles suspension of 10 ml was centrifuged at 154350 g (approx. 35000 rpm) using Beckman Coulter Ultra Centrifuge for 30 mins. The pellet of nanoparticles was formed. The supernatant was removed and the supernatant was then observed under spectrophotometer at 276 nm for absorbance and the amount of the drug in the supernatant was estimated.

\[
\text{Entrapment Efficiency (\%) = } \frac{(\text{amount of drug added - unentrapped Drug})}{\text{amount of drug added}} \times 100
\]

**Particle Size Measurement**\(^{[13]}\)

The average particle size and the size distribution of the obtained nanoparticles was determined by photon correlation spectroscopy (PCS) using zetasizer. The analysis was performed at a temperature of 25 °C using samples appropriately diluted with filtrated and double distilled water in order to avoid multiscattering events.

**Zeta-Potential measurement**\(^{[13]}\)

The Zeta-Potential was measured by laser Doppler anemometry (LDA) using a Zetasizer. The analysis is performed at a temperature of 25°C using samples appropriately diluted with 1.54 mM NaCl solution in order to maintain a constant ionic strength.

**Transmission Electron Microscopy (TEM) of Gelatin Nanoparticles formulation**

The morphology of gelatin nanoparticles of terbutalin sulfate was studied by transmission electron microscopy (TEM) on a Philips EM268D instrument (Philips, Netherlands) at SAIF Panjab University, Chandigarh. The aqueous dispersion of nanoparticles (one drop) was placed over a 400-mesh carbon-coated copper grid followed by negative staining with phosphotungstic acid solution (3% w/v, adjusted to pH 4.7 with KOH) and placed at the accelerating voltage of 95 kV for TEM.

**In-Vitro Release Study**\(^{[13]}\)

Drug release from known amount of Terbutaline sulfate loaded nanoparticles was determined using the dialysis tube diffusion method at 37 ± 1°C. Gelatin nanoparticulate formulation GNps3 (2 mL) and pure terbutaline sulfate were placed into the dialysis tube (Sigma Aldrich) tied at both the ends and suspended in a beaker containing 50 mL of Phosphate Buffer Saline (pH-7.4) with 200 mg/mL ascorbic acid, an anti-oxidant to prevent oxidative
degradation of terbutaline Sulfate. The pure terbutaline sulfate of same amount as present in gelatin nanoparticle formulations were magnetically stirred at 50 rpm and the temperature was maintained at 37 ± 1°C throughout the procedure. Samples(1 ml) were withdrawn at definite time intervals and replaced with same volume of Phosphate Buffer Saline (pH-7.4). The samples were then analyzed spectrophotometrically (UV-1601 Shimadzu, Japan) for drug content.

RESULTS AND DISCUSSION
The prepared gelatin nanoparticle formulations were evaluated for entrapment efficiency. The results of the entrapment efficiency are shown in table 2.

The effect of the concentration on the entrapment efficiency of terbutaline sulfate had found to increase with increase in the concentration(i.e. GNPs4>GNPs3>GNPs2>GNPs1) . The particle size and the polydispersity index of gelatin nanoparticle formulations are shown in the table 3.

The particle size of the gelatin nanoparticle formulation GNPs4 was found to be maximum and the particle size of the gelatin nanoparticle formulation GNPs1 was found to be minimum. The effect of the concentration on the particle size of gelatin nanoparticles observed that the particle size of the gelatin nanoparticle increase with increase in the concentration. The sequence of particle size was found to be GNPs4>GNPs3>GNPs2>GNPs1. The polydispersity index of the gelatin nanoparticles of formulation GNPs1 and GNPs2 are good as compared to the formulation GNPs3 and GNPs4.

The gelatin nanoparticle formulation GNPs3 was selected best formulation depending upon the particle size less then 500nm and higher entrapment efficiency as compared to GNPs1 and GNPs2. The zeta potential of gelatin nanoparticle formulations are shown in the table 4 .

The results of Zeta Potential of gelatin nanoparticles of all formulation showed slight positive charge on the surface. The gelatin nanoparticle formulation were found to be stable depending upon the charges present on the surface of the gelatin nanoparticle which prevent the aggregation of the particle suspended in suspension of gelatin nanoparticle formulations.

The selected gelatin nanoparticles formulation (i.e GNPs3) was also evaluated for Transmission Electron Microscopy(TEM). The results of the Transmission Electron
Microscopy (TEM) of gelatin nanoparticle formulation (GNps3) are shown in the figure 1 and figure 2.

TEM micrographs of prepared gelatin nanoparticle formulation (GNps3) showed that the gelatin nanoparticles were spherical in shape. The gelatin nanoparticles loaded with terbutaline sulfate were found to be black in color and the gelatin nanoparticles without terbutaline sulfate were found to be white in color. The Transmission Electron Microscopy (TEM) confirmed the preparation of smooth and spherical nature of the gelatin nanoparticles. The comparison of in-vitro release of free terbutaline sulfate (pure drug) and gelatin nanoparticle formulations are shown in the graph below (figure 3).

In vitro release of terbutaline sulfate from all gelatin nanoparticle formulation exhibited sustained release of the terbutaline sulfate as compared to the pure drug. The release of the terbutaline sulfate from the gelatin nanoparticle formulations found to be best fit in higuchi plot as compared to zero order plot, first order plot and Krosmeyer peppas plot because the regression co-efficient in case of higuchi plot was found to higher as compared to the regression co-efficient in case of zero order plot, first order plot and Krosmeyer peppas plot.

**Stability Study of Gelatin Nanoparticles**

The optimized formulation (GNps3) of Gelatin Nanoparticles was selected for stability study to be performed at refrigerated condition (5°±3°C), at room temperature (25°±2°C/65%±5% RH) and at accelerated condition (40°±2°C/75%±5% RH) according to ICH guidelines for 6 months (180 days). The samples were withdrawn after 0, 45, 90, 135 and 180 days and were checked for particle size, entrapment efficiency and in vitro drug release. The formulation at refrigerated condition serve as control and was used to compare the results of formulation kept at room temperature and at accelerated condition. The results of stability study of Gelatin Nanoparticle formulation (GNps3) are shown in Table 5.

The results of stability studies showed that the selected formulation of gelatin nanoparticle formulation GNps3 was stable at refrigerated condition (5°±3°C), at room temperature (25°±2°C/65%±5% RH) and at accelerated condition (40°±2°C/75%±5% RH) according to ICH guidelines for 6 months (180 days).
In-Vivo Study Using Rat Model \textsuperscript{[14,15]}

Direct Intratracheal administration technique was used in rats. In this technique, rats were anaesthised with Ketamine intraperitoneal injection (50 mg/kg). After anaesthesia, the animal was laid in a supine position, attached by its superior incisors to a board and tilted at an angle of 45 degrees. The mouth was kept open to locate the vocal cords and for delivering selected nanoparticle formulation (GNps3) in trachea. The administration was performed by inserting a modified oral gavage needle No. – 16 (which had thin plastic tube at tip of needle and 1 ml of disposable syringe at the bottom of the needle) in the trachea, between the vocal cords. Nanoparticle formulations containing the drug (upto 25 – 30 µl) was delivered into the trachea as a liquid bolus by intratracheal instillation. For the deepest administration within the lung and for the highest bioavailability, the instillation was followed by the administration of 3 ml of air bolus by using microsyringe. The drug deposition was determined by taking blood samples 0.5 ml at different time intervals (i.e. 0, 1, 2, 4, 6, 12, 24, 36 and 48 hrs) from tail vein of the rats into heparinized Eppendorf tubes. The blood samples were centrifuged at 15000 rpm for 15 mins and obtained plasma was examined for Terbutaline Sulfate concentration using HPLC method and pharmacokinetic of Terbutaline Sulfate was studied from different selected nanoparticle formulations.

The results of the in-vivo studies indicated that the drug plasma concentration of Terbutaline sulfate found to be nil in the blood of the rat at different time interval of sampling which indicated that the gelatin nanoparticles are localized in the lungs of the rat and no drug present in the blood of the rat upto 48 hrs of blood sampling and whole of the drug is releasing from gelatin nanoparticles in lungs in sustained manner. The localization of gelatin nanoparticles are confirmed from the results of the in-vivo study as whole of the drug is present in the lung and no drug was present in the blood of the rat.

Table 1: Composition of different Gelatin Nanoparticle formulations

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation Code</th>
<th>Gelatin Concentration (%)</th>
<th>Amount of drug (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GNps1</td>
<td>1.25</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>GNps2</td>
<td>2.5</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>GNps3</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>GNps4</td>
<td>7.5</td>
<td>60</td>
</tr>
</tbody>
</table>
Table 2: Entrapment efficiencies of gelatin nanoparticle formulations (GNPs1, GNPs2, GNPs3 and GNPs4)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation code</th>
<th>Entrapment Efficiency(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GNPs1</td>
<td>46.16 ± 0.53</td>
</tr>
<tr>
<td>2</td>
<td>GNPs2</td>
<td>53.89 ± 0.27</td>
</tr>
<tr>
<td>3</td>
<td>GNPs3</td>
<td>56.75 ± 0.85</td>
</tr>
<tr>
<td>4</td>
<td>GNPs4</td>
<td>58.60 ± 0.68</td>
</tr>
</tbody>
</table>

Table 3: Particle size and polydispersity index of gelatin nanoparticle formulations (GNPs1, GNPs2, GNPs3 and GNPs4)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation code</th>
<th>Particle Size (nm)</th>
<th>Polydispersity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GNPs1</td>
<td>179.9 ± 0.86</td>
<td>0.061 ± 0.014</td>
</tr>
<tr>
<td>2</td>
<td>GNPs2</td>
<td>198.4 ± 1.04</td>
<td>0.110 ± 0.008</td>
</tr>
<tr>
<td>3</td>
<td>GNPs3</td>
<td>252.4 ± 0.65</td>
<td>0.116 ± 0.019</td>
</tr>
<tr>
<td>4</td>
<td>GNPs4</td>
<td>1545 ± 0.97</td>
<td>0.338 ± 0.026</td>
</tr>
</tbody>
</table>

Table 4: Zeta Potential of gelatin nanoparticle formulations (GNPs1, GNPs2, GNPs3 and GNPs4)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation code</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GNPs1</td>
<td>14.2 ± 0.75</td>
</tr>
<tr>
<td>2</td>
<td>GNPs2</td>
<td>3.81 ± 0.41</td>
</tr>
<tr>
<td>3</td>
<td>GNPs3</td>
<td>10.4 ± 0.63</td>
</tr>
<tr>
<td>4</td>
<td>GNPs4</td>
<td>12.6 ± 0.96</td>
</tr>
</tbody>
</table>

Table 5: Stability Study of Gelatin Nanoparticles formulation (GNPs3) at Refrigerated Condition (5°±3°C), Room temperature (25°±2°C/65%±5% RH) and Accelerated Condition (40°±2°C/75%±5% RH)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Days</th>
<th>Refrigerated Condition (5°±3°C)</th>
<th>Room Temperature (25°±2°C/65%±5% RH)</th>
<th>Accelerated Condition (40°±2°C/75%±5% RH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Particle size (nm)</td>
<td>0</td>
<td>252.4 ± 0.65</td>
<td>252.0 ± 0.7</td>
<td>252.9 ± 0.3</td>
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<tr>
<td></td>
<td></td>
<td>45</td>
<td>252.4 ± 0.65</td>
<td>252.7 ± 0.5</td>
<td>253.1 ± 0.8</td>
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<td></td>
<td></td>
<td>90</td>
<td>252.4 ± 0.65</td>
<td>252.9 ± 0.2</td>
<td>253.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>135</td>
<td>252.4 ± 0.65</td>
<td>253.0 ± 0.5</td>
<td>253.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180</td>
<td>252.4 ± 0.65</td>
<td>253.2 ± 0.6</td>
<td>254.5 ± 0.8</td>
</tr>
<tr>
<td>2</td>
<td>Entrapment Efficiency (%)</td>
<td>0</td>
<td>56.75 ± 0.85</td>
<td>56.64 ± 0.33</td>
<td>56.45 ± 0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45</td>
<td>56.75 ± 0.85</td>
<td>56.52 ± 0.28</td>
<td>56.09 ± 0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>56.75 ± 0.85</td>
<td>56.36 ± 0.12</td>
<td>55.74 ± 0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>135</td>
<td>56.75 ± 0.85</td>
<td>56.16 ± 0.85</td>
<td>55.46 ± 0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180</td>
<td>56.75 ± 0.85</td>
<td>56.02 ± 0.37</td>
<td>55.17 ± 0.31</td>
</tr>
<tr>
<td>3</td>
<td>In vitro Drug Release (%)</td>
<td>0</td>
<td>78.22 ± 1.15</td>
<td>78.10 ± 0.94</td>
<td>77.86 ± 0.22</td>
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<td>45</td>
<td>78.22 ± 1.15</td>
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<td>90</td>
<td>78.22 ± 1.15</td>
<td>77.84 ± 1.03</td>
<td>77.37 ± 1.46</td>
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<tr>
<td></td>
<td></td>
<td>135</td>
<td>78.22 ± 1.15</td>
<td>77.66 ± 0.85</td>
<td>76.90 ± 1.23</td>
</tr>
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<td></td>
<td></td>
<td>180</td>
<td>78.22 ± 1.15</td>
<td>77.52 ± 0.74</td>
<td>76.63 ± 0.65</td>
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</table>
Figure 1: TEM diagram of Gelatin nanoparticle formulation, GNps3

Figure 2: TEM diagram of Gelatin nanoparticle formulation, GNps3
CONCLUSION

Pulmonary drug delivery is an effective route to deliver the drug directly in the lungs which overcome various side effects of systemic delivery of the drugs. Exploiting pulmonary route for delivery of drugs indicated that the gelatin nanoparticle with size less than 500 nm were localized effectively in the pulmonary route to treat the lung disease such as asthma, chronic obstructive pulmonary diseases (COPD) etc. The results indicated that the prepared gelatin nanoparticles effectively sustain the release of the drug (terbutaline sulfate) as compared to the pure terbutaline sulfate. The prepared gelatin nanoparticles were found to be stable when tested for entrapment efficiency, particle size and in-vitro release at different conditions temperature according to ICH guidelines for 6 months. The in-vivo data using rat lung model indicated that the gelatin nanoparticles were effectively localized in the lungs and there were no trace of drug in the blood samples of rat for upto 48 hrs which confirmed the presence of terbutaline sulfate loaded gelatin nanoparticles in the lungs of rat.

ACKNOWLEDGMENT

The Punjab university, Chandigarh offered handsome support to carry out the research work in there institution by providing necessary facilities for research work. The rayat bahra institution of pharmacy, kharar also extended support in my research work. I am highly obliged to Dr. S.L. Harikumar, Dr. Shishu, and Dr. Abhishek buddiraja for their directions and continuous encouragement during the course of my research work.
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10. Drug index, 2004; 7: 4 – 357


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Original Article

Development and characterization of albumin nanoparticles for pulmonary drug delivery

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ABSTRACT

Pulmonary drug delivery is a non-invasive, non-systemic delivery approach for both local and systemic drugs and a method to directly target disorders of lung. The albumin nanoparticles were prepared by modified desolvation method using different concentration of albumin as biodegradable polymer for lungs. The prepared albumin nanoparticles were evaluated for entrapment efficiency, particle size, polydispersity index, zeta potential, transmission electron microscopy, in-vitro drug release, stability study and in-vivo study. The entrapment efficiency of all albumin nanoparticles formulations were found to be in the range of 61.74% - 73.45%. The particle size of albumin nanoparticle formulations were found to be in range 81.6 nm - 148.4 nm. The zeta potential of albumin nanoparticles found to exhibit stability due to negative charge on the surface. The transmission electron microscopy indicated the spherical surface of the albumin nanoparticles. The in vitro release was found to follow higuchi plot as compared to zero order plot, first order plot and krosmeyer peppas plot. Stability studies showed that albumin nanoparticle formulation ANps5 was stable according to ICH guidelines for 6 months. In-vivo study using rat model indicated the localization of albumin nanoparticles in the lungs of rat.

Keywords: albumin nanoparticles, pulmonary drug delivery, terbutaline sulfate, particle size, entrapment efficiency, stability

INTRODUCTION

Lungs are an attractive target for the pulmonary administration of active pharmaceutical ingredients (APIs) in the form of various drug delivery systems [1-3]. Additionally, pulmonary route offers many advantages over conventional oral administration, such as a high surface area with rapid absorption due to high vascularization and circumvention of the first pass effect [2]. Pulmonary drug delivery is a non-invasive, non-systemic delivery approach for both local and systemic drugs and a method to directly target disorders of lung such as asthma, chronic obstructive pulmonary diseases (COPD), emphysema, cystic fibrosis, lung cancer, tuberculosis, pulmonary hypertension and diabetes. The principal of pulmonary drug delivery system is aerosolization which means reduction in the drug particle size delivered to alveoli and bronchioles.

The advantages of pulmonary drug delivery system[4,5] are

1. Allow efficient drug targeting to the lungs
2. It gives very fast onset of action comparable to the i.v. Route
3. Inhaling helps to avoid gastrointestinal tract problems such as poor solubility, low bioavailability, gut irritation, unwanted metabolites, food effects and dosing variability.
4. It requires low and fraction of oral dose
5. Pulmonary drug delivery having very negligible side effects since rest of body is not exposed to drug.

Nanoparticles are solid, colloidal particles which vary in size from 10 to 1000 nanometers. A drug can be dissolved, entrapped, adsorbed, attached or encapsulated into a nanoparticle matrix. Nanoparticles can protect the drug from degradation, enhance its transport and distribution.
and prolong its release; hence, the plasma half-life of the drug entrapped can be improved [6]. The advantage of using polymeric nanoparticles as colloidal carriers for advanced drug delivery is mainly their small size, which allows nanoparticles to penetrate even small capillaries and be taken up within cells, allowing efficient drug accumulation at targeted sites in the body. Biodegradable polymers used for nanoparticle preparation allow for sustained drug release at the targeted site over a period of days or even weeks after administration [7]. Nanocarrier systems can provide the advantage of sustained release in the lung tissue, resulting in reduced dosing frequency and improved patient compliance [8].

In the present research, albumin was used as biodegradable polymer to form albumin nanoparticles. Albumin nanoparticles were used to target lungs locally to treat various lung diseases such as asthma, chronic obstructive pulmonary diseases (COPD), cystic fibrosis, tuberculosis, lung cancer, pulmonary hypertension etc. Albumin nanoparticles administered in lungs were inert to the surrounding tissue as they contain no irritating or toxic additives and degrade when applicable within an acceptable period of time without producing toxic by-products. Therefore, it was possible to locally target the lung tissue thereby reducing the dose as well as increasing the patient compliance by integration of nanotechnology and pulmonary drug delivery systems and provide a sustained effect of more than 12 hrs.

MATERIALS AND METHODS
Terbutaline sulfate obtained as gift sample from cipla, Baddi (H.P.), Bovine serum albumin obtained from the qualikem, Mumbai, acetone purchased from SD Fine Chemical, chandigarh, glutaraldehyde purchased from the Qualikem, Mumbai, disodium hydrogen ortho Phosphate and potassium dihydrogen ortho phosphate purchased from Central Drug House, New Delhi, Sodium Chloride is purchased from Qualikem, Mumbai, Sodium hydroxide is purchased from SD Fine Chemicals, Chandigarh.

Preparation of Albumin Nanoparticles[9]: Bovine serum albumin (BSA) nanoparticles were prepared by using a desolvation method with minor modifications. Bovine serum albumin (BSA) powder of required amount was added to distilled water to prepare aqueous solutions of different concentrations (1 - 5% w/v), 100 mg of terbutaline sulfate was added to aqueous solutions and prepared aqueous solutions were adjusted to pH - 9 by using 0.1 N NaOH with continuous stirring at 500 rpm. The solutions were stirred overnight at 500 rpm using a magnetic stirrer for complete hydration. A desolvating agent, acetone, was added drop wise at a rate of 1 ml/min into the BSA solutions until the solutions became just turbid. Finally, 0.01 ml of a 4% glutaraldehyde-ethanol solution was mixed to induce nano-particle cross-linking. The solutions were stirred continuously at 500 rpm and kept for 3 hrs till albumin nanoparticles get formed. The composition of different albumin nanoparticles formulations are shown in Table 1.

Entrapment Efficiency [10]: The prepared nanoparticles suspension of 10 ml was centrifuged at 154350 g (approx. 35000 rpm) using Beckman Coulter Ultra Centrifuge for 30 mins. The pellet of nanoparticles was formed. The supernatant was removed and the supernatant was then observed under spectrophotometer at 276 nm for absorbance and the amount of the drug in the supernatant was estimated.

Particle size measurement [10]: The average particle size and the size distribution of the obtained nanoparticles was determined by photon correlation spectroscopy (PCS) using zetasizer. The analysis was performed at a temperature of 25°C using samples appropriately diluted with filtered and double distilled water in order to avoid multiscattering events.

Zeta-Potential measurement [10]: The Zeta-Potential was measured by laser Doppler anemometry (LDA) using a Zetasizer. The analysis is performed at a temperature of 25°C using samples appropriately diluted with 1.54 mM NaCl solution in order to maintain a constant ionic strength.

Transmission Electron Microscopy (TEM) of Gelatin Nanoparticles formulation: The morphology of gelatin nanoparticles of terbutaline sulfate was studied by transmission electron microscopy (TEM) on a Philips EM268D instrument (Philips, Netherlands) at SAIF Panjab University, Chandigarh. The aqueous dispersion of nanoparticles (one drop) was placed over a 400 mesh carbon-coated copper grid followed by negative staining with phosphotungstic acid solution (3% w/v, adjusted to pH 4.7 with KOH) and placed at the accelerating voltage of 95 kV for TEM.

In-vitro release study[10]: Drug release from known amount of Terbutaline sulfate loaded nanoparticles was determined using the dialysis tube diffusion method at 37 ± 1°C. Gelatin nanoparticulate formulation GNPs3 (2 ml) and pure terbutaline sulfate were placed into the dialysis tube (Sigma Aldrich) tied at both the ends.
and suspended in a beaker containing 50 mL of Phosphate Buffer Saline (pH-7.4) with 200 mg/mL ascorbic acid, an anti-oxidant to prevent oxidative degradation of Terbutaline Sulfate. The pure terbutaline sulfate of same amount as present in gelatin nanoparticles formulations were magnetically stirred at 50 rpm and the temperature was maintained at 37 ± 1°C throughout the procedure. Samples(1 ml) were withdrawn at definite time intervals and replaced with same volume of Phosphate Buffer Saline (pH-7.4). The samples were then analyzed spectrophotometrically (UV-1601 Shimadzu, Japan) for drug content.

RESULTS AND DISCUSSION

The prepared Albumin nanoparticle formulations were evaluated for entrapment efficiency. The results of the entrapment efficiency are shown in table – 2. The entrapment efficiency of different albumin nanoparticles formulations found to increase with increase in the concentration of albumin in different nanoparticle formulations. The entrapment efficiency found to be maximum for albumin nanoparticle formulation ANps5 and minimum for ANps1. The particle size and the polydispersity index of albumin nanoparticle formulations are shown in the Table -3. The particle size of the Albumin nanoparticle formulation ANps5 was found to be maximum and the particle size of the Albumin nanoparticle formulation ANps1 was found to be minimum. The effect of the albumin concentration on the particle size of Albumin nanoparticles observed that the particle size of the Albumin nanoparticle increase with increase in the albumin concentration. The sequence of particle size was found to be ANps5>ANps4>ANps3>ANps2>ANps1.

The Albumin nanoparticle formulation ANps5 was selected as a best formulation depending upon the particle size less than 500 nm required to get entrapped in the alveoli of lungs and possess higher entrapment efficiency as compared to ANps1, ANps2, ANps3 and ANps4.

The zeta potential of albumin nanoparticles formulations are shown in the Table 4. The results of Zeta Potential of Albumin nanoparticles of all formulation showed slight negative charge on the surface. The albumin nanoparticle formulation were found to be stable depending upon the charges present on the surface of the albumin nanoparticles which prevent the aggregation of the particle suspended in suspension of albumin nanoparticle formulations. The selected albumin nanoparticles formulation (i.e ANps5) was also evaluated for Transmission Electron Microscopy(TEM). The results of the Transmission Electron Microscopy(TEM) of albumin nanoparticle formulation (ANps5) are shown in the figure 1 and figure 2.

TEM micrographs of prepared albumin nanoparticle formulation (ANps5) showed that the albumin nanoparticles were spherical in shape. The albumin nanoparticles loaded with terbutaline sulfate were found to be black in color. The Transmission Electron Microscopy(TEM) confirmed the preparation of smooth and spherical nature of the albumin nanoparticles. The comparison of in-vitro release of free terbutaline sulfate (pure drug) and albumin nanoparticle formulations are shown in the graph below(figure 3).

In vitro release of terbutaline sulfate from all albumin nanoparticle formulation exhibited sustained release of the terbutaline sulfate as compared to the pure drug. Albumin nanoparticles formulations exhibited a biphasic pattern of drug release, an initial burst effect due to immediate release of the surface associated drug and prolonged release in the later stage due to the slow diffusion of drug from the matrix. The release of the terbutaline sulfate from the albumin nanoparticle formulations found to be best fit in higuchi plot as compared to zero order plot, first order plot and krosmeyer peppas plot because the regression co-efficient in case of higuchi plot was found to higher as compared to the regression co-efficient in case of zero order plot, first order plot and krosmeyer peppas plot.

Stability Study of Albumin Nanoparticles: The optimized formulation (ANps5) of Albumin Nanoparticles was selected for stability study to be performed at refrigerated condition (5°±3°C), at room temperature (25°±2°C/65±5% RH) and at accelerated condition (40°±2°C/75±5% RH) according to ICH guidelines for 6 months(180 days). The samples were withdrawn after 0, 45, 90, 135 and 180 days and were checked for particle size, entrapment efficiency and in vitro drug release. The formulation at refrigerated condition serve as control and was used to compare the results of formulation kept at room temperature and at accelerated condition. The results of stability study of albumin Nanoparticle formulation (ANps5) are shown in Table 5. The results of stability studies showed that the selected formulation of albumin nanoparticle formulation ANps5 was stable at refrigerated condition (5°±3°C), at room temperature (25°±2°C/65±5% RH) and at accelerated condition (40°±2°C/75±5% RH) according to ICH guidelines for 6 months(180 days) because
there was negligible change in the values of particle size, entrapment efficiency and in-vitro drug release of albumin nanoparticle formulation ANps3.

**In-Vivo Study Using Rat Model[11,12]:** Direct Intratracheal administration technique was used in rats. In this technique, rats were anaesthetized with Ketamine intraperitoneal injection (50 mg/kg). After anaesthesia, the animal was laid in a supine position, attached by its superior incisors to a board and tilted at an angle of 45 degrees. The mouth was kept open to locate the vocal cords and for delivering selected nanoparticle formulation (ANps3) in trachea. The administration was performed by inserting a modified oral gavage needle No. – 16 (which had thin plastic tube at tip of needle and 1 ml of disposable syringe at the bottom of the needle) in the trachea, between the vocal cords. Nanoparticle formulations containing the drug (upto 25 – 30 µl) was delivered into the trachea as a liquid bolus by intratracheal instillation. For the deepest administration within the lung and for the highest bioavailability, the instillation was followed by the administration of 3 ml of air bolus by using micorsyringe. The drug deposition was determined by taking blood samples 0.5 ml at different time intervals (i.e. 0, 1, 2, 4, 6, 12, 24, 36 and 48 hrs) from tail vein of the rats into heparinized Eppendorff tubes. The blood samples were centrifuged at 15000 rpm for 15 mins and obtained plasma was examined for Terbutaline Sulfate concentration using HPLC method and pharcomkinetic of Terbutaline Sulfate was studied from different selected nanoparticle formulations.

The results of the in-vivo studies indicated that the drug plasma concentration of Terbutaline sulfate found to be insignificant in the blood of the rat at different time interval of sampling which indicated that the albumin nanoparticles are localized in the lungs of the rat and no drug present in the blood of the rat upto 48 hrs of blood sampling and whole of the drug is releasing from albumin nanoparticles in lungs in sustained manner. The localization of gelatin nanoparticles are confirmed from the results of the in-vivo study as whole of the drug is present in the lung and no drug was present in the blood of the rat.

**CONCLUSION**

Pulmonary drug delivery is a attractive route to deliver the drug directly in the lungs which overcome various side effects of systemic delivery of the drugs. Exploiting pulmonary route for delivery of drugs indicated that the albumin nanoparticle with size less then 500 nm were localized effectively in the pulmonary route to treat the lung disease such as asthma, chronic obstructive pulmonary diseases (COPD) etc. The results indicated that the prepared albumin nanoparticles effectively sustain the release of the drug(terbutaline sulfate) as compared to the pure terbutaline sulfate. The prepared albumin nanoparticles were found to be stable when tested for entrapment efficiency, particle size and in-vitro release at different conditions temperature according to ICH guidelines for 6 months. The in-vivo data using rat lung model indicated that the albumin nanoparticles were effectively localized in the lungs and there were insignificant trace of drug in the blood samples of rat for upto 48 hrs which confirmed the presence of terbutaline sulfate loaded albumin nanoparticles in the lungs of rat.

**ACKNOWLEDGMENT**

The Punjab university, Chandigarh offered handsome support to carry out the research work in there institution by providing necessary facilities for research work. The ranyb bhatta institution of Pharmacy, kharar also extended support in my research work. I am highly obliged to Dr. S.L.Harikumar, Dr. Shishin, and Dr. Abhishek baddiraj for their directions and continuous encouragement during the course of my research work.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Formulation code</th>
<th>Amount of Albumin used (mg)</th>
<th>Amount of terbutaline sulfate used (mg)</th>
<th>Concentration of albumin (w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ANps1</td>
<td>200</td>
<td>100</td>
<td>1%</td>
</tr>
<tr>
<td>2</td>
<td>ANps2</td>
<td>400</td>
<td>100</td>
<td>2%</td>
</tr>
<tr>
<td>3</td>
<td>ANps3</td>
<td>600</td>
<td>100</td>
<td>3%</td>
</tr>
<tr>
<td>4</td>
<td>ANps4</td>
<td>800</td>
<td>100</td>
<td>4%</td>
</tr>
<tr>
<td>5</td>
<td>ANps5</td>
<td>1000</td>
<td>100</td>
<td>5%</td>
</tr>
</tbody>
</table>
## Chapter 9

### Publications

#### Table 2: Entrapment Efficiencies of Albumin nanoparticle formulations

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation code</th>
<th>Entrapment Efficiency(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ANps1</td>
<td>61.74 ± 0.65</td>
</tr>
<tr>
<td>2</td>
<td>ANps2</td>
<td>64.82 ± 0.54</td>
</tr>
<tr>
<td>3</td>
<td>ANps3</td>
<td>68.36 ± 0.94</td>
</tr>
<tr>
<td>4</td>
<td>ANps4</td>
<td>69.22 ± 1.08</td>
</tr>
<tr>
<td>5</td>
<td>ANps5</td>
<td>73.45 ± 0.77</td>
</tr>
</tbody>
</table>

#### Table 3: Particle size and polydispersity index of Albumin nanoparticle formulations

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation code</th>
<th>Particle Size(nm)</th>
<th>Polydispersity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ANps1</td>
<td>81.6 ± 0.8</td>
<td>0.207 ± 0.011</td>
</tr>
<tr>
<td>2</td>
<td>ANps2</td>
<td>99.4 ± 0.3</td>
<td>0.247 ± 0.008</td>
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<tr>
<td>3</td>
<td>ANps3</td>
<td>116.5 ± 0.7</td>
<td>0.238 ± 0.024</td>
</tr>
<tr>
<td>4</td>
<td>ANps4</td>
<td>127.2 ± 0.5</td>
<td>0.165 ± 0.035</td>
</tr>
<tr>
<td>5</td>
<td>ANps5</td>
<td>148.4 ± 0.6</td>
<td>0.280 ± 0.018</td>
</tr>
</tbody>
</table>

#### Table 4: Zeta Potential of Albumin nanoparticle formulations

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation code</th>
<th>Zeta Potential</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>ANps1</td>
<td>-4.64 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>ANps2</td>
<td>-31.03 ± 0.11</td>
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<tr>
<td>3</td>
<td>ANps3</td>
<td>-21.81 ± 0.08</td>
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<tr>
<td>4</td>
<td>ANps4</td>
<td>-15.21 ± 0.15</td>
</tr>
<tr>
<td>5</td>
<td>ANps5</td>
<td>-18.44 ± 0.27</td>
</tr>
</tbody>
</table>

#### Table 5: Stability Study of Albumin Nanoparticles formulation (ANps5) at Refrigerated Condition (5°±3°C), Room temperature (25°±2°C/65%±5% RH) and Accelerated Condition (40°±2°C/75%±5% RH)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Days</th>
<th>Refrigerated Condition (5°±3°C)</th>
<th>Room Temperature (25°±2°C/65%±5% RH)</th>
<th>Accelerated Condition (40°±2°C/75%±5% RH)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Particle size (nm)</td>
<td>0</td>
<td>148.4 ± 0.6</td>
<td>148.6 ± 0.4</td>
<td>148.9 ± 1.3</td>
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<td></td>
<td></td>
<td>45</td>
<td>148.4 ± 0.6</td>
<td>148.8 ± 0.7</td>
<td>149.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>148.4 ± 0.6</td>
<td>149.1 ± 0.9</td>
<td>149.7 ± 0.8</td>
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<tr>
<td></td>
<td></td>
<td>135</td>
<td>148.4 ± 0.6</td>
<td>149.3 ± 1.5</td>
<td>150.2 ± 1.1</td>
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<tr>
<td></td>
<td></td>
<td>180</td>
<td>148.4 ± 0.6</td>
<td>149.6 ± 0.9</td>
<td>150.8 ± 0.5</td>
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<tr>
<td>2</td>
<td>Entrapment Efficiency (%)</td>
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<td>73.45 ± 0.77</td>
<td>73.31 ± 0.47</td>
<td>73.09 ± 0.80</td>
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<td>73.45 ± 0.77</td>
<td>73.20 ± 0.75</td>
<td>72.65 ± 0.35</td>
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<td></td>
<td>90</td>
<td>73.45 ± 0.77</td>
<td>73.06 ± 0.32</td>
<td>72.30 ± 0.97</td>
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<td>135</td>
<td>73.45 ± 0.77</td>
<td>72.83 ± 0.45</td>
<td>71.96 ± 0.67</td>
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<td></td>
<td></td>
<td>180</td>
<td>73.45 ± 0.77</td>
<td>72.60 ± 0.37</td>
<td>71.57 ± 0.82</td>
</tr>
<tr>
<td>3</td>
<td>In vitro Drug Release (%)</td>
<td>0</td>
<td>68.26 ± 0.55</td>
<td>68.15 ± 1.51</td>
<td>68.04 ± 0.64</td>
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<td>68.26 ± 0.55</td>
<td>68.02 ± 0.45</td>
<td>67.57 ± 1.33</td>
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<td>90</td>
<td>68.26 ± 0.55</td>
<td>67.83 ± 1.26</td>
<td>67.24 ± 0.58</td>
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<td>135</td>
<td>68.26 ± 0.55</td>
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<td>66.91 ± 1.39</td>
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<td>68.26 ± 0.55</td>
<td>67.48 ± 0.83</td>
<td>66.57 ± 1.42</td>
</tr>
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</table>

Figure 1: TEM diagram of Albumin nanoparticle formulation, ANps5

Figure 2: TEM diagram of Albumin nanoparticle formulation, ANps5
Figure 3: Comparison of In-vitro drug Release of Pure drug (Terbutaline sulfate) and Albumin Nanoparticle formulations (ANps1, ANps2, ANps3, ANps4 and ANps5) in Phosphate Buffer Saline (PBS) pH- 7.4

REFERENCE
PULMONARY DRUG DELIVERY AS A VITAL ROUTE FOR DELIVERING NANOPARTICLES – A REVIEW

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3 School of Pharmaceutical Sciences, Shoolini University, Solan, Himachal Pradesh, India-173212.

ABSTRACT
The nanotechnology has rekindled interest in the lungs as a main route of drug delivery for both systemic and local treatments. The large alveolar surface area of lung coupled with the thin epithelial barrier and extensive vascularization might enhance drug transport and uptake. Such an avenue for drug delivery would greatly improve the treatment of pulmonary diseases such as asthma, chronic obstructive pulmonary diseases, lung cancer, tuberculosis and cystic fibrosis etc. The alteration of particle characteristics including size, surface chemistry, surface charge, and surface area, allows for the possibility of synthesizing particles specific for targeting to various cellular populations and organs including systems such as the respiratory tract. Pulmonary drug delivery is an area extensively investigated through the use of nanoparticles (particles that have a mean diameter of 300 nm or less). Polymeric nanoparticulate formulations have many advantages over traditional dosage forms which include enhanced dissolution properties and vast potential for intracellular drug delivery. It is analysed that in the near future pulmonary diseases may be treated effectively and efficiently using inhalable therapeutic agents loaded nanoparticles. Such an approach might minimize drug related systemic side effects. After identification of the various modes of interaction, it will be possible to control and minimize such toxicological effects allowing for nanoparticles to be safely utilized as therapeutic agents for site-specific drug delivery.

Keywords: Nanoparticles, pulmonary drug delivery, aerosol, alveoli, bronchioles, inhalation.
INTRODUCTION
The pulmonary route has recently gained importance as a suitable target for drug delivery. The large alveolar surface area, the thin epithelial barrier and extensive vascularization have the tendency to enhance drug transport and uptake. Pulmonary drug delivery is a non-invasive, non-systemic delivery approach to directly target disorders of lung (such as asthma, chronic obstructive pulmonary diseases (COPD), emphysema, cystic fibrosis, lung cancer, tuberculosis, pulmonary hypertension and diabetes etc) for both systemic and local drugs. It offers several advantages\(^1\) over oral, intranasal and transdermal delivery systems such as large absorptive surface area with extensive vasculature, easily permeable membrane, immense capacity for solute exchange due to thinness of the alveolar epithelium, low extra cellular and intracellular enzymatic activity, rapid and predictable onset of action with minimum side effects, reduced costs due to need of low dose, avoid first pass metabolism and improved patience compliance\(^2\).

![Diagram of the human respiratory tract](image_url)

**Figure 1: Different regions of the human respiratory tract**

The human lung (figure-1) consists of two functional parts, the airways (trachea, bronchi and bronchioles) and the alveoli (gas exchange areas). It contains about 2300 km of airways and 500 million alveoli. The surface area of the human lungs is estimated to be approximately 75-140 m\(^2\) in adults\(^3\). The pseudostratified epithelia that constitute the barrier to absorption into the blood-stream are markedly different in airways and alveoli of the lungs. The airways are
composed of a gradually thinning columnar epithelium, with the bronchial epithelium of 3-5 mm and bronchiolar epithelium of 0.5 – 1 mm in thickness. In the tracheobronchial region the epithelium is protected by a mucous layer. Any particle deposited in this area is transported away from the lung by mucociliary clearance or diffuse through the thick mucus to reach the epithelium cells. In contrast, the alveoli have a thin, single cell layer. The distance from the air in the alveolar lumen to the capillary blood flow is less than 400 nm. The large surface area of the alveoli and the intimate air-blood contact in this region make the alveoli less protected against inhaled substances, such as nanoparticles as compared to the airways. The pulmonary drug delivery system is based on the principle of aerosolization. Aerosols containing a uniformly sized particle matrix, which carry a drug or a single drug-containing matrix, may provide uniform dose delivery and drug release kinetics. Pulmonary lung targeting finds applications in drug delivery to (1) lung itself (i.e., local delivery) (2) other body organs after dissolution and subsequent absorption into blood circulation following transfer across alveolar membranes (i.e., Systemic delivery) (3) metabolism by alveolar macrophages. To generate aerosols containing nanometer-sized dry particles from suspensions, there are two methodologies (airjet atomization and electrohydrodynamic atomization). In air jet atomization, atomizer is used to aerosolize suspensions and the generation of nanometer sized dry particles results from the evaporation of drops. Electrohydrodynamic atomization (EHDA) allows control or tuning of the droplet size, through control of conductivity of the liquid. Various advantages of aerosol administration are
(i) Rapid drug absorption into the systemic circulation
(ii) Higher bioavailability than other non-invasive route of administration because of delivery of peptides and proteins as compared to oral administration and for many small molecules where first-pass metabolism limit oral bioavailability.

Inhalation devices for pulmonary drug delivery are of three types which as follows
A. Meter dose inhalers
B. Dry powder inhalers
C. Nebulizers

A. Meter dose inhalers: A metered dose inhaler (MDI) (Figure-2) is a complex system designed to provide a fine mist of medicament, generally with an aerodynamic particle size of 2.5 µm in the breath flow.
less than 5 microns, for inhalation directly to the airways for the treatment of respiratory diseases such as asthma and COPD.\textsuperscript{8}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{meter_dose_inhalers.png}
\caption{Meter dose inhalers}
\end{figure}

B. \textbf{Dry powder inhalers:} Dry powder inhalers (DPI) (Figure-3) are frequently highly soluble and quickly dissolve in the fluid layer lining the surface of the deep lung before passing through the thin cytoplasm of the type I alveolar cells, the interstitial space and capillary endothelium. The main advantages of dry powder systems include product and formulation stability, the potential for delivering a low or high mass of drug per puff, low susceptibility to microbial growth, and applicability to both soluble and insoluble drugs. Current challenges facing the development of these systems for macromolecules involve moisture control, efficient powder manufacturing, reproducible powder filling, unit dose packaging and development of efficient reliable aerosol dispersion and delivery devices.\textsuperscript{8,9} There are two types of devices

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{dry_powder_inhalers.png}
\caption{Dry powder Inhalers}
\end{figure}

i. \textbf{Unit-Dose:} Devices Single-dose powder inhalers are devices in which a powder contained capsule is placed in a holder. The capsule is made to open within the device and the powder is inhaled.

ii. \textbf{Multi-dose Devices:} Multi-dose device uses a circular disk that contains either four or eight powder doses on a single disk. The doses are stored in separate aluminum blister reservoirs until just before inspiration.
C. Nebulizers: There are two types of nebulizer systems, the ultrasonic and the air jet. In ultrasonic nebulizers, ultrasound waves are generated in an ultrasonic nebulizer chamber by a ceramic piezoelectric crystal that vibrates when electrically excited. These set up high energy waves in the solution, within the device chamber, of a precise frequency that generates an aerosol cloud at the solution surface. The aerosol produced by an air jet nebulizer is generated when compressed air is forced through an orifice; an area of low pressure is formed where the air jet exists. Nebulizers (Figure-4) are particularly useful for the treatment of hospitalized or non-ambulatory patients.

![Figure 4: Air jet nebulizer](image)

Many efforts have been focused for specific delivery of drugs to the target tissues by the development of nanoparticles, solid lipid nanoparticles, liposomes, nanoemulsions, or dendrimers. Nanoparticles are particulate dispersions with a size in the range of 1-1000 nm and the drug is dissolved, entrapped, encapsulated or attached to nanoparticle matrix. In drug delivery system, the roles of polymeric nanoparticles are to carry the drug molecules, to protect drugs from degradation, and to control drug release. Polymeric nanoparticles used therapeutically are composed of biodegradable or biocompatible materials, such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), algic acid, gelatin and chitosan. Nanoparticles as a drug delivery system have several advantages:

a) An achievement of enhanced solubility of the drug than its own aqueous solubility

b) Site-specific targeting can be carried out by attaching targeting ligands to surface of particles or use of magnetic guidance.
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c) Sustain release of the drug at the time of transportation and at the site of localization to obtain increase in drug therapeutic efficacy and reduction in side effects by changing distribution of the drug in organ and clearance of the drug.

d) Increased potential of drug internalization by cells.

e) Particles degradation and controlled release characteristics can be easily modulated by the choice of matrix constituents.

f) Drug loading is high and drugs can be incorporated into the systems without any chemical reaction which helps in preserving the drug activity.

g) Improved patient compliance.

CAPABILITY OF NANOPARTICLES TO TARGET LUNG TISSUES

(A) Mechanism of deposition of nanoparticles in the respiratory tract: Size particularly is an important determinant of whether or not nanoparticles will be efficiently deposited deep into the lungs or if they will simply be exhaled. The sizes of particles which are used for inhalation therapy are usually expressed in terms of the mass median aerodynamic diameter (MMAD)\(^{[12]}\). The aerodynamic diameter is usually defined as the diameter of a sphere of unit density, which reaches the same velocity in the air stream as a non-spherical particle of arbitrary density. This diameter defines the mechanism of particle deposition in the respiratory system\(^{[13]}\). In general, aerosol particle size is considered to be the mass median aerodynamic diameter (MMAD). The MMAD is used to explain the particle size distribution of any aerosol statistically based on the weight and size of the particles. Thus, a group of very dense particles will exhibit a larger MMAD than that of a group of less dense particles, despite an identical geometric size\(^{[14]}\). The criterion for successful deposition requires that the particles must be small enough to avoid deposition by impaction in the upper respiratory tract and enable them to pass through the mouth, larynx, pharynx, and lower airways while simultaneously being large enough (or having sufficient inertia) to avoid exhalation\(^{[1]}\). Consequently, particle size and density reflected in the MMAD of a particle are important characteristics for lung delivery. The nanoparticle diameter ranging from 30-50 nm and 1-3 \(\mu\)m have found to exhibit high deposition in pulmonary region\(^{[13]}\). Different studies have shown that particles containing diameters ranging from 100–500 nm might be successfully deposited into various regions within the respiratory tract when they are incorporated into suitable vehicles such as aerosols or dry powders\(^{[10,13]}\).

Particle deposition occurs via one of the following principal mechanisms(Figure-5):

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1) Impaction: Each time the airflow changes due to a bifurcation in the airways, the suspended particles tend to travel along their original path due to inertia and may impact on an airway surface. This mechanism is highly dependent on aerodynamic diameter, since the stopping distance for very small particles is quite low. Impaction occurs mostly in the case of larger particles that are very close to airway walls, near the first airway bifurcations. Therefore, deposition by impaction is greatest in the bronchial region. Impaction accounts for the majority of particle deposition on a mass basis\textsuperscript{[16]}.

2) Sedimentation: Sedimentation is the settling out of particles in the smaller airways of the bronchioles and alveoli, where the air flow is low and airway dimensions are small. The rate of sedimentation is dependent on the terminal settling velocity of the particles, so sedimentation plays a greater role in the deposition of particles with larger aerodynamic diameters. Hygroscopic particles may grow in size as they pass through the warm, humid air passages, thus increasing the probability of deposition by sedimentation\textsuperscript{[16]}.

3) Interception: Interception occurs when a particle contacts an airway surface due to its physical size or shape. Unlike impaction, particles that are deposited by interception do not deviate from their air streamlines. Interception is most likely to occur in small airways or when the air streamline is close to an airway wall. Interception is most essential for fibers, which easily contact airway surfaces do to their length. Furthermore, fibers have small aerodynamic diameters relative to their size, so they can often reach the smallest airways\textsuperscript{[16]}.

4) Diffusion: Diffusion is the primary mechanism of deposition for particles less than 0.5 microns in diameter and is governed by geometric rather than aerodynamic size. Diffusion is the net transport of particles from a region of high concentration to a region of lower concentration due to Brownian motion. Brownian motion is the random wiggling motion of a particle due to the constant bombardment of air molecules\textsuperscript{[16]}. Breath holding is essentially for deposition of nanometer particles where the mechanism of deposition is predominantly diffusion\textsuperscript{[17]}.

5) Electrostatic precipitation: Electrification normally occurs in aerosol generating processes. The unipolar charge carried by the particle may have a significant effect on the deposition efficiency of particles in the lungs during breathing. The deposition of the particles in the lungs shows a relatively small fractional deposition in the lungs under same conditions when condensation aerosols are used. Since, the condensation, aerosols are free of electric
charges, the difference in fractional deposition may be caused by the additional deposition from electrostatic forces. This type of effect is quite significant for submicron and micron particles carrying unipolar charge of magnitude of hundred electrons. The observed changes are caused primarily by electrostatic precipitation resulting from the image force between the particle and the wall\textsuperscript{[18]}

Figure 5: Deposition mechanisms of particles

The area in the respiratory tract for where the deposition of nanoparticles will occur primarily depends on their size\textsuperscript{[3]}. 1 nm particles deposition will primarily occur within the upper airways including the nose, pharynx, and larynx\textsuperscript{[3]}. Optimal deposition into the tracheal and bronchi regions requires the use of 5 nm particles and 20 nm particles are optimal for deposition into the deeper alveolar regions of the lungs\textsuperscript{[19]}. The inertial impaction occurs during the passage through the oropharynx and large conducting airways if the particles possess a mass median aerodynamic diameter (MMAD) more than 5 μm. When the MMAD of particles ranges from 1 to 5 μm, they are subjected to sedimentation by gravitational force that occurs in smaller airways and respiratory bronchioles. Sedimentation is influenced by breath holding. Particles with a MMAD of less than or equal to 0.5 μm, they are deposited significantly by diffusion, based on the Brownian motion\textsuperscript{[4]}

(B) Interaction of nanoparticles with lung epithelia and macrophages

Depending upon the location of deposition, the nanoparticles will interact with specific cell population within the lungs (Figure – 6). Ciliated epithelial cells as well as type I and II pneumocytes are the primary cells within the deep respiratory tract that interact with
nanoparticles\textsuperscript{[19]}. However, the mechanisms of interaction are not well known and very little exists in the literature.

\begin{center}
\includegraphics[width=\textwidth]{figure6.png}
\end{center}

\textbf{Figure 6: Interaction of nanoparticles with lung epithelia and macrophages}

Receptor-mediated endocytosis is the mechanism most likely responsible for the intracellular uptake of the nanoparticles whereby opsonins (such as proteins, glycoproteins, and glycolipids) precipitate onto the particle surface forming a complex recognizable by receptors of macrophages which then may bind to the complex with their cell surface allowing for particle uptake through pseudopod extensions. Phagosomes then fuse with lysosomes (containing acid hydrolases) which have the ability to degrade the drug delivery vehicle. During this process, however, the drug itself as well as the particle may be destroyed by the action of enzymes and thus the nanoparticles must first emigrate the lysosomes in order to retain activity. Nanoparticle delivery to the alveolar region allows drug targeting to the alveolar macrophage population and has important magnitude for treating diseases which involve or are caused by these immune cells. Furthermore, nanoparticles have potential to be used for delivery of antigens and DNA and may be important for vaccine delivery through the respiratory route\textsuperscript{[20]}. 

Macrophages are central in defending the lungs against the assaults of particles and pathogens in inspired air. Particles are not only ingested but undergo gradual dissolution within the phagolysosomes of macrophages. The phagocytic and microbicidal potential of macrophages is one of the major reasons to keep lungs remain clean and sterile. Macrophages
may also prevent allergy by ingesting and catabolizing inhaled foreign particles. During lung infections macrophages may preserve and present antigens to lymphocytes and act cooperatively with other components of the immune system to enhance the immune response. Lung macrophages recognize and destroy neoplastic cells, thus preventing the development of cancer\cite{21}. Macrophages can secrete such diverse substances as lysosomal enzymes, interferon, components of complement, angiogenesis factor, plasminogen activator, cyclic nucleotides, leukotrienes, prostaglandins, inflammatory cytokines, and granulopoietins. Diverse agents such as viruses, silica, immunosuppressives, ethanol intoxication, cigarette smoke, air pollution, hypoxia, and hyperoxia can depress the ability of pulmonary macrophages to protect their host. There are also situations in which pulmonary macrophages not only fail but are themselves implicated in the pathogenesis of pulmonary diseases. For example, the ingestion of particles (e.g., cigarette smoke), microbes, or endotoxin causes the release of lysosomal enzymes and oxygen radicals into the macrophage cytoplasm or the external environment. These substances may damage surrounding cells or other macrophages; then dead or dying macrophages release substances that can attract fibroblasts and elicit fibrogenic responses. This extracellular release of proteases and oxygen radicals can also alter the extracellular matrix or the activity of a variety of enzymes, then macrophages may be centrally involved in the development of lung disease\cite{21}.

\textbf{(c) Interaction of nanoparticles with lung surfactant:} Once nanoparticles are deposited onto the lining of the respiratory tract, they first contact the mucous layer within the airways or the surfactant – lining fluid layer within the alveolar region (Figure-7). Airway mucous (about 5 \textmu m in depth) is a complex aqueous secretion of airways, comprising electrolytes, proteins, glycoproteins (mucins) and debris of cells. The components vary much depending on environmental and disease states. The surfactant lining layer (10-20 nm in thickness) that covers the alveolar surface is composed of 90% in weight of phospholipids and 10% in weight of specific proteins\cite{22}. Both airways and alveolar surface liquids are coated with at least a monolayer of highly surface active lung surfactant, which are primarily water insoluble long –chain phospholipids. They form liquid crystals but not micelles in aqueous media to maintain the functions of the lungs such as facilitation of gas exchange and prevention of alveoli collapse by reducing the lung air interface surface tension\cite{23}. 

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Figure 7: Synthesis and secretion of surfactant which coats the alveolar surface of the air

Water interphase.
An analysis of the interaction between pulmonary surfactant and nanoparticles is of utmost importance as dire consequences would result if such drug delivery systems destabilize the surfactant film coating the alveoli\(^{24}\). Accordingly, many surfactant film studies are performed using a Langmuir Blodgett trough which allows one to mimic the physiological situation present within the respiratory tract\(^{18}\). A recent study by Stuart et al. investigated the interaction of different nanoparticles with the lung surfactant film\(^{24}\). Accordingly, the interaction between nanoparticles and dipalmitoylphosphatidylycholine (DPPC) a major component of native pulmonary surfactant was investigated\(^{18}\). A DPPC lipid monolayer was used to simulate the surfactant layer of the respiratory tract\(^{18,24}\). The aim of this study was to investigate if the deposition of nanoparticles in the alveolar region will compromise the integrity of the surfactant film. Such interactions might cause dosage form related incompatibilities and are important for the preclinical evaluation of the feasibility of nanoparticle delivery of nanomedical devices. The incorporation of the particles into the lipid film was size dependent and had a measurable impact on the surface tension of the lipid layer. However, the study also showed that nanoparticles do not significantly destabilize the surfactant film. The method outlined in this study might be a suitable test to set limits for nanoparticle deposition and to evaluate dosage form related nanotoxicological properties of inhalable nanoscaled drug delivery systems. In another study, nanoparticles comprised of \(\text{DO}_{-\infty}-\text{Tocopheryl polyethylene glycol} 1000 \text{ succinate (TPGS)}\) as well as other biodegradable substances as carrier matrix were investigated. TPGS is also known to stabilize pulmonary surfactant thereby making its use in pulmonary drug delivery more attractive. The study also
concluded that the TPGS coated nanoparticles do not destabilize the model surfactant film and have great potential for pulmonary drug delivery\cite{13}.

(d) **Retention and clearance mechanisms of nanoparticles deposited within the respiratory tract:** The nanoparticles must first overcome the clearance and defense mechanisms associated with the pulmonary route for effective deposition. The particles must have the ability to escape the mucociliary clearance within the upper bronchi-tracheal region in order to reach the epithelial cells\cite{10}. The primary function of the mucociliary clearance is to trap inhaled dust particles removing them from the respiratory tract and involves ciliated epithelial cells located within the upper airway (bronchi and tracheal regions). The cells cover between 30–65% of the airway epithelium and each of the epithelial cells bear approximately 200 cilia on their surface, greatly enhancing particle elimination\cite{3}.

These ciliated epithelial cells are surrounded by periciliary fluid which includes a mucous Layer\cite{3} and this fluid enhances the mucociliary clearance by effectively trapping particles. The mucus is pushed towards the glottis by the mucociliary clearance and from here the particles will either be removed via excretion through the mouth or through the gastrointestinal tract. If particles are able to enter the lower airways such as alveoli, then the particles will be cleared through the action of alveolar macrophages. They reside within the surfactant monolayer \cite{3} and nanoparticles are able to interact with them as mentioned earlier\cite{3}. Upon activation the alveolar macrophages release immunological response mediators such as cytokines and chemokines\cite{25} which ultimately allow for phagocytosis and particle elimination. The subsequent pulmonary inflammation caused by such mediators is however disadvantageous as a result of the adverse health effects and toxicity effects observed with the use of ultra fine particles. The inflammation can lead to oxidative stress due to depletion of alveolar anti-oxidants. Pulmonary surfactant also plays a vital role in protecting and eliminating inhaled particles and experiments have shown that the surfactant plays a role in the particle clearance upon expiration. During expiration, a pressure gradient is established which facilitates particle elimination where the high surface tension within the trachea and bronchi (in comparison to the alveoli) forces particles towards the surfactant, facilitating their elimination. Alveolar clearance is instigated as the surfactant film promotes the deposition of these particles within the aqueous region where the resident macrophages await. Once deposited within the lung lining fluid, there are separate biokinetics for lung absorption and non-absorptive clearances. The kinetics of dissolution of inhaled particulates
determines whether the inhaled nanomaterials will dissolve in the epithelial lining fluid for lung absorption or whether such nanomaterials will undergo non-absorptive clearances\textsuperscript{26}. The kinetics of diffusion in the alveoli is much faster than that in the small airways, mainly because lung absorption mostly occurs from the air-side surface of the alveoli to the pulmonary capillaries. The alveoli has thin monolayer (0.1 – 0.4μm) composed of extremely broad and thin type I cells and small compact type II cells, and a large surface area (more than 100 m\textsuperscript{2}). only a small portion of inhaled nanoparticles is absorbed from the tracheobronchial airways which have a much thicker layer of column-shaped epithelial cells (10-60 μm) and lower surface area (1-2 m\textsuperscript{2}). This is supported by Fick’s law. Low molecular weight hydrophilic molecules can be absorbed by the active transport via specific transporters or by passing through the tight junctions. The kinetics of active absorption should depend upon the lung regional expression and functionality of receptors or transporters\textsuperscript{27}.

Inhaled nanomaterials that are insoluble in mucus and lining fluid are not able to be rapidly absorbed and may undergo physical translocation. This is different depending on the lung region in which the nanoparticles have been deposited. Immersion of the inhaled, slowly dissolving or insoluble nanomaterials in the fluid lining the lungs may enable them to be closely associated with epithelial cells and cells of the host-defence system for particle-cell interaction. Subsequently, several post-defense mechanisms, including the mucociliary escalator transport, phagocytosis by macrophages and endocytosis are involved in the removal of deposited nanoparticles and to maintain the lung mucosal surfaces\textsuperscript{28}.

The mucociliary escalator dominates clearance of nanoparticles from the upper airways. Nanoparticles that consist of slowly dissolving or insoluble materials in the airway mucus will be partly moved by action of the ciliated epithelial cells pushing the mucus along with the nanoparticles that deposited on the airway wall to the larynx, where they are swallowed to the gastro-intestinal tract or excreted through the mouth. The deposited nanoparticles may also be removed by coughing within 1-2 days\textsuperscript{4}.

Clearance of the slowly dissolving and insoluble nanoparticles from the alveoli is predominantly by macrophage phagocytosis and endocytosis. The airside surface of each of the 500 million alveoli in the human lung is routinely monitored by 12-14 alveolar macrophages in the lung lining fluid. The uptake of deposited particles by alveolar macrophages depends upon the particle size and composition of coating material. Particles of
1-3 μm in diameter are far better taken up than those of 6 μm by macrophages, which have cell diameters about 15-22 μm. Size of particles less than 0.26 μm can escape from the phagocytosis by macrophages. Due to the small size, the chance of nanoparticles undergoing phagocytosis in the alveoli is much lower than micron-sized particles. The remaining nanoparticles will interact with the non-phagocytic cells of the epithelium and the endocytic events are regulated by clathrin-coated pits and caveolae, as well as scavenger receptors. It has been suggested that caveolae and coated pits preferentially transport small and large particles, respectively, but this need to be further verified in vivo. Caveolae are indentations of the plasma membrane lined with caveolin-1, and are abundantly expressed on lung capillaries and type I alveolar cells. Macromolecules or particles of several nanometers in radii may be transported within caveolae from lung to blood.

PROCESSING METHODS OF NANOPARTICLE FOR PULMONARY DRUG FORMULATIONS
(1) Emulsion-Solvent Evaporation: In this method, the polymer is first dissolved in a water-immiscible, volatile, organic solvent such as chloroform, dichloromethane, or ethyl acetate. The drug is added to this polymer solution and the mixture is emulsified into an outer water phase containing an emulsifier, such as poly(vinyl alcohol) (PVA), gelatin, polysorbate 80, or poloxamer-188 to yield an o/w emulsion. To harden the nanoemulsion droplets into solid nanoparticles, the organic solvent is evaporated or extracted from the system after it diffuses into the external aqueous phase. Emulsification is facilitated by high-speed homogenization or sonication. For the removal of solvent, the stirring process may be continued for several hours at one of the challenges encountered in this method is the poor entrapment and burst release effect of moderately—water-soluble and hydrophilic drugs.

(2) Emulsification Solvent Diffusion Method: In this technique, the solvent and water are mutually saturated at room temperature before use to ensure the initial thermodynamic equilibrium of both liquids. Later, the organic solvent containing the dissolved polymer and the drug is emulsified in an aqueous surfactant solution (usually with PVA as a stabilizing agent) by using a high-speed homogenizer. Water is subsequently added under constant stirring to the o/w emulsion system, thus causing phase transformation and outward diffusion of the solvent from the internal phase, leading to the nanoprecipitation of the polymer and the formation of colloidal nanoparticles. Finally, the solvent can be eliminated by vacuum steam distillation or evaporation.
(3) Emulsion Polymerization: This method has been used to prepare poly (alkyl cyanoacrylate) nanoparticles with an approximate diameter of 200 nm. The alkyl cyanoacrylate monomer is dispersed in an aqueous acidic medium containing stabilizers such as dextrans and poloxamers. Surfactants such as polysorbates can be used as well. The low pH favors the formation of stable and high molecular mass nanoparticles. Under vigorous mechanical stirring, polymerization follows the anionic mechanism since it is initiated usually by nucleophilic initiators such as OH\(^{-}\), CH\(_3\)O\(^{-}\), and CH\(_3\)COO\(^{-}\) and proceeds at ambient temperature. The nonpolar ends within the interior of the surfactant micelles help solubilize the monomer. In the presence of water-soluble initiators, chain growth commences at the hydrophilic surface of the micelle. When the monomer in the interior of the micelle gets depleted, more monomer droplets from the exterior aqueous phase enter inside; thus, the polymerization reaction proceeds inward and continues until it is terminated by the free radicals. The drug can be solubilized in the polymerization medium either before the monomer is added or later when the reaction has ended. Finally, the nanoparticulate suspension is purified either by ultracentrifugation or by redispersing the nanoparticles in an isotonic medium. The various factors affecting the formation of particles, their size, and molecular mass include monomer concentration, stirring speed, surfactant/stabilizer type and concentration, and the pH of the polymerization medium\(^{[31]}\).

(4) Supercritical Fluid Technology: This technology is advantageous in that the use of an organic solvent/surfactant can be avoided or minimized, thus producing nanoparticles that are free from toxic impurities. Carbon dioxide is nontoxic, nonflammable, and environmentally acceptable, and supercritical CO\(_2\) can be easily obtained by pressurizing and heating the CO\(_2\) system to a minimum of 73.8 bars and 31.05\(^\circ\)C, respectively.

In the supercritical antisolvent method, both the drug and the polymer are dissolved in a suitable organic solvent and are atomized through a nozzle into supercritical CO\(_2\). The dispersed organic solvent phase and the antisolvent CO\(_2\) phase diffuse into each other and since CO\(_2\) is miscible only with the solvent, the solvent gets extracted causing the supercritical fluid–insoluble solid to precipitate as nanoparticles. The rates of two-way mass transfer are much faster than those for conventional organic antisolvents. When the density of CO\(_2\) decreases, the atomization of the spray is intensified, resulting in faster mass transfer rates associated with high surface area of the associated droplets, thus rapid nucleation and
smaller particle sizes. The dry, micronized powder is then collected following the depressurization of CO\textsubscript{2}[24].

In the rapid expansion of supercritical solutions technique, the solute is dissolved in supercritical CO\textsubscript{2} and this solution is atomized through a nozzle into a collection chamber at atmospheric conditions. When expanded, CO\textsubscript{2} immediately evaporates and the solute precipitates as a coprecipitate of the drug embedded in the polymer matrix. Various parameters that affect the resulting particle size and morphology are the pre- and postexpansion temperature and pressure, nozzle geometry, and solution concentration. The disadvantages of this method include the use of higher temperatures to form homogenous precipitates (thus degrading thermally labile drugs) and the limited solubility of the polymers and drugs that result in low drug loading[35].

(5) Phase Separation in Aqueous System: This technique depends on the precipitation of the drug-entrapping polymer either by the addition of a third compound to the polymer solution or by some other physical means. The point has to be reached where two liquid phases are formed, the polymer-rich coacervate and the supernatant liquid phase, which is depleted in the polymer. Briefly, two steps are involved in the process: (i) the formation of liquid droplets of the polymer from the complete solution phase, which depends on the solubility parameters of the polymer, and (ii) subsequent hardening of the polymer droplets due to extraction or evaporation of the polymer solvent. A number of organic solvents, such as dichloromethane, isopropanol, and heptanes, have been used as solvent, coacervating agent, and hardening agent. If a drug is initially dispersed in the polymer solution, it can be coated by the coacervate. Phase separation could occur as a result of changes in pH or counterions or as a result of the aqueous phase acting as a nonsolvent for the polymer. Both hydrophilic and hydrophobic drugs can be entrapped. The main advantage of phase-separation method is that it protects active drugs from partitioning out into the dispersed phase[35].

(5) Spray Freeze Drying: A typical Spray Freeze Drying (SFD) technique involves the atomization of an aqueous drug solution via a two-fluid or an ultrasonic nozzle into a spray chamber filled with a cryogenic liquid (liquid nitrogen) or halocarbon refrigerant such as chlorofluorocarbon or fluorocarbon. The spraying process can be performed beneath (spray-freezing into liquid) or above the surface of the cryogenic liquid, depending on the position of nozzle. It is also possible to use a nozzle arrangement for introducing liquid nitrogen directly into the spraying solution although the application of such a method for inhaled particles has
not been discussed. Since the level of the cryogenic liquid will inevitably drop due to evaporation, continuous addition of fresh cryogenic liquid is required, especially when a lengthy atomization process or a large spray volume is used. Upon contact with the cryogenic medium, the liquid droplets solidify rapidly (in milliseconds time scale) because of the high heat-transfer rate. Stiring of cryogenic liquid may be required to prevent the possible aggregation of newly formed frozen particles. Once the spraying process is completed, the whole content can be lyophilized, as with conventional freeze-drying\cite{37}.

**TOXICITY OF INHALED NANO PARTICLES**

With respect to the pulmonary route of drug delivery, toxicologists are primarily interested in the mechanisms by which smaller sized inhaled nanoparticles can cause lung injury and inflammation\cite{38}. When comparing the surface area of larger sized particles and very small sized nanoparticles the surface area of the smaller sized nanoparticles has been shown to promote interactions with biological systems leading to plausible negative effects\cite{38}. Specifically, it has been shown that nanoparticles have a larger inflammatory potential per unit mass compared to particles of larger sizes within rat lungs, however experiments pertinent to the toxicity of nanoparticles have mainly been investigated in vitro and only few in vivo studies are found in the literature\cite{28}. The primary means by which nanoparticles induce toxicological effects is through the production of reactive oxygen species thereby causing oxidative stress within the biological system. To fully explain the role of oxidative stress this process in pulmonary toxicity, the hierarchical oxidative stress model has been proposed\cite{38}. According to this model, reactive oxygen species (ROS) are produced as by-products during normal cellular respiration. Cells contain sufficient antioxidants such as glutathione and antioxidant enzymes and are thus able to neutralize these reactive species. In the case of severe lung injury (which might also be caused by inspiration of very small nanoparticles), the amount of ROS is greatly increased and consequently the cells do not contain enough antioxidants. This results in the accumulation of reactive molecules such as oxidized glutathione within the cells. Production of these ROS is also known to increase within the mitochondria in the presence of nanoparticles. An accumulation of oxide ions (O$_2^-$) is believed to be the consequence of particles becoming stalled within the mitochondria, hindering the electron transport chain and subsequent energy production. In such a situation, cells are able to detect the low ratio of glutathione to oxidized glutathione and subsequently induce inflammation\cite{38}.
Experiments were conducted by Oberdörster et al. (Oberdörster, 1995) displaying the importance of the effect of surface chemistry on particle toxicity. An in vivo study was performed by exposing rats to polytetrafluoroethylene (PTFE) fumes which were heated to 480 °C. The application of heat resulted in the formation of PTFE nanoparticles with a median diameter of 18 nm[39]. It was observed that inhalation of these fumes caused severe acute lung injury. Furthermore, high rates of mortality were found within four hours post inhalation. A subsequent study by Oberdörster explored the impact of inhaled ultrafine and fine titanium oxide (TiO2) particles in the lungs of rats and mice. Introduction of ultrafine (20 nm) TiO2 particles within the respiratory tract resulted in an exaggerated inflammatory response, determined by the increased number of neutrophils present within the lungs after 24 hours. Interestingly, exposure of the animals to an equivalent dose of fine sized TiO2 particles (250 nm) did not result in the exaggerated immune response that was observed with the ultrafine TiO2 particles thus emphasizing the importance of particle size for toxicity. Further data analysis revealed that the response elicited by both ultrafine and fine TiO2 particles was similar upon comparing their surface areas (rather than mass) to the percent of neutrophils found within the lungs. The results of the study served to indicate the notion that surface area is directly correlated with toxicity and may be employed to predict pulmonary toxicity[38].

APPLICATIONS OF PULMONARY NANO PARTICLES

Due to rapid advances in nanotechnology and biotechnology, nanoparticles have been considered as an effective form for delivery of the new generation of proteins and gene based macromolecular therapeutic agents into the body, since many of the components of living cells are constructed at the nano-level, such as ribosomes, membrane transporters, receptors and cell signaling systems. Nanoparticles fall in the same size range of the biological entities; therefore they can readily interact with molecules on both the cell surface and within the cell. Drugs that are deposited within the lungs in the nanoparticulate form have a greater chance to escape from the clearance mechanisms by the lung defense systems, compared to microparticulate form. Thus, drug bearing nanoparticles have the potential to deliver the drugs efficiently to the epithelium, while avoiding unwanted mucociliary clearance. Nanoparticles are useful to deliver water-insoluble drugs. Despite high potency, the effectiveness of water insoluble drugs can be severely limited because the solubility is too low to reach therapeutic systemic concentrations. However when their size is reduced to nano-level, the increased particle surface to volume ratio helps to enhance solubility and dissolution rate in an aqueous environment.
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environment. Nanoparticulate forms of drug could have an enormous benefit by significantly improving systemic bioavailability and allowing a more rapid onset of therapeutic action. The therapeutic applications of nanoparticles in respiratory and systemic diseases are numerous. Recent research has been focused on determining nanoparticles of different types to serve as vectors for the pulmonary drugs or genes delivery through inhalation or systemic administration, whereas various efforts have been undertaken for developing and delivering drug particles of nano-sized to the lung. Most of the studies reported have focus on the use of these strategies for the treatment of pulmonary infection. For example, transfer of gene using intranasal administration of chitosan-DNA nanospheres was shown to inhibit respiratory syncytial virus infection and to reduce allergic inflammation of airway in mice when given prophylactically or therapeutically. Moreover, nanoparticle-mediated intranasal delivery of short interfering RNA (siRNA) is required to target against a specific viral gene, NS1 which has also been shown to inhibit respiratory infection of syncytial virus in mice and rats. The significance of nano-sized drug particles for treatment of pulmonary infection has also been investigated. Aerosolized nano-sized itraconazole as inhalation resulted in significantly high lung concentrations, prophylactically inhibit invasive pulmonary aspergillosis and reduce infection-related deaths in mice, whereas oral drug administration did not. Pandey et al.\textsuperscript{[41]} demonstrated that a single inhalation of aerosolized poly (DL-lactide-co-glycolide) nanoparticles loaded with antitubercular drugs (isoniazid, rifampicin, or pyrazinamide) provided therapeutic plasma drug concentration for up to 6 days in guinea pigs and investigated that repeated inhalations were almost similar to oral administrations of free drug for treatment of experimental tuberculosis.

Anti-tubercular drugs have been successfully entrapped and delivered in biodegradable and biocompatible polymers. Zahoor et al.\textsuperscript{[42]} have developed inhalable alginate nano-particles as antitubercular drug carriers against experimental tuberculosis. The relative bioavailability of all drugs from the formulation have found significantly higher compared with oral free drugs when tested in guinea pigs. In another study, Justo et al.\textsuperscript{[43]} prepared the kanamycin-loaded lipid vesicles by ethanol injection method for administration by inhalation route. The selected drug was indicated for multiresistant tuberculosis, and administration through inhalation allows both local delivery of the drug to the lungs and systemic therapy. In a study by Garcia-Contreras et al.\textsuperscript{[44]} reported systemic delivery of insulin administered by the pulmonary route. The insulin formulations were administered by intratracheal instillation, spray instillation, and subcutaneous route. The plasma concentration of insulin and glucose were determined.
and pharmacokinetic analysis suggested that the drug had longer mean residence time when administered to the lungs of Sprague-Dawley rats. Glucocorticoids such as budesonide, triamcinolone acetonide, and fluticasone, have a high degree of hepatic first-pass inactivation of the swallowed fraction of the inhaled dose, whereas there is no evidence of first-pass metabolism of these drugs in lung and when administered by inhalation are effective and widely used as anti-inflammatory agents of patient with asthma allergic rhinitis and advanced chronic obstructive pulmonary disease.

CONCLUSIONS
The pulmonary route of drug delivery has several advantages when compared to other routes for drug administration and benefits include a large surface area, extensive vascularization, avoiding first pass metabolism, and presence of a thin epithelial barrier. Research within the pharmaceutical industry towards the pulmonary route for drug delivery is gaining momentum and persistently being investigated for site-specific drug delivery of inhaled particles. Nanomedicine is gaining interest within the drug delivery field. Through the use of nanoparticles (particles with a mean diameter below 300 nm) it is expected that there will be improvements in site-specific drug delivery which will minimize side-effects resulting from the use of nonspecific drug carriers. Deposition of drugs to specific areas within the lungs may be enhanced and improved through the use of appropriate sized nanoparticles and may prove instrumental for treatment of pulmonary conditions such as asthma, chronic obstructive pulmonary diseases, lung cancer, tuberculosis and cystic fibrosis etc. With the perfection of pulmonary nanoparticle drug formulations, the lungs may become a preferred route of drug delivery for many local and systemic therapeutic agents.

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