Formulation and Evaluation of Terbutaline Sulphate Loaded Inhalation Nanoparticles for Pulmonary Drug Delivery

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ABSTRACT

The main aim of present research work is to design Terbutaline sulphate loaded inhalation nanoparticles employing eudrgit RSPO and poloxamer 188 as polymers and their effect on various physico-chemical parameters were studied. Terbutaline sulphate nanoparticles were prepared using double emulsion solvent evaporation technique by employing different concentrations of eudragit RSPO polymer. Drug was uniformly mixed and loaded in the polymeric solution. Formulated nanoparticles were studied for parameters such as Particle size, % entrapment efficiency, swelling index and *in-vitro* drug release studies. FT-IR analysis did not indicate any interaction of drug with the polymer. Encapsulation efficiency, particle size and drug release parameters varied considerably with change in polymer concentrations. However, appreciable particle size and % entrapment efficiency were observed with 1% eudragit concentration and significant sustained action of nanoparticles was produced with 0.2% polymer. With solvent evaporation technique, we successfully synthesized and evaluated sustained release inhalation nanoparticles with poloxamer 188 and eudragit RSPO as polymers.

Keywords: Terbutaline sulphate, double emulsion, inhalation nanoparticles, pulmonary, drug delivery, solvent evaporation.

INTRODUCTION

Pulmonary drug delivery has become an interesting approach and gained lot of importance as well in targeting lungs. Pulmonary drug delivery system is defined as the delivery system where the drug is directly delivered to lungs for treating lung disorder.[1] This route offers many advantages over other routes because of its high vascular nature, large surface area, avoidance or circumvention of first pass effect etc.[2] Usually inhalation nanoparticles are administered through intravenous route. But for the treatment of diseases localized in the respiratory tract or respiratory system, pulmonary delivery via inhalation becomes prospective, nanoparticles cannot be delivered efficiently via pulmonary route due to their poor aerolization properties. Even after inhalation nanoparticles exhibit ineffective deposition in the alveolar region of lungs. Hence some additional polymers or vehicles will be required that enhance the delivery of nanoparticles to the target site.[3]

Pulmonary drug delivery is an a non-invasive, non-systemic delivery approach for both local and systemic drugs and a method to directly target disorders of lungs such as asthma, chronic obstructive pulmonary diseases (COPD), emphysema, cystic fibrosis, lung cancer, tuberculosis, pulmonary hypertension and diabetes.

The main principle of pulmonary drug delivery system is aerolization which means reduction in the drug particle size delivered to alveoli and bronchioles of the lungs.[4]

Inhalation Nanoparticles

Nanotechnology is the science of small and tiny molecules. Pharmaceutical nanoparticles may be defined as the solid and sub-micron sized drug carriers that are less than 100nm in diameter. The main aim behind the synthesis of nanoparticles is to control the drug release from the formulations by altering the pharmacokinetic properties to enhance pharmacodynamic properties of drugs at the target site.[5,6] Nanoparticulate formulations include biodegradable and biocompatible polymers in their formulations as they play a vital role. In case of inhalation nanoparticles, Polymers used should fulfill the following delivery requirements. Firstly, Polymers for pulmonary drug delivery should degrade within a short period of time. If not, polymers accumulate in the lungs upon repeated doses and may cause toxicity. For this purpose poly vinyl alcohols grafted with short PLGA chains can be used. Such a polymer degrades rapidly without any signs of toxicity. Ether-anhydride terpolymers with sebacic acid, poly ethylene glycol and 1, 3-bis (carboxyphenoxy) propane, are the other polymers with faster degradation rates that suits pulmonary application and form aerolizable particles.[7]

Terbutaline sulphate, an anti-asthmatic drug used to target lung locally. Terbutaline causes bronco dilation by direct stimulation of beta-2 adrenergic receptors present in bronchial smooth muscles. Terbutaline is variably absorbed from the gastro intestinal tract and about 60% of the absorbed dose undergoes first-pass metabolism by sulfate conjugation in the liver and the gut wall, so as to avoid first-pass metabolism[8] it is delivered via pulmonary

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route and sustained release of the drug can be achieved by incorporating them in the polymeric nanoparticles.[9]

MATERIALS AND METHODS

All chemicals used were either analytical or pharmaceutical grades, such as Terbutaline sulphate was procured from Yarrow chem. products Mumbai, eudragit RSPO and poloxamer 188 were obtained from Yarrow chem. products Mumbai, Span80 from Finar chemicals Ahamadabad, dichloro methane and ethanol from Tarun scientifics.

Preparation of Inhalation Nanoparticles

Terbutaline sulphate (TBS) loaded Inhalation nanoparticles (INPs) were prepared by using double emulsion solvent evaporation technique. Various formulations were prepared to know the effect of polymer and surfactants concentration and were assigned formulation code F1 to F7 as shown in Table 1. Firstly polymeric solution is prepared by dissolving specific amount of eudragit RSPO in 10 ml of organic mixture consisting of span80 (2%w/v) dissolved in dichloromethane. Above obtained solution is emulsified by the drop wise addition of aqueous drug solution under magnetic stirring at 1000-1200 rpm for 15 min to get primary W/O emulsion. Further this is added to 10 ml distilled water containing poloxamer188 under stirring for 10 min to achieve a stable W/O/W double emulsion. The W/O/W emulsion is separated by ultracentrifugation at 11000 rpm for 40 min. Finally the obtained product is freeze dried or lyophilized which leads to the formation of nanoparticles.[10,11]

Table 1: Composition of Formulations of Inhalation Nanoparticles

S. No.	Ingredients	F1	F2	F3	F4	F5	F6	F7
1	Terbutaline sulphate (mg)	500	500	500	500	500	500	500
2	Eudragit RSPO (mg)	250	375	500	625	750	875	1000
3	Poloxomer 188 (mg)	10	10	10	10	10	10	10
4	Dichloro Methane (ml)	10	10	10	10	10	10	10
5	Span 80 (ml)	0.02	0.02	0.02	0.02	0.02	0.02	0.02

F= formulation, mg= milligram, ml= millilitre

Evaluation Studies

FT-IR Studies

Fourier transform infrared spectra were obtained using infrared spectrophotometer (Bruker, Germany). Samples of terbutaline and its physical mixture with polymers were pressed to a pellet under hydraulic press.

Obtained pellet was scanned for a spectrum at a scanning range of 4000 to 400 cm-1 with a resolution of 4 cm^{-1}.

Particle Size Analysis

Size of nanoparticles can be determined using optical microscope. Designed nanoparticles were diluted with ultra purified water and analysed at a scattering angle of 90° and temperature of 25° C under a optical microscope, whose ocular micrometer was previously calibrated with stage micrometer.

The mean diameter of each particle and mean hydrodynamic diameter was generated by cumulative analysis in triplicate.[12]

Zeta Potential

Zeta potential of terbutaline loaded inhalation nanoparticles was determined using zeta sizer.[13] It is an indicator of stability of nanoparticles. Ususlly small particles in a formulation possess large zeta potential values, offering stability to the preparation whereas large particles have small values rendering the formulation unstable.

Swelling Index

Synthesized nanoparticles were initially weighed (W_D) and immersed in deionized water for about 24 h. The weight of swollen nanoparticles (W_S) can be obtained by taking away the surface water using blotting paper. Swelling index was then calculated using the formula.

$$\text{Swelling index} = \frac{WS - WD}{WD} \times 100$$

Drug Entrapment Efficiency

Entrapment efficiency of nanoparticles can be determined using centrifugation method. Here, 100 mg of nanoparticle suspension was subjected to centrifugation at 300 rpm for 10 min.

The sediment in the centrifugation tube was diluted to 100 ml with dichloromethane and the absorbance of the same was recorded at 277 nm by using UV-Visible spectrophotometer (Systronics, India). Amount of Terbutaline sulphate in supernatant and sediment gave a total amount of terbutaline sulphate in 1ml dispersion. Percentage entrapment of drug was calculated by the following formula.[14]

$$\text{Entrapment efficiency} = \frac{\text{Amount of entraped d drug}}{\text{Total amount of drug}} \times 100$$

In vitro Drug Release Studies

In vitro drug release of prepared terbutaline sulphate nanoparticles using eudragit RSPO polymer was performed in phosphate buffer pH 6.8 under sink condition. The drug loaded nanoparticles equivalent to 5 mg of drug were placed in dialysis membrane.

The bag was suspended in 50 ml of dissolution media (phosphate buffer pH 6.8) in a beaker and the placed in magnetic stirrer (Remi equipment, Pvt. Ltd.) with temp maintained at 37°C and rotated at 100 rpm.

Aliquots of 1 ml of sample were withdrawn at respective time intervals (0.5h, 1h, 2h, 3h, 4h, 6h, 8h, 10h, 12h and 24h) and replaced with fresh 1ml of dissolution media (phosphate buffer pH 6.8) to maintain the sink condition.

The amount of drug released at respective time points were estimated by Double beam UV-Visible spectrophotometer (Systronics, India) at 277nm.[15]

RESULTS AND DISCUSSION

FT-IR Spectroscopy Studies

Drug-polymer compatibility studies were studied using FT-IR spectrophotometer (Bruker, Germany).

An IR spectrum of pure terbutaline sulfate showed characteristic peaks at 1344.44 cm^{-1} (O-H bending), 2974.36 cm^{-1} (C-H stretch), 1610.06 cm^{-1} (C=C stretch), 1100.11cm^{-1}(C-O stretch), 1205 cm^{-1} (C-N stretch) and 3333.39 cm^{-1} (N-H bending vibration).

These peaks are considered as the characteristic peaks of terbutaline and were found unaffected when observed in FT-IR spectra of terbutaline with other excipients.
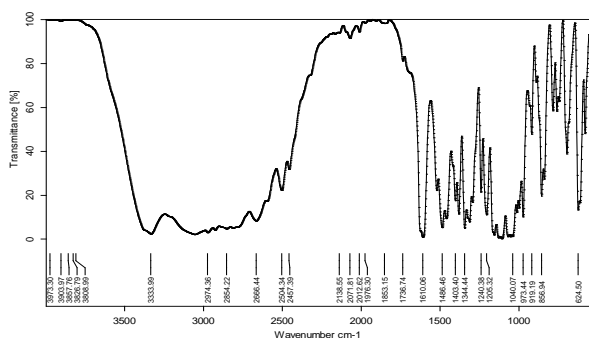

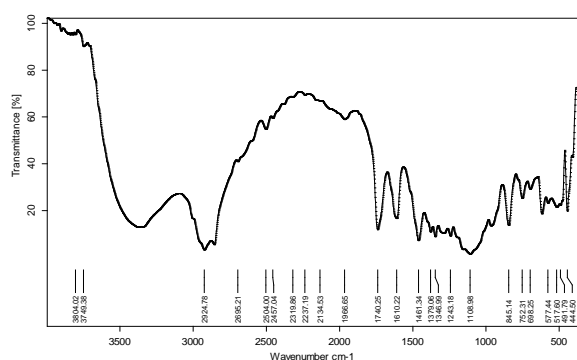**Figure 1:** FT-IR Spectrum of Pure Drug, Terbutaline Sulfate

Figure 2: FT-IR Spectrum of Pure Drug with all Excipients

Entrapment Efficiency

Entrapment efficiency of Terbutaline loaded Inhalation nanoparticles were carried by UV spectroscopic method. Results of the study demonstrated that F7 formulation possess maximum entrapment efficiency and F1, the minimum with 68%.

Entrapment efficiency gives information regarding the percentage of drug encapsulated with respect to the total drug introduced into the polymeric solution. Role of polymer concentration on entrapment efficiency was studied.

The entrapment efficiency of terbutaline nanoparticles was in the range 68-93%. This is due to the fact that as polymer concentration increases entrapment efficiency also increases.

Cross-linking nature of polymer might have prevented drug leakage from the nanoparticles. F7 formulation has shown maximum entrapment efficiency of 93%.
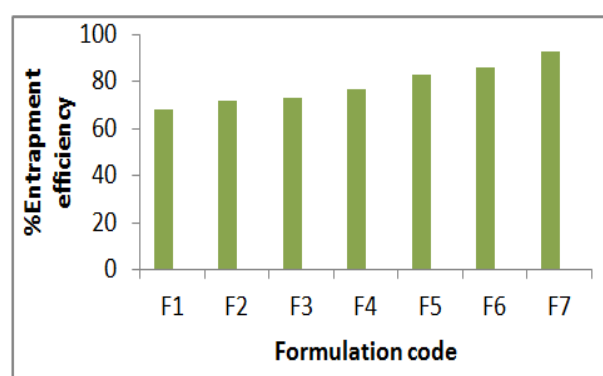

Figure 3: Entrapment Efficiency of Formulations F1 to F7
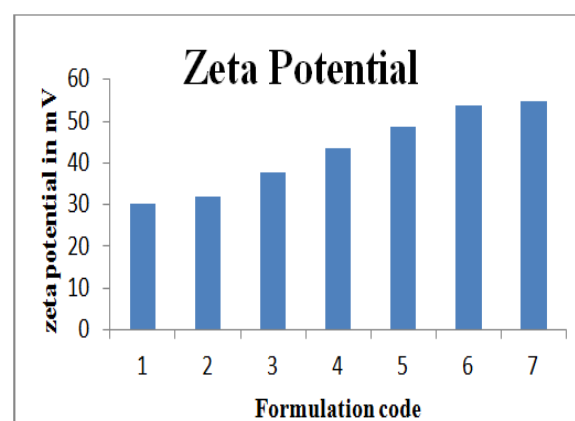
Zetapotential

Figure 4: Zeta Potential of Nanoparticles

Particle Size Analysis

Particle size of prepared nanoparticles was in the range of 50nm to 120nm. F7 formulation has maximum particle size and F1, the minimum. As the concentration of polymer increases, particle size also gets increases. Nanoparticles are studied for their size using optical microscope.

From the results, it was clear that as the polymeric content increases, size of nanoparticles also increases. Particle size of designed formulations was in the range of 50-120 nm.

Zeta potential of inhalation nanoparticles was determined using Zeta sizer and the obtained values were in the range 30.27 to 54.93mV and all the formulations carried slight positive charge on the surface.

This indicates the good stability of synthesized nanoparticles.

All formulations were found stable, this might be because of the small size of the particles and existence of electrorepulsive forces between the drug and polymer particles.

When the particle size is small, the particles remain dispersed throughout the dispersion without settling and when repulsive forces exist, and then the particles do not experience any attractive forces and remain without forming any flocs and aggregates.
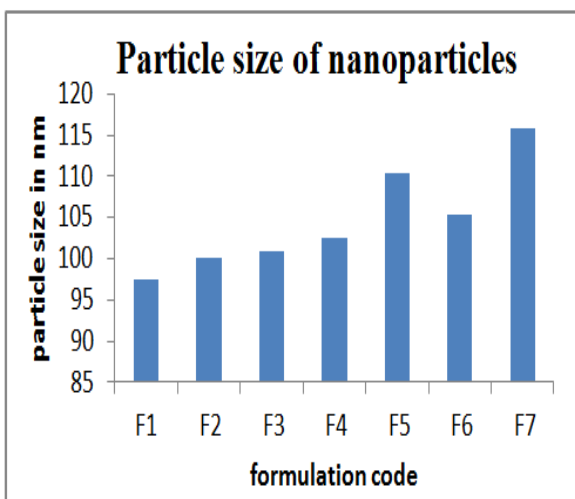

Figure 5: Particle size of nanoparticles

Swelling Index

The swelling index of prepared nanoparticles was in the range 34% to 78%.

Formulation F7 has shown maximum swelling index value, whereas F1 formulation the minimum. Swelling index is the capability of a polymer to swell when dropped in a suitable solvent.

As the polymer ratio increases, swelling capability of nanoparticles also increases.

However, for the nanoparticles with more polymer content, the more hydrophilic property of polymer binds with water and finally swells to give a gelatinous structure.

Thus, F7 formulation found to have maximum swelling index as compared to other formulations.
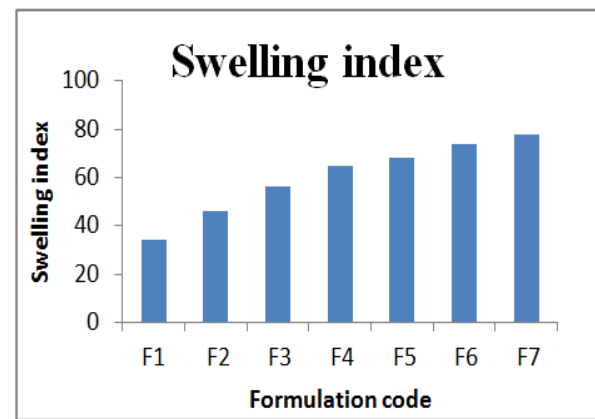

Figure 6: % Swelling Index of Nanoparticles

***In-vitro* drug release**

In-vitro drug release studies were performed using UV-spectrophotometer for a period of 24h.

At the end of first hour of the diffusion studies, drug release for all the formulations was in the range, 20.6% to 16.9%. After 24h of diffusion studies, all the formulations showed drug release in the range, 96.3% to 78.2%.

F7 formulation sustained the drug release than other formulations. Inhalation nanaoparticles were subjected to in-vitro drug release studies using diffusion test apparatus. Results of diffusion studies are shown in Fig no. 7.

Drug release was found to be decreased with increase in the polymer concentration.

Terbutaline release from nanaoparticles was studied in phosphate buffer of pH 6.8 for 24h. As the eudragit concentration is increased, more polymer will be available to bind with water and forms a viscous gel like structure that blocks the pores on the surface of nanoparticles and aid in delaying drug release from the formulation. Among all designed formulations, F7 was selected as an optimized formulation as it showed an extended drug release of 78.2% over a period of 24h.
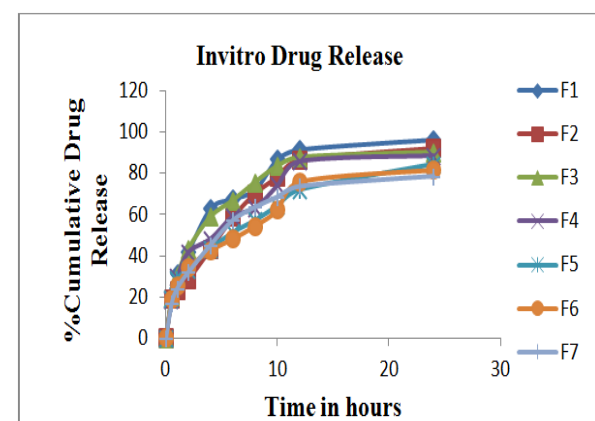

Figure 7: In vitro drug release of Inhalation nanoparticles

Formulation F7 was considered as the best formulation based on the results of particle size (less than 200 nm), % entrapment efficiency and *in-vitro* drug release studies.

CONCLUSION

Pulmonary drug delivery is a non-invasive delivery approach and considered an effective route as it avoids side effects encountered with systemic drug delivery. Nanoparticles made of eudragit RSPO were effectively localized to lungs so as to treat lung disorders such as asthma, chronic obstructive pulmonary disease (COPD). The present work confirms that double emulsion solvent evaporation technique is suitable for the development of inhalation nanoparticles. Polymer and surfactant ratios are considered important factors for the development of Terbutaline sulphate loaded inhalation nanoparticles. Results of the study indicated that synthesized inhalation nanoparticles sustained the drug release from formulations as compared to the pure drug. Thus it can be concluded that terbutaline sulphate inhalation nanoparticles serve as an effective carrier for the controlled delivery of drug to the target site, lungs.

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