ABSTRACT
Nanoparticulate drug delivery has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for biologically active agents. They show a wide range of possibilities to obtain desirable drug properties by altering or converting the biopharmaceutical and pharmacokinetic properties of the molecule. Isoniazid is first line medication in the prevention and treatment of tuberculosis. In the following laboratory studies solid lipid Isoniazid nanoparticles were prepared using Stearic acid and Tween 20 by w/o/w double emulsion-solvent evaporation method. The isoniazid nanoparticles were characterized by using Scanning electron microscopy(SEM), particle size distribution by Zeta sizer. The nanoparticles were evaluated for their drug loading efficiency, drug diffusion. The prepared SLNs were found in spherical shape. Present Work is focused on increase incorporation of drug into SLN. Work indicates that SLN of isoniazid can give reliable therapeutic effect for the treatment of tuberculosis by prolonging its action.

KEYWORDS: Solid Lipid Nanoparticles, Isoniazid, Tween20, Stearic acid.

INTRODUCTION
The recent past has witnessed the advancements of nano drug delivery technologies that can increase efficacy and safety, extend patient lives and provide competitive differentiation for biopharmaceuticals. The large size of most dosage forms, along with their other properties, shows physical and chemical instability within the body and limited membrane permeability and severe toxicity when applied systematically. Therefore researchers are developing a range of new delivery technologies and materials to enable these new drugs to be delivered
intact to their target sites.\textsuperscript{[2]} The majority of conventional drug products are in large size, so that nanoparticles are expected to have a broad impact on new trends in drug designing. Nanoparticles are defined as particles with a diameter smaller than 100 nm and are increasingly used in different applications, majorly in systems carrying drug and to pass organ blood-brain barrier.\textsuperscript{[3,4]} drug product designing.\textsuperscript{[5-8]} The fundamental properties for Nanoparticulate drug delivery systems are particle size, surface area, dispersing stability, magnetic and optical properties. As the particle size decreases, the number of molecules present on the particle surface increases and also the dissolution rate, stated by B.E.T equation.\textsuperscript{[9]} The solid lipid nanoparticles (SLNs) possess a lipid core matrix in the nanometer range stabilized by a layer of surface active agents. They have been used as delivery systems for the proteins vaccines and other drugs for controlled release compared to other colloidal drug delivery systems. Their capacity to enter through many biological barriers, sustained release of their drugs\textsuperscript{[18]}, and their small nano size range makes the implementation of SLNs as successful drug delivery systems.\textsuperscript{[14]}

Tuberculosis, or TB (tubercle bacillus/phthisis pulmonalis/consumption) is a common, and in many cases fatal, infectious disease caused by different species of mycobacterium usually Mycobacterium tuberculosis. TB mainly attacks the lungs, it also infect different other body organs. It spread’s through air, when people who have an active TB infection cough, sneeze, or otherwise transmitting their saliva through the air, hence its air bourne disease.\textsuperscript{[10]} Isoniazid also known as isonicotinyl hydrazine (INH) is an organic compound that is the first-line medication in prevention and treatment of tuberculosis.\textsuperscript{[13]} INH which is bactericidal for both extracellular and intracellular organisms. It is the primary drug for the treatment of tuberculosis when the disease is caused by isoniazid-sensitive strains of the M. tuberculosis.\textsuperscript{[11]} INH is a hydrophilic drug, which is effective drug for the treatment of tuberculosis. Isoniazid is a class three drug according to B.C.S classification(high solubility and low permeability) having an aqueous solubility of approximately 125 mg/ml. The drug is characterized by a short half-life ranging from 1h to 4h, depending on the rate of metabolism. INH has high absorption from three sections of the small intestine and from intramuscular injection sites. INH is less permeated through the stomach and is mainly absorbed through the intestine.\textsuperscript{[12]}
MATERIALS AND METHOD
Isoniazid was a gift sample from M/s. Calyx Pharmaceutical Ltd., Mumbai. Tween 20 is obtained from M/s. Hi-media laboratories, Mumbai. Stearic acid is obtained from M/s. Molychem, Mumbai. All other materials used in this study are of analytical grade. Stearic Acid and Tween 20 of variable conc. were studied to optimize the formulation for maximum entrapment efficiency (EE). Eight different formulations were prepared by using different concentrations of tween 20 and stearic acid. The different parameters used in formulation development are given in Table No.1.

Table No 1: Formulation table of Isoniazide SLN’s

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity Required (mg)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td></td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>PVA</td>
<td></td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Stearic acid</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Tween 20</td>
<td></td>
<td>0.5ml</td>
<td>1ml</td>
<td>1.5ml</td>
<td>2ml</td>
<td>0.5ml</td>
<td>1ml</td>
<td>1.5ml</td>
<td>2ml</td>
</tr>
</tbody>
</table>

METHODS OF PREPARATION OF SLNS
Solvent injection method was used to prepare SLN loaded Isoniazid Nanoparticles. Stearic acid, the solid lipid, was dissolved in methanol. The surfactant containing drug is slowly added to the organic solvent mixture through an injection needle stirred in to PVA solution at 1000 rpm for 3 hrs. The presence of surfactant within the aqueous phase helps to produce lipid droplets and also helps in stabilizing the formed SLNs by reducing the surface tension. Drug polymer ratios were taken 1:5, 1:10 and different formulations were prepared as shown in Table 1.

CHARACTERIZATION AND EVALUATION
The prepared formulation are characterized to ensure their predictable in vitro and in-vivo performances. The nanoparticle formulation produced by different polymer ratios may have different physicochemical characteristics. These differences do have an impact on their behavior in vivo and in vitro. The characterization parameters for the purpose of evaluation could be classified into three broad categories, which include physical, chemical and microbiological parameters. Parameters characterized in product development are size distribution, surface topology and diffusion rate profile.
Scanning electron Microscopy (SEM)

SEM\textsuperscript{[7-8]} was conducted to characterize the surface morphology of the prepared formulation (colloidal solution and precipitate). One drop of formulation was mounted on a clear-glass stub, air-dried, coated with Polaron E5100 sputter coater (Polaron, Watford, United Kingdom), and visualized under a scanning electron microscope.

Size determination

The mean particle size was obtained by particle size analyzer (Malvern). The instrument measures the particle size based on the laser diffraction theory using Fourier lens to a point at the center of multi element detector and a sample holding unit (Su cell). The sample was stirred using a stirrer before determining the vesicle size. The nano suspension was diluted about 100 times in the deionized water\textsuperscript{[4]} Diluted nanosuspension was added to sample dispersion unit containing stirrer and stirred at high speed in order to reduce inter particles aggregation and laser beam was focused.

Drug loading efficiency

Entrapment efficiency of isoniazid loaded SLNs was determined by measuring the concentration of unentrapped drug in aqueous medium by centrifugation method in a high speed cooling centrifuge (C-24, Remi) at 5,000 rpm for 15min at 4°C, and the supernatant was separated. The amount of isoniazid in the supernatant was determined by using UV-Vis spectrophotometer after appropriate dilution. The percentage entrapment efficiency (% EE) was calculated by using the following formula\textsuperscript{[20]}

\[
\%EE = \frac{\text{Total drug content} - \text{Free drug}}{\text{Total drug content}} \times 100.
\]

In vitro diffusion Studies

In vitro\textsuperscript{[19]} diffusion studies were done by using Franz diffusion cell. The capacity of the receptor compartment was 20 ml. The area of the donor compartment exposed to receptor compartment was 1.41 cm\textsuperscript{2}. Dialysis membrane was soaked overnight in phosphate buffer pH 7.4 to conduct diffusion studies for the Solid lipid nanoparticles. 10 mg from prepared formulations were taken and placed in the donor cell. Dialysis membrane was placed in between donor cell and receptor cell. In receptor cell approximately 20 ml of phosphate buffer pH 7.4 until it touches the dialysis membrane.\textsuperscript{[17]} The temperature of the receptor phase was maintained at
37 ± 0.5 °C and the receptor compartment was stirred with magnetic stirrer to maintain homogeneous condition. The aliquots of 3 ml were withdrawn at the time interval of (30 min, 60 min, 2hrs, 3hrs, 4hrs, 5hrs, 6hrs, 7hrs, 8hrs and so on up to 48hrs) and replaced with equal volume of dissolution medium. The samples were analysed in a UV-Visible spectrophotometer and amount of drug release at various time intervals were calculated.\textsuperscript{[16]}

**RESULTS AND DISCUSSION**

a. **SLN Nanoparticles**

Solid lipid nanoparticles were prepared by Solvent injection method. Drug can be effectively loaded in lipid which resulting less toxic and that can exhibit enhanced efficiency.

b. **SEM observation**

The surface morphology of Solid lipid nanoparticles loaded with isoniazid i.e. F-1 and F-5 were studied by SEM (Figures 1). Surface morphology showed spherical shape with encapsulated drug molecules.

![Figure 1: SEM analysis of (a)isoniazid loaded SLN (F-1) (b) isoniazid loaded SLN (F-5)](image)

**Particle size distribution analysis**

The particle size distribution analysis was performed by using particle size analyzer (Malvern) and the results in Figure 2 showed that the average particle size of the nanoparticles for F1 was 203.7 d.nm. The particle size distribution curve indicated that they are in uniform size.
Drug loading efficiency
The drug encapsulation was calculated among all formulations and results of formulation f1,f2,f3 and f4 showed drug content of 73.1%, 74.2, 78.5%, respectively whereas among the formulation-f5,f6,f7 and f 8 the % drug content was found to be 74.6%, 72.7%, 71.5% and 76.5% respectively.

In vitro diffusion studies
In vitro diffusion studies have been performed for all the prepared nanoparticles. The diffusion studies were also performed for pure drug for comparison. The drug release for the pure drug was found to be 46.3±1.22%. The drug release for formulations F1-F8 was uniform and extended for a period of 48 hours. The percentage drug release was in the range of 55.96±1.26% to 95.33±1.45% Among all the Colloidal solution formulations, F3 and F8 showed maximum drug release of 74.85±1.15% and 95.33±1.45% respectively in 48 hours.

CONCLUSION
The objective of the present work was to formulate SLN of antitubercular drug Isoniazid. SLN was prepared by Solvent injection method by using Stearic acid and Tween 20. Results show that an increase in ratio of drug: Stearic acid from 1:5 and 1:10 Tween 20 conc. 0.5 -2 ml increase in entrapment efficiency was observed. Two independent variable conc. of
Stearic acid and Tween 20 were found to have significant effect on dependent variable entrapment efficiency but not on drug release profile. The major outcome of this work was the successfully entrapment of a hydrophilic drug within lipid core. It can be concluded that using drug: Stearic acid ratio 1:10 ($f_0$) concentrations in optimum concentration and Tween 20 2.0 ml during the process of formulation better entrapment and drug release profile achieved and by this SLN approach prolong the action of the drug.

REFERENCES


