Sodium Alginate Nanoparticles of Isoniazide: Preparation and Evaluation

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ABSTRACT

Fabrication and evaluation of the Isoniazide loaded sodium alginate nanoparticles (NPs) was main objective of current investigation. These NPs were engineered using ionotropic gelation technique. The NPs fabricated, were evaluated for average particle size, encapsulation efficiency, drug loading, and FTIR spectroscopy along with in vitro drug release. The particle size, drug loading and encapsulation efficiency of fabricated nanoparticles were ranging from 230.7 to 532.1 nm, 5.88% to 11.37% and 30.29% to 59.70% respectively. Amongst all batches studied formulation F-8 showed the best sustained release of drug at the end of 24 hours.

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INTRODUCTION

Mycobacterium tuberculosis is second, next to HIV, in causing mortality from infectious disorder which affects 1/3 of the total world population. [1] The biopharmaceutical and pharmacokinetics properties of antitubercular drug necessitate the daily intake of these for tuberculosis management. A pronounced incidence of adverse effects, daily dosing and low patient compliance may result in discontinuation of therapy, and increase in multidrug-resistant strains of Mycobacterium tuberculosis. [2-3] Hence, the major objectives of tuberculosis research are to minimize the dose and dosing frequency. By using various drug delivery systems the pharmacokinetics of antitubercular drugs can be enhanced, that may lead to an improvement in the therapeutic strategy for management of tuberculosis. During the last decade, several researches were carried out with some modifications in drug delivery systems to enhance the pharmacokinetics of antitubercular drugs. These drug delivery systems with some modifications have been combined with some disadvantages such as increased consumption of polymer [4], low drug encapsulation [5] and use of organic solvents [6], etc. This investigation was designed for the development of nanoparticles drug delivery system using polymer of natural origin. This developed novel drug delivery system would have the potential to overcome the drawbacks of modified
drug delivery systems mentioned above. The properties like biocompatibility biodegradability and non-toxicity of Sodium alginate made it suitable for its use in the field of drug delivery. The United States Food and Drug Administration has recommended Sodium alginate (SA) for oral use [7-8] and is enriched with the property of hydrophilicity (which protect it from fast uptake by the reticuloendothelial system). It is a natural polysaccharide, rich in carboxyl group and is easy to bind with positive charge cations such as Ca²⁺. It is of low toxicity, good biocompatibility and relatively low cost, therefore it is widely used in medicine and food industry. [9,10] Therefore, it was employed to fabricate nanoparticles entrapping antitubercular drugs. [11] Further, research will look into designing of novel drug delivery systems with drug targeting to lungs. Our research group has explored the fabrication of Sodium alginate nanoparticles by simple and fast ionotropic gelation between the SA (polymer) and its counter ion calcium chloride which exhibit a significant ability in protein association (about 90%), along with peptide absorption improvement through numerous epithelia, like nasal, intestinal and ocular. [12] Recently, the fabrication of sodium alginate nanoparticles as carriers for doxorubicin to increase efficacy of active moiety has been reported. [13] In view of the above, it was planned to prepare and characterize sodium alginate nanoparticles encapsulating Isoniazid, followed by evaluation for the sustained release of the drug.

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**Characterization of sodium alginate nanoparticles**

**Particle size**

Freeze-dried nanoparticles were dispersed into HPLC grade water. The particle size of the Isoniazid loaded sodium alginate nanoparticles were evaluated by Particle Size Analyzer (Malvern Instruments Ltd, Malvern, UK). The particle sizes of various fabricated nanoparticle formulations are shown in table 2.

**Determination of Encapsulation Efficiency (EE) of nanoparticles**

10 ml suspension of nanoparticles was centrifuged at 10,000 rpm (Remi, Mumbai) for 90 minutes at 10°C. After centrifugation, the clear supernatant procured was diluted 10 times with double distilled water to quantify the amount of unbound isoniazid using UV-Visible spectrophotometer at λmax = 263 nm. Encapsulation Efficiency & drug loading of nanoparticles were determined using equation given below. [15]

\[
\text{Encapsulation efficiency} = \frac{\text{Total amount of isoniazid} - \text{Amount of free isoniazid}}{\text{Total amount of isoniazid}} \times 100
\]

\[
\text{Drug loading} = \frac{\text{Total amount of isoniazid} - \text{Amount of free isoniazid}}{\text{Total weight of nanoparticles}} \times 100
\]

**Fourier transform infrared spectroscopy (FTIR)**

The infrared spectrum of pure isoniazid, sodium alginate, physical mixture (sodium alginate and isoniazide) and isoniazide loaded nanoparticles was carried out to advocate drug loading and drug-excipients interaction. [16]

**In vitro drug release study of sodium alginate nanoparticles**

The drug release studies were carried out using dissolution medium (250 ml of 7.4 pH phosphate buffer) at 50 rpm at 37 ± 0.5°C temperature. The nanoparticles containing drug equivalent to 50 mg were taken in a dialysis bag and put into flask containing dissolution medium. 5 ml aliquots of samples were withdrawn at specific interval i.e. 1, 2, 4, 6, 12, 14, 16, 18, 20, 22 & 24 hours. After suitable dilutions, absorbance of the samples was determined by UV-Visible Spectrophotometer at λmax 263 nm. Absorbance for the samples withdrawn was recorded and percentage drug release at different time intervals was plotted against time.

**RESULTS AND DISCUSSION**

**Optimization using 3² factorial design**

The factorial design was applied for the determination of appropriate amount of sodium alginate and calcium chloride on the basis of loading capacity, encapsulation...
efficiency and average particle diameter measurements. 13 formulations were fabricated as per experimental design and influence of 2 factors, i.e. SA (A) and calcium chloride (B) was examined on 3 responses viz. loading capacity, encapsulation efficiency and average particle diameter. The loading capacity and encapsulation efficiency of all fabricated formulations was found to be in the range of 5.88% to 11.37% and 30.29% to 59.70%, respectively, whereas mean particle size was observed between 230.7 to 532.1 nm. The obtained polynomial equations were employed for the calculation of the variance and responses were evaluated for parameters such as degree of freedom, F value, sum of squares & mean sum of squares applying the software. The polynomial equations were obtained through regression analysis for responses as follows

Loading capacity = +9.51-1.08*A+1.27*B (1)

Encapsulation efficiency = +49.49+5.82*A+6.81*B (2)

Particle size = +324.40+96.63*A+25.72*B (3)

Response surface curve depicting the combined effect of both factors on loading capacity, encapsulation efficiency and particle size of nanoparticles is given below in Figures 1, 2 and 3, respectively.

![Fig. 1: Response surface plot depicting the combined effect of Sodium alginate & calcium chloride on loading capacity (%) of Isoniazide nanoparticles](image1.png)

![Fig. 2: Response surface plot depicting the combined effect of Sodium alginate & Calcium chloride on encapsulation efficiency (%) of Isoniazide nanoparticles](image2.png)

![Fig. 3: Response surface plot depicting the combined effect of Sodium alginate & Calcium chloride on particle size of Isoniazide nanoparticles](image3.png)

![Fig. 4: Particle size distribution curve of the Isoniazide loaded sodium alginate nanoparticles (F-8).](image4.png)

The importance was designated to mean particle size, loading capacity and encapsulation efficiency. The variables according to Design Expert software for optimized nanoformulation were sodium alginate 3.0% w/v and calcium chloride 2% w/v which led to formulation of nanoparticles with average particle diameter of 245.5 nm

**Particle size**

Particle size of the prepared formulations is shown in Table 2. It was observed that particle size varied from 230.7 and 532.1 nm for sodium alginate nanoparticles and particle size of optimized formulation (F-8) was shown in Figure 4. For SA nanoparticles, an increase in

Table 2: Experimental Design for Isoniazide nanoparticles and results for the measured responses

<table>
<thead>
<tr>
<th>Std</th>
<th>Run</th>
<th>Factor 1 Sodium alginate</th>
<th>Factor 2 Calcium chloride</th>
<th>Response 1 Drug loading (%) w/v ± SD</th>
<th>Response 2 Entrapment efficiency (%) ± SD</th>
<th>Response 3 Average particle size (nm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1</td>
<td>0</td>
<td>-1</td>
<td>7.97 ± 0.18</td>
<td>41.25 ± 0.08</td>
<td>230.7 ± 0.08</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>10.82 ± 0.19</td>
<td>56.33 ± 0.20</td>
<td>386.8 ± 0.08</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>10.40 ± 0.13</td>
<td>54.38 ± 0.24</td>
<td>284.2 ± 0.08</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>8.99 ± 0.13</td>
<td>46.78 ± 0.19</td>
<td>231.9 ± 0.08</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>9.91 ± 0.10</td>
<td>51.55 ± 0.82</td>
<td>285.3 ± 0.08</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>-1</td>
<td>-1</td>
<td>5.98 ± 0.09</td>
<td>36.29 ± 0.25</td>
<td>253.7 ± 0.08</td>
</tr>
<tr>
<td>3</td>
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<td>-1</td>
<td>1</td>
<td>9.31 ± 0.10</td>
<td>48.43 ± 0.26</td>
<td>240.9 ± 0.08</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>11.37 ± 0.11</td>
<td>59.70 ± 0.15</td>
<td>245.5 ± 0.08</td>
</tr>
<tr>
<td>2</td>
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<td>1</td>
<td>-1</td>
<td>9.62 ± 0.05</td>
<td>58.06 ± 0.07</td>
<td>416.3 ± 0.08</td>
</tr>
<tr>
<td>5</td>
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<td>-1</td>
<td>0</td>
<td>10.17 ± 0.15</td>
<td>52.63 ± 0.18</td>
<td>532.1 ± 0.08</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>10.62 ± 0.09</td>
<td>55.26 ± 0.17</td>
<td>334.4 ± 0.08</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>9.84 ± 0.10</td>
<td>51.21 ± 0.13</td>
<td>382.6 ± 0.08</td>
</tr>
<tr>
<td>12</td>
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<td>0</td>
<td>8.70 ± 0.10</td>
<td>45.26 ± 0.08</td>
<td>381.4 ± 0.08</td>
</tr>
</tbody>
</table>

concentration of the calcium chloride led to reduction in average size of nanoparticles. Quantity of calcium chloride has significant role in the protection of nanoparticles because it hinders the cluster formation of nanoparticles.

**Determination of Encapsulation Efficiency (EE) of nanoparticles**

The drug loading and encapsulation efficiency of fabricated nanoparticles is shown in Table 2. It was observed that with increase in the concentration of sodium alginate, encapsulation efficiency and drug loading were also found to increase, owing to increase in viscosity of the aqueous medium. But the encapsulation efficacy decreased with increasing in calcium chloride concentration. When quantity of calcium chloride was increased, it promoted the solubilisation of drug in the aqueous phase.

**Fourier transform infrared spectroscopy (FTIR)**

Drug compatibility studies using FTIR were conducted for the pure drug, sodium alginate and the physical mixture. The spectral data are given in Figure 5-7. The results indicated no chemical incompatibilities between Isoniazide and sodium alginate used in nanoparticles.

![FTIR spectrum of Sodium alginate](image1)

![FTIR spectrum of physical mixture of Isoniazide and sodium alginate](image2)

![Fish: FTIR spectrum of Isoniazide](image3)

![In vitro drug release profiles of Isoniazide loaded sodium alginate nanoparticles (F1-F13)](image4)

**In vitro drug release studies**

The pattern of drug release from nanoformulation displayed cumulative drug release in the range 66.56%–83.53% as shown in Figure 8. The Isoniazide loaded nanoparticles showed a biphasic drug release profile initially with outburst release of Isoniazide followed by sustained release of Isoniazide. The initially outburst release may be due to association of Isoniazide with surface of nanoparticles. Initial release of Isoniazide is linked with those Isoniazide moieties dispersing from near the nanoparticles surface.

The drug release may depend upon the sodium alginate amount. In optimized formulation (F8) sodium alginate showed drug release of 66.56% within 24 hour showing a sustained release profile, during the first hour nanoparticles formulation gave outburst release and after that it showed a sustained. Literature reports suggest that macrophages take 2 h to achieve their maximum engulfment capacity. Therefore, it can be deduced that the majority of drug would be released inside the cell following endocytosis of the carrier system. [17-18]

The objective of current investigation was fabrication and evaluation of isoniazid loaded sodium alginate nanoformulations. The Isoniazid-loaded sodium
alginic nanoformulations were evaluated by particle size analyzer and Fourier transform infrared spectroscopy. Existence of Isoniazide in loaded nanoparticles was confirmed by Fourier transform infrared spectroscopy studies. Sodium alginate nanoparticles with properties like biodegradability, biocompatibility, more stability, low toxicity, convenient and simple preparation technique, offers an important and valuable tool for the Isoniazide delivery through novel drug delivery system. The sodium alginate & calcium chloride concentration role on loading capacity, encapsulation efficiency and particle size was evaluated by optimization. The observed parameters for formulation F-8 were significant as compared to software predicted values given by design expert. The in vitro drug release for the optimized formulation was 66.56% in 24 h. The development of this Isoniazide loaded sodium alginate nanoformulation has the potential to provide enhanced efficacy of Isoniazide.

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**REFERENCES**
