Preparation and characterization of biopolymeric nanoparticles used in drug delivery

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Nanotechnology plays an important role in advanced biology and medicine research particularly in the development of potential site-specific delivery systems with lower drug toxicity and greater efficiency. These include microcapsules, liposomes, polymeric microspheres, microemulsions, polymer micelles, hydrogels, solid nanoparticles etc. In the present study, preparation and characterization of biopolymeric gelatin nanoparticles for encapsulating the antimicrobial drug sulfadiazine and its in vivo drug release in phosphate buffer saline (PBS) have been investigated. The nanoparticles prepared by second desolvation process varied in a size range 200 nm and 600 nm with a drug entrapment efficiency of 50% characterized by atomic force microscopy and dynamic light scattering. The drug release from the nanoparticles occurred up to 30% in a controlled manner.

Keywords: Gelatin, Nanoparticles, Nanotechnology, Sulfadiazine, Cross-linker, Cysteine

Nanotechnology has various applications in different fields like fiber and textiles, agriculture, electronics, forensic science, space and medical therapeutics. Polymeric nanoparticles hold significant promise as potential drug carriers for treating diseases and disorders due to their attractive physicochemical properties such as size, surface potential and hydrophilic-hydrophobic balance. The various forms of natural and synthetic polymers are used for drug encapsulation and to deliver compounds. The chitosan, a natural and antioxidative polymer obtained from crustacean shells and the synthetic polymers L-, D-, and D.L. -polylactic acid (PLA), polyglycolic acid (PGA), and polycaprolact acid (PCL), polyvinyl alcohol, polyethylene glycol (PEG), poly-N-vinyl pyrrolidone, polyethyleneimine (PEI), polyethylene oxide (PEO), phosphatidyl-ethanolamine (PE), methoxypoly-ethylene glycol (MPEG)-PLA copolymer and pluronic PEO-polypropyleneoxide – PEO triblock copolymer have been used for controlled release of drug, thereby reducing unwanted side effects and improving therapy.

Although biodegradable nanoparticles of natural polymers such as starch and chitosan are largely in use as drug carriers, gelatin nanoparticles (G-NPs) represent a promising carrier system for controlled drug delivery. Gelatin is a natural polymer derived from collagen and is commonly used for pharmaceutical and medical applications, because of its biodegradability and biocompatibility in physiological environments. The mechanical properties, swelling behavior and thermal properties depend significantly on the cross-linking degree of gelatin. The gelatin nanoparticles have been prepared by desolvation/coacervation or emulsion method.

Sulfadiazine (SDZ) has been proved to be an effective treatment for meningococcal meningitis and as a preventive medication for diseases among army recruits and other susceptible groups. It is a white or yellow crystalline powder, insoluble in water and very slightly soluble in ethanol and slowly darkens on exposure to light with decomposition. In this study, SDZ has been taken as a model drug. In the present study, preparation and characterization of biopolymeric gelatin nanoparticles for encapsulating the antimicrobial drug sulfadiazine and its in vivo drug release in phosphate buffer saline (PBS) have been investigated.

Materials and Methods

The biodegradable polymer type A gelatin 175 Bloom (Mol. wt. 40-50 kD), pI (7.0-9.0), pH (3.8-5.5) from porcine skin and type B gelatin 75 bloom (Mol. wt. 20-25 kD), pI (4.7-5.2), pH (5.0-7.5) from bovine skin were purchased from Sigma-Aldrich, Steinheim, Germany. Hydrochloric acid (N/10), NaOH (0.1 N) and glutaraldehyde (8% v/v and 10% v/v) from Thomas Bakers, India, calcium chloride dihydrate (1 M) from Spectrochem.
Pvt. Ltd., India, sulfadiazine (100 µl of 10 mg/ml from Sigma-Aldrich, USA) and L-cysteine (from Sisco Research Laboratories Pvt. Ltd, India) and acetone (Qualigens Fine Chemicals, India) were used.

Preparation and characterization of nanoparticles

The preparation of biodegradable nanoparticles was achieved by the two step method called desolvation. Gelatin type A 175 bloom

Gelatin type A (0.25 g) from porcine skin (175 Bloom Sigma-Aldrich, Steinheim, Germany) was dissolved in 25 ml water and heated below 40°C along with magnetic stirring. About 25 ml of acetone was added to the gelatin for complete desolvation and rapid sedimentation. The precipitate formed in the sample was redissolved in 25 ml water under heating and the pH was adjusted to 2.5. The gelatin was again desolvated by addition of 25 ml acetone. After 10 min of stirring, 500 µl of glutaraldehyde (8% v/v) and glutaraldehyde (10% v/v) was added to 25 ml of the gelatin sample for cross-linking the molecules. After 30 min of stirring, 500 mg L-cysteine was added. The sample was centrifuged for 2 min to remove the cysteine and purified three-fold by centrifugation for 12 min at 10,000 rpm and redispersion in acetone/water mixture (30/70). The sample obtained after last dispersion was magnetically stirred with slight warming to remove the acetone. The particle size was measured using dynamic light scattering to be 300 nm at both 8% (v/v) and 10% (v/v) and later by AFM.

Gelatin type B 75 bloom

About 0.25 g gelatin type B 75 bloom was dissolved in 25 ml water and about 25 ml acetone was added for completely desolvating and sedimenting the gelatin molecules. The pH was adjusted to 8.76 in the gelatin samples. The gelatin was again desolvated by adding acetone about 25 ml and then 500 µl CaCl₂ (1 M) was added to cross-link the particles by magnetic stirring. The gelatin nanoparticles were made free of acetone by evaporation method. The particle size was determined by dynamic light scattering to be 110 nm at both 8% (v/v) and 10% (v/v) and later by AFM.

Drug delivery

Drug loading and entrapment

The drug loading and entrapment was studied as discussed previously. Briefly, the drug dissolved in distilled water at a concentration of 10 mg/ml was loaded in gelatin nanoparticles by adding in a small aliquot of 10 µl at a time under mild ultrasonication for 10 s. Thus, 100 µl of drug from the stock of 10 mg/ml was loaded in 2.5 ml of nanoparticles dispersion. In order to know the drug entrapment, 100 µl of drug-loaded nanoparticle (DLNP) was separated from the unentrapped drug by passing the drug loaded solution through a Millipore filter (0.22 µm Cut-off) and the absorbance of free drug in the filtrate was measured by UV spectrophotometer (Synergy HT, Bio-Tek Instrument Inc., USA) using KC4 v3.4 software.

The percentage of entrapment efficiency was calculated by the formula

\[
\text{Entrapment efficiency} = \left( \frac{A_{\text{total drug}} - A_{\text{free drug}}}{A_{\text{total drug}}} \right) \times 100
\]

where \(A_{\text{total drug}}\) is the optical density (OD) of drug loaded nanoparticles and \(A_{\text{free drug}}\) is the OD of filtrate containing the free drug.

Release studies

The drug release was evaluated using the equilibrium dynamic dialysis technique. In brief, 2.5 ml of drug-loaded nanoparticles was taken in a dialysis bag (Spectra Por, USA; cut-off size 10 kD) and then dialyzed against 350 ml of phosphate buffer saline (PBS) at pH 7.4 and kept under constant magnetic stirring at a rotation speed of 50 rpm. The experiment was carried at both room temperature and 37°C to check if different temperature conditions made any difference in the release pattern. Periodically, aliquot of 1 ml was collected and analyzed for presence of free drug by UV-visible spectrophotometry. The peak absorbance of the drug was found to be \(\varepsilon_{\text{max}} = 259\) nm. The drug concentration was measured by spectrophotometer (Bio-Tek Instruments Inc, USA) using KC4 v 3.4 software. The release experiment was monitored for over a period of 72 h.

Results and Discussion

Nanoparticle preparation and characterization

The Fig. 1 depicts the atomic force microscopy (AFM) photograph of gelatin nanoparticle of 4 µm scan size. The nanoparticles were prepared by using the 75 and 175 gelatin bloom. The second desolvation method used for the synthesis of nanoparticles was found to be well suited using a biodegradable biopolymer for encapsulation of compounds. Table 1 represents nanoparticle sizes measured using dynamic
light scattering to be in the range 100-300 nm. Then they were determined to be in the range 200-600 nm using AFM after stabilization studies. Gelatin and PEGylated gelatin nanoparticles approximately 200 nm in diameter with spherical shape were determined by scanning electron microscopy. In the present study, the particle size was determined using dynamic light scattering and AFM.

Effect of cross-linker concentration

Gelatin may have either positive or negative charge, depending upon the number of amino acids. In glutaraldehyde, NH₂ groups are cross-linked with two (-CHO) groups. The variation in concentration of the glutaraldehyde for 175 bloom type B made no significant changes in the size of gelatin nanoparticles and their size was measured by dynamic light scattering.

Effect of pH

The pH 5.16 was an acidic pH for both type A and B gelatin. The pH was changed to 8.76 for calcium chloride to make carboxyl (-COOH) groups predominant for cross-linking with Ca²⁺. At pH 5.16, the size of nanoparticles was found undefined, because it was the pl of 75 bloom (4.7-5.2). The variation in pH value resulted in nanoparticles of smaller size. The pH value 5.16 produced no results, as it was near the isoelectric point of 75 bloom gelatin (pl 4.7-5.2). At pH 8.76, nanoparticles were formed of size less than 100 nm. For the cross-linker calcium chloride in 75 bloom type A, pH was varied at values 8.76 and 5.16. The corresponding nanoparticle sizes were measured using the dynamic light scattering.

Table 1—Nanoparticle size determination by the variation of pH and cross-linker concentration for type A and B gelatin

<table>
<thead>
<tr>
<th>Gelatin bloom</th>
<th>Cross-linker</th>
<th>pH</th>
<th>Conc.</th>
<th>Nanoparticle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 bloom Type B</td>
<td>CaCl₂</td>
<td>8.76</td>
<td>1 M</td>
<td>100 nm</td>
</tr>
<tr>
<td>75 bloom Type B</td>
<td>CaCl₂</td>
<td>5.16</td>
<td>1 M</td>
<td>Undefined</td>
</tr>
<tr>
<td>175 bloom Type A</td>
<td>Glutaraldehyde</td>
<td>4.15</td>
<td>8% (v/v)</td>
<td>300 nm</td>
</tr>
<tr>
<td>175 bloom Type A</td>
<td>Glutaraldehyde</td>
<td>4.15</td>
<td>10% (v/v)</td>
<td>300 nm</td>
</tr>
</tbody>
</table>

Drug release studies

The nanoparticle-mediated drug delivery has been found to greatly enhance the therapeutic index of a drug. A drug when administered inside a human body along with a high molecular weight polymer performs better in terms of its increased bioavailability with reduced side effects. The drug masked by the polymer is not available for interaction with other blood factors and it is released slowly in a controlled fashion as the polymer swells/degrades in aqueous media. The gelatin nanoparticles were applied as a drug delivery vehicle for steady and controlled release of sulfadiazine in aqueous media.

The entrapment efficiency of drug loaded in biopolymeric gelatin nanoparticles was found to be 50% and the percentage of drug release was approximately 30%. The drug entrapment and its controlled release might have occurred for longer time, if a hydrophobic nanoparticle was used for a hydrophobic drug, instead of hydrophilic one. The design of biodegradable nanocarriers and the drugs to be released should be considered with greater significance. The release of drug was studied at both room and body temperature and was found to increase with temperature. Fig. 2 shows the release kinetics of sulfadiazine.
of drug sulfadiazine for 72 h at room temperature and at 37°C.

Earlier, the drugs isoniazid (INH) and rifampin (RIF) were entrapped in PLG polymers, and the microparticles having a diameter ranging from 11.75-71.95 µm have been found to provide sustained release of drugs over 6-7 weeks in mice\textsuperscript{13}. In the present study, the drug sulfadiazine was entrapped in biodegradable gelatin nanoparticles of size 600 nm and its release was studied over 72 h in PBS 7.4. The \textit{in vitro} release of drug from the mixed micelles in PBS 7.4 at different temperatures was done at an incubation of 72 h at 4°C, room temperature and 37°C and the respective drug release presented\textsuperscript{12}. In the present study, the drug release was found to be higher at body temperature than room temperature.

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**References**