Gelatin Coated Albumin Nano Particles Bearing Acyclovir for Effective Management of Visceral Leishmaniasis by Targeting Liver Kupffer Cells

Bhatt Akanksha1* Bhatt Ganesh1 Kothiyal Preeti 1

1Shri Guru Ram Rai Institute of Technology and Sciences, Patel Nagar, Dehradun
1Shri Guru Ram Rai Institute of Technology and Sciences, Patel Nagar, Dehradun

Abstract: To make drug comfortable for administration to patients it is converted to dosage form by mixing with recipients, additives. Nano particles are one of the novel colloidal drug delivery systems that hold great promise for reaching the goal of controlled drug delivery as well as site-specific delivery. In the present study, an attempt was made to develop gelatin coated albumin nano particles of acyclovir for the treatment of visceral leishmaniasis with a view to see the targeting of nano particles at the required site for action and helps to provide sustain release and thus reduces the dose frequency and increase the patient compliance. Pre formulation testing is the first step in the rationale development of dosage form of a drug. The method was able to produce the free flowing nano particles. Bovine serum albumin is a biodegradable polymer for preparing targeted nano particle. FTIR studies revealed that there was no interaction between the selected drug and polymer. The particle size analysis revealed that the particle size was found to be in the size range of 200-800 nm, that comes under nano range. The zeta potential of nano particle was found to be -59.3, having good stability. The maximum entrapment efficiency was found to be 86.78±0.5 % for the formulation F3. The entrapment efficiency increases with increasing polymer concentration but up to a certain ratio. The maximum percentage yield was found to be 51.13±0.53 % for formulation F4, where the concentration of albumin is highest while nano particles yield is lowest in F1 19.10±0.79, as the concentration of albumin is lowest. In vitro release shown as, cumulative release of drug was determined up to 8 hrs for formulation F1, F2, F3, F4 was 62.48± 0.38, 51.67±0.55, 48.34±0.29, 34.98±0.32 respectively. The receptor ligand binding is 95.89%. And it was confirmed by checking the amount of drug release from formulation is 3.89% while prior it was 48.34%, so the remaining drug was bind with receptor cells.

Keywords: Desorption Method, Receptor Ligand Binding, Zeta Potential, Pre formulation Studies.

I. INTRODUCTION

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Development of new drug molecule is expensive and time consuming. Improving safety efficacy ratio of “old” drugs has been attempted using different methods such as individualizing drug therapy, dose titration, and therapeutic drug monitoring. Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body.

Targeted drug delivery system (T.D.D.S.) is the goal oriented drug delivery in which the delivery is designed in such a way that it only signifies targeted compartment, either with the help of carrier, ligand, polymer, proteins (components through which the drug can be targeted) or by altering the molecular weight, size or shape of formulation the science of targeted drug delivery is burgeoning with new information and explosive growth of technology and methodology in this area. The delivery of drug can be done with the help of molecule that bind either with receptor or antigens targeting [1][2]. The concept of targeting was first specified by Paul enrliech, who

1 Corresponding Author: bhattakanksha261@gmail.com
proposed drug delivery as magic bullets. Gregoriadls, 1981 described drug targeting using novel drug as old drug in new clothes.\[3\] Colloidal drug carrier system such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10–400 nm diameter show great promise as drug delivery systems\[6\]

1.1. Methods of Drug Targeting

1.1.1. Passive Targeting

Results in the formation of complex i.e. drug carrier complex, which avoid elimination due to body defense mechanism like metabolism, opsonization and excretion this complex will circulate in the blood and get attached to its targeted site due to property of drug. The properties are molecular weight, molecular size, nature of surface and surface charge\[5,6\]

1.1.2. Inverse Targeting

In this the RE System take colloidal carrier to achieve its targeting, where pre administration of blank colloidal carrier is done, for saturation of RES.

1.1.3. Active Targeting

Active targeting includes the delivery of drug through antibody, antigens, receptors and proteins. Which are either uniquely expressed or over expressed on target cell population ligand such as peptide for neovascularure targeting\[7\] and folate receptors in certain cancers.\[8,9\] Antibodies have higher specificity however the production of antibodies remain expensive and time consuming and problems with stability and storage of resulting product is still exist.\[10\]

Nanoparticles (NPs) are one of the novel colloidal drug delivery systems that hold great promise for reaching the goal of controlled drug delivery as well as site-specific delivery. Nanoparticles are a type of colloidal drug delivery systems where the particle size ranges from 10-1000 nm in diameter. Nanoparticles are made of non-biodegradable and biodegradable polymers. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nano particles, nano spheres or nano capsules can be obtained Nanoparticles are solid and spherical structures ranging in size and prepared from natural or synthetic polymers. A wide variety of drugs can be delivered using nanoparticles such as hydrophilic small drugs, hydrophobic small drugs, vaccines and biological macromolecules. Nano particles were able to protect drugs against chemical and enzymatic degradation and were able to reduce side effects of some active drugs\[11-13\]

1.2. Preparation of Nano Particles

The choice of preparation method essentially depends on the raw materials intended to be used and on the solubility characteristics of active compound to be associated with the particles. Regarding raw material, criteria such as biocompatibility, the degradation behavior, choice of administrative route, desired release profile of the drug and finally the type of biomedical application determine its selection.

Nanoparticles have been prepared most frequency by three methods:

- Dispersion of preformed polymers
- Polymerization of monomers
- Ionic gelation or coacervation of hydrophilic polymers.

The internal structure of the liver is made of around 100,000 small hexagonal functional units known as lobules. Lobules are the functional unit, which made many lobes of liver. Liver consisting of various cells i.e. Hepatocytes and kupffer cells, blood vessels connected by various capillary like tubes and have larger space lined by endothelium called sinusoids\[14\]
**Table 1. Nanoparticle Classification**

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanotubes</td>
<td>carbon, (fullerenes)</td>
</tr>
<tr>
<td>Nanowires</td>
<td>metals, semiconductors, oxides, sulfides</td>
</tr>
<tr>
<td>Nanocrystals</td>
<td>nitrides</td>
</tr>
<tr>
<td>Other nanoparticles</td>
<td>quantum dots insulators, semiconductors</td>
</tr>
<tr>
<td></td>
<td>metals, magnetic materials, ceramic oxides,</td>
</tr>
<tr>
<td></td>
<td>metals</td>
</tr>
<tr>
<td>Nanobots</td>
<td>biochip, nubots</td>
</tr>
</tbody>
</table>

1.3. Kupffer Cell

Kupffer cells are first protective cellular line in liver sinusoid and localized in the vicinity of hepatocytes. It is a location in liver where macrophages resides, about 80-90% of macrophages are present in liver. Function of kupffer cells is essential in liver injury as macrophages engulf the foreign material (bacteria, virus or any infectious material), in inflammation, KCs are important source of various inflammatory-related mediators, such as poor anti-inflammatory cytokines (TNF-α, IL-6, IL-10), chemokines (MCP-2, IL-8, IP-10) and other inflammatory-related mediators (prostanoids, nitric oxide, reactive oxygen species) [15].

As discussed above those liver macrophages resides in kupffer cells and an extent to RES, so by targeting to these cells macrophages targeted automatically. Mainly targeted anti inflammatory and immunosuppressive drugs. Importance of liver macrophages, as liver has a simple structure composed of hepatic parenchymal cell and sinusoidal cell: kupffer cells (macrophages) are present in hepatic sinusoidal lumen and monocytes circulate in peripheral blood. Called blood monocytes, which are around 20% of the peripheral blood mononuclear cell (PBMC) fraction. The macrophages are professional phagocytes as having the self defense mechanism. [16-20]

Opsonization is a process by which particular antigens are rendered more susceptible to phagocytosis, foreign particle attaches to protein and prone to increase phagocytosis called opsonins, which are easily recognized by macrophages. E.g. of opsonins are immunoglobulin, bovine serum albumin, gelatin, human fibrinoinctin, bovine tuftsin, enhance phagocytosis [21]. Visceral Leishmaniasis is closely interrelated to socioeconomic, climatic, and environmental factors, the latter including land use/land cover, and topography, rainfall, temperature, and vegetation coverage [22]. As visceral leishmaniasis is a protozoa disease which infected the macrophages of body and kupffer cells (KCs) in the liver resident macrophages, which constitute 80% of tissue macrophages of the body kupffer cells. KCs are the first cellular protective line in liver sinusoid, which come into contact with gut derived pathogenic microbes, microbial debris and senescent erythrocytes and impede the exogenous materials by producing cytokines and directly phagocytosing and degrading them [23].

II. MATERIAL AND METHODOLOGY

2.1. Materials

Acyclovir (Mediflora pharmacy, Gurgaon), Bovine Serum albumin (Central drug house Ltd. New Delhi), glutaraldehyde (25%), methanol, trypsin, sodium chloride (Central drug house Ltd. New Delhi).

2.1.1. Preparation of BSA Nanoparticles by Desolvation Method

BSA nanoparticles were prepared by a desolvation technique. The different amount of bovine serum albumin (i.e. 50, 150, 250, 350) was dissolved in sufficient amount of 0.9% Nacl, titrated to pH 7-10. The drug was added which transformed into nanoparticles by the continuous addition of 8.0 ml of the desolving agent ethanol under the stirring (500 rpm) at room temperature. After the desolvation process, few drops of 2% glutaraldehyde were added to induce particle cross linking. The cross linking process was performed under stirring of the suspension over a time period of 24 h.
2.1.2. Coating of Nanoparticles

20 mg of gelatin was added to 10 mg of bovine serum albumin loaded nanoparticles which is dispersed in 5ml acidic phosphate buffer saline (pH 5.0) and the mixture was then stirred at room temperature overnight. The resulting nanoparticles were purified by three cycles of differential centrifugation and followed by redispersion of pellet to the original volume in NaCl. Each redispersion step was performed in an ultrasonication bath over 5 min. The solvent was evaporated and the nanoparticles were collected and stored in refrigerator at 2-8°C

2.2. Characterization of Nanoparticles

2.2.1. Shape and Size

Morphology and size of gelatin coated nanoparticles was determined by scanning electron microscopy (SEM).

2.2.2. Zeta Potential

The zeta potential and surface charge of nanoparticle was determined by the zeta potential analyzer. The zeta potential of nanoparticle is commonly used to characterize the surface charge property of nanoparticles.

2.2.3. Percentage Yield

It is calculated to know about the efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of nanoparticles recovered from each batch in relation to the sum of starting material.

2.2.4. Entrapment Efficiency

200 mg of nanoparticles were crushed in mortar and pestle. 10 mg powder were taken and introduced in 10 ml volumetric flask and volume makeup by phosphate buffer pH 7.4. 1 ml of above solution was taken and dilute up to 10 ml. The above solution was analyzed in U.V. spectrum at 250 nm. The entrapment efficiency of the prepared nanoparticle was calculated.

2.2.5. In Vitro Drug Release

In vitro drug release study was carried out by modified diffusion apparatus consists of beaker containing 50 ml of phosphate buffer pH 7.4.

2.3. Receptor Ligand Binding Study

Goat liver was purchased from shaulter house, liver was excised and liver was washed twice with saline phosphate buffer. The organ was minced into small pieces and digested using 30 ml of 1mg/ml trypsin at room temperature. After putting it aside for 30 min the liver was homogenate and filtered to remove undigested tissue fragment. Filtrate was transferred into centrifuge tubes and centrifuge twice for 5 min at 300 rpm to wash out the residual enzymatic solution. The supernatant was discarded and pellet was resuspended and differential centrifuge was performed to separate non parenchymal from parenchymal cells. After centrifuged cell suspension was kept aside for 3 min .the supernatant was put into another tube .finally the cell suspension was centrifuged at 300 rpm for 5 min and supernatant was discarded and the pellet was redispersed in buffer solution. 10 mg of nanoparticles were added into the above final solution and kept aside for 1 hr. the solution was filter after 30 min and filtrate was diluted and analyzed by UV spectrometer at 250 nm.

III. RESULTS

3.1. Particle size

The SEM photomicrographs of nanoparticle are shown in Fig, shows the scanning electron microscopy of albumin nanoparticle at different magnification and area. The particle size of plane nanoparticle was found to be in the size
range of 200-800 nm. And that of gelatin coated nanoparticles were in the range of 300-900. It was observed from these photomicrographs that all samples of particles forms small cluster and are sub spherical in shape.

Fig1. A. Scanning electron microscopy (SEM) photomicrograph of albumin nanoparticles at 20µm area at 531X

Fig2. C. Scanning electron microscopy (SEM) photomicrograph of gelatin coated albumin nanoparticles at 1 µm area at 5X
D. Scanning electron microscopy (SEM) photomicrograph of gelatin coated albumin nanoparticles at 100 µm area at 329X

3.2. Zeta Potential

The graph of zeta potential of gelatin coated nanoparticles is shown in Fig 6.10. The zeta potential of nanoparticle was found to be -59.3. The gelatin coated nano particle shows the value of zeta potential -59.3, which shows that the nanoparticle having good stability as per table 6.7 and the negative charge of the zeta potential shows that the gelatin coating will enhance the phagocytosing property of nanoparticle.

![Zeta Potential Graph]

Fig3. zeta Potential of gelatin coated nanoparticle

3.3. Percentage Yield

The percentage yield of different formulation F1 to F4 was calculated and the yield was found to be in range of 19.10±0.79 to 51.13±0.53%. Percentage yield of all batches is shown in table 6.10. The maximum percentage yield was found to be 51.13±0.53% for formulation F4, where the concentration of albumin is highest while nanoparticles yield is lowest in F1 19.10±0.79 as the concentration of albumin is lowest.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Total amount of ingredients (mg)</th>
<th>Percentage yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>570</td>
<td>19.10±0.79</td>
</tr>
<tr>
<td>F2</td>
<td>670</td>
<td>21.10±0.30</td>
</tr>
<tr>
<td>F3</td>
<td>770</td>
<td>34.28±0.83</td>
</tr>
<tr>
<td>F4</td>
<td>870</td>
<td>51.13±0.53</td>
</tr>
</tbody>
</table>

3.4. Entrapment efficiency

The encapsulation efficiencies of all four formulations were given in table 6.9 and the entrapment efficiency was
found to be in range of 72.34±0.45 to 86.78±0.5 %. The maximum entrapment efficiency was found to be 86.78±0.5 % for the formulation F3. The entrapment efficiency increases with increasing polymer concentration but up to a certain ratio.

**Table3. Entrapment efficiency of gelatin coated acyclovir nanoparticle. (for n=3)**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Entrapment efficiency (%) ±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>72.34±0.45</td>
</tr>
<tr>
<td>F2</td>
<td>78.33±0.76</td>
</tr>
<tr>
<td>F3</td>
<td>86.78±0.5</td>
</tr>
<tr>
<td>F4</td>
<td>70.34±0.38</td>
</tr>
</tbody>
</table>

3.5. In-vitro drug release

*In vitro* drug release study was carried out by modified diffusion apparatus using egg membrane, consists of beaker containing 50 ml of phosphate buffer pH 7.4. The dissolution study was done on all four formulations of gelatin coated nanoparticles using phosphate buffer pH 7.4 as dissolution media. *In vitro* dissolution was carried by egg membrane modified method. The readings of *in-vitro* permeation studies are given in Table. *In vitro* release is done to see how drug behaves *in-vivo*. The cumulative release of drug was determined up to 8 hrs for formulation F1,F2,F3,F4 was 62.48,51.67,48.34,34.98 respectively.

![Fig3. Zero order release plot of gelatin coated nanoparticles](image)

![Fig4. First order release plot of gelatin coated nanoparticle](image)
IV. MATHEMATICAL MODELING

The data obtained from in-vitro release study was treated by various convectional mathematical models to determine the release mechanism from designed nano particle formulations. Selection of suitable release model was based on values of R (correlation coefficient), k (release constant) and n (diffusion exponent) obtained from curve fitting of release data. The drug release mechanism and kinetics are two important characteristics of delivery system for the drug dissolution profile based on mathematical models.

Mathematical Models are:

- Zero order model
- First order model
- Higuchi’s square root of time equation model
- Korsmeyer-peppas power law equation model
The data obtained from in-vitro release study was treated by various mathematical models (zero order, first order, higuchi and korsmeyer peppa’s) to determine the release mechanism from designed nanoparticle formulation. Selection of a suitable release model was based on the values of R (correlation coefficient) and k (release constant) and n (diffusion exponent) obtained from curve fitting of release data.

It was found that all formulations follow first order kinetics.

The regression coefficient for all formulation of Higuchi plot was found to be almost linear, suggesting that the formulation was diffusion controlled. Korsmeyer peppas model was used when the release mechanism is not well known or when more than one type of phenomenon is involved. The value of n is used to characterize the different release mechanism. The value of n for F1 to F4 formulation is found in between 0.65 to 0.81, that all the formulation indicates that the release is non-Fickian diffusion method.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Regression coefficient(R²)</th>
<th>Slope (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>zero order</td>
<td>first order</td>
</tr>
<tr>
<td>F1</td>
<td>0.987</td>
<td>0.994</td>
</tr>
<tr>
<td>F2</td>
<td>0.981</td>
<td>0.996</td>
</tr>
<tr>
<td>F3</td>
<td>0.987</td>
<td>0.995</td>
</tr>
<tr>
<td>F4</td>
<td>0.972</td>
<td>0.973</td>
</tr>
</tbody>
</table>

V. RECEPTOR LIGAND BINDING STUDY

By considering the absorbance of formulation F3 at U.V. (250 nm) it was found that the receptor ligand binding is 95.89 %. And it was confirmed by checking the amount of drug release from formulation is 3.89% while prior it was 48.34%, so the remaining drug was bind with receptor cells.

VI. DISCUSSION

In the present study, an attempt was made to develop gelatin coated albumin nanoparticles of acyclovir for the treatment of visceral leishmaniasis with a view to see the targeting of nanoparticles at the required site for action and helps to provide sustain release and thus reduces the dose frequency and increase the patient compliance. Nanoparticles were successfully prepared by desolvation method. The method was able to produce the free flowing nanoparticle. Bovine serum albumin is a biodegradable polymer for preparing targeted nanoparticle. FTIR studies were carried out to find out the possible interactions between the drug and polymer. The study revealed that there was no interaction between the selected drug and polymer. The particle size analysis revealed that the particle size was found to be in the size range of 200-800 nm, that comes under nano range. The zeta potential of nanoparticle was found to be -59.3, which shows that the nanoparticle having good stability, the negative charge of the zeta potential shows that the gelatin coating will enhance the phagocytosing property of nanoparticle. The maximum entrapment efficiency was found to be 86.78±0.5 %. For the formulation F3. The entrapment efficiency increases with increasing polymer concentration but up to a certain ratio. The maximum entrapment efficiency was found to be 86.78±0.5 % for the formulation F3. The entrapment efficiency increases with increasing polymer concentration but up to a certain ratio. The maximum percentage yield was found to be 51.13±0.53% for formulation F4, where the concentration of albumin is highest while nanoparticles yield is lowest in F1 19.10±0.79 as the concentration of albumin is lowest. In vitro release shown as, cumulative release of drug was determined up to 8 hrs for formulation F1, F2, F3, F4 was 62.48, 51.67, 48.34, 34.98 respectively. From result it was concluded that on increasing the concentration of polymer release rate of formulation decreased. The receptor ligand binding is 95.89 %. And it was confirmed by checking the amount of drug release from formulation is 3.89% while prior it was 48.34%, so the remaining drug was bind with receptor cells.
VII. ACKNOWLEDGEMENTS

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