Amphotericin-B loaded Chitosan Nanoparticles Intended for Oral Delivery: Biodistribution, Pharmacokinetic and Scintigraphy Study in Rabbit Model

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ABSTRACT
The objective of this work was to explore the targeting efficiency of Amphotericin B (AmpB) following oral administration by introducing chitosan (CH) nanoparticles (NPs) as a delivery system. The AmpB loaded chitosan nanoparticles (CHNPs) were prepared by ionic gelation of CH with tripolyphosphate anions and had a mean size, zeta potential, loading capacity and entrapment efficiency of 440±7 nm, +22 ± .2 mV, 9.02% ± .07% and 58% respectively. The strong positive charge at the NP’s surface suggests that the proposed delivery system has interaction with the mucus membrane of intestine and absorbs the drug AmpB at intestinal site after oral administration. For the successful oral delivery of AmpB, biodistribution, pharmacokinetic and scintigraphy study were performed in rabbits. Biodistribution of Amp-ChNP formulation in the rabbit’s blood following oral administration was performed using optimized technetium labeled (99mTclabeled) AmpB formulation. The high values in stomach and intestine for AmpB loaded CHNPs are suggestive that the major part of the drug was reaching in the gastrointestinal tract (GIT). Gamma scintigraphy imaging of rabbit following oral administration were performed to determine the localization of drug in body. The results clearly suggest that the formulation remains for a longer period of time in GIT which initially was in the anterior part of GIT and later migrates to the posterior part of GIT. These encouraging results confirmed the development of an oral delivery system of AmpB which bypass the side effects occurring from the traditional parenteral delivery system of the antibiotic AmpB.

KEYWORDS
Amphotericin B, Chitosan, Bovine Serum Albumin, Tripolyphosphate, AmpB loaded Chitosan Nanoparticles, Dimethyl sulfoxide, Polydispersity Index, Oral delivery.

1. INTRODUCTION
The sustained release of drugs is obtained by drug delivery systems. Ionic cross-linking of chitosan can be used for drug delivery by the association with tripolyphosphate (TPP) [1-4]. The poly peptides can be successfully delivered [5-10] by using CH nanoparticles (NPs) which have high loading capacity, adsorption to GIT, long shelf life etc. AmpB was isolated from Streptomyces nodosus in 1955, is the classical broad-spectrum agent for systemic fungal infections and it remains the gold standard therapy for fungal infections. However, the poor water solubility of AmpB limits its application [11].

The present study aims in the development of oral formulation with CH as a polymer with bovine serum albumin as stabilizer, along with TPP as a poly anion cross linking agent. Biodistribution, Pharmacokinetic and Scintigraphy studies are done for AmpB loaded CHNPs which shows that this type of formulation is potential and promising carrier for the delivery of AmpB drug.

Rest of the paper is organized as follows. Section 2 discusses material and methods while section 3 presents the results. Discussion and conclusions are drawn in section 4 and 5 respectively.
2. MATERIALS AND METHODS

2.1. Materials
AmpB was supplied by Synbiotics Ltd. (Vadodara, Gujarat- India) while Chitosan and tripolyphosphate (TPP) were obtained from Sigma–Aldrich (MO, USA). Bovine Serum Albumin, Di Methyl Sulfoxide (DMSO) and Glacial Acetic Acid were purchased from Central Drug House (CDH) Analytical Reagent (New Delhi, India).

2.2. Animals
The animals have been provided by the Institute of Nuclear Medicine & Allied Sciences, Delhi, India. All the experiments have been done according to the Govt. of India guidelines.

2.3. Preparation of AmpB loaded chitosan nanoparticles
CHNPs were fabricated by using Ionic gelatin process. Firstly CH solution was prepared in acetic acid solution and later dissolved in BSA. TPP was then dissolved in distilled water, added with the solution containing BSA and then AmpB drug was dissolved to finally form NPs.

2.4. Physicochemical characterization
The physicochemical characterization was done by determining diameters, PDI s, zeta potential and morphology of NPs.

2.5. Preparation of radiolabeled formulations
Radiolabeled 99mTc-pertechnetate AmpB loaded CHNPs were prepared by AmpB formulation in glass vial with stannous chloride as reducing agent. The radio labeling efficiency was then estimated [13].

2.6. Radiolabeling efficiency determination and in vitro stability
The thin layer chromatography method was used for estimating the radiolabeling efficiency. Here silica gel (ITLC–SG) strips was used as stationary phase while acetone was used as mobile phase. The radiolabeling efficiency was determined according to equation [14]

\[
\%\text{Radioactivity/g of tissue} = \frac{\text{Radioactivity counts retained in the lower half of the strip}}{\text{Initial radioactivity associated (total count present with strip)}} \times 100
\]

2.7. In-vivo studies

2.7.1 Biodistribution and pharmacokinetics studies
Radiolabeled drug formulation containing AmpB was administered to rabbits and radioactivity was measured.

2.7.2 Gamma scintigraphy imaging
The localization of drug in different organs was studied using Gamma scintigraphy imaging and images following oral administration of radiolabeled AmpB loaded CH NPs were recorded.

2.7.3 Statistical analysis
It is done with the help of software named Graph pad prism 3.0. The values were significant for probability less than 0.05 while a difference of 0.01 was considered highly significant.

3. Results

3.1. Preparation
CHNPs (AmpB-CHNPs) were prepared in different proportions by using Ionic gelatin process having cationic core composed of CH polymer and BSA as aqueous stabilizer while TPP was used as a polyanion cross linking agent. The formulations were optimized on the basis of size, PDI and zeta-potential of NP dispersions. It is observed that by the incorporation of the increased ratio of cationic CH polymer, the size of the intermediate NPs decreases and charge migrates towards positive. A 1:0.5:0.5 ratio of drug with polymer and stabilizer was used to prepare the hybrid NPs. The results are mentioned in Table 1 while Fig. 1 shows the TEM image.
Table 1: Particle characterization

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zeta potential (mV)</th>
<th>Size (nm)</th>
<th>Poly dispersity index (PDI)</th>
<th>Encapsulation eff. (% wt/wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmpB-CHNPs</td>
<td>22±0.2</td>
<td>440±7</td>
<td>0.4±0.01</td>
<td>58</td>
</tr>
</tbody>
</table>

3.2. Preparation and optimization of radiolabeled formulations

When AmpB loaded CHNPs were labeled with 99mTc-pertechnetate, it exhibited 78% radio labeling efficiency as shown in Table 2. The pH of the radiolabeled formulations was found up to 4.2 of free 99mTc-pertechnetate for AmpB loaded CHNPs as determined by ITLC-SG and shown in Table 2. The labeled formulation were found to be stable in normal saline solution up to 24 h (degradation <10% w/w) as shown in Table 3.

Table 2: Radiolabeling Parameters for AmpB formulation

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Concentration of SnCl₂, 2H₂O (µg)</th>
<th>pH</th>
<th>Labeling efficiency (%)</th>
<th>Reduced /hydrolyzed (%)</th>
<th>Free 99mTc-pertechnetate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>300</td>
<td>4.2</td>
<td>78</td>
<td>0.7±0.08</td>
<td>6.3±0.7</td>
</tr>
</tbody>
</table>

Table 3: In-vitro stability of 99mTc–AmpB loaded CHNPs in saline at different time intervals.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Radiolabeling efficiency (%) in saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
</tr>
<tr>
<td>8</td>
<td>65</td>
</tr>
<tr>
<td>24</td>
<td>60</td>
</tr>
</tbody>
</table>

Thus these formulations were found to be suitable for conducting biodistribution and pharmacokinetic studies of the drug in rabbit.

3.3. Biodistribution and pharmacokinetic study

Biodistribution studies of 99mTc–AmpB formulation in organ of interest were observed and the radioactivity was estimated (Fig. 2). The results reveal that AmpB loaded CHNPs administered orally showed larger accumulation of AmpB in stomach and intestine as compared to other tissues. The blood concentrations of

Fig. 1 TEM image of AmpB-CHNPs
AmpB versus time following administration are shown in Fig. 3. When AmpB loaded CH NPs was administered orally the $C_{\text{max}}$ (3.2) %/g are reached at 4 h in blood (Fig. 3), followed by an exponential decline depending on the time.

![Fig. 2 Biodistribution studies on organ of interest with AmpB loaded CHNPs](image)

![Fig. 3 Mean blood concentration-time curve of AmpB in Rabbit](image)

The results of selected pharmacokinetic parameters like $C_{\text{max}}$, $T_{\text{max}}$, $K_e$, half-life, and AUC, by oral administration were calculated from the blood concentration versus time- profiles from the pharmacokinetic experiment and are shown in Table 4. The $t_{\text{max}}$ of AmpB loaded CHNPs was attained at 4 h which conveys that the drug has taken 4 h to reach maximum concentration in blood, and was further lowered and thus shows a better existence of drug in blood upto 10 h.

![Table 4 Pharmacokinetic parameters of AmpB-CH NPs formulation after oral administration in rabbit](image)
4. Discussion

CH nanoparticulate systems have been developed for the complex deliverable drug like Amp-B which has issues like poor absorption from GIT. In the present research work (CHNPs consisting TPP and BSA), CH-tripolyphosphate (CH-TPP) NP’s have been used to encapsulate AmpB. The CHNPs were developed by adding a cross-linking agent, i.e. tripolyphosphate (TPP), into the aqueous phase containing CH. CH-TPP nanoparticles with entrapped AmpB have been found to be better delivery vehicle with BSA as stabilizer. The HRTEM images (Fig. 1) revealed that the fabricated polymeric NPs have spherical morphology and were well dispersed. These studies have proven that the drug remains at the anterior part of body in the initial period of drug administration and later migrates into the posterior part of body. The results have proved that upto 10h the formulation remains in the intestine part and showed its maximum value over there. The studies clearly revealed that the formulation becomes maximum available to the GIT and hence the objective of oral absorption has been achieved.

However the value of $C_{\text{max}}$ is very high which may be due to the reason that drug is localized in the chitosan and taking more time to diffuse through the shell of polymer. The high size of NPs may also be the reason for the high $C_{\text{max}}$ value. Moreover the drug is absorbing with slow rate at the target site which may be due to the intercalation of drug within the NPs and it is also not eliminating as it should be for the ideal case. This necessitates for further improvement in experimental ratio of drug with polymer and stabilizer.

5. Conclusions

The development of nanoparticulate delivery system for amphotericin B using ionic gelation technique has been done in the proposed work. A fruitful approach combining the polymer, polyanion cross linker and aqueous stabilizer has improved the oral bioavailability of AmpB. CH loaded NPs demonstrated the safe and effective use of AmpB for oral administration. All the obtained results reveals good safety profile of AmpB-CHNPs and the therapeutic outcome of the proposed formulation make it more prominent than existing AmpB-formulations.

Acknowledgement

The authors would like to thank Institute of Nuclear Medicine & Allied Sciences, Delhi, India for conducting the scintigraphy study and All India Institute of Medical Sciences, New Delhi India for providing the support in doing TEM study.

References