FORMULATION AND EVALUATION OF CHITOSAN NANOPARTICLE LOADED WITH PHYLLANTHIN

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Abstract:
Objective: The aim of the work was to formulate and evaluate chitosan nanoparticle loaded with Phyllanthin.
Methods: Chitosan nanoparticle loaded with phyllanthin were prepared by ionic chelation method using chitosan solution, prepared by dissolving chitosan flakes in 1.5% glacial acetic acid to this STPP (0.10 to 0.60 %/V), phyllanthin and was added to chitosan solution. The prepared chitosan nanoparticle loaded with phyllanthin from all batches (F1-F6) were characterized for its morphology, particle size, drug loading capacity, entrapment efficiency, invitro release study, stability study.
The chitosan nanoparticle have an average particle size of 1.0 ± 5.0µm. The % entrapment efficiency of phyllanthin was found to be 34.4-74.5% w/w. The % drug loading capacity was 34-60.5% w/w. The drug content in chitosan nanoparticle was found to be 69-80.5% w/w. The invitro release studies of all the formulations F1-F6 was carried out and result have shows that be F6 were most stable formulation and good release.
Conclusion: The evaluation results have shown that formulation F6 was found to be more stable with good release characteristics invitro and have good bioavailability.
Keywords: Nanoparticle, Phyllanthus niruri, Chitosan, Ionic chelation

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Please cite this article in press Praseena K and Junise.V., Formulation and Evaluation of Chitosan Nanoparticle Loaded With Phyllanthin, Indo Am. J. P. Sci, 2018; 05(04).
INTRODUCTION:
Nanotechnology is the emerging field in medicine which are expected to elicit significant therapeutic benefits. The attempt were made to reformulate add new technologies to existing blockbuster drugs for getting a positive outcome which could give better therapeutic outcomes [1].

In phyto-formulation research, developing nano dosage forms with phytoconstituents have several advantages, which include enhance solubility and bioavailability, avoid toxicity toxicity, increase pharmacological activity,good stability, improve tissue macrophages distribution, avoid frequent dosing and finally protection from physical and chemical degradation.Preparing nanoparticles various techniques are involved.[2] The standardization of herbal drug are very crucial for proving the safety and quality based on the concentration of the active moiety. The drug manufactures and research organizations dealing with phytoconstituents are required to perform the quality check of phytoconstituents for established the therapeutic efficacy.[3]

Here we have tried to formulate and evaluate chitosan nanoparticle loaded with phyllanthin. Phyllanthin is the major active phytoconstituent of Phyllanthus niruri. The whole parts of P. niruri have reported to have various therapeutic activities. The nanoparticle approach will release the drug in a controlled fashion and also can be used for site specific drug delivery [4].

The chitosan nanoparticle loaded with phyllanthin was prepared by ionotropic gelation method, using chitosan as the polymer and STPP as the crosslinker, the phyllanthin was dissolve directly to the chitosan solution. Phyllanthin loaded nanoparticle evaluated for invitro release.[5]

MATERIALS AND METHODS:
MATERIALS
Phyllanthus niruri plant was collected from local areas of Calicut, Kerala and the extract was prepared. Chitosan, Sodium tripolyphosphate were procured from LOBA Chemie Labotaratory Mumbai. Glacial acetic acid, Hexane, Ethyl acetate, Conc. Sulphuric acid, Hydrochloric acid, Sodium hydroxide, Nitricacid, Pyridine, Sodium nitroprusside, Sodium picrate, Lead acetate, Copper sulphate purchased from (Nice chemicals pvt. Ltd Coimbatore.)

Preparation of extracts
The phyllanthus niruri extract was prepared by continuous soxhlet extraction using successive solvents based on their increasing polarity. 50gms of powdered drug was placed inside a thimble made of thick filter paper, and was loaded into the Soxhlet extractor. The solvent pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the soxhlet chamber and was heated to boil. The extraction was carried out continuously until the colour of the solvent changes. The extracts was concentrated after recovering the solvents. The phyllanthus niruri extract was prepared with successive solvents with increase in polarity namely hexane, ethyl acetate, methanol, water. The prepared extracts were subjected to qualitative test for identify various phytochemical constituents as per standard procedures.[6]

The isolation of phytoconstituent from phyllanthus niruri was carried out by chromatographic method.[7]

Compatibility study by FTIR
The compatibility study was carried out to find any interactions between the herbal drug and polymer. The samples were placed in IR sample holding window. The spectra were scanned for with individual drug, polymer and its combination. These spectra were compared and interpreted for shifting of major functional peaks and disappearance or appearance of new functional peaks. FTIR spectra of samples were carried out using FTIR spectrophotometer (Shimadzu FTIR 8400S, Japan) by comparing the standard functional frequencies.[8]

Formulation of Herbal Nanoparticle
The Chitosan nanoparticles loaded with phyllanthin was prepared by Ionic gelation method. The ionic gelation is an ionic interaction between positively charged chitosan solution and negatively charged TPP solution. The Chitosan solution was prepared by dissolving chitosan flakes of concentration 0.10 to 0.60% w/v 1.5% in glacial acetic acid solution, and STPP as a cross linker with concentration 0.10 to 0.60% w/v dissolved in distilled water. The extract was dissolved directly in chitosan solution. To this TPP solution was added in drop and stirred at 1000 rpm on a stirrer at room temperature. The resulting mixture sonicated for 20 minute. The resulting suspension was subsequently centrifuged at 15000 rpm for 10 min. The pellets obtained were re-suspended in deionised water by sonication, centrifuged and dried at room temperature (about 25 °C).[9]
Table 1: Different batches of phyllanthin loaded chitosan nanoparticle

<table>
<thead>
<tr>
<th>SL NO</th>
<th>INGREDIENTS</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Phyllanthin (w/v)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2.</td>
<td>Chitosan (% w/v)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>3.</td>
<td>STPP (% w/v)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>4.</td>
<td>Glacial acetic acid (% v/v)</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Entrapment efficiency**
The entrapment efficiency was determined by measuring the concentration of free drug in the dispersion medium. The free drug was determined by adding 2ml of the nano suspension to 8ml water for dissolving the free drug. The resulting suspension was centrifuged for 90 min at 15,000 rpm. The supernatants were examined by UV spectrophotometer. The amount of free drug was detected in the supernatant. The entrapment efficiency was calculated by subtracting initial drug from the free drug. [12]

**In vitro drug release study**
The invitro drug release study was carried out in phosphate buffer pH 6.8 in dialysis bag method using dialysis membrane of 12,000-14,000 molecular weight. The membrane was washed with warm double distilled water (70°) for 1 h and then rinsed thrice with water to remove the glycerin. 5ml of suspension was placed inside the dialysis bag, tied at both ends and dipped in a receptor compartment containing the dissolution medium. The medium was stirred at 100 rpm using a magnetic bead at temperature at 37±0.2°. 2ml aliquot were withdrawn at preset time intervals and replaced by an equal volume of a fresh dissolution medium. The samples were analyzed spectrophotometrically at 254 nm. The concentration of drugs in test samples were measured by UV spectrophotometer and calculated by using the regression equation of the calibration curve.[13]

**DLS and zeta potential**
The average hydrodynamic diameter and the the polydispersity index of drug loaded nanoparticles were determined by non-invasive Scatter technology (DLS)(Zetasizer, modelo ZS, Malvern Instruments, UK). The zeta potential was measured in duplicate with laser Doppler velocimetry at 25°C, the nanoparticles were concentrated at 0.5 mg/ml with deionized distilled water. The nanoparticulate suspension was added to the sample dispersion holder which was stirred to minimize the particle aggregation by inter particle aggregations. The analysis were performed thrice and average hydrodynamic particle size was expressed as the value of z-average size ± SD. The width of size distribution was indicated by the poly dispersion index. Distilled water was used as the dispersion medium. [14]

**Measurement of pH**
The pH of nanoparticle formulations were determined by using digital pH meter. 10ml of suspension was checked after calibrating the pH meter. The measurement of pH of each formulation was done in triplicate and average values was calculated.[15]

**Stability studies**
The stability studies were carried out on formulations according to International Conference on Harmonization (ICH) guidelines. Short term accelerated stability study was carried out for a period of 45 days for the formulations. The stability
study was carried out to assess the stability of chitosan nanoparticles containing the drugs. The samples were taken in borosilicate sealed glass vials. The vials were then stored at room temperature 25±2°C /60% RH and 40±2°C /70±5% RH, over a period of 3 months instability chamber and the samples were withdrawn at 0, 1, 2 and 3 months to analyse the drug content and any change physical appearance. 

**RESULTS AND DISCUSSION:**

**Incompatibility studies**

The compatibility studies were carried out and the result have shown that the FTIR spectra analysis did not show any interactions between drug and the polymer.

**Drug content of P. niruri in nanoparticle**

The percentage drug content of chitosan nanoparticle loaded with phyllanthin was found to be 69% to 80.5%. The maximum drug content was shown by F6 formulation.

**Entrapment efficiency of P. niruri in nanoparticle at $\lambda_{max}$-254nm**

The entrapment efficiency of the prepared formulation F1 to F6 was found to be 34.4% to 74.5% respectively. The maximum entrapment efficiency was shown by F6 formulation.

**DLS and zeta potential of formulation F6**

The average particle diameter of drug loaded nanoparticle was found to be 479.9 nm using DLS and the zeta potential was found to be 30.2 mV. The zeta potential was found to increase with the particles surface charge also will increase. The results have also shows that the zeta potential was found to get increase with increase in particle surface charge.

**Morphology of formulation F6**

The morphology of phyllanthin loaded chitosan nanoparticle was studied by SEM and results have revealed that the particle were in nano scale range with a smooth surface.
In vitro drug release study
The percentage cumulative drug release of chitosan nanoparticle loaded with phyllanthin of all formulation were noted. The maximum drug release showed by F6 that was 74.80%

Table 2: Regression co-efficient ($r^2$) values of kinetic models for formulations F6

<table>
<thead>
<tr>
<th>Formulation</th>
<th>KINETIC DRUG RELEASE</th>
<th>MECHANISM OF RELEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero Order</td>
<td>First Order</td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient ($r^2$)</td>
<td>Correlation coefficient ($r^2$)</td>
</tr>
<tr>
<td>F6</td>
<td>0.920</td>
<td>0.968</td>
</tr>
</tbody>
</table>
The results obtained from in vitro release studies of the selected formulations, F6 were plotted in different kinetic models. Regression coefficient (r^2) values of kinetic models of formulations are shown in the table 2. The result have suggested that the release data and best fit with Higuchi model kinetics. Higuchi equation explains the diffusion release mechanism, so formulations follow the diffusion mechanism of drug release.

**Stability studies**
The stability and chemical interaction of the chitosan nanoparticles loaded with phyllanthin was carried. The result have shown no change in physical appearance. The total drug content in the formulations was determined at time 0, 1, 2 and 3 months of storage at room temperature (25-30°C), refrigerator temperature (3-5°C) and 40°C with relative humidity of 75%. The results indicated that the formulated chitosan nanoparticles loaded with phyllanthin were physically and chemically stable.

**CONCLUSION:**
The research findings have concluded that all formulations F1-F6 evaluated. All formulation F1-F6 have an average nano size of 1.0 ± 5.0µm. The % entrapment efficiency range of 34.4 - 74.5%w/w. The % drug loading capacity range of 34% - 60.5%w/w. Formula F6 shows maximum results. The invitro release studies were conducted; maximum release was shown by F6, which follows Higuchi diffusion model. Stability studies revealed that there was no significant difference in the physical and chemical parameters. It was finally concluded that the formulations F6 was found to be more promising formulation as it shows better physicochemical characteristics compared to other formulations.

**ACKNOWLEDGEMENT:**
The authors gratefully thanks to Dr. Sujith Varma, associate Professor in National college of pharmacy Calicut and also gratefully acknowledge the support for the study by Faculty of Al shifa college of pharmacy, Botany department of University of Calicut.

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