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Preparation and characterization of doxorubicin HCl loadedchitosan nanoparticles by w/o emulsion method

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Abstract

Controlled and specific medication delivery using polymeric nanoparticles has received considerable attention lately. The goal of this research was to create doxorubicin-loaded chitosan nanoparticles and test their efficacy. Nanoparticles lacked aggregation, according to scanning electron microscopy. There was no evidence of a chemical reaction between the medication and polymer in the FTIR spectra. Research conducted in vitro on medication release has provided evidence that it is possible to prolong drug release. Drug release studies demonstrate the prolonged release after vigorous agitation produces nano-sized particles.

Key-Words: Nano particles, FTIR, SEM, Agglomeration

Introduction

Recently, there has been a lot of focus on improving medication delivery via the creation of colloidal carrier systems. There has been a great deal of study dedicated to the use of nanoparticles in the pharmaceutical industry in recent decades.1 Polymeric nanoparticles have received the greatest attention among carriers. Because of their simple manufacturing, biodegradability, ability to modify bio distribution, and decreased toxicity, chitosan nanoparticles have lately received attention in targeted medication administration. These particles' ability to bind to negatively charged cell membranes and, maybe, to be taken up by cells, is promising for the treatment of solid malignancies. When considering intravenous delivery, it's important to consider how positively and negatively charged particles will interact with various components of the blood. Doxorubicin administration via the mucosa is an attractive method because of the possibility that these modifications would modify the drug's biodistribution and/or organ membranes' interactions with cells and/or membranes.2 Chitosan has showed excellent biocompatible feature 3 and breakdown by lysosome enzyme in serum.4 The current investigation used a no-emulsion approach to producing nanoparticles.

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Material and Methods

Materials

Doxorubicin HCL was supplied as a gift sample from Khandelwal Laboratory Ltd., Mumbai, India. Chitosan was gifted from Central Institute of Fisheries Technology (Cochin, India), Sodium tripolyphosphate (TPP) was purchased from National Chemicals, Vadodara. Span 20 and Hexane – LR was purchased from S.D. Fine chemicals Ltd., Mumbai. Acetic acid glacial was purchased from Allied chemicals, Vadodara.

Preparation of Nanoparticles

Preparation of plain nanoparticles

The preparation had been done in two steps as follows: Step – I: In 25 ml liquid paraffin 5% w/v span 20 was added and stirred under magnetic stirrer for 15-20 min.1% w/v solution of chitosan was added drop wise intoabove solution under the Ultra Turrax at 9500 rpm for5 min.

Step II: 0.5 ml of 0.5%w/v solution of sodium TPP wasadded drop wise in it under the UT-at the speed of 9500 rpm for 5 min that is plain Nanoparticles.

Preparation of Drug loaded nanoparticles Doxorubicin HCL (30% w/w Drug: Polymer) is dissolved in distilled water added into Step I, then theprocedure for step II was followed.

Characterization of Nanoparticles Particle size analysis

Particle size measurement was carried out by laser scattering technique using Malvern Hydro 2000 SM particle size analyzer (Malvern Instruments, UK).

Aqueous Nanoparticulate dispersion was added to the sample dispersion unit containing stirrer and stirred in order to minimize inter particle interactions, the laser obscuration range was maintained between 10-2-%. The analysis was performed thrice and average values were taken.

The number of particles (Np) was calculated by the following equation

$$Np = 6\ M0\ Xm/\pi\ p\ Dn^3$$

Where Dn is a number average diameter of the polymer particles obtained from dynamic light scattering, Xm fractional conversion, p is the density of the polymer in g/cm^3 and Np a number of particles/cm^3.

% Entrapment [5]

The entrapment was found out using Gel chromatography. 400 mg Sephadex G-25 was added in 10 ml distilled water and was soaked for 30 min for swelling. 0.4 µ filter was kept at the bottom of the syringe. The swollen Sephadex G- 25 was packed in column (2.0 ml syringe). Nanoparticulate dispersion was added in syringe (5 mm diameter), amount of elute was measured, and optimize the volume require to saturate the column. The length of the column was kept constant. Nanosuspension was passed through the column. Eluted Nanosuspension was subjected to 0.1 N HCl and was kept overnight. It was filtered and filtrate was taken for analysis. The analysis was done using spectrophotometer at λ = 480 nm. The % entrapment was found out using the following formula.

$$\% \text{ entrapment} = \frac{B-A}{\text{Total amount of drug added}} \times 100$$

B-A = Amount of drug entrapped

Scanning electron microscopy[6]

Scanning electron microscopy studies was done for Nanoparticles. The aim was to study the particle size, shape and surface characteristics. The lyophilized powder was then visualized under a Scanning Electron Microscope (JSM 5610 LV, SEM, JEOL, DATUM,LTD, JAPAN).

Differential Scanning calorimetry (DSC) [7]

DSC analysis of the nanoparticles was carried out in Mettler Toledo differential Scanning calorimeter (Mettler Toledo, Switzerland) at heating rate of 10ºC/minute in the range of 50ºC to 250º C.

Infrared (IR) spectrum[8]

The IR spectra of the formulations were carried out for Nanoparticles as well for chitosan and doxorubicin hydrochloride in Shimadzu, FTIR, at Vaibhav Analytical Lab., Ahmedabad., at the wavelength ranging from 400 cm^{-1} to 4000 cm^{-1}

Nanoparticle drug release kinetics research, 9, 10.

Bags for dialysis are prepared.

Dialysis cellulose bag (Cutt off 12000 Hi Media) soaked in phosphate-buffered saline (PBS) overnight.

Dialysis equipment was prepared by carefully opening the wet sac, washing it well with PBS, filling it with PBS, and checking it for leaks. Next, 1 ml of the Nanosuspension under study was carefully placed into the empty sac, which served as the donor area. The sac was checked for leaks once more before being suspended in 20 ml of phosphate-buffered saline (PBS) in a glass beaker. During the experiment, the beaker's contents were swirled with a Teflon-coated bar magnet, and the beaker was covered with aluminum foil to avoid loss of liquid due to evaporation.

Sampling

Aliquots of 1 ml were taken from the receptor section at regular intervals for testing. The receptor

compartment was refilled with new buffer. Immediately upon removal, analysis was performed. All tests were performed three times, and the average was recorded for each.

Drug Release Analysis, by Percentage

The methodology used to calculate the drug's release percentage

CrVr multiplied by 100 is the percentage of drug that was spread.

CdVd

Cr = Receptor Drug Concentration. Vr = Receptor Compartment Volume

Cd = Donor Compartment Drug Concentration Where: Vd = Donor Volume Discussion and Results

As shown in table no. 1 total 11 batches of nanoparticles were generated using variable concentration of oil, span 20, Chitosan and TPP where varied concentration of chitosan 2.0% to 1.0% and Span 20 from 10% to 1% were taken by using 1 to 0.25% of TPP. Eleven batches were produced from the various combinations of ingredients. When making nanoparticles using the water/oil emulsion approach, the average particle size was reduced. Particle size rose when Ultraturrax was run at 9500 rpm for 5 minutes. Particle size decreased to 9500 rpm (for 5 minutes), whereas particle size increased no further beyond this speed (for 5 minutes). Therefore, it was thought to be the best possible rate for the operation. One concentration is chosen from all of them to produce a particles in nano range with a good particle sizedistribution that is shown in fig. 1.

Characterization of nanoparticles Particle size analysis

In particle size analysis of Nanoparticles prepared by w/o method the d (0,9) = 210 nm which shows formation of nanoparticles with uniform size distribution. As high shear force applied during preparation the reduction of particle size occurred.

Drug entrapment efficiency

Amount of drug entrapped is shown in following table

2. The formulations showed to be in nano range were characterized for the entrapment efficiency from whichfour batches got good entrapment from 50 to 54% which was found to be highest.

Scanning electron microscopy

For the imaging of NPs, three viewing fields were selected at different magnification. The magnification giving best resolution was selected. The images are shown in fig. 2 which shows that nanoparticles arespherical in shape.

Differential Scanning calorimetry (DSC)

DSC analysis of the nanoparticles was carried out in Mettler Toledo differential Scanning calorimeter (Mettler Toledo, Switzerland) at heating rate of 10ºC/minute in the range of 50ºC to 250º C. Fig 3 shows DSC thermograms of Nanoparticles prepared by w/o emulsion method. The Prepared Nanoparticles showed glass transition temp. (Tg) at 90.6 ºC and ΔH value = 1613mJ .

Infrared (IR) spectrum

The IR spectra of the formulations were carried out for Nanoparticles as well for chitosan and doxorubicin hydrochloride in Shimadzu, FTIR, at Vaibhav Analytical Lab., Ahmedabad. At the wavelength ranging from 400 cm^{-1} to 4000 cm^{-1}

Fig 4, Fig 5 and Figure 6 show IR spectrum of chitosan, Nanoparticles and doxorubicin Hydrochloride respectively. The IR spectra of naoparticles were compared with chitosan and with doxorubicinhydrochloride. It was showed that there is noadditional peak of drug in the nanoparticles IR spectra. It indicates that the drug is completely entrapped into the nanoparticles.

Kinetic of Release

The order of drug release was determined by plotting graphically % cumulative drug release → Time (Fig 7 & 8)

In vitro diffusion studies for all the two type of Nanoparticles up to 120 hours were carried out using a cellophane membrane and the results were compared with each other. When graphing the percent cumulative drug release against time for both formulation types, a linear curve is not formed (figure 7), showing that the release is not zero order. However, when plotting Mt/M versus Square root of time (T 12), the regression coefficient ranged from 0.81 to 0.971, indicating a linear relationship between these two parameters and confirming that the release followed Peppas's diffusion-controlled model and that the drug was entrapped within the matrix.

Conclusion

Doxorubicin nanoparticles prepared using a water-in-oil (W/O) emulsion technique resulted in prolonged drug release. The only nanoparticles produced when the two liquids were combined immediately. In particle size examination it was determined to be in nano range 210 nm which demonstrates creation of nanoparticle with uniform size distribution. In the best-performing batch, drug

entrapment was measured at 53.12%. Peppas's diffusion controlled model, which describes the release, demonstrates that the drug was successfully entrapped inside the polymeric matrix.

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