



## Preparation and characterization of doxorubicin HCl loaded chitosan nanoparticles by w/o emulsion method

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### Abstract

Polymeric nanoparticles are recently more investigated for controlled and targeted drug delivery. The aim of this study was to prepare and evaluate chitosan nanoparticles containing doxorubicin by w/o emulsion method. SEM indicated spherical structure of nanoparticle without agglomeration. FTIR spectra indicated no chemical interaction between drug and polymer. In vitro drug release study suggests sustain drug release for longer period of time. Vigorous agitation leads to formation of smaller particle which lies in nano size and its drug release study confirms its sustained release.

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

### Introduction

Development of colloidal carrier systems has now been an area of great interest in the field of drug delivery. Nanoparticles in pharmaceutical applications have gained plenty of research attention during recent decades.<sup>1</sup> Amongst carrier, polymeric nanoparticles are the most extensively investigated. Chitosan nanoparticles have recently gained interest in targeting drug delivery, because of ease of synthesis, biodegradability, ability to alter bio distribution and lower toxicity. Cell adhesion and potentially cell uptake of such particles should be favored due to their attraction of negatively charged cell membrane, attractive features for the treatment of solid tumors. From the perspective of intravenous administration, positively charged particles would interact with different blood components as compare to negatively charged particles. These changes could potentially create a different bio distribution and /or organ membranes would be expected to interact with cells and/or membranes would be desirable for testing alternatives modes of administration of Doxorubicin, i.e. mucosal administration.<sup>2</sup> Chitosan has shown favorable biocompatible characteristic<sup>3</sup> and degrade by lysosome enzyme in serum.<sup>4</sup> In this present study, nanoparticles were prepared by w/o emulsion method.

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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 into above solution under the Ultra Turrax at 9500 rpm for 5 min.

Step II: 0.5 ml of 0.5%w/v solution of sodium TPP was added 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 the procedure 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

$$N_p = 6 M_0 X_m / \pi p D_n^3$$

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<sup>3</sup> and Np a number of particles/cm<sup>3</sup>.

#### **% Entrapment<sup>5</sup>**

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.

$$\% \text{ entrapment} = \frac{B-A}{A} \times 100$$

Total amount of drug added B-A = Amount of drug entrapped

#### **Scanning electron microscopy<sup>6</sup>**

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)<sup>7</sup>**

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

#### **Infrared (IR) spectrum<sup>8</sup>**

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<sup>-1</sup> to 4000 cm<sup>-1</sup>

#### **Studies of kinetics of drug release from nanoparticles<sup>9,10</sup>**

##### **Preparation of Dialysis bag**

Cellulose dialysis bag (Cutt off 12000 Hi Media) soaked overnight in PBS.

Dialysis set up: The wet sac was gently open and wash copiously with PBS then it was filled with PBS and examined for leaks. The sac was then emptied and 1 ml of Nanosuspension to be investigated was accurately transferred into the sac, which thus became the donor compartment. The sac was once again examined for any leaks and then was suspended in glass beaker containing 20 ml of PBS, which acted as receptor compartment. The content of the beaker was stirred using Teflon coated bar magnet and the beaker was closed with aluminum foil to prevent any evaporative losses during the experimental work.

##### **Sampling**

At predetermined interval of time 1 ml aliquots were withdrawn from the receptor compartment and subjected to analysis. Fresh buffer was used to replenish the receptor compartment. Analysis was carried out immediately after withdrawal. All experiments were repeated thrice and the average values were taken.

##### **Data analysis**

##### **Percent Drug Release**

The % drug release was determined by the formula

$$\% \text{ Drug diffused} = \frac{CrV_r \times 100}{CdV_d}$$

Cr = Conc. of drug in the receptor compartment

Vr = Volume of the receptor compartment

Cd = Conc. of drug in the donor compartment

Vd = Volume of donor compartment

#### **Results and Discussion**

##### **Preparation of nanoparticles**

As shown in table no. 1 total 11 batches of nanoparticles were prepared using different 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 taking 1 to 0.25% of TPP. From all these different ratios total 11 batches were made. In case of nanoparticles prepared by W/O emulsion method there was a decrease in average particle diameter. When the Ultraturax speed was increased upto 9500 rpm for 5 min, there was increase in particles size. Below 9500 rpm (for 5 min), there was decrease in particle size while Above speed of this 9500 rpm (for 5 min), there was no further increase in particle size. So, it was considered as an optimum speed for the process. From all these above batches a concentration is selected which gives a



particles in nano range with a good particle size distribution 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 which four 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 are spherical 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  $10^{\circ}\text{C}/\text{minute}$  in the range of  $50^{\circ}\text{C}$  to  $250^{\circ}\text{C}$ . Fig 3 shows DSC thermograms of Nanoparticles prepared by w/o emulsion method. The Prepared Nanoparticles showed glass transition temp. ( $T_g$ ) at  $90.6^{\circ}\text{C}$  and  $\Delta H$  value =  $1613\text{mJ}$ .

##### **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  $400\text{ cm}^{-1}$  to  $4000\text{ cm}^{-1}$

Fig 4, Fig 5 and Figure 6 show IR spectrum of chitosan, Nanoparticles and doxorubicin Hydrochloride respectively. The IR spectra of nanoparticles were compared with chitosan and with doxorubicin hydrochloride. It was showed that there is no additional 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  $\rightarrow$  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.

Linear curve are not obtained upon plotting the % cumulative drug release versus time for all the two type of formulations, indicating that the release is not zero order (figure 7). However, the regression coefficient of the plot of  $M_t/M_{\infty}$  versus Square root of time ( $T^{1/2}$ ) was found to be laying 0.81- 0.971 indicating that a linear relationship exists between these two parameters and that the release obeys peppas diffusion controlled model and also indicated that drug entrapped within the matrix.

##### **Conclusion**

Nanoparticles of doxorubicin by W/O emulsion method were able to provide sustained drug release. when both the solutions were mixed immediately, than only the particle formed in nano range In particle size analysis it was found to be in nano range 210 nm which shows formation of nanoparticle with uniform size distribution. The drug entrapment was found to be 53.12% in optimized batch. The release shows that the drug entrapped within the polymeric matrix and it follows peppas diffusion controlled model.

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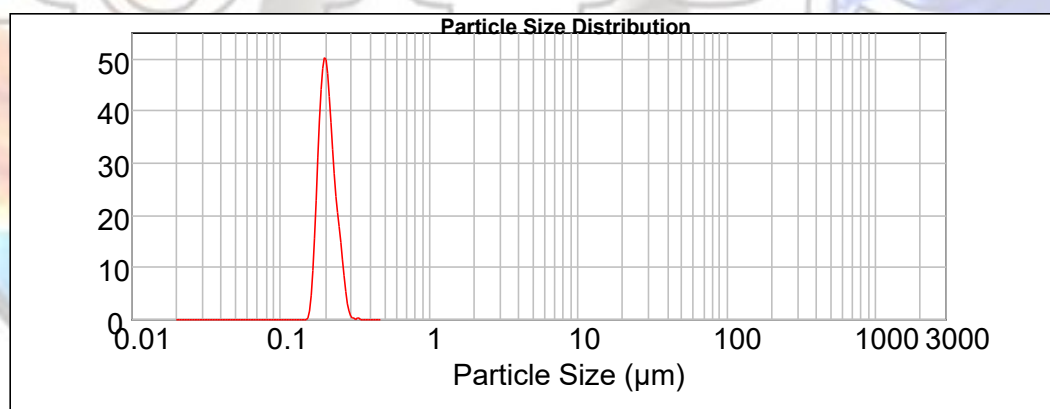
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**Table 1: Formulations with different ratios**

Batch No.	Quantity of oil	Conc. of span – 20	Conc. of Chitosan	Conc. of TPP
B1	25 ml	10%	2.0%	1.0%
B2	25 ml	7.5%	2.0%	1.0%
B3	25 ml	5 %	2.0%	1.0%
B4	25 ml	2.0%	2.0 %	1.0%
B5	25 ml	1%	2.0%	1.0%
B6	25 ml	5%	1.5%	1.0%
B7	25 ml	5%	1.0%	1.0%
B8	25 ml	5%	1.0%	0.75%
B9	25 ml	5%	1.0%	0.5%
<b>B10</b>	<b>25 ml</b>	<b>5%</b>	<b>1.0%</b>	<b>0.5%</b>
B11	25 ml	5%	1.0%	0.25%

**Table 2: % drug entrapment**

Drug : Polymer ratio % W/W	% Entrapment
10	49.78%
20	50.76%
<b>25</b>	<b>53.12%</b>
30	53.6%



$d(0,1) = 186 \text{ nm}$   $d(0,5) = 199 \text{ nm}$   $d(0,9) = 210 \text{ nm}$  uniformity=0.106

**Fig. 1: Particle size analysis of nanoparticles**

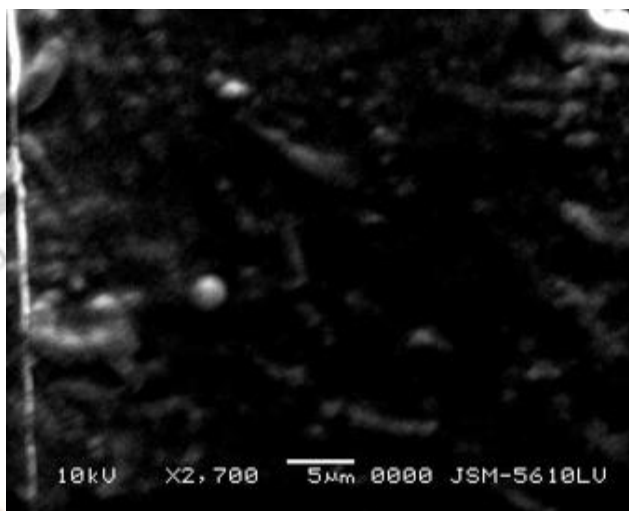


Fig. 2: Scanning Electron Micrograph of Nanoparticles

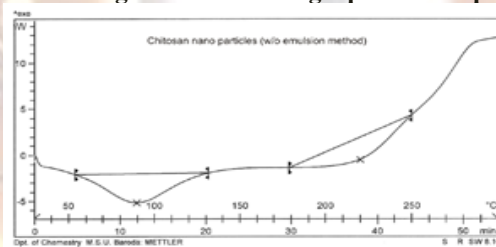


Fig. 3: DSC of Nanoparticles

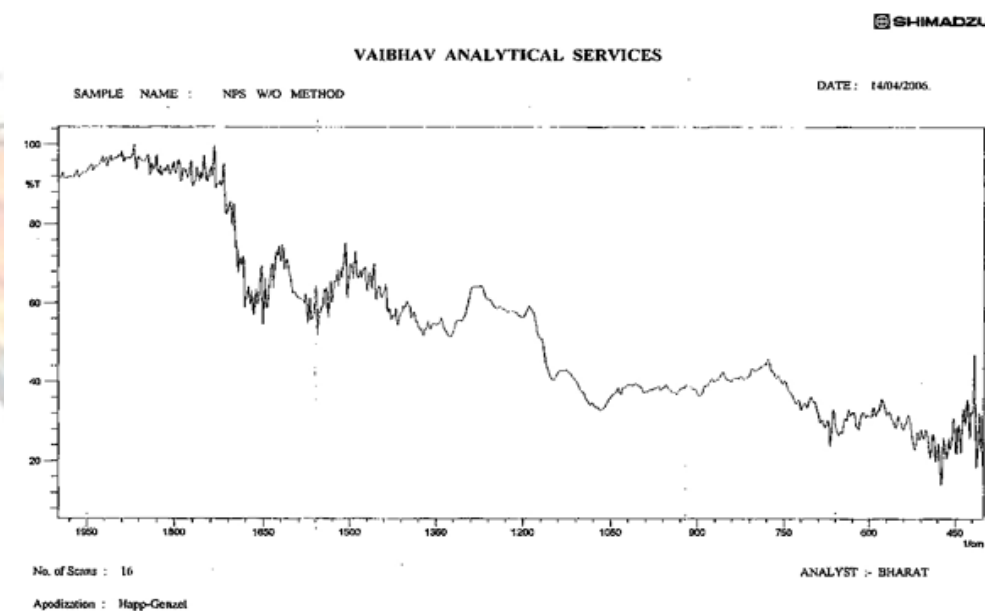


Fig. 4: IR spectrum of chitosan

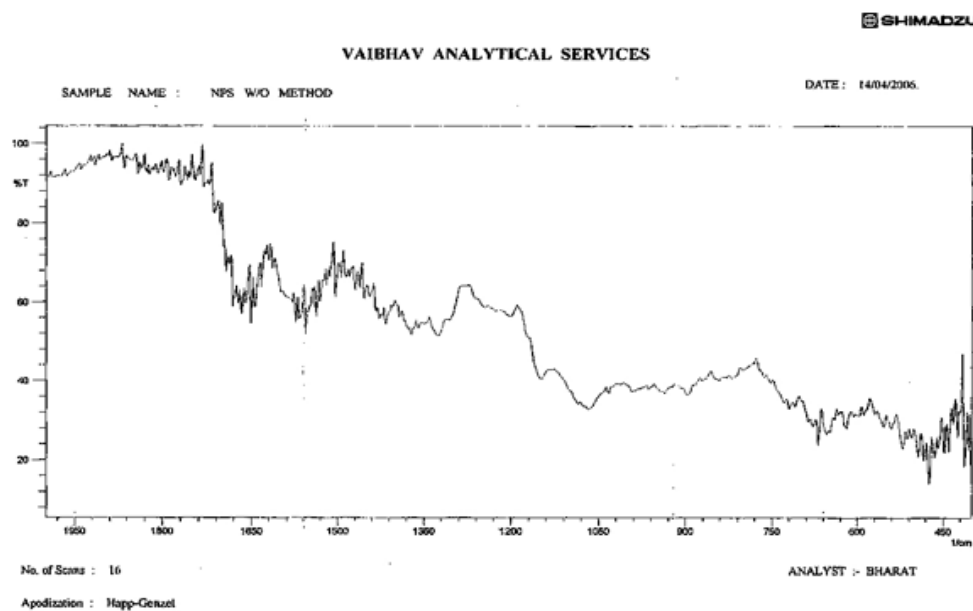


Fig. 5: IR spectrum of Nanoparticle

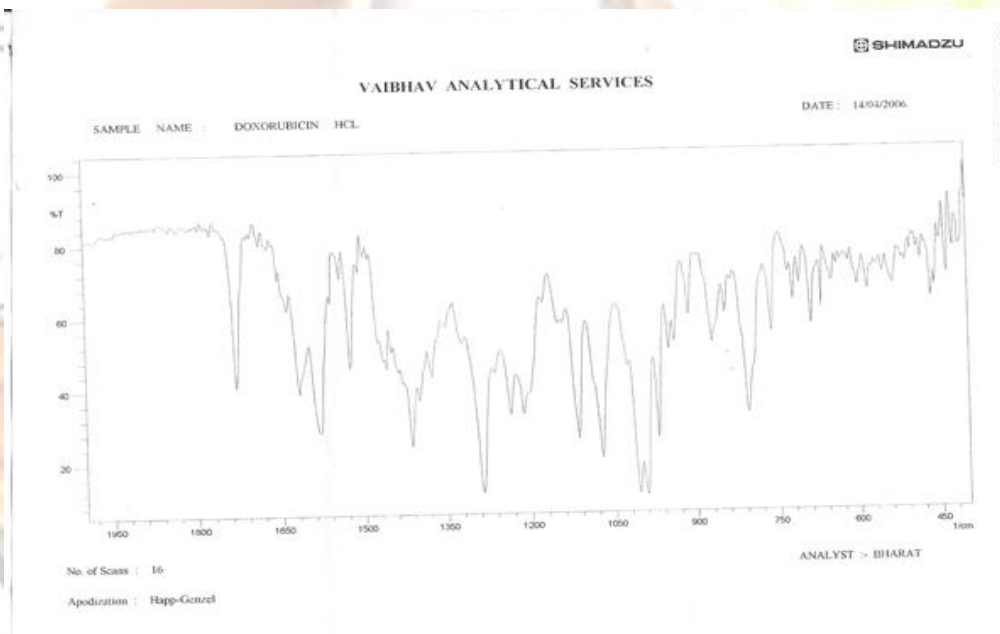


Fig. 6: IR spectrum of Doxorubicin Hydrochloride



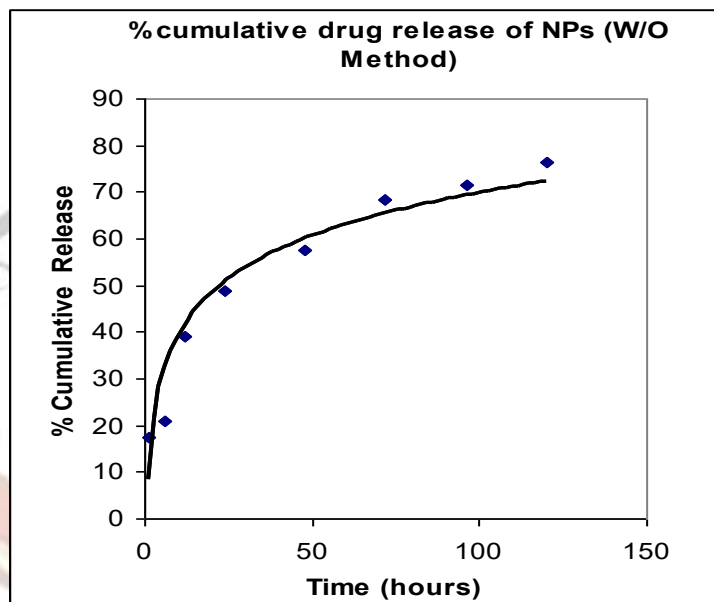


Fig. 7: %Cumulative Drug Release→ Time

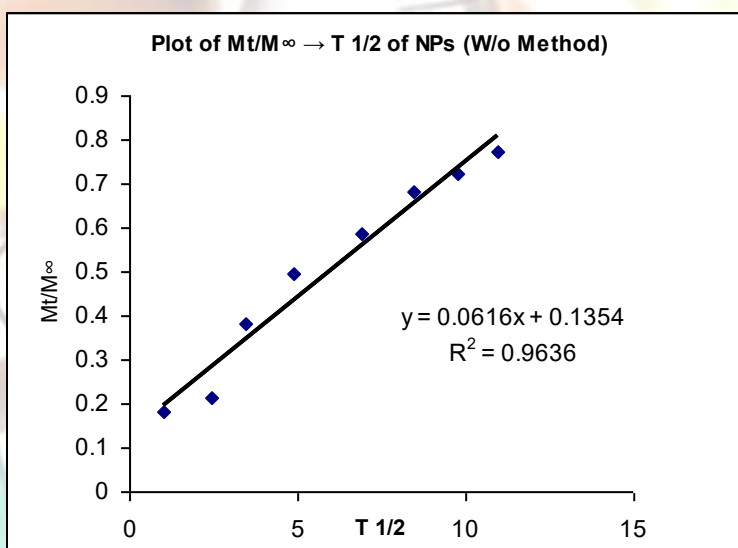


Fig. 8:  $M_t/M_\infty \rightarrow T_{1/2}$  of NPs