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Preparation of bovine serum albumin loaded chitosan nanoparticle using reverse micelle method

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ABSTRACT

To prepare Bovine serum albumin loaded chitosan nanoparticles by reverse micellar method. Bovine serum albumin loaded chitosan nanoparticles were prepared by using Cetyl trimethyl ammonium bromide as surfactant, Isooctane as oil, 1-Hexanol as Co-solvent, BSA as the model drug, and chitosan-BSA solution as aqueous phase. The resultant nanoparticles were characterized for size, mean diameter, uniform distribution and electrophoretic mobility. Particle size was measured by Scanning Electron Microscope. Mean diameter of the particle was measured by Photon Correlation spectroscopy. Electrophoretic mobility of the particle was measured by Zetasizer. Amount of drug and polymer has been changed keeping all the remaining parameters constant. Effect of polymer concentration on the resultant nanoparticles size, mean diameter, particle distribution and electrophoretic mobility has been studied. .Effect of drug concentration on the resultant nanoparticles size, mean diameter, particle distribution and electrophoretic mobility has been studied. Increased drug concentration has decreased the particle size. Increased surfactant concentration decreased particle size. Increased polymer concentration has no significant impact on particle size distribution. Higher the drug and surfactant concentrations lower the polymer concentrations resulted in uniform particle size distribution.

Keywords: Bovine serum albumin (BSA), Cetyltrymethylammoniumbromide (CTAB), Scanning electron microscope (SEM), Chitosan (CT).

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INTRODUCTION

Naturally occurring polymers, specifically, polysaccharides like chitosan and alginate, have been widely studied as primary material carriers of drug and proteins [1, 2]. Chitosan is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters [3, 4]. Chitosan is biodegradable, biocompatible, and non toxic material allowing its widespread applications in biomedical fields [5, 6]. Reverse micelles present multimolecular and self-assembly entities, formed as dispersed colloid phases of microemulsions at particular compositional ranges thereof [7, 8]. In 1982, Boutonnet et al. reported the first synthesis of a material via using reverse micelles. Microemulsions are transparent, isotropic, thermodynamically stable dispersions of oil and water, stabilized by surfactant molecules [9, 10]. The main goal of this study was to prepare bovine serum albumin (BSA) loaded in chitosan nanoparticles with Cetyltrimethylammonium Bromide (CTAB) based water-in-oil reverse micelle [11].

MATERIALS AND METHODS

Materials

BSA (Bovine serum albumin) supplied by ottochemicals, Chitosan supplied by Himedia laboratories, Isooctane supplied by sigma laboratories, Hexanol supplied by SD-fine chemicals, CTAB supplied by SD-fine chemicals.

Methodology

In order to prepare the reversed micelles, CTAB, oil and co-solvent were poured into test tube. The chitosan-BSA solution was then added to the mixture of CTAB and solvent while stirring. The formation of reversed micelles was inferred the mixed emulsion became transparent or semi-transparent. The BSA-loaded chitosan particles were prepared by adding aqueous NaOH solution into the reverse micelles, the completion of the reaction was recognized when; the reverse micelle solvent becomes turbid. The BSA-loaded chitosan particles were separated using a laboratory centrifuge at 6000 rpm for 15 min, in which the precipitated solids was collected in the bottom of test tubes. Finally, the obtained nanoparticles were dried for 24 h in air at the laboratory ambient temperature**.** The concentration of drug and polymer are varied keeping all the remaining parameters constant. Effect of polymer concentration on the resultant nanoparticles size, mean diameter, particle distribution and electrophoretic mobility has been studied. Effect of drug concentration on the resultant nanoparticles size, mean diameter, particle distribution and electrophoretic mobility has been studied.

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RESULTS

Determining the size of nanoparticles

Size of nemesulide loaded CAHP nanoparticles was determined by scanning electron microscope. In order to perform the SEM observation, nanoparticle suspension was fist diluted with ultrapure water (1/5), and then a drop of the diluted nanoparticle suspension was then directly deposited on a polished aluminum sample holder. Samples were dried in vacuum. The morphology of nanoparticles was observed at 15 kV using a scanning electron microscope (SEM; S-3700 N, Hitachi, Japan).

Particle Size Analysis

Mean particle size of the nanoparticles was determined by Photon Correlation Spectroscopy (PCS) with a Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK). Measurements were realized in triplicate at a 90º angle at 25ºC under suitable dilution conditions. Particle size distribution was expressed as mean diameter (nm) ± standard deviation and polydispersity index. Mean particle size was found to be 548.2 nm.

Zeta Potential Measurement

Zeta potential of nanoparticle dispersions was measured in mV by Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) in triplicate to determine the surface charge and the potential physical stability of the nanosystem. Zeta potential of nanoparticles was measured in aqueous dispersion. Measurements were realized in triplicate at a 120º angle at 25ºC.

Determination of crystallinity of BSA loaded chitosan nanoparticles by X-ray diffraction study

Physical status of BSA loaded chitosan nanoparticles:

An X-ray diffractometer (Philips, Xpert-Pro, The Netherlands) was used to determine the physical status of chitosan in the nanoparticles. The diffraction angle (2θ) was recorded from 3° to 80° with a scanning speed of 5°/minute. CuKa radiation was used as the X-ray source at 40 kV and 30 mA.

DISCUSSION

In this study, BSA was encapsulated into chitosan nanoparticles via reverse micelle method. The size of particles was obtained in range 80 to 180 nanometres. Here the effect of polymer (chitosan) concentration on the particle size was studied. The chitosan concentration was varied from 2.5mg/ml to 5mg/ml. At 2.5 mg/ml concentration the particle size varied from 80nm to 280nm.When the concentration of chitosan has increased from 2.5 mg/ml to 5mg/ml the particles are distributed in between 100-120nm. Then the effect of Drug (bovine serum

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albumin) concentration on the particle size was studied. The BSA concentration was varied from 2.5mg/ml to 5mg/ml. At 2.5 mg/ml concentration the particles are very tightly packed when the concentration of BSA has increased from 2.5 mg/ml to 5mg/ml the particles are distributed in between 100-120nm.Increased drug concentration decreased the particle size. After that effect of surfactant (CTAB) concentration on the particle size was studied. The CTAB concentration was varied from 10mg/ml to 20mg/ml.At10mg/ml concentration the particles are distributed in-between 80nm to 280nm.When the concentration of surfactant has increased from 10 mg/ml to 20mg/ml the particles are distributed in between 100-120nm.Increased surfactant concentration decreased the particle size.

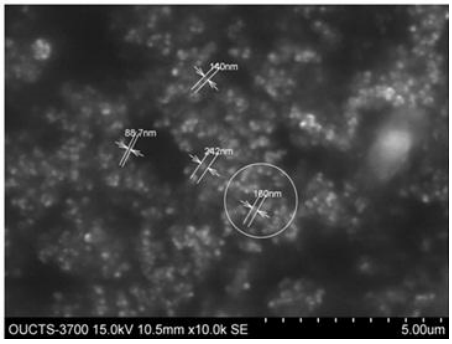

Figure1 SEM Images of BSA loaded chitosan nanoparticles (5 mg/ml BSA, 2.5 mg/ml CT, 10 mg CTAB)

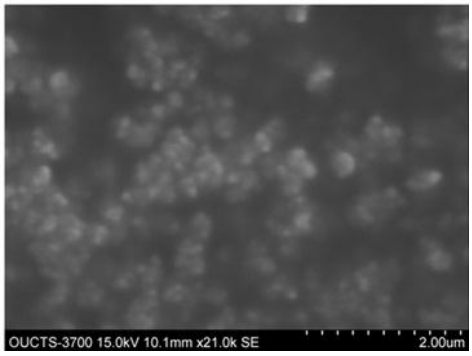

Figure 2 SEM Images of BSA loaded chitosan nanoparticles (5 mg/ml BSA, 2.5 mg/ml CT, 10 mg CTAB)

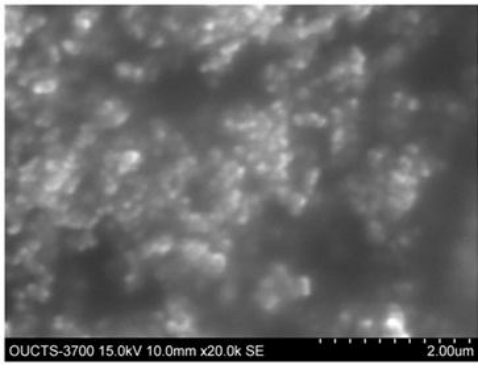

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Figure3 SEM Images of BSA loaded chitosan nanoparticles (5 mg/ml BSA, 5 mg/ml CT, 20 mg CTAB)

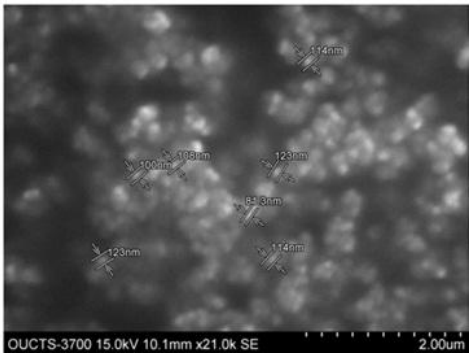

Figure 4 SEM Images of BSA loaded chitosan nanoparticles (5 mg/ml BSA, 5 mg/ml CT, 20 mg CTAB)

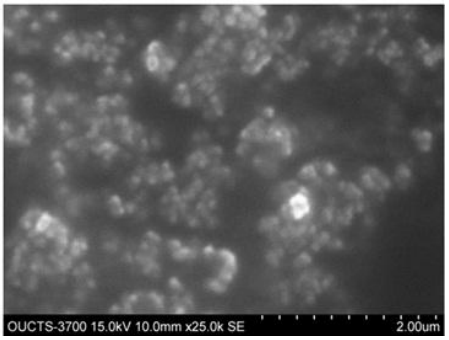

Figure 5 SEM Images of BSA loaded chitosan nanoparticles (5 mg/ml BSA, 5 mg/ml CT, 10 mg CTAB)

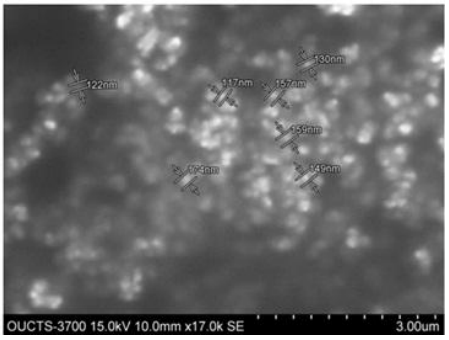

Figure 6 SEM Images of BSA loaded chitosan nanoparticles (5 mg/ml BSA, 5 mg/ml CT, 10 mg CTAB)

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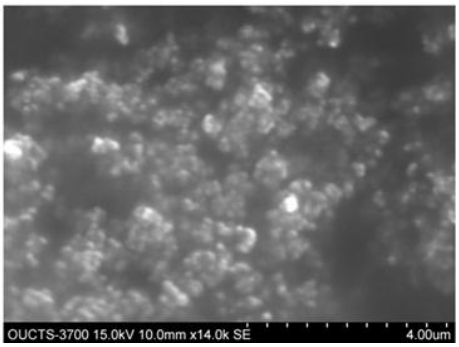

Figure 7 SEM Images of BSA loaded chitosan nanoparticles (5 mg/ml CT, 2.5 mg/ml BSA, 10 mg CTAB)

An X-ray diffractometer (Philips, Xpert-Pro, The Netherlands) was used to determine the physical status of chitosan in the nanoparticles.

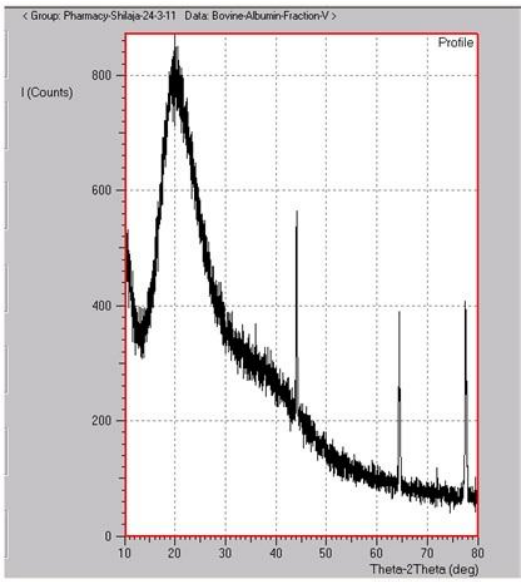

Figure 8 X-RD of pure drug

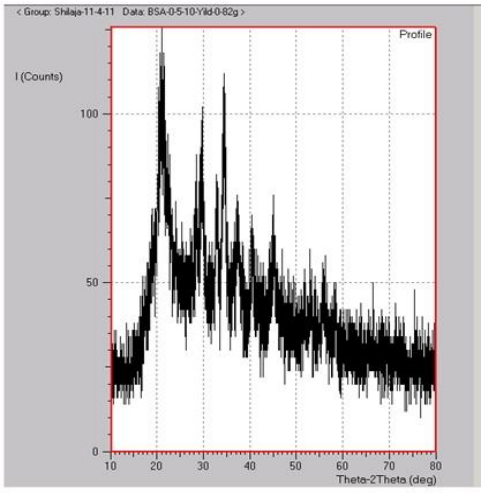

Figure 9 X-ray diffraction study of Bovine serum albumin (5 mg BSA, 2.5 mg chitosan, 10 mg CTAB)

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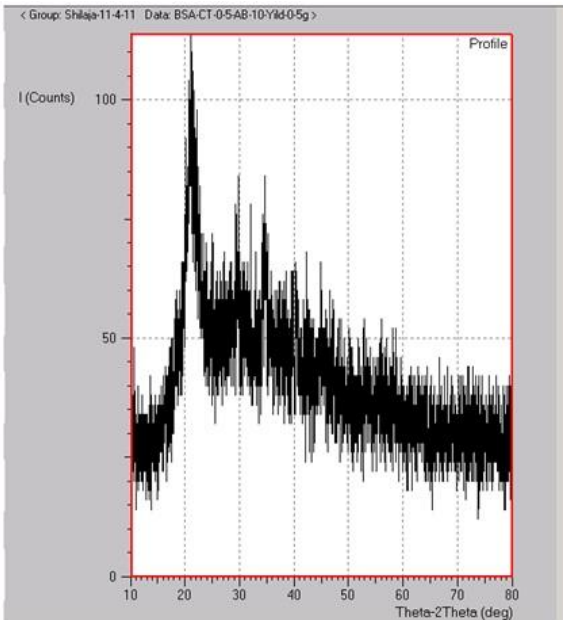

Figure 10 X-ray diffraction study of Bovine serum albumin (5 mg BSA, 2.5 mg chitosan, 20 mg CTAB)

Mean particle size of the nanoparticles was determined by Photon Correlation Spectroscopy (PCS) with a Malvern Zetasizer. Mean particle size was found to be 725.2 nm. Zeta potential of nanoparticle dispersions was measured in mV by Malvern Zetasizer. Zetapotential of the nemesulide loaded Cellulose acetate hydrogen phthalate nanoparticles was found to be -19.5 mV indicating good stability.

CONCLUSIONS

Here the effect of drug, polymer and surfactant concentration on the particle size distribution was studied. Increased drug concentration has decreased the particle size. Increased surfactant concentration decreased particle size. Increased polymer concentration has no significant impact on particle size distribution. Higher the drug and surfactant concentrations lower the polymer concentrations results in uniform particle size distribution. Further study can be done on entrapment efficiency and drug release from polymeric nanoparticles.

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This work was supported by the University Grants commission (UGC) New Delhi

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Figure 11 Size distribution report by intensity

Size Distribution Report by Intensity

v2.0

Malvern

Sample Details

Sample Name: SME1 1
SOP Name: SME1 SIZE.sop
General Notes:

File Name:	a2.dts	**Dispersant Name:**	Water
Record Number:	1	**Dispersant RI:**	1.330
Material RI:	1.50	**Viscosity (cP):**	0.8864
Material Absorbtion:	0.10	**Measurement Date and Time:**	Thursday, September 23, 201...

System

Temperature (°C):	25.0	**Duration Used (s):**	80
Count Rate (kcps):	168.5	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	10

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	725.3	**Peak 1:**	571.7	68.8	185.4
PdI:	0.636	**Peak 2:**	2350	18.0	669.3
Intercept:	0.962	**Peak 3:**	4791	13.3	728.9

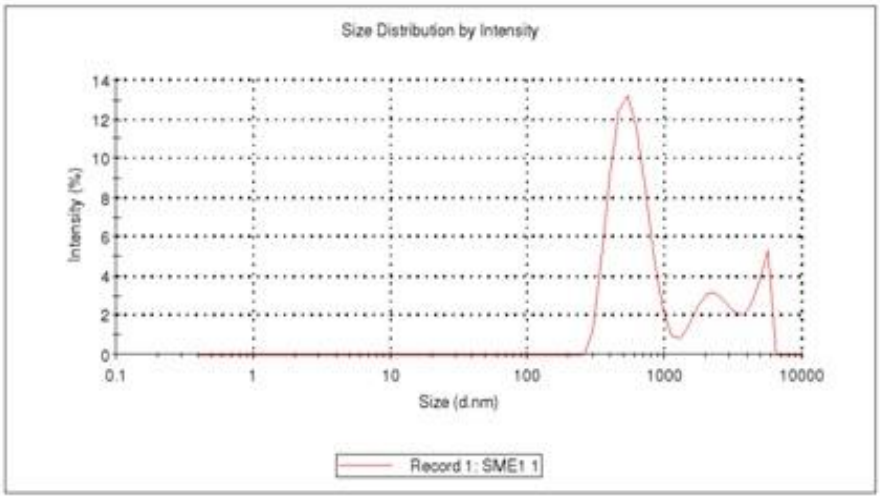

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Fig 12: zeta potential report

Zeta Potential Report

v2.2

Malvern

Sample Details

Sample Name: Plain Emulsion 1
SOP Name: Plain Emulsion Zeta Potential.sop
General Notes:

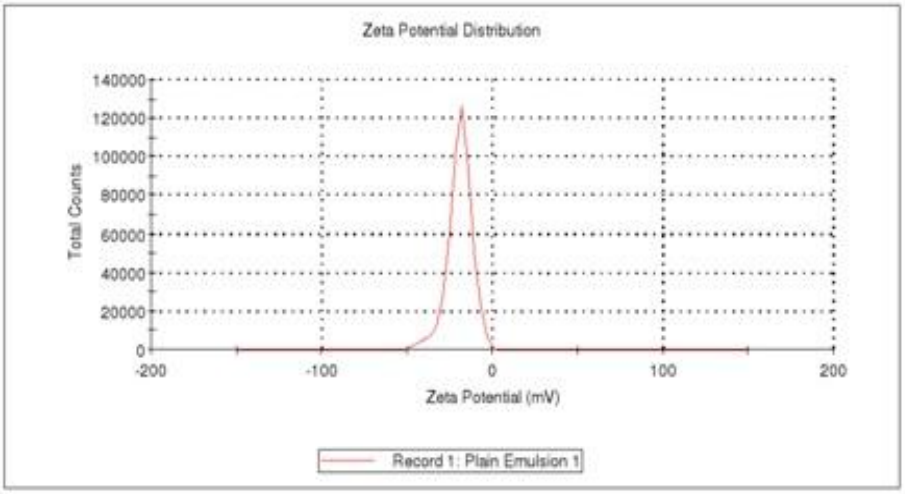
File Name: a2z.dts
Record Number: 1
Date and Time: Thursday, September 23, 2010 1...
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8875
Dispersant Dielectric Constant: 78.6

System

Temperature (°C): 25.0
Count Rate (kcps): 341.8
Cell Description: Clear disposable zeta cell
Zeta Runs: 20
Measurement Position (mm): 2.00
Attenuator: 7

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -19.4	Peak 1:	-19.4	100.0	7.52
Zeta Deviation (mV): 7.52	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 2.01	Peak 3:	0.00	0.0	0.00



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