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Research Article

Formulation and evaluation studies of BSA loaded chitosan nanoparticles by polymerization technique

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Keywords:
Polymerization,
Zeta potential,
Scannig Electron microscope,
Methyl Methacrylate,
Ammonium Per Sulphate.

Abstract

Aim: To prepare BSA loaded chitosan nanoparticles by polymerization technique and to study the effect of initiator concentration upon particle size, product yield, entrapment efficiency, loading capacity and drug release from the formulation.

Methodology: In the present study BSA loaded chitosan nanoparticles were prepared by polymerization technique. Three formulations were prepared by varying the concentration of Initiator. The concentration of Initiator (Ammonium per sulphate) was maintained 1%, 2% 3% in formulation 1, Formulation 2 and Formulation 3 respectively. The effect of initiator concentration on Mean particle diameter, Drug content, entrapment efficiency, loading capacity, electrophoretic mobility and zeta potential was studied.

Results and discussion: Best nanoformulations were obtained with Ammonium per sulphate 2 % concentration with Mean particle diameter of 441.7 nm. Electrophoretic mobility and Zeta potential value (-3.304 and -42.1) was also more among all chitosan formulations indicating greater stability.

Conclusion: Hence Formulation 2 was considered to be the best Formulation for the preparation of BSA loaded Chitosan nanoparticles.

1. Introduction

Nowadays there has been considerable interest in developing new routes alternative to injection for delivering macromolecules such as proteins and peptides. However, peptides and protein drugs are degraded before they reach the blood stream and cannot cross the mucosal barrier. The mucoadhesive polymer coated nanoparticles can solve these problems [9]. They were prepared by polymerization technique. Methyl methacrylate polymerized in the presence of polysaccharide such as chitosan leads to formation of mucoadhesive polymer coated nanoparticles. The mucoadhesive polymers could interact with the mucus glycoproteins which allow the mucoadhesive system to remain adhesive for an extended period of time. Coating nanoparticles with them improved their mucoadhesion. These mucoadhesive polymer coated nanoparticles are suitable for carrying hydrophilic drugs [11,12,20].

Nanoparticles used as drug delivery vehicles are generally < 100 nm in at least one dimension and consist of different biodegradable materials such as natural or synthetic polymer lipids or metals. Nanoparticles are taken up by cells more efficiently than larger micromolecules so can be used as effective transport and delivery systems [5,6,21].

For therapeutic applications drugs can either be integrated in the matrix of the particle attached or to the particle surface. A dug targeting system should be able to control the fate of drug entering the biological environment [1,2]. An effective approach for achieving efficient drug delivery would be to rationally develop nanosystems based on the understanding of their interactions with the biological environment, target cell population, target cell surface receptors, changes in cell receptors that occur with progression of disease, mechanism and site of drug action, drug retention, multiple drug administration, molecular mechanisms and pathobiology of disease under consideration. Reduced drug efficacy could

be due to multiple drug targeting, chemical properties of delivering molecules, alterations in genetic makeup of cell surface receptors, over expression of efflux pumps, changes in the signalling pathways with the progression of disease or drug degradation [3,4]. Most of the nanoparticles prepared from water insoluble polymers are involved heat, organic solvent or high shear force that can be harmful to the drug stability. In contrast water soluble polymers offer mild and simple preparation methods without use of organic solvent and high shear force [24].

Chitosan is a polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine and can be derived by the partial deacetylation of chitin. It is a biodegradable, biocompatible and hydrophilic polymer of low toxicity [7,8]. It is a material found in abundance in shells of crustacean such as lobsters, prawns and crabs. It is insoluble under alkaline and neutral conditions, but can react with inorganic and organic acids such as hydrochloric acid, lactic acid, acetic acid and glutamic acid under acidic conditions [18,19]. It has OH and NH2 groups that give rise to hydrogen bonding and these groups could act as nucleophilic agent to initiate the polymerization of methylmethacrylate leading to an irreversible attachment between chitosan and methylmethacrylate through different multipoint linkages [13-15]. The cationic polyelectrolytic nature of chitosan could interact with a negatively charged mucosal surface. It was also confirmed that coating liposomes with chitosan improved their adsorption to mucosal surfaces [16,17].

2. Materials and Methods

2.1 Materials: Methylmethacrylate, Chitosan, Ammoniumpersulphate

2.2 Preparation of chitosan coated nanoparticles

Chitosan coated nanoparticles were prepared by emulsion polymerization technique in a closed 100ml flask. Chitosan was dissolved in 100 ml 1% acetic acid solution under magnetic stirring at 400-500 rpm. The pH value was adjusted to 4-5. One percent (w/v) of the monomer methylmethacrylate was dissolved in the above mixture at 75^0C and APS solution was added. The reaction was completed after 5 hrs. Three formulations were prepared by varying the concentration of Initiator [22,23]. The concentration of Initiator (Ammonium per sulphate) was maintained 1%, 2% 3% in formulation 1, Formulation 2 and Formulation 3 respectively. The effect of initiator concentration on Mean particle diameter, Drug content, entrapment efficiency, loading capacity, electrophoretic mobility and zetapotential was studied. Comparative study was performed to determine the sustained release effect of the formulations [10,25].

3. Results and Discussion

The obtained formulations were evaluated for size, Product yield, Drug content, Entrapment efficiency, Loading capacity and drug release.

3.1 Percentage Yield

The yields of the prepared nanoparticles were calculated. Nanoparticles dried at room temperature were weighed and the yield of nanoparticles was calculated using the formula:

$$\text{Percent Yield} = \frac{\text{the amount of nanoparticles obtained (g)}}{\text{The theoretical amount (g)}} \times 100$$

Product yields of Chitosan Formulations 1, 2 and 3 prepared by polymerization technique were found to be 40 % and 96.6% and 40% respectively. From the results it was found that Product yield of Formulation 2 was more when compared with other two formulations.

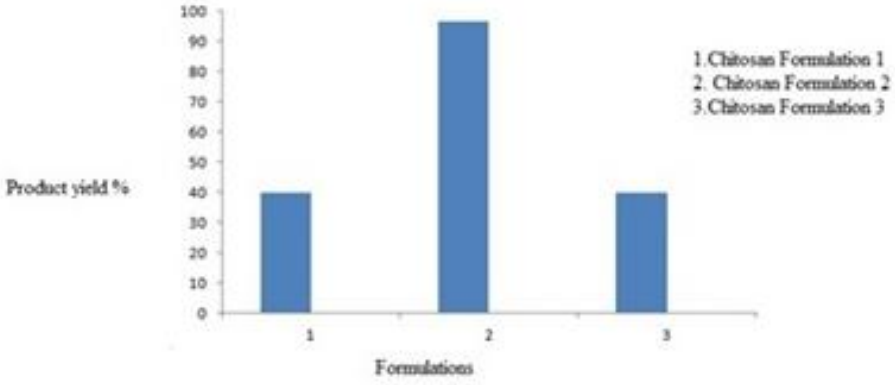


Fig 1: Comparison of product yields of Chitosan formulations

3.2 Fourier Transforms infrared Spectroscopy (FT-IR)

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and formulations were studied. The characteristic absorption peaks of BSA were obtained at wave numbers 3306.32cm^{-1}, 2872cm^{-1}, 1170cm^{-1}, 3109.35cm^{-1}, 1696 cm^{-1}. (K Br disk).The characteric absorption peaks for chitosan were obtained at wave numbers 3200 cm^{-1}, 2920 cm^{-1}, 1100 cm^{-1}. The peaks obtained in the spectra's of each formulation correlates with the peaks of drug spectrum. This indicates that the drug was compatible with the formulation components.

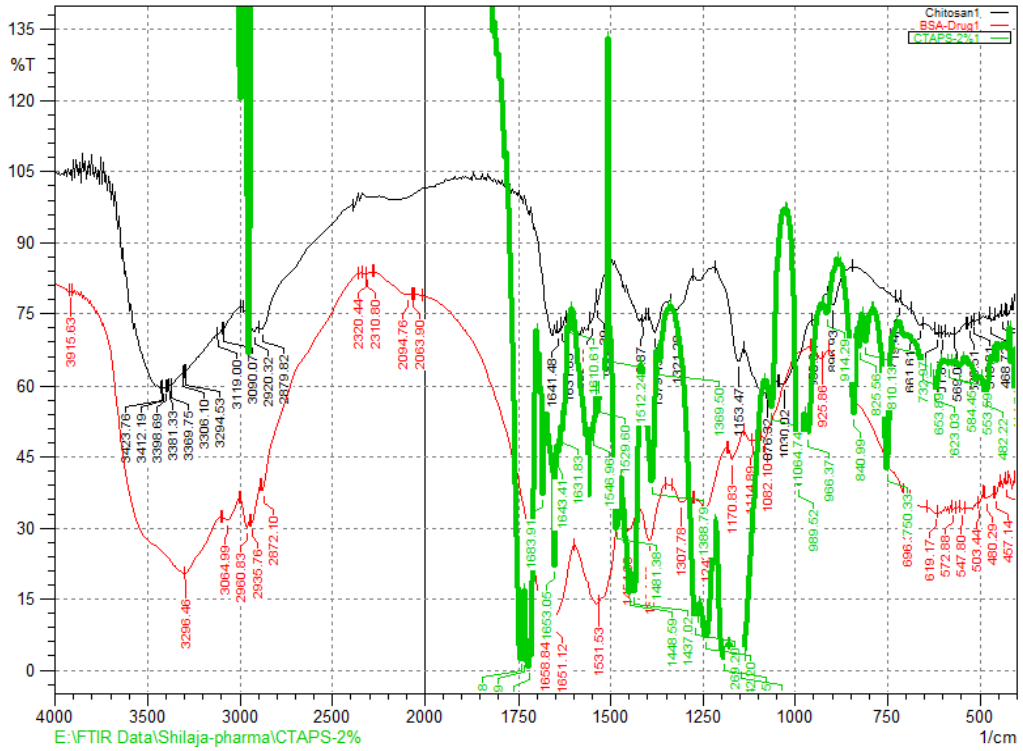


Fig 2: FTIR Spectra of Chitosan Formulation 2

3.3 Scanning electron microscopy (SEM)

Morphological characterization of the nanoparticles was carried using scanning electron microscopy (SEM-S-3700N). For SEM the double – sided sticking tape, and coated with gold film (thickness 200nm) under the reduced pressure (0.001torr) was used. The sample for the SEM analysis was prepared by sprinkling the nanoparticles on one side of double adhesive stub. The nanoparticles were viewed at an accelerating voltage of 15-20kv.

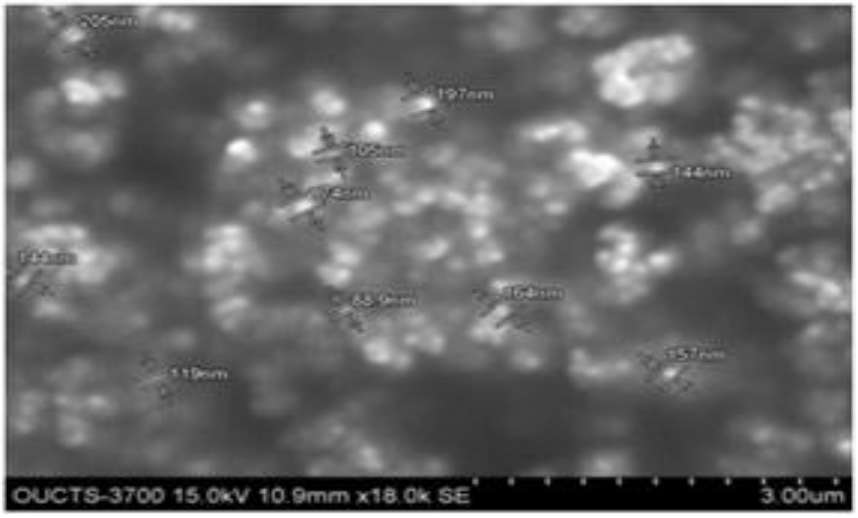
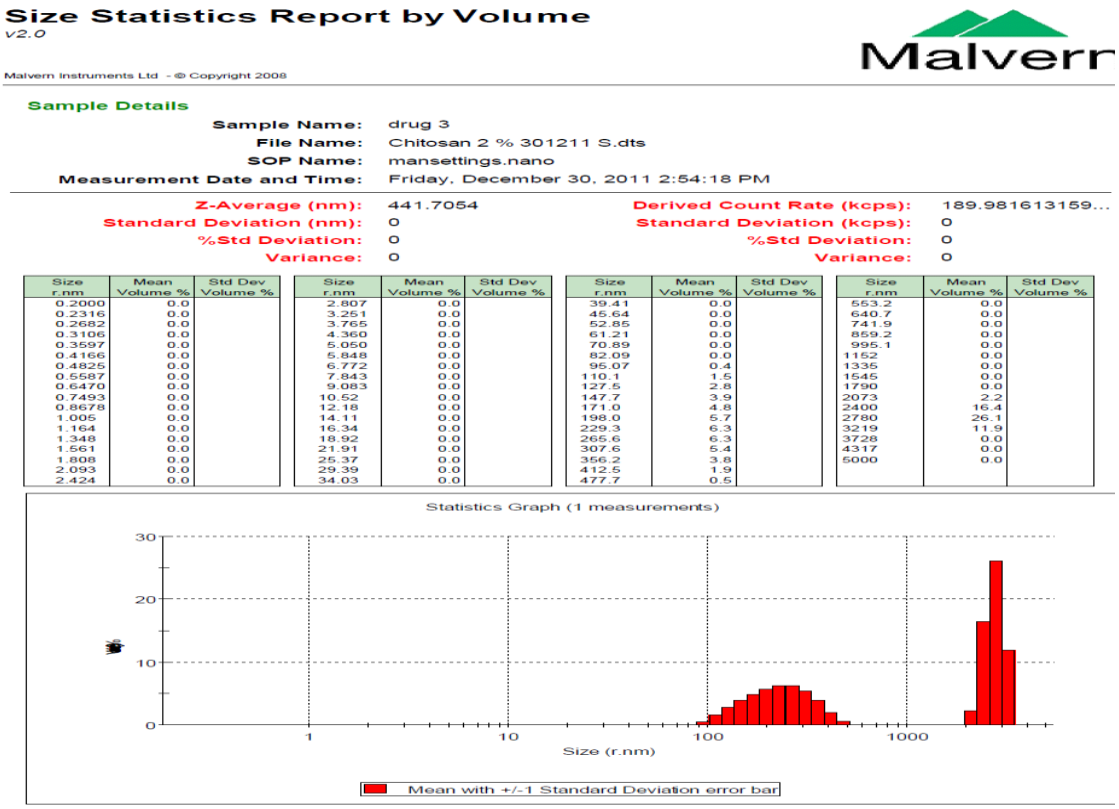


Fig 3: SEM images of Chitosan formulation 2

3.4 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.

Particle sizes of chitosan 1%, Chitosan 2 % and chitosan 3% formulations prepared by polymerization technique were found to be 484.15 nm and 441.7 nm and 1005.75 nm respectively. From the results it was found that Formulation chitosan 2% was resulting particles in the nanorange when compared with other two formulations.



Size Statistics Report by Volume

v2.0

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Sample Details

Sample Name:	drug 3
File Name:	Chitosan 2 % 301211 S.dts
SOP Name:	mansettings.nano
Measurement Date and Time:	Friday, December 30, 2011 2:54:18 PM

Z-Average (nm):	441.7054	Derived Count Rate (kcps):	189.981613159...
Standard Deviation (nm):	0	Standard Deviation (kcps):	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size r.nm	Mean Volume %	Std Dev Volume %	Size r.nm	Mean Volume %	Std Dev Volume %	Size r.nm	Mean Volume %	Std Dev Volume %	Size r.nm	Mean Volume %	Std Dev Volume %
0.2000	0.0		2.807	0.0		39.41	0.0		553.2	0.0	
0.2316	0.0		3.251	0.0		45.64	0.0		640.7	0.0	
0.2682	0.0		3.765	0.0		52.85	0.0		741.9	0.0	
0.3106	0.0		4.360	0.0		61.21	0.0		859.2	0.0	
0.3597	0.0		5.050	0.0		70.89	0.0		995.1	0.0	
0.4166	0.0		5.848	0.0		82.09	0.0		1152	0.0	
0.4825	0.0		6.772	0.0		95.07	0.4		1336	0.0	
0.5587	0.0		7.843	0.0		110.1	1.5		1545	0.0	
0.6470	0.0		9.083	0.0		127.5	2.8		1790	0.0	
0.7493	0.0		10.52	0.0		147.7	3.9		2073	2.2	
0.8678	0.0		12.18	0.0		171.0	4.8		2400	16.4	
1.005	0.0		14.11	0.0		198.0	5.7		2780	26.1	
1.164	0.0		16.34	0.0		229.3	6.3		3219	11.9	
1.348	0.0		18.92	0.0		265.6	6.3		3728	0.0	
1.561	0.0		21.91	0.0		307.6	5.4		4317	0.0	
1.808	0.0		25.37	0.0		356.2	3.8		5000	0.0	
2.093	0.0		29.39	0.0		412.5	1.9				
2.424	0.0		34.03	0.0		477.7	0.5				

Fig 4: Particle size distribution report of Chitosan 2 % nanoparticles prepared by Polymerization technique

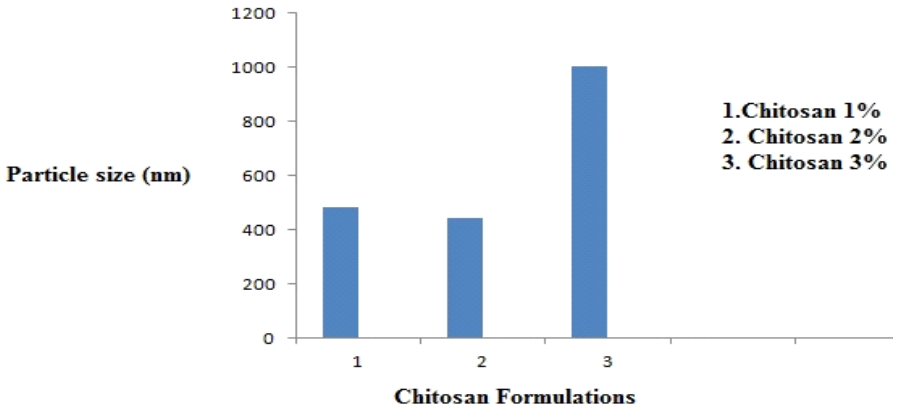

Fig 5: Comparison of particle sizes of chitosan formulations

3.5 Drug content

Drug loaded nanoparticles were weighed, then grinded to fine powder and dissolved in a solvent in which the drug is completely soluble. It was subjected to stirring around 700 rpm for 3 hrs. Amount of drug in the supernatent was determined by UV-Spectrophotometric method.

Drug contents of chitosan 1%, Chitosan 2 % and chitosan 3% formulations prepared by polymerization technique were found to be 86.6% , 53.07% 76.6% respectively. From the results it was found that Drug content of Chitosan 1% was more when compared with other two formulations.

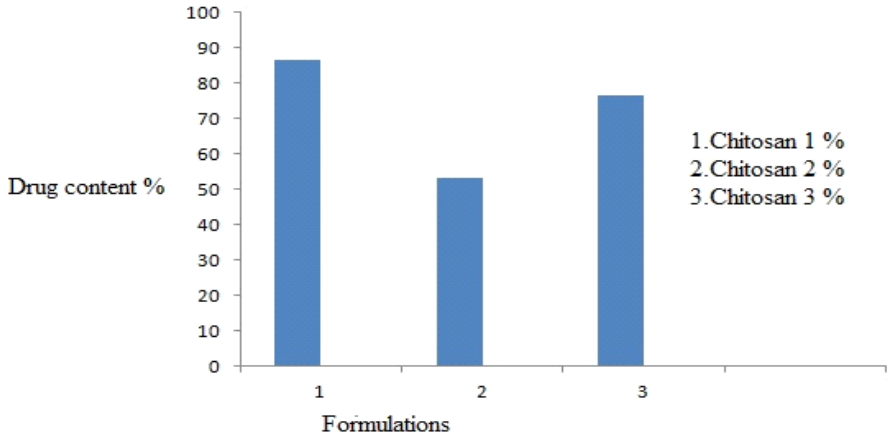

Fig 6: Comparison of drug contents of chitosan Formulations

3.6 Encapsulation efficiency (EE)

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (*w*) by UV-spectrophotometry. A standard calibration curve of concentration versus absorbance was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (*W*). Effectively, (*W*-*w*) will give the amount of drug entrapped in the pellet. Then percentage entrapment is given by

$$\frac{(W - w)}{W} \times 100$$

Loading capacity was calculated by the Following equation

$$\frac{(W - w)}{\text{Nanoparticle weight}} \times 100$$

The Entrapentment efficiencies of chitosan 1%, Chitosan 2 % and chitosan 3% formulations prepared by polymerization technique were found to be 29% ,67.53 % 48.07% respectively. From the results it was found that entrapment efficiency of Chitosan 2% was more when compared with other two formulations.

Loading capacities of chitosan 1%, Chitosan 2 % and chitosan 3% formulations prepared by polymerization technique were found to be 27.84% , 54.8 % 34.76% respectively. From the results it was found that loading capacity of Chitosan 2% was more when compared with other two formulations.

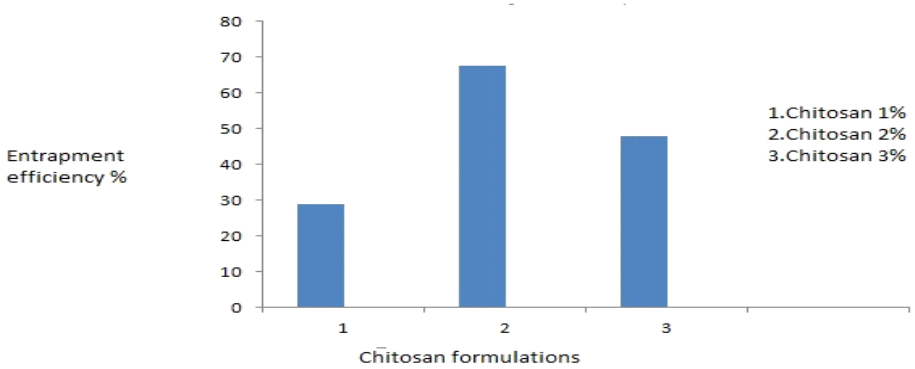

Fig 7: Comparison of entrapment efficiencies of Chitosan Formulations

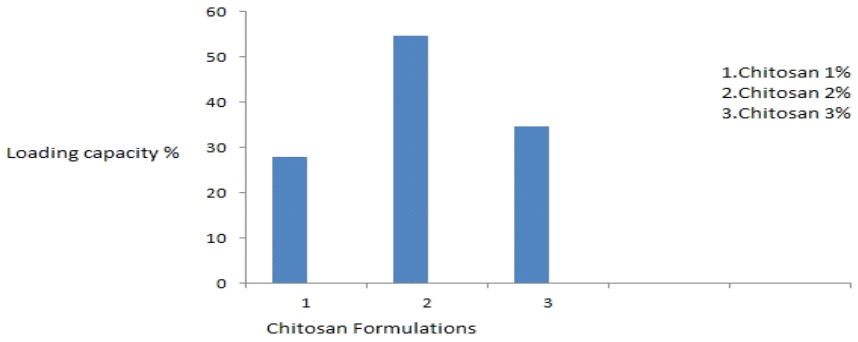

Fig 8: Comparison of loading capacities of chitosan formulations

3.7 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.

Electrophoretic mobility values of chitosan 1%, Chitosan 2 % and chitosan 3% formulations were found to be -2.286,-3.304 and -1.423 respectively. From the results it was found that Electrophoretic mobility value of Chitosan 2% was higher when compared with other two formulations. The Zetapotential values of chitosan formulations 1, 2 and 3 technique were found to be -28.8,-42.1and -18.4 respectively. From the results it was found that zetapotential value of Chitosan 2% was higher when compared with other two formulations.

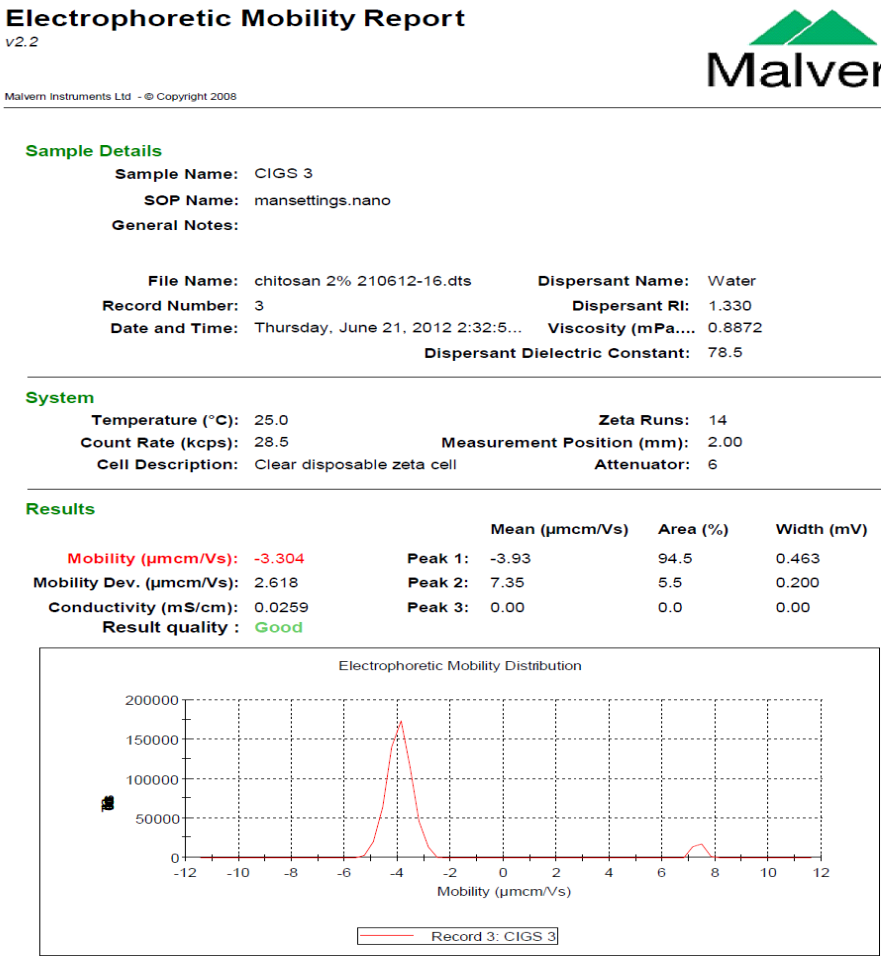

Fig 9: Electrophoretic mobility report of Chitosan 2 % nanoparticles prepared by Polymerization technique

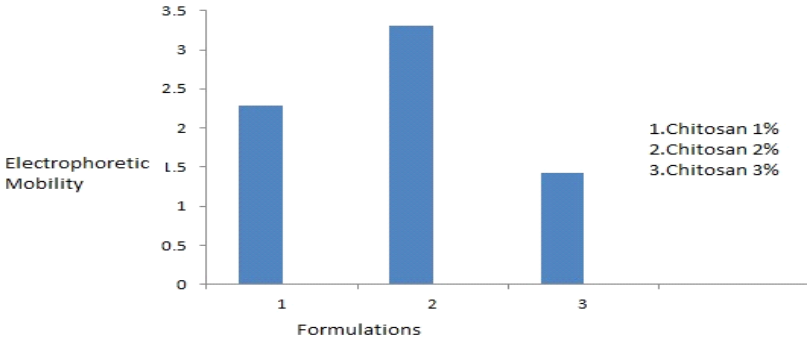

Fig 10: Comparison of electrophoretic mobility values of chitosan formulations

Zeta Potential Report

v2.2

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Sample Details

Sample Name: CIGS 3
SOP Name: mansettings.nano
General Notes:

File Name: chitosan 2% 210612-16.dts
Record Number: 3
Date and Time: Thursday, June 21, 2012 2:32:57...
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (mPa.s): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Count Rate (kcps): 28.5
Cell Description: Clear disposable zeta cell
Zeta Runs: 14
Measurement Position (mm): 2.00
Attenuator: 6

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -42.1	**Peak 1:**	-50.1	94.5	5.90
Zeta Deviation (mV): 33.4	**Peak 2:**	93.7	5.5	2.55
Conductivity (mS/cm): 0.0259	**Peak 3:**	0.00	0.0	0.00

Result quality : Good

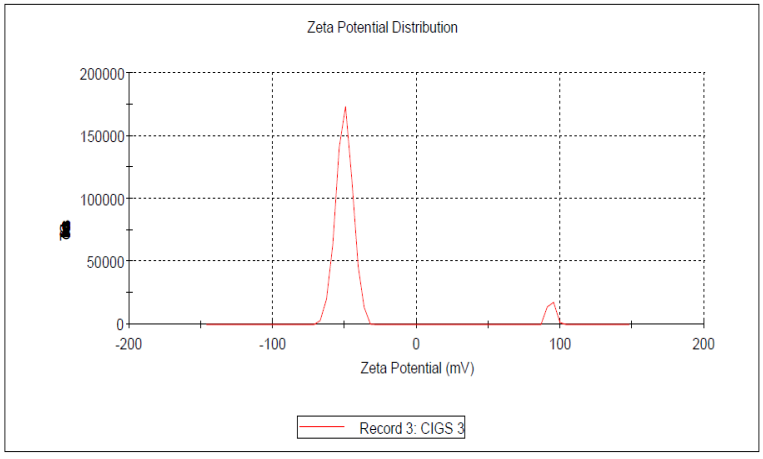

Fig 11: Zeta potential report of Chitosan 2 % nanoparticles

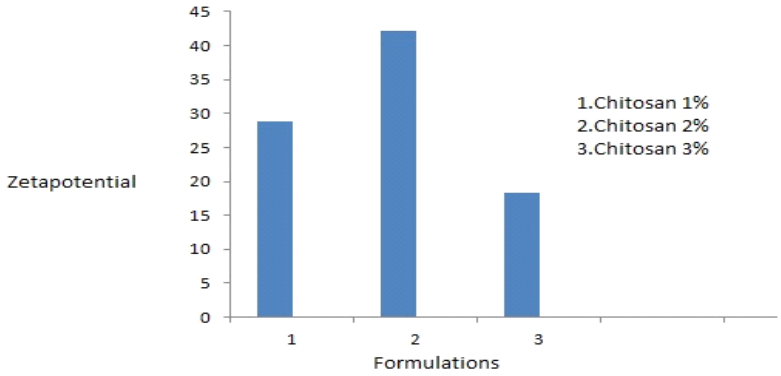

Fig 12: Comparison of Zetapotential values of chitosan formulations

3.8 Drug release studies

Drug release studies were performed by means of orbitary shaker. Drug release from polymeric nanoparticles was determined as follows. A known amount of nanoparticles was transferred to a conical flask and 50 mL of the Phosphate buffer pH 7 was added to the tube. The temperature and rotation were adjusted to 37^0C and 90 rpm, respectively. At predetermined time of 0.5, 2, 4, 6, 8, 10, 12, and 24, 36, 48 hours. 5mL of sample was removed and ultracentrifuged at 15, 000 × *r* for 60 minutes, and 5mL of the supernatant were replaced by fresh medium. The samples were further analyzed by UV Spectrophotometer.

In all chitosan Formulations the drug release was slow, extended over a period of 48 hrs.In a time period of 48 hrs 98.6 %, 96.4 % and 97.12 % of drug has been released from chitosan 1 %, chitosan 2 % and chitosan 3 % formulations respectively. Among all formulations the drug release was maximum in chitosan 1 % formulation.This may be because of maximum drug content in formulation 1. The curve Fitting data revealed that the release followed First order kinetics and Higuchis and Peppas plots stated Fickian diffusion controlled pattern in all the three formulations.

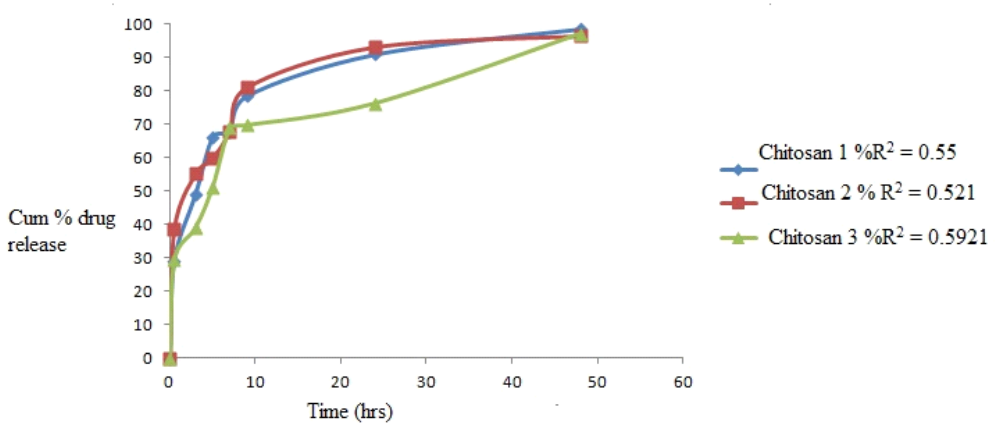

Fig 13: Comparison of *In vitro* drug release pattern of Chitosan Formulations

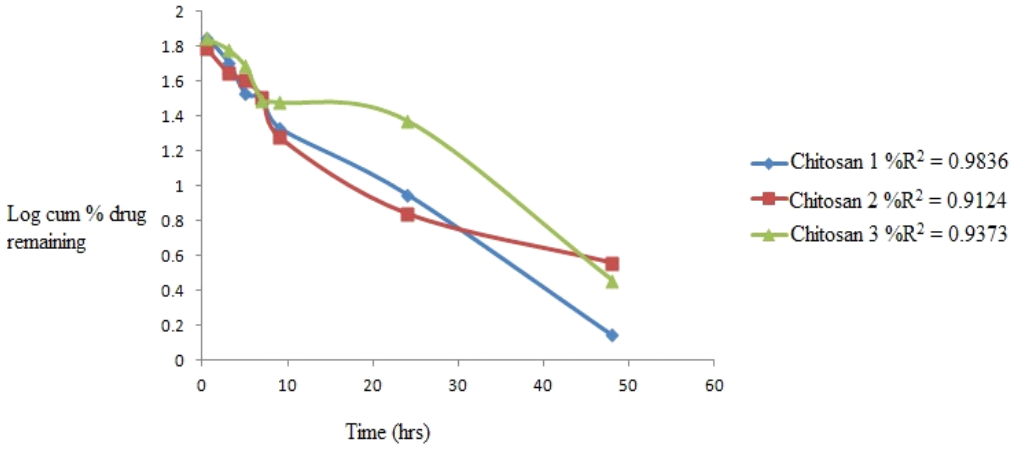

Fig 14: Comparison of First order release pattern of Chitosan Formulations

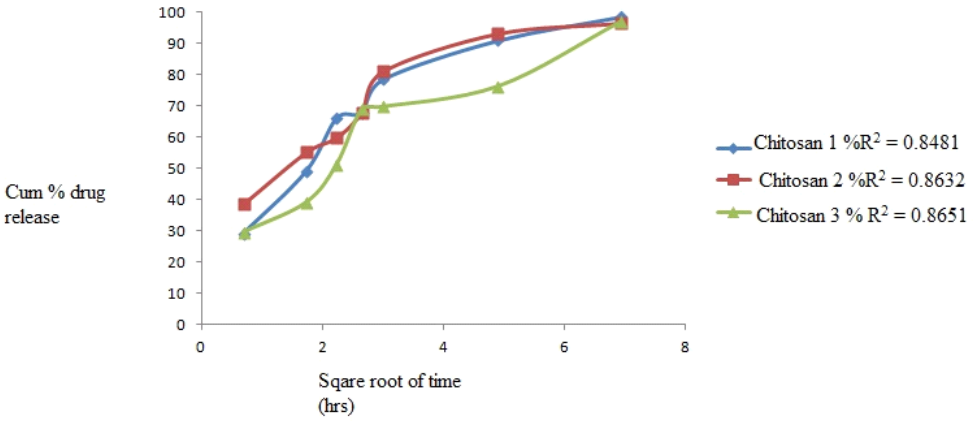

Fig 15: Comparison of Higuchis square root time dependent plots of chitosan Formulations

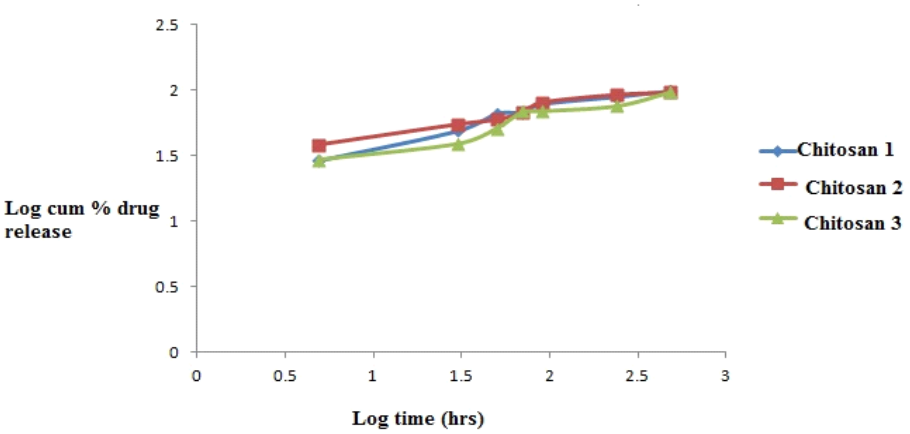

Fig 16: Comparison of peppas double log plots of chitosan formulations

4. Discussion

Three Formulations were prepared by varying the concentration of initiator. When the concentration of initiator was maintained at 2 %, maximum product yield was obtained. Ammonium per sulphate directly attacks the characteristic group of alcohol and amine group of chitosan polymer backbone producing free radicals. These free radicals initiate the graft copolymerization with methyl methacrylate. It was found that at lower level of APS concentration yield was very low. Moreover, by increasing the initiator concentration, there might be increase in free radical formation randomly; Hence the yield increases significantly. Further increase in the initiator concentration resulted in a decrease of the polymerization reaction. It might be due to increase in the number of free radicals terminated prior to MMA addition.

Entrapment efficiency and loading capacity of Formulation 2 was more when compared with other two formulations.

Electrophoretic mobility and zetapotential values of formulation 2 were higher indicating good stability. It may be because of small particle size of the formulation. Zeta potential is a measure of the charge of the particle, as such the larger the absolute value of the zetapotential the larger the amount of charge of the surface. In a sense, the zeta potential represents an index for particle stability. For the case of charged particles, as the zeta potential increases, the repulsive interactions will be larger leading to the formation of more stable particles with a more uniform size distribution. A physically stable nanosuspension solely stabilized by electrostatic repulsion will have a minimum zeta potential of ± 20 mV. Zeta potential value was found to be -42.1 mV for Formulation 2.

In vitro drug release studies were performed by means of orbitary shaker. There are several factors which affect the release rate of the entrapped drug. Larger particles have a smaller initial burst release and longer sustained release than smaller particles. Among all formulations the drug release was maximum in chitosan 1 % formulation.This may be because of maximum drug content in formulation 1.

5. Conclusions

From the results it can be conclude that Formulation 2 (APS 2%) can be considered as the best formulation for the preparation of BSA loaded mucoadhesive nanoparticles Because of its small particle size, good stability and sustained release property.

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