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Research Article

IN VITRO DISSOLUTION STUDIES OF BSA LOADED CARBAPOL NANOPARTICLES BY POLYMERIZATION TECHNIQUE

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ABSTRACT:-
Aim of the study was to prepare BSA loaded carbapol 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. Methyl methacrylate was polymerized in the presence of polysaccharide such as carbapol leads to formation of mucoadhesive polymer coated nanoparticles. Coating nanoparticles with them improved their mucoadhesion. These mucoadhesive polymer coated nanoparticles are suitable for carrying hydrophilic drugs In present study BSA loaded carbapol 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 zetapotential was studied. Best nanoformulations were obtained with Ammonium per sulphate 2 % concentration with Mean particle diameter of 512 nm. Electrophoretic mobility and Zeta potential value (-1.702 and -21.7) was more among all carbapol formulations indicating greater stability. Hence Formulation 2 was considered to be the best Formulation for the preparation of BSA loaded Carbapol nanoparticles.

Keywords: *Methylmethacrylate, Carbapol, Ammonium Persulphate*

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 (Anne *et al.*, 2006). The mucoadhesive polymer coated nanoparticles can solve these problems. They were prepared by polymerization technique. Methyl methacrylate polymerized in the presence of polysaccharide such as carbapol 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 (Cui *et al.*, 2006). 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 (Krishnasailaja *et al.*, 2011).

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 (Soppimath *et al.*, 2001). 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 (Mora-Huertas *et al.*, 2010). 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. 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 (Dash *et al.*, 2008).
Carbapol is a high molecular weight Poly (acrylicacid) copolymer, loosely cross linked with allyl sucrose. This mucoadhesive polymer could interact with mucus glycoproteins by forming physical entanglements followed by hydrogen with sugar residues on oligosaccharide chains resulting in the formation of a strengthened mucus gel network, which allows the mucoadhesive system to remain adhesive for an extended period of times. It was also showed that coating nanoparticles with them improved their mucoadhesion (Hoffman *et al.*, 1983).

MATERIALS AND METHODS

Methylmethacrylate purchased from Sigma Aldrich (St.Louis MO, USA).
Carbapol is supplied from Fisher Scientifics.
Ammoniumpersulphate purchased from Sigma Aldrich (St.Louis MO, USA).
Preparation of Carbapol coated nanoparticles: Carbapol coated nanoparticles were prepared by emulsion polymerization technique in a closed 100ml flask. Carbapol was dissolved in 100 ml water under magnetic stirring at 400-500 rpm. 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 24 hrs. 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 zetapotential was studied. Comparative study was performed to determine the sustained release effect of the formulations (Krishna *et al*., 2011).

RESULTS AND DISCUSSION

The obtained formulations were evaluated for size, Product yield, Drug content, Entrapment efficiency, Loading capacity and drug release (Patil *et al.*,). *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:

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

Product yields of Carbapol 1, 2 and 3 formulations were found to be 80.45%, 95.6% and 86.9 % respectively. From the results it was found that product yield of Formulation 2 was more when compared with other two formulations.

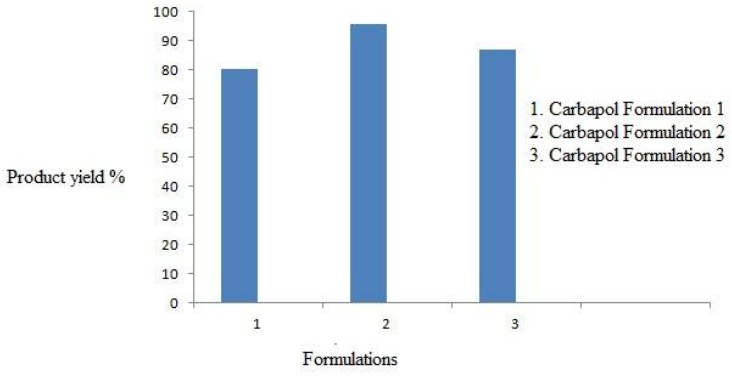

Figure 1: Comparison of product yields of carbapol formulations

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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 3306.32 cm^{-1}, 2872cm^{-1}, 1170 cm^{-1}, 3109.35cm^{-1}, 1696 cm^{-1}. (K Br disk).The characteristic absorption peaks for carabapol were obtained at wave numbers at 2955 cm^{-1}, 1700 cm^{-1}, 1230.63 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.

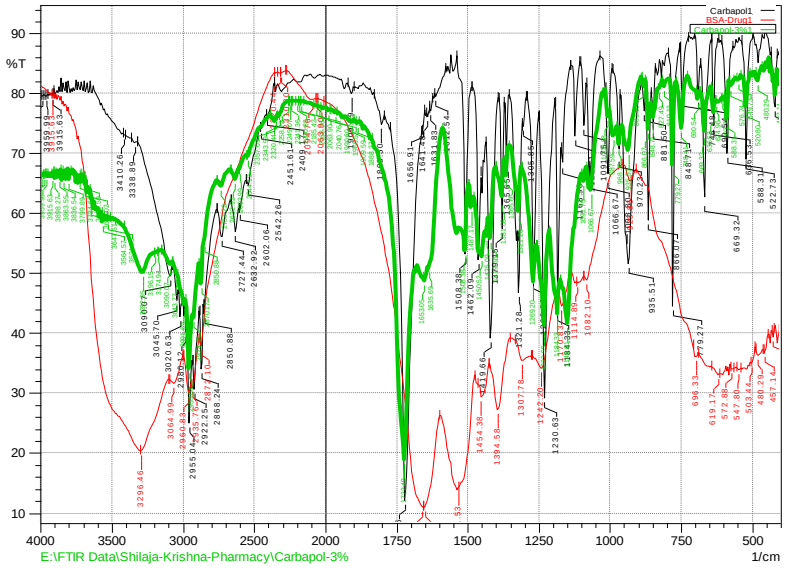

Figure 2: FTIR Spectra of carbapol Formulation 2

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). 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.

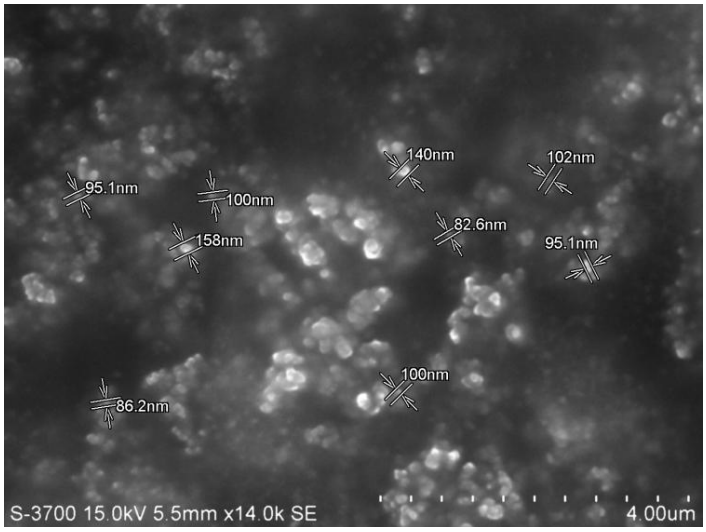

Figure 3: SEM Images of carbapol nanoparticles Formulation 2

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 (Lin *et al.*, 2006).

Particle sizes of carbapol 1%, Carbapol 2 % and carbapol 3% formulations were found to be 996.2 nm and 528 nm and 2761.9 nm respectively. From the results it was found that carbapol 2% Formulation was resulting particles in the nanorange when compared with other two formulations.

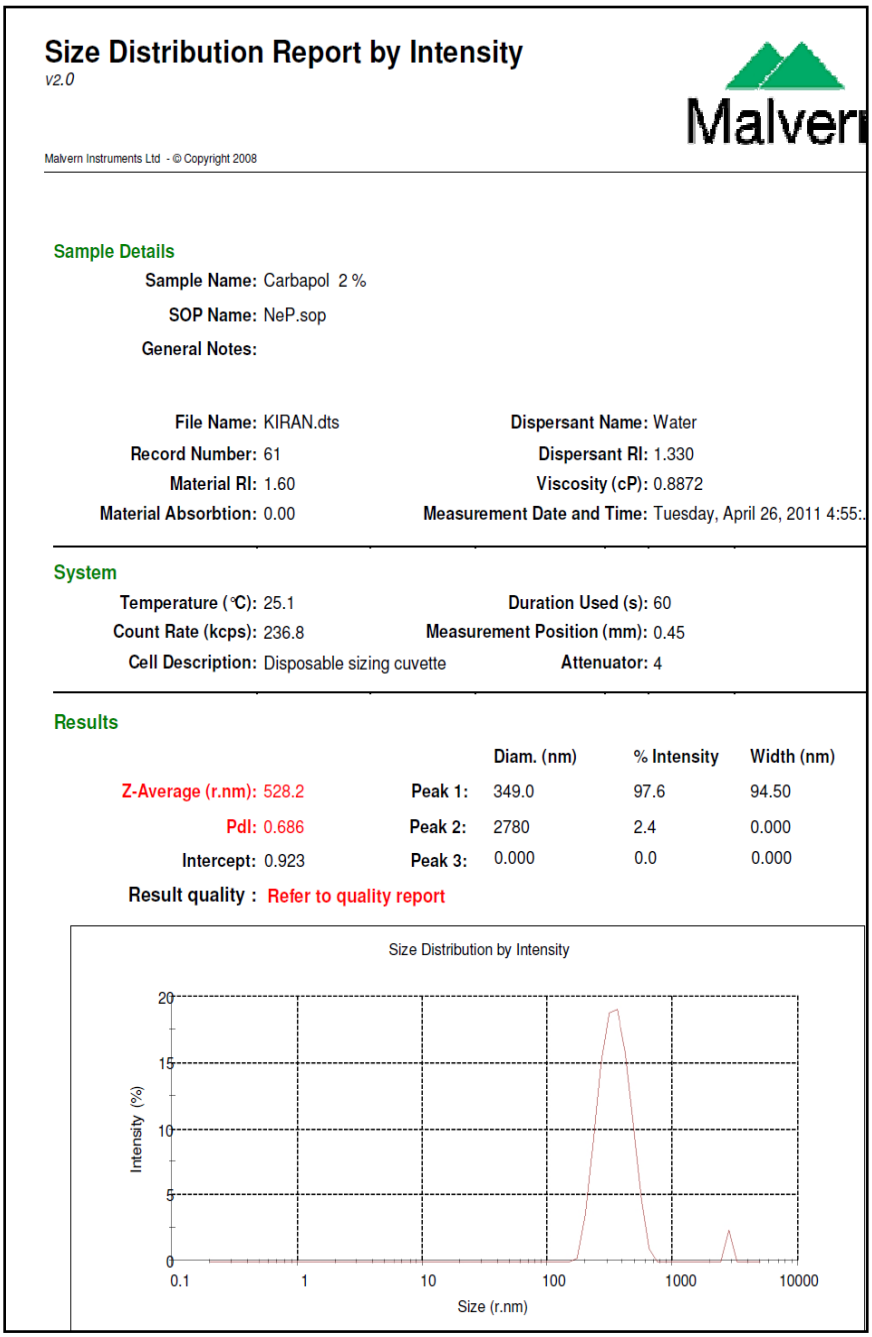

Figure 4: Particle size distribution report of carbapol 2 % nanoparticles prepared by Polymerization technique

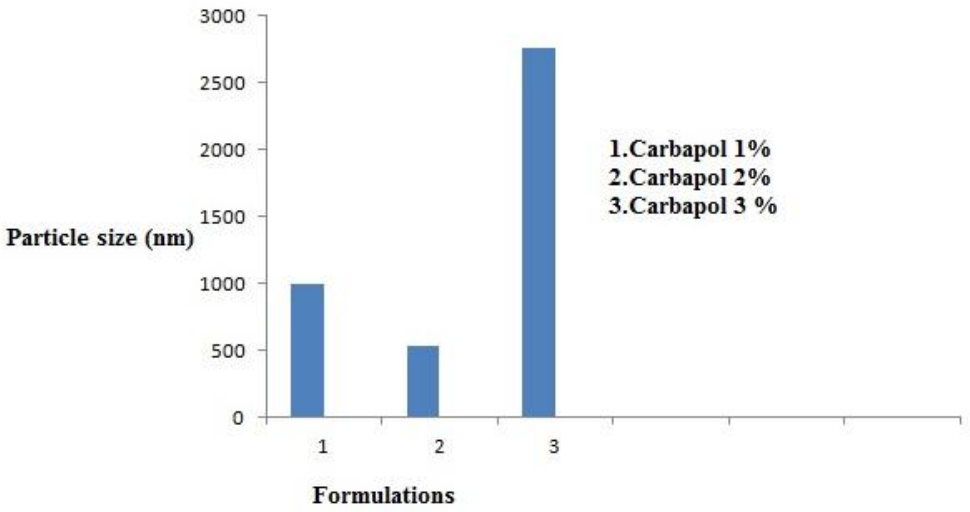

Figure 5: Comparison of particle sizes of carbapol formulations

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 carbapol 1%, Carbapol 2 % and carbapol 3% formulations were found to be 88.76 %, 77.79% and 72.25% respectively. From the results it was found that drug content of Carbapol 1% was more when compared with other two formulations (Le *et al.,* 2009).

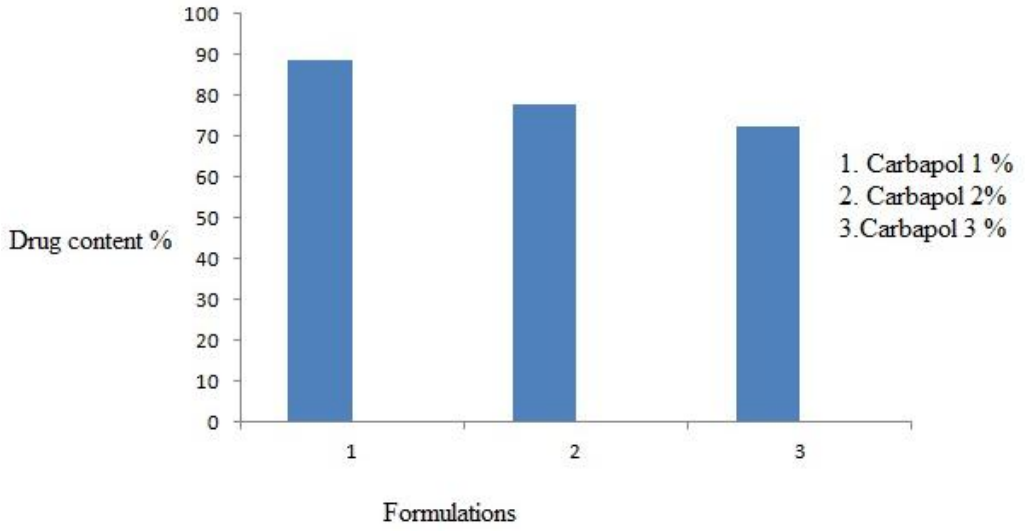

Figure 6: Comparison of drug contents of Carbapol Formulations

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 (Susmita *et al*., 2013). 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 Entrapent efficiencies of carbapol 1%, Carbapol 2 % and carbapol 3% formulations were found to be 44.8%, 39.27 % and 64.42% respectively. From the results it was found that entrapment efficiency of Carbapol 3% was more when compared with other two formulations.

Loading capacities of carbapol 1%, Carbapol 2 % and carbapol 3% formulations were found to be 19.72%, 15.84 %, 44.58% respectively. From the results it was found that loading capacity of Carbapol 3% was more when compared with other two formulations.

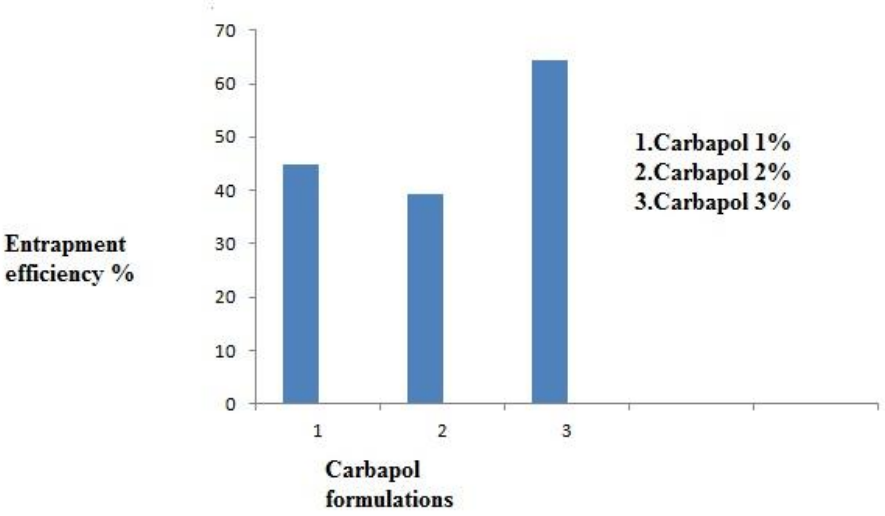

Figure 7: Comparison of Entrapment efficiencies of carbapol formulations

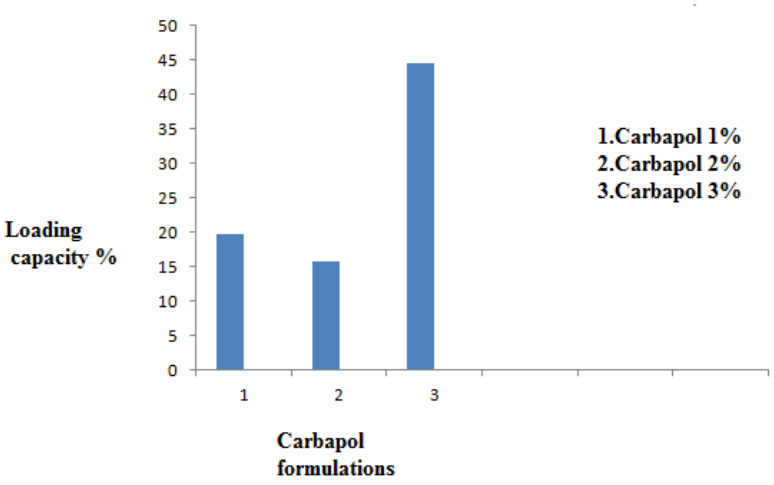

Figure 8: Comparison of loading capacities of carbapol formulations

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.

The Electrophoretic mobility values of carbapol 1%, Carbapol 2 % and carbapol 3% formulations prepared by polymerization technique were found to be -1.113,-1.702 and 0.9985 respectively and the Zetapotential values were found to be -14.44,-21.7 and -12.7 respectively. From the results it was found that Electrophoretic mobility and Zetapotential value of Carbapol 2% was higher when compared with other two formulations.

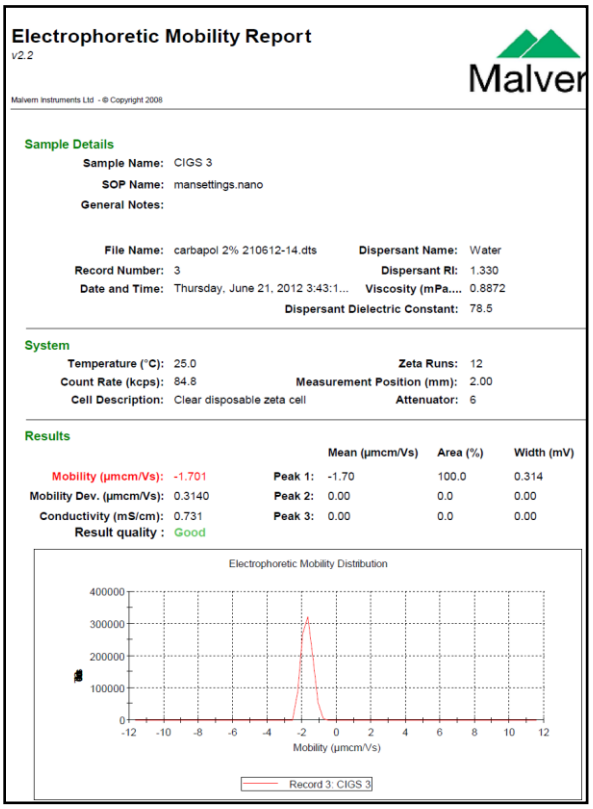

Figure 9: Electrophoretic mobility of carbapol 2% Formulation

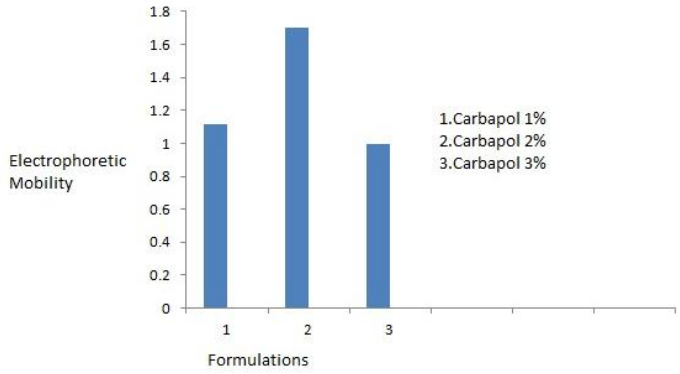

Figure 10: Comparison of electrophoretic mobility values of carbapol formulations

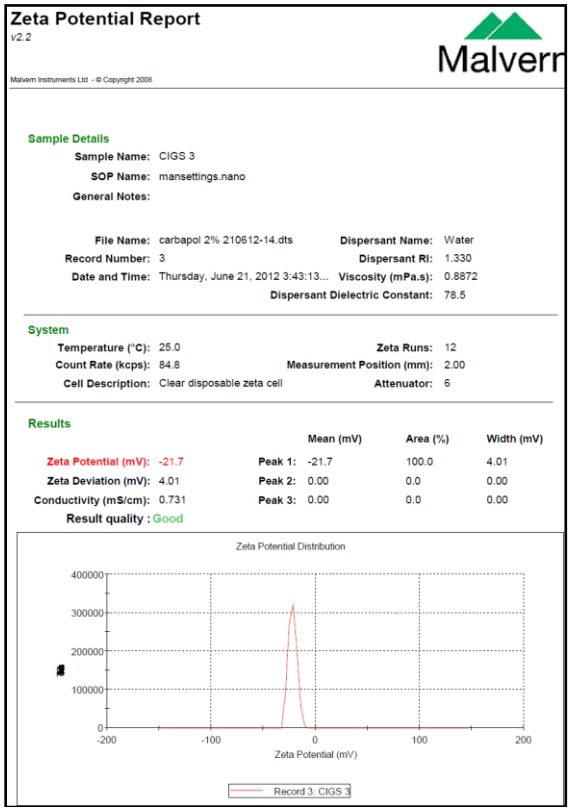

Figure 11: Zeta potential reports of carbapol 2 % nanoparticles prepared by Polymerization technique

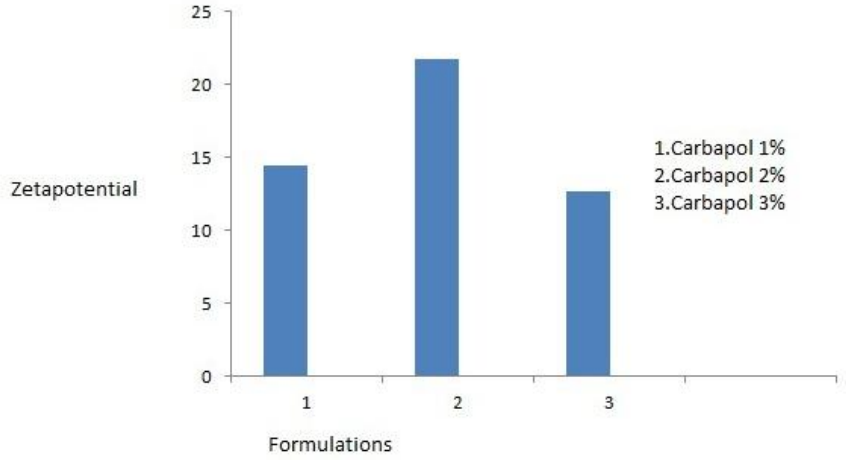

Figure 12: Comparison of Zetapotential values of carbapol formulations

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 using UV Spectrophotometer. In all carbapol Formulations the drug release was slow, extended over a period of 24 hrs. In a time period of 9 hrs 85.4 %, 93.29 % of drug has been released from carbapol

1 %, carbapol 2 % formulations respectively. In a time period of 24 hrs 93.29 % of drug has been released from carbapol 3 % formulation.

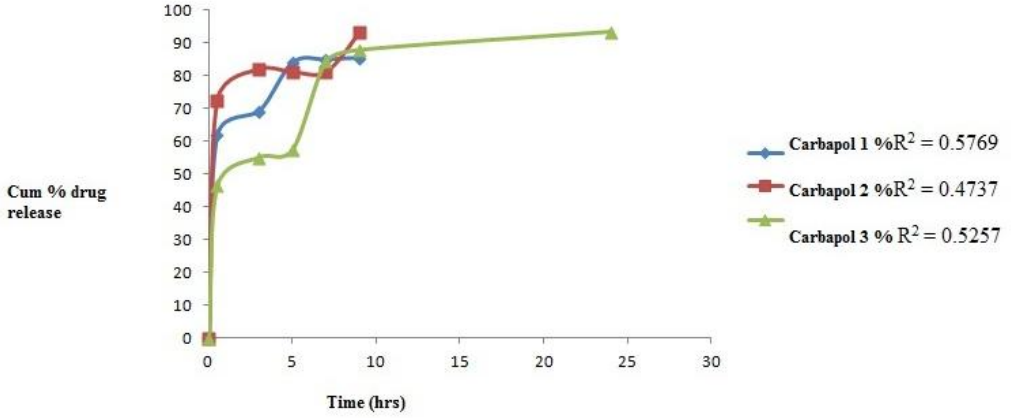

Figure 13: Comparison of invitro drug release profiles of carbapol formulations

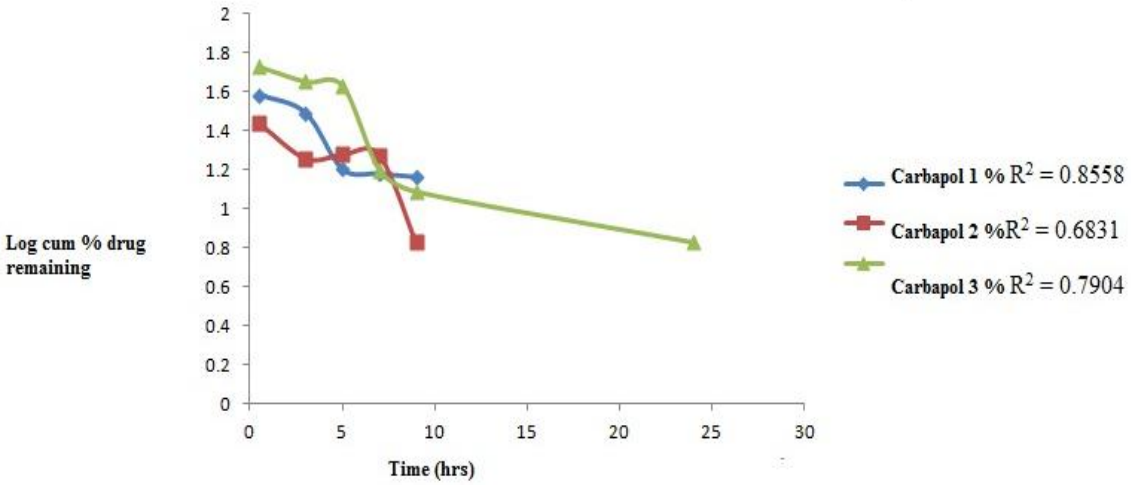

Figure 14: Comparison of First order release of carbapol formulations

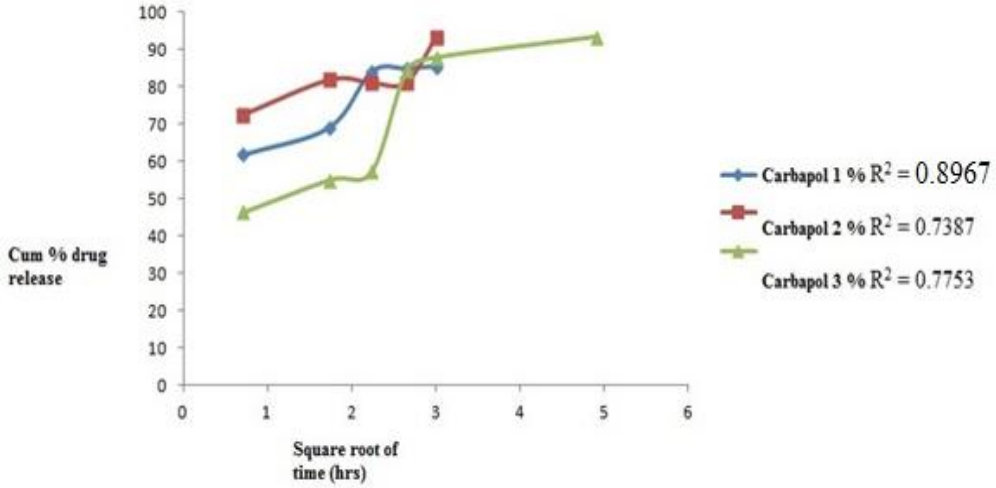

Figure 15: Comparison of Higuchis square root time dependent plots of carbapol formulations

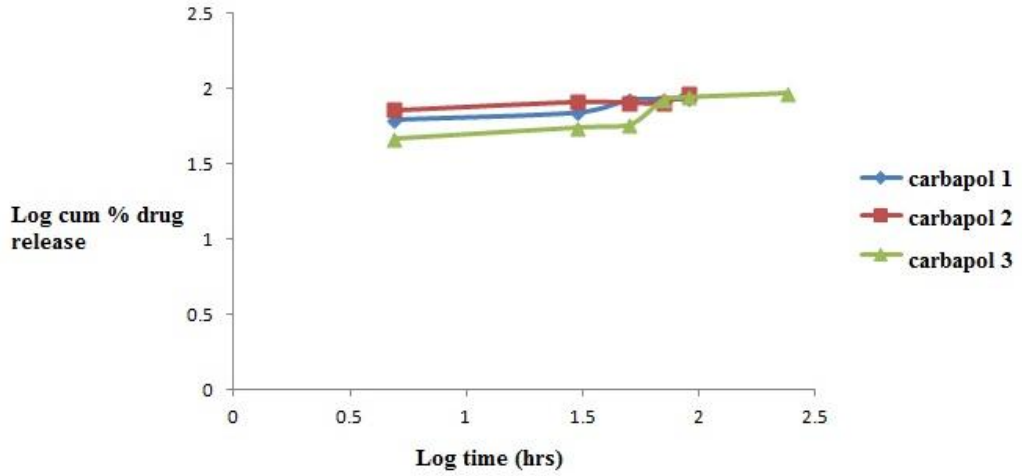

Figure 16: Comparison of peppas double log plots of Carbapol formuations

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 carbapol 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.

Drug content, entrapment efficiency and loading capacity of Formulation 3 was more when compared with other two formulations.

This may be because of the large diameter of the particles in Formulation 3.This can be explained from the fact that a grater the amount of drug results in more viscous dispersed phase, making the mutual dispersion of the phases more difficult and originating larger particles. Drug content, entrapment efficiency and loading capacity was more in Formulation 3.

Electrophoresis 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 -21.7mV for Formulation 2.

Invitro 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. Particle diameter of Carbapol 3 % formulation was more (2761nm) when compared with remaining two formulations. So drug release was extended up to 24 hrs in carbapol 3 % Formulation. When compared Formulations 1 and 2, in a time period of 9 hrs the drug release was more in formulation 2.

Conclusion

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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