Preparation, Characterization and Water Uptake Behavior of Polysaccharide Based Nanoparticles

Swelling behavior of nanoparticles

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Abstract- **In most of the cases where conventional dosage forms are used, only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its biochemical properties. Therefore, one of the most attractive areas of research in drug delivery today is the design of nanosystems that are able to deliver drugs to the right place, at appropriate times and at the right dosage. The present study reports a novel strategy for the preparation of nanoparticles of alginate and chitosan by microemulsion crosslinking technique. Morphology and structural characterization of prepared nanoparticles were investigated by transmission electron microscope (TEM) analysis, and Fourier transform infrared spectra (FTIR), respectively. Nanoparticles were tested for cytotoxicity with human foreskin fibroblast (HFF) cells. The particles were allowed to swell in phosphate buffer saline (PBS) and the influence of factors such as chemical composition of nanoparticles; pH and temperature of swelling bath were studied on water sorption capacity of nanoparticles. The prepared nanoparticles could prove to be an excellent option as carrier for controlled and targeted delivery of anticancer drugs.**

Keywords- Chitosan; Alginate; Swelling; Nanoparticles

I. INTRODUCTION

Nanocarriers have been receiving a lot of attention recently and their utilization in treating complex diseases like tumor has transformed into a growing Pharma industry. The recent Food and Drug Administration approval of Abraxane (ABI-007) and analbumin-taxol nanoparticles for the treatment of breast cancer has opened the doors for the development of other nanoscale drug delivery devices with the aim of concentrating more of a drug onto the target tissue and less onto healthy tissues [1]. Nanotechnology is already in use in more than 600 products in the market, yet the U.S. Food and Drug Administration still has relatively limited knowledge of ability to assess the safety and impact of the technology that is already being used in consumer products. Nanotechnology refers to research and technology development at the atomic, molecular, and macromolecular scales, leading to the controlled manipulation and study of structures and devices with length scales in the 1 to 100 nanometers range. The reason why these nanoparticles are attractive for medical purposes is based on their important and unique features, such as their much large surface to mass ratio, their quantum properties and their ability to adsorb and carry other compounds. Nanoparticles have a relatively large (functional) surface which is able to bind, adsorb and carry other bioactive agents such as drugs, probes and proteins. The primary goals for research of nano-bio-technologies in drug delivery includes, developments of new safe medicines and more specific drug delivery systems having reduced toxicity, greater safety, biocompatibility while maintaining therapeutic effects [2]. These favorable properties can be achieved by using naturalling occurring polymers for nanoparticles like polysaccharides.

Chitosan is a natural cationic polysaccharide derived by deacetylation of chitin, a copolymer consisting of combined units of glucosamine and N-acetyl glucosamine [3, 4]. In the pharmaceutical field advantageous biological properties of chitosan have promoted its extensive studies as carriers both of drugs [5, 6] and of proteins [7, 8]. Chitosan is a low molecular naturally occurring polymer. It is the most promising biodegradable polymer for nanoparticle preparation and their subsequent therapeutic applications. The average molecular weight of chitosan is 80 kDa. Other benefits of chitosan nanoparticles include their small size, low cytotoxicity, less inflammatory responses, and no cellular deposition [9]. Chitosan is prone to chemical and physical modifications, and is very responsive to environmental stimuli such as temperature and especially pH. These features make chitosan a smart material with great potential for developing multifunctional nanocarriers systems to deliver large varieties of therapeutic agents administrated in multiple ways with reduced side effects [10, 11].

Nowadays, it has become customary to use the naturally occurring polymers as source materials for drug carriers. Many other natural polymers have also been used for nanoparticles formulation that included starch, carboxymethyl cellulose [12], alginate [13], etc. which are biocompatible, non-toxic, non-carcinogenic and biodegradable also. Recently, many biochemists have identified that chitosan is not rejected by the body and it can improve the effective and safe delivery of drugs and vaccines with its absorptive power. Although chitosan alone has been extensively used in making drug delivery systems, its formulations with other natural polymers could give rise

to drug carrier systems with enhanced performance and wider applicability. Nanoparticles consisting of synthetic biodegradable polymers, natural biopolymers, lipids and polysaccharides have been developed and tested over the past decades. Recently, the idea of using nanoparticles made from natural biodegradable polymers to deliver drugs has provoked great interests. Among them, alginate is very promising and has been widely exploited in pharmaceutical industry for controlling drug release. Chitosan and alginate have highly pH-dependent swelling properties, which could be used to control the release of drugs [14, 15] and may be used to treat colon cancers.

Polyelectrolyte complexes, an important class of macromolecular materials, are formed by electrostatic interactions between polymers that carry oppositely charged ionizable groups [16]. In the present study chitosan and alginate have been chosen as the constituent biopolymers to design nanoparticle because of their different chemical properties as linear polyamine, reactive amino and, reactive hydroxyl groups, chelating potential with many transitional ions and biological properties as biocompatible, biodegradable, safe and non-toxic, etc. Most importantly, both chitosan and alginate are opposite in their charges and exhibit pH dependence in acid and alkaline pH ranges, respectively. In the present study nanoparticles of both chitosan and alginate have been prepared by ionotropic gelation method in their microemulsion and their water imbibition capacity has been evaluated under varying experimental conditions.

II. METHODOLOGY

A. Materials

Chitin {$(C_8H_{13}NO_5)_n$, (Sunchem, India)}, sodium alginate, a sodium salt of alginic acid, of medium viscosity (Chemika- biochemical, India) were employed as constituent biopolymers for preparing nanoparticles and used as received. The crosslinkers used in the present study were $CaCl_2$ and tripolyphosphate (TPP), (Research Lab, Pune India) for crosslinking alginate and chitosan, respectively. Paraffin oil of medium viscosity was used for preparing emulsions of the biopolymers and crosslinkers.

B. Methods

1) Conversion of Chitin to Chitosan:

Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (crowns, shrimps, etc.) and cell wall of fungi. Common method for synthesis of chitosan is deacetylation of chitin using sodium hydroxide in excess as a reagent and water as a solvent, this is kept at 50ºC for 8-10 days and then washing is done. This reaction pathway, when allowed to go to completion (complete deacetylation) yields upto 98% product [17]. Deacetylation reaction of conversion of chitin to chitosan is shown in Fig. 1.

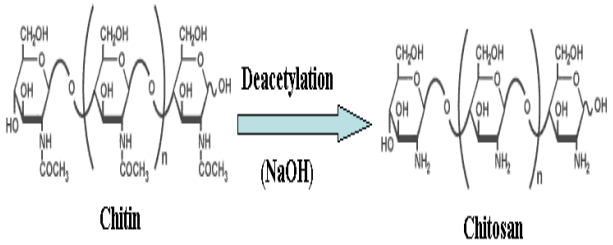

Fig. 1 Deacetylation reaction of conversion of chitin to chitosan

2) Synthesis of Nanoparticles:

The methods for nanoparticles of pharmaceutical use are broadly divided into two categories viz. phase separation and solvent evaporation. In the present investigation, however, a microemulsion crosslinking technique has been followed as published by Cascone et al [18]. In brief, the method may be described as below:

An ‘aqueous phase’ was prepared by dissolving a definite amount of chitosan in 2% acetic acid solution at concentration of 8 mg/mL and sodium alginate solution at concentration of 15 mg/mL, respectively. The above two solutions were mixed into the ‘oil phase’ i.e. paraffin oil (6 mL) with vigorous shaking (shaking speed 300 rpm, 0.5 HP motor capacity) for 30 min, and to these emulsions fixed volumes of $CaCl_2$ (0.4M) and TPP (0.3M) were added dropwise. The crosslinking reaction, as shown in Fig. 2, was allowed to take place for 4 hours. The nanoparticles prepared were cleaned by centrifugation (Remi Centrifuge, India) and resuspending them in toluene three times and then twice in acetone. The final product was dried at room temperature to obtain a fine cream colored powder.

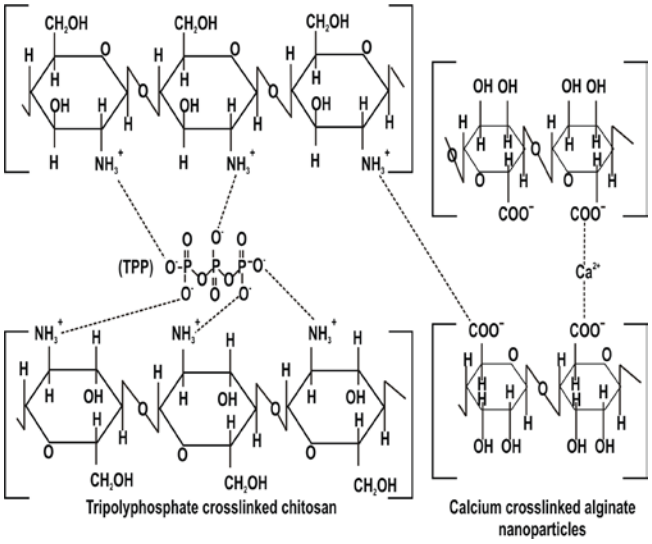

Fig. 2 Scheme of crosslinking reactions of chitosan and alginate

III. CHARACTERIZATION

A. FTIR Spectral Analysis

FTIR spectral analysis was carried out for structural characterization of prepared nanoparticles. The FTIR spectra of crosslinked nanoparticles were recorded on a

FTIR spectrophotometer in the range of 400-5000 cm^{-1} (FTIR-8400S, Shimadzu Spectrophotometer).

B. TEM (Transmission Electron Microscopy) Analysis

The TEM images of the prepared alginate and chitosan nanoparticles were recorded to determine internal particle distribution and morphology of the nanoparticles. Transmission electron microscopy (TEM) was performed by using a Morgagni-268-D transmission electron microscope with an acceleration voltage of 80.0 W. The TEM measurements were done by dispersing a drop of the sample solution on Formvar-coated C grids.

C. *Cytotoxicity Test*

In order to determine cytotoxicity, the nanoparticles were suspended in culture medium, serially diluted across 96-well micro titer plates, and incubated at 37°C with 5% CO_2 .Two sets of exposure times were carried out. These included 4 ends of each exposure period a MTS (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium) mixture was added. After the completion of exposure period, the plates were then placed on a microcell plate reader, shaken for 10 sec and the absorbance of the formazan product was read at 492 nm. Each experiment was repeated on three separate occasions. Two internal controls were set up for each experiment: (1) an ICo consisting of cell only; and (2) IC100 consisting of medium only. Background absorbance due to the non-specific reaction between test compounds and the MTS reagent was deducted from exposed cell values [19].

D. Swelling Studies of Nanoparticles

The swelling behavior of polymeric nanoparticles depends on the nature of the solvent and internal structure of polymer network. Water intake capacity of nanoparticles was determined by a conventional gravimetric procedure as reported in the literature [20]. In brief, 0.1 g of nanoparticles were allowed to swell in a definite volume (10 mL) of phosphate buffer saline (PBS, pH 7.4) taken in a preweighed sintered glass crucible (pore size 5-10 µm) and weighed after 24 hours by removing excess phosphate buffer saline by vacuum filtration. The swelling of nanoparticles was monitored continuously up to 15 min after which no weight gain of swollen nanoparticles was recorded. This clearly indicated the arrival of equilibrium swelling conditions. The amount of water imbibed by the nanoparticles was calculated by the following equation,

$$\text{Swelling ratio} = Wo / Wt$$

Where, Wo and Wt are the weight of dry and swollen nanoparticles at zero and time t, respectively.

E. *Swelling Studies in Physiological Fluids*

In order to study the swelling of nanoparticles in simulated biological media, the following aqueous fluids (100 mL) were prepared: Saline water (0.9 g NaCl), synthetic urine (0.8 g NaCl, 0.10 g $MgSO_4$, 2.0 g urea, 0.6 g $CaCl_2$), Urea (5 g), D-glucose (5 g),KI (15 g).

F. Evaluation of in Vitro Blood Compatibility

1) Protein Adsorption:

The foremost event occurring at the interface of the blood- material contact is the adsorption of plasma proteins (bovine serum albumin, fibrinogen, etc.) which subsequently influences the adhesion of leukocytes, macrophages or platelets and ultimately leads to fibrous encapsulation. Thus, the adsorption of proteins could be one of the determinants of biocompatible nature of the material. The adsorption of proteins was studied as below:

A known volume of protein solution of definite concentration was mildly shaken with the known weight of nanoparticles for a definite time period and the reaming concentration of protein was monitored in the supernatant solution spetrophotometrically. The amount of the adsorbed protein was calculated with the help of the following mass balance equation.

$$\text{Adsorbed amount (mg m}^{-2}) = (Co-Ca)\ V / A$$

where, Co and Ca are the concentrations of protein solution (mg per mL) before and after adsorption, respectively. V is the volume of the protein solution and A is the surface area of the adsorbent.

2) % Hemolysis:

Hemolysis experiments were performed on the surfaces of the prepared nanoparticles as reported elsewhere [21]. In a typical experiment, dry particles were equilibrated in normal saline water (0.9% NaCl solution) for 24 h at 37°C and human blood was added. After 20 min, 20 mL of saline was added to stop haemolysis and the sample is incubated for 60 min at 37°C. Positive and negative controls were obtained by adding 0.025 mL of human ACD blood and saline solution, respectively to 2.0 mL of distilled water. Incubated samples were centrifuged for 45 min, the supernatant was taken out and its absorbance was recorded on a spectrophotometer at 545 nm. The percent haemolysis may be calculated using the following relationship.

$$\%\ \text{Hemolysis} = A\ \text{test sample} - A\ (-)\ \text{control} / A\ (+)\ \text{control} - A\ (-)\ \text{control}$$

Where A = Absorbance.

G. Statistical Analysis

All the experiments were done at least thrice and data and Figs. have been shown along with S.D. and error bars, respectively.

IV. RESULTS AND DISCUSSION

A. Crosslinking between Alginate and Chitosan

In the present study both chitosan and alginate were crosslinked in two ways, viz; (i) both the biopolymers were crosslinked with tripolyphosphate and calcium chloride, respectively and (ii) the polymers were mutually crosslinked due to electrostatic attraction between them. In this way, chitosan and alginate are tightly held with each other via strong electrostatic attractive forces. The proposed

crosslinking reaction between alginate and chitosan is shown in Figure 2.

B. FTIR Analysis

The potential interactions between the biopolymers of the nanoparticles could be explicitly explained by spectral evidences revealed from the FTIR spectra. The spectra of native alginate and chitosan, and prepared nanoparticles are shown in Fig. 3. In the spectra of chitosan, a broad band at 3424 cm^{-1} corresponds to the amine and hydroxyl groups; the peak at 2876 cm^{-1} is due to -OH stretching; the absorption band of the carbonyl (C=O) stretching of the secondary amide (amide I band) appears at 1655 cm^{-1}, and the bending vibrations of the N–H (N acetylated residues, amide II band) at 1599 cm^{-1} [22]. The peaks at 1423 and 1381 cm^{-1} belong to the N–H stretching of the amide and ether bonds and N–H stretching (amide III band), respectively. The peaks observed at 1081 and 1033 cm^{-1} are due to secondary hydroxyl group (characteristic peak of -CH-OH in cyclic alcohols and C-O stretch) and the primary hydroxyl group (characteristic peak of $-CH_2$-OH in primary alcohols and C-O stretch) [23]. The bands around 1030 cm^{-1} (C-O-C stretching) present in the IR spectrum of sodium alginate are attributed to its saccharide structure. In addition, the bands at 1617 and 1417 cm^{-1} are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups [24]. Furthermore, the absorption band of chitosan at 3424 cm^{-1} (stretching vibration of -OH and $-NH_2$) shifts to 3421 cm^{-1} after reaction with alginate and becomes broad. These results indicate that the carboxylic groups of alginate associate with positively charged ammonium groups of chitosan through electrostatic interactions to form the polyelectrolyte complex. Moreover, the ionotropic crosslinking of chitosan and alginate by tripolyphosphate and calcium chloride also occurs via electrostatic forces.

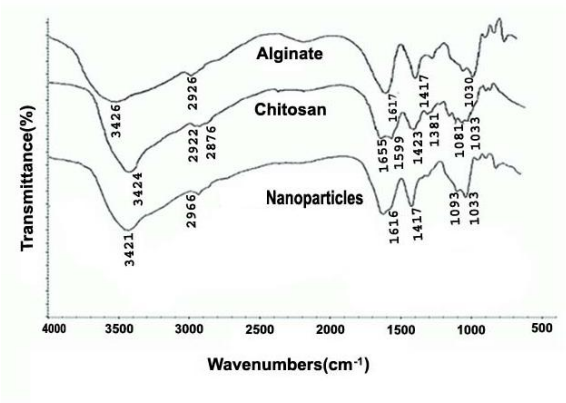

Fig. 3 FTIR spectra of alginate, chitosan and crosslinked alginate-chitosan nanoparticles

C. TEM (Transmission Electron Microscopy) Analysis

The size of nanoparticles plays an important role in deciding whether the particles are eventually utilizable for any pharmaceutical purpose or not. It is known that the particles larger than 400 nm (i.e. the minimal diameter of the capillaries), will be filtered by the lung. In addition, it has been reported that particles which exceed than 200 nm tend to eliminate immediately by one of the organs from MPS. Thus, the smaller the size of nanoparticles is, the larger their circulation time in blood vessel would be.

In the present study the TEM image of the nanoparticles is shown in Fig. 4 which clearly reveals that the particles are almost spherical in size and are present as aggregated ones. The size of the particles does not vary significantly from each other and falls within the approximate range of 10 to 30 nm. Thus, the nanoparticles prepared in this study may be of great use as drug carrier.

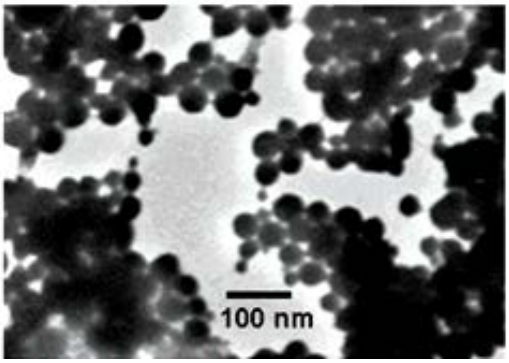

Fig. 4 The TEM image of alginate-chitosan nanoparticles

D. Cytotoxicity

The cytotoxicity reactivity were graded based on zone of lysis, vacuolization, detachment and membrane disintegration as 0, 1, 2 ,3 and 4 represent ting none, slight, mild, moderate and severe, respectively. The quantitative evaluation of reactivity for negative and positive controls and test samples are summarized in shown through the microscopic observations as depicted in Fig. 5 (a), (b), and (c), respectively. It was found that the test sample showed none reactivity to fibroblasts cells after 24 h of contact. The achievement of numerical grade more than 2 is considered as cytotoxic effect. Since the polymer network material in the present work achieved a reactivity grade not more than 2, the material is considered as not cytotoxic. Negative control gave none cytotoxic reactivity and positive control gave severe cytotoxic reactivity as expected.

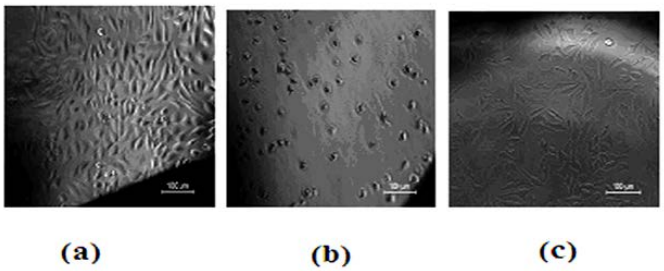

Fig. 5 Microscopic images showing L-929 cells around (a) negative control (b) positive control and (c) polymer network sample, respectively

E. Effect of Chemical Compositions

The swelling of polymer networks greatly depends on the internal structural parameters of the swelling network such as the amounts of constituent polymers present, crosslink density of the hydrogel, presence of hydrophilic and/or hydrophobic functional groups, etc. In the present

study, the influence of the chemical composition of alginate-chitosan nanoparticles on their swelling ratio has been investigated by varying the amounts of chitosan, alginate and crosslinkers ($CaCl_2$ and TPP), in the feed mixture, respectively. The observed results may be discussed as below:

1) Effect of Variation of Chitosan:

When the amount of chitosan is varied in the range of 0.2 g to 0.5 g in the feed mixture of the nanoparticles, the swelling ratio is significantly affected. The results obtained are shown in Fig. 6, that shows an increase in swelling with increasing amount of chitosan up to 0.3 g, whereas, further increase in the amount of chitosan (i.e. beyond 0.3 g) results in a fall in the swelling ratio. The observed initial increase in the swelling ratio may be attributed to the fact that with increasing amount of chitosan, the hydrophilicity of nanospheres also increases and, consequently, more water sorption is observed. However, beyond 0.3 g of chitosan content, the decrease obtained in swelling may be due to the reason that at much higher content of chitosan, the nanospheres become morphologically and structurally compact and as a result the diffusion of water molecules into the particles get hindered. This clearly lowers the swelling ratio as reported elsewhere too [25].

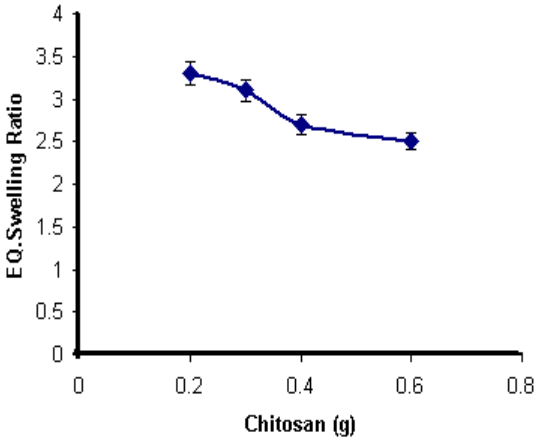

Fig. 6 Effect of chitosan variation on the swelling ratio of the nanoparticles

2) Effect of Variation of Alginate:

The influence of alginate content in the nanoparticles on their swelling ratio has been investigated by increasing the amount of alginate in the range of 0.2 to 0.4 g in the feed mixture of the nanoparticles. The results are shown in Fig. 7, which indicate that in the whole studied range of alginate, the swelling ratio constantly increases. The observed increase in water sorption capacity could be attributed to the fact that due to hydrophilic and anionic nature of alginate, it's increasing amount results in an enhanced hydrophilicity of the network and electrostatic repulsions between the alginate chains. These two factors collectively result in greater sorption of water molecules [26].

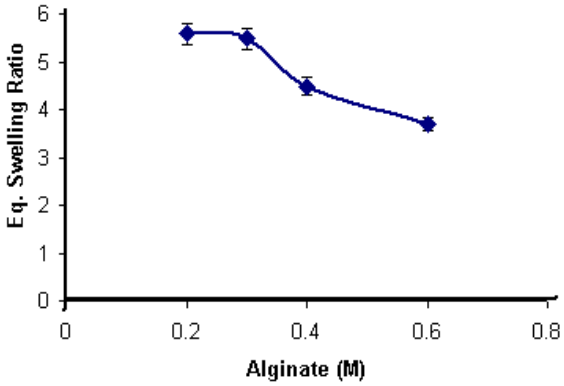

Fig. 7 Effect of Alginate variation on the swelling ratio of the nanoparticles

3) Effect of Variation of Crosslinkers:

Calcium chloride and tripolyphosphate are well known crosslinking agents of chitosan and alginate, respectively. When the concentrations of $CaCl_2$ and TPP increase in the range from 0.1 to 0.6 M, the swelling ratio also increases as shown in Figures 8 and 9, respectively. The increase in swelling ratio with increasing crosslinker concentration is an unusual finding and may be explained due to the reason that because of increasing degree of cross-linking of nanospheres, their size gets smaller and this results in an increase in external as well as internal surface area of nanospheres [27] which consequently results in a greater interaction between the constituent biopolymer and water molecules. Another possible reason may be that the increasing amounts of calcium chloride and TPP in the feed mixture of the nanoparticles may produce electrostatic repulsions between positively charged chitosan molecules and calcium ions, and negatively charged alginate molecules and TPP molecules. Thus due to an enhanced repulsion the biopolymer chains of the nanoparticles network get relaxed causing an increase in water sorption capacity.

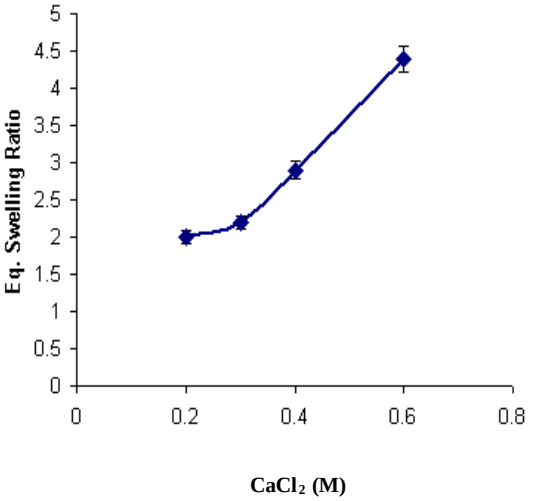

Fig. 8 Effect of $CaCl_2$ variation on the swelling ratio of the nanoparticles

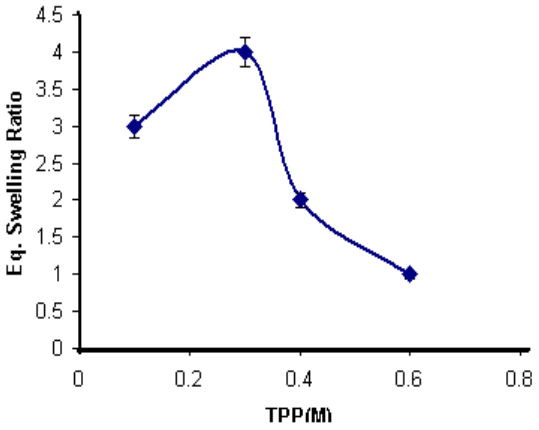

Fig. 9 Effect of TPP variation on the swelling ratio of the nanoparticles

4) Effect of Biological Fluids:

It is well established that the equilibrium swelling behavior of a polymer network in a solvent is the eventual result of a balance between osmotic and restoring elastic pressure. The presence of salts in the surrounding aqueous medium is capable of tilting this balance which may results in either an increase or decrease in swelling of polymer network. The effect of simulated biological fluids on swelling of nanoparticles has been examined by performing swelling experiments in the presence of urea, D-glucose (5%w/v), potassium iodide (KI) (15%w/v) and the physiological fluids such as saline water (0.9%NaCl) and artificial urine. The results are shown in Figure 10 which clearly shows that the presence of solute suppresses the swelling ratio due to a decrease in osmotic pressure of the external solution [28]. The possible interactions between the solute and biopolymer molecules may also cause a decrease in swelling ratio.

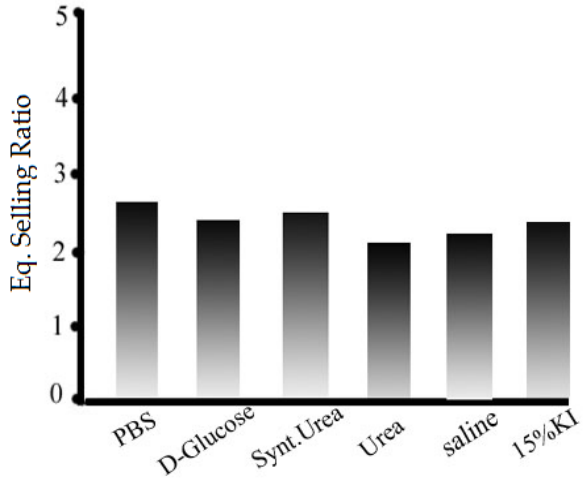

Fig. 10 Effect of biological fluids on the swelling ratio of the nanoparticles

5) Effect of pH:

The pH of the solution is one of the prime factors that drastically influence the swelling behavior of a system. pH responsive macromolecular devices have been most frequently used to design controlled release formulations for oral administration of macro drug, like peptides and insulin, which remains the most clinically acceptable way of drug delivery [29]. In the present study the effect of pH on the swelling ratio of nanoparticles was investigated by performing water sorption experiments at pH 1.7, 7.4 and 8.6 which represent the pH conditions of stomach, blood and large intestine, respectively.

The swelling results are shown in Figure 11 which indicates a significant dependence of swelling behavior of the nanoparticles on different pH values. The result reveals that at pH 1.8, a lower swelling ratio was found, because PKa of alginate is about 3.2 [30] and most of the carboxyl groups in the alginate exist in the form of COOH in the lower pH medium (pH 1.7). In the nanoparticle network, the *H* bonding constructed by –COOH groups of alginate led to the stronger interaction between polymer chains. Accordingly, the swelling ratio in pH 1.7 is relatively lower. At higher pH, the carboxylic acid group gets ionized and acquires $–COO^-$ form. Thus, the weak H-bonding interaction between polymer chains and electrostatic repulsion between $–COO^-$ groups resulted in the higher swelling ratio [31]. Therefore, based upon pKa of alginate 3.4 and chitosan 6.2 [32], the species involved in interactions are NH^{3+} and COOH (at pH 1-3), NH_2 and COO^- (at pH 7-13). In acidic conditions, the swelling is controlled mainly by the amino group (NH_2). It is a weak base with an intrinsic pKa of about 6.2 and so it gets protonated and the increased charge density on the polymer should enhance the osmotic pressure inside the particles because of the NH^{+3}-NH^{+3} electrostatic repulsion. This osmotic pressure difference between the internal and external solutions of the network is balanced by the swelling of the gel. However, under very highly acidic conditions (pH<3), a screening effect of the counter ions, i.e. Cl^-, shield the charges of the ammonium cations and prevent an efficient repulsion. As a result, a remarkable decrease in equilibrium swelling is observed. Again, the screening effect of the counter ions (Na^+) limits the swelling at pH>8.5. When the ionic strength of the solution is increased, the difference in osmotic pressure between bulk of the particles and the medium decreases which results in lower the swelling capacity [33].

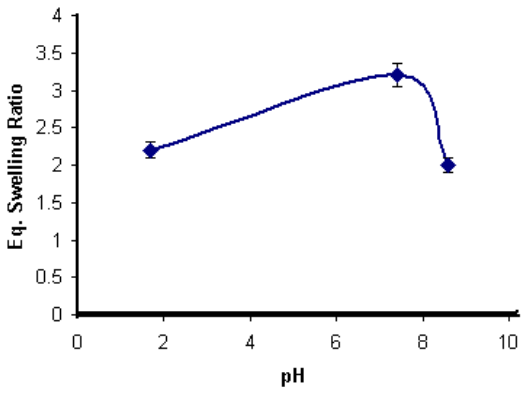

Fig. 11 Effect of varying pH on the swelling ratio of the nanoparticles

6) Effect of Temperature:

The effect of temperature on the degree of water sorption has been investigated by carrying out water sorption experiments in the range 15° to 45°C. The results are presented in Figure 12, which clearly indicate that the swelling ratio marginally increases in the temperature range from 15°C to 30°C, while beyond 30°C a sudden fall in swelling ratio is noticed. The observed initial increase in swelling of nanoparticles may be attributed to the fact that a rise in temperature enhances rates of water diffusion and segmental mobility of macromolecular chains, which consequently result in a greater degree of swelling. However, beyond 30°C the swelling ratio decreases, it may be due to the reason that at higher temperature the hydrogen bonds holding water molecules and the polymer chains get broken and, therefore, the swelling ratio decreases [34].

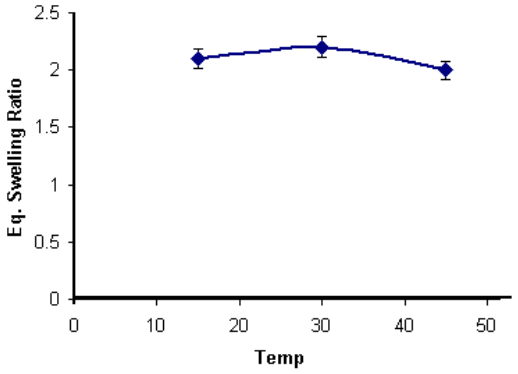

Fig. 12 Effect of temperature on the swelling ratio of the nanoparticles

F. Evaluation of Biocompatibility

The selection of a material to be employed as a biomaterial for a specific end use must meet several criteria such as favorable physicochemical properties, function desired, nature of the physiological environments, adverse effects in case of failure, expected durability and considerations relating to cost and ease of production. Whatever being the type of materials the biocompatibility is the foremost requirement for all biomaterials. In the present study, the assessment of in vitro biocompatibility has been made on the basis of two in vitro tests viz. BSA (bovine serum albumin) adsorption test, and hemolysis assay as discussed below:

1) BSA Adsorption:

In the present investigation, the biocompatibility of prepared nanoparticles has been judged by monitoring the amount of protein (BSA) adsorbed by the nanoparticles. The results are shown in Table 1, which indicate that the amount of adsorbed BSA decreases with increasing amount of chitosan, alginate, TPP and $CaCl_2$ in the feed mixture of nanoparticles. The observed results may be explained on the basis of fact that chitosan, alginate, TPP and $CaCl_2$ are hydrophilic in nature and do not provoke either any damage to blood cells or the surface of plasma proteins which are the main requisites for biocompatibility. Furthermore, it is also known that protein adsorption is more favorable onto hydrophobic surface rather than hydrophilic one. Thus, an enhanced hydrophilic nature of nanoparticles with increasing content of chitosan, alginate, and TPP and $CaCl_2$ results in lower protein adsorption.

TABLE I DATA SHOWING THE BIOCOMPATIBILITY PARAMETERS WITH VARYING COMPOSITION OF NANOPARTICLES

Alginate(g)	*Chitosan(g)*	*$CaCl_2$(M)*	*TPP(M)*	*%Hemolysis*	*BSA Adsorption (mg g^{-1})*
0.2	*0.3*	*0.4*	*0.3*	*27.10 ± 0.81*	*0.48 ± 0.014*
0.3	*0.3*	*0.4*	*0.3*	*26.45 ± 0.73*	*0.35 ± 0.010*
0.4	*0.3*	*0.4*	*0.3*	*24.30 ± 0.72*	*0.24 ± 0.007*
0.3	*0.2*	*0.4*	*0.3*	*27.12 ± 0.81*	*0.35 ± 0.010*
0.3	*0.4*	*0.4*	*0.3*	*26.11 ± 0.78*	*0.21 ± 0.006*
0.3	*0.3*	*0.3*	*0.3*	*27.00 ± 0.81*	*0.49 ± 0.014*
0.3	*0.3*	*0.6*	*0.3*	*21.45 ± 0.64*	*0.32 ± 0.009*
0.3	*0.3*	*0.4*	*0.1*	*31.67 ± 0.95*	*0.62 ± 0.018*
0.3	*0.3*	*0.4*	*0.4*	*34.10 ± 1.0*	*0.22 ± 0.006*

2) % Hemolysis Test:

Hemolysis studies were performed on nanoparticles of various composition and the results are summarized in Table 1 which indicate that with increasing chitosan, alginate and $CaCl_2$ content, the extent of hemolysis decreases. The observed results may be attributed to the reason that with change in chitosan, alginate and $CaCl_2$ concentration in the feed mixture of the nanoparticles, the surface composition favorably changes, which improves the blood compatible quality of the material.

V. CONCLUSIONS

Nanoparticles of alginate and chitosan were prepared by microemulsion crosslinking technique and their swelling behavior had been investigated in this study. It is noticed that the water sorption property is greatly determined by the chemical composition of the nanoparticles. When the amount of chitosan increases upto 0.3 g, the adsorption of PBS increases, whereas, a further increase in chitosan results in a fall in the swelling ratio, While in the case of increasing alginate content, the degree of swelling also increases. The extent of water sorption by the nanoparticle is also found to increase from acidic to natural pH range while a fall in the swelling ratio is noticed with increasing pH in the alkaline pH range. The swelling ratio increases with increasing temperature upto 30ºC and then it decreases. A lower degree of swelling is also observed in simulated biological fluids like saline water, artificial urine, KI solution and D-glucose solution.

The nanoparticles were also characterized by various analytical techniques, such as FTIR spectroscopy, SEM, TEM and XRD, which confirmed the crosslinking of

nanoparticles. The polymeric nanoparticles clearly show the characteristic groups of alginate and chitosan in their FTIR spectra. Cytotoxicity results show that the prepared particles have no cytotoxicity at all.

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