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Thiolated chitosan coated poly hydroxyethyl methacrylate nanoparticles: Synthesis and characterization

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Abstract

The purpose of the present study was to prepare and characterize poly hydroxyethyl methacrylate (pHEMA) nanoparticles coated by thiolated chitosan (chitosan–glutathione conjugate), using radical polymerization method and cerium (IV) ammonium nitrate as initiator. The effects of different parameters such as molecular weight of thiolated chitosan, concentration of initiator and monomer on physicochemical properties of nanoparticles were studied. FT-IR, dynamic light scattering, scanning electron microscope (SEM), transmission electron microscope (TEM) and zeta potential determination were used for characterization of nanoparticles. Nanoparticles were spherical with a size range of 150–750 nm. The size of nanoparticles reduced when the concentration of initiator was increased, while the concentration of monomer influenced directly the size of nanoparticles. The nanoparticles carried positive charge and a clear layer surrounded the poly (hydroxyethyl methacrylate) core of nanoparticles observed using TEM confirms the presence of thiolated chitosan as the coating layer.

Keywords: Thiolated chitosan; Poly hydroxyethyl methacrylate; Nanoparticles; Radical polymerization; Zeta potential

1. Introduction

It is well known that the bioavailability of orally administered peptide and protein drugs is very low because of their instability in the gastrointestinal (GI) tract and low permeability through the intestinal mucosa owing to their molecular weight and hydrophilicity (Sakuma, Hayashi, & Akashi, 2001; Takeuchi, Yamamoto, & Kawashima, 2001).

Recently, nanoparticulate systems have been identified as suitable peptide/protein carriers (Dinarvand, Dorkoosh, Hamidi, & Moghadam, 2004). Nanoparticles are expected to be adsorbed as intact form in the gastrointestinal tract after oral administration (Florence, Hillery, Hussain, & Jani, 1995; Takeuchi et al., 2001). They are able to protect drugs from degradation, to improve permeation and/or penetration of the drugs across mucosal surface and also to control the release of the encapsulated or adsorbed drug (Janes, Calvo, & Alonso, 2001; Sandri, 2007; Takeuchi et al., 2001). Major physicochemical parameters influencing the elimination of colloidal drug delivery systems from blood circulation are the particle size, hydrophilicity and recognition by macrophages or the complement system (Aumelas, Serrero, Durand, Dellacherie, & Leonard, 2007). In addition, it has been demonstrated that surface properties of nanoparticles are a key factor in determination of their in vivo fate (Chauvierre, Labarre, Couvreur, & Vauthier, 2003). To prevent the uptake of hydrophobic particles by

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macrophages and thus increase their presence in plasma as well as prolonging their therapeutic effects, one strategy may be to coat nanoparticles with hydrophilic polymers providing a steric barrier against non-specific protein adsorption. The use of poly ethylene glycol (PEG) or polysaccharides such as dextran, chitosan, pectin and hyaluronic acid at the carrier surface grafting are among the most popular approaches (Aumelas et al., 2007; Chauvierre et al., 2003; Choi & Kim, 2007; Esmaeili et al., 2008). Besides, such surface modification of nanoparticles, can improve the hydrophilic properties of nanoparticles prepared by synthetic biodegradable polymers, and make them ideal carriers for hydrophilic drugs such as peptides, proteins and hydrophilic molecules such as anticancer drugs (Esmaeili et al., 2008; Hu et al., 2002; Uyama, Kato, & Ikada, 1998). Polyethylene glycol modified nanoparticles have been extensively prepared by different researchers (De Jaeghere et al.,1999; Esmaeili et al., 2008; Heald et al., 2001; Peracchia, 1998; Ryu et al., 2000). These hydrophobic–hydrophilic nanoparticles have a limitation in their preparation procedure, which requires the use of organic solvents and surfactants as well as sonication or homogenization (Hu et al., 2002).

In view of targeting, PEG-coated nanoparticles are passive systems because their modification of tissue distribution is basically a result of the difference in microvascular permeability between healthy and altered tissue and of their long circulating properties (Chauvierre et al., 2003). For these reasons polysaccharide coatings have been considered as an appropriate alternative to the PEG coatings. Polysaccharide grafting of nanoparticles may achieve active targeting by itself since they have specific receptors in certain cells or tissues (Listinsky, Siegal, & Listinsky, 1998; Stahn & Zeisig, 2000).

In addition it has been demonstrated that nanoparticles with mucoadhesion properties can increase oral bioavailability of drugs (Ponchel & Irache, 1998; Takeuchi et al., 2001). The coating of nanoparticles using polysaccharide has been achieved through adsorption, incorporation and copolymerization or by covalent grafting (Lemarchand, Grefa, & Couvreur, 2004).

One of the most widely used natural polymers for different pharmaceutical purposes is chitosan and its derivatives (Thanou, Verhoef, & Junginger, 2001). Chitosan is a biodegradable, biocompatible and non-toxic polysaccharide, which can adhere to the mucus layer by establishment of electrostatic interactions with sialic groups of mucin (Hassan & Gallo, 1990). It can enhance the absorption of hydrophilic molecules by promoting a structural reorganization of the tight junction-associated proteins (Bernkop-Schnürch & Kast, 2001; Illum, Farraj, & Davis, 1994). Thiolated chitosans, derived from chitosan, were shown to further improve the mucoadhesive properties by forming disulfide bonds with cysteine-rich domains of mucus glycoproteins (Bernkop-Schnürch, 2005). In addition, those products develop additional mucosal permeation-enhancing properties by glutathione (GSH) regeneration mechanism (Bernkop-Schnürch, Kast, & Guggi, 2003). They have potential antiprotease activity due to their ability to bind divalent cations such as Zn^{2+} or Mg^{2+}, which are cofactors of many proteases (Bernkop-Schnürch, & Kast, 2001). Thiolated chitosans are also employed as matrices for controlled drug release due to in situ cross-linking properties (Bernkop-Schnürch, Hornof, & Guggi, 2004). All these characteristics make thiolated chitosans very promising materials for the mucosal administration of peptides and proteins (Bravo-Osuna, Vauthier, Farabollini, Palmieri, & Ponchel, 2007). Many approaches have been developed to prepare the chitosan particles as drug delivery systems for anticancer drugs or proteins (Hu et al., 2002).

Chitosan coated PMMA nanoparticles were prepared by Cui, Qian, and Yin (2006), and the effect of pH value of the reaction medium, chitosan and initiator concentration on the nanoparticles diameter were investigated. However, PMMA is a non-biodegradable polymer, which is not acceptable for the development of a systemic drug delivery to humans. Therefore Chauvierre et al. (2003) prepared polysaccharide-decorated biodegradable nanoparticles using poly (alkyl cyanoacrylate). Recently Bravo-Osuna, Schmitz, Bernkop-Schnürch, Vauthier, and Ponchel, 2006; Bravo-Osuna, Ponchel, and Vauthier, 2007 have elaborated thiolated chitosan coated acrylic nanoparticles. They utilized different molecular weight of chitosan-TBA for surface modification of poly (isobutyl cyanoacrylate) nanoparticles. The developed system possessed the advantages of both thiolated polymers and the colloidal systems.

The aim of the present study was to develop and characterize poly hydroxyethyl methacrylate (pHEMA) nanoparticles coated with chitosan–glutathione conjugate.

2. Materials and methods

2.1. Materials

Chitosan (ChitoClear) with a medium molecular weight and degree of deacetylation of about 89% was kindly provided as a gift by Primex (Norway). L-Glutathione reduced form (GSH), 1-ethyl-3-(3-dimethyl amino-propyl) carbodiimide hydrochloride (EDAC), *N*-hydroxysuccinimide (NHS), 2-hydroxyethyl methacrylate (HEMA), ammonium cerium (IV) nitrate, sodium nitrite, hydrochloric acid, glacial acetic acid, sodium hydroxide and potassium hydrogen phosphate were all purchased from Merck (Germany). Ellman's reagent, 5,5′-dithiobis (2-nitro benzoic acid), was obtained from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Depolymerization of chitosan

Chitosan was depolymerized by chemical reaction following the method developed by Peniston and Johnson (1975) and Huang, Khor, and Lim (2004). Briefly 100 ml of 2% (w/v) chitosan solution in 6% (v/v) acetic acid was prepared and then 10 ml of $NaNO_2$ solutions in Aquamax® (Youglin, Korea) water at different concentrations (7.0, 2.7 and

1.6 mg/ml) were added to obtain the desired final molecular weight of 20000 (cht.20), 50,000 (cht.50) and 100,000 (cht.100) g/mol, respectively, and stirred for 1 h at room temperature. The depolymerized chitosan was precipitated by raising the pH to 9 by addition of 4 M NaOH dropwise. The white-yellowish solid was filtered and washed thoroughly with acetone and redissolved in a minimum volume of acetic acid 0.1 N. Purification was carried out by subsequent dialyses (Sigma dialyses tubes M_w cutoff 12 kDa) against Aquamax® water (2 × 1 l for 90 min and 1 × 1 l over night). The dialyzed product was freeze dried (LyoTrap plus, LTE Scientific, UK) and then stored at 4 °C.

2.3. Measurement of molecular weight

The average molecular weight of chitosan was determined by the viscometric method on the basis of intrinsic viscosity [η]. Briefly depending on the expected molecular weight, various concentrations of chitosan in the range of 0.1–7.5 mg/ml in a solution of sodium chloride:acetic acid (0.2:0.1 M) were prepared. Viscosity of solutions was measured in an Ubbelohde tube (53713/Ic Schott Gerate) at 25 °C. The intrinsic viscosity [η] was then deduced from the reduced viscosity measured for each solution of chitosan by extrapolation at zero concentration. The molecular weight was determined by using the Mark–Houwink Sakurada equation:

$$[\eta] = K \times M^a$$

whereas $K = 1.81 \times 10^{-3}$ and $a = 0.93$ (Knaul, Kasaai, Bui, & Creber, 1998).

2.4. Preparation of chitosan–glutathione conjugate

The covalent attachment of reduced glutathione to different molecular weight of chitosan was carried out according to slightly modified method described by Kafedjiiski, Föger, Werle, and Bernkop-Schnürch (2005) as follows:

One gram of chitosan was hydrated in 8 ml HCl (1 M) and dissolved by the addition of demineralized water to obtain a 1% (w/v) polymer solution. The pH was adjusted to 6.0 by the addition of NaOH (5 M). Afterwards, 5 g of reduced glutathione in 10 ml demineralized water was added to above solution under continues stirring. Then EDAC dissolved in 5 ml demineralized water was added in a final concentration of 200 mM. Thereafter, NHS dissolved in 5 ml demineralized water was added into the reaction mixture under vigorous stirring in a final concentration of 200 mM. The pH was readjusted to 6.0 with NaOH (5 M). The reaction mixture was incubated for 15 h at room temperature under permanent stirring. In order to eliminate unbound reagents, the resulting polymer conjugate was dialyzed in tubing (molecular weight cutoff 12 kDa) first against 5 mM HCl, twice against 5 mM HCl containing 1% NaCl, and finally twice against 1 mM HCl. Controls were prepared in the same way but omitting EDAC and NHS during the coupling reaction. Finally, the frozen aqueous polymer solutions were lyophilized at −50 °C and 0.01 mbar (LyoTrap plus, LTE Scientific, UK) and stored at 4 °C until further use.

2.5. Preparation of coated nanoparticles

The graft of chitosan or chitosan–GSH conjugate onto pHEMA and nanoparticles preparation was performed by radical polymerization.75 mg either chitosan or conjugated chitosan were dissolved in a definite amount of nitric acid (0.2 M) in a two necked flask at 40 °C, under gentle stirring and argon bubbling. After 10 min, under vigorous magnetic stirring, solution of 0.08 M cerium IV ammonium nitrate (CAN) in 0.2 M nitric acid and HEMA were added to obtain a 10 ml solution. The added amount of CAN solution was in the range from (0.004–0.02 mol/l) and that of HEMA was between (0.04–0.16 mol/l). Argon bubbling was kept for additional 10 min. The reaction was allowed to continue at 40 °C under gentle stirring for 40 min. After cooling to room temperature and raising the pH to 4.5 by addition of sodium hydroxide (1 N), nanoparticles suspension was purified by dialysis (Sigma dialyses tubes M_w cutoff 12 kDa) against 1 l of acetic acid solution (16 μmol/l) in Aquamax® water twice for 90 min and once overnight.

2.6. Determination of the thiol group content of chitosan–GSH conjugate

The amount of thiol groups immobilized on chitosan–GSH conjugate was determined spectrophotometrically using Ellman's reagent quantifying free thiol groups as described by Kafedjiiski et al. (2005) and Bernkop-Schnürch, Schwarz, and Steininger (1999). Briefly, 5 mg of each conjugate was dissolved in 2.5 ml of demineralized water. To aliquots (250 μl) of the conjugate solutions, 250 μl of 0.5 M phosphate buffer pH 8.0 and 500 μl of Ellman's reagent (3 mg dissolved in 10 ml of 0.5 M phosphate buffer pH 8.0) were added. The reaction was allowed to proceed for 2 h at room temperature. The precipitated polymer was removed after centrifugation (24,000*g*; 5 min) and 300 μl of the supernatant fluid was transferred to a microtitration plate. The absorbance was immediately measured at a wavelength of 450 nm (PharmaSpec 1700, Shimadzu, Japan). The amount of thiol moieties was calculated from a standard curve obtained from solutions with increasing amounts of reduced glutathione.

The total amount of sulfhydryl groups fixed on the polymer is represented by the summation of reduced thiol groups and of oxidized thiol moieties available in form of disulfide bonds. Disulfide content was determined after reduction with $NaBH_4$ and addition of Ellman's reagent as described by Guggi and Bernkop-Schnürch (2005).

2.7. Determination of the thiol group content of chitosan–GSH coated pHEMA (cht-GSH-pHEMA) nanoparticles

To quantify thiol groups content of the nanoparticles, iodine titration was performed as described by Bernkop

Schnürch & Steininger (2000) and Kast and Bernkop-Schnürch (2001). Twenty milligrams of the lyophilized nanoparticles were hydrated in 6 ml of demineralized water. Following adjusting the pH value to 2–3 with 1 M HCl the solution became clear. Then 500 μl of aqueous starch solution (1%) was added. The samples were titrated with an aqueous iodine solution (1 mM) until a permanent light blue color was maintained.

2.8. Nanoparticles characterization

2.8.1. FT-IR spectroscopy

In order to investigate the chemical reactions between glutathione, chitosan and HEMA, FT-IR spectra were measured by a Nicolet spectrometer (Magna IR 550, USA). Also FT-IR spectra of different molecular weight of chitosan were obtained. The freeze dried nanoparticles were mixed with KBr and pressed to a plate for this reason.

2.8.2. Measurement of particle size

The hydrodynamic mean diameter of nanoparticles were determined by dynamic light scattering using Zetasizer® (Nano-ZS, Malvern, UK). All dynamic light scattering measurements were performed with a wavelength of 633 nm at 25 °C with an angle detection of 90°. The samples were diluted in acetic acid (16 μmol/l) in Aquamax® water and three subsequent measurements were determined for each sample.

2.8.3. Determination of zeta potential

The zeta potential measurements were performed by Laser Doppler Electrophoresis using Zetasizer® (Nano-ZS, Malvern, UK). As Arangoa et al. (2000) demonstrated, in order to maintain a constant ionic strength the samples were diluted (1:50 v/v) in NaCl 1 mM (pH ~ 6.5). Each sample repeatedly measured three times.

2.8.4. Scanning electron microscopy (SEM)

The morphological characteristics of nanoparticles were observed by using a scanning electron microscope (XL 30, Philips, The Netherlands). Nanoparticle suspensions were successively diluted in Aquamax® water to 1/100 (v/v). The dilutions were spread on an aluminium disc and dried at room temperature. The dried nanoparticles were then coated with gold metal using a sputter coater (SCD 005, Bal-Tec, Swiss).

2.8.5. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) (CEM 902A, Zeiss, Germany) was used to observe the morphology of chitosan coated pHEMA nanoparticles. Nanoparticle suspensions were diluted in Aquamax® water to 1/100 (v/v) and prior to observation nanoparticles were negatively stained with solution of 1% phosphotungstic acid.

3. Results and discussion

3.1. Chitosan depolymerization and characterization

Depolymerization of chitosan was carried out via oxidative process by $NaNO_2$ as a simple and reproducible method. Molecular weights of depolymerized chitosans determined by capillary viscometry measurement are shown in Table 1. Janes and Alonso (2003) and Mao et al. (2004), demonstrated that a large series of chitosans with desired molecular weights could be obtained by changing chitosan/$NaNO_2$ molar ratio. Similar to the results obtained by Bravo-Osuna, Ponchel, et al. (2007), present results demonstrated that using distinct amounts of $NaNO_2$ produces chitosans with molecular weights in accordance with expected theoretical values. As shown in Fig. 1, FT-IR spectra of different molecular weights of chitosans were not affected by the depolymerization process, which is in agreement with the results previously obtained by Huang et al. (2004). This, in turn, indicates that all produced chitosans with different molecular weight show the same characteristics (general structure, degree of deacetylation) of the parent chitosan (Mao et al. 2004).

3.2. Determination of thiol groups in chitosan–GSH conjugate and its coated pHEMA nanoparticles

The amount of free thiol groups, disulfide bonds and total sulfhydryl groups immobilized on chitosan–GSH conjugates are summarized in Table 2. In conjugate including parent chitosan with medium molecular weight (cht.med-GSH) the free thiol content (280 μmol/g) is closely similar to the results (265 μmol/g) obtained by Kafedjiiski et al. (2005). However, our results for thiol content of different molecular weight of chitosan differ significantly from those published previously by Bravo-Osuna, Ponchel, et al. (2007). The conjugates with lower molecular weight chitosan, presented less free thiol groups and more disulfide bonds.

Results of total sulfhydryl groups on various molecular weight of chitosan, as suggested earlier by Bravo-Osuna, Ponchel, et al. (2007), indicated that when the molecular weight of chitosan increased, the difficulty of thiol bearing agent to reach the amino groups of chitosan increased and less sulfhydryl groups could be fixed on the polymer. Glutathione, susceptible to oxidation and disulfide bond for-

Table 1
Molecular weights of depolymerized chitosan

Chitosan	$NaNO_2$ (mg/ml)	Expected M_w (g/mol)	Determined M_w (g/mol)
Parent (medium molecular weight)	–	400,000	489,700
Chitosan 100	1.6	100,000	97,050
Chitosan 50	2.7	50,000	52,480
Chitosan 20	7.0	200,000	19,760

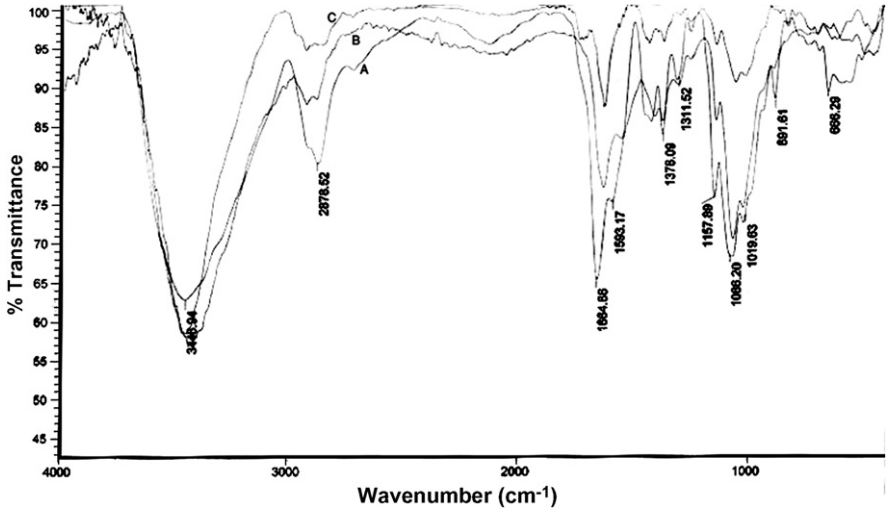

Fig. 1. FT-IR spectra of chitosan with different molecular weights (A, cht.med; B, cht.100, C, cht.20).

Table 2
Thiolated chitosan charecterization

Chitosan type	Free thiol (μmol/g)	Disulfide content	Total amount of sulfhydryl groups
Parent chitosan	280	264	544
Cht.100	88.86	497	585.86
Cht.50	78.14	540.17	618.31
Cht.20	65.5	564.6	630.1

mation, is a tripeptide with high conformational flexibility, the Gly residue is particularly mobile, and the Glu part of the GSH molecule is the most rigid (Kafedjiiski et al., 2005; Krezel & Bal, 2003). In parent chitosan (cht.med-GSH) the glutathione can be fixed on the NH_2 positions which are far enough from each other to prevent disulfide bonds formation. In lower molecular weight chitosan, glutathione is immobilized on closer positions and its mobility and polymer short chains results in the formation of inter- and intra-molecular disulfide bonds. However, presence of a cross-linked structure is not necessarily a disadvantage in the development of drug delivery systems. They can be very useful in the control of the drug release or in the modulation of the gel viscosity (Bernkop-Schnürch, Krauland, Leitner, & Palmberger, 2004; Bravo-Osuna, Teutonico, Arpicco, Vauthier, & Ponchel, 2007).

Chitosan-GSH-pHEMA nanoparticles made by cht.med, cht.100, cht.50 and cht.20 after titration by iodine solution, respectively, presented 238, 75.53, 64 and 51.74 μmol/g of free thiol content. This minute reduction of free thiol content might be related to either determination method or disulfide bond formation during polymerization procedure.

3.3. Nanoparticles preparation and characterization

Nanoparticles of pHEMA coated by chitosan–GSH prepared in this study were spherical (Fig. 2) with a size range

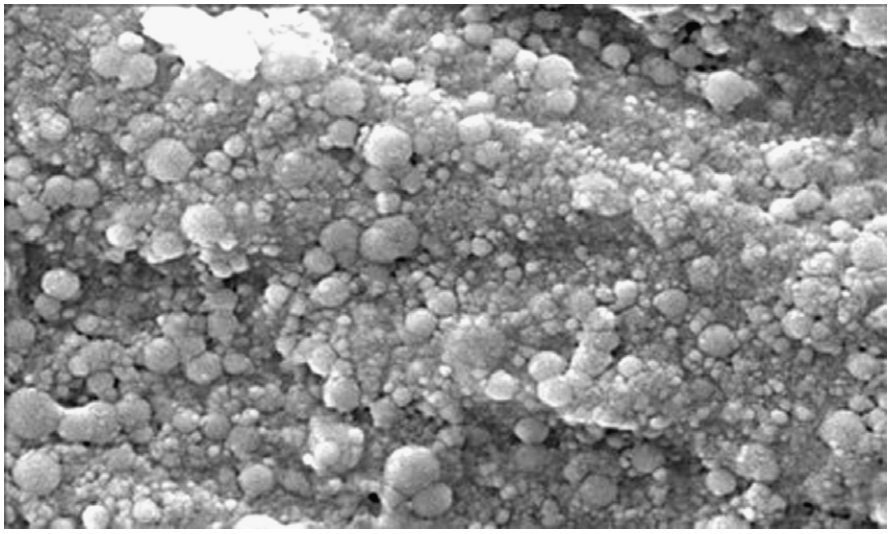

Fig. 2. Scanning electron micrograph of nanoparticles.

Table 3
Mean hydrodynamic diameter, polydispersity index and zeta potential of nanoparticles made by different molecular weight of chitosan and chitosan–GSH

Nanoparticles formulation	Mean hydrodynamic diameter (nm)	Polydispersity index	Zeta potential
Cht.med-pHEMA	748	0.278	57
Cht.100-pHEMA	562	0.367	51
Cht.50-pHEMA	422	0.363	33.6
Cht.20-pHEMA	374	0.427	29.6
Cht.med-GSH-pHEMA	268	0.272	66.1
Cht.100-GSH-pHEMA	309	0.382	55
Cht.50-GSH-pHEMA	347	0.357	49.6
Cht.20-GSH-pHEMA	398	0.391	43.5

of 150–750 nm (Table 3). Chitosan coated acrylic nanoparticles using commercial and depolymerized chitosan have been prepared by different researchers. Hu et al. (2002) synthesized chitosan-polyacrylic acid nanoparticles by polymerization and dropping method. Nanoparticles carried positive charge and showed the size in the range from 50 to 400 nm. Chauvierre et al. (2003) prepared chitosan coated poly (isobutyl cyanoacrylate) (PIBCA) nanoparticles by radical emulsion polymerization method initiated by CAN. Cui et al. (2006) employed emulsion polymerization method in order to prepare poly (methyl methacrylate) nanoparticles coated by chitosan. Bravo-Osuna et al. (2006) elaborated thiolated chitosan coated PIBCA nanoparticles. They prepared different molecular weights of chitosan via oxidative procedure and modified them with 2-iminothiolane (TBA). In another study they modified the PIBCA core by the inclusion of methyl methacrylate as second monomer and coated them with chitosan/thiolated chitosan of different molecular weights. Also, because of partial insolubilization of chitosan-TBA they could not elaborate nanoparticles with 100 percent of chito50-TBA and chito100-TBA (Bravo-Osuna, Ponchel, et al., 2007).

At present work pHEMA nanoparticles coated by chitosan–GSH conjugate were prepared. In comparison with 2-imminothiolane, owing to possession of several carboxyl and amine groups, glutathione has more hydrophilic properties which following conjugation improves solubility of chitosan in a manner that using 100 percent of them for nanoparticles preparation is made possible.

pHEMA is one of the most frequently used polymers in medicine, and its good performance is well known (non-toxicity, biocompatibility and high resistance to degradation and hydrolysis under physiological conditions). pHEMA hydrogels have been considered for implantable applications, including biohybrid artificial organs, drug delivery devices and insulin delivery systems. It has been proved that pHEMA is a good carrier for hydrophilic drugs (Kim, Shin, Shin, Kim, & Kim, 2005).

In addition, all graft copolymers of pHEMA coated with chitosan prepared by various scientists improved the hydrophilicity and biocompatibility of the nanoparticles (El-Tahlawy & Hudson, 2001; Li, Liu, & Fang, 2003).

FT-IR spectra of chitosan–GSH and its graft with HEMA (cht-GSH-pHEMA) are depicted in Fig. 3. The spectrum of conjugate showed strong amide characteristic peaks at 1664 cm^{-1} assigned to the stretching vibrations of C=O and peak at 3344 cm^{-1} due to the stretching vibrations and at 1629 cm^{-1} related to the bending vibrations of N—H moiety. The absorption band due to S—H stretching vibrations of thiol group was observed at 2525 cm^{-1}. In the spectrum of chitosan-GSH-HEMA the absorption at 1731 cm^{-1} is attributed to the C=O asymmetric stretching the ester of pHEMA, confirming that the conjugate was grafted by this polymer.

Fig. 4 shows TEM photograph of thiolated chitosan coated pHEMA nanoparticles. As can be observed the dye retained in the external layer, exhibited a clear layer that surrounded the compact core.

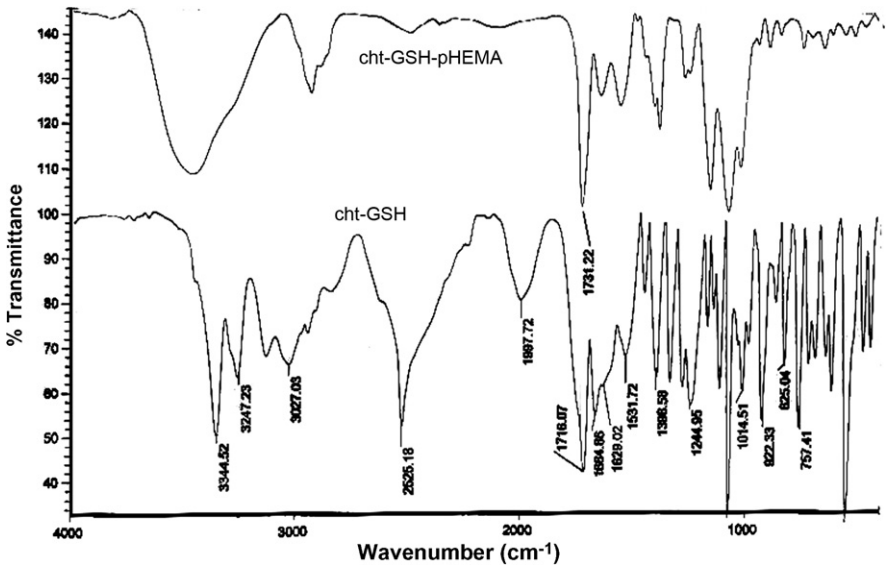

Fig. 3. FT-IR spectra of chitosan–GSH and its graft with HEMA (cht-GSH-pHEMA).

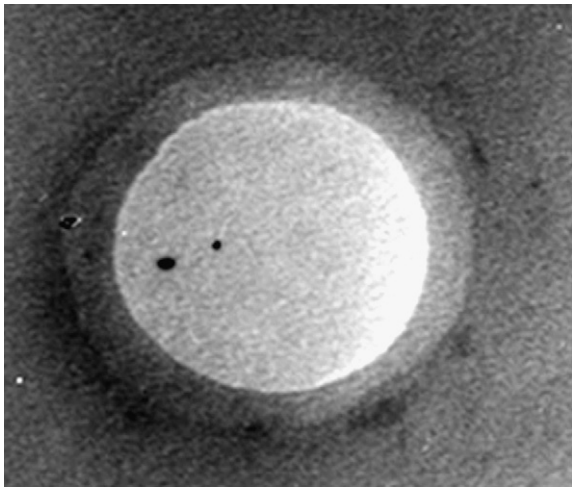

Fig. 4. Transmission electron micrograph of a nanoparticle.

Mean hydrodynamic diameter, polydispersity index and zeta potential of nanoparticles prepared by different molecular weights of chitosan and chitosan–GSH in a fixed amount of HEMA and CAN were presented in Table 3. Positive value of zeta potential is clearly related to the presence of chitosan at the nanoparticles surface. Raising chitosan molecular weight in chitosan-pHEMA nanoparticles increased hydrodynamic mean diameter and zeta potential. The same results were obtained by Bravo-Osuna, Vauthier, et al. (2007), Bravo-Osuna, Ponchel, et al. (2007). However, behavior of nanoparticles consisted of thiolated chitosans were to the contrary. All molecular weight of chitosan-pHEMA nanoparticles showed greater diameter in comparison with the corresponding thiolated chitosan. CAN directly attacks the characteristic NH_2 group of chitosan, producing free radicals which in turn, initiate the graft copolymerization (Joshi & Sinha, 2007). Hence, the free accessible amine in unmodified chitosan is more than thiolated one and addition of more pHEMA causes greater particle diameter.

As can be observed in Table 3, there is an inverse relationship between the molecular weight of chitosan and mean diameter of cht-GSH-pHEMA nanoparticles. Presumably in lower molecular weight existence of vastly intra- and inter-molecular disulfide bonds leading to a higher cross-linked structure and more rigid chain that produce nanoparticles with larger diameter.

Chitosan free amino groups could be responsible for the positive zeta potential values observed in all formulations, which might ensure the electrostatic interactions of preparations with the anionic groups of the mucus resulting in augmentation of their mucoadhesion properties (Bravo-Osuna, Vauthier, et al., 2007). Although in thiolated chitosan a number of amine groups are bound to GSH and therefore lower zeta potentials are expected, the free NH_2 of GSH could compensate it and all cht-GSH-pHEMA nanoparticles carried greater positive zeta potential than cht-pHEMA nanoparticles.

Result showed increasing the concentration of the initiator at a fixed concentration of HEMA monomer and chitosan, the diameter of nanoparticles decreased (Fig. 5). The mechanism of initiation for chitosan is believed to begin with a complex formation of Ce^{4+} with the primary amine and the hydroxyl groups at the C-2 and C-3 positions, respectively. The radicals responsible for the initiation of grafted copolymer chains are produced from the complex dissociation (Zohuriaan-Mehr, 2005). Higher CAN concentration increased the number of radicals resulting in a reduction in the molecular weight of copolymers. Correlations between initiator concentration, molecular weights and particle size have been previously reported. Cui et al. (2006) described, by increasing the concentrations of the initiator, APS, up to 0.2% at a fixed concentration of monomer methyl methacrylate and definite temperature, the diameters of the resulting nanoparticles

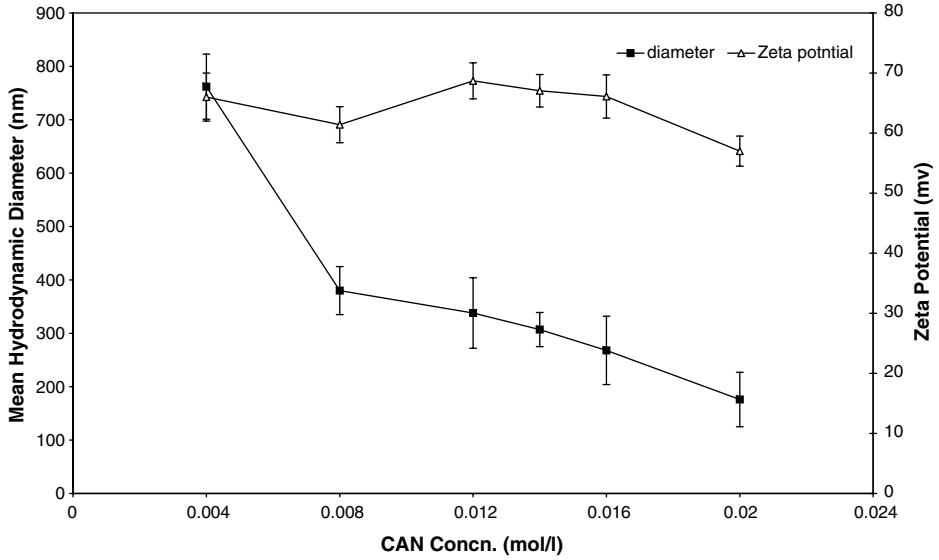

Fig. 5. Effect of the concentration of CAN on the hydrodynamic diameter of nanoparticles and zeta potential.

decreased slightly. It was related to the higher APS concentrations which increased the number of the radicals causing a reduction in the molecular weight of the copolymers. In another study Langer, Marburger, Berthold, Kreuter, and Stieneker (1996), showed strong relationship between particle diameters and molecular weights for methylmethacrylate sulfopropylmethacrylate copolymer nanoparticles.

Increasing the concentration of HEMA monomer at a fixed amount of CAN and chitosan resulted in rising diameter of nanoparticles. One can say when large amount of HEMA applies, the length of produced polymeric chains are more and cause large nanoparticles.

The results of zeta potential of cht-GSH-pHEMA nanoparticles fabricated by various amounts of CAN concentration showed approximately similar values (Table 3). As a general rule, decreasing the nanoparticles diameter causes increase in the ratio of surface to volume. Besides, spreading of charge density in larger particle surface resulted in lower zeta potential. At present study results were on the contrary regarding the small nanoparticles. One can say, while fixed amount of chitosan was applied in all formulations, in case of smaller nanoparticles more chitosan may coat the pHEMA cores because of larger particle surface. Hence represents more zeta potential in spite of expected small value in proportion to the small particle size approximately near to greater particles.

4. Conclusion

The thiolated chitosan coated pHEMA nanoparticles were prepared by radical polymerization method. The prominent advantage of this system is that it is solely made of hydrophilic polymers: thiolated chitosan and polyHEMA, which are non-toxic and biocompatible. The size of nanoparticles can be easily altered by changing the initiator and monomer concentration. Such hydrophilic and mucoadhesive nanoparticles seem to be very promising vehicles for hydrophilic drugs like peptides and proteins.

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