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Synthesis and characterisation of chemically modified chitosan microspheres

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Abstract

Chemically modified chitosan was synthesised by graft copolymerisation of poly(ethylene glycol) diacrylate macromonomer onto chitosan backbone. The polymerisation was initiated by ceric ammonium nitrate. Graft copolymers were prepared by varying the concentration of chitosan and the macromonomer. These graft copolymers were characterised by FT-IR, ^{1}H-NMR and DSC techniques. Microspheres based on chitosan and polymer grafted chitosan were prepared by a polymer dispersion technique. Microspheres were characterised by scanning electron microscopy, (SEM) particle size distribution analysis and DSC methods. A comparative study in relation to the structural deviation among chitosan and modified chitosan microspheres is discussed. These chemially modified chitosans appeared to be hydrophilic and form aggregates during microsphere preparation.

Keywords: Chitosan; Poly(ethylene glycol) diacrylate; Graft copolymerisation; Microspheres

1. Introduction

Various studies on chemical modification of chitin and chitosan with high molecular weight poly(ethylene glycols) (PEG) have been reported (Sugimoto, Morimoto, Sashiwa, Saimoto & Shigemasa, 1998; Tokura et al., 1998; Mo, Aiba, Wang, Hayashi & Xu, 1998). PEG is a highly water soluble amphipathic polymer and frequently used for chemical modification of natural and artificial macromolecules for biomedical applications. It is important to impart stealth properties to materials in contact with blood to prevent the immune system from recognising them as foreign objects. PEG is known to reduce immunoreaction of the body against polymers (Sofia, Premnath & Merill, 1988). Thus grafts of PEG were attached to the backbone sugar chain. Dal Pozzo, Vanini, Fagnoni, Guerrini, De Benedittis and Muzzarelli (2000) studied the preparation and characterisation of poly(ethylene glycol)-crosslinked reacetylated chitosans. Semi-interpenetrating polymer networks composed of β-chitin and poly(ethylene glycol) macromer were also studied by Kim, Lee and Cho (1995). A variety of graft copolymers of chitosan and vinyl monomers were synthesised (Kojima, Yoshikuni & Suzuki, 1979; Kurita, Yoshida & Koyama, 1988; Shantha, Bala & Panduranga Rao, 1995; Shigeno, Kondo & Takemoto, 1982) and evaluated as flocculants, ion-exchangers and drug delivery systems. In the current study, we have synthesised graft copolymers of chitosan and PEG diacrylate (PDA). The copolymerisation was initiated by a free-radical technique using ceric ammonium nitrate [$(NH_4)_2Ce(NO_3)_6$]. The graft copolymer was characterised by Fourier transform-infrared spectroscopy (FT-IR), proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) and differential scanning calorimetry (DSC). Poly[chitosan-g-PDA] (CPE) microspheres were prepared by polymer dispersion technique. These microspheres were characterised by DSC, scanning electron microscopy (SEM) and particle size analysis techniques. Chitosan microspheres were also prepared by the similar technique and the effect of grafting on the structural variation is subjected to a comparative analysis.

2. Experimental

2.1. Materials

Chitosan (medium molecular weight) was obtained from Aldrich and used without further purification. PDA (Average M_n ca. 575, Aldrich, St. Louis, USA) and $(NH_4)_2Ce(NO_3)_6$ (Ajax chemicals, Sydney, Australia) were procured. Poly(methylmethacrylate) (PMMA) was synthesised by a method as reported previously (Jeyanthi & Panduranga Rao, 1987). Glutaraldehyde (25% aq. solution)

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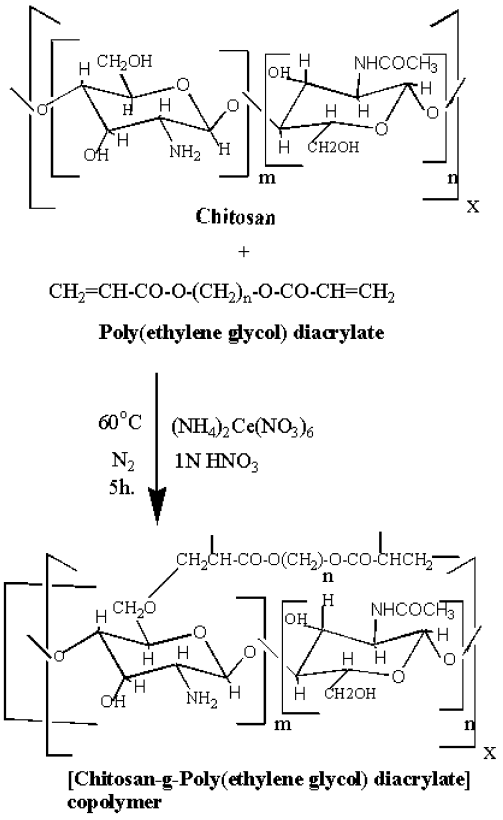

Fig. 1. Synthesis of Poly(chitosan-g-PDA) copolymer.

was from Scharlan, Barcelona, Spain. All other chemicals used were of reagent grade and used as obtained.

2.2. Methods

2.2.1. Synthesis of poly[Chitosan-g-PDA]

Chitosan (2.5 g) dissolved in 1 M hydrochloric acid was taken into a flask fitted with a condensor. $(NH_4)_2Ce(NO_3)_6$ (1×10^{-3} M) dissolved in 1 M nitric acid was added to the chitosan solution and the flask was flushed with nitrogen for 30 min. PDA (5 g) was added dropwise and the reaction mixture was stirred for 5 h at 60°C under nitrogen. The reaction mixture was then filtered through a wet cheese cloth. The product was then precipitated with 5% sodium hydroxide. The precipitate was obtained by centrifugation and then washed free of alkali with distilled water. The homopolymer formed was removed by extensive extraction with methanol. Finally the graft copolymer was washed with distilled water and lyophilised. Graft copolymers were prepared by varying the ratio of chitosan to the PDA monomer. Copolymers with chitosan: PDA, in 1:1 (CPE-1), 1:1.5 (CPE-1.5) and 1:2 (CPE-2) ratio were prepared and characterised. Fig. 1 shows the schematic preparation of the copolymer.

2.2.2. Preparation of microspheres

A known weight of graft copolymer was dissolved in 5% aqueous acetic acid to which was added 10% of PMMA solution in a toluene/chloroform mixture (1:1). This reaction mixture was stirred vigorously for 20 min after the addition of 25% aqueous glutaraldehyde. The mixture was left for 24 h to undergo the cross-linking reaction. This cross-linked product was then washed several times with toluene and isopropanol to remove the PMMA. The microspheres formed were finally rinsed with distilled water and freeze-dried.

2.2.3. Characterisation

The FT-IR spectra of chitosan and its graft copolymer were recorded on Perkin Elmer Paragon1000 FT-IR spectrometer. ^{1}H-NMR spectra were obtained on BRUKER 400 FT-NMR in trifluroacetic acid and deuterium oxide. The thermal analysis of the chitosan, polymer grafted chitosan and microsphere samples was carried out on Perkin Elmer series – 7 thermal analyser in a nitrogen atmosphere at a heating rate of 10°C min^{-1}.

The morphology of the chitosan and polymer grafted chitosan mirospheres were studied by SEM. SEM was carried out on Cambridge Stereoscan S-250 mk 3 SEM. The samples for SEM analysis were prepared by dispersing the microspheres in isopropanol and spraying onto a microscope coverslip. The coverslip was mounted onto the aluminium stub using a double adhesive tape. The stubs were coated with gold using Bal-tec scd.050 sputter coater. Particle size analysis of the microspheres was carried out on a Malvern Mastersizer 2000 instrument. The microspheres were dispersed in an aqeous medium and particle size analysed after sonication of the sample.

3. Results and discussion

3.1. Preparation and characterisation of copolymers

PDA was covalently grafted onto chitosan backbone in order to impart stealth properties to the microspheres prepared using these copolymers. PDA was grafted onto the chitosan chain by ceric ion initiation technique. Ceric ions are known to liberate free radicals on the backbone (Rao, Joseph & Nayudamma, 1968). The level of grafting of the polymer on to the chitosan was found to increase with the increasing concentration of the monomer used upto a certain extent. The amount of homopolymer formed was minimum when Ch/PDA was reacted in 1:1.5 ratio. In case of CPE-2, the termination reactions may occur after the saturation of grafting sites on the backbone leading to the formation of excess homopolymer when the monomer concentration was increased. This was also reported in an earlier investigation in which acrylic and methacrylic acids were grafted onto chitosan backbone (Shantha et al., 1995). In the present study aimed at the development of stealth particles from modified chitosans, we were interested in the development of PDA grafted chitosans with optimum

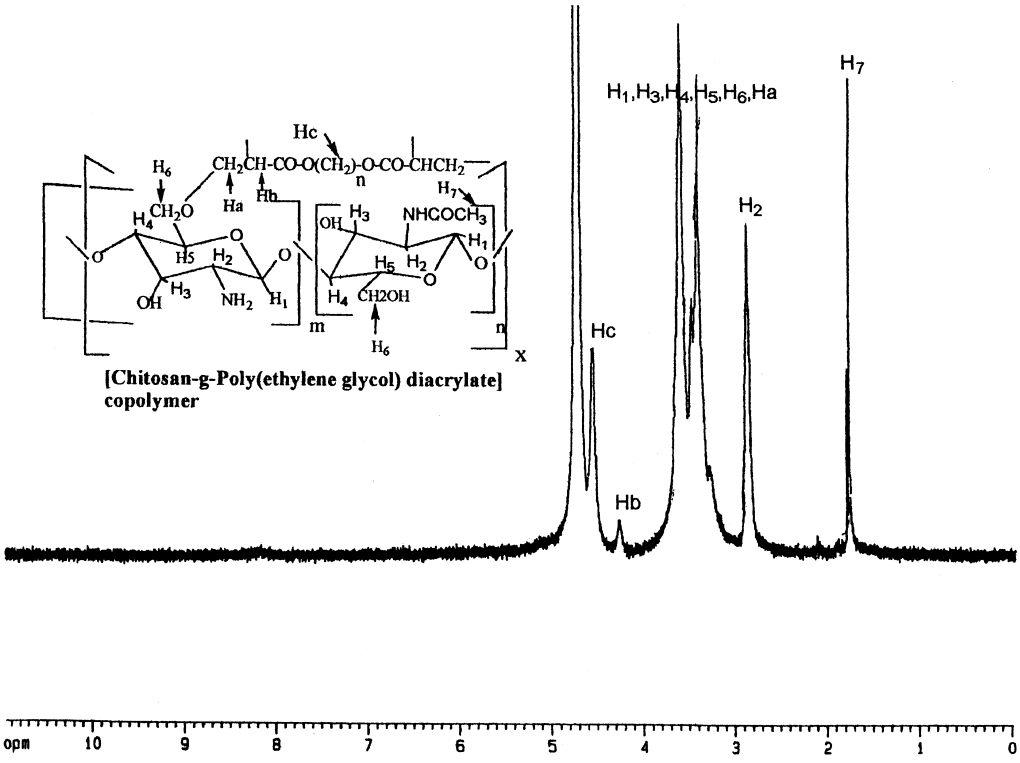

Fig. 2. ^{1}H-NMR of CPE-1.5.

amount of polymer grafted onto the sugar backbone. PDA grafted chitosan is also expected to be highly hydrophilic in nature when compared to unmodified chitosan.

The FT-IR of the copolymer shows sharper peaks when compared to the chitosan. The absorbances at 2879 (υ_s CH_2), 1382 (δ CH_2), 1157 (twisting vibration of CH_2) cm^{-1} were stronger in the copolymer. The copolymer also shows the presence of a peak at 1736 cm^{-1} indicating the presence of ester carbonyl group from the PDA side chains. The absorption due to vinyl unsaturation at 1640 cm^{-1} is absent. This indicates that PDA was completely polymerised during the reaction.

Fig. 2 shows the ^{1}H-NMR of CPE-1.5 copolymer. The peak at 1.76 ppm is due to the acetyl protons of the *N*-acetyl glucosamine units. A singlet at 2.8 ppm and multiplets at 3.3–3.5 ppm corresponding to the ring methine protons and also the methylene protons (H_6 and Ha). The copolymer shows the additional signals due to the methine and methylene protons of the PDA moiety at 4.2 and 4.5 ppm. The absence of the vinyl group signals between 6.2 and 5.8 ppm confirms the completion of polymerisation of the PDA macromonomer.

The DSC of chitosan and the copolymers are shown in Fig. 3. Chitosan shows a small endothermic transition between 80 and 100°C which may be due to the loss of moisture content in the polysaccharide. There is an exothermic peak at 295°C which may be attributed to the decomposition of the chitosan. The DSC of CPE-1 shows a broad transition due to loss of water between 80 and 110°C and has an exothermic peak at 301°C. CPE-1.5 shows an almost continuous transition between 260 and 335°C with a peak at 333°C. In case of CPE-2, the exothermic peak was around 313°C. These results demonstrate that CPE-1.5 undergoes thermal transition over a wide range of temperature. The exothermic peaks correspond to the thermal decomposition temperatures of the respective copolymers. The PDA grafted chitosans exhibit higher decomposition temperatures when compared to the chitosan molecule. This variation in thermal events proved the copolymer formation. The PDA grafted chains may also be involved in increased cross-linking which leads to chain rigidity. The higher decomposition temperature of CPE-1.5 copolymer indicates the enhanced thermal stability. This may be attributed to the significant amount of PDA grafted onto the chitosan backbone when compared to the other two copolymers.

3.2. Preparation and characterisation of microspheres

Microspheres were prepared and characterised from chitosan and CPE-1.5 copolymer under similar reaction conditions. CPE-1.5 was chosen to prepare the microspheres as it was found to have optimum grafting of PDA when compared to the other two copolymers. The geometry of the formed particles was observed by optical microscopy. Chitosan formed very good spherical particles when compared to the CPE particles even though equal amounts of cross-linking agent was employed and subjected to the same period of cross-linking. In the case of CPE-1.5

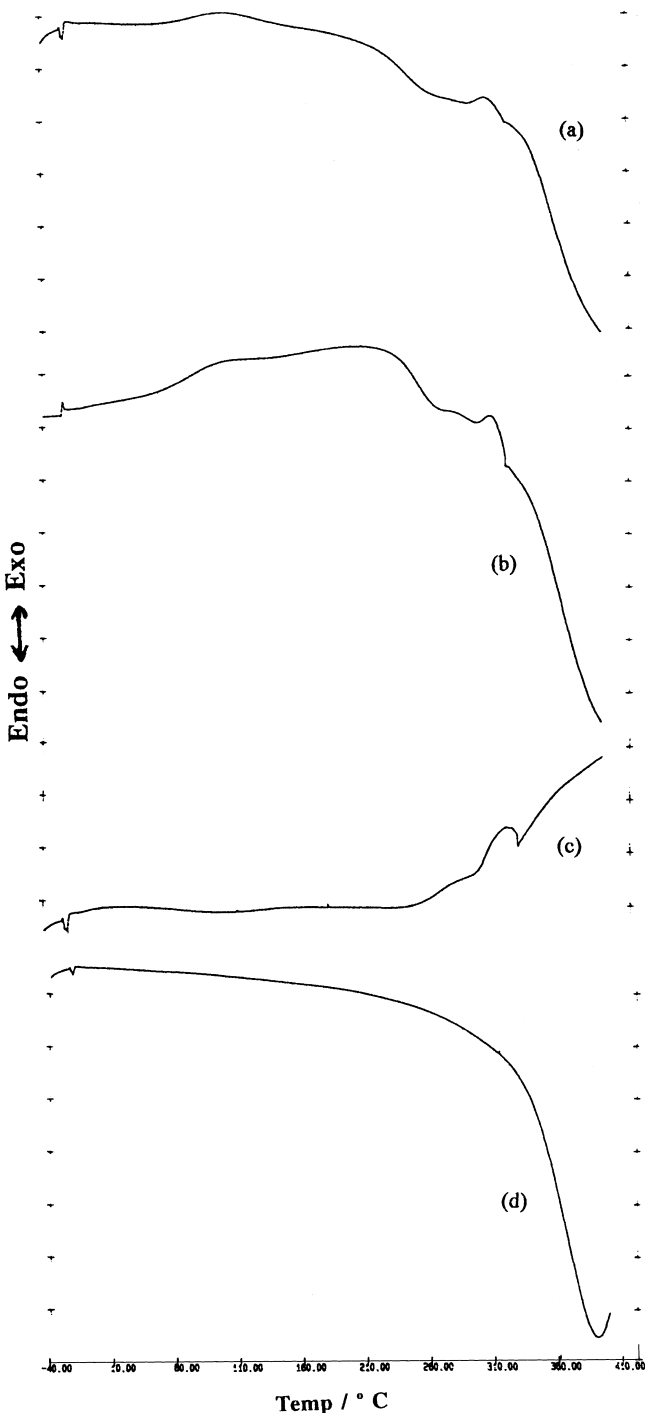

Fig. 3. DSC of chitosan (a), CPE-1 (b), CPE-1.5 (c), CPE-2 (d).

microparticle formation, the failure to achieve uniform spherical geometry could be attributed to the steric hindrance of the grafted chains. These grafted chains may cross-link the sugar chains and in turn hinder the level of cross-linking required for uniformity in sphere formation. This was confirmed from repeated trials of the preparation where all the preparation parameters were kept constant. Further these particles were characterised by different techniques and the results are discussed comparatively.

3.3. DSC of microspheres

Fig. 4 shows the DSC of chitosan and CPE-1.5 microspheres. Chitosan microspheres show a sharp glass transition at 195°C. The DSC of CPE-1.5 microspheres did not

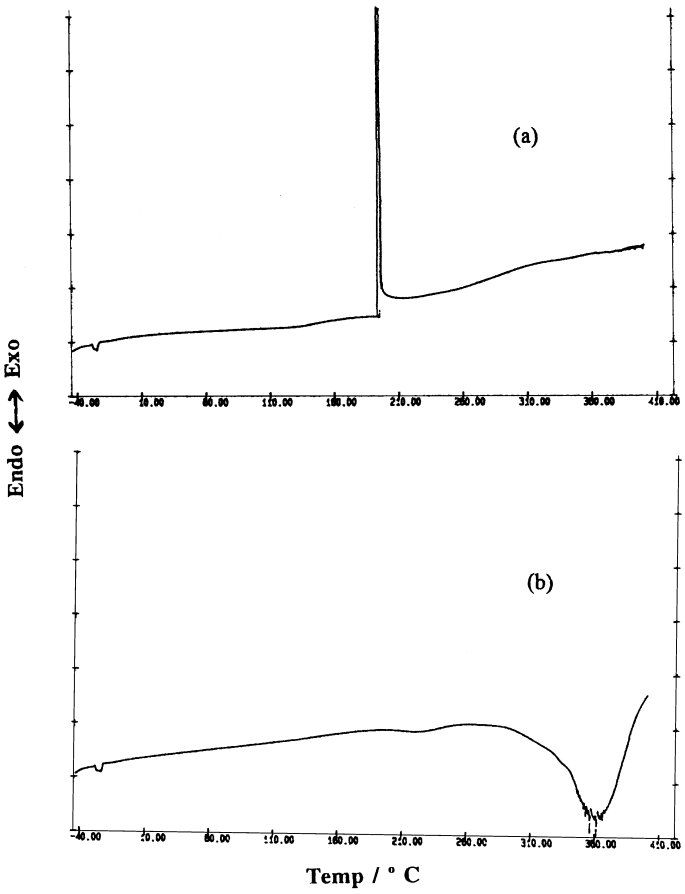

Fig. 4. DSC of chitosan (a) and CPE-1.5 (b) microspheres.

exhibit any significant glass transition temperature. A broad and weak transition between 200 and 230°C was observed. Another strong transition was found at 360°C with associated chitosan backbone. This may be due to the ineffective cross-linking of the polymer-grafted chitosans that leads to faster decomposition. As such the polymer-grafted microspheres were found to have higher thermal stability when compared to the decomposition temperature of chitosan. This indicates that the polymer grafted chains on the chitosan backbone remain unaffected even after being subjected to the cross-linking reaction during the preparation of microspheres.

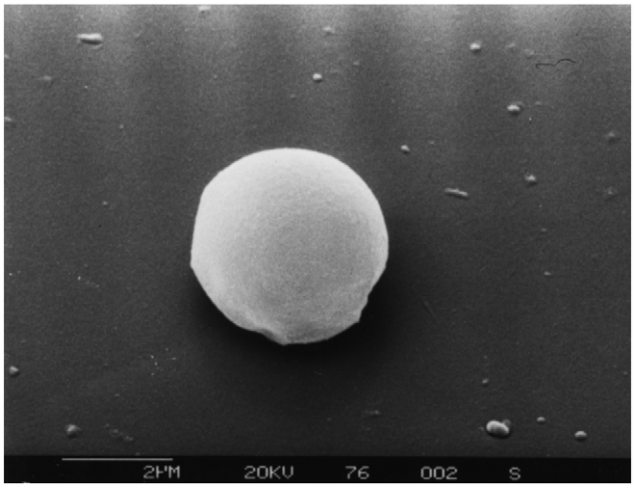

Fig. 5. SEM of chitosan (a) and CPE-1.5 (b) microspheres.

3.4. SEM of the microspheres

Fig. 5 shows the SEM of chitosan and CPE-1.5 microspheres. Chitosan microspheres exhibit a smooth surface morphology. These spheres were found to have regular spherical geometry and were dense when compared to the CPE-1.5 microspheres. This may be due to the higher cross-linking density of the chitosan microspheres. The SEM of CPE-1.5 microspheres shows a rough surface morphology that may be due to the polymer grafted on the chitosan. The lack of uniformity in geometry of these spheres is evident in the SEM pictures. The insufficient cross-linking of these particles has lead to the uneven formation of spheres. Aggregation, a characteristic phenomenon observed in the CPE microspheres, clearly distinguishes them from the chitosan microspheres. Grafting PDA onto chitosan has increased the hydrophilicity of the copolymer. This leads to the formation of aggregates. Some of the

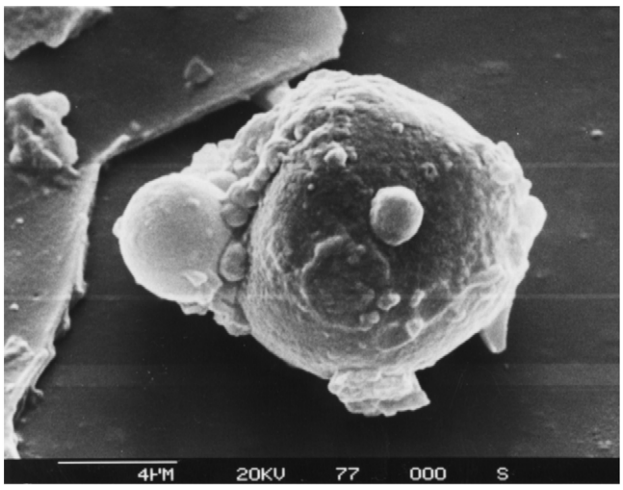

Fig. 5. (*continued*)

poorly cross-linked smaller particles formed a plaque like structure on some of the spheres leading to increased non-uniformity of the surface structure. These smaller particles associated with the plaque formation may also contribute to the larger particle sizes as evident from the particle size distribution curve.

3.5. Particle size analysis

The particle size distribution curve for CPE-1.5 microspheres is shown in Fig. 6. About 50% of the measured particles were found to have a mean particle size under 54 μm. About 10% of the microspheres were below 17 μm in size range and 90% were below 119 μm. In the case of the chitosan microspheres, the particle size of 50% of the spheres was below 50 μm and 10% was below 13 μm. About 90% of the spheres was below 125 μm. Although the particle size distribution of the chitosan microspheres indicated the formation of smaller spheres below 50%, the range of spheres above 50% was found to be larger than the CPE-1.5 microspheres. Aggregation of smaller spheres could be attributed to the very small amounts of very large particles in the size distribution curve of the CPE-1.5 microspheres. The increased hydrophilicity of the PDA grafted chitosans may lead to aggregation between the smaller particles as shown in SEM. This aggregation was not observed in the case of the chitosan microspheres. The non-uniformity in cross-linking of these particles may also lead to increased inter-molecular hydrogen bonding between the unmodified acetyl glucosamine groups of the polysaccharide which induces higher levels of aggregation.

4. Conclusions

Chemically modified chitosans were synthesised by graft copolymerisation technique. Ceric ion was employed in the synthesis of chitosan and PDA graft copolymer. These copolymers were characterised by spectral techniques. Microspheres prepared from both chitosan and modified chitosans were characterised. The structural diversity

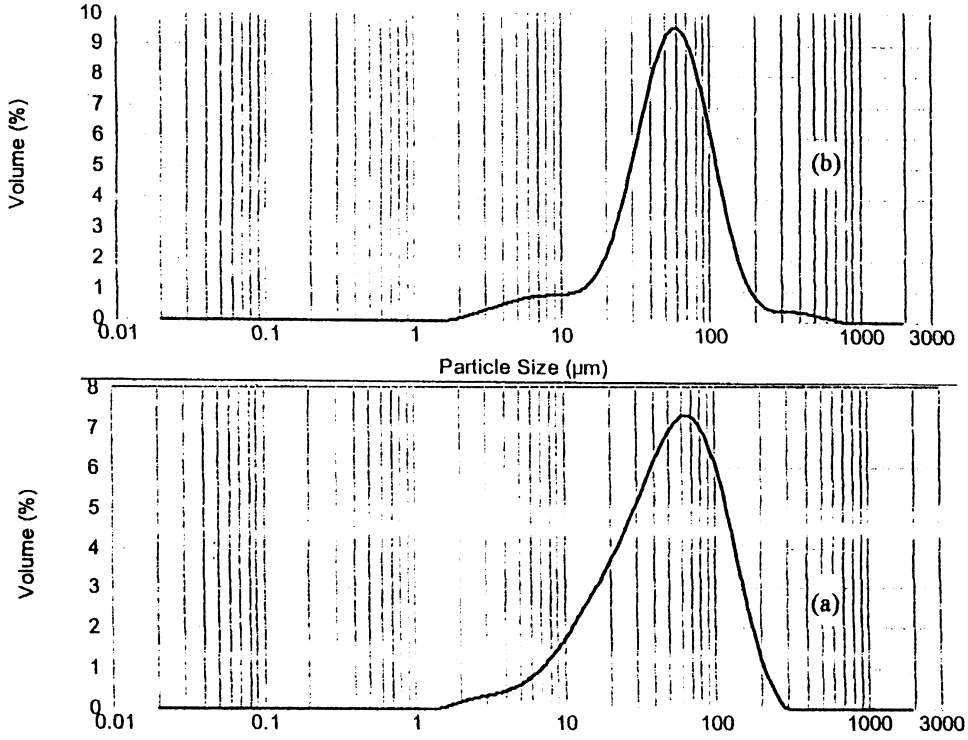

Fig. 6. Particle size distribution of chitosan (a) and CPE-1.5 (b) microspheres.

attained due to the chemical modification of chitosan was studied systematically by thermal analysis, surface morphology and particle size distribution of the microspheres. The optimum amount of polymer grafted to chitosan was found to enhance the hydrophilicity of the chitosan although steric hindrance resulted in ineffective cross-linking. By appropriate modifications, custom-made chitosan stealth particles may be successfully achieved and exploited in the design and delivery of novel active ingredients.

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