Study on Radiation-Induced Grafting of Hydrophilic Monomers onto Chitosan

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ABSTRACT: The graft polymerization of 2-Hydroxyethyl acrylate, 2-Hydroxyethyl methacrylate, and N-vinylpyrrolidone onto chitosan in aqueous solution initiated by γ-ray irradiation was carried out. The effect of various conditions such as the absorbed dose, concentration of monomer, and solvent on grafting was investigated. The grafting yield increased with the increase in absorbed dose. The degree of grafting increased with increase in the radiation dose. The obtained graft copolymers showed the solubility in water in wide pH interval. The interactions between grafted chitosan copolymers with sodium dodecyl sulfate (SDS) were studied in an aqueous solution. It was found that there is a narrow molar ratio of SDS/cation ($\sim$ 0.40–0.70) depending on different grafted copolymers, at which the turbidity of SDS-grafted chitosan complex has a maximum due to the formation of water insoluble interpolymer aggregates via the SDS attached on the polymer chain. The turbidity falls sharply with the further addition of excessive SDS, which forms micelle in the solution and causes the de-aggregation of the interpolymer aggregates and also because of the precipitation of complexes and returns to the original level. The morphologic properties of thin films of SDS- grafted chitosan complex were investigated. It was observed that upon heating the insoluble complex of SDS and grafted chitosan in water, superstructures were formed. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 558–563, 2008

Key words: graft copolymers; irradiation; surfactants; water-soluble polymers; chitosan

INTRODUCTION

Chemical modifications of chitin and chitosan have been studied widely, but till recent years, only a small number of the researches on their irradiation modification, including radiation degradation[1] and radiation grafting, were reported.[2–8] Some monomers, such as 2-hydroxyethyl methacrylate, methyl methacrylate, butyl acrylate, N-isopropylacrylamide, N-maleamic acid, and N,N'-dimethylaminoethylmethacrylate,[9–12] using gamma irradiation have been investigated, but few studies on the method of measurement of grafting yield have been reported.

The interaction of polyelectrolytes and its gels with oppositely charged surfactants has been extensively studied in recent decades.[13,14] Hayakawa et al.[15] investigated the interaction of polyelectrolytes with oppositely charged surfactants, revealing preaggregate formation at far lower surfactant concentrations than critical micelle concentration.

Early investigators focused on water-soluble polymers interacting with simple surfactants, but in recent years, macromolecules with pronounced amphiphilic character have become of greater interest. In fact, amphiphilic synthetic polymers comprised a wide range of graft, block and star copolymers with a variety of functional groups and an almost unlimited number of their combinations. That opens up a possibility of close imitation of polymer-surfactant interactions in biological tissues. In particular, membrane cells are inter- and intracellularly stabilized by biopolymers like proteins and polysaccharides.[16] The study of the interaction between analogous polymers and bilayer forming surfactants is therefore highly relevant to a better understanding of the organization of these complex structures.[17,18]

In this work, the grafting of 2-hydroxyethyl acrylate (HEA), 2-hydroxyethyl methacrylate (HEMA), and N-vinylpyrrolidone (N-VP) onto chitosan (CS) in aqueous solutions using ^{60}Co gamma radiation technique and the interaction with surfactant were studied.

EXPERIMENTAL

Materials

Water-soluble chitosan (hydrochloride form, which in the following text will be called merely ''chitosan'') was purchased from Jakwang Co., Korea.

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The weight-average molecular weight of the sample was 200 kDa. The deacetylation degree of the chitosan was examined by FTIR spectroscopy[19] on Satellite 3000 spectrometer (Mattson, USA) and was determined as 87%. 2-Hydroxyethyl acrylate, 2-Hydroxyethyl methacrylate and *N*-vinylpyrrolidone were obtained from Aldrich and purified by distillation under a vacuum. All other chemicals and reagents were of analytical grade.

Synthesis

In a typical grafting reaction, an exact amount of dry chitosan is first dissolved in distilled water and a perfectly transparent solution is obtained. Then HEA, HEMA, and *N*-VP are added to a chitosan solution (pH of solution 3.6, 3.8, and 4.0, respectively) to form a homogeneous blend. The grafting reaction is performed in the sealed glass ampoules with ~ 9.5 mm internal diameter in atmosphere of argon. Before synthesis the feed mixture is saturated by argon. The copolymerization is performed in sealed glass ampoules using MRX-γ-25M (Russia) setup equipped by ^{60}Co source with a dose rate 0.11 Gy/s. The dose rate is determined by the Fricke dosimeter. The contents of the ampoules are poured into a 100-mL flask, and then the reaction mixture is adjusted to pH 11.0 by adding dilute NaOH solution slowly. Then the products are precipitated in acetone, separated by filtration, washed with water to remove not reacted monomer, and dried under a vacuum to a constant weight. The exhaustive extraction of the product with ethanol allowed the separation of homopolymers formed during the grafting reaction. The degree of purification has been controlled by the detection of homopolymer content in the wastewater with the help of the FTIR spectroscopy.

Characterization

Grafting parameters, such as grafting degree (%G) and efficiency percentage (%E), were determined as follows:[20] %$G = (W_1 - W_0)/W_0 \times 100\%$ and %$E = (W_1 - W_0)/W_2 \times 100\%$. Concentration of grafted copolymers of chitosan (C_x, unit-mol/L) was determined as follows:[21]

$$C_x = \frac{q \times 1000 \times (\text{Mr} \times W_0 + 163 \times W_2)}{\text{Mr} \times 163 \times W_1 \times V}$$

where q is the mass of copolymer in g, W_0 is the weight of initial chitosan in g, W_1 is the weight of grafted chitosan after acetone extraction and dialysis in g, W_2 is the weight of grafted monomer in g, V is the volume of solution in mL, Mr and 163 denote the molecular mass of synthetic monomers and chitosan, respectively.

The solubility of polymers in water has been controlled by measuring the optical density (D) of solutions at various pH-values with the help of UV-VIS spectrometer (UV-2401 PC Shimadzu, Japan) at 400 nm. The final products were stored in desiccators for future analysis.

The samples for microscopic investigation were prepared as follows: a solution of sodium dodecyl sulfate (SDS 0.01M) and grafted chitosan (0.1M) in water was cast onto a glass microscope slide to give a thin film. After drying, this film was covered with pure water and subsequently heated at 60°C for 1 min on a hot stage. No corrections of the pH were made (pH = 4). After cooling to room temperature the turbid films, which were still covered with water, were inspected by optical microscopy using a Carl Zeiss Axiostar plus Transmitted-Light microscope (Carl Zeiss Light Microscopy, Germany).

The presence of HEA, HEMA, and *N*-VP on the chitosan was studied by the FTIR spectroscopy. Samples were ground to powder and mixed with exhaustively dried KBr. The infrared spectra of irradiated chitosan and grafted chitosan (CS-*g*-PHEA, CS-*g*-PVP, and CS-*g*-HEMA) were recorded as KBr pellets on a FTIR-Satellite 3000 spectrometer (Mattson, USA). The spectra were taken with resolution of 4 cm^{-1} and were averaged over 120 scans. The scanning range lied within the limits 4000 and 400 cm^{-1}.

RESULTS AND DISCUSSION

The grafted chitosan copolymers were prepared by the reaction of synthetic monomers (HEA, HEMA, N-VP) with chitosan. The polymerization in various molar ratios took place easily under the designated conditions. The results are shown in Figures 1 and 2.

Figure 1 shows the effect of radiation dose (D) on grafting onto chitosan in an aqueous solution. The degree of grafting tended to increase with an increase in the radiation dose in the dose range studied here. Therefore, it seems that the number of activated sites on chitosan should increase in proportion to the radiation dose. The grafting degree increased with the increasing of the total radiation dose. After the reaching of the radiation dose, about 3 kGy forming grafted copolymers of chitosan with HEA are crosslinked to form high swelling hydrogels. With the further increasing of the radiation dose, the hydrogels are predominantly formed. In the case of grafting of HEMA onto chitosan, the degree of grafting with the increase of the total radiation dose increased significantly due to the precipitation of grafted samples. Grafting efficiency in all systems was approximately 30–50% independent of the grafted monomer.
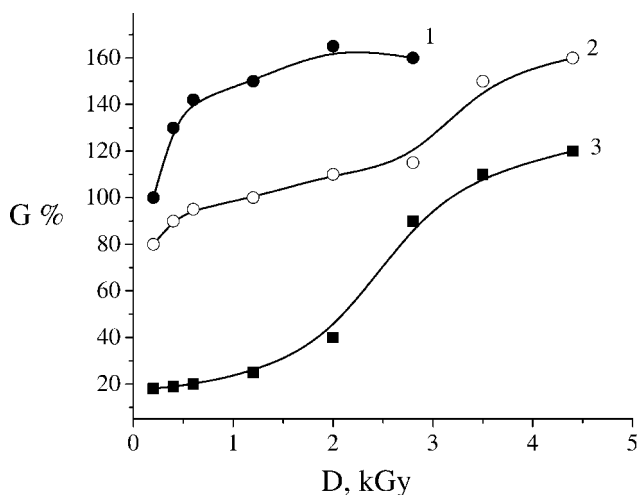
Figure 1 The effect of dose on grafting yield of CS-*g*-PHEA (1), CS-*g*-PVP (2), and CS-*g*-PHEMA (3). $C_{monomer}$ = 0.35 mol L^{-1}, C_{CS} = 0.2 wt %.

The grafting percentage and the amount of synthetic polymer introduced to chitosan increased with the concentration of monomer are shown in Figure 2. When the concentration of monomer in the feed mixture increased from 0.1 to 0.4 mol L^{-1}, the grafting percentage rose from 150 to 400%, from 95 to 330%, and from 30 to 170% for HEA, N-VP, and HEMA, respectively. When the HEA concentration increased more than 0.4 mol L^{-1}, the gelation of the reaction system took place during the polymerization, so that the isolation of the grafted chitosan was difficult. In the case of N-VP the degree of grafting slightly increased at the high content of monomer, but when the N-VP concentration increased considerably, the homopolymer was formed predominantly. On the other hand, HEMA showed an extremely high degree of grafting up to 400% with
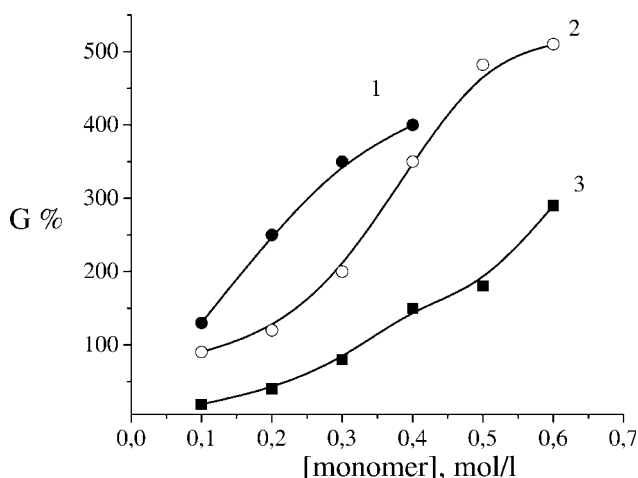
the increase of the HEMA concentration, but all of the obtained grafted samples with a high degree of grafting were insoluble in water.

For the characterization of grafted chitosan, the FTIR spectra were recorded. Observed differences between the FTIR spectra of chitosan and those of graft copolymer verified the existence of grafting. The FTIR spectra of irradiated chitosan (a), CS-*g*-PHEA (b), CS-*g*-PVP (c), and CS-*g*-HEMA (d) are shown in Figure 3. The chitosan spectrum shows the characteristic absorption bands at 1636 (Amide I), 1528 (—NH$_2$ bending) and 1381 cm^{-1} (—CH$_2$ bending). The absorption bands at 1156 cm^{-1} (antisymmetric stretching of the C—O—C bridge), 1084 and 1028 cm^{-1} (skeletal vibrations involving the C—O stretching) are characteristics of its polysaccharide structure. Compared to the FTIR spectrum of chitosan, the CS-*g*-PHEA has a new absorption peak appearing around 1726 cm^{-1} corresponding to the carbonyl stretching vibration of carboxylic ester moieties, while the strong absorption band at 1275 and 1075 cm^{-1} results from its hydroxyl character (primary alcohol group). Furthermore, in the grafted products the peak at 1636 cm^{-1} (characteristic peak for amide I group in pure chitosan) was shifted slightly to 1653 cm^{-1}, and there are also shifts of the peaks in the region 1229–915 cm^{-1}. The increasing of the feed ratio of HEA to chitosan made the absorption at 1726 cm^{-1} rise, which means that more
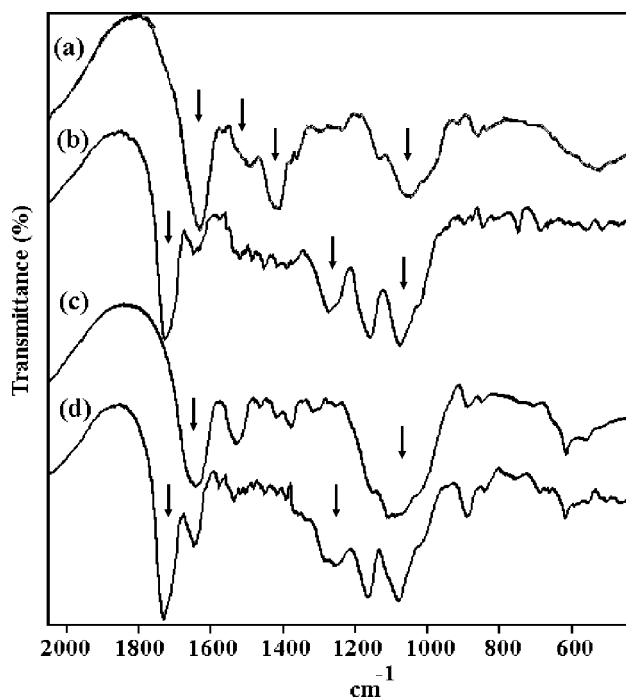

Figure 2 The effect of monomer concentration on grafting yield of CS-*g*-PHEA (1), CS-*g*-PVP (2), and CS-*g*-PHEMA (3). C_{CS} = 0.2 wt %, D = 0.5 kGy.

Figure 3 FTIR spectra in the region 500–2000 cm^{-1} for irradiated chitosan (a), CS-*g*-PHEA (b), CS-*g*-PVP (c), and CS-*g*-PHEMA (d). $C_{monomer}$ = 0.35 mol L^{-1}, C_{CS} = 0.2 wt %, D = 0.5 kGy.

monomer had been grafted to chitosan. There were characteristic absorption peaks of CS-*g*-PVP (Fig. 3, curve c) at 881, 1270–1370, 1381, 1530, and 1667 cm^{-1} due to symmetric and asymmetric stretching amide in polyvinyl pyrrolidone. Furthermore, in the grafted sample amid the peak of PVP shifted slightly from 1653 (characteristic peak for amid group in pure PVP) to 1667 cm^{-1} and become broadened. This is probably due to the influence of polysaccharide to the amid group of N-VP. Characteristic FTIR spectrum of CS-g-PHEMA was similar to that in CS-*g*-PHEA and was observed shifts in the region of 1390–1490 cm^{-1} due to the presence of branching methyl groups in the structure of HEMA. The observed differences between the FTIR spectra of the chitosan and grafted copolymers of chitosan verify the existence of synthetic polymer chains. A possible mechanism of grafting onto chitosan is proposed in our previous research[22] in analogy with one suggested by Ding et al.[23] Radicals, likely to be attracted to the hydroxyl groups (OH) at the side carbon of the chitosan ring or α-methylenic (CH$_2$) groups with tearing off H-atom forming free radicals as was previously showed for high molecular alcohols.[24] At those sites, polymer chain starts and propagates as regular radical polymerization of polyacrylates.

It was found that the grafted chitosan, except CS-*g*-PHEMA with high grafting degree (%G > 50%), are completely soluble in typical solvents for chitosan, such as dilute organic acids, hydrochloric acid in all cases independently from the specific grafting degree. Also, the grafted products became soluble in distilled water. This appreciation was based on solubility tests carried out on the grafted products. The chains of chitosan in the grafted copolymers provided for pH-sensitivity, and the presence of HEA, HEMA, and N-VP chains provide for solubility of polymers in wider pH interval. The pH range in which the grafted copolymers are soluble depends on the specific grafting value.[22] The increase of %G provides for the solubility of grafted copolymers in a wider pH interval in comparison with the initial unmodified chitosan because HEA, HEMA, and N-VP chains are nonsensitive to pH. This behavior expands potential applications of chitosan derivatives.

An important feature of water-soluble polyelectrolytes is their capability to form aggregates with suitable counterpart species. The combination of aqueous solutions of polycations with polyanions or anionic surfactants results in water-insoluble precipitates, so-called polyelectrolyte complexes (PELC) or polyelectrolyte surfactant complexes (PELSC). The copolymers, which include in their structure except ionic group hydrophilic groups another nature are the most suitable for investigation of PELC. It is providing the solubility of obtained PELC in water at any respects of detergent and polymer.
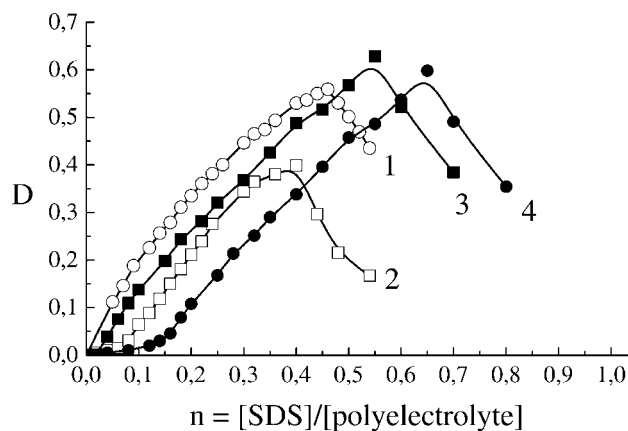

Figure 4 Turbidimetric titration curves of chitosan (1), CS-*g*-PHEA (2), CS-*g*-PVP (3), and CS-*g*-PHEMA (4) with grafting degree 40, 27, and 10%, respectively, by SDS solution. $C_{polymers} = 0.01$ unit-mol L^{-1}, $C_{SDS} = 0.005$ mol L^{-1}.

In present work, complexation between grafted copolymers and sodium dodecyl sulfate (SDS) were investigated. Figure 4 shows the dependence of turbidity D on the polymeric components ratio [SDS]/[grafted chitosan]. Upon the addition of oppositely charged surfactant to the polymer solution, immediate interaction takes place in the solution largely because of the electrostatic attraction among many possible forces such as van der Waals, hydrogen bonding and hydrophobic interaction between the polymer electrolyte and the surfactant molecules. First, this binding phenomenon will neutralize the ionic charge of the system and cause change in the polymer conformation. As a result, the physical and chemical properties of the polymer solution will be greatly altered. Upon the addition of surfactants, the turbidity of the grafted chitosan solution drastically increases for all cases. The maximum on the turbidimetric titration curve corresponds to the polyelectrolyte complexes. The result shows that the turbidity increases in a different region of [SDS]/[cation] for various grafted samples. A considerable increase of turbidity is observed upon addition of SDS to CS-*g*-PVP and CS-*g*-PHEMA solutions (Fig. 4, curves 3, 4), in this case, the composition of PELSC shifts to the increasing of SDS. This can be explained by the additional hydrophobic interaction between the hydrophobic tails of the surfactant molecules with hydrophobic portion of polymer chains of N-VP or HEMA, in contrast with the interaction of hydrophilic CS-*g*-PHEA with SDS.[25] With the further addition of SDS, the turbidity falls rapidly because of partly solubilization and precipitation of complexes and returns to the original level. For a grafted copolymer, with a high grafting degree (∼ 120%), the turbidity of solutions does not increase significantly. It probably can be explained by the enormous quantity of nonionic groups in the structure of copolymer

and formation of PELSC stabilized by hydrophobic bonds.

The structure of PELSC formed by grafted chitosan and SDS in the solid state was studied by FTIR spectroscopy. For this purpose the PELSC was obtained as a precipitate, which then was separated and dried. Figure 5 shows FTIR spectra of CS-*g*-PHEA, SDS, and PELSC. It can clearly be seen that the spectrum of PELSC is characterized by the presence of bands typical of both initial components. The main difference observed in FTIR spectrum of PELSC as compared with those of grafted chitosan and SDS is the distinctive peak at about 1520 cm^{-1}, which is not observed in FTIR spectra of starting components or even in their physical mixture; furthermore, in the PELSC, the peak at 1490 cm^{-1} was shifted slightly to 1450 cm^{-1}. This is indicative of the existence of a specific interaction between CS-*g*-PHEA and SDS. Similar results were also observed for SDS-CS-*g*-PVP and SDS-CS-*g*-PHEMA complexes (data not shown).

To investigate the morphological properties, a thin film of SDS-CS-*g*-PHEA complex was prepared by casting an aqueous solution of SDS and CS-*g*-PHEA onto a glass microscope slide. After the addition of water, this initially clear film became turbid and remained tightly adsorbed onto the microscope slide. The subsequent heating of the water-covered film
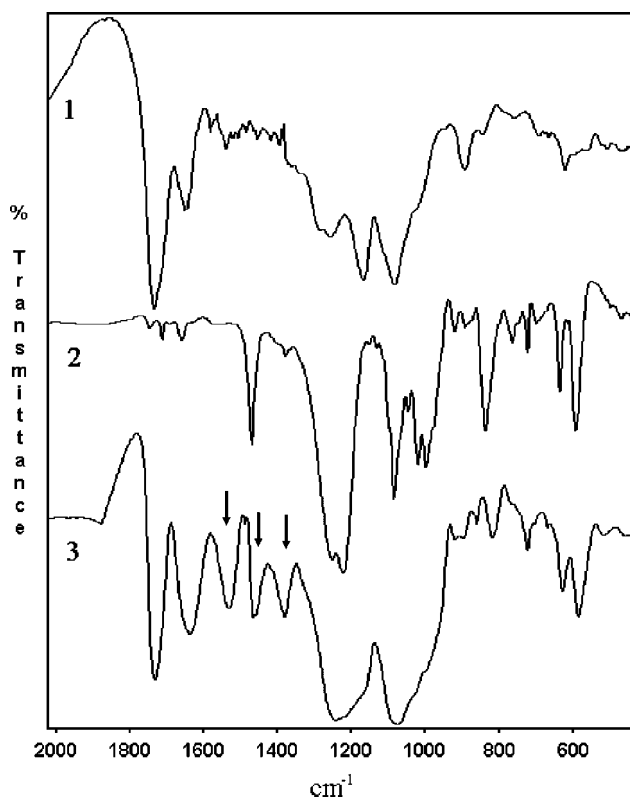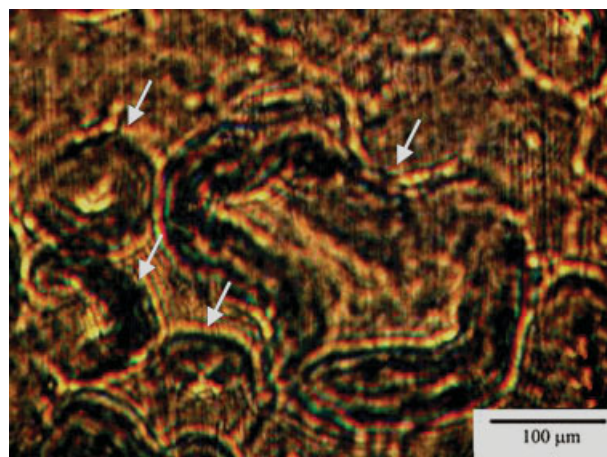

Figure 6 Optical micrograph of PELSC complexes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

for 1 min at 60°C results in the spontaneous formation of immense numbers of vesicles. The heating of the films in the absence of water does not result in the formation of superstructures; upon evaporation of water the structures collapsed. The superstructures are immobilized in the surrounding matrix of SDS-CS-*g*-PHEA (Fig. 6). Sometimes spherical matrix particles, including one ore more vesicles, can be observed. Since the unmodified chitosan does not form some superstructures, it cannot be used as a control (data not shown). Good quality, tissue-like samples are obtained when CS-*g*-PHEA and SDS are mixed in a ratio of ∼ 10 glucoseamine units to one SDS molecule. Under optimal conditions, the binding sites of the polymer are only partially occupied by SDS anions. The SDS anions are thus able to migrate along the polymer backbone, facilitating the reorganization of the material into multivesicular superstructures. A similar morphology was also observed in SDS-CS-*g*-PVP, and SDS-CS-*g*-PHEMA complexes (data not shown).

The SDS-CS-*g*-PHEA complexes have thus been shown to form ordered multivesicular assemblies which resemble the architecture of biological tissues. In biological tissues, polypeptides and polysaccharides are known to keep the phospholipids walled cells together. It is suggested that in our system surfactant vesicles are joined by a CS-*g*-PHEA containing a matrix.

CONCLUSIONS

Chitosan was a graft copolymerized with synthetic monomers, viz. hydroxyethyl acrylate, hydroxyethyl methacrylate, and *N*-vinylpyrrolidone in an aqueous solution initiated by γ-ray irradiation. The grafted copolymers are soluble in water and diluted acid

Figure 5 FTIR spectra in the region 500–2000 cm^{-1} for CS-*g*-PHEA (1), SDS (2) and PELSC (3).

solutions, describing an enhanced hydrophilic character as compared with control chitosan. Graft polymerization of chitosan is very helpful in preparing polysaccharide-based multifunctional advanced materials for wide applications. The interaction of grafted chitosan polyelectrolyte with SDS was studied. It was found that turbidity of aqueous solutions of grafted chitosan with added SDS exhibits of a sudden rise and subsequent a sharp fall was due to the aggregation, deaggregation and precipitation of complexes depending on the SDS/cation molar ratio.

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