Physiochemical, Circular Dichroism-Induced Helical Conformation and Optical Property of Chitosan Azo-Based Amino Methanesulfonate Complex

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ABSTRACT: We have demonstrated the efficient procedure for the preparation of chitosan azo-based amino methanesulfonate complex in isopropyl alcohol under mild condition. The ionic complexation between chitosan and {4-[(4-nitrophenyl)azo]phenyl}amino methanesulfonate is confirmed by ultraviolet (UV) and Fourier transform infrared spectroscopy. The circular dichroism spectra of chitosan complex showed positive at $\lambda = 298$ nm band with 3.88 mdeg having UV absorbance maximum up to a narrow band at $\lambda = 290$ nm in dimethyl sulfoxide, indicating that the polymer adopted helical (right handed) secondary structure. Physical properties, thermal stability, and surface morphology were analyzed by X-ray diffraction, thermogravimetric analysis, differential scanning calorimetry and scanning electron microscopy. Optical properties of chitosan complex are evaluated by photoluminescence spectroscopy that showed blue shift (λ_{em}) peak at 400 nm at excitation wavelength 325 nm. Overall, it may open new perspectives for the use of chitosan azo-based biohybrid in biomedical applications. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 4897–4903, 2012

Key words: chitosan; {4-[(4-nitrophenyl)azo]phenyl}amino methanesulfonate; complex; optical property

INTRODUCTION

Chitosan is a linear polysaccharide produced by deacetylation of chitin closely related to cellulose. Due to its accessibility, biodegradability, nontoxicity, and biocompatibility have applications in various areas, such as textiles, pharmaceutics, medicine, food industry, cosmetics, antimicrobial agents, optical, adhesives, and other industrial applications.[1–7] Chitosan can sequestrate azodyes in a process that depends on the chemical structure of the dyes and on pH.[8] To overcome the susceptibility and improve the stability of bioactive compounds during processing and storage, the emerging technology of nanoencapsulation/microencapsulation has been recently applied in food, drug, and nutraceutical industries. In the case of colloids, the bioactive molecule to deliver can be encapsulated into the particles or adsorbed at their surface, according to the therapeutic strategy or the nature of the bioactive compound.[9–11] The optical property when induced into biopolymer makes the material much more important from the biotechnological application point of views.[12] Due to the easy availability of free amino group in chitosan, it carries a positive charge and thus in turn reacts with many negatively charged surface polymers and also undergoes chelation with metal ions.[10,13]

Many studies have been carried out to investigate the preparation, characterization, and application of chitosan with azo dyes.[13,14–18] Nakamura and Fukao[19] reported systematic dielectric relaxation study of solid-like polyelectrolyte surfactant complexes formed between poly(styrenesulfonate) and cationic surfactants. The inherent importance of chiroptical phenomenon and chirality, measurement of optical activity is one of the most underutilized of all spectroscopy techniques. The preferred technique for measuring chiroptical data, circular dichroism (CD) spectroscopy, is making remarkable progress on all fronts from a better understanding of principals to new applications.[20] CD is the important technique for the study and investigating the structural, functional, and dynamic interaction between macromolecules and low-molecular weight species in solution.

In continuation of our interest in the modification of chitosan and azo dyes,[4,6,10,21–24] the present work is intended to the preparation of chitosan azo-based amino methanesulfonate complex with CD-induced conformation, optical properties, and physiochemical properties for beneficial applications in biomedical fields.

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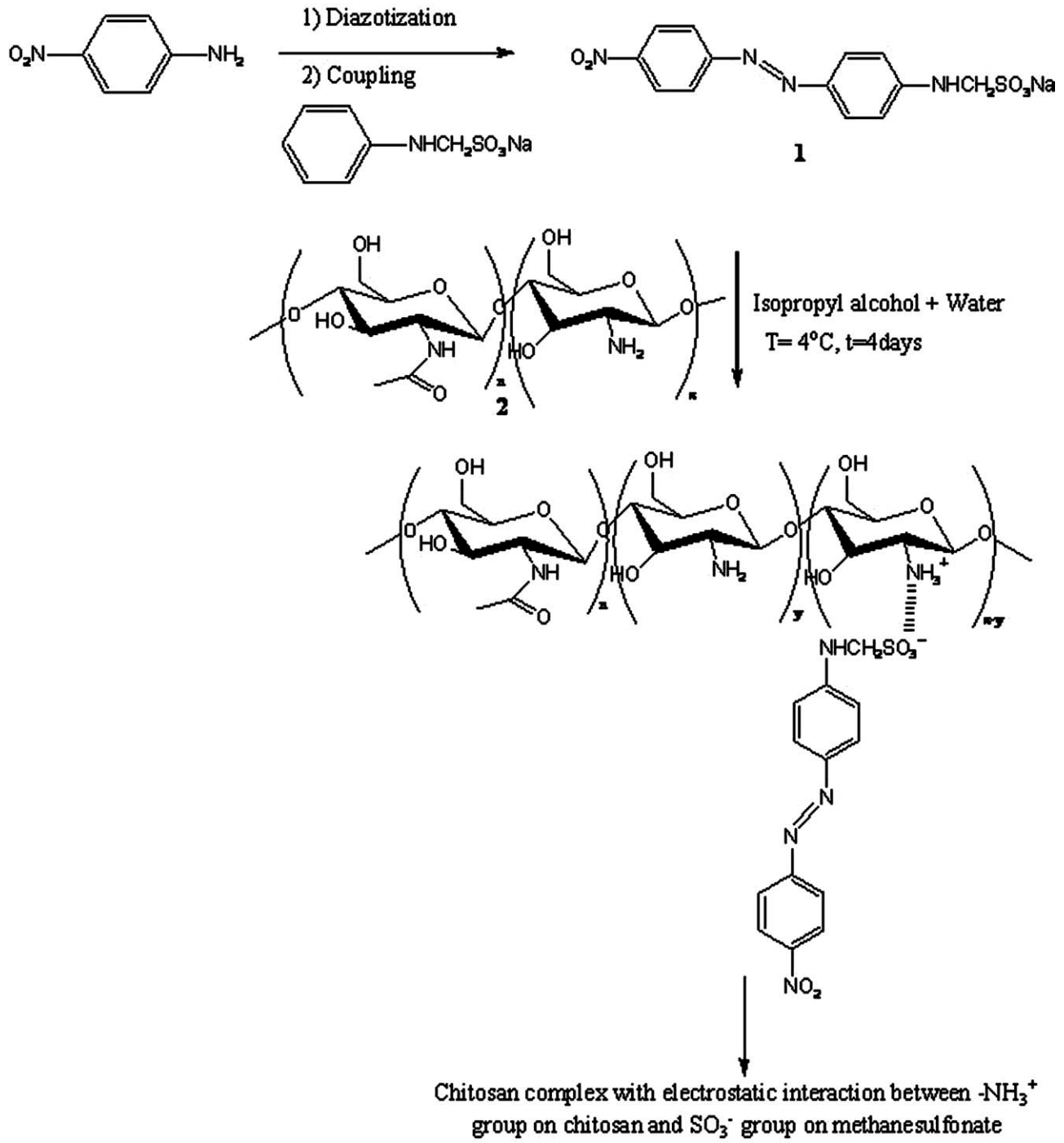

Scheme 1 Synthesis of chitosan azo-based amino methanesulfonate complex.

EXPERIMENTAL

Materials

The chitosan powder (95% degree of deacetylation) was obtained from Qingdao Yunzhou Biochemistry (China), average molecular weight was about <5000 g mole^{-1} (*Mv*), and a degree of deacetylation (DA) ≥90%. Sodium (anilinomethane) sulfonate, ethanol, hydrochloric acid (37%), sodium nitrite, sodium acetate, isopropyl alcohol, and glacial acetic acid (Samchun Chemicals, Korea) were used without further purification. The purity of all the synthesized compounds has been checked by Thin Layer Chromatography (TLC) using silica gel with different solvent systems.

Preparation

Synthesis of sodium {4-[(4-nitrophenyl)azo]phenyl} amino methanesulfonate (1)

This synthesis method was adapted from a procedure reported in Ref. 23. In a 250-mL three-necked

flask equipped with a mechanical stirrer, *p*-nitroaniline (4 mmol) was suspended in water (20 mL) at 0–5°C and then HCl (35%, 10 mmol) and sodium nitrite (4 mmol) were slowly added. The mixture was stirred for 1 h to complete diazotization. Sodium (anilinomethane)sulfate was used as a coupling component and then hydrolyzed under aqueous alkaline solution to form 4-[(4-nitrophenyl)diazenyl]benzenamine. $NaHSO_3$ (4 mmol) was added to the solution of 50 mL of water and 60 mL of ethanol and heated to 60°C. After cooling the reaction mixture to room temperature, aniline (4 mmol) and HCHO (4 mmol) were added to the solution, followed by stirring for 1 h. The coupling solution was slowly added to the diazotization solution and stirred at room temperature overnight. After reaction, the pH of the solution was adjusted to 5 by adding sodium acetate and the crude product was collected by filtration. The product (Scheme 1) was purified by recrystallization in ethanol (yield 50%). ^{1}H NMR (400 MHz, dimethyl sulfoxide [DMSO]): δ_H = 8.3 (d, 2H), 7.9 (d, 2H), 7.8 (d, 2H), 6.8 (d, 2H), and 5.2 (s, 2H).

Preparation of chitosan azo-based amino methanesulfonate complex

Chitosan powder (50 mg) was added to 1 mL of 1% acetic acid, 6 mL of isopropyl alcohol, and 1 mL of distilled water, and the mixture was stirred until the polymer was completely dissolved. Then, {4-[(4-nitrophenyl)azo]phenyl}amino methanesulfonate (0.286 g) was added to the above solution. Then the mixture was stirred for 4 h at room temperature (22°C) and kept at 4°C for 4 days. The obtained product was successively washed with an isopropyl alcohol and water mixture (5 : 1, v/v) and dried in air. The product was recrystallized from the *N,N*-dimethylacetamide and isopropyl alcohol solution and washed several times with the isopropyl alcohol/water mixsolvent. The final product (Scheme 1) was dried and kept in desiccators until the analysis was performed.

Measurements

Spectroscopy analysis

Fourier transform infrared (FTIR) spectra were recorded on JASCO FT-IR 300E device using KBr. Ultraviolet (UV)–visible absorption spectra were measured on an Agilent 8453 spectrophotometer (USA). ^{1}H NMR spectra of the sample was recorded on a Bruker 400 MHz NMR spectrometer using tetramethylsilane as an internal standard DMSO-d_6 as a solvent.

XRD analysis

X-ray diffraction (XRD) pattern of the samples were recorded on a 2100 series X-ray diffractometer with CuKα radiation (wavelength λ = 0.154 nm) at a voltage of 40 kV and a current of 50 mA. The scanning rate was 3° per minute, and the scanning scope of 2θ was from 3° to 40° at room temperature (25°C).

Thermal study

Thermogravimetric analysis (TGA) was carried out in a TA Q 50 system TGA. The samples were scanned from 0 to 700°C at a heating rate of 10°C min^{-1} under flow of nitrogen. Differential scanning calorimetry (DSC) with DSC Q1000V7.0 Universal V3.6C TA instrument, heating and cooling rates of 10°C min^{-1} was used.

Morphology study

The surface morphology was analyzed by scanning electron microscopy (SEM) JEOLJSM-6490LA.

PL spectroscopy

The photoluminescence (PL) spectra were recorded on a Perkin-Elmer LS55 Fluorescence spectrometer.

CD study

CD spectrum was recorded on a Jasco-J-715 spectrometer in DMSO.

RESULTS AND DISCUSSION

FTIR spectroscopy

The FTIR spectrum of native chitosan (a) and chitosan azo-based amino methanesulfonate complex (b) was measured and presented in Figure 1. The characteristic peaks of native chitosan [Fig. 1(a)] located at 3355 cm^{-1} are assigned to the hydrogen bonded

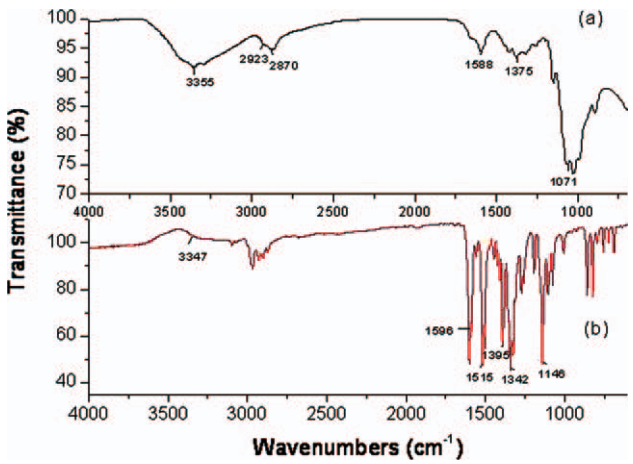

Figure 1 FTIR spectra of chitosan (a) and chitosan azo-based amino methanesulfonate complex (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

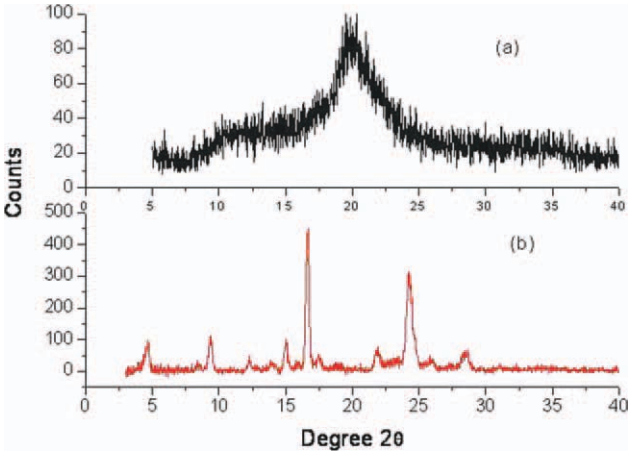

Figure 2 XRD spectra of chitosan (a) and chitosan azo-based amino methanesulfonate complex (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

O—H stretching vibrations. Other important peaks are 2923 and 2870 cm^{-1} (C—H stretching), 1588 cm^{-1} (amide band, N—H stretching of amino group) ,1375 cm^{-1} (asymmetric C—H bending of CH_2 group), and 1071 cm^{-1} (skeletal vibration involving the bridge C—O stretching) of glucosamine residue. After modification in the amino group of chitosan with [4-[(4-nitrophenyl)azo]phenyl]amino methanesulfonate, this band shifted to 1596 cm^{-1} due to symmetrical bending stretching of amine salt NH_3^+. The characteristic vibration bands of the dye appearing at 1515 and 1395 cm^{-1} are due to the asymmetric and symmetric N=O stretching of nitro group and at 1342 and 1146 cm^{-1} are due to the asymmetric and symmetric S=O stretching of sulfonic acid group.[25] It has been known that the protonation of the amine groups is responsible for the capture of anionic dyes through electrostatic attraction.[26] This is the reason for the low capacity of the chitosan under such conditions. When the chitosan solution were added to with [4-[(4-nitrophenyl)azo]phenyl]amino methanesulfonate, it is suggested that $-SO_3^-$ group from [4-[(4-nitrophenyl)azo]phenyl]amino methanesulfonate is electrostatically attached to $-NH_3^+$ group of chitosan.[24] The ionic interaction between chitosan (NH_3^+) and methanesulfonate (SO_3^-) is responsible for a new signal at 1596 cm^{-1}, which has been assigned to the absorption characteristic of symmetrical stretching of amine salt.

XRD spectroscopy

XRD spectra of native chitosan (a) and chitosan azo-based amino methanesulfonate complex (b) are shown in Figure 2. XRD studies of chitosan [Fig. 2(a)] exhibits very broad peaks at $2\theta = 10°$ and $2\theta = 20°$. Diffractive region of chitosan complex [Fig. 2(b)] is observed at 2θ of 4°, 9.47°, 15°, 21°, and 28° and very strong sharp peaks are observed at 2θ of 16.5° and 24°. Chitosan shows very broad lines especially for the smaller diffraction angles, thereby indicating long range disorder. The intense small angle peaks in chitosan complex exhibit higher long range order. In this complex, multiple scanned angles at lower intensity could be due to impurities. Intensity is not a routine predictor of crystal structure; it can be obtained from XRD pattern and reflects the unit cell dimensions.[27] Therefore, intensity is a means of obtaining structural information from powder diffraction. In addition, the lattice dimension is also related to the interplanar distance, *d*, that can easily be described for the particle size and geometry of the unit cell. The crystalline size *d* was calculated corresponding to the most intense peak by using Debye Sherrer formula.[28] The crystalline size was obtained to vary between 38 and 75 nm. XRD studies proved here that the crystal lattice has transformed from an amorphous structure into a relatively more cryatalline structure in chitosan to chitosan azo-based amino methanesulfonate complex.

Thermogravimetric analysis

The TGA thermograms of chitosan and chitosan azo-based amino methanesulfonate complex (b) are shown in Figure 3. TGA of pure chitosan in Figure 3(a) showed two different stages of weight loss. The first stage weight loss starting from 47 to 100°C, this may corresponds to the loss of adsorbed water. The second stage of weight loss starts at 247°C and continues up to 330°C due to the degradation of chitosan biopolymer. The TGA thermogram of chitosan azo-based amino methanesulfonate complex in Figure 3(b) indicates the two weight loss, first occurs

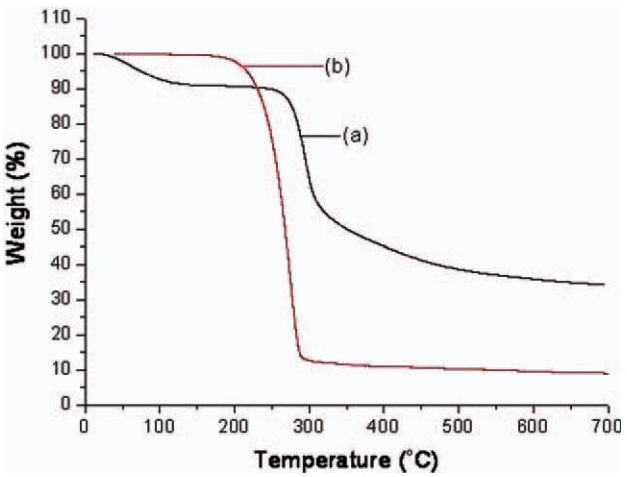

Figure 3 TGA of chitosan (a) and chitosan azo-based amino methanesulfonate complex (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

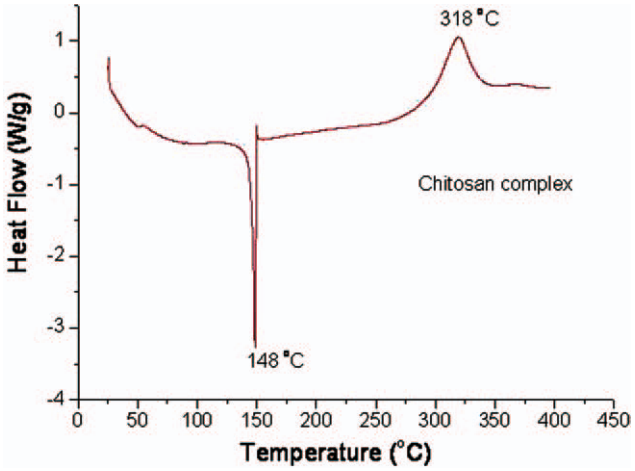

Figure 4 Differential scanning calorimetry (DSC) of chitosan azo-based amino methanesulfonate complex. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

at the 100 °C due to the evaporation of moisture. The second stage, it began to fast process of weight loss appears in chitosan complex decomposing from 195 to 296 °C, is may be due to the lower grafting of azo-based methane-sulfonate. The results demonstrate the lower thermal stability of chitosan azo-based amino methanesulfonate complex to the chitosan. Introduction of azo-based methanesulfonate group into polysaccharide structure should disrupt the crystalline structure of chitosan.

Differential scanning calorimetry

DSC thermogram of chitosan azo-based amino methanesulfonate complex showed in Figure 4, characteristic sharp endothermic peaks at 148°C due to the structural arrangement of polysaccharide. There is one broad exothermic peak at 318°C due to the thermal decomposition. The results indicated that the structure of chitosan chains has been changed due to the introduction of azo-based methanesulfonate moieties and the reduced ability of crystallization.

SEM analysis

The SEMs of the native chitosan and chitosan azo-based amino methanesulfonate complex are shown in Figure 5. The native chitosan [Fig. 5(a,b)] exhibited a nonporous, smooth, membranous phase consisting of dome shaped orifices, microfibrils, and crystallites. The scanning electron micrographs of the chitosan azo-based amino methanesulfonate complex are shown in Figure 5(c,d). It exhibited a numerous rose

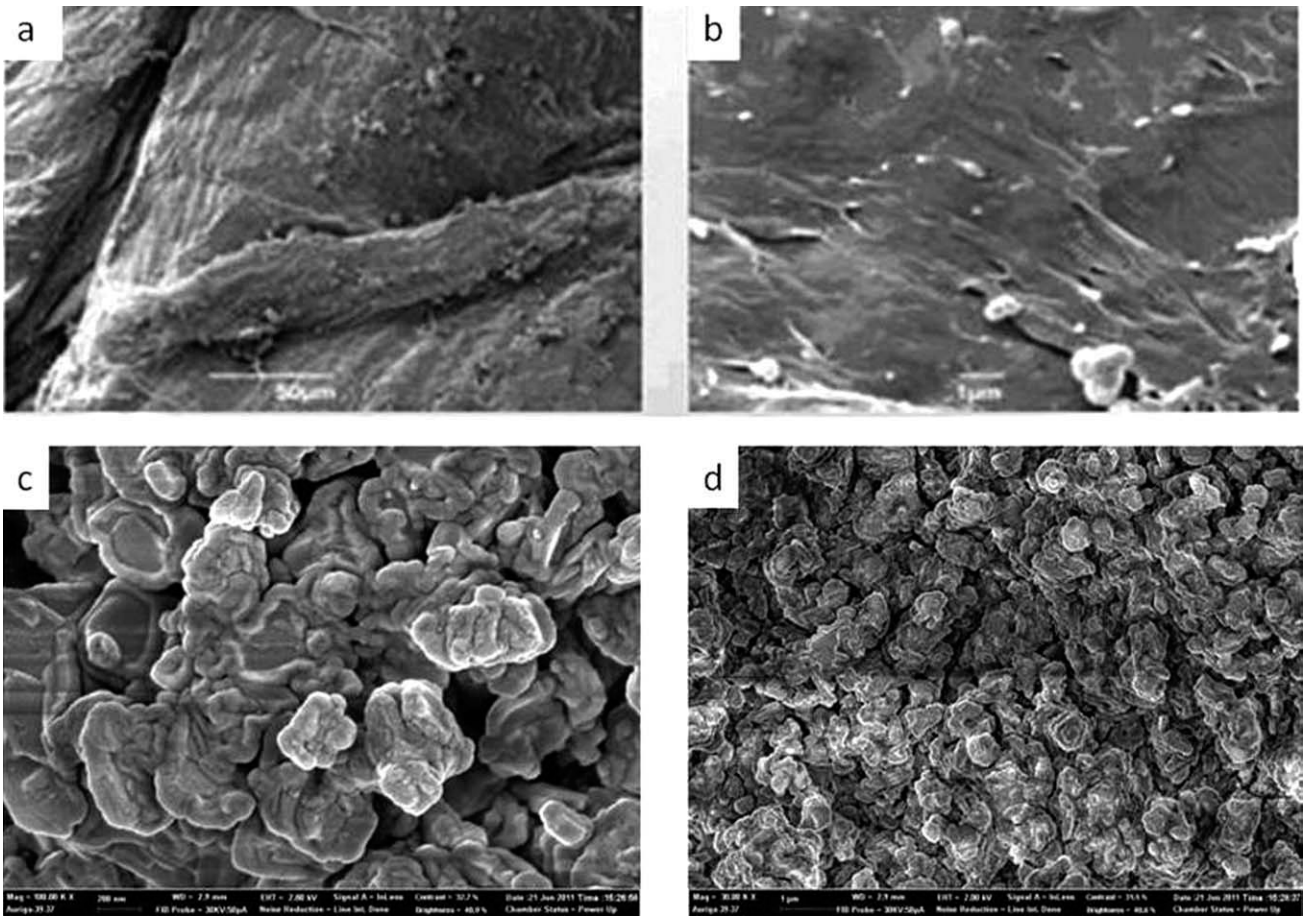

Figure 5 SEM images of chitosan (a, b) and chitosan azo-based amino methanesulfonate complex (c, d).

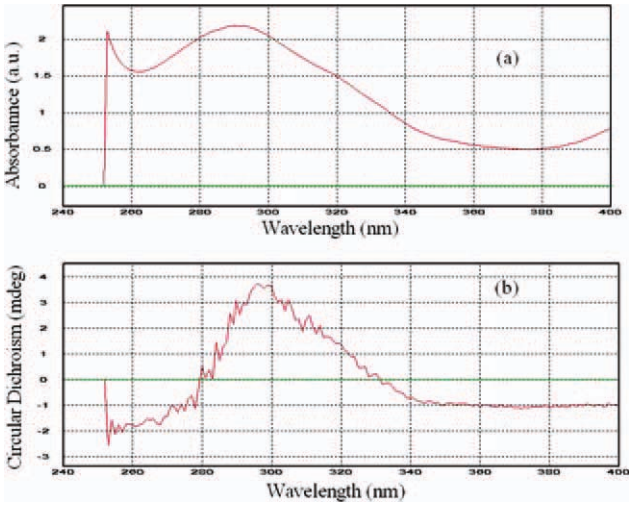

Figure 6 UV (a) and CD (b) spectra of chitosan azo-based amino methanesulfonate complex. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

flower shape structure and numerous microvilli. The result shows azo-based methanesulfonate was successfully integrated into matrix with no visible agglomerate formation at low particle amounts.

CD and UV–visible spectra of chitosan azo-based amino methanesulfonate complex

CD and UV spectra of biopolymer complex in DMSO solution with concentration 30 mg L^{-1} are shown in Figure 6. The CD and UV spectra of chitosan azo-based amino methanesulfonate complex with positive CD band at $\lambda = 298$ nm with 3.88 mdeg having UV absorbance maximum up to a narrow band at $\lambda = 290$ nm. The strong positive CD spectral band observed for the chitosan complex is obviously due to the coupling of azo dyes containing an azo-chromophore group (N=N) belonging to glucosamine units. Therefore, observed splitting patterns of CD suggest a helical (right handed) conformation. The inversion of the CD pattern in chitosan complex (negative to positive) at $\lambda = 260$–298 nm with -2 to 3.88 mdeg suggests that there is a change in the chiral structure of the polymer system. The CD sign also occurs regardless of the configuration of the chiral carbon atoms in the repeating glucosamine unit, because the configurations cannot be interconverted into one another without the rupture of chemical bonds. Therefore, the observed CD reversion can only be assigned to the helix sense reversion of the polymer conformation (chirality on the molecular level) because the polymer may adopt a different helical conformation by twisting the neighboring glucose rings around the glucosidic linkage.[20] The driving force for such a conformational reversion is related to the enhanced interaction of the hydrophobic groups appended to the chains.

PL properties

The PL spectra of the native chitosan and chitosan azo-based amino methanesulfonate complex are shown in Figure 7. The emission spectra and fluorescent intensity of chitosan and chitosan azo-based amino methanesulfonate complex are performed at an excitation wavelength of 325 nm. The emission spectra of pure chitosan exhibit a broad and relatively weak band maximum (λ_{em}) peak at 425 nm and chitosan azo-based amino methanesulfonate complex band maximum (λ_{em}) peak at 400 nm at excitation wavelength of 325 nm. When 4-[(4-nitrophenyl)azo]phenyl amino methanesulfonate is introduced into the pure chitosan, the absorption of chitosan azo-based amino methanesulfonate complex is found shift to a shorter wavelength (400 nm) showed blue shift emission maxima due to shield light on the pronounced effects of azo-based methanesulfonate with bulky molecular size in the side chain of polymers to finely perturb the resulted torsion angle and the relative movement of the phenyl segments, which in turns tunes the general electro-optical properties of parent polymeric matrix.[29]

CONCLUSIONS

In this work, we prepared chitosan azo-based amino methanesulfonate complex and CD study reveals that the chitosan complex adopts helical (right-handed) confirmation in positive band in DMSO. Physiochemical and thermal stability of chitosan azo-based complex have resulted because of amorphous structure and the drop in thermal stability due to the disruption of the intermolecular hydrogen bonds in polymer chain. The chitosan azo-based amino methanesulfonate complex shows PL properties. These new and

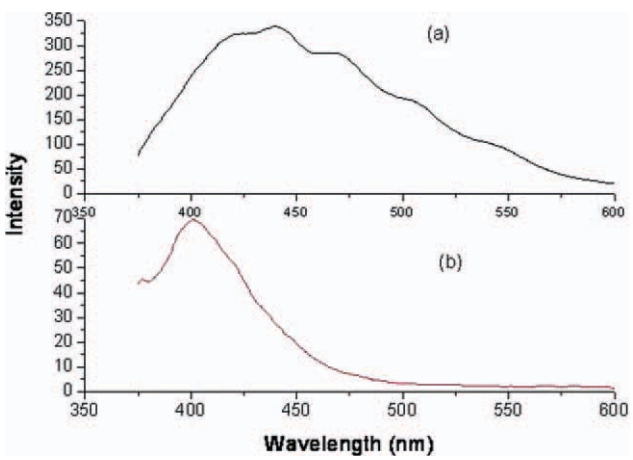

Figure 7 PL spectra of chitosan (a) and chitosan azo-based amino methanesulfonate complex (b) at excitation wavelength 325 nm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

safe colloids offer wide perspectives of development in biomedical applications.

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