Gel–Sol Transition and Thermotropic Behavior of a Chitosan Derivative in Lyotropic Solution

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ABSTRACT: A chitosan derivative which has a structure that approximates *N*-phthaloyl-3,6-di-*O*-acetylchitosan (AcPhCh) was used to investigate the thermal behavior of lyotropic gel liquid crystalline phases in dimethylsulfoxide (DMSO). A distinct gel–sol transition in addition to a liquid crystal–liquid (or chiral nematic–isotropic) transition was observed in the AcPhCh–DMSO system by differential scanning calorimetry (DSC). The detected gel–sol transition was reversible with a considerable amount of temporal hysteresis. Mesophase ordering occurred prior to gel formation for AcPhCh–DMSO samples while cooling from above the order–disorder "clearing" transition temperature. Similarly, a transition to a liquid crystal phase takes place prior to gelation while increasing the polymer concentration in the isotropic solution at ambient temperature. Circular dichroism (CD) spectra in addition to polarized light microscopy (PLM) observations as a function of temperature supported the existence of liquid crystal–liquid transitions at the temperatures recorded by DSC. A ball-drop experiment carried out on the lyotropic gel confirmed that the DSC step transition was indeed a gel–sol transition and provided additional mechanistic information. The above studies demonstrate, contrary to previous reports, that development of a lyotropic gel by rodlike polymers may occur first by forming an anisotropic liquid followed by gelation.

Introduction

Recently, there have been reports that concentrated solutions of chitosan (β-1–4-linked 2-amino-2-deoxy-D-glucopyranose structural repeat units), Ch, and its derivatives (acetoxypropyl)- (APCh) and (hydroxypropyl) chitosan (HPCh) show lyotropic liquid crystalline phases when dissolved in suitable solvents.[1,2] It has also been demonstrated that APCh exhibited thermotropic behavior.[1] The formation of mesophases by these polymers was believed to occur due to the rigidity of the polymer backbone.[2] In our laboratory, we have recently synthesized *N*-phthalolylchitosan (PhCh) with a degree of phthaloylation equal to 1.45 (containing significant *O*-phthaloylation) and *N*-phthaloyl-3,6-di-*O*-acetylchitosan (AcPhCh) with a degree of phthaloylation and acetylation of 1.45 and 1.50, respectively.[3] It was demonstrated that PhCh and AcPhCh form lyotropic liquid crystalline phases by circular dichroic (CD) spectroscopy and polarized light microscopy (PLM).[3]

The presence of both gel–sol and liquid crystalline-liquid transitions has been reported previously for relatively rigid polymeric systems, specifically, poly(γ-benzyl α-L-glutamate (PBLG),[4–6] poly(p-phenylenebenzobisthiazole) (PBZT) in concentrated sulfuric acid,[7] and a polyimide (3,6-diphenylpyromellitic dianhydride (DPP-MDA) reacted with (trifluoromethyl)-4,4′-diaminobiphenyl (PFMB)) in *m*-cresol.[8] A comprehensive review on the problem of gelation of rodlike polymers was published by Russo and co-workers.[9] They presented a provisional map of gelling behavior consisting of four regions of distinct polymer concentrations, near the biphasic gap on a binary phase diagram of temperature and polymer weight fraction. However, experimental observation of these four regions, according to the authors, depends upon the presence of a nonsolvent (such as water) in the PBLG–DMF system. According to their observation, liquid crystalline (cholesteric) organization takes place very slowly in a gelled sample, where gelation takes place from isotropic solution, indicating that self molecular organization, though slow,

is present even in a gel network consisting of rigid-rod polymers. Similarly, gelation from a liquid crystal phase is only rapid in region IV, which is the highest concentration regime, but the authors could not explain this behavior. Interestingly, studies carried out with DPP-MDA–PFMB by Cheng et al.[8] indicate that the liquid crystalline–liquid and gel–sol transitions occur almost simultaneously in the heating cycle, while for the cooling cycle the sol–gel transition occurs first through a nucleation-free liquid–liquid phase separation. Furthermore, Cheng et al. presented a speculative mechanism for ordering of DPPMDA–PFMB into an anisotropic gel which involves two processes: (i) gelation through a liquid–liquid phase separation process where a bicontinuous phase forms that contains "polymer-rich" regions in the matrix and (ii) growth of anisotropic domains from regions rich in polymer that have obtained a critical polymer concentration to form the liquid crystalline ordered gel. The gelation mechanism as a function of temperature and quenching concentration for synthetic random coil polymers and for some biological systems in dilute solution was also studied.[5] These studies conclude that the gelation occurs through nucleation of small crystallites, which also act as cross-link points, followed by aggregation. All of the above studies on the gelation mechanism of polymers which form lyotropic gels suggest a two-step mechanism where the first step could be either (i) a nucleation and growth process or (ii) a spinodal-like phase separation process. The second step involves reorganization of the gel to form a liquid crystalline gel material.

The present paper reports on the gel liquid crystal phase of AcPhCh in dimethyl sulfoxide (DMSO). An investigation was carried out to document the unique thermal characteristics and route to formation of the anisotropic AcPhCh–DMSO gels as a function of the polymer weight fraction. The gel–sol as well as the clearing transitions were observed by differential scanning calorimetry (DSC). The gel–sol transition was further substantiated by a ball-drop experiment which measures the softening temperature of the gel. The clearing transition was verified by polarized light microscopy (PLM) observations as well as by circular dichroism (CD) spectral changes as a function of temperature. The demonstration herein that AcPhCh–

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DMSO gel liquid crystals have a thermally reversible gel–sol transition which is well below the clearing transition stimulated further studies reported in this paper which explore the relative kinetics and mechanistic features involved in the formation of an anisotropic gel by this rodlike modified biological macromolecule–solvent liquid crystalline system.

Experimental Section

Polymer Synthesis. The polymer was synthesized by a modification of a literature procedure[5] which has been reported in detail elsewhere.[3,10] Molecular weight averages (M_w and M_w/M_n) of the polymer were determined by GPC and were found to be 111 000 and 2.6, respectively. The degrees of N-phthaloylation, N-acetylation, O-phthaloylation, and O-acetylation were determined from elemental analysis and ^{1}H NMR spectral integration as was previously described[3,10] and are 0.93, 0.07, 0.57, and 1.4, respectively.

Sample Preparation. Different concentrations of the polymer solutions were prepared separately in small glass vials. When desired, the solutions were homogenized by heating them in a thermostated oven at ca. 100 °C for ca. 10 min (well below the boiling point of the solvent and the decomposition temperature of the respective polymers). The vials were tightly capped with Parafilm so that the solvent did not evaporate during this process. The homogenized solutions were then stored at room temperature for ca. 1 month before being analyzed by differential scanning calorimetry (DSC; see below).

The homogenized solutions were sandwiched between glass plates to form the sample cells. The thickness of the cells was a function of the quantity of sample added between the plates. The cell thickness was determined using a micrometer with an accuracy of ±5 µm. The sandwiched cells were sealed from all sides using epoxy to prevent evaporation of the solvent.

Instrumental Methods. Differential scanning calorimetry (DSC) experiments were performed using a Dupont DSC 2910 and 2000 thermal analyzer system under a N_2 purge and a heating rate of 5 °C/min (unless otherwise indicated). The sample pans for the DSC measurements contained between 6 and 10 mg of sample and were hermitically sealed. The gel–sol and liquid crystal–liquid transition temperatures were recorded from the heating thermograms as the midpoint of the step transition and the end point of the endothermic transition, respectively. Polarized light microscopy (PLM) was performed using a Leitz Ortholux II microscope (with X320 magnification) equipped with a Mettler FP 50/52 hot stage. The ball-drop experiment was performed by placing a steel ball of diameter and weight of 0.64 cm and 1.065 g, respectively, on the top of the gel.[8] The gel and ball were confined in a vial which was capped tightly with Teflon tape. With subsequent heating, the gel softens and the ball drops into the gel. The depth of the ball drop was measured using a microscope eyepiece with a graduated scale focused onto the bottom surface of the ball. The temperature accuracy of the bath for this experiment was ±1 °C. Circular dichroic (CD) spectra were recorded using a Jasco J-710 spectropolarimeter interfaced with a programmable temperature controller (RTE 110, Neslab) under a N_2 atmosphere.

Results and Discussion

The occurrence of a liquid–liquid crystal transition in the chitosan derivative AcPhCh in DMSO (see Experimental Section) with a polymer weight fraction, x, equal to 0.09 (AcPhCh–0.09DMSO) has been investigated by CD and PLM. The results of the previous study suggested that this sample formed a right-handed cholesteric mesophase structure. Further investigations are described herein to study the thermal characteristics of the AcPhCh–DMSO mesophase.

The DSC first heating scan (thermogram a) of the AcPhCh–0.19DMSO sample which was aged for longer than 30 days is shown in Figure 1. The thermogram clearly shows two distinct endothermic transitions at 66 and 100–120 °C, respectively. The lower temperature steplike transition appeared similar to a glass–liquid (T_g) transition,
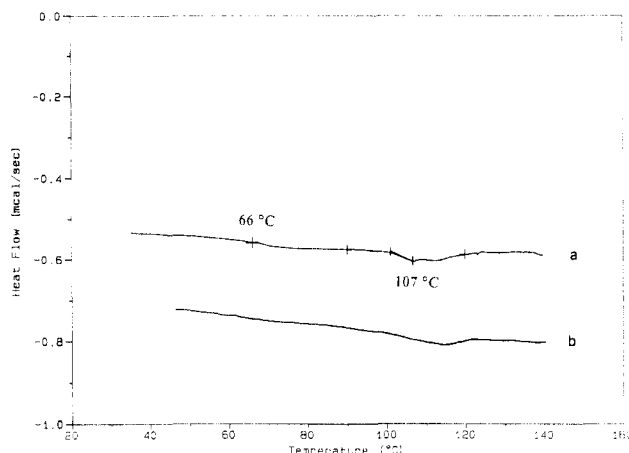

Figure 1. DSC thermograms of AcPhCh–0.19DMSO recorded at a heating rate of 5 °C/min. Thermogram a corresponds to the first heating scan, whereas thermogram b corresponds to the second heating scan recorded immediately after a rapid quench of the isotropic liquid from above the clearing transition temperature.
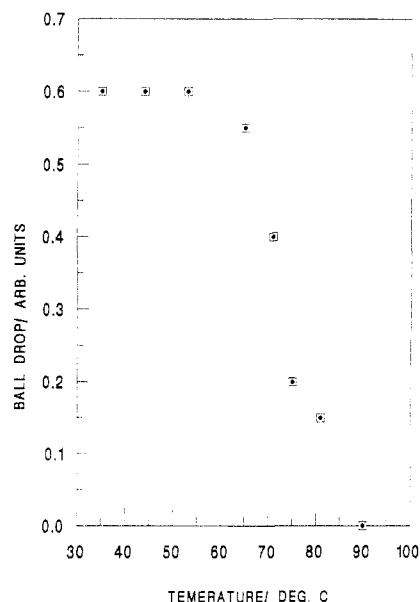

Figure 2. Recording of the distance by which a steel ball (see Experimental Section) has dropped into an AcPhCh–0.19DMSO anisotropic gel at various temperatures.

which led us to suspect that it was due to a gel–sol transition. The higher temperature endotherm is characteristic of that seen for crystalline melting and was therefore assigned to the order–disorder "clearing" transition (liquid crystalline–liquid transition).

The clearing transition, determined above by DSC measurements, was confirmed from PLM visual observations during sample heating (see Experimental Section). The existence of a gel–sol transition, also indicated by the DSC thermogram in Figure 1 (thermogram a), was verified using the ball-drop technique (see Experimental Section). The principle of this experiment is that the gel loses its mechanical strength ("softens") at the gel–sol transition. This can be visually observed by measuring the distance which a steel ball placed on the surface of the gel falls into the gel as a function of the gel temperature. Figure 2 shows the results of the ball-drop experiment performed on the AcPhCh–0.19DMSO sample. It was observed that the lyotropic gel softens at ~60 °C. Furthermore, the gel has lost a considerable degree of its initial strength by 70 °C and becomes a solution by 80 °C as the ball drops to the bottom of the vial. At this point, visible fluid flow was observed in the polymer solution when the vial was tilted.
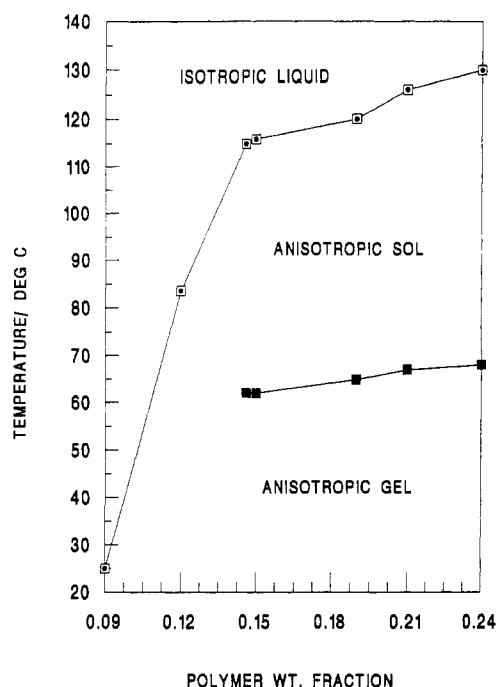
Figure 3. Phase diagram of AcPhCh–*x*DMSO showing the anisotropic gel, anisotropic sol, and isotropic liquid states as determined from DSC and PLM studies in the heating cycle. Observation of the gel–sol transition by DSC measurements required some equilibration time though the isotropic–liquid crystal transition ΔH and peak temperature were unaffected by a heating and cooling cycle.

Therefore, the gel–sol transition determined from the ball-drop experiment agrees well with the value determined from the DSC experiment (see Figure 1). It may be emphasized here that the polymer solution is still aniso-tropic at 80 °C, which was verified from PLM studies with samples confined between two glass plates. The isotropic transition starts taking place at ca. 100 °C and is completed at 120 °C. Therefore, in the AcPhCh–0.19DMSO system, the gel–sol transition and the clearing transition take place between 60 and 70 °C and between 100 and 120 °C, respectively.

Further DSC measurements were performed to study the effects of the AcPhCh polymer concentration on the thermal transitions discussed above. Specifically, DSC analyses were carried out on AcPhCh–*x*DMSO samples with polymer weight fraction (*x*) values of 0.09, 0.12, 0.14, 0.18, 0.19, 0.22, and 0.24. Following the first DSC heating scan, these lyotropic samples were equilibrated at room temperature for a minimum of 24 h and subsequently at 35 °C for 15 min. The second DSC heating scan was then performed from which the gel–sol transition (midpoint of the step transition) and clearing transition values (taken as the end point of the endothermic transition) were recorded. The values thus obtained are shown in the phase diagram in Figure 3. The critical concentration for the AcPhCh–DMSO polymer–solvent system at 22 °C to form a liquid crystal is 0.09 as noted in Figure 3 and determined previously by PLM and CD studies.[3] At polymer weight fraction values between 0.12 and 0.24, all the solutions showed distinct gel–sol transitions and clearing temper-atures ranging between 60 and 70 °C and between 82 and 130 °C, respectively. It appears that the gel–sol transition temperature shows only a small dependence on the polymer weight fraction, whereas the clearing temperature shows a significant increase for more concentrated samples. The temperature difference between the gel–sol and clearing transitions becomes as large as 58 °C for *x* = 0.24. This behavior of AcPhCh lyotropic gels where a distinct anisotropic gel–sol transition can be used to provide a
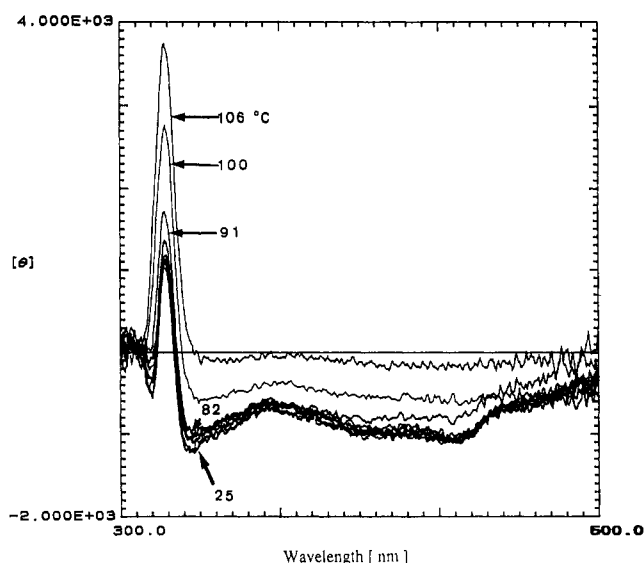

Figure 4. Circular dichroism spectra of AcPhCh–0.14DMSO recorded at different temperatures.

liquid crystalline solution has not been reported so far in any other lyotropic system.

Since AcPhCh–DMSO samples undergo a gel–sol tran-sition forming an anisotropic liquid upon heating and then show a broad clearing transition at higher temperatures, an investigation of changes in the mesophase order as a function of temperature was undertaken employing CD spectroscopy. CD spectra for AcPhCh–0.14DMSO re-corded at different temperatures (see Experimental Sec-tion) are shown in Figure 4. The major CD spectral features are negative CD bands with peaks at 320, 340, and 510 nm in addition to the positive band with a peak at 329 nm. It is believed that the peak at 510 nm is the apparent absorption band resulting from selective reflec-tion of the cholesteric helix,[11] whereas the negative and positive bands with peaks at 320 and 329 nm, respectively, are associated with the phthalolyl chromophoric groups. The negative band at 340 nm is liquid crystal induced.[3] Interestingly, spectra recorded between 25 and 82 °C showed little to no change in the position or magnitude of the CD bands. Therefore, based on the CD spectral results, it can be stated that there is no significant change in the mesophase order in passing through the gel–sol transition in this lyotropic system. This also suggests that the rodlike molecules still retain their orientational order, whereas the intermolecular interactions responsible for maintenance of the gel network are weakened. Indeed, it would be expected that the physical gel network, which is maintained with variable gel strength up to ca. 82 °C (see Figures 2 and 4), can stabilize the order present in the mesophase by decreasing the diffusivity of macromolecular chains. The intensity of the absorption bands at 510 and 340 nm started decreasing at temperatures above 82 °C, which is expected since disruption of the liquid crystalline phase begins at approximately the same temperature, based on DSC and PLM investigations. On the other hand, the absorption band due to the phthaloyl chromophoric group at 329 nm increases further as the isotropic transition approaches. This is believed to occur since, at higher temperatures, the electronic dipoles associated with the pathaloyl group are no longer aligned along a preferential direction. A change in orientational order can cause an increased coupling of the polarization vector of the incident light beam with the electronic dipole of the chromophoric group, resulting in a higher CD signal. It also should be noted that the apparent CD band at 510 nm does not show any significant shift in the λ_{max} with temperature.

Additional investigations were carried out to understand the kinetics of mesophase formation as well as gelation in the AcPhCh–0.19DMSO system. A second DSC heating scan, shown in Figure 1 (thermogram b), was performed immediately after quenching the sample to 20 °C from its isotropic state. The gel–sol transition (observed in thermogram a; see above) was not discernible above the background signals, although the clearing transition was observed and appeared similar to that seen in the first heating scan (~0.2 cal/g for thermograms a and b shown in Figure 1). Therefore, it appears that upon heating this sample above the clearing transition, rapid cooling, and subsequent heating, the liquid crystalline ordering achieved is almost identical to the original sample which had been aged for longer than 30 days. Moreover, the steplike gel-sol transition could only be observed in the same sample after it had been stored for a day or two (DSC thermogram not shown), which indicates that the development of a rigid free-standing gel took considerably longer than the relatively rapid mesophase formation described above.

Observation of mesophase formation during the cooling cycle by PLM was also carried out for the AcPhCh–0.19DMSO sample. From this experiment, it appeared that mesophase formation occurred well above the sol–gel transition at ~90 °C. Therefore, in agreement with the discussion above, liquid crystalline ordering of this mesophase occurs readily by a mechanism which is independent of gel formation. Further support of mesophase formation prior to gelation for the AcPhCh–DMSO polymer–solvent system has been obtained by observation of solutions at the critical polymer concentration x_c. At x_c, a biphase consisting of isotropic and anisotropic areas first appeared under the PLM at ambient temperature. With subsequent increase of x to 0.12, whole areas of the polymer solution in the cell became birefingent, but the solution was yet to form a free-standing gel. Significant gelation occurred only at higher polymer weight fraction values of ca. $x = 0.14$.

The difference in the gelation behavior observed for the AcPhCh–xDMSO system relative to other rodlike polymers studied in the past is extremely interesting and raises many questions and comments. Previous reports for rodlike polymers showed that chain entanglements as well as other association mechanisms lead to gelation which occurs more rapidly than mesophase formation.[8,9] Since gelation occurred only after the formation of the AcPhCh–DMSO mesophase, it follows that substantially less energy must be required to form a mesophase prior to gelation as opposed to after gelation, since molecular reorganization within a gel matrix becomes relatively restricted. Indeed, Cheng et al.[8] stated that mesophase formation in their polymer solution system is somewhat decelerated by the rapid gelation. This explains why in that study the introduction of thermal energy by elevated temperature annealing is required to accelerate molecular reorientation and subsequent mesophase formation. Therefore, if the kinetics for mesophase formation is significantly faster than gelation for a rodlike polymer system, then, under similar thermal conditions, it is likely that the formation of the anisotropic gel where molecular orientation has reached its equilibrium value will occur at a relatively faster rate than when gelation precedes mesophase formation.

Of related interest is the observation that the formation of an anisotropic liquid from the isotropic state is enhanced by the acetylation of phthalolylchitosan.[3] By replacing the hydroxy groups of PhCh with acetyl groups, it is expected that intra- and interchain H-bonding interactions are destroyed. However, interchain association is still maintained which could be due to steric hindrance. This,

in the absence of additional information, may explain the lower x_c value observed for AcPhCh relative to PhCh. Further studies are in progress to investigate changes in chain dynamics of modified chitosan polymers as a function of the chemical modification that was performed. In this way, an understanding on a molecular level will be pursured to explain many of the macroscopic observations described above.

Conclusions

The lyotropic gels of AcPhCh obtained from site-selective chitosan modification show a gel–sol transition well below the clearing transition. The magnitude of the heat of transition associated with the clearing transition is of the order of 0.1–0.4 cal/g depending upon the polymer concentration in the gel. From DSC, PLM, and ball-drop experiments it was demonstrated that after heating the samples above the clearing transition and subsequent cooling, the formation of the lyotropic mesophase occurred prior to gelation. This directly implies that the mesophase ordering during cooling from above the clearing transition of the rodlike AcPhCh polymer in DMSO occurs by a mechanism which does not require the degree of interchain interactions which is associated with gel formation. The kinetic preference for liquid crystal formation prior to gelation for AcPhCh–DMSO systems stands in contrast to earlier reports by other investigators that rodlike polymer systems, enroute to anisotropic gels, first form isotropic gels which then reorganize to form liquid crystalline gel systems.

References and Notes

(1) Ogura, K.; Kanamoto, T.; Sanan, T.; Tanaka, K.; Iwakura, Y. *Proc. 2nd Int. Conf. Chitin/Chitosan, Tottori, Japan*, 1982, p 39.

(2) Terbojevich, M.; Cosani, A.; Conio, G.; Marsano, E.; Bianchi, E. *Carbohydr. Res.* 1991, 209, 251.

(3) Rout, D. K.; Pulapura, S. K.; Gross, R. A. *Macromolecules*, accompanying paper in this issue.

(4) Russo, P. S.; Magestro, P.; Miller, W. G. In *Reversible Polymeric Gels and Related Systems*; Russo, P. S., Ed.; American Chemical Society: Washington, DC, 1987, Chapter 11, p 152.

(5) Hill, A.; Donald, A. M. *Polymer* 1988, 28, 1426.

(6) Jackson, C. L.; Shaw, M. T. *Polymer* 1990, 31, 1070.

(7) Cohen, Y.; Frost, H. H.; Thomas, E. L. In *Reversible Polymeric Gels and Related Systems*; Russo, P. S., Ed.; American Chemical Society: Washington, DC, 1987; Chapter 12 and references therein.

(8) Cheng, S. Z. D.; Lee, S. K.; Barley, J. S.; Hsu, S. L. C.; Harris, F. W. *Macromolecules* 1991, 24, 1883.

(9) Russo, P. S.; Chowdhury, A. H.; Mustafa, M. In *Mater. Res. Symp. Proc.* 1989, 134, 207.

(10) Pulapura, S. K.; Rout, D. K.; Gross, R. A., manuscript in preparation.

(11) It may be pointed out here that for the AcPhCh sample studied in ref 3 a distinct CD peak at 510 nm was not observed. In that work, the AcPhCh obtained had degrees of phthaloylation and acetylation of 1.45 and 1.57, respectively, and M_w and M_w/M_n values of 143 000 and 1.58, respectively. In the present study, the AcPhCh sample used was analyzed in a manner similar to that of the above sample and was found to have degrees of phthaloylation and acetylation of 1.50 and 1.57, respectively, and M_w and M_w/M_n values of 111 000 and 2.6, respectively. Therefore, it appears that minor changes in molecular weight and/or chitosan substitution resulted in observable changes in the mesophase order (as detected by CD measurements) for these respective chitosan samples.