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Liquid crystalline behaviour of chitooligosaccharides

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Abstract

Two chitooligosaccharide samples (COS1 and COS2) were prepared by enzymatic degradation of chitosan. The molecular weight of the main component was determined by mass spectrometry to be 2340 and 4303, respectively. They showed cholesteric liquid crystal structures in concentrated solutions. The critical concentrations to form a lyotropic liquid crystalline phase in formic acid were measured by means of polarized optical microscopy, refractometry and Fourier transform infrared spectroscopy. The results from the three methods agreed with each other. The experimental value of COS2 was quantitatively consistent with that calculated according to Khokhlov and Semenov theory. But the experimental value of COS1 diverged from the theoretical one, which is explained by the inaccuracy at higher concentrations of the second virial approximation underlying this theory.

Keywords: Chitosan; Chitooligosaccharides; Liquid crystals; Critical concentration; Molecular weight

1. Introduction

Chitosan is the deacetylated product of chitin, which is the second most plentiful structural biopolymer (after cellulose) and is found in the exoskeleton of crustaceans, in fungal cell walls and in other biological materials. It is a cationic heteropolysaccharide composed mainly of β-(1,4)-2-deoxy-2-amino-D-glucopyranose units, and partially of β-(1,4)-2-deoxy-2-acetamido-D-glucopyranose. Chitosan is a nontoxic, bioactive, biocompatible and biodegradable polymer, which has recently found a wide application as a functional material (Muzzaralli, 1977; Roberts, 1992).

Chitosan exhibits its biological activity only in acid medium because of its poor solubility above pH 6.5. Therefore, recent studies on chitosan have attracted interest in converting it to more soluble chitooligosaccharides (COS) (Kuroiwa, Ichikawa, Sato, & Mukataka, 2003; Park, Je, & Kim, 2003a,b). COS possess a number of interesting biological activities, such as antibacterial and antifungal properties (Jeon, Park, & Kim, 2001), as well as antitumor properties (Tokoro, Tatewaki, Suzuki, Mikami, & Suzuki, 1988), and immunoenhancing effects (Hirano et al., 1991).

This paper concentrates on another property of COS, i.e. liquid crystalline behaviour, which has not been reported. It is well known that high molecular weight chitosan and its derivatives often display lyotropic liquid crystalline behaviour (Dong & Li, 1999; Dong, Wu, Wang, & Wang, 2001b; Dong et al., 2002; Ogura, Kanamoto, Sannan, Tanaka, & Iwakura, 1982; Rout, Li, Pulapura, & Gross, 1993; Sakurai, Shibano, Kimura, & Takahashi, 1985; Wu, Dong, Chen, Huang, & Li, 2002). Some authors have reported the influence of molecular weight on the critical concentration for formation of a liquid crystalline phase (Dong, et al., 2001a; Hu et al., 2000; Terbojevich, Cosani, Conio, Marsano, & Bianchi, 1991; Wang, Dong, Liu, Yuan, & Mei, 1999). All these studies investigate the region of high or medium molecular weight (10^4-10^6), but neglect the low molecular weight region ($<10^4$), which is just the most sensitive region (Fig. 1). COS with the molecular weights of 2341 and 4303 was used in this study to disclose the relation between critical concentration of the lyotropic liquid crystal and the molecular weight of chitosan in this sensitive region of the molecular weight dependence of the concentration where liquid crystalline behaviour is observed.

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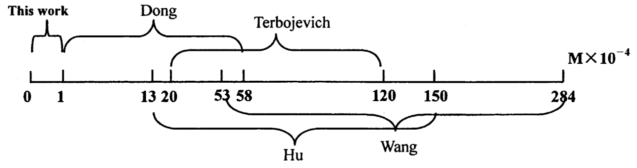

Fig. 1. Chitosan molecular weight range studied for determination of liquid crystalline behaviour (Dong et al., 2001a,b, 2002).

Table 1
Description of samples

Sample	Claimed molecular weight	Degree of deacetylation[a] (%)	Properties
COS1	<3000	79.2	Yellow powder
COS2	<5000	73.2	Yellow powder

[a] Measured with FTIR method (Sannan, Kurita, Ogura, & Iwakura, 1978).

2. Experimental

2.1. Materials

Two chitooligosaccharide samples were supplied by the Haidebei Marine Biological Product Co., Ltd. The properties of the samples are listed in Table 1.

2.2. Analyses

The Fourier transform infrared (FTIR) spectra were measured using a Nicolet Avatar 360 spectrophotometer. Mass spectra (MS) were performed on a Bruker Dalton Esquire 3000 plus instrument (range: 50–6000 *m/z*). Positive ions were generated by chemical ionization by using acetic acid as the ionization gas. The micrographs were obtained with an Olympus BH-2 polarized optical microscope (POM). The refractive index was measured using a A. Krüss Optronic Abbe refractometer.

2.3. Determination of critical concentration

Chitooligosaccharide solutions in formic acid at a range of concentrations with a concentration intervals of 1 wt% were prepared in small glass vials. The vials were tightly sealed after stirring. The solutions were aged for 3 days at 20 °C before use. The solutions were then sandwiched between two glass slides to form the sample cells. The cells were observed at 20 °C with POM. The lowest concentration at which birefringence appeared was defined as the critical concentration C_1 of the liquid crystal-isotropic phase transition. The solutions were also used to detect the C_1 by means of FTIR and refractometry.

3. Results and discussion

3.1. Determination of molecular weight

The molecular weight (MW) of COS is hard to detect using the normal methods for measuring MW of high polymers. MS has been used extensively for MW estimation of oligosaccharides (Bahrke et al., 2002). In this paper, MS was employed to measure the value of MW for the mean component of both COS1 and COS2 (Fig. 2), which were 2340 and 4303, respectively. Fig. 2 also illustrates a distribution of sizes from the peak heights of components. It can be seen that COS2 is more polydisperse than COS1.

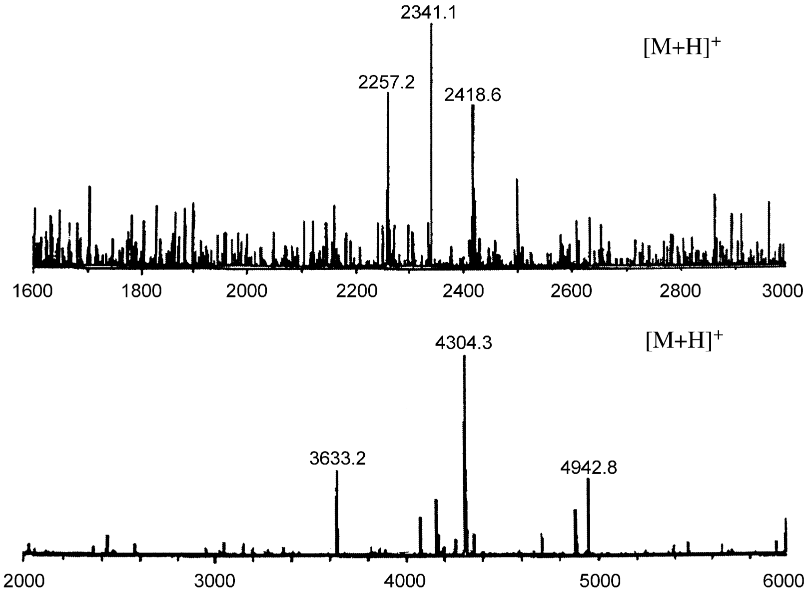

Fig. 2. Mass spectra of COS1 (a) and COS2 (b) (Dong et al., 2001a,b, 2002).

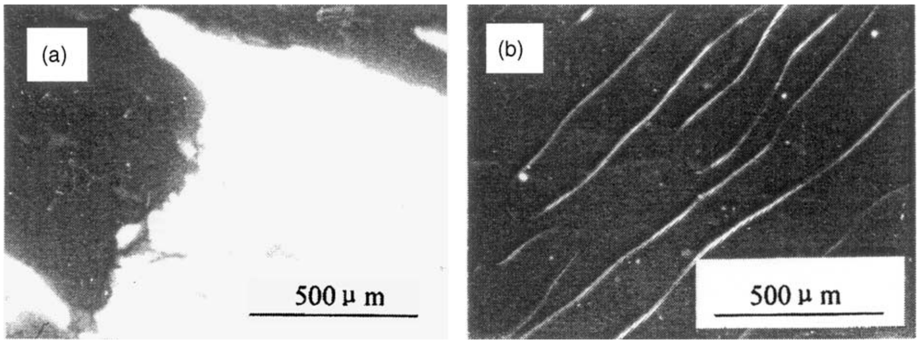

Fig. 3. Photomicrographs of the planar texture (a) and the oily streak texture (b), seen in a 45 wt% COS2/FA solution and a 35 wt% COS2/FA solution, respectively, under crossed polars (Dong et al., 2001a,b, 2002).

3.2. Liquid crystalline textures of COS

Both COS1 and COS2 solutions in formic acid (FA) formed mesophases at suitably high concentrations. Fig. 3a shows a planar texture for a 45 wt% COS2/FA solution. It is well known that the cholesteric phase usually demonstrates a planar texture, while the axes of spirals are almost vertical to the sample plane. Fig. 3b shows another planar texture, an oily streak texture, for a 35 wt% COS2/FA solution. The oily streak texture is a typical cholesteric texture for small molecule liquid crystals. This is the first time it has been observed for chitosan, probably because of the low molecular weight used in this work.

In general, the system formed a lyotropic liquid crystal with a molecular weight distribution at two critical concentrations: C_1 and C_2. Below C_1, the solution was isotropic, between C_1 and C_2, the solution was a mixture of isotropic and anisotropic, and above C_2, and the solution was fully anisotropic. The results of C_1 and C_2 measured by POM were listed in Table 2. Normally C_2 is more difficult to measure than C_1. It is of interest to observe that the coexistence of isotropic and anisotropic occurred in droplet textures (Fig. 4). At lower concentration in the biphase region of COS2, white drops (anisotropic) and black background (isotropic) can be seen (Fig. 4a); but at higher concentrations, black drops (isotropic) and white background (anisotropic) can be observed (Fig. 4b). A similar situation can be observed in the biphase region of COS1 (Fig. 4c and d). From Table 2, it can be noted that the biphase region of COS2 was wider than that of COS1. That may be due to both the greater polydispersity of COS2 and higher molecular weight of COS2.

3.3. Refractometry measurement of the C_1

Refractometry has been used to detect the critical concentration of polysaccharide (Suto & Maruyama, 1990; Wu et al., 2002). The average refractive indexes of each of the prepared solutions were measured at a constant temperature of 25 °C. Then, curves of refractive index vs. concentration were plotted. The curves (Fig. 5) show an inflection because of different refractive indexes of isotropic and anisotropic phase. Because the C_1 is defined as the concentration where birefringence begins to appear, the concentration that is just more than the inflection point is the C_1. Therefore, the C_1 was 35 and 31 wt% for COS1 and COS2, respectively.

3.4. FTIR measurement of the C_1

The FTIR spectroscopy was employed as an auxiliary technique to measure C_1. In the mesophase, the molecular chain spontaneously ordered, therefore more intermolecular hydrogen bonds form. As a result, the characteristic FTIR bands of groups, which correspond to the formation of hydrogen bands, will move more or less when the liquid crystalline phase appears. The strong interactions between the chains and solvent can be shown by the widening of bands in FTIR spectra (Fig. 6).

From Fig. 7, it can be seen that seven bands shifted at the C_1. The largest change was $\sim 20\ \mathrm{cm}^{-1}$. These bands were attributed to –OH, –NH, –NHCO– of chitooligosaccharide, including C=O of formic acid. They all corresponded to the formation of hydrogen bonds. Although from the shift of only one band it is difficult to tell the C_1, the shift of all seven bands makes it clear. From FTIR measurement, the C_1 were 31–32 and 35–36 wt% for COS1 and COS2, respectively.

3.5. Influence of molecular weight on C_1

Khokhlov and Semenov (1981, 1982) use finite stiffness in their theoretical formulation of semi-flexible polymer,

Table 2
Critical concentrations for liquid crystal formation in formic acid

Sample	C_1 (wt%)	C_2 (wt%)
COS1	36	~59
COS2	32	~58

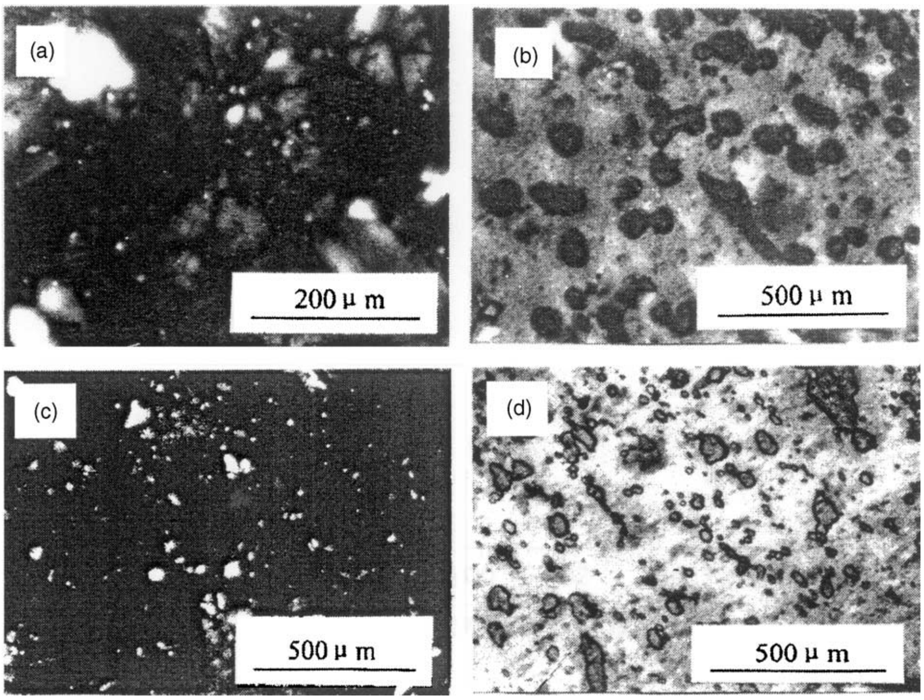

Fig. 4. Photomicrographs of the droplet texture under crossed polars in (a) a 38 wt% COS2/FA solution; (b) a 50 wt% COS2/FA solution; (c) a 35 wt% COS1/FA solution and (d) a 50 wt% COS1/FA solution (Dong et al., 2001a,b, 2002).

where they have extended the theory of Onsager and Ann (1949) for rods, to worm-like chains. The persistence length q of the chain plays a central role.The expression deduced by Khokhlov and Semenov is as follows

$$V' = \frac{d}{2q}\left\{\frac{3.34 + 11.94(L/2q) + 6.34(L/2q)^2}{[1 + 0.586(L/2q)](L/2q)}\right\};$$

$$d = \left(\frac{M_0}{\rho \times N_A \times L_0}\right)^{1/2};$$

where ρ is density of polymer; M_0 is molar mass of repeat unit; N_A is Avogadro constant; L_0 is the length of repeat unit along chain (taken as 0.515 nm, half of b in unit cell of chitosan crystal (Sakurai et al., 1985)); d is the diameter of chain; $q = 22$ nm (Terbojevich et al., 1991); L is the contour length of chain. V' is the critical concentration in v/v%, which can be calculated from C_1 (w/w%) with the density of chitosan (1.5 g ml^{-3}) and formic acid (1.22 g ml^{-3}).

Calculated data are shown in Table 3, comparing with C_1 value of COS2 was quantitatively consistent with the theoretical one, but for COS1 the two values were somewhat different. This suggests that the second virial approximation underlying this theory is inaccurate at higher critical concentrations for lower molecular weight chitooligosaccharide system. But it appears that the, Khokhlov and Semonov theory can still be used for higher molecular weight chitooligosaccharide system.

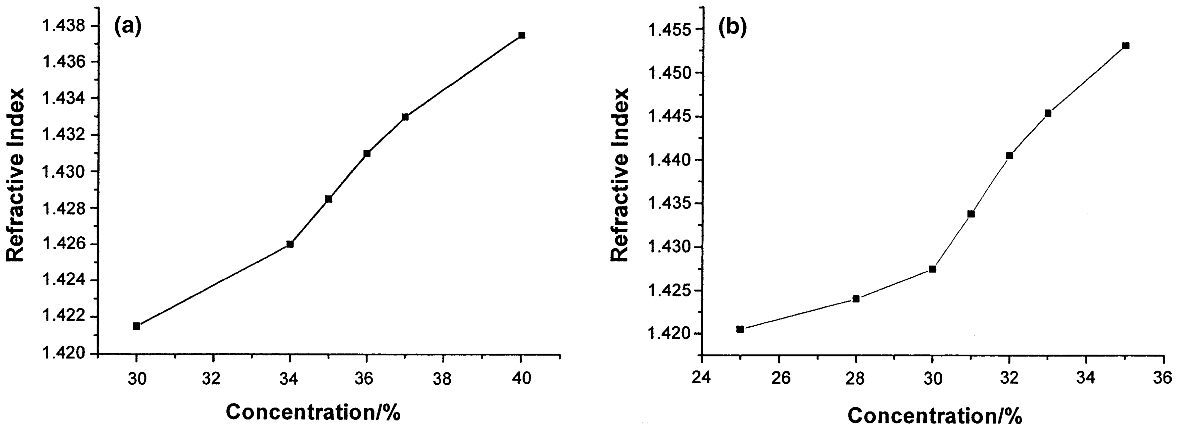

Fig. 5. Measurements of C_1 by refractometry for a COS1/FA solution (a) and a COS2/FA solution (b) (Dong et al., 2001a,b, 2002).

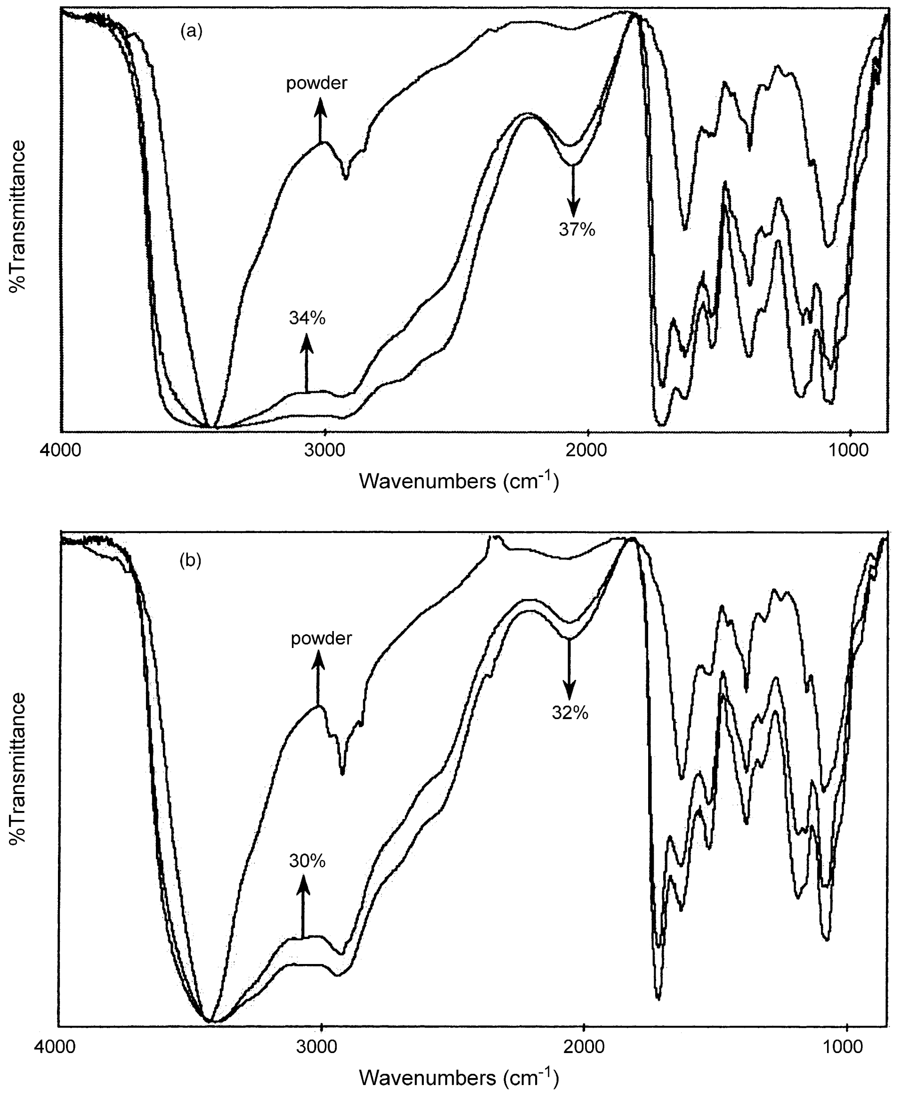

Fig. 6. FTIR spectra of chitooligosaccharide/FA solutions of different concentrations, compared with the raw material powder. (a) COS1; (b) COS2 (Dong et al., 2001a,b, 2002).

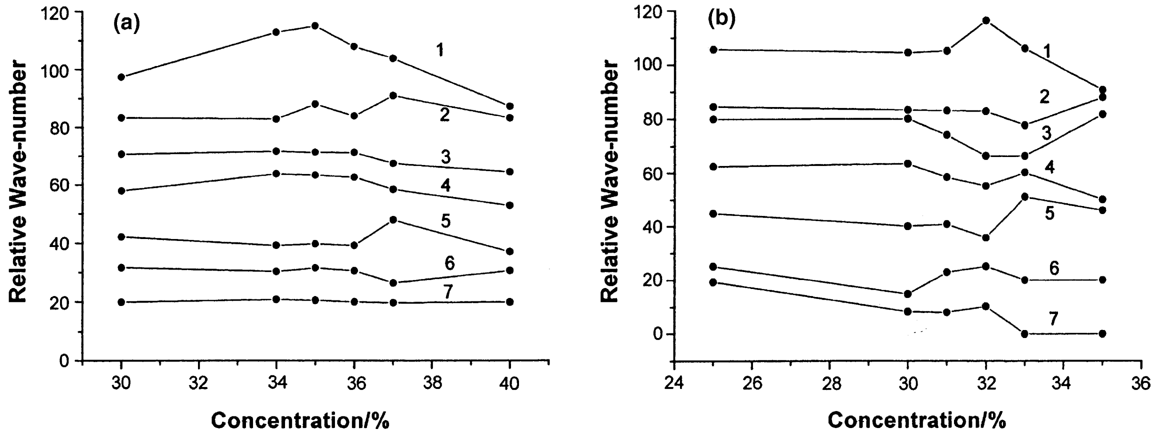

Fig. 7. The shift in wavenumber of characteristic bands of FTIR vs. concentration of chitooligosaccharide. (a) COS1/FA solutions, 1. $-NH_2$, $-OH$ (3390–3418 cm^{-1}), 2. C=O of formic acid (1716–1724 cm^{-1}), 3. amide I (1626–1633 cm^{-1}), 4. amide II (1520–1531 cm^{-1}), 5. 6. C_3-OH (double peaks, 1178 ~ 1189 cm^{-1} and 1148 ~ 1153 cm^{-1}), 7. C_6-OH (1073–1074 cm^{-1}); (b) COS2/FA solutions. 1. $-NH_2$, $-OH$ (3385 ~ 3411 cm^{-1}), 2. C=O of formic acid (1711–1721 cm^{-1}), 3. amide I (1626–1634 cm^{-1}), 4. amide II (1511–1525 cm^{-1}), 5. 6. C_3-OH (double peaks, 1178–1193 cm^{-1} and 1148–1158 cm^{-1}), 7. C_6-OH (1066–1085 cm^{-1}) (Dong et al., 2001a,b, 2002).

Table 3
The calculated data for critical concentration (wt%) of chitooligoaccharides in formic acid

M	L (nm)	$L/2q$	V'_2 (%)	C_1 (%)
501	1.5994	0.03635	136.2	–
805	2.575	0.05852	89.7	91.5
966	3.09	0.07023	77.0	80.5
1000	3.199	0.0727	74.8	78.5
2000	6.398	0.1454	44.2	49.3
2340[a]	7.488	0.1702	39.7	44.7
2500	7.997	0.1817	38.0	43.4
3000	9.596	0.2181	34.0	38.7
4303[a]	13.764	0.3128	27.8	32.1
5000	15.994	0.3635	25.8	30.0
8000	25.59	0.5816	21.3	25.0
1×10^4	31.988	0.727	19.8	23.3
5×10^4	159.938	3.635	15.3	18.2
10×10^4	319.876	7.27	14.9	17.7
20×10^4	639.752	14.54	14.7	17.4
50×10^4	1599.379	36.35	14.5	17.3
70×10^4	2239.13	50.889	14.5	17.3

[a] Values corresponding to measured for COS1 and COS2.

4. Summary of conclusions

Chitooligosaccharides were found to form mesophases at high concentrations. Further, the influence of molecular weight on the boundary concentration between isotropic and biphasic phases, i.e. critical concentration was investigated. COS2 with a molecular weight of 4303 had a critical concentration of 31–32 wt%, which was predicted by Khokhlov and Semonov theory. However, COS1 with a molecular weight of 2340 had a critical concentration of 35–36 wt%, which was somewhat lower than that anticipated by this theory.

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