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Structural study of polysaccharide films by small-angle neutron scattering

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Abstract

A simple procedure to reveal the distinctive features of the polysaccharide polymer structure in bulk based on using heavy water (D_2O) at small-angle neutron scattering (SANS) is proposed. The use of heavy water allowed us to increase the contrast of the internal structure of the samples capable of taking up this solvent and to trace structural changes depending on the degree of swelling in water vapour. As an example of this technique, SANS was used to study the structural characteristics of chitin–cellulose films. The results were suggestive for conclusions about the inner structure of these films and showed that the proposed approach in combination with SANS technique is an efficient and useful tool for structural characterisation of polymer materials in a solid state. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Small-angle neutron scattering; Supermolecular structure; Chitin–cellulose films

1. Introduction

In the last ten years much attention has been paid to investigations of natural polysaccharides both in biochemistry and in polymer physics and chemistry [1]. An increasing interest in such materials is due to the wide range of their applications and invites further investigations of their structure. The availability of structural heterogeneity makes it possible to perform experimental procedures based on scattering of the incident radiation. Among these methods the small-angle neutron scattering technique (SANS) is a convenient and efficient tool for the determination of structural characteristics in a scale from tens up to thousand of angstroms. This is due to a variety of capabilities of deuteration labelling increasing contrast. However, the structural study of these polymers in a solid state presents substantial difficulty.

In Ref. [2] we showed the efficiency of using heavy water (D_2O) for an increase in the contrast of internal structure of chitosan films, that allowed variations in the supermolecular structure of chitosan to be followed in the course of swelling. An analysis of the laws of chitosan film swelling performed within the framework of the fractal modelling, established that the swelling proceeded via the stage of heavy water cluster formation.

In the present work, we employed this procedure based on using D_2O at SANS to characterise the distinctive features of structure of chitin–cellulose films. Chitin and its partially deacethylated product—chitosan—are biodegradable and biocompatible polymers, the sources of which are constantly renewed in nature [3,4]. The modification of chitin films by the addition of cellulose molecules is important for their practical application as sutures in surgery. It has been found that the abnormal dependence of viscosity and energy of activation of viscous flow of chitin–cellulose mixtures is dependent on their composition [5]. Also it has been shown that the films with a small cellulose content (about 5%) have stronger mechanical properties, which can be due to the changes in their supermolecular organisation. Although the properties of these polymers have been extensively studied in recent decades, the data obtained are by no means exhaustive and the design of these polymers for many applications requires the knowledge of the supermolecular structure of chitin and its compounds with other polymers.

2. Experimental

2.1. Materials

The molecular and physical characteristics of the polymer used in this study are given below. Chitin was obtained from the prawn of North Sea, chemical formula is $C_8H_{13}O_5N$, its density is $d = 1.3753$. The molecular weight of chitin, $M_v = 135000$, was calculated according to equation:

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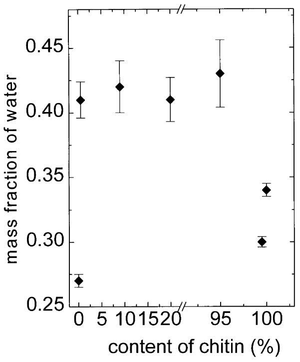

Fig. 1. The dependence of water mass fraction on the chitin content in the chitin–cellulose films swollen relative to heavy water vapour of saturated solution $K_2Cr_2O_7$ in D_2O up to achievement of conditions of equilibrium absorption of heavy water.

$[\eta] = 2.4 \times 10^{-3} M^{0.69}$, where $[\eta] = 8.3$ dl/g was determined in dimethylacetamide +5% LiCl at 298 K. The molecular and physical characteristics of cellulose (sulphite): $C_6H_{10}O_5$, $d = 1.4867$ g/ml. $M_v = 110\,000$ was calculated according to equation: $[\eta] = 5.93 \times 10^{-5} M^{0.94}$, where $[\eta] = 3.0$ dl/g was determined in cadoxen at 293 K.

Mixtures preparation: 3 g of chitin was dissolved in 97 g of dimethylacetamide (DMAA) plus 9% LiCl and 3 g of cellulose was dissolved in 97 g of DMAA and 9% LiCl and then weighted portions of chitin and cellulose solutions were gently mixed by agitation during 2 weeks at 293 K. Mixed films of chitin and cellulose were made by pouring the casting mixtures onto a glass plate and immersing in *n*-BuOH; then films were washed from Cl-ions and dried at 293 K.

Films of chitin–cellulose were allowed to stay in a desiccator relative to saturated solution $K_2Cr_2O_7$ in D_2O at 20°C for equilibrium absorption of heavy water vapour. The approach of equilibrium was monitored by periodic weighing of samples. The equilibrium was established within 3 weeks. The content of water in films after achievement of equilibrium was determined by weighing them. The dependence of a mass fraction of water on the chitin content in the films is presented in Fig. 1, from which it is clear that the small additive of cellulose to chitin films (0.5% of cellulose) results in their sorption ability reduction. The sorption ability grows with the further increase in the content of cellulose. Also, the share of the absorbed water exceeds its value for a pure component. Such abnormal behaviour of absorption of water vapour reflects structural changes occurring with large and with small additives of cellulose. It is possible to assume, that the mixes of chitin with the large contents of cellulose are thermodynamically less compatible than the mixes of chitin with the small additive of cellulose, that results in the formation of a more heterogeneous structure (“microphase separation”). The method of SANS was used for the further analysis of the film structure within the framework of prospective heterogeneity

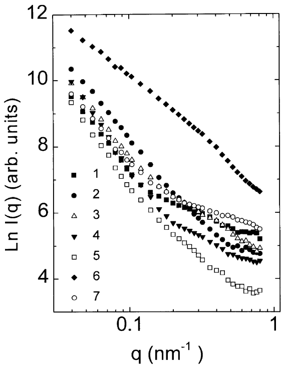

Fig. 2. SANS curves in a double logarithmic scale, as ln *I* versus ln *q*, for the chitin–cellulose films swollen in equilibrium conditions relative to heavy water vapour of saturated solution $K_2Cr_2O_7$ in D_2O: 1–0%, 2–0.5%, 3–9%, 4–20%, 5–95%, 5–95%, 6–99.5%, 7–100% of chitin.

2.2. Methods

The SANS experiments were performed at the small-angle neutron diffractometer “Membrana-2” [6]. The average wavelength of the incident beam was $\lambda = 0.3$ nm, the spectral half-width was $\Delta\lambda/\lambda = 0.35$. The observed scattering curves from chitin–cellulose films under study were compared with simulated curves corrected for the spectral and collimation distortions of the diffractometer, detector efficiency, sample transmission and incoherent background scattering.

3. Results and discussion

SANS techniques were used to study the structural changes of chitosan films in our previous work [2]. The use of heavy water opened the way to increase the contrast of the internal structure of samples under study and to trace structural changes of the chitin–cellulose films depending on the degree of swelling in water vapour. To find out if this can be a source of small-angle scattering in films under consideration, it is necessary to determine the neutron scattering length densities of the film components. The scattering density of heavy water is known: $\rho = 6.34 \times 10^{10}$ cm^{-2}. The scattering length density for other substances is calculated according to the formula:

$$\rho = \left(\sum b_i\right) dN_A/M, \qquad (1)$$

where b_i is the neutron coherent scattering length for *i*th atom (the summarisation is carried out over all atoms included into the monomer), N_A is Avogadro number, M is the molecular mass of the monomer. The calculations have shown that the density of scattering amplitude for chitin and cellulose is practically the same: 1.67×10^{10} cm^{-2} and 1.74×10^{10} cm^{-2}, respectively. Because chitin and cellulose are practically not soluble in water, this provides a sufficient reason to consider the phase of D_2O in chitin–cellulose films as distributed homogeneous

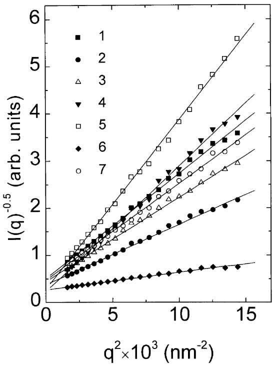

Fig. 3. The initial parts of SANS scattered intensity, as a function of $I^{-0.5}$ against q^2, for the chitin–cellulose films swollen in equilibrium conditions relative to heavy water vapour of saturated solution $K_2Cr_2O_7$ in D_2O: 1–0%, 2–0.5%, 3–9%, 4–20%, 5–95%, 5–95%, 6–99.5%, 7–100% of chitin. The solid lines correspond to the best linear fits to the experimental data points in accordance with Eq. (1) with the parameters listed in Table 1.

particles that have sharp boundaries. This means that the films containing D_2O can be considered as a two-phase system, in which the small-angle scattering is formed by the clusters of heavy water against the background of the chitin–cellulose matrix.

Fig. 2 displays the SANS profiles of chitin–cellulose films of various composition swollen in water vapour. The scattering curves are given in a double logarithmic scale, as a log–log plot of SANS scattered intensity, $I(q)$, plotted versus the scattering vector **q** ($q = 4\pi \sin\theta/\lambda$ is the magnitude of the scattering vector, 2θ is the scattering angle). One can see that two regimes with the different behaviour characteristic of the various length scales can be distinguished. The practically identical scaling dependence $I(q) \backsim q^{-3}$ is observed for low values of $q < 0.12$–$0.15\ \mathrm{nm}^{-1}$, i.e. in the so-called Guinier regime, for all samples, with the exception of films with a very small content of one component. At large values of q, $I(q)$ decays with a power law of the form $I(q) \backsim q^{-1}$, which is typical of locally cylindrical structures. The essential change of the SANS profile is observed for chitin–cellulose films with a chitin or cellulose content of the order 0.5%. In the first case the range of scaling law of q^{-3} is expanded up to $q = 0.3\ \mathrm{nm}^{-1}$. Additionally, the value of exponent for the relation $I(q) \backsim q^{-\alpha}$ is changed from $\alpha \sim 3$ to $\alpha \sim 2$ in the second case. This fact can be explained by the changes in form of scattering heterogeneities as far as such a power law $I(q) \sim q^{-2}$ is characteristic of the swollen branched chains [7] and may be indicative of the scattering on water molecules distributed along the polymer chains of one component in the matrix of another component (an analogue of random walk chain model with the definite cross section size).

Table 1
The structural parameters of chitin–cellulose films on dependence of film composition

Sample	Fraction of chitin	$I(0)$	ξ (nm)
1	0	$(3.8 \pm 0.4) \times 10^4$	21 ± 1
2	0.5	$(5.8 \pm 0.6) \times 10^4$	19 ± 1
3	9	$(4.9 \pm 0.4) \times 10^4$	21 ± 1
4	20	$(19 \pm 1) \times 10^4$	30 ± 2
5	95	$(11.9 \pm 0.8) \times 10^4$	28 ± 2
6	99.5	$(12.7 \pm 0.8) \times 10^4$	12.3 ± 0.3
7	100	$(4.3 \pm 0.3) \times 10^4$	19.3 ± 0.5

Debye and co-workers [8,9] developed an expression for the scattering law for two-phase system in the case of perfectly random form and distribution of phases:

$$I(q) = \frac{I(0)}{(1 + q^2\xi^2)^2}, \tag{2}$$

where ξ is known as the Debye–Bueche correlation length, $I(0)$ is a constant being determined by extrapolation of the coherent scattering intensity to zero angle. The plot of $I^{-0.5}(q)$ against q^2 should yield a straight line where the value of the correlation length, ξ, can be found from the slope/intercept ratio. In our case, in which one phase is a heavy water component distributed in the second phase (chitin–cellulose matrix), ξ, may be taken as a measure of the size of the clusters of D_2O.

Initial parts of the SANS curves from chitin–cellulose films of various compositions swollen in water vapour are given in Fig. 3 in the range of $q < 0.12\ \mathrm{nm}^{-1}$ as function $I^{-0.5}(q^2)$. The solid lines are the linear fit to the experimental data in accordance with Eq. (1) with the results given in Table 1. The dependence of the sizes of heterogeneities (the correlation length ξ) in chitin–cellulose films depending on the ratio of the components is presented in Fig. 4.

SANS investigations of chitin–cellulose films show that their supermolecular structure is rather heterogeneous and is characterised by the average effective size of static heterogeneities of the order 20–30 nm. At the same time the dependence of these values on the film composition has an abnormal form. It is clear that there is a reduction in the size of the heterogeneities, content of cellulose being of the order 0.5%. This cannot be attributed only to the lowered sorption of water in comparison with pure chitin. The ratio of fractions of the absorbed water for these films is 0.88, while the estimation of the change of volume of heterogeneities, proportional to ξ^3, gives 0.25 (i.e. their amount grows approximately 3.5 times in the latter case along with the reduction of the sizes of scattered heterogeneities). It makes clear that the supermolecular structure of the chitin films with the small content of cellulose molecules represents a very diluted polymer system of cellulose chains randomly distributed in the chitin matrix. Such a structure of the polymer film involves the presence of D_2O clusters distributed over the chitin matrix (along the cellulose chains) to a greater degree than in the case of the pure chitin. The form of these heterogeneities is changed and becomes rather extended, which involves the fractal-like law of the asymptotic behaviour of the scattering intensity $I(q) \sim q^{-2}$. The swelling proceeds via the accumulation of water molecules in the chitin–cellulose interphase regions.

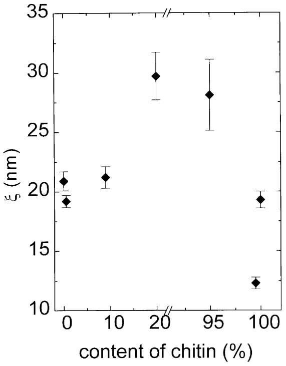

Fig. 4. The dependence of mean size spatial heterogeneities for swollen chitin–cellulose films on content of chitin.

Furthermore, the chitin matrix does not represent a homogeneous medium for water. There are sites (for example, NH_3^+ groups of chitin) which selectively bind water [2]. Hence, the scattering law indicates that heavy water is distributed over the polymer matrix and the form of this distribution has a mass-fractal type. As a result, a more homogeneous absorption of water takes place in contrast to heavy-water cluster formation which is characteristic of pure chitin films or chitin–cellulose films with the larger fraction of cellulose. These results indicate that the changes in mechanical properties of the modified chitin films containing small fraction (~0.5%) of cellulose chains reported in the previous study may be associated with the structural changes shown in this paper.

Another characteristic point is the inverse composition of the films: 0.5% chitin and 99.5% cellulose. A less significant downturn of the sizes of heterogeneities is observed in comparison with pure cellulose. However, it is necessary to take into account the increase in the amount of water absorbed by this film (almost two times), probably favouring structural reorganisations occurring in the system.

From the experimental data we can extract the following information about the changes in the concentration of the scattering centres (heterogeneities). Firstly, this value can be extracted from the value of $I(0)$ which can be written in the form [10]:

$$I_G(0) \sim NV^2 \cdot \langle \Delta\rho^2 \rangle, \tag{3}$$

where V is the volume of heterogeneity. Secondly, N is obtained from the known mass fraction of water in films and the measured sizes of heterogeneities (on the supposition of their globular form). Then, the relative change in concentration of the scattering heterogeneities in the films with the different composition can be calculated in relation to the concentration of heterogeneities in the pure chitin film, N_{chitin}. These relative estimations δN are shown in Table 2. One can see that these values coincide practically for all films, except for the chitin film with 0.5% cellulose (it is a difference in order of 10 times). Such an agreement attests that our approach based on application of Eq. (1) is appropriate. A discrepancy of structural parameters for the chitin films with 0.5% cellulose is well explained by the most pronounced changes in supermolecular structure in swollen films. It is important to mention that such behaviour occurs in the characteristic point of composition of the films (as it follows from the mechanical measurements [5]) and the specific properties of the films in this point result from their structure in which the chitin film is penetrated by polymer chains of cellulose.

4. Conclusions

The results of this study demonstrate that using heavy water in SANS measurements of polymer films is a simple and convenient tool of deuteration labelling increasing the contrast of internal structure that opens the way to stand out of its heterogeneity and to receive additional information about structural regularities observed in such polymer systems. This approach can be particularly advantageous for structural investigations of mixed films, one component of which is hydrophobic and the other component is hydrophilic.

Acknowledgements

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Table 2
The relative estimations of changes in concentration of scattering heterogeneities δN obtained from SANS data and water mass fraction

Fraction of chitin	Mass fraction of water	δN (from water fraction)	δN (from $I(0)$)
0	0.27 ± 0.01	1	1
0.5	0.41 ± 0.01	2.0 ± 0.2	3.0 ± 0.8
9	0.42 ± 0.02	1.6 ± 0.2	1.4 ± 0.4
20	0.41 ± 0.02	0.6 ± 0.1	0.6 ± 0.2
95	0.43 ± 0.03	0.7 ± 0.2	0.5 ± 0.2
99.5	0.30 ± 0.01	5.5 ± 0.6	80 ± 15
100	0.34 ± 0.01	1.5 ± 0.2	1.7 ± 0.4

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