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An ESR and PGSE-NMR evaluation of the molecular accessibility of poly(vinyl alcohol) hydrogels

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Abstract

Electron spin resonance spectroscopy (ESR) and pulsed-gradient-spin-echo nuclear magnetic resonance (PGSE-NMR) measurements on poly(vinyl alcohol) (PVA) hydrogels reveal that nanostructure is not appreciably affected by the number of freezing–thawing cycles.

Keywords: PVA hydrogel; Nanostructure; Supported enzymatic catalysis

1. Introduction

Poly(vinyl alcohol) (PVA) is a material practically insoluble in water at room temperature. After dissolution at 80–90°C, it is known to give exceedingly metastable solution at room temperature, from which highly elastic PVA hydrogels do precipitate after letting the solution stand at −18°C for a few hours.

Upon repeated freezing–melting cycles in the −18–+20°C range, PVA hydrogels are reported to gradually evolve to a material featured by complex macro- and microporosity, in which macropores (micrometer in size) decorate an overall structure that, in the swollen state, is built up with an array of micropores (nanometers in size) which is produced by a network of extensively solvated PVA polymer chains cross-linked by PVA crystalline nanophases [1].

In view of the peculiar synthetic procedure to PVA hydrogels, this material has been very extensively utilized by many authors as support for cells [2–4] and enzymes [5,6] since long for a variety of technical applications, including biocatalysis [2,3,7]. Thus, the cells or biomolecules are suspended or dissolved in the metastable solution at room temperature and subsequently trapped in the PVA hydrogel.

We are currently interested [8,9] in the investigation of the nanostructure and molecular accessibility of gel-type synthetic functional resins under operational conditions (swollen state) investigated on the basis of a combination of

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Table 1
ESR and PGSE-NMR analysis of PVA hydrogels

Entry	Hydrogel	Cycle	D/D_o	τ_o/τ	E_a^a (kcal/mol)
1	H-5	0	0.77	0.96	4.6
		1–5	0.81	0.96	
2	H-10	0	0.63	0.83	4.7
		1–5	0.65	0.83	
3	H-15	0	0.55	0.72	4.8
		1–5	0.54	0.72	
4	H-20	0	0.47	0.65	5.1
		1–5	0.47	0.65	
5	H-25	0	0.40	0.53	nd
		1–5	0.41	0.53	
6	H-30	0	0.34	0.42	nd
		1–5	0.34	0.42	

[a] E_a in bulk water: 4.3 kcal/mol.

conceptually independent analytical techniques, i.e., inverse steric exclusion chromatography (ISEC) [10], electron spin resonance spectroscopy (ESR) of solution of stable radical (TEMPONE), which let us pervade the polymer network [8] and pulsed-gradient-spin-echo nuclear magnetic resonance (PGSE-NMR) of solvent to be able to swell the network itself [9,11]. In this connection, PVA hydrogels have been so far investigated by many different techniques as X-ray diffraction [1], infrared spectroscopy [12], scanning electron and light optical microscopy [1,13–15], dynamic viscoelasticity [15], tension text [1] and swelling ability measurements [15,16], and we became interested to evaluate with our approach their nanomorphology and molecular accessibility. In this letter, we like to present our ESR and PGSE-NMR results.

2. Experimental

Six PVA (Aldrich, Mw = 50,000, 99 + % hydrolyzed) hydrogels were prepared with PVA percentages (w/w) ranging from 5 to 30% (H-5 ÷ H-30) and each one was worked out with five −18°C (12 h) + 20°C (6 h) freezing–melting cycles upon starting from a +90°C dissolution step. The maximum number of cycles utilized (five) has been suggested from Ref. [16] and it is considered sufficient for producing a material of satisfactory robustness and rigidity. The essential spectroscopic data are collected in Table 1. The symbols D and τ refer to the water diffusion coefficient and the TEMPONE rotational correlation time, respectively, the subscript 'o' refers to the values in bulk water. The activation energies of the diffusion process E_a have been calculated from Arrhenius plot in the temperature range 5–35°C.

3. Results and discussion

It is seen that the translational mobility of water and the rotational mobility of the spinophore are appreciably affected by the presence of dissolved PVA in the metastable solution obtained at room temperature (cycle 0) and that this effect turns out to be gradually more marked with the increase of PVA percentage. The activation energy of the diffusion process does not change significantly with the PVA concentration, thus showing that the role of the polymer chain is that of inert obstacles to the diffusion of water molecules. Normalized diffusion coefficient as a function of PVA weight fraction (ω) for the PVA solution is reported in Fig. 1. Up to approximately $\omega = 0.5$, the free-

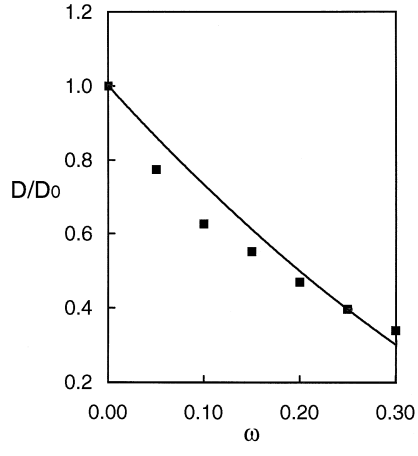

Fig. 1. Water self-diffusion coefficient data as a function of PVA weight fraction and comparison with the prediction of the free volume theory (Eq. (1)).

volume theory gives the concentration dependence of D as [17]:

$$\frac{D}{D_o} = \exp\frac{A\omega}{1-\omega} \quad (1)$$

with A being a system-dependent parameter. The fit of Eq. (1) to the experimental data is shown as a solid curve. In agreement with the values obtained for numerous polymer–solvent systems, an A value close to -1 ($A = -1.2$) is observed [17]. Experimental τ values vs. ω for the PVA solution are reported in Fig. 2. In a polymer solution, the viscosity η can be expressed as [18]:

$$\eta = \eta_o \exp(\nu\phi) \quad (2)$$

where ϕ is the volume fraction of polymer chains, η_o is the viscosity of the bulk solvent, and ν is a convenient parameter. ϕ can be related to ω by means of the expression:

$$\phi = \frac{1}{1 + \left(\frac{1}{\omega} - 1\right)\frac{d_p}{d_w}} \quad (3)$$

where d_p and d_w are the density of dry polymer and water, respectively. From the combination of the Stokes–Einstein equation with Eqs. (2) and (3), we obtain:

$$\tau = \left(\frac{4\pi a^3}{3kT}\right)\eta = \left(\frac{4\pi a^3}{3kT}\right)\eta_o \exp\left(\frac{\nu}{1 + \left(\frac{1}{\omega} - 1\right)\frac{d_p}{d_w}}\right) \quad (4)$$

where a is the hydrodynamic radius of the rotating particle. The fit of Eq. (4) (with $d_p = 1.35$ and $d_w = 1.00$ g/cm^3) to the experimental data is shown as a solid line in Fig. 2.

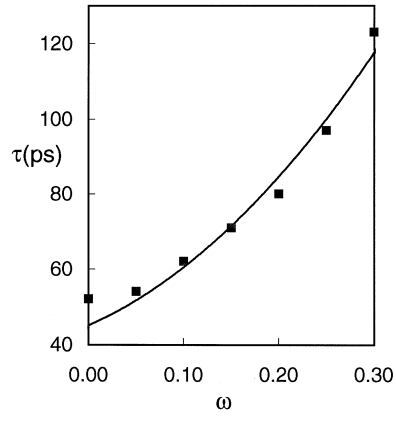

Fig. 2. TEMPONE rotational correlation time data as a function of PVA weight fraction and comparison with the prediction of Eq. (4).

Remarkably, no appreciable further change of D/D_o and τ_o/τ is observed as the consequence of the following five thermal cycles. This means that the mobilities of water and of TEMPONE inside the hydrogels formed in cycle 1 are equal to those observed in the metastable starting solution and that apparently, they are not influenced by hydrogel aging. Moreover, while D appears to be already appreciably reduced (compared with bulk water) in the metastable starting solution, τ is not so drastically affected for 5% PVA, but the reduction of rotational mobility does indeed become gradually apparent with the increase of PVA percentage.

In view of the evident consideration that in solution referring to cycle 0 no solid polymer network is apparently yet formed, we should conclude that the moderate reduction of D and τ is due to a pure macromolecular solute effect well-known in the literature [17]. But if so, it has to be remarked that the occurrence of the physical cross-linking effect which produces the hydrogel state suitable to cause the trapping of cells and enzymes does not affect, in fact, the translational and rotational dynamics of species pervading the just-formed polymer network.

This observation was not at all predictable at the start of this work and it appears to be of some practical interest. In fact, the major task of any supporting approach in biocatalysis is to ‘solidify’ (vide infra) cells and enzymes to make them comfortably handy in their employment in

bioreactor. The implicit two requisites are (i) lack of any biological and chemical damage to the bioactive species and (ii) the lowest possible effect on the reagents and products molecular mobility [19].

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