Indirect Measurement of the Cooperative Hydrogen Bonding of Polymers Using NMR Quadrupole Relaxation and PFG Methods

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Summary: A general method of measurement of polymer hydrogen bond (HB) cooperativity using a low-molecular weight model ligand named marker and two independent methods of ^{2}H NMR is presented. As marker, a deutero-compound chemically similar to the functional groups of one of the polymers is used, e.g. pyridine-d_5 in the investigated interaction of *poly*(4-vinylpyridine) with *poly*(4-vinylphenol) or acetic acid-d_4 in the interaction of polyacrylic acid with *poly*(4-vinylphenol) reported here. The method is based on the fact that a substantial fraction of the marker, originally bound to the groups of one of the polymers, is liberated by the cooperative interaction between the two polymers. For the establishment of the fraction of the bound marker before and after mixing the polymers, ^{2}H NMR quadrupolar relaxation or ^{2}H PFG NMR diffusion measurement can be used with comparable precision. In both these methods, the results must be normalized to a standard viscosity using the relaxation or diffusion of an added inert compound such as $CDCl_3$.

Keywords: cooperative bonding; hydrogen bond; NMR; *poly*(4-vinylphenol); *poly*(4-vinylpyridine)

Introduction

The cooperativity of non-covalent electrostatic, hydrogen bond (HB) or hydrophobic interactions is crucial in the formation of natural or synthetic macromolecular complexes.[1–4] Many individual examples of such cooperative binding were recognized and studied, in particular in the case of HB,[5–10] but systematic work has just started in this field. Several principles governing cooperativity were discovered in the study of electrostatic interactions between oppositely charged macromolecules.[10–17] One of them, dubbed the proximity effect, was found to be operative in the HB cooperativity as well.[18–20] However, systematic verification of this principle as well as the discovery of others needs quantitative studies of the binding degree between the macromolecules in question. This meets serious obstacles in the case of HB.

It is certainly true that a sufficiently strong HB usually changes both frequency and intensity of some infrared bands corresponding to the stretching vibration of the bond bearing the hydrogen. It also changes the position of NMR signals of at least the protons involved in the bond. However, the quantification of these changes is not as easy as it could seem to be. The main difficulty lies in the fact that the polymers involved in HB interactions usually are soluble only in solvents forming HB with them so that no (or almost no) spectroscopic change is brought about by the binding between the polymers.

This difficulty could be seemingly avoided by turning from individual atomic groups of the polymers to their molecular shape or bulkiness, e.g. by measuring their

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hydrodynamic radius using DLS or PFG NMR. Ironically, the effective size of the macromolecular complexes often decreases with increased degree of their inner binding, obscuring thus the quantitative information we are looking for.

There is an indirect way of circumventing these difficulties, which we already suggested for a special case[18,19] but found to be rather generally applicable in our newer studies. The principle, explained in detail in this article, is the following. The solution of one of the macromolecules in an appropriate solvent is mixed with a low-molecular-weight analogue of the other macromolecule (let us call it marker), in a quantity sufficient to block most (say 98%) of its binding sites. It is preferable to have the marker in a deuterated form. The marker molecules bound to the polymer have a diffusion coefficient (measured by PFG NMR) very different from those in the excess free molecules and, usually, there is also a large difference in the quadrupolar relaxation rate (measured by ^{2}H NMR) of the corresponding deuterons so that, even under conditions of a very fast exchange, the binding degree of the marker can be established by two independent methods. After adding the complementing macromolecule, some of the bound marker molecules are liberated due to the cooperative interaction between the macromolecules. The degree of the freed molecules can be measured by the same method as above and the binding degree between the macromolecules can be calculated.

Materials and Methods

Materials

Poly(4-vinylpyridine) ($M_n = 5100$) and polyacrylic acid ($M_n = 432, 720, 1070, 1300, 1500$ and 1800) were purchased from Polymer Source, Canada. Acetic acid-d_4 (AcD), and chloroform-d ($CDCl_3$) were products of Eurorad. These solvents were dried with molecular sieve before use. The measured solutions were transferred into a NMR tube, degassed and sealed.

NMR Relaxation and PFG Measurements

All NMR measurements were done with an upgraded Bruker Avance DPX 300 spectrometer (^{1}H frequency at 300.13 MHz) equipped with a Bruker PFG unit BGU2 and inverse-detection water-cooled PFG probe with a gradient range up to 1500 G/cm. ^{2}H longitudinal relaxation measurements were done at 46.071 MHz with a broadband probe using an inverse-recovery pulse sequence d_1-π_x-d_2-$\pi/2_{x,-x}$-FID, with $d_1 = 8$ s and d_2 incremented in 16 steps from 0.1 to 1.6 s. 2048 points were collected in each of the 48 scans. The measurements were done without lock, usually at night to minimize outer magnetic influences. Each measurement was repeated at least five times and the arithmetic mean of the resulting T_1 values (scattered less than +/− 2%rel.) was taken. PFG diffusion experiments of the deutero-compounds were measured at the ^{2}H resonance, using the decoupler channel of a z-gradient inverse-detection probe. The pulse sequences were both those of pulsed gradient spin-echo (PGSE), i.e. d_1-$\pi/2_x$-gr-d_2-π_y-d_2-gr-FID and pulsed gradient stimulated echo (PGSTE),[21,22] i.e. d_1-$\pi/2_x$-gr-$\pi/2_x$ -d_2-d_2-$\pi/2_x$-gr-FID, with $d_1 = 8$ s and $d_2 = 0.2$ s. The gradient of a 2 ms pulse was incremented from 10 to 50 G/cm in 16 steps. 2048 points were collected in each of the 64 scans. Again, the measurements were done without lock and at least 5 times repeated (scatter +/− 2%rel.). Diffusion experiments with the polymer PVP were done on ^{1}H resonance with a deuterium lock using PGSTE sequence on a special water-cooled inverse-detection z-gradient probe, $d_2 = 0.02$ s and the 1 ms gradient pulse incremented from 5 to 500 G/cm in 16 steps. The measurements were repeated 3 times with the scatter $< 3\%$.

Results and Discussion

As indicated in the Introduction, the indirect method of measuring the degree of binding α of polymeric HB-donor (HBD) groups to complementary HB-acceptor (HBA) groups or vice versa is based on

measuring the fraction of a low-molecular-weight ligand (called marker in this paper) liberated from its binding to one of the polymers due to its substitution by the groups of the other polymer. The choice of the marker depends on its availability but, for physical clarity of the results, it should be chemically as similar as possible to the polymer groups by which it is substituted. In such case, the quantity of its liberation by the analogous polymer substantially exceeding that given by statistics is a measure of *net cooperation* of bonds between the polymers.

Theoretically, the competition between the marker in large excess and the analogous groups of the polymer could disrupt the bonding pattern between the polymers to such degree that a weak cooperativity could not be observed in this way. Such case is not very probable, however, because a substantial part of the driving force of cooperative binding is the entropy gain due to the liberation of low-molecular-weight ligands. Such ligands are always present, at least as solvent molecules (it is virtually impossible to dissolve hydrogen-bonding polymers in a solvent, which would not interact with them in some way). Hence, if cooperative binding does occur in usual solution of the polymers, it will very probably occur in the presence of the marker, too.

It is preferable to have it in a fully deuterated form for two main reasons: (1) its signals do not interfere with the ^{1}H NMR spectra of the interacting polymers and (2) longitudinal ^{2}H NMR relaxation is mostly quadrupolar, its rate being dependent exclusively on the local dynamics and usually sharply differing between the free and bound states. In a recent study[18,19] of cooperative binding of *poly*(4-vinylpyridine) (PVP) to *poly*(4-vinylphenol) (PVF), pyridine-d_5 (PD) was added as marker to the solution of PVF. Here, we present a complementary example: in the study of cooperative binding of polyacrylic acid (PAA) to PVP, acetic acid-d_4 (AC) is added as marker to the solution of PVP.

After having explored various media containing THF-d_8, DMSO-d_6, dioxane-d_8, and chloroform-d (CD) in various proportions, in which either PVP was not properly soluble or some of the components interfered as a too strong HB acceptor, we found the solution of 2.6 mol/L of AC in CD as a viable medium for our study (so AC serves both as marker and as co-solvent; at the same time, CD serves as co-solvent and inert for viscosity correction, see below). In the following, we first report on the extraction of necessary diffusion and relaxation parameters of the marker AC under its interaction with PVP and, subsequently, show an example of the study of cooperative binding between PVP and PAA.

PFG Study of AC Diffusion in the System with PVP

We applied the PGSE pulse sequence[21] in ^{2}H NMR for the measure of the diffusion coefficient of AC. The first prerequisite of the applicability of our method is that the exchange between the bound and free AC is much faster than the signal decay due to diffusion.[18] The sure criterion of it is the mono-exponential dependence of the signal intensity on the square of the field gradient applied. Figure 1 shows in a semi-logarithmic graph that this requirement is fulfilled in various systems. Being it so, the molar fraction φ of AC bound by HB to PVP can be obtained from the formula

$$\varphi = \frac{D_F^N - D^N}{D_F^N - D_B^N} \tag{1}$$

where the individual quantities are the diffusion coefficients of AC in the current system (D^N), that of the free AC (D_F^N) and that of AC bound to PVP (D_B^N). The subscript N means normalization, i.e. correction[31] for the influence of viscosity of the system. In our case, D_F^N is by definition the diffusion coefficient of AC in its 2.6 mol/L solution in CD at 295 K; its actual value is 2.001×10^{-9} m^{2}s^{-1}. Let D_C^S be the diffusion coefficient of CD in the same solution. Then

$$D^N = DD_C^S/D_C \tag{2}$$

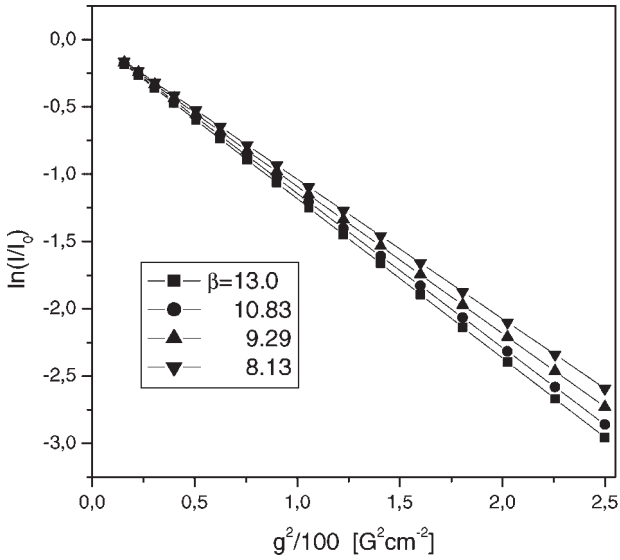

Figure 1.
Logarithmic dependences of relative signal intensity of ^{2}H NMR signals of acetic acid-d_4 (AC) on the square of field gradient in PGSE of PVP-AC systems with the indicated AC/VP molar ratios at 295 K.

where D and D_C are the diffusion coefficients of the given species (AC or PVP) and CD, respectively, measured in the actual system. If diffusion of PVP is measured by ^{1}H PFG NMR, we get D_B of the given system (thanks to quite narrow M_w distribution, we get a single quantity rather than a distribution).

The results of PFG measurements in AC-PVP systems are given in Table 1. For further use but also as a check of the regularity of measurement, the calculated effective equilibrium constant K is added. It is defined as

$$K = \frac{[\mathrm{AC}\cdot\mathrm{VP}]}{[\mathrm{AC}][\mathrm{VP}]} = \frac{\varphi}{[\mathrm{VP}]_0(1-\varphi\beta)(1-\varphi)} \quad (3)$$

where $[\mathrm{AC}\cdot\mathrm{VP}]$, $[\mathrm{AC}]$, and $[\mathrm{VP}]$ are the actual concentrations of hydrogen-bound groups, free acetic acid and vinylpyridine groups, respectively. As in other papers, $\beta = [\mathrm{AC}]_0/[\mathrm{VP}]_0$.

As it can be seen, the value of K varies only 1.6%rel. around 18.9 L·mol^{-1}, which gives assurance of the reliability of measurement. Calculated from this value (or from φ), the fraction α of the VP groups blocked by the marker is between 0.978 and 0.977 for all examined ratios β, i.e. high enough for our purposes.

Study of ^{2}H NMR Quadrupolar Relaxation of AC in the System with PVP

Like in the previous case, the independent relaxation method of measuring φ presupposes that the exchange between free and bound AC is much faster than the actual relaxation rate of the deuterons in the system. Figure 2 shows in a semi-logarithmic that

Table 1.
Diffusion coefficients ($m^2s^{-1} \times 10^9$) of AC (D) and PVP (D_B), the calculated fraction φ of bound AC and the effective equilibrium constant K in various solutions of AC and PVP in CD at 295 K (concentration of PVP given in equivalents of VP groups $[VP]_0$).

$[VP]_0$	β	D_B	D_B^N	D	D^N	φ	K
0.20	13.00	0.1833	0.2098	1.630	1.866	0.0753	19.2
0.24	10.83	0.1802	0.2102	1.576	1.839	0.0903	18.8
0.28	9.29	0.1742	0.2099	1.504	1.812	0.1053	19.1
0.32	8.13	0.1682	0.2103	1.429	1.786	0.1202	18.7
0.36	7.22	0.1614	0.2097	1.354	1.759	0.1353	18.8
0.40	6.50	0.1553	0.2098	1.282	1.732	0.1502	18.6

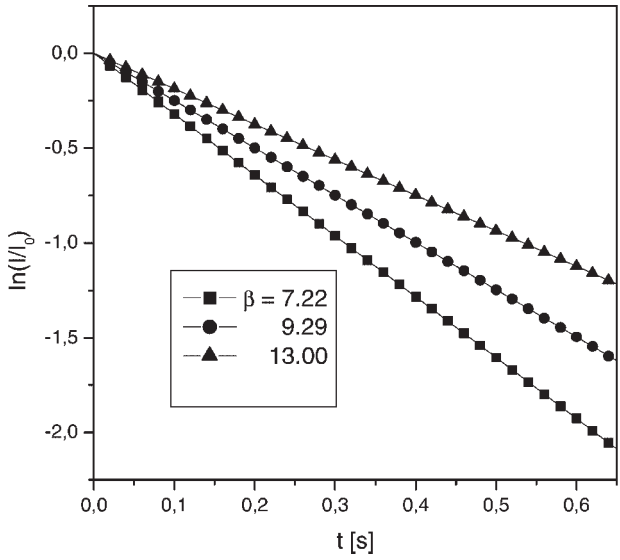

Figure 2.
Logarithmic dependences of relative signal intensity of ^{2}H NMR signals of acetic acid-d_4 (AC) on time in their quadrupolar relaxation in PVP-AC systems with the indicated AC/VP molar ratios at 295 K.

the intensity decay of the NMR signals is strictly mono-exponential, i.e. this requirement is fulfilled, too. Under these conditions, the bound fraction φ of AC can be independently obtained from

$$\varphi = \frac{R_1^{\mathrm{N}} - R_{1\mathrm{F}}^{\mathrm{N}}}{R_{1\mathrm{B}}^{\mathrm{N}} - R_{1\mathrm{F}}^{\mathrm{N}}} \tag{4}$$

where R_1, $R_{1\mathrm{B}}$ and $R_{1\mathrm{F}}$ are the longitudinal relaxation rates actually measured and in the bound and free state, respectively. Again, the superscript N means normalization to the same viscosity of the standard solution of 2.6 mol/L AC in CD, using in all cases the formula[18]

$$R_1^{\mathrm{N}} = R_1 R_{1\mathrm{C}}^{\mathrm{S}} / R_{1\mathrm{C}} \tag{5}$$

where $R_{1\mathrm{C}}$ and $R_{1\mathrm{C}}^{\mathrm{S}}$ are the relaxation rates of CD in the actual and the standard solution, respectively.

In Equation (4), R_1 is actually measured in the given system, $R_{1\mathrm{F}}$ corresponds to the standard solution and was established to be $R_{1\mathrm{F}}^N = 0.661\ \mathrm{s}^{-1}$. However, $R_{1\mathrm{B}}^N$ is not accessible so easily. The extrapolation method devised earlier[18] does not give reliable results in the present case as the range of β is limited by PVP solubility. Relinquishing partially the independence of both methods, we can utilize the values of φ obtained by diffusion measurements to get $R_{1\mathrm{B}}^N$. Table 2 shows the results for the same systems as above. The values of $R_{1\mathrm{B}}^N$ vary less than 1% around 14.309 s^{-1} giving us thus the assurance that this is the right value for the present systems. The use of φ from PFG measurements makes the relaxation method somewhat less independent than desirable. Nonetheless, it is still valuable as it is somewhat more sensitive; it can serve as a check of the PFG method as well.

Table 2.
Longitudinal relaxation rates R_1 (s^{-1}) of AC deuterons in AC-PVP solutions in CD and the calculated values of $R_{1\mathrm{B}}^N$.

$[\mathrm{VP}]_0$	β	φ	R_1	R_1^N	$R_{1\mathrm{B}}^N$
0.20	13.00	0.075	1.872	1.685	14.314
0.24	10.83	0.090	2.172	1.890	14.297
0.28	9.29	0.105	2.494	2.095	14.288
0.32	8.13	0.120	2.838	2.299	14.311
0.36	7.22	0.135	3.210	2.504	14.323
0.40	6.50	0.150	3.612	2.709	14.314

Table 3.
Marker relaxation rates R_1, R_1^N (s^{-1}) and diffusion coefficients D, D^N ($m^2s^{-1} \times 10^9$), calculated binding fractions φ of the marker and α of the PAA groups and the resulting cooperativity coefficient ζ for the given polymerization degrees P_n of PAA.

P_n	R_1	R_1^N	φ	D	D^N	φ	α	ζ
5.99	2.632	2.289	0.120	1.483	1.779	0.124	0.183	2.62
10.01	2.791	2.326	0.122	1.488	1.786	0.120	0.190	2.73
14.85	2.856	2.285	0.119	1.496	1.795	0.115	0.214	3.11
18.04	2.754	2.203	0.113	1.439	1.800	0.112	0.244	3.64
20.82	2.669	2.135	0.108	1.443	1.804	0.110	0.268	4.12
24.98	2.635	2.108	0.106	1.447	1.809	0.107	0.281	4.40

The Study of HB Cooperativity in the PVP-PAA System

In the following, we present one series of measurements as an illustration of the method. In this series, $[VP]_0 = 0.40$ eq/L and $[AC]_0 = 2.6$ mol/L as above, i.e. $\beta = 6.5$. The concentration of PAA was always $[AA]_0 = 0.40$ eq/L, i.e. the ratio of AA and VP groups was 1.0. The binding fraction φ of the marker was measured in each case both by the diffusion and relaxation methods. Naming φ_0 the fraction of the marker bound in the system without added PAA (0.1502 in the present case), the fraction α of binding of the AA to VP groups can be obtained from the simple relation

$$\alpha = (\varphi_0 - \varphi)[AC]_0/[AA]_0. \quad (6)$$

Defining the cooperativity coefficient as the ratio of the effective equilibrium constants of binding of PAA groups (K_{AA}) and marker groups (K_{AC}), it easy to show that

$$\zeta = \frac{K_{AA}}{K_{AC}} = \frac{\alpha(1-\varphi)}{\varphi(1-\alpha)}. \quad (7)$$

Table 3 shows the results obtained in this series.

Although the obtained cooperativity coefficients ζ are lower than those, which

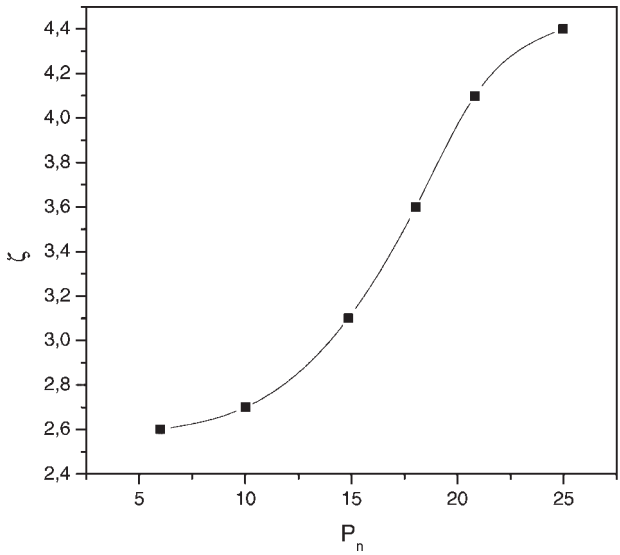

Figure 3.
Dependence of the cooperativity coefficient ζ of PAA groups in their hydrogen bond interaction with PVP on P_n (acetic acid-d_4/chloroform-d, 295 K).

could be calculated for the interaction between PVP and PVF[32], they are still quite substantial. This could be expected from the already studied interaction of PVP with bivalent HB donors[20] crudely modeling the dimer of acrylic acid. Moreover, as shown in Figure 3, the dependence of ζ on P_n appears to have slightly sigmoidal shape indicating[32] that the cooperativity is of a higher order, i.e. does not result from a mere sum of ΔG of individual hydrogen bonds. The reason for this is clearly the proximity effect[20] of neighboring hydrogen bonds.

Conclusions

We have presented two complementary examples of the method quantitatively evaluating the cooperativity of hydrogen bond interaction between two complementary polymers, one of them a HB polymer donor (*poly*(4-vinylphenol) or polyacrylic acid), the other a HB acceptor (*poly*(4-vinylpyridine)). We demonstrated that meaningful results can be obtained with chemically quite different markers as pyridine-d_5 (as crude model of PVP groups) and acetic acid-d_4 (as crude analogue of PAA groups). The basic principle of the method is the measurement of the fraction of the marker bound to one of the polymers before and after adding the second polymer. It evidently can be fulfilled by the two demonstrated ^{2}H NMR methods, namely quadrupolar relaxation of the marker's deuterons and PFG measurement of self-diffusion of the marker molecules, provided that the precision in both cases is high (the scatter of results being 2% rel. or less) and the results are normalized to a standard viscosity using relaxation or diffusion of an added inert compound such as $CDCl_3$.

If the marker is a close low-molecular-weight model of the HB donor or acceptor groups of one of the polymers then the quantity of its liberation by the analogous polymer substantially exceeding that given by statistics is a measure of *net cooperation* of bonds between the polymers.

Although further studies have to be done for its full establishment in other, more difficult fields of use, the presented method can already be considered to be general and, at the same time, probably unique for quantitative evaluation of the type of hydrogen bond cooperativity.

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[1] J.-M. Lehn, *Supramolecular Chemistry*, VCH Publishers, Weinheim, Germany, **1995**.
[2] E. Tsuchida, *Macromolecular Complexes, Dynamic Interactions and Electronic Processes*, VCH Publishers, New York **1991**.
[3] J. Mich, Ed., *Modular Chemistry*, Proceedings, NATO ASI Series, Series C, Vol. 499; Kluwer Academic Publishers, **1997**.
[4] A. Weber, Ed., *Structure and Dynamics of Weakly Bound Molecular Complexes*, Kluwer Academic Publishers, **1987**.
[5] G. C. Pimentel, A. L. McClellan, *The Hydrogen Bond*, W.H. Freeman and Co., San Francisco and New York **1960**.
[6] G. R. Desiraju, T. Steiner, *The Weak Hydrogen Bond*, Oxford University Press, Oxford **1999**.
[7] R. Otero, M. Schock, L. M. Molina, E. Laegsgaard, I. Stensgaard, B. Hammer, F. Besenbacher, Angewand. Chemie. Int. Ed. **2005**, *44*, 2270.
[8] P. M. Tolstoy, P. Schah-Mohammedi, S. N. Smirnov, N. S. Golubev, G. S. Denisov, H. H. Limbach, *J. Am. Chem. Soc.* **2004**, *126*, 5621.
[9] M. Arno, L. R. Domingo, *Org. & Biomol. Chem.* **2003**, *1*, 637.
[10] A. G. Fraile, D. G. Morris, A. G. Martinez, S. D. Cerero, K. W. Muir, K. S. Ryder, E. T. Vilar, *Org. & Biomol. Chem.* **2003** *1*, 700.
[11] J. Kříž D. Kurková, J. Dybal, D. Oupický, *J. Phys. Chem. A* **2000** *104*, 10972.
[12] J. Kříž, H. Dautzenberg, *J. Phys. Chem. A* **2001** *105*, 3846.
[13] J. Kříž, J. Dybal, H. Dautzenberg, *J. Phys. Chem. A* **2001** *105*, 7486.
[14] J. Kříž, J. Dybal, D. Kurková, *J. Phys. Chem. B* **2002** *106*, 2175.
[15] J. Kříž, J. Dybal, D. Kurková, *J. Phys. Chem. A* **2002** *106*, 7971–7981.
[16] J. Kříž, H. Dautzenberg, J. Dybal, D. Kurková, *Langmuir* **2002** *18*, 9594.

[17] J. Kříž, J. Dybal, D. Kurková, *J. Phys. Chem. B* **2003** *107*, 12165.
[18] J. Kříž, J. Dybal, *J. Phys. Chem. B* **2005** *109*, 13436.
[19] J. Kříž, J. Dybal, J. Brus, *J. Phys. Chem. B* **2006** *110*, 18338.
[20] J. Kříž, J. Dybal, *J. Phys. Chem. B* **2007**, *111*, 6118–6126.
[21] E. O. Stejskal, J. E. Tanner, *J. Chem. Phys.* **1965**, *42*, 288.
[22] J. E. Tanner, *J. Chem. Phys.* **1970**, *52*, 2523.

