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# A STUDY OF DEXTRAN HYDRATION IN DILUTE, AQUEOUS SOLUTION BY THE PROTON MAGNETIC RELAXATION METHOD

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## ABSTRACT

The times of proton magnetic relaxation in dilute (<1%), aqueous solutions of dextrans, having a molecular weight range of $17 \times 10^3$–$500 \times 10^3$, are highly sensitive to the temperature–time prehistory of the samples investigated. Reliable results have been obtained only after preliminary heating of the solutions at 100° for 30 min. On the basis of the model of "two states of water in a solution", the dependence of the degree of hydration of a dextran on its molecular weight has been obtained. In the molecular weight range $17 \times 10^3$–$110 \times 10^3$, only a fraction of the D-glucose residues are hydrated, the degree of hydration increasing with the molecular weight. The data obtained are considered to be a consequence of intersegmentary interaction in a dextran macromolecule.

## INTRODUCTION

The method of proton magnetic relaxation, which is being widely used to study the hydration of biopolymers in aqueous solution, is based on the fact that the relaxation times of the protons of water ($T_1$ and $T_2$) decrease with increasing polymer concentration, *i.e.*, as the degree of water-structuring grows.

To a first approximation, it is assumed that, in a polymer solution, water molecules, on the average, exist in two states: the "free" state ($f$) and the "bound" state ($b$), each of which has a characteristic relaxation time, $T_{if}$ and $T_{ib}$ ($i = 1,2$), respectively, with $T_{ib} \ll T_{if}$. Further, on the assumption that water is bound by a polymer in a stoichiometric ratio ($W$), and using the theory of relaxation in multiphase systems[2], the relaxation times of the system as a whole can be expressed in terms of the relaxation times of the $f$- and $b$-states. If the polymer concentrations ($c$) are sufficiently low, these relations take the form

$$1/T_i = 1/T_{if} + Wc/T_{ib}, \qquad i = 1,2. \tag{1}$$

Thus, in accordance with the concepts outlined above, the experimental dependence $1/T_i(c)$ should be linear. From the angular coefficients of these dependences, $K_i = W/T_{ib}$ ($i = 1,2$), the values of $W$ and $T_{ib}$ can be determined by using the well-known Solomon equations[3] (*2*)–(*4*):

$$1/T_{1b} = \{\tau_{cb}/(1+\omega^2\tau_{cb}^2)+2\tau_{cb}/(1+4\omega^2\tau_{cb}^2)\}\cdot 3\gamma^4\hbar^2/10r^6 \quad (2)$$

$$1/T_{2b} = \{3\tau_{cb}+5\tau_{cb}/(1+\omega^2\tau_{cb}^2)+2\tau_{cb}/(1+4\omega^2\tau_{cb}^2)\}\cdot 3\gamma^4\hbar^2/20r^4 \quad (3)$$

$$\tau_{cb} = \{6K-37+(72K^2-300K+889)^{0.5}\}^{0.5}/2\omega, \quad (4)$$

where $\gamma$ is the gyromagnetic ratio, $\hbar$ the Planck constant, $r$ the distance between the protons in a water molecule, $\omega$ the resonance frequency, $\tau_{cb}$ the correlation time of the rotational motion of water in the $b$-state, and $K = K_2/0.5K_1$.

An essential shortcoming of the above concepts is the identification of the hydration shell as a monomolecular layer of water on the surface of a polymer chain. In reality, the hydration shell is thicker than the size of a molecule of water[4], and the degree of "boundness" of hydrated water decreases, and its mobility increases, with the distance from the polymer chain. This effect (the multiplicity of states of "bound" water) can be taken into account by introducing a spectrum of relaxation times characteristic of the "bound" water as a whole[5,6]. Analysis, however, shows[5] that, at a sufficient dilution, the "model of multiple states" is formally identical with the "model of two states". In this case, both these models will be physically adequate if one considers relaxation times $T_{ib}$ ($i = 1,2$) in the model of two states as averages over some distribution. On this basis, one may consider that the use of the above-mentioned relationships to describe polymer hydration in a sufficiently dilute solution is justified.

In the present communication, the results of a study into the hydration of dextrans of various molecular weights are given. In addition, some data on dextran aggregation in concentrated solutions and on the stability of the dextran aggregates being diluted are described.

## RESULTS AND DISCUSSION

### *Dextran aggregation*

The values of relaxation times of water protons in dilute solutions of dextran are highly sensitive to the temperature–time prehistory of the samples investigated. Thus, for example, the results of measurements will be low if the stock solutions of dextran are allowed to stand for about two weeks at 4° and only then diluted to the working concentrations at room temperature. It is characteristic that these low values remain unchanged during 15 or 20 days at temperatures of 4–25°. If, however, the diluted solution is heated at 100° for 30 min, the relaxation times increase noticeably up to their equilibrium values. A repeated heat treatment and subsequent prolonged storage at 4° did not influence noticeably the values of relaxation times.

The above-mentioned peculiarities of dextran dilution are typical of all the dextran samples studied, with the exception of sample *D5*. These peculiarities indicate that, in sufficiently concentrated solutions, dextran is associated. The aggregates of dextran molecules are highly stable to dilution at comparatively low temperatures. At temperatures of the order of 100°, these aggregates rapidly decompose into individual molecules.

According to published data[7], dextran aggregation is explicitly observed only for samples having a rather low-molecular weight ($\sim 10^4$). Concentrated solutions of dextrans of low molecular weight grow perceptibly turbid with time. The original state of the solutions is restored after brief heating at 100°.

Aggregation of dextrans having a higher molecular weight in solution is not accompanied by visual effects. This seems to be the reason why so little attention has been paid to dextran aggregation in studies of the physico-chemical properties of dextran solutions. However, our data show that it is very important to take this aspect into consideration.

If the proton magnetic relaxation is measured using solutions of dextran obtained at room temperature by diluting a stock solution, then the degree of hydration, which is calculated from these data, will depend unsystematically upon the molecular weight of the dextran (Fig. 1, I). For heat-treated samples the molecular-weight dependence of the degree of dextran hydration assumes a normal monotonic shape (Fig. 1, II).

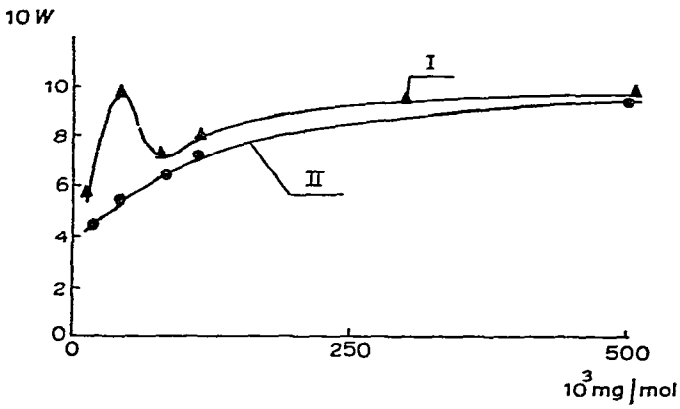



Fig. 1. Degree of hydration of dextran as a function of its molecular weight: I, before heat treatment at 100°; II, after heat treatment at 100°.

At room temperature, the processes of disaggregation of the different samples in a diluted solution probably proceed at different rates. Consequently, by the time at which measurements are started, these solutions are found to be in different states; naturally, this hinders a comparison of their properties. A normal comparison is possible only after the samples have been heated at 100° to bring the solutions into the equilibrium state.

*The influence of the molecular weight on dextran hydration*

The dependence of the degree of hydration of dextran on its molecular weight is shown in Fig. 1 (II). The hydration degree ($W$) is expressed as the mean number of water molecules linked by a single monomer unit of dextran. In the analysis of the

data presented, two features are noteworthy: (*a*) in the molecular weight range $17\times10^3$–$110\times10^3$, $W<1$, *i.e.*, some of the monomer units of dextran remain unhydrated; (*b*) if the chemical similarity of monomer units holds, $W$ approaches unity with increase in molecular weight.

The first feature may be regarded as a consequence of considerable intersegmentary interaction in the dextran molecule, which makes it impossible for a part of the segments to form bonds with water molecules. Semi-quantitative consideration of this interaction is possible on the basis of concepts developed by Kurata[8], who carried out a detailed analysis of the influence of intersegmentary interaction on the thermodynamic properties of polymer solutions. In accordance with these concepts[8], for a separate macromolecule having a degree of polymerization $P$ in a sufficiently diluted solution, the total number of contacts with the participation of segments in the volume of the macromolecule may be represented as:

$$N = N_{22} + N_{21}, \tag{5}$$

where $N_{22}$ is the number of segment–segment contacts, and $N_{21}$ is the number of segment–solvent molecule contacts. By definition,

$$N_{22} = Ng_{22}, \tag{6}$$

where $g_{22}$ is the probability of a segment–segment contact.

In order to calculate $g_{22}$, the following, approximate, but illustrative, method[8] can be used.

A macromolecule can be approximated to a sphere whose radius is equal to the root-mean-square radius of gyration of the macromolecule ($\langle\bar{R}^2\rangle^{0.5}$). It is assumed that the segments are randomly distributed in the volume of this sphere ($V^*$). In this case, $g_{22}$ may be regarded as the probability of the segment collision, which is proportional to the segment concentration in the macromolecule volume, *i.e.*,

$$g_{22} \propto P/V^*(P). \tag{7}$$

Then, taking into account that $V^*(P) \propto \langle\bar{R}^2\rangle^{1.5}$ and $\langle\bar{R}^2\rangle \propto P^x$, where $x \neq f(P)$,

$$g_{22} = AP^{1-1.5x}, \tag{8}$$

where $A$ is a constant. Combining equations (5)–(8),

$$N_{21} = N(1 - AP^{1-1.5x}). \tag{9}$$

To a first approximation, each segment–solvent molecule contact may be considered as an elementary act of hydration. Therefore, it may be assumed that $W = N_{21}/P$.

Hence, $$W = (1 - AP^{1-1.5x})N/P \tag{10}$$

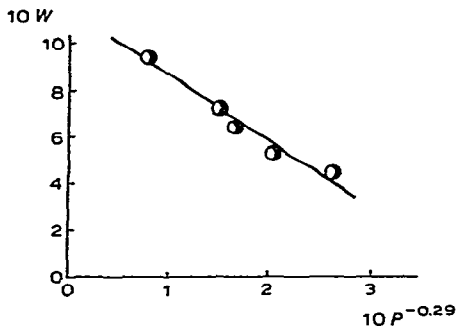
or $$W \propto (1 - AP^{1-1.5x}) \tag{11}$$

because, in a homologous series, $N \propto P$ provided that the structural similarity is retained[8].

According to data reported[9] for dextran, $\langle \bar{R}^2 \rangle \propto P^{0.86}$, *i.e.*, $x = 0.86$. Thus, in conformity with the concepts described above, the dependence of the degree of hydration of dextran on its degree of polymerization should satisfy the following equation

$$W \propto (1 - AP^{-0.29}). \qquad (12)$$

As can be seen from Fig. 2, this equation adequately describes the experimental data. Thus it may be assumed that the concepts presented above reflect, in general, the real picture of the phenomenon.



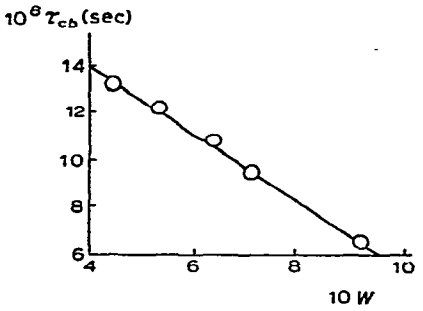




Fig. 2. Degree of hydration of dextran as a function of its degree of polymerization, as given in (*12*).

Fig. 3. Correlation times of rotational motion of "bound" water molecules as a function of the degree of hydration of dextran.

The intersegmentary interaction which takes place in a dextran macromolecule interferes with its complete hydration. As the molecular weight increases, the concentration of segments in a macromolecule decreases, since the volume of the macromolecule increases more rapidly than does its weight. This implies that, with increasing molecular weight, the mean distance between segments increases and, therefore, their interaction weakens, thus leading to an increase in the degree of hydration.

Apart from the degree of hydration, the proton magnetic relaxation method enables the evaluation of another important parameter, namely, the correlation time of the rotational motion of water molecules in the *b*-state [$\tau_{cb}$ in (*3*)]. This parameter is closely associated with the rotational mobility of the segments. The dependence of $\tau_{cb}$ on the degree of hydration, which has been averaged over all the samples investigated, is presented in Fig. 3. From these data, it follows that, as the degree of hydration increases, $\tau_{cb}$ decreases, *i.e.*, the internal rotation of segments is facilitated. This fact suggests that the hydration (*i.e.*, screening by water molecules) of neighbouring monomer units, the probability of which increases with the degree of hydration, leads to a weakening of their interaction, thereby favouring an increase in the rotational mobility of monomer units.

## EXPERIMENTAL

*Materials.* — Commercial preparations of dextrans of various molecular weights were used without any additional purification. The molecular weights ($\times 10^{-3}$) were as follows: *D1* (Koch–Light), 17–20; *D2–D4* (Polfa, Poland), 40, 80, and 110, respectively; *D5* (Serva), 500. Prior to using, the preparations were kept in a desiccator over calcium chloride for a week.

*Preparation of solutions.* — Stock solutions (20%) of dextrans were prepared at room temperature by weight, using preparations dried over calcium chloride. These solutions were kept for 14 days at 4°, and then they were diluted by weight to a concentration of 1%. Subsequent dilutions to the working concentrations (0.2–1%) were carried out by volume. As a rule, before measuring the proton magnetic relaxation, the solutions were kept in a boiling-water bath for ~30 min. Ordinary distilled water was used for the preparation of the stock solutions and for their subsequent dilutions.

*P.m. relaxation.* — The proton magnetic relaxation times were measured for dextran solutions, using an "SPX" spectrometer (Brucker–Physik) at a resonance frequency of 90.004 MHz and a temperature of 30°. The spin–lattice relaxation times ($T_1$) were determined acccording to Hahn[10], making use of the following pulse sequences: 180°–90° and 90°–90°–180°. The error in the determination of $T_1$ did not exceed $\pm 2.5\%$.

The spin–spin relaxation times ($T_2$) were determined according to Carr–Purcell–Meiboom–Gill[11,12], with an accuracy of not less than $\pm 2\%$.

An averager and a chart recorder were used to register the pulse sequences. In all experimental runs, the duration of the 90° pulses was 3–5 μsec, while that of the 180° pulses was 8–10 μsec.

For all the dextran preparations in the range of concentrations studied (0.2–1%), the concentration dependences of the inverse relaxation times are well described by equation (*1*). As an illustration, these dependences for *D1* are shown in Fig. 4.

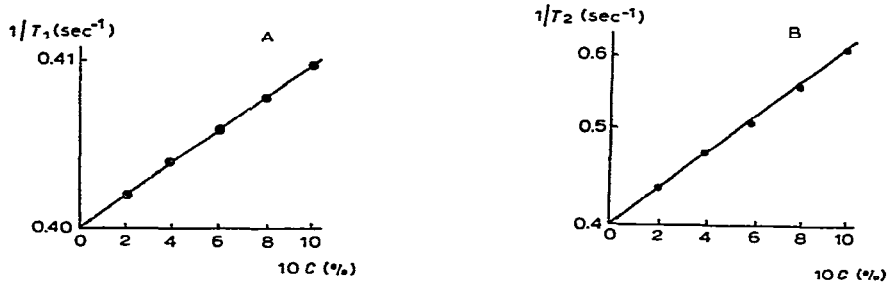



Fig. 4. Concentration dependences of p.m. relaxation times ($T_1$ and $T_2$) in solutions of dextran, as given in (*1*).

The angular coefficients of the concentration dependences of relaxation times were calculated by the least-squares method. In calculating the degree of hydration $W$

and the degree of polymerization of dextran, the unit molecular weight was taken to be equal to 162, without taking into account the branching.

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