SELF-DIFFUSION OF WATER IN TOBACCO MOSAIC VIRUS SOLUTIONS

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SUMMARY

Self-diffusion coefficients for water in solutions of tobacco mosaic virus have been measured near the critical concentration of the solutions. The diffusion coefficients of the water in the solutions is very close to the diffusion coefficient of water in pure water. It is concluded from this data that no more than 3 % of the water in a 4 % tobacco mosaic virus solution is in a bound ice-like state. This statement is in disagreement with JARDETSKY and JARDETSKY who conclude from nuclear magnetic resonance measurements that as much as 20 % of the water in a 1.9 % tobacco mosaic virus solution exists in an ice-like structure.

The self-diffusion coefficients of protons in aqueous solutions of T.M.V. near the critical concentration have been measured using a nuclear magnetic resonance spin-echo technique. The spin-echo apparatus, which uses high intensity pulsed radio frequency resonant radiation, as opposed to the usual steady state N.M.R., which uses very small and steady intensity resonant radiation, is described in ref. 1. The spin-echo technique is described in ref. 2, 3 and details of the technique used herein for the measurement of self-diffusion coefficients are discussed in ref. 4.

The sample of 80–85 % monodisperse T.M.V. was obtained through the courtesy of Dr. W. M. STANLEY at the University of California. The initial concentration of the sample was 26.9 mg T.M.V./ml of solution containing traces of phosphate buffer at pH 7. At this concentration, no phase separation appeared during six months at 4°. A part of the initial sample was diluted with distilled water to a concentration of 10.8 mg T.M.V./ml of solution. A second part of the sample was concentrated by slow evaporation of water on a house vacuum line to a concentration of about 40.4 mg T.M.V./ml of solution. After several weeks the 40.4 g/ml solution separated with about 0.2 volume fraction of dense phase. After completion of the measurements at 40.4 mg/ml, this same sample was concentrated further until it was all dense phase.

Proton signal intensity measurements relative to a water sample were made and indicated that no appreciable fraction of the protons in the sample resided in an ice-like structure with relaxation times so short that they were not observed.

The data in Fig. 1 shows that the self-diffusion coefficients of protons in all the T.M.V. solutions are within 2–3 % of the self-diffusion coefficients of protons in pure

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Abbreviation: T.M.V. tobacco mosaic virus.

water. In both water and T.M.V. the proton diffusion coefficients almost surely represent the diffusion coefficient of water molecules[5].

The magnitudes of the differences in proton diffusion coefficients in pure water and T.M.V. solutions are about equal to the estimated magnitude of the experimental error. Nevertheless, it is worth noting that diffusion coefficients of water in T.M.V. solutions are consistently lower than the diffusion coefficient of water in pure water. The activation energy of the diffusion coefficient in pure water agrees well with that obtained by SIMPSON AND CARR[6]. Fig. 2 shows the temperature-dependence of the proton diffusion coefficient for pure water and for the most concentrated T.M.V. solution.

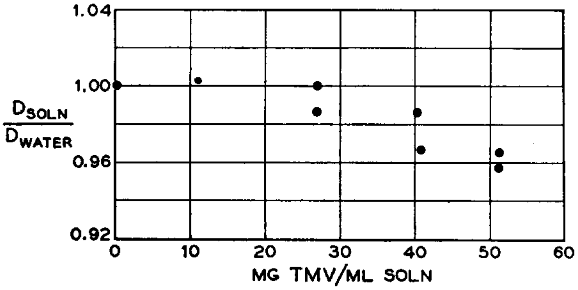

Fig. 1. Diffusion coefficients of T.M.V. solutions relative to water at room temperature *vs.* concentration of the T.M.V. solution.

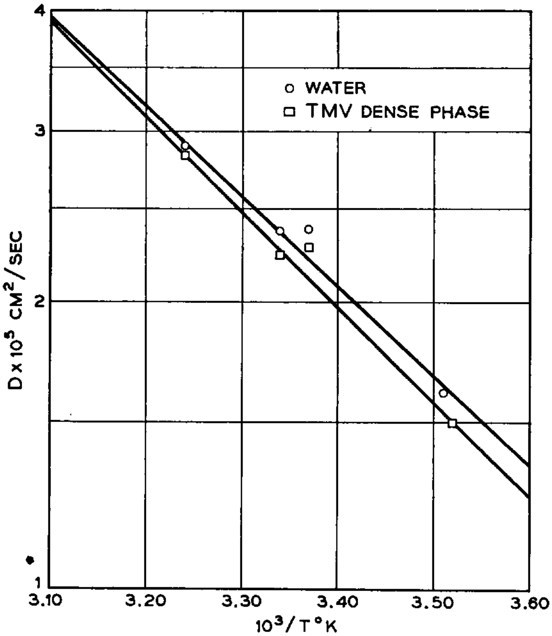

Fig. 2. Logarithm of the self-diffusion coefficients of water in a 51 mg/ml solution of T.M.V. *vs.* reciprocal temperature. The activation energies are 4.3 kcal/mole for water and 4.5 kcal/mole for the T.M.V. solution. This difference is not significant. The set of points which deviates most from the lines drawn on the graph was obtained on a different day and in a different region of the magnetic field than the remaining points. The deviation represents error in the absolute value of the diffusion coefficients owing to a change of calibration from day to day. The relative error for the diffusion coefficients is much less than the error in the absolute values.

It has been suggested that long range order of solutions of T.M.V. in water arises from the interaction of hydration shells of the virus particles[7,8]. This attitude is in contrast with the theory of organization of rod-shaped particles advanced by ONSAGER[9] and elaborated for T.M.V. solutions by OSTER[10]. Indeed, JARDETZKY AND JARDETZKY[11] suggested that their N.M.R. results are consistent with an interpretation in which about 20 % of all the water in the solution is organized into an ice-like structure around the T.M.V. particles.

If water tends to associate into an ice-like structure, or even a more weakly associated complex, an average molecule is immobilized[*] for a certain fraction, f, of the time of observation in the experiment. On the basis of this rather simple model one can write the self-diffusion coefficient, D, of the water in the T.M.V. solution as

$$D = D_0 \{ 1 - [1 - ({}^{v}b/{}^{v}0) f] \}$$

where D_0 is the self-diffusion coefficient of water in pure water, v_b is the diffusion jump frequency of the water of hydration (bound water) and v_0 is the jump frequency of water in pure water. If the binding is at all appreciable v_b/v_0 is much less than one and $D/D_0 \cong 1-f$. Since the system is at equilibrium, the time average f is also the average fraction of bound water molecules at any given time. The data and this result give a lower bound for $1-f$ and hence an upper bound for f of about 3 % bound water. Taking the molecular weight of the T.M.V. to be about 40 million, one finds that this corresponds to $1.6 \cdot 10^6$ water molecules per virus molecule in a 4 % T.M.V. solution. This number of water molecules per virus molecule does not exclude what might be considered the normal hydration of the roughly 50,000 ionic groups per T.M.V. molecule[**].

The spin–lattice relaxation time T_1 and the spin–spin relaxation time T_2 were also measured and are approximately 0.75 and 0.20 sec respectively for the sample containing 26.9 mg T.M.V./ml. We consider it probable that these relaxation times are to a large degree determined by paramagnetic effects and, as such, are difficult to relate to the problem discussed here.

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[*] The random translational degrees of freedom of the center of mass of the water molecule are converted into multiply-periodic vibrational degrees of freedom.

[**] We are indebted to Professor I. M. Klotz for pointing this fact out to us and thereby correcting an erroneous statement in our manuscript.