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Stability of Dextran Solutions

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In order to clarify the accessibility of dextran to both water and dextran the onset of turbidity in dextran solution was studied by means of viscosimetry and near-infrared and PMR measurements. Mole fraction of monomeric and hydrogen-bonded water species in dextran solutions were calculated for various concentrations of dextran solutions from $2\nu_1+\nu_3$ and $\nu_1+\nu_2+\nu_3$ bands where ν_1, ν_2, and ν_3 are the fundamental vibrations of symmetric stretching, bending and asymmetric stretching, respectively. The extent of hydrogen bonds was estimated from PMR chemical shift of water proton. A dextran solution was characterized by a low concentration in monomeric water. An anomalous time dependence of viscosity and PMR chemical shift was observed for concentrated solutions. A mechanism has been proposed for gelation and the onset of turbidity, stress being laid on comparable accessibility of dextran to both water and dextran.

Certain molecular weight fractions of dextran are of significant use as blood plasma substitutes. This brings forward the hydration of dextran as an important physiological subject. However, little attention has been paid to the stability of dextran solutions. A 40% dextran 40 (a fraction of 40000 molecular weight) solution loses transparency with time and turns into a gel.[1] A 10% dextran 40 solution used as blood plasma substitute gives rise to the formation of insoluble substance in an injection vial during the course of sotrage.[2,3] This indicates peculiarity in the stability of a dextran solution. In this paper, the results of studies on the transition from a solution to a sol or a gel are given.

Onset of turbidity and gelation are generally accomapanied by an intermolecular cross-linking of polymer chains.[4] Accessibility of polymer chain to another one and water might play a key role to determine the state of a solution. Accessibility of dextran to water has been characterized by estimating the extent of hydrogen bonds in water by means of near-infrared and proton magnetic resonance (PMR) investigations. Intermolecular interaction of dextran has been evaluated from viscometric data.

Experimental

Dextran 40 from *Leuconostoc* strain cultured in sucrose media was obtained from Fison Ltd., Pharmaceutical Division (London). The average molecular weight was 40000.

Viscosity measurements were carried out with a Tokyo Keiki Type B Viscometer. A Shimadzu Model SV-20 automatic spectrophotometer was used for obtaining near-infrared spectra. A 1 cm-path of cell was jacketed with circulating temperature-controlled water. For PMR a JEOL Model MH-60 was used. Samples were injected in a standard tube and were kept at 25 °C for a fixed time. Measurements were conducted with and without spining a sample tube. Chemical shift was referred to external tetramethylsilane (TMS).

Results

Turbidity and Gelation of Dextran Solutions. Dilute dextran solution remains clear even after storage for a long time. Insoluble substance is formed in a 10% dextran solution during storage. When dextran concentration exceeds 20%, the solution gradually turns turbid. In the concentration range 40—50%, the occurrence of turbidity is followed by gelation. The change is accompanied by an increase in viscosity. The change of viscosity with time for a 48% dextran solution is shown in Fig. 1. Viscosity was determined at 25 °C with a Type B viscometer. Viscosity decreases at first, then increases, attaining more than ten times the initial value in 160 h after preparation.

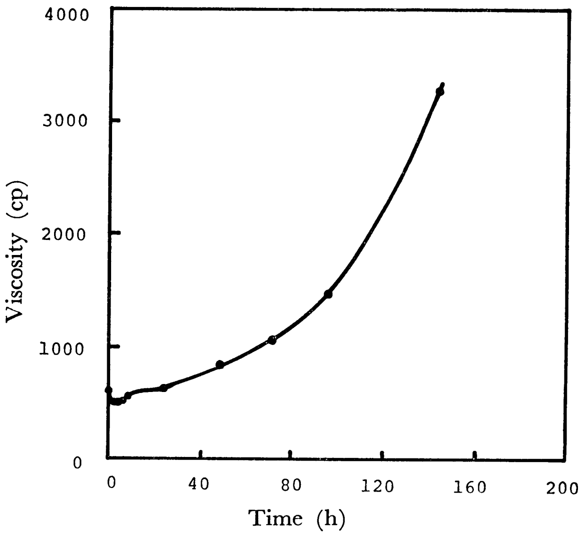

Fig. 1. Time dependence of viscosity of a dextran solution. Viscosity was determined for a 48% of dextran 40 solution at 25 °C.

Increase in viscosity is shown for solutions of various concentrations stored at 25 °C for 7 days. A marked increase is observed for the solution (concn>30%) which exhibited conspicuous turbidity.

The results suggest that an increase in viscosity is induced by intermolecular cross-linking of dextran during the course of storage. Dextran might be dissolved in an unstable state, turning gradually to a stable state. Such a transition would be accelerated in a concentrated solution. The following investigation was performed in order to characterize the transition from an unstable solution to a stable solution.

Hydrogen-bonds in a Stable State of Dextran Solutions. In order to estimate the extent of hydrogen-bond formation, near-infrared and PMR investigations were carried out for a dextran solution stored for a long time. An aqueous dextran solution contains several types of hydrogen-bond: (1) intra-dextran molecule, (2) inter-dextran molecule, (3) dextran–water molecule, and (4) inter-water molecule. Hydrogen-bonds related to water, (3) and (4), were studied with particular attention.

Hydrogen-bond formation of water molecules can be determined by various methods such as vibration spectroscopy and nuclear magnetic resonance (NMR). For an aqueous solution, near-infrared spectroscopy and NMR are effective.

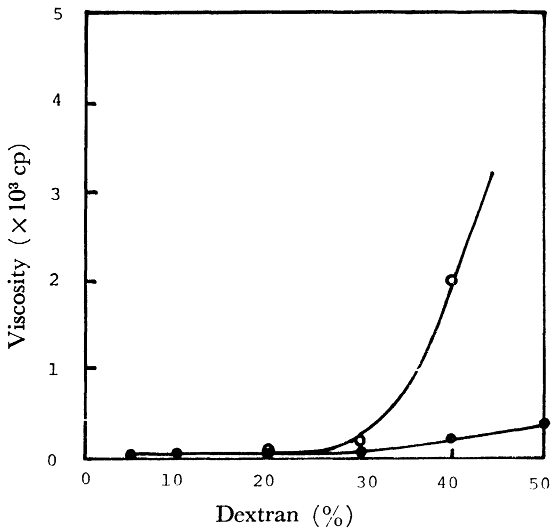

Fig. 2. Viscosity-concentration curves for dextran solutions. Viscosity was determined for a respective concentration of dextran 40 solution at 25 °C, 0 h (—●—) and 7 days (—○—) after preparation.

Water has three fundamental vibrations; ν_1 of symmetric stretching, ν_2 of bending and ν_3 of asymmetric stretching. Combination bands such as $\nu_1+\nu_2+\nu_3$ and $2\nu_1+\nu_3$ appear in near-infrared in the range 900—1300 nm. These two combination bands are sensitive to hydrogen-bond formation.

Water gives absorbance maximum at 977 nm for the $2\nu_1+\nu_3$ combination band at 25 °C. The peak is estimated to shift to 958 nm for monomeric water and 988 nm for entirely hydrogen-bonded water.[5] On the other hand, the $\nu_1+\nu_2+\nu_3$ combination band consists of three peaks, attributed to monomeric water (1160 nm), single hydrogen-bonded water (1200 nm) and double hydrogen-bonded water (1240 nm).[6] Relative intensity of these three peaks depends on the extent of respective hydrogen-bonded water species.

Near-infrared spectra at 25 °C are shown in Fig. 3 for water, 10% and 50% dextran solutions. The $2\nu_1+\nu_3$ peak shifted to a longer wavelength with an increase in the extent of hydrogen-bonded water species. A decrease in intensity for 50% dextran solution might be due to a decrease in the concentration of water.

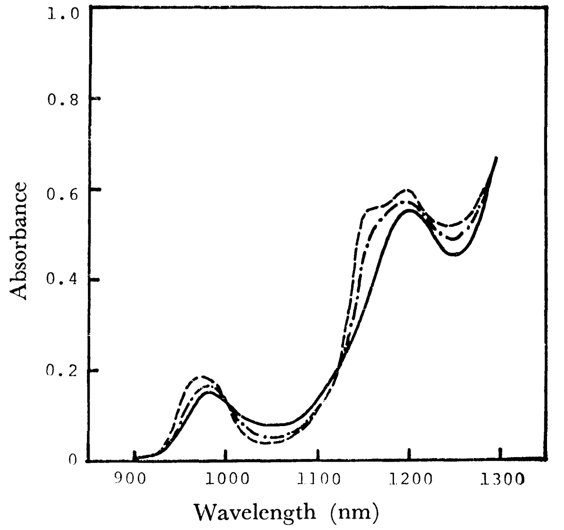

Fig. 3. Near-infrared spectra of water in dextran solutions. Spectra were obtained for a 50% (——), a 10% (—·—) of dextran solutions and water (----) at 25 °C.

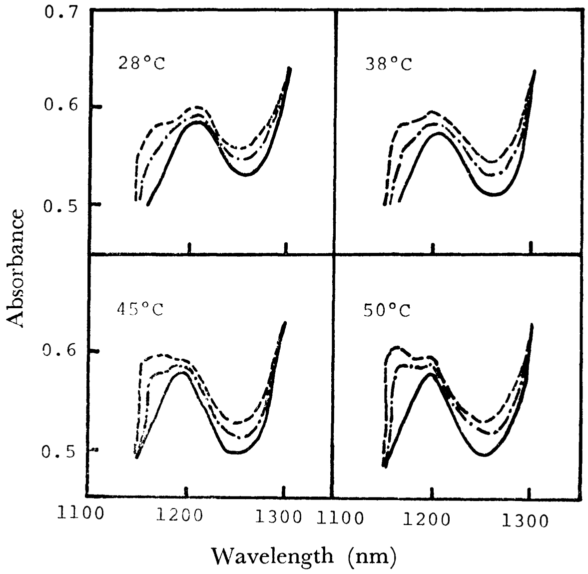

Fig. 4. Temperature dependence of near-infrared spectra of water in dextran solutions.
Spectran were determined for a 50% (——), a 10% (—·—) of dextran solutions and water (----) at respective temperature.

A complicated change was observed for a $\nu_1+\nu_2+\nu_3$ combination band caused by addition of dextran to water. Absorbance at 1160 nm decreased a great deal for dextran solutions. The effect of dextran on relative intensity at 1160, 1200, and 1240 nm is more obvious with rise in temperature. Temperature dependence of the $\nu_1+\nu_2+\nu_3$ band is shown in Fig. 4 for water, 10% and 50% dextran solutions. For water, absorbance at 1160 nm increased markedly with an increase in temperature, which was caused by breakingdown of hydrogen-bonds. Formation of monomeric water species might be slightly depressed for the 10% dextran solution with increasing temperature. No obvious decrease in absorbance at 1160 nm was found for the 50% dextran solution even at 50 °C. This suggests that release of monomeric water species is extremely retarded, and that in concentrated solutions hydration becomes very stable against temperature.

The extent of monomeric and hydrogen-bonded

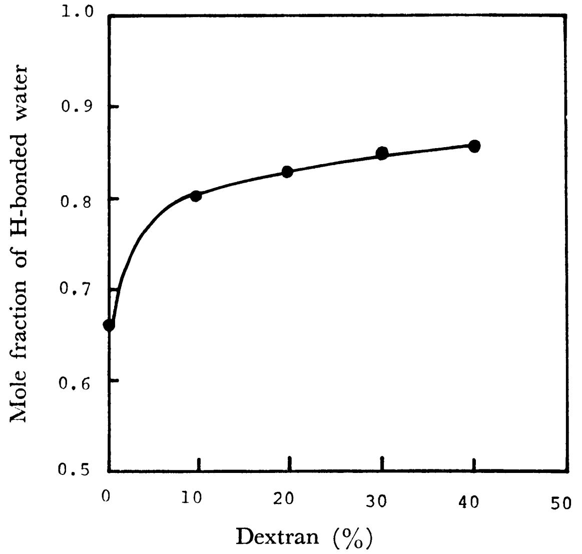

Fig. 5. Molar fraction of hydrogen bonded water species in various concentrations of dextran solutions. Molar fraction of hydrogen bonded water species was calculated from absorbance at 1160 and 1200mm at 28 °C.

water species can be estimated from intensity at 1160 and 1200 nm for dextran solutions.[7,8] Hydrogen-bonded water species should be classified into single and double hydrogen-bonded ones. We have calculated both types as hydrogen-bonded water species. Mole fraction of hydrogen-bonded water species is plotted for each concentration of dextran solution at 28 °C in Fig. 5. Hydrogen-bonds might be significantly formed with an increase in concentration of dextran. As an example the extent of monomeric water involved in 40% dextran solution is less than half in pure water at 28 °C.

A similar result was obtained from PMR studies on the state of water in dextran solution. Water proton gives a sharp single peak around 5 ppm *vs.* tetramethylsilane (TMS). Hydrogen-bond formation shifts the peak to lower magnetic field. A 10% dextran solution exhibited a slightly broadened but still sharp single peak at 6 ppm when a sample tube was spinned. Non-spinned sample, however, gave a very broadened signal. At 50% dextran concentration, another broad signal was detected at a higher magnetic field than that of the main signal. The broad signal at higher magnetic field was obtained either with or without spinning of the sample tube (Fig. 6). The signal was attributed to the protons of dextran. Broadening in water proton signal for a 50% dextran solution might be caused by immobilization of water.

The concentration dependence of PMR chemical shift is shown in Fig. 7 for water proton of dextran solutions of various concentrations. Measurements were carried out with a spinned sample tube 24 h after the samples had been prepared. Chemical shift moved sharply to lower magnetic field with an increase in concentration of dextran and reached a constant value at approximately 10%, which indicated a marked increase in the extent of hydrogen-bonds of water.

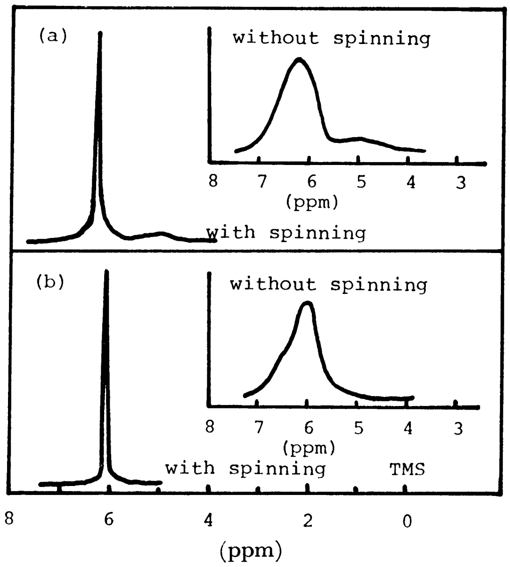

Fig. 6. Proton magnetic resonance spectra of water in dextran solutions.
Measurements were conducted for a 10% (b) and a 40% (a) of dextran solutions with and without spinning a sample tube at 25 °C using tetramethylsilane (TMS) as an external standard.

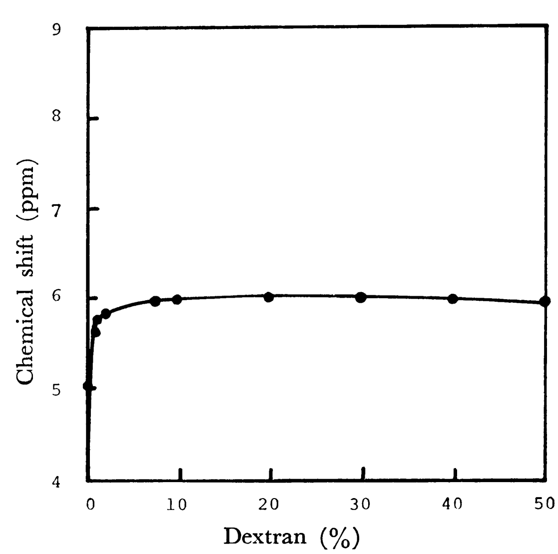

Fig. 7. Concentration dependence of PMR chamical shift for dextran solutions.
Chemical shift was determined from PMR spectrum for respective concentration of dextran solution measured with spinning a sample tube at 25 °C using TMS as an external standard.

Anomalous Time Dependence of Viscosity and PMR Chemical Shift. Investigations on the process of hydration of dextran are necessary to clarify the mechanism of the occurrence of turbidity and gelation. Time dependence of viscosity and PMR chemical shift was examined. An anomalous time dependence was observed in concentrated solutions.

Anomality in viscosity is shown in Fig. 8. Viscosity was measured at 25 °C for a 48% dextran solution. Viscosity was found to decrease sharply in the first few hours, reaching the minimum, and then to in-

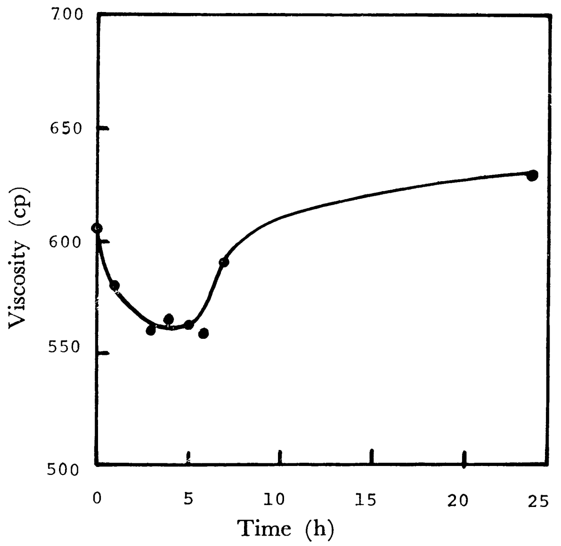

Fig. 8. Viscosity change of a dextran solution.
Measurements were made for a 48% of dextran solution at 25 °C.

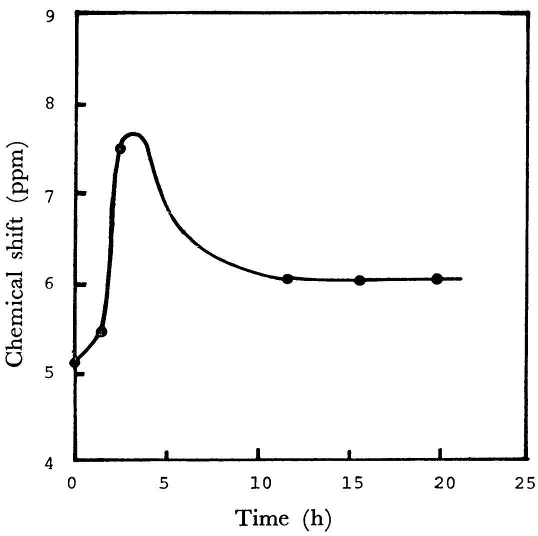

Fig. 9. Time dependence of PMR chemical shift of water in a dextran solution.
A 40% of dextran solution was injected into a standard sample tube with an external reference of TMS. The sample tube was spinned for measurement at 25 °C.

crease over the initial value. No such anomaly was observed for a dilute solution which showed constant viscosity irrespective of time. A 10% dextran solution showed a gradual increase in viscosity with no minimum. A minimum in viscosity–time curve was observed when the dextran concentration exceeds 30%.

Time dependence of PMR chemical shift is shown for a 40% dextran solution in Fig. 9. A marked shift to lower magnetic field in chemical shift took place in a few hours. This was followed by a sharp change to higher magnetic field up to a steady value. The steady value of chemical shift lies at fairly lower magnetic field as compared with that of the initial one. The maximum in a time–chemical shift curve appears in a solution with dextran concentration exceeding 30%. This is in line with viscosity.

Discussion

Maximum viscosity is associated with a PMR chemical shift *vs.* time curve for water proton of a dextran solution. Anomalous time dependences of viscosity and PMR chemical shift suggest that at least two competitive reactions such as hydration and dehydration take place simultaneously.

The viscosity of a dextran solution seems to be closely related to the states of polymer chains. The increase in viscosity might result from the intermolecular cross-linking of dextran, which causes turbidity and gelation. The decrease in viscosity in the first stage might be caused by an opposite reaction such as decoupling of intermolecular interaction followed by intermolecular linking. It seems that several processes such as (1) intermolecular cross-linking, (2) decoupling, (3) hydration, and (4) dehydration of dextran take place simultaneously and competitively. The combined effect of these processes might be determined by competitive accessibility of dextran to water and dextran. Dextran has comparable accessibility to both water and dextran. That is why a dextran solution exhibits a complicated behavior.

Dextrans are highly branched polymers of D-glucose. The linkages between D-glucose units at the branching points are α-1,6, α-1,4, and α-1,3. Taylor *et al.*[9,10] have extensively investigated the effects of variation in branching structure on the water sorption. Dextrans with a high α-1,4 content exhibited the greatest sorption, those with appreciable α-1,3 content exhibited intermediate sorption, while those with predominantly α-1,6 linkages were least hygroscopic. The results of powder X-ray diffraction measurements indicate that the structural correlation is related to the onset of crystalization or crystallinity. Linear polymers are crystallized with the greatest ease, while cross branching tends to prevent the alignment of proximal linear chains.

Dextran 40, in which the α-1,6 linkage predominates, can be characterized by the least hygroscopicity and the greatest ease in crystallization of dextran. Dextran is soluble in water because of an extreme accessibility to water. If dextran has a chance to associate with other polymer chains, intermolecular cross-linking through hydrogen-bonding would be induced due to high accessibility to dextran, giving rise to turbidity and gelation. Although a dextran solution displays turbidity and makes a gel, a neat and clear solution can be obtained by a small driving force to break intermolecular coupling, *e.g.*, heating, since the strength of intermolecular coupling would be no more than that of hydration. A turbid dextran solution can be transformed into a clear one by heating.

References

1) N. Nakajima, Y. Iwao, T. Kuoka, S. Suzuki, and M. Aizawa, *Yakugaku Zasshi*, **95**, 749 (1975).

2) R. A. Edwald, A. A. Young, and W. H. Crosby, *Military Med.*, **129**, 952 (1964).

3) D. E. Cadwallader, Jr., C. H. Becker, J. H. Winter, and D. Marcus, *J. Am. Pharm. Assoc.* (*Sci.*), **47**, 894 (1958).

4) M. Aizawa, M. Takahashi, and S. Suzuki, *Chem. Lett.*, **1974**, 193.

5) O. D. Bonner, G. B. Woolsey, *J. Phys. Chem.*, **72**, 899 (1968).

6) G. Herzberg, "Molecular Spectra and Molecular Structure. II," D. Van Northrand Co., Ind., Princeton, N. J. (1959), p. 281.

7) M. Aizawa, and S. Suzuki, *Bull. Chem. Soc. Jpn.*, **46**, 2634 (1973).

8) K. Buijs, and G. R. Choppin, *J. Chem. Phys.*, **39**, 2035 (1963).

9) N. W. Taylor, H. F. Zobel, N. N. Hillman, and F. R. Senti, *J. Phys. Chem.*, **63**, 599 (1959).

10) N. W. Taylor, J. E. Clusby, F. R. Senti, *J. Phys. Chem.*, **65**, 1810 (1961).