Solution Dynamics of the Dextran/Crosslinking Agent Systems

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ABSTRACT: The interaction of some selective Cl- and *N*-containing functional crosslinking agents such as epichlorohydrin (ECH), *N*,*N*′-methylenebisacrylamide (MBAM), and bifunctional agents such as glutaraldehyde (GA) and glycidylmethacrylate (GM) with dextran (Dx) in aqueous solutions were studied by viscometric and spectroscopic methods. The dynamic viscosities of Dx-crosslinker aqueous solutions have been measured at physiological temperature, 37°C and in the concentration range of 0.22–0.4 g/dL. Concentration of crosslinkers were kept constant at 0.001–0.35 mol/L. Viscosity behavior of the solutions was interpreted using the Huggins and Kraemer equations. Moreover, the interaction between hydroxyl groups of the Dx with crosslinkers in aqueous solutions, structure properties was also confirmed thereby use of Raman and FTIR spectroscopy. For the Dx/crosslinker systems, the decreasing order of interaction was determined as ECH > GA > MBAM > GM. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 1177–1187, 2007

Key words: dextran/water/crosslinker system; viscometry; solution dynamics; FTIR spectroscopy; Raman spectroscopy

INTRODUCTION

Dextran (Dx) is a bacterial polysaccharide, which consists mainly of α-1,6-linked D-glycopyranose residues with a few percent of α-1,2-,α-1,3-, or α-1,4-linked side chains[1] (Scheme 1). This polymer consists of repeating units of a single monomer-glucose and can be activated through its hydroxyl groups by a number of chemical methods that are efficient for coupling with other molecules. Dx can be activated at multiple sites throughout its chain, since each monomer contains hydroxyl residues.

Dx has been used extensively as a crosslinking agent for proteins and other molecules. It has been used as a drug carrier to transport greater concentrations of pharmaceuticals to tumor sites *in vivo*, as a hapten carrier to elicit an immune response against coupled molecules, as a multifunctional linker to crosslink monoclonal antibody conjugates with chemotherapeutic agents and as a stabilizer of enzymes and other proteins.[2] Both Dx and multifunctional monomers (crosslinkers) have great potential lonely as biomaterials. In addition, combining their properties can give a hydrogel that may be selectively and enzymatically degradable but not hydrolytically degradable. In the whole of pharmaceutical formulations, an important point is understood how the diffusion process of the drug is influenced by the structure of the polymer network. Depending on the chemistry used, crosslinked Dx can be stable in the stomach and small intestine but degradable in the large intestine.[3] The objective of this study was initially to investigate the dynamics of crosslinkers with Dx in aqueous solutions to develop Dx capsules for delivery of drugs to colon in the gastrointestinal tract.

Recently, some attention has been focused on the study of dynamic[4–6] and thermodynamic[6–11] aspects of Dx aqueous solutions in the presence (or absence) of certain denaturing agents, and the unperturbed dynamisms and the θ temperatures of Dx solutions in water, dimethyl sulfoxide, ethylene glycol, and methoxy ethylene glycol. The reaction kinetics of epichlorohydrin (ECH) with carbohydrate polymers were also investigated.[12] Functional polymer hydrogels forming from self-crosslinkable Dx/ECH, Dx/*N*,*N*′-methylenebisacrylamide (MBAM), Dx/$POCl_3$, and Dx/γ-ray systems are synthesized and mechanism of crosslinking reactions are proposed.[13] Gelation behavior of poly(*N*-vinyl-2-pyrrolidone) (PVP) in aqueous solutions has been explained in the presence of different amounts of persulphate content.[14] The kinetic investigations of PVP/persulphate system have revealed that significant changes occur in the hydrodynamic volume of the polymer which may be inter-

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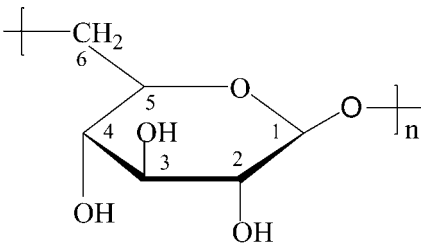


Scheme 1 Structure of Dx.

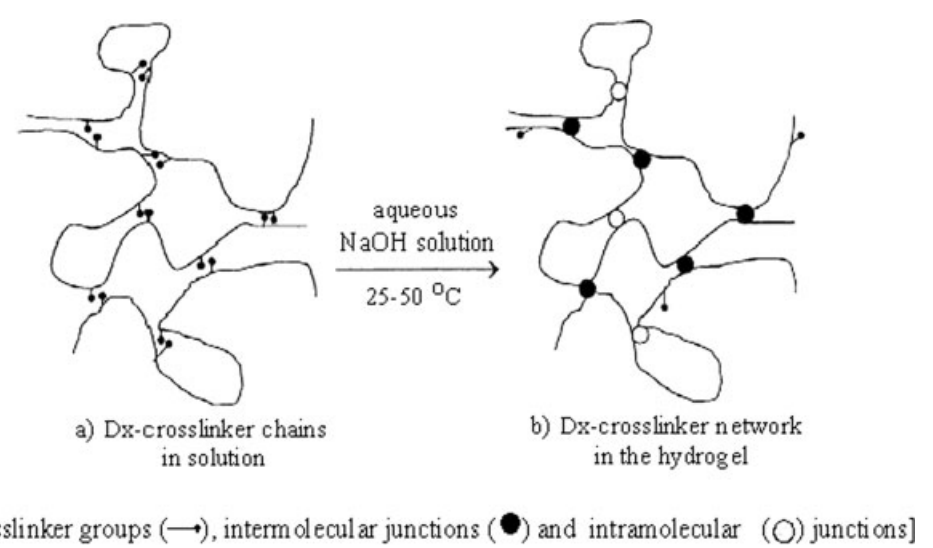
preted by probable chain scission and/or degradation in the polymer chain.[15] Functionalized Dxs were synthesized by macromolecular reactions of hydroxyl group with various reagents.[16–18] The obtained results were discussed in a review.[19]

A substantial portion of literature is interested in preparation of hydrogels and crosslinking of Dx. The properties of their aqueous solutions have been little investigated. Because of the intensive research on polymer networks, the term ''gel'' is used so frequently that we feel it necessary to define the meaning of the term ''hydrogel.''[20] The term ''relaxed hydrogel'' is used for the Dx-crosslinker network immediately after crosslinking but before swelling. After swelling, a ''swollen hydrogel'' will be formed.[21]

A schematic representation of the hydrogel formation is shown in Scheme 2(a,b). It indicates a polymer network created by chemical crosslinking of an aqueous solution of crosslinker substituted Dx polymer chains. Polymeric hydrogels from Dx/crosslinker mixtures are carried out by intermolecular side-chain reaction of Dx hydroxyl groups with monomeric crosslinking agents in aqueous solutions.[13] Crosslinking may occur only at high crosslinker concentrations. However, no interpretation is present for studies achieved at low crosslinker concentrations. Dx/crosslinker aqueous solutions have been commonly used in various technologies, as well as its hydrogels. The aim of the present work is to report the interaction dynamics of Dx/crosslinker chains in solutions [Scheme 2(a)].

The aim of this research was also to investigate the dynamics of Dx/crosslinker/chains in solution as they differ from Dx/crosslinker/network in the hydrogels. Hydrogels based on crosslinked Dxs are very suitable systems for the controlled release of proteins. These materials have great potential alone as biomaterials, but the literature published in journals is incomplete for understanding the chemistry of Dx/crosslinker interactions in solution. Reaction mechanism of Dx gels has been elucidated but interactions in Dx/crosslinker aqueous system have not been. This paper describes the interaction dynamics of Dx/crosslinker in aqueous solutions and represents the results of viscometric and spectroscopic studies carried out to elucidate the interactions of Dx's hydroxyl group with polyfunctional crosslinking agents in aqueous solutions. In this study, the interactions between Dx and different crosslinkers in homogeneous aqueous solutions at physiological temperature (37°C) are investigated.

To elucidate of reaction mechanism and interactions between crosslinker and Dx, molecular spectroscopy, in particular vibrational spectroscopy is useful. Vibrational spectra are especially sensitive to the geometry of molecules, system of intramolecular and intermolecular interactions. A comparative study was made by using FTIR and Raman spectroscopy to determine the role of crosslinker aqueous solutions to Dx. The normal vibrations of Dx molecules have, with few exceptions, close or coinciding frequencies; however, they differ greatly in the shape and contribution of inter- and intramolecular H-bonding.



Scheme 2 Schematic representation of the crosslinking reaction.

EXPERIMENTAL

The Dx T-500 from Leuconostoc Mesenteroides, used in this study was purchased from Sigma. The molecular weight characteristics were determined by the manufacturer as average molecular weight, 413,000 g/mol. It was dried and stored in a vacuum desiccator at 40°C and used without any further purification.

ECH, MBAM, glycidylmethacrylate (GM), glutaraldehyde (GA) were supplied from Merck and they were used without further purification. Dx-crosslinker solutions were prepared in double distilled and deionized water.

The dynamic viscosity measurements were performed at 37°C, using an Ubbelohde type of capillary viscometer. The temperature of thermostate was controlled within a range of ±0.1°C and the flow times were measured with digital accuracy of ±0.1 s. Dynamic viscosities were always measured twenty hours after the preparation of solutions. First, intrinsic viscosity of Dx solution in water ($c = 0.4$ g/dL) was determined. Second, intrinsic viscosities of Dx/crosslinker/water solutions were found. Dx is the solute and crosslinker/water solution (0.001–0.35 mol/L) is the solvent of solutions used in viscosity measurements, The final concentration of Dx solutions is same with concentration of solution that not contain a crosslinker; 0.4 g/dL.

The concentration of Dx solutions in water and in crosslinker solutions (0.001–0.35 mol/L in water) were 0.4 g/dL. The polymer samples for FTIR and Raman spectra were prepared by solvent evaporation at room temperature and dried at 25°C under vacuum for a week.

Fourier transform infrared (FTIR) spectra were recorded on an ATI UNICAM Mattson 1000 FTIR spectrometer at room temperature. The spectra were collected over the range 4000–400 cm^{-1} by averaging 40 scan at a maximum resolution of 4 cm^{-1}. Solid samples (0.01 g) were finely ground and analyzed by dispersing them in 0.09 g of dried spectroscopic grade KBr (Merck) by pressed-disc technique.

Raman spectra of samples were recorded using Labrom 800 HR Raman spectrometer (Jobin Yvon) with a He-Ne Laser source emitting at 633 nm, 600–1200 grooves/mm holographic grating and a change coupled device (CCD) detector. Raman spectra were obtained in 250 s. integrations with an average of three scans. Spectra were recorded with reproducibility within 1 cm^{-1}, Hole: 400 μm, Slit: 150 μm, Resolution: 0.1 μm.

RESULTS

The effects of concentration and type of crosslinker on the viscosity of diluted Dx solutions were explored by applying the well-known Huggins and Kraemer eqs. (1) and (2), respectively, to experimental data.[22,23]

$$\eta_{sp}/c = [\eta] + k_H[\eta]^2 c \quad (1)$$

$$\ln(\eta_r)/c = [\eta] - k_K[\eta]^2 c \quad (2)$$

Here, η_{sp}/c is the reduced specific viscosity, k_H is the Huggins constant (or Huggins slope coefficient). $\ln(\eta_r)/c$ is logarithmic viscosity number. k_K is the Kraemer constant. It is easily shown that both equations should extrapolate to a common intercept equal to $[\eta]$ that $k_H + k_K$ should equal 0.5. The usual calculation procedure involves a double extrapolation of eqs. (1) and (2) on the same plot. The intrinsic viscosities $[\eta]$ were determined from the average values of the intercepts of the plots of η_{sp}/c and $\ln(\eta_r)/c$ versus c. The dimension of $[\eta]$ is a measure of the effective hydrodynamic volume of the polymer in the solution. Moreover, it was found that

$$k_H + k_K \approx 0.5 \quad (3)$$

which was to be expected theoretically. But many systems appear not to follow this relationship. If either of these requirements is not met, molecular aggregation, ionic effects, or other problems may be indicated. In this study, it is observed sum of k_H and k_K are mathematically not related by eq. (3) (≈ 0.7 experimentally).

Noting the negative sign in eq. (2), k_K is actually a negative number.[24]

Kraemer equation can also given as:[25,26]

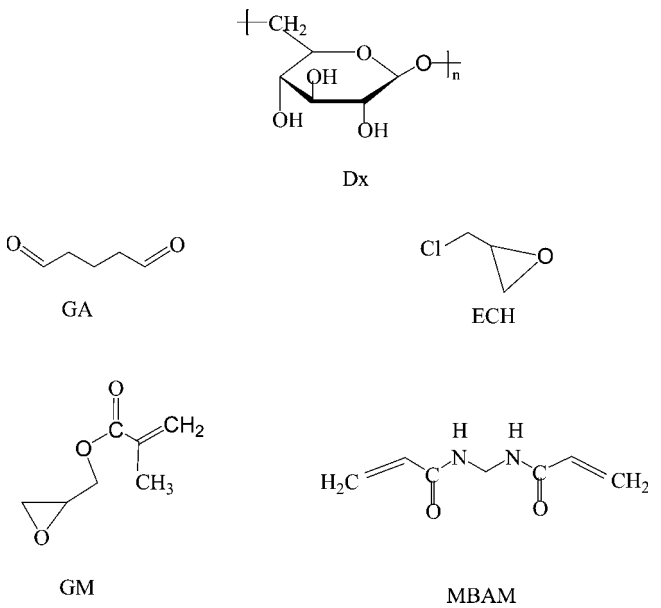
$$\ln(\eta_r)/c = [\eta] + k_K[\eta]^2 c \quad (4)$$

In this case, relation k_H and k_K was given as

$$k_H - k_K \approx 0.5 \quad (5)$$

Several authors have used both extrapolation methods (Huggins and Kraemer) for the calculation of $[\eta]$. In several cases this led to identical values of $[\eta]$. It was also found same $[\eta]$ value (0.343 dL/g) thereby both extrapolation methods in this study.

The aim of this work was to determine the behavior of Dx aqueous solutions in the presence of crosslinker employed at low concentrations and to provide an interpretation to changes occurring in the structure of the polymer chain. In this work, concentration of crosslinkers was kept constant at 0.001–0.35 mol/L since an increase in crosslinker concentration resulted in the formation of insoluble polymer fragments in solution which gave rise to difficulties during viscosity measurements. The facility and sensitivity of viscometric method have made it preferably available for the hydrodynamic volume measurements of the polymer in the solution. $[\eta]$, it also reflects the dynamism,



Scheme 3 Structures of Dx and crosslinkers.

H-bonding, dipole–dipole, and hydrophobic interactions ability of the polymer in the presence of different additives.

The addition of crosslinking agents to solution affects the dynamism of Dx/water systems depending on the nature and the concentration of the crosslinker. Dx has H-acceptor oxygen (ring- and bridge-) atoms and H-acceptor and donor three hydroxyl groups in each repeating unit. It is strongly expected that H-bonding will occur between these groups (as can be seen in Scheme 3).

Possibilities of H-bonding for Dx are also given in literature[27]: (1) the ring oxygen atom is invariably a H-bond acceptor; (2) the most common situation is that each hydroxyl group is associated with two H-bonds, one a donor and one an acceptor bond; (3) an environment of one H-bond, as donor only, less common; (4) an environment of three H-bonds, with one donor and two acceptors, is least common; (5) in a polysaccharide, there may be intramolecular H-bonding between residues; (6) hydroxyl groups not involved in H-bonding to other oxygen atoms can be present.

According to Pimentel and McClellan's classification,[28] all molecules can be conveniently classified into four types with respect to their ability to participate in H-bonding. Dx, water, and crosslinkers used in this study are Type III molecules (molecules with both donor and acceptor groups). Type III molecules can self-associate by H-bonding with themselves. Two types of H-bonded complexes may be formed: (1) intermolecular, involving two or more separate molecules; and (2) intramolecular, involving donor and acceptor sites within the same molecule. The strength of H-bonding depends on the relative acidities and basicities of the donor and the acceptor sides and in the case on intramolecular H-bonds, on the spatial arrangement present. Self association through intermolecular H-bonds can form a large variety of open/linear and cyclic/closed polymers. An intramolecular H-bonding is a favorable spatial configuration, that is, the distance between the H of the donor group and acceptor site is between 1.4 and 2.5 Å, and the angular orientation of the acceptor site does not deviate greatly from the bond axis of the donor group A–H.

As can be seen in Scheme 3, GA and MBAM are symmetric molecules. But GM is an asymmetric molecule. MBAM has H-acceptor four groups (C=O and NH) and H-acceptor two π-electron systems. ECH has H-acceptor etheric oxygen and H-acceptor halide. GA has H-acceptor two groups (C=O). Although GM has H-acceptor one group (C=O), H-acceptor two etheric oxygen and H-acceptor one π-electron system, its hydrophobic interactions are also dominant.

In Figure 1, it is represented the reduced viscosity (η_{sp}/c) and inherent viscosity ($\ln(\eta_r)/c$) values as a function of concentration by using Huggins and Kraemer equations to obtain intrinsic viscosity, [η] of the Dx/water system (no crosslinker agent). Intrinsic viscosity (0.343 dL/g) of Dx/water system is obtained by extrapolation to zero concentration.

For Dx/water system, constants and slopes determined by Huggins and Kraemer equations are given in Table I. From this table, Huggins and Kraemer constants-concentration profiles are observed to be nonlinear. k_H decreases with Dx/water concentration up to 0.33 but after that upon further dilution it shows increase again. The decrease of k_H with increasing coil expansion is a general phenomenon, well established by experiments but correlations of k_H with coil expansion factor (it is proportional with intrinsic viscosity) found by different authors often do not overlap.[29] This result is thought to be due to relative influences of the equilibrium association constant, the association number, the molecular weight, the size and shape of

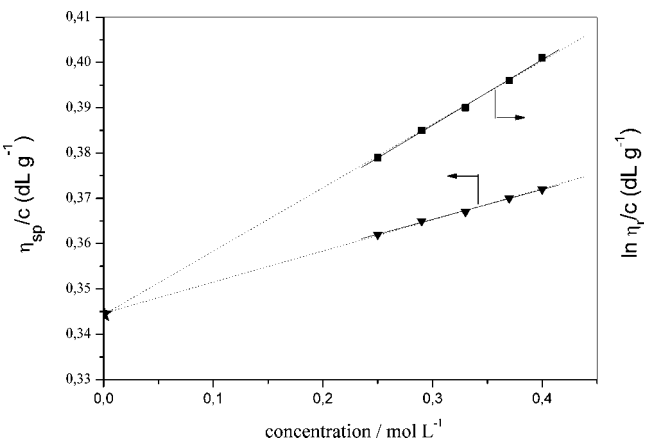


Figure 1 Plot of reduced viscosity η_{sp}/c and inherent viscosity ($\ln(\eta_r)/c$) versus concentration of the Dx/water system.

TABLE I
Numerical Values of Huggins and Kraemer Constants and Slopes Determined by Equations

[Dx-water] (g/dL)	Huggins and Kraemer constants		Huggins and Kraemer slopes	
	k_H	k_K	$k_H[\eta]^2$	$k_K[\eta]^2$
0.40	1.232	−0.543	0.145	−0.0650
0.37	1.218	−0.542	0.143	−0.0649
0.33	1.211	−0.532	0.142	−0.0636
0.29	1.231	−0.547	0.145	−0.0655
0.25	1.224	−0.535	0.144	−0.0640

the molecules and associates, and the solvent interaction. The estimation of the Huggins coefficient for polymer molecules is involved because of the interpenetration of polymer coils, the extent of which is a function of the segment–segment and segment–solvent interactions, and which affects the intramolecular hydrodynamic interaction and the molecular dynamic.

Figure 2 illustrates the viscosity behaviors of Dx/crosslinker systems in aqueous solution. The interaction of crosslinkers in aqueous solutions with Dx was studied by viscometric methods and compared with the interaction of Dx-water systems. Correspond the structure of Dx, it is strongly expected that molecular association/interaction will form between the polymer segments and solvent molecules through H-bonding. H-bonding is an association phenomenon. It causes a decrease in the total number of free molecules. In H-bonding, a specific covalent A-H group interacts with a specific acceptor site. The A-H bond is thereby weakened but not broken, and the properties of the acceptor group are also affected. All of these interactions affect viscosity.

As the MBAM and ECH concentrations were increased, the viscosity of the Dx solution was also increased. Here, polymer–solvent (crosslinker solution) associations are more dominant.

However, the viscosity was decreased when GM concentration was increased. This decrement results in the break of polymer–solvent associations, i.e., H-bonds between polymer and solvent molecules as well as in rupture of the interactions between the polymer segments. Despite GM is a molecule with acceptor group, its hydrophobic interactions are also present. Besides it is an asymmetric molecule, so interactions are dependent on the spatial arrangement of molecule.

In the presence of GA, the shape of the plot changes remarkably. There is a significant increase in [η], especially at higher concentrations of water/GA system. When the concentration of this additive system is below 0.23 mol/L, the plot becomes almost a straight line. It is obvious that [η] is nearly independent of c below 0.23 mol/L. In the concentrated solutions of GA, the intermolecular junctions of Dx-GA chains in solution aggregate.

There is a competition among Dx, crosslinker and water in Dx/crosslinker chains in solution. From Table II, the lowest intrinsic viscosity for Dx solution was observed when no crosslinker was added (except for ECH). A decreasing trend in the hydrodynamic volume of the polymer molecule may be interpreted by chain scission and/or intramolecular crosslinking. The highest intrinsic viscosity was observed when GM was added to Dx solution. The increased molecu-

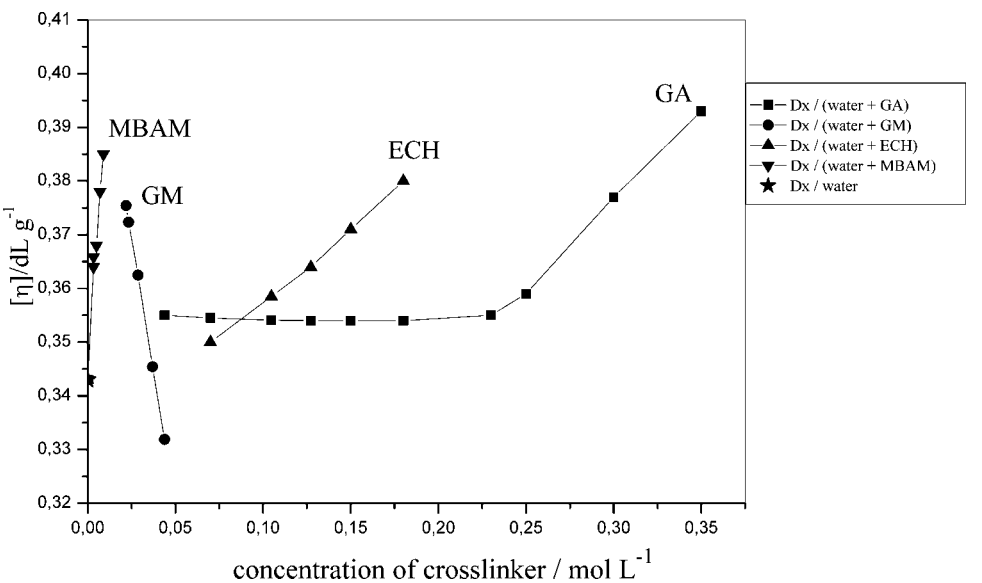


Figure 2 Viscosity behaviors of the interaction of ECH, MBAM, GA and GM in aqueous solutions with Dx.

TABLE II
Numeric Values of Huggins and Kraemer Constants, Intrinsic Viscosities of the Dx Solutions in both with and without Crosslinker Medium

Additives	[η]	Huggins and Kraemer constants		Huggins and Kraemer slopes	
		k_H	k_K	$k_H[\eta]^2$	$k_K[\eta]^2$
Water + ECH	0.333	1.741	−1.086	0.193	−0.120
Water	0.343	1.232	−0.543	0.145	−0.065
Water + GA[a]	0.355	1.145	−0.470	0.144	−0.059
Water + MBAM	0.362	0.887	−0.176	0.116	−0.023
Water + GM	0.406	0.246	0.604	0.041	0.100

[a] Numeric values of Huggins and Kraemer constants, intrinsic viscosities of the Dx solutions are calculated in the concentrations of this additive system (water + GA) is below 0.23 mol/L.

lar structure in H-bonded systems decreases freedom of molecular motion, causes more ''entanglements'' between molecules, and therefore tends to increase viscosity.[27]

The intrinsic viscosities of Dx/crosslinker systems are higher than those of the native Dx/water system except ECH. For the aqueous solutions of Dx, the decreasing order of interaction was determined as below:

$$(\text{ECH} + \text{water}) > \text{water} > (\text{GA} + \text{water}) > (\text{MBAM} + \text{water}) > (\text{GM} + \text{water})$$

GM, MBAM, and GA increased the intrinsic viscosity of Dx but ECH had an effect decreasing.

In the presence of the additive (water/crosslinker system), polymer-solvent interactions arise. Huggins and Kraemer slopes are generally considered as a measure of the degree of interaction between polymer and solvent. A large slope value indicates a much more complete solvation of the polymer, thus the presence of a better solvent. Huggins and Kraemer's constants give also idea about the interaction between Dx and the added crosslinker solution (Table II). Huggins and Kraemer constants as presented in Figures 3 and 4 have importance for the elucidate solution dynamics of the Dx/crosslinker systems. Concentration of crosslinker-Kraemer constant variation (Fig. 4) is similar to the concentration of crosslinker-intrinsic viscosity graph (Fig. 2). Concentration of crosslinker-Huggins constant change is on the contrary display a reverse order (Fig. 3). For the Dx/crosslinker systems, the decreasing order of interaction is also same to decreasing order of Huggins constant:

$$(\text{ECH} + \text{water}) > \text{water} > (\text{GA} + \text{water}) > (\text{MBAM} + \text{water}) > (\text{GM} + \text{water})$$

Decreasing order of Kraemer's constant is changed according to adverse order of interaction.

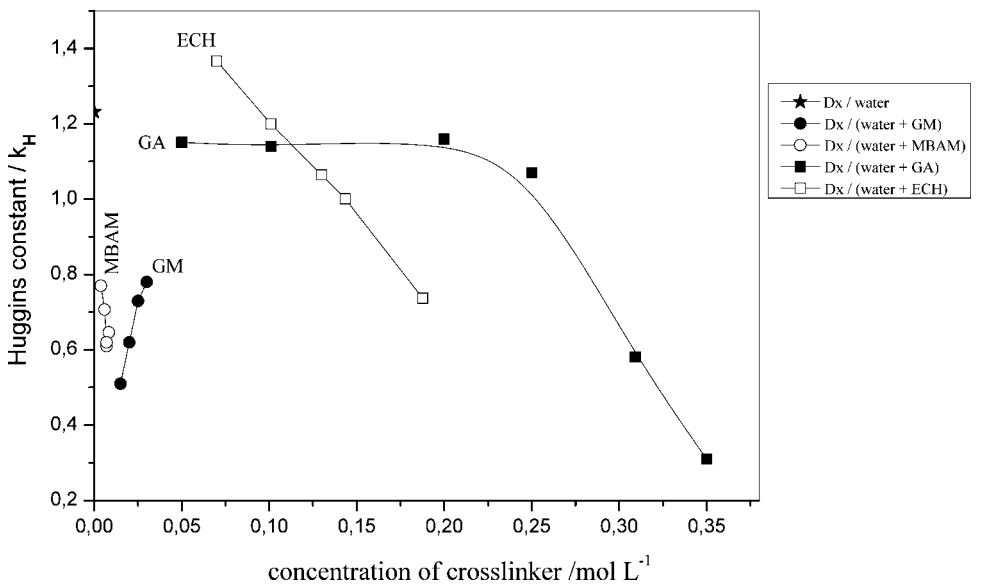


Figure 3 Variation of the Huggins constants (k_H) of Dx solutions with concentration of solvent.

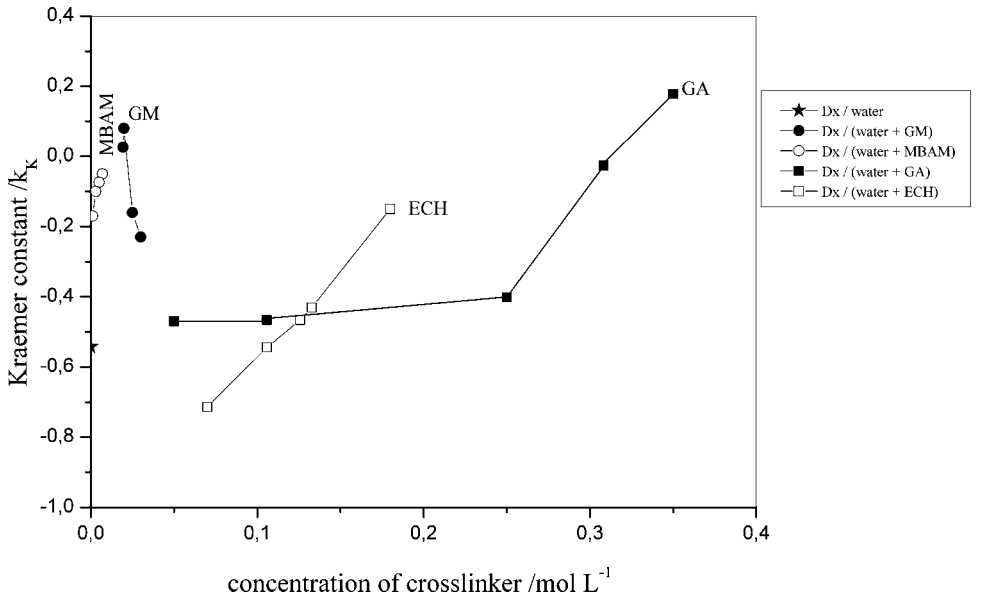


Figure 4 Variation of the Kraemer constant (k_K) of Dx solutions with concentration of solvent.

Although it is difficult to distinguish quantitatively between intra- and intermolecular interactions, the data obtained from viscosity measurements suggest that the intramolecular interactions of hydrophobic side chains are predominant in the studied polymer concentration (0.4 g/dL).

The viscometric magnitudes showed that the interactions involving mainly the H-bond formation, both between the polymer segments and polymer–crosslinker molecules are in competition with each other. Interactions between polymer and solvent (crosslinker solutions) seem to be more dominant than those interactions between the polymer segments. If crosslinking occurs, a decrease in intrinsic viscosity is observed since the crosslinker tends to break the present H-bonds and to form intramolecular junctions penetrating into the polymer chain. As can be seen from the Table II, ECH is decreased intrinsic viscosity of Dx/water system. The effective role of ECH in the crosslinking of Dx has been observed and confirmed here by Raman and FTIR spectroscopy as well.

Viscometric method gives information about interactions but does not give a valid reason about more interaction in MBAM and ECH while decrease of interaction in GM and different behavior in case of GA. Why these crosslinkers have different effects? For more comprehensive and deep compare, Infrared and Raman spectra have been used as solid evidence for the direct association of the crosslinker aqueous solutions with the Dx hydroxyl groups.

The aim of spectroscopic studies is to observe changes in polymer conformation related with crosslinker character and the interpretation of the characteristic bands appearing at different frequencies. The investigation of spectral evidences with viscometric studies presents another purpose of spectroscopic studies. Spectral differences have been grouped with respect to characteristic field (O—H stretching, C—H stretching, C—O stretching, C—C stretching) and thus evaluated.

The choice of Raman spectroscopy for analysis of chemical composition and structure is based on the high sensitivity of the Raman Effect for certain nonpolar chemical groups. In polymers, these groups are primarily the nearly homonuclear single and multiple C—C bonds that are weak or absent in the FTIR spectra. In this study, the characteristic group frequencies for Raman spectroscopy have been tabulated.

The difference in wavelength between the incident and scattered radiation corresponds to wavelengths in the mid-infrared region. Indeed, the Raman scattering spectrum and infrared absorption spectrum for a given species often resemble one another quite closely. There are, however, enough differences between the kinds of groups that are infrared active and those that are Raman active to make the techniques complementary rather than competitive. For some problems, the infrared method is the superior tool; for others, whereas the Raman procedure offers more useful spectra. For the other, an important advantage of Raman procedure over infrared lies in the fact that water does not cause interference.[30]

The bands in the vibrational spectra of Dx/crosslinker systems are complex. In normal vibrations, with certain frequencies, various groups and bands participate. Despite the equal or close frequencies of the normal vibrations, considerable differences are observed in the shape and band intensities. In addition, vibrational spectra are especially sensitive to the geometry of molecules, system of intramolecular and intermolecular interactions.[31] This has determined the signifi-

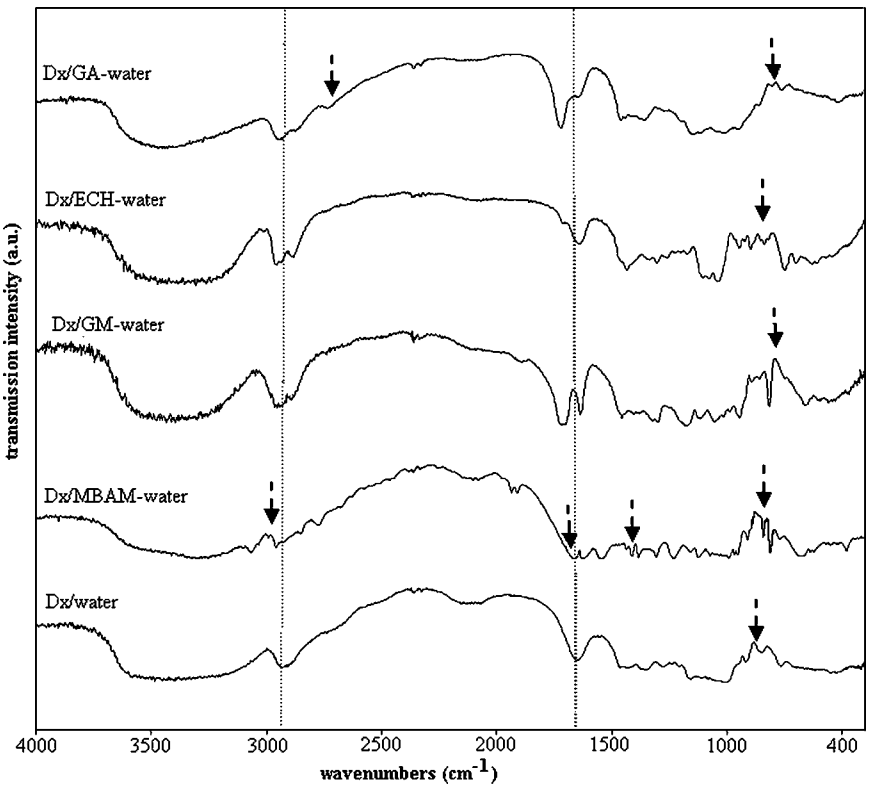


Figure 5 FTIR spectra of Dx/water and Dx/crosslinker aqueous solution.

cance of vibrational spectroscopy in investigating the structure and properties of carbohydrates. The high sensitivity of vibrational spectra to the steric factors in carbohydrates also leads to significant methodological difficulties. Separate definition and interpretation are necessary of spectral features caused by: (1) differences in the skeletal base configurations of molecules determined by the chemical composition; (2) realization of certain types of rotamer of lateral hydroxyl and oxymethylene groups; (3) differences in the three-dimensional order of molecules in the system of hydrogen bonds.

Comparison of FTIR spectra for Dx/water and Dx/crosslinker systems (Fig. 5), reveals characteristic shifts of the respective band wavelengths, after engagement of OH groups in covalent links. The skeleton of Dx molecules is composed of a successive combination of C—O and C—C bonds. The changes in the band positions and intensities observed from the FTIR spectra of parent Dx/water and Dx/crosslinker solutions are summarized in Table III. As evident from these data, the following changes of the main absorption bands are observed: (a) Increasing areas of characteristic hydroxyl group bands in field of 3300–

TABLE III
Some Characteristics FTIR Band Wavelengths (cm^{-1}) for Dx and Dx/Crosslinker System

	FTIR frequencies and relative intensities				
		Dx/			
Assignments	Water	GA-water	MBAM-water	ECH-water	GM-water
OH stretching	3375 (s)	3471 (s)	3298 (m)	3307 (s)	3301 (s)
C—H stretching	2941 (m)	2932 (s)	2956 (w)	2959 (m)	2931 (s)
		2880 (w)	2851 (w)	2881 (w)	2885 (w)
OH bending	1650 (s)	1721 (s)	1544 (m)	1720 (sh)	1707 (s)
		1648 (sh)	1657 (m)	1637 (m)	1637 (s)
			1620 (m)		
CH_2 scissoring	1431 (w)	1458 (w)	1430 (w)	1430 (w)	1455 (w)
C—C stretching	1160 (w)	1143 (m)	1157 (w)	1172 (w)	1181 (w)
C—O stretching	1029 (w)	1015 (w)	1060 (w)	1040 (w)	1052 (w)

s: strong, sh: shoulder, m: medium, w: weak.

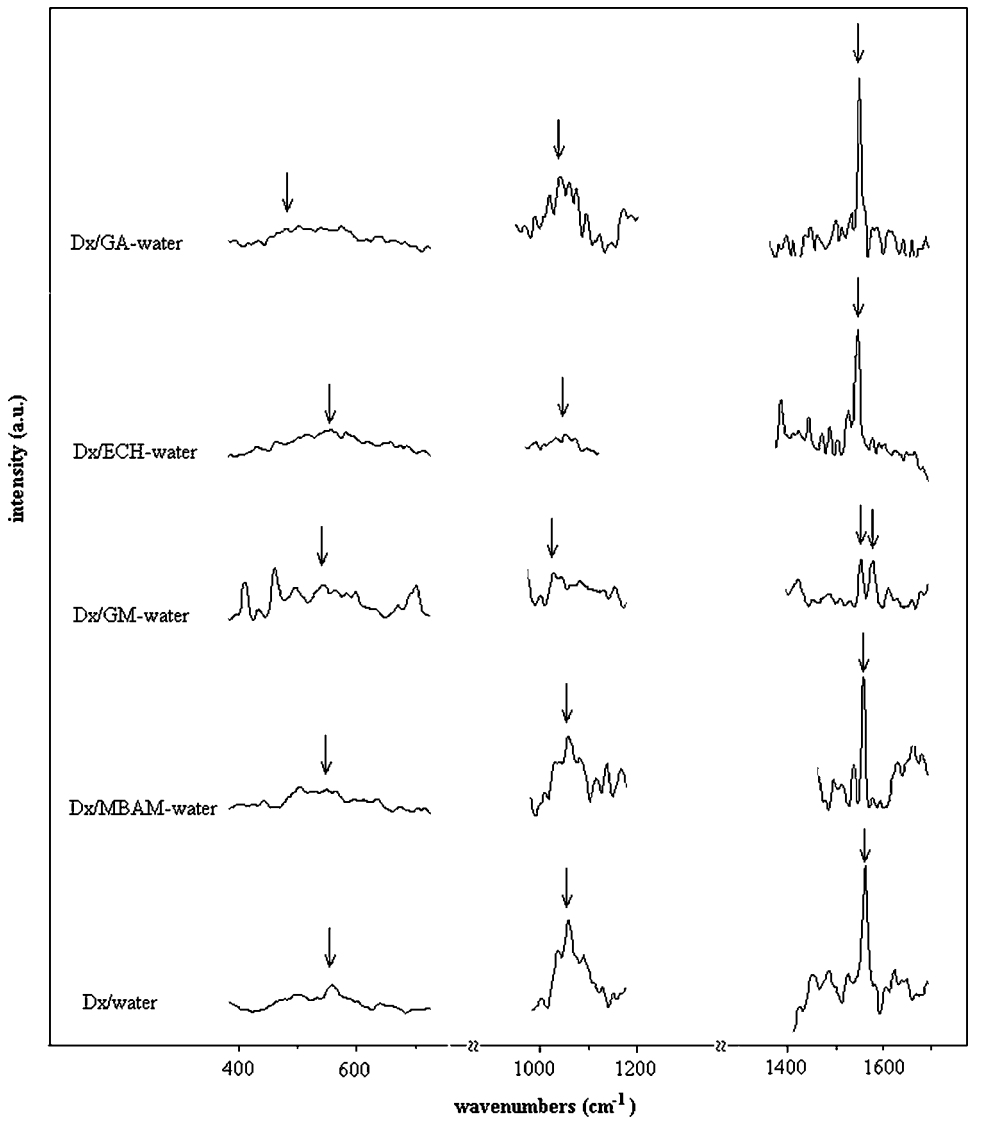


Figure 6 Raman spectra of Dx/water and Dx/crosslinker aqueous solution.

3470 cm^{-1} (broad and symmetric OH peak in Dx/ECH-water and Dx/GM-water systems); (b) Appearing the new absorption bands (doublet or new bands) of 2850–2880 cm^{-1} as a result of interaction of Dx hydroxyl groups with crosslinkers; (c) Appearing the changes (shoulder, doublet, or triplet) in the presence of crosslinker band of 1650 cm^{-1} corresponding to OH bending of Dx/water system; (d) Disappearing of 3250 cm^{-1} (NH) and 1440 (CN) bands through interaction of Dx hydroxyl groups with amine groups of MBAM. Decreasing intensity and appearing triplet peak of 1640 cm^{-1} (C=C in free acrylic groups) and $\sim$ 3000 cm^{-1} (CH_2=) absorption bands in Dx/MBAM-water system; (e) Appearing of 2932 cm^{-1} (C—H stretching) asymmetric peak by interaction of GA-water solution with Dx.

As a known fact, the stretching vibrations of free OH groups not added to H-bond are observed at higher wavenumbers. As H-bond formation is realized more the followed band shifts to lower wavenumbers and the band broadens. Free OH stretching vibrations may be observed at $\sim$ 3500 cm^{-1} or higher wavenumbers.

The decreasing order of interaction for the solutions of Dx GA > water > ECH > GM > MBAM may also be observed in the spectra as well. The H-bonded OH stretching vibrations for the Dx in GA solution are observed at 3471 cm^{-1} whereas those of the sample prepared in MBAM shift towards 3298 cm^{-1}.

The very strong, wide and nearly symmetrical peak observed at 3375 cm^{-1} in Dx/water is due to OH stretching vibrations. Stable H-bonds are found between the OH groups in Dx. Introduction of the GA into Dx structure causes disruption of these H-bonds and a hypsochromic shift is observed with GA to 3471 cm^{-1}. The medium intensity peak at $\sim$ 1650 cm^{-1} in Dx is due to OH bending vibrations.[32] This peak shows a number of splitting in the crosslinkers and

TABLE IV
Some Characteristics Raman Band Wavelengths (cm^{-1}) for Dx and Dx/Crosslinker System

	Raman frequencies and relative intensities				
		Dx/			
Assignments	Water	GA-water	MBAM-water	ECH-water	GM-water
C—O rotation, C—C—O angle bending	96 (s)	93 (s)	95 (s)	91 (s)	89 (m)
C—O—C bending, CH_2 rocking	554 (w)	549 (w)	553 (w)	550 (w)	553 (w)
C—O, C—C asym. stretching, CH_2 rocking	1053 (m)	1046 (w)	1050 (m)	1044 (w)	1050 (w)
CH_2 out-of-plane bending				1229 (w)	
	1243 (w)	1262 (w)	1333 (m)	1277 (w)	1224 (m)
C—C, C—O stretching,	1556 (w)	1558 (m)	1562 (m)	1568 (m)	1564 (w)
C—H bending,	1520 (sh)	1545 (sh)	1540 (sh)	1535 (sh)	1587 (w)
OH bending					
Aromatic C—H	2330 (s)	2329 (s)	2333 (m)	2327 (s)	2333 (m)
	2775 (m)	2613 (s)	2761 (m)	2546 (s)	2453 (m)
	3083 (m)	3088 (m)	2816 (m)	3120 (m)	3102 (m)
	–	3250 (m)	3249 (m)	3247 (m)	3217 (m)
	3323 (m)	3324 (m)	3318 (m)	3314 (m)	–
	3827 (m)	3834 (m)	3846 (m)	3835 (m)	3841 (m)

s: strong, sh: shoulder, m: medium, w: weak.

appears the changes (shoulder or new bands) in the presence of crosslinker. Similar findings were made for Dx with ECH.[33] For instance, three closely placed FTIR bands in the region 1060–1010 cm^{-1} are connected with valency vibrations of ν_{CO} in OH groups of glycosidic unit. Especially, high shifts were observed between the Dx/water band at 1029 cm^{-1} and the respective bonds of GA (1015 cm^{-1}), MBAM (1060 cm^{-1}), ECH (1040 cm^{-1}), and GM (1052 cm^{-1}) systems (Table III).

As antisymmetrical CH_2 stretching vibration are observed at high frequency values ($\sim$ 2950 cm^{-1}), symmetrical CH_2 stretching may be displayed at lower frequency values (2850–2890 cm^{-1}) on the other hand, in crosslinker systems this frequency shifts to lower fields and may occur almost $\sim$ 80 cm^{-1} (except for GA) (Table III). This is clear evidence implying that the hydrophobic interactions between the methylene groups of Dx are being destructed in the presence of crosslinker. This behavior implies that symmetrical stretching will weaken as a consequence of the hydrophobic interactions of methylene groups.

C—O stretching vibrations may be evidently observed at $\sim$ 1050 cm^{-1} in FTIR. It is a quite widely known fact that as H-acceptor O atom participates actively in the formation of H-bond, C—O bond energy is expected to weaken and shift toward lower frequencies.

On the other hand, the 916 cm^{-1} band in the original (nocrosslinker) Dx, corresponding to the δ (C—H) + ν (C—C) + δ (C—C—H) vibrations in the glycosidic unit, is shifted to higher frequencies after interaction with crosslinkers. The shift is greater after association of hydroxylic groups by crosslinkers (Fig. 5 and Table III).

The corresponding band at 1650 cm^{-1} is much more intense in the FTIR spectrum than band at 1560 cm^{-1} in the Raman (Fig. 6 and Table IV). The 1053 cm^{-1} band of Dx/water system in the Raman spectrum corresponds to the 1029 cm^{-1} band in the FTIR spectrum. The Raman spectra of Dx/water system is distinguished by the presence of band at 553 cm^{-1}. Zhbankov et al.[31] (1997) have reported that the Raman spectra of α-D-glucose polymers (amylase, amylopectin, Dx, and pullulan) are characterized by an essential property in the region up to 600 cm^{-1} and are distinguished by the presence of strong bonds at 479–483 cm^{-1} (amylase, amylopectin), 543 cm^{-1} (Dx), and by the absence of such intense bands in the case of pullulan.

C—C—O and C—O—C bending vibrations may well be observed in far-Raman region and right at the outside of this region (<400 cm^{-1}). As a consequence of the H-bonding with etheric oxygen of OH groups of Dx both two bending vibrations are violated and subsequently, the spectrum shifts towards lower frequency values. In Raman study, a prominent peak is easily illustrated in this region at 549–553 cm^{-1}. The band appearing at 554 cm^{-1} for Dx/water system will shift towards upper-field at a magnitude of $\sim$ 5 cm^{-1} (Table IV).

In this study C—O rotation and C—C—O angle bending ($\sim$ 90 cm^{-1}) spectral evaluations highly illustrate the spectral distinctions in the behavior of Dx/water system and those cast from different crosslinker solutions. This characteristic band shifts toward lower

frequency values for the samples prepared in crosslinker medium (Table IV). The highest frequency shift value is displayed for the samples cast from GM. Within the measure of the H-bond formation it is expected that C—C—O angle bending vibrations will shift towards lower wavenumbers.

In spectra obtained from Dx sample in GM solution that was H-bond acceptors, the band sharpness and intensity has been increased (Fig. 5). As the solvent polarity increased, intermolecular H-bonding decrease and band assignments observed to shift downwards.

CONCLUSIONS

In the present study, the effects of different crosslinkers in aqueous solution on the dynamic behavior of Dx have been investigated. The interaction of ECH, MBAM, GA, and GM in aqueous solution with Dx was studied by viscometric and spectroscopic methods. The conclusion of viscometric studies indicate that this interaction is favored in the sequence of GM $<$ MBAM $<$ GA $<$ ECH. The decreasing order of interaction with crosslinker solutions of Dx has been clearly observed as MBAM $<$ GM $<$ ECH $<$ water $<$ GA thereby use of Raman and FTIR spectroscopy. When viscometric results are taken as the basis; GM appear to the best crosslinker whereas, according to spectroscopic results, MBAM is the best crosslinkers for Dx. On the other hand, GA and ECH are weakest crosslinkers for Dx. But they have dissolving capability of the polymer. As a consequence of the evaluation of viscometric and spectroscopic methods, MBAM and GM sometimes exchange positions during the crosslinking capability of the polymer or from time to time they seem to display similar crosslinking behaviors. We have provided only macroscopic information about this topic by viscometry. By using spectroscopic methods, molecular level information obtained and it was found a correlation of molecular and macroscopic level.

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