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ORIGINAL CONTRIBUTION

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Aqueous biphase formation by mixtures of dextran and hydrophobically modified dextran

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Abstract A series of modified dextrans was prepared by condensation of straight chain saturated C3, C4 and C6 fatty acids and the phase behavior of aqueous solutions of these materials with unmodified dextran was measured as a function of temperature, concentration and degree of substitution. At a constant degree of substitution the tendency towards aqueous biphase formation increased with the length of the hydrophobic substituent, the temperature and the molecular weight. Fluorescence studies of the modified dextrans with pyrene as a probe indicated the presence of hydrophobic micro-domains. Rheological study showed that there was no large-scale association for C3 and C4 substituted dextran, mainly intramolecular association, however some intermolecular association existed for C6 substituted dextran. The results are compared with the behavior of the classical PEG/dextran biphase systems, and mechanisms driving phase separation are discussed.

Key words Biphase formation – hydrophobic modification – dextran

Introduction

Aqueous mixtures of dextran and polyethylene glycol (PEG) form two dilute aqueous phases, one containing mainly PEG and the other aqueous dextran [1]. These aqueous biphase systems have been extensively studied because of applications in separations of biological materials [2, 3]. Dextran also forms aqueous biphases with poly(vinyl alcohol) and with block copolymers of PEG and poly(propylene oxide) (Pluronics). The factors influencing biphase formation which have been reported include the effects of salts, solvents [4–8], temperature [9–12], and molecular weight distributions [13–15].

Thermodynamic predictions of biphase formation have been based on group contribution methods [16] and variations of Flory Huggins theory [17–21]. Wennerström and coworkers state that since there is relatively little entropy cost in de-mixing macromolecules, biphase formation occurs when there are segment-to-segment repulsive interactions between the two types of polymer [17].

In this communication, we report the phase behavior of aqueous mixtures of dextran and hydrophobically modified dextran as functions of the degree and type of modification. This work provides background information for other adhesion studies of the interactions of two surfaces, one bearing dextran and the other bearing modified dextran. The ultimate goal is to link solution phase behavior reported herein with adhesive characteristics; this work is in progress.

The ternary system used in this work is dextran(DEX)/hydrophobically modified dextran(HDEX)/water. Modified dextran was synthesized by esterification of dextran with propionic acid (C3), butyric acid (C4) and hexanoic acid (C6). Using this approach a family of biphase systems was prepared by varying the properties of the hydrophobically modified dextran. This approach allows control of the phase boundary defining the onset biphase formation

as well as the solvency properties of the more hydrophobic phase. A couple of related biphase systems have been reported and, where possible, there are compared with the new results from this work. Hydroxypropyl-modified dextran is a biphase system when mixed with aqueous dextran [2]. Lu and coworkers have hydrophobically modified dextran by ester formation with pentanoic acid and have reported the biphase behavior for mixtures with aqueous PEG [22, 23].

Experimental

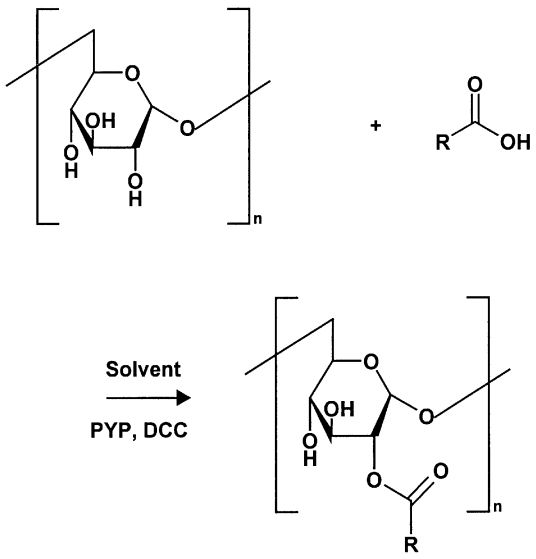
Materials

Analytical grade solvents were used as received. Millipore water was used for all experimental preparation. Two dextrans (Sigma) were used; the mass average molecular weights, given by the supplier were 74 000 and 167 000 Da. The dextran samples were dried under vacuum at 40 °C for 24 h before use. Formamide, N,N-dimethylformamide was from BDH. 1,3-dicyclohexylcarbodiimide (DCC), 4-pyrrolidinopyridine (PYP), PYP, DCC, and the carboxylic acids were obtained from Aldrich. Pyrene (Sigma) was recrystallized in ethanol before use.

Synthesis of modified dextran

Hydrophobically modified dextran (HDEX) was prepared by esterification of dextran with carboxylic acids in the mixture of formamide and N,N-dimethylformamide using the procedure reported by Bamford et al. [24] (see Scheme 1). In a typical reaction, dextran dissolved in a mixture 40/60 (v/v) of DMF and formamide to give an anhydroglucose concentration of 0.65 mol/l. PYP (0.025 mol/l), carboxylic acid (0.25 mol/l) and DCC were added in sequence. The DCC was added as a 50 wt% solution in dichloromethane to give a DCC concentration of 0.25 mol/l in the reaction mixture. The molar ratios of anhydroglucose units (MW = 162) to fatty acid in the reactions were 0.1, 1.0 and 1.1. The composition of the resulting HDEX was expressed as a degree of substitution, which was the moles of ester groups divided by the number of moles of anhydroglucose units.

DCC was dissolved in dichloromethane to form 50 wt% solution and added dropwise into the flask. The reaction was conducted at 30 °C for 16 h with stirring, after which the reaction mixture was filtered to remove the dicyclohexylurea, formed by DCC during the reaction. The product was precipitated by acetone and washed four times in acetone to remove unreacted carboxylic acid and solvent. The product was vacuum dried at 40 °C and stored at 5 °C.



Scheme 1 Reaction route for the synthesis of modified dextran. The ester is shown to form on the C2 carbon of the anhydroglucose ring. The R groups are hydrocarbon chains with 2, 3, and 5 carbons

Six samples of modified dextran were prepared from two dextran and three fatty acids. The compositions are summarized in Table 1.

Characterization of modified dextran

FTIR was carried out to confirm ester formation after reaction of DEX with fatty acids. Samples of KBr pellet were prepared. Infra-red spectra were recorded on a Bio-Rad FT S-40 FTIR spectrometer from 400 to 4000 nm with a resolution of 8.

A Bruker AC 200 NMR Spectrometer was used to record both ^{13}C and ^{1}H NMR spectra. Degrees of fatty acid substitution on DEX were determined by proton spectra of NMR. ^{1}H chemical shifts were reported relative to the HDO peak at 4.6 ppm.

Molecular weight distributions were obtained by gel permeation chromatography using a Waters 401 Differential Refractive index detector. The column used was TSK gel PW_{XL} 4000 and 3000 with the temperature at 40 °C. Water was used as solvent with a flow rate of 0.5 cm^3/min. Calibration curves were obtained from poly(ethylene glycol) and glucose. Mass average molecular weights estimated from the GPC traces were about 2/3 of the LALLs values reported by Sigma. The GPC curves showed no evidence of either scission or crosslinking due to conversion of DEX to HDEX.

The hydrophobic characteristics of the modified dextrans were probed by fluorescence measurements using

Table 1 Properties of modified dextrans

Sample code	Parent DEX molecular weight	Feed composition[a)]	% Acid conversion	Degree of substitution[a)]	Fraction C2[b)]
C6-HDEX 9–23	74000	2.8	64	0.23	0.5
C4-HDEX 11–20	74000	1.8	67	0.37	0.68
C3-HDEX 10–31	74000	1.2	73	0.61	0.52
C6-HDEX 6–22	167000	2.6	59	0.23	0.53
C6-HDEX 7–23	167000	3.2	58	0.18	0.60
C6-HDEX 7–12	167000	4.2	46	0.11	0.75

[a)] Moles of fatty acid/moles of anhydroglucose units.
[b)] Fraction of total number of ester groups which are on C2.

pyrene as a probe. Samples were prepared using filtered (45 μm Millipore) stock solution of water saturated with pyrene. Steady-state fluorescence spectrums were recorded with an Aminco Luminescence Spectrometer (SLM Instruments Co.) at 23 °C and 50 °C using an excitation wavelength 330 nm. The excitation slit width is 4 and 1 for excitation and emission, respectively. The ratio of the intensities, I1/I3, was used as an indication of the polarity of the environment surrounding the pyrene probes [25].

A Bohlin VOR Rheometer (Bohlin Instruments) fitted with a C14 concentric cylinder and a 1.6 g cm torsion bar was used to measure viscosity. The experiments were conducted at 25 °C at constant shear rate of 20 s^{-1}.

Densities were measured with a DMA 45 (AP PAAR) vibrating U tube densitometer. Temperatures were maintained at 25 °C controlled with a water bath (RM6 LAUDA, Brinkmann).

Phase information

Cloud points were determined by titration of DEX solutions with HDEX until the onset of visible turbidity. Binodal curves were constructed from sets of cloud point data collected as a function of the initial DEX concentration.

Tie lines were determined by mixing polymer solutions in separatory funnels and after 24 h equilibration, samples from each phase were removed for analysis. Experiments with C6-HDEX were performed at room temperature (~23 °C) and 50 ± 0.5 °C.

NMR was used to determine the molar ratio of two polymers in each phase. The total concentration of carbohydrate in each phase was determined by optical rotation (Perkin-Elmer 241 MC). Calibration curves of rotation vs. concentration were made for each polymer. Control experiments confirmed that for mixtures the optical rotation was a linear combination of the contribution of DEX and HDEX. The weight fraction of polymers in each phase was calculated from the feed composition and composition of upper and lower phases.

Results

A series of hydrophobically modified dextrans (HDEX) was prepared by esterification of dextran (DEX) with propionic acid (C3), butyric acid (C4) or hexanoic acid (C6). The chemistry of the modification is summarized in Scheme 1 and the properties of the modified polymers are summarized in Table 1. The esters had characteristic IR adsorption bands at 1735 and 1260 cm^{-1}. The esterification yields, based on fatty acid feed, ranged from 50% to 73%. The corresponding degrees of substitution (i.e. the number of ester groups per anhydroglucose ring) ranged from 0.1 to 0.6.

^{1}H-NMR was used to measure the composition of purified HDEX polymers. Figure 1 shows spectra for C6-HDEX together with the parent DEX. Peaks centered at 4.8, 3.8, 3.5, 3.4 ppm, were due to anhydroglucose protons and the peak at 4.8 ppm was due to the anomeric proton on C_1 [26]. After modification new peaks appeared at 0.7, 1.1, 1.5, and 2.3 ppm for hexionate modified dextran; the peak assignments are shown in Fig. 1 [27].

HDEX showed two anomeric proton peaks, one at 4.8 ppm corresponding to DEX and the other at 4.9 ppm. The latter peak was assigned to anomeric protons (C_1) in which the hydroxyl on the neighboring C_2 carbon reacted to form an ester with the fatty acid [28]. The fraction of the total ester groups that were on C_2 was calculated from the total degrees of substitution and relative areas of the 4.8 and 4.9 peak. The results listed in Table 1 indicate that

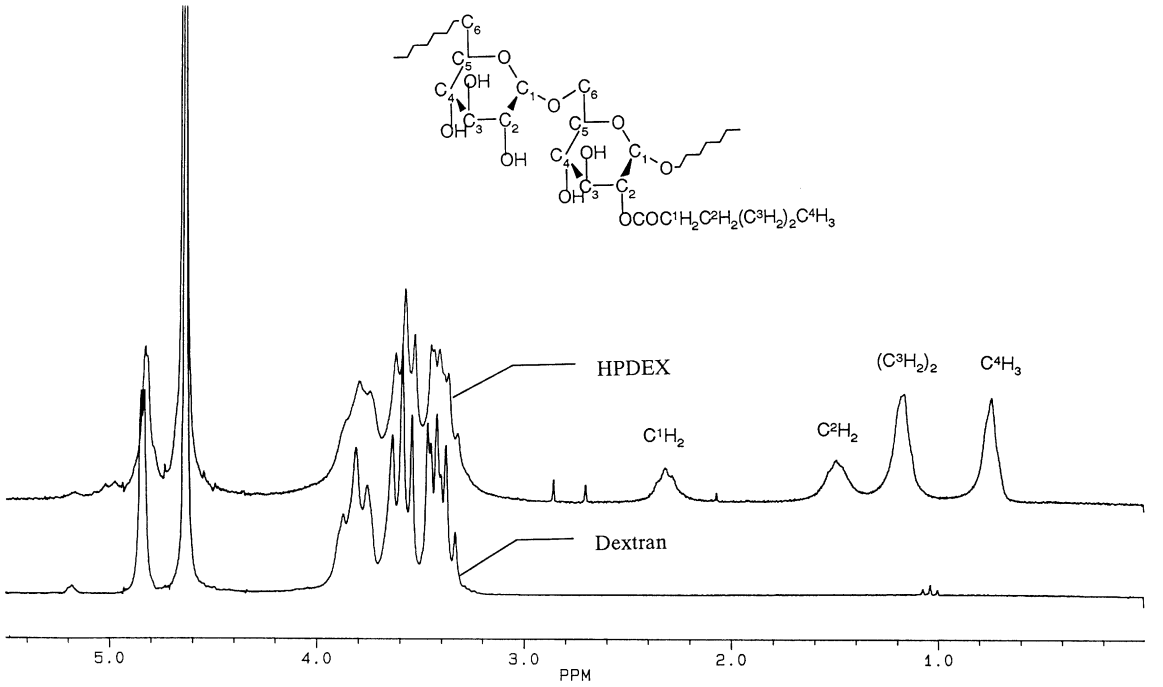


Fig. 1 ^{1}H-NMR spectra of C6-HDEX and DEX dissolved in D_2O

esterification preferentially occurred on C2; this is consistent with published information [12, 29].

The solubility of modified dextran in water varied with the degree of fatty acid substitution (DS) and the chain length of substitute. Preliminary work showed that the maximum DS values while maintaining water solubility were 0.26 ± 0.02 for C6 and 0.50 ± 0.02 for C4 modified dextran. These values seem in accord with literature values of 0.81 and 0.69 with ethyl and butyl carbonate substituted dextran [30].

Fluorescence was used to probe hydrophobicity of HDEX. Unmodified dextran is a highly hydrophilic polymer with I1/I3 = 2.3. By contrast, the modified HDEX solutions had I1/I3 values as low as 1.3. For comparison, sodium dodecyl sulfate micelles gave an I1/I3 value of 1.21 in our laboratory. Figure 2 shows results for three types of HDEX with various DS and at various concentrations. When plotted as I1/I3 vs. the molar concentration of fatty acid carbons, the C3 and C4 modified dextrans fell on the same curve whereas the C6 solutions gave low I1/I3 values at lower concentrations. The I1/I3 results were not sensitive to temperature. For example, for C6-HDEX the values were 1.47 and 1.45 at 25 °C and 50 °C, respectively.

Hydrophobically modified water-soluble polymers have the potential to form intermolecular aggregates [31, 32]. Such behavior is usually evidenced by high viscosities. Reduced viscosities of DEX and HDEX are shown as a function of concentration in Fig. 3. The reduced viscosity of C6 modified dextran showed a much stronger dependency on concentration than did DEX, C4-HDEX or C3-HDEX. Results for C3-DEX were slightly above DEX whereas at high concentrations the viscosities of C4-HDEX were slightly less than DEX.

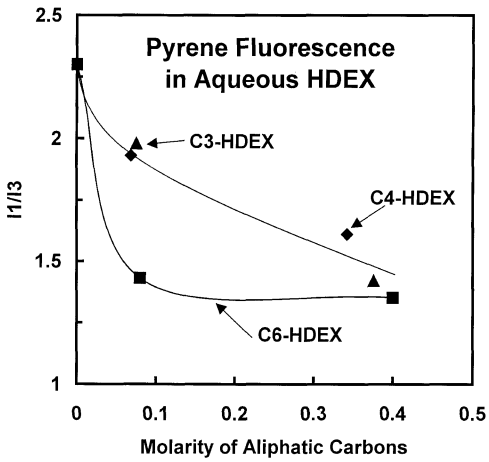


Fig. 2 The influence of fatty acid carbon concentration (not including carbonyl carbon) on the I1/I3 value for HDEX. The polymers were 9–23, 11–20 and 10–31; see Table 1 for polymer properties

Figure 4 shows part of the binodal phase boundary for two ternary systems both containing water, C6-HDEX and DEX. The two curves correspond to low and high

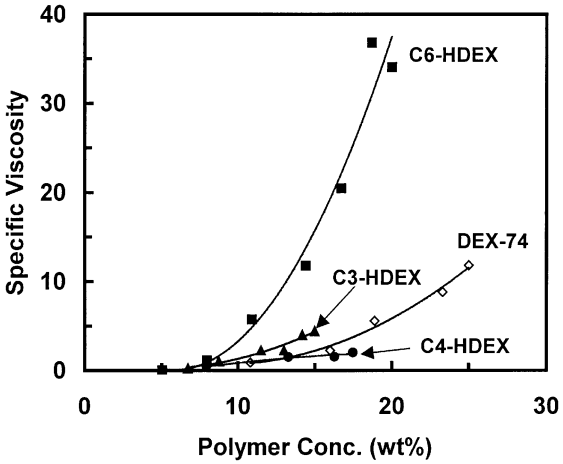


Fig. 3 The specific viscosity of aqueous HDEX as functions of concentration at 25 °C with a shear rate of 20 1/s. The polymers were 9–23, 11–20 and 10–31

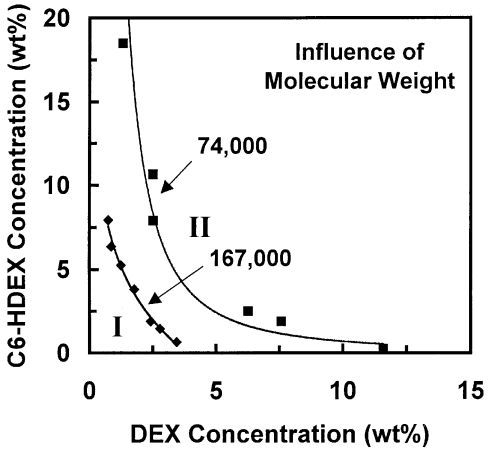


Fig. 4 Phase boundaries for C6 modified dextran. For both systems the DS was 0.23 and the temperature was 23 °C. The labels denote the molecular weight of the parent dextrans. The I region corresponds to a single phase region and II the biphase region

molecular weight dextrans, and the corresponding modified dextrans. In both cases biphase formation occurred at low polymer concentrations. However, the higher molecular polymers phase separated at concentrations a few weight percent lower than the lower molecular weight pair.

Biphase formation was not observed for low degrees of substitution with short chains. For example, with C4-HDEX/DEX-74/water was one phase for DS values less than 0.32 ± 0.02 for total polymer concentrations less than 35 wt%. Similarly, C3-HDEX/DEX-74/water formed a single phase up to DS values 0.60 ± 0.02, the highest that we were able to synthesize.

The compatibility of dextran with modified dextran decreased when the degree of hydrophobic modification was increased. The binodal curves for the DEX-176/C6-HDEX-176/water system, shown in Fig. 5, illustrate this point. Similarly, the results in Fig. 6 show that the two polymers become less compatible when the hydrophobic chain length is increased. For the results in Fig. 6 the single phase region for the C6-HDEX is smaller than that of the C4-HDEX even though the C4 containing polymer had nearly twice as many grafted chains (i.e. 0.37 vs. 0.23).

The temperature sensitivity of the DEX-74/C6-HDEX/water system was studied and the results are summarized in Fig. 7. Comparing 23 °C to 50 °C there was little difference in the position of the binodal curves. However the slopes of the tie lines were greater at high temperature, indicating a greater partitioning of water into the dextran phase. Similar behavior has been reported in the PEG/DEX/water system [11] and the explanation is that water becomes a poorer solvent with increasing temperature for polymers containing small hydrophobic domains such as PEG or HDEX [33, 34].

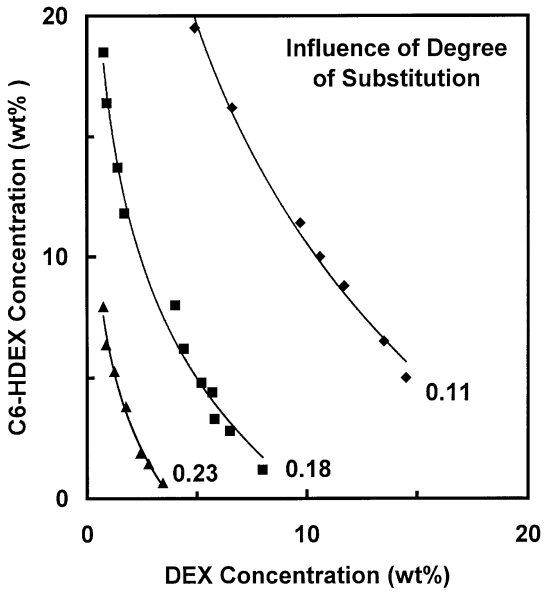


Fig. 5 The influence of the degree of hydrophobic modification on the DEX-76/C6-HDEX/water binodal curves at 23 °C

Some of phase composition and density data are given in Table 2. In the DEX-74/C6-HDEX results, the upper phases were more dilute and DEX rich whereas the lower phase was concentrated and HDEX rich. The density difference between the two phases was in the second decimal place. By contrast, in the HDEX/C4-HDEX results, the upper phase was HDEX rich, the total concentration was slightly higher than the lower phase, and the density differences were in the third decimal place. It seems

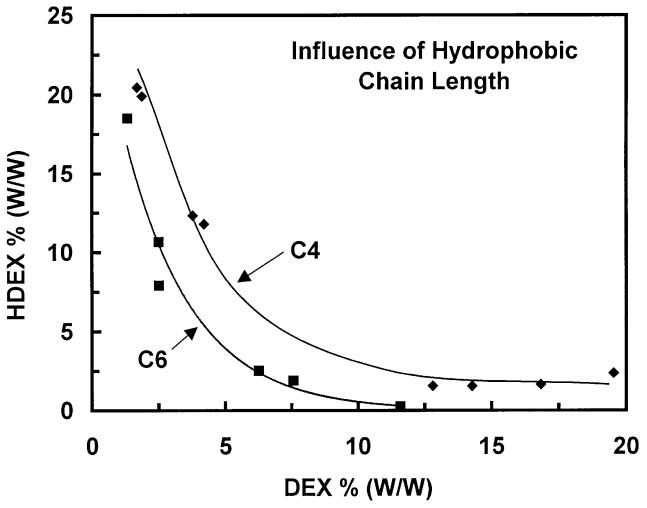


Fig. 6 The influence of hydrophobic chain length on phases separation. The systems were aqueous mixtures of DEX-74/C4-HDEX (11–20, DS = 0.37) and DEX74-/C6-HDEX (9–23, DS = 0.23) both at room temperature

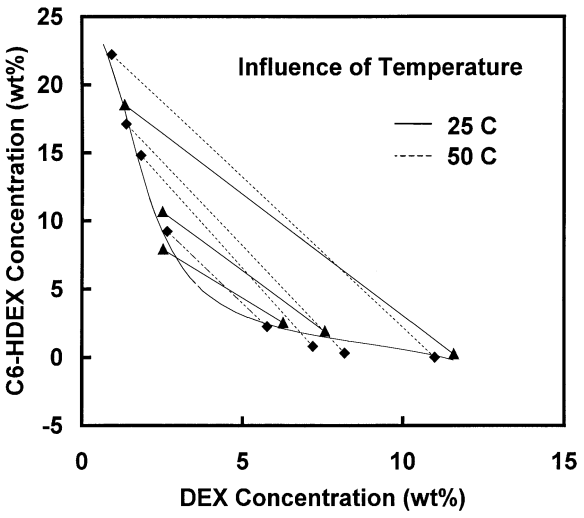


Fig. 7 Phase diagrams for DEX-74/C6-HDEX/water at two temperatures. The degree of substitution of C6-HDEX was 0.23

reasonable to speculate that the HDEX/C4-HDEX system is near a transition after which no biphase separation occurs and that HDEX/C3-HDEX is beyond the transition and thus does not phase separate.

Concluding remarks

The objectives of this work were to prepare a family of polymer pairs in which the compatibly in aqueous solution could be varied in subtle ways. The HDEX/DEX/water systems fulfill these requirements. By varying either the hydrophobic chain or the degree of substitution on HDEX, the compatibility with DEX can be modified. This system is interesting because the chemical structures of DEX and HDEX are so similar. Indeed by employing butyric acid (C4) for the modification, the densities and total concentrations of the equilibrium phases are also nearly equal.

Table 2 Composition of density of upper and lower phases

Upper phase			Lower phase		
HDEX [wt %]	DEX [wt %]	Density [g/cm^3]	HDEX [wt %]	DEX [wt %]	Density [g/cm^3]
C6-HDEX(9–23) and DEX-74 at 23 °C					
0.25	11.59	1.0466	18.5	1.32	1.0601
2.52	6.27	1.0296	7.9	2.52	1.0455
1.89	7.57	1.0338	10.66	2.5	1.0413
C6-HDEX(9–23) and DEX-74 at 50 °C					
0	11.0	1.0411	22.2	0.92	1.0666
0.3	8.19	1.0306	17.1	1.37	1.0536
0.8	7.20	1.0314	14.8	1.83	1.0484
2.22	5.77	1.0269	9.21	3.25	1.0383
C4-HDEX(11–20) and DEX-74 at 23 °C					
11.78	4.16	1.0492	1.55	12.81	1.0519
19.89	1.87	1.0645	1.64	16.84	1.0685
20.44	1.68	1.0743	2.37	19.54	1.0834
12.31	3.78	1.0563	1.53	14.28	1.0587

Associative thickener structures such as hydrophobically modified cellulose have been extensively reported in the recent literature. These molecules employ a few (≤ 10 hydrophobes per polymer chain), long (more than 10 aliphatic carbons) hydrophobes [35]. By contrast, the HDEX prepared in this work have about one short (i.e. less than 6 aliphatic carbons) hydrophobic chains for every two anhydroglucose rings. Viscosity and pyrene fluorescence results indicate possible intermolecular association with C6-HDEX, whereas the rheological behavior of C4-HDEX and C3-HDEX were similar to the parent dextran, indicating no intermolecular association. Similarly, the GPC traces of C3 and C4 modified dextran were similar to the parent dextran, indicating that the short hydrophobic chains did not significantly influence the polymer configuration.

The compatibility of HDEX with DEX was characterized in terms of the tendency for aqueous biphase formation. Most of the results were consistent with intuition. Increasing the molecular weight, the hydrophobic chain length or the degree of substitution increased the tendency for biphase formation. The behaviors of HDEX/DEX/water were also consistent with other DEX biphase systems. Figure 8 compares the phase behavior of the present system with two other dextran-based biphase systems

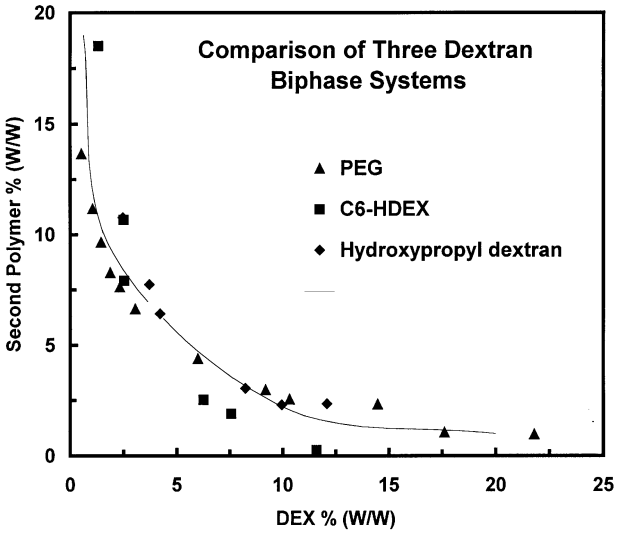


Fig. 8 Comparison of phase boundaries of three dextran-based biphase systems. The PEG/DEX and hydroxypropyl-DEX/DEX data were taken from Albertsson [2]

taken from the literature. Therefore, it seems reasonable to conclude that the factor governing phase separation in the HDEX/DEX/water are similar to the other biphase systems.

The main conclusions from this work are:

1) The addition of small hydrophobic groups to dextran decreases the affinity of the polymer for water. The modified dextrans become water insoluble when the degree of substitution is around 0.50 ± 0.02 and 0.26 ± 0.02 for C4 and C6 substituted dextran at the molecular weight of 167 000.

2) Pyrene partitions into hydrophobic domains in aqueous solution of HDEX. C3 and C4 modified dextran did not display evidence for intermolecular association whereas C6 modified dextran did.

3) Aqueous mixtures of dextran and hydrophobically modified dextran display aqueous biphase formation at polymer concentrations greater than 5–10%. The tendency for phase separation increases with the molecular weight, degree of substitution and the hydrophobic chain length.

4) Increasing temperature from 23 °C to 50 °C drives water from the HDEX-rich phase to the dextran-rich phase.

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