SOLVENT MODULATION OF POLYSACHARIDE CONFORMATION

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Introduction

Polymers are widely used in solution to provide structure and function, e.g., viscoelasticity, colloidal stability, and delivery of actives. Underlying these functions are inter- and intra-molecular interactions that are modulated by the solvent used. Solvents can have a great impact on polymer conformation [Alexandridis and Yang, 2000]. A solvent may enable the dissolution of a certain polymer or cause a polymer to phase separate depending on the interactions between the polymer and the solvent molecules. Addition of a second solvent (cosolvent) in a polymer solution can enhance or reduce the polymer solubility in the solution. An important motivation to consider mixtures of solvents is to enhance the solubility of substances that may be insoluble in a neat solvent [Lin and Alexandridis, 2002]. This is especially applicable in the pharmaceutical industry.

Our group has a longstanding interest in the conformation of polymers as influenced by the solvents in which they are dissolved [Alexandridis, 1998; Alexandridis and Yang 2000; Alexandridis, 2006]. We recently considered the common polysaccharide dextran in single polar solvents (water, formamide, dimethylsulfoxide, ethanolamine), and found hydrogen bonding to be an important interaction contributing to the solubility of dextran [Antoniou et al., 2008 (a)]. We further investigated the dextran coil conformation in binary mixtures of a good solvent (water, formamide, or ethanolamine) and a non-solvent (ethanol) as a minor component [Antoniou et al., 2008 (b)]. The observed effects were related to the fractional solubility parameters of the solvent mixture, and, again, hydrogen bonding emerged as a main interaction between the polymer and the molecules of the mixed solvent. Here we address binary mixtures of water and a polar organic solvent (ethanol, glycerol, formamide, or dimethylsulfoxide). The solvent effects on the dextran coils are discussed in terms of intermolecular interactions and are correlated to the solvent cohesive forces expressed through their surface tension.

Materials & Methods

Materials

We consider the T500 dextran fraction, with weight average (M_w) and number average (M_n) molecular weights 500,000 and 191,500 respectively, purchased from Amersham Biosciences AB (Uppsala, Sweden) and Pharmcosmos A/S (Holbaek, Denmark). Dimethylsulfoxide (DMSO) and glycerol (purified grade, 99% min) were purchased from Fisher

Scientific (Fair Lawn, NJ). Formamide (molecular biology grade) was purchased from VWR International (West Chester, PA) and ethanol from Decon Labs, Inc. (King of Prussia, PA). The water used was purified with a MilliQ system. Given volumes of water with one of the following polar organic solvents: glycerol, formamide, DMSO and ethanol were mixed to create a mixed solvent of desired volume % composition. Individual dextran samples were prepared for every concentration by dissolving appropriate amount of polymer in the given solvent.

Methods

The viscosity of dilute dextran solutions was determined using Cannon Fenske Routine type viscometers [Hiemenz and Rajagopalan, 1997; Sperling, 2001]. Dextran solutions exhibit Newtonian flow characteristics [Tirtaatmadja et al., 2001]. The viscosity data analysis procedure has been presented previously [Antoniou et al., 2005]. Briefly, focusing on data in the dilute regime, we obtain the intrinsic viscosity for dextran and extract the coil dimensions using the Einstein viscosity relation (see below). The intrinsic viscosity [η] of dextran in different solvents is determined using the Huggins (1) and Kramer (2) equations [Flory, 1953; Sperling, 2001] by plotting η_{sp}/C and $\ln(\eta_{rel})/C$, respectively, against the polymer concentration, and subsequent extrapolation to zero concentration (infinite dilution).

$$\eta_{sp}/C=[\eta] + k'[\eta]^2C \qquad (1)$$

$$(\ln \eta_{rel})/C=[\eta] - k''[\eta]^2C \qquad (2)$$

η_{rel} is the relative viscosity ($\eta_{rel}=\eta/\eta_0$, η and η_0 are the kinematic viscosity of the solution and the pure solvent, respectively, in mPa·s or cSt), η_{sp} is the specific viscosity ($\eta_{sp}=\eta_{rel}-1$), $k'$and k'' are the Huggins and Kramer constants, respectively, and C is the concentration (g/dl) of the polymer solution.

Dextran coil adopts the random coil conformation in single solvents as established previously [Antoniou et al., 2007]. The volume of dextran coils V_{coil} is determined using [η] and the Einstein equation (3) for the contribution of dispersed spheres to the solution viscosity [Sperling, 2001]. A hydrodynamic sphere of volume V_{coil} is used to represent the polymer coil:

$$\eta-\eta_o=2.5\eta_o\Phi \qquad (3)$$

$$[\eta]=2.5\Phi/C \text{ or } [\eta]=2.5N_{AV}V_{coil}/M_w \qquad (4)$$

where Φ = volume fraction of dispersed phase, C = polymer concentration, V_{coil} = volume of a spherical particle, and N_{AV} = Avogadro's number.

Results & Discussion

Solvent Quality

An initial assessment of solvent quality was made using the interaction parameter, χ_{12}, and the free energy of interfacial interaction, ΔG_{121} (**Table 1**). χ_{12} values for the different single solvents were determined following procedures in [Hansen, 2000] outlined below. Using the Hansen solubility parameters (δ_D, δ_P δ_H) we first calculate the solubility parameter distance Ra:

$$(Ra)^2 = 4(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2$$

The radius of interaction sphere in the Hansen space, Ro, is determined using the computer generated RED (Relative energy difference: RED=Ra/Ro) values given by Hansen and the calculated Ra values: Ro=Ra/RED. The distance in Hansen space is RA=Ra/2, the maximum distance in Hansen space allowing solubility is R_M=Ro/2, the hydrogen bonding cohesion (solubility) parameter equals $H=(RA/R_M)^2$, and finally $\chi_{12}=H/2$.

A polymer dissolves in a solvent when their χ_{12} value is lower than 0.5. We observe in **Table 1** that the solvents with χ_{12} parameter values close or lower than the 0.5 in which dextran is expected to dissolve in are glycerol, formamide, and DMSO. The value of the dextran-ethanol χ_{12} parameter was significantly higher than 0.5, which shows ethanol to be a bad solvent.

Another approach for predicting interactions between molecules of dextran (1) immersed in a solvent (2) is the van Oss [1994] analysis. The estimated free energy of interfacial interaction, ΔG_{121}, when positive, suggests that molecules (1) will repel each other in solvent (2) and will spontaneously dissolve in it. If ΔG_{121} is negative, then molecules (1) will attract each other in solution, and so will tend to precipitate from the solvent (2) [van Oss, 1994].

The surface tension (or surface free energy per unit area), γ_i, of a liquid or solid is defined as half of the free energy change due to cohesion of the material in vacuo, ΔG_{ii}.

$$\gamma_i = -\tfrac{1}{2}\Delta G_{ii}$$

Since the free energy of cohesion is contributed by a number of more or less independent forces, the surface tension can also be broken down into its separate components, i.e.:

$$\gamma_i = \Sigma\, \gamma_i^j$$

where j stands for dispersion, dipolar, induction, H-bonding and metallic interactions, and γ_i^j stands for the component of the surface tension arising from the j'th type of interaction.

The free energy of interfacial interaction, ΔG_{121}, between molecules or particles of substance (1), immersed in liquid (2), comprises of the additive apolar, ΔG^{LW}, and polar, ΔG^{AB}, free energy components. The apolar component describes the Lifshitz-van der Waals (LW) interactions, and the polar component the electron-acceptor – electron-donor interactions (AB) [van Oss, 1994].

$$\Delta G = \Delta G^{LW} + \Delta G^{AB}$$

Given that: $$\Delta G_{ii} = -2\gamma_i$$

And $$\gamma_i = \gamma_i^{LW} + \gamma_i^{AB}$$

Then:

$$\Delta G_{121} = -2\gamma_{12}$$
$$\gamma_{12} = \gamma_{12}^{LW} + \gamma_{12}^{AB}$$
$$\gamma_{12}^{LW} = (\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}})^2$$
$$\gamma_{12}^{AB} = 2(\sqrt{\gamma_1^{+}} - \sqrt{\gamma_2^{+}})(\sqrt{\gamma_1^{-}} - \sqrt{\gamma_2^{-}})$$

So: $$\Delta G_{121} = -2(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}})^2 - 4(\sqrt{\gamma_1^{+}}\cdot\sqrt{\gamma_1^{-}} + \sqrt{\gamma_2^{+}}\cdot\sqrt{\gamma_2^{-}} - \sqrt{\gamma_1^{+}}\cdot\sqrt{\gamma_2^{-}} - \sqrt{\gamma_1^{-}}\cdot\sqrt{\gamma_2^{+}})$$

where γ^+ and γ^- are electron-acceptor and electron-donor parameters of the surface tension of substances 1 and 2.

According to the ΔG_{121} values resulting from this method (**Table 1**), dextran is expected to dissolve in formamide, DMSO or water (the ΔG_{121} values are positive), but not in ethanol or glycerol (negative values). These two different approaches (Hansen [2000] and van Oss [1994]) concur that formamide, DMSO and water are good solvents for dextran and ethanol is a bad solvent. Glycerol appears to be a good solvent on the basis of the χ_{12} data and a bad solvent if we use the ΔG_{121} values. However, the χ_{12} value of glycerol 0.491 is very close to the limiting value of 0.5, and the ΔG_{121}= -2.11 is relatively close to 0 in comparison to the ΔG_{121} value for ethanol -9.66. Clearly glycerol is a better solvent for dextran than ethanol.

Mixed solvents

Water, ethanol, glycerol, and formamide are protic, hydrogen bond donor (donor groups: -O-H or >N-H) strongly associated solvents [Reichardt, 1990], and DMSO is an aprotic, highly dipolar H-bond acceptor solvent (acceptor group: S=O). The use of mixed solvents entails knowing their properties as a function of the composition. Some physical, chemical and thermodynamic properties of certain mixed solvents have been measured [Reichardt, 1990], and equations have been generated to estimate solvent properties in terms of composition [Marcus, 2002]. In order to rationalize the solubility of dextran in mixed solvents we use here the surface tension of the solvent mixture and examine its relationship to the dextran solution properties.

Table 1: Hildebrand (δ), and Hansen (δ_D, δ_P δ_H) solubility parameters of the single solvents and polymer used in this study, free energy of interfacial interaction, ΔG_{121}, estimated with the van Oss [1994] procedure for the single solvents used in this study, and Flory-Huggins parameter, χ_{12}, estimated using the Hansen [2000] procedure and data.

Solvent	δ	δ_D	δ_P	δ_H	ΔG_{121}	χ_{12}
Dimethylsulfoxide	26.7	18.4	16.4	10.2	4.51	0.500
Formamide	36.7	17.2	26.2	19.0	6.67	0.419
Glycerol	36.2	17.4	12.1	29.3	-2.11	0.491
Ethanol	26.5	15.8	8.8	19.4	-9.66	0.696
Water	47.8	15.5	16.0	42.3	41.24	
Dextran	38.6	24.3	19.9	22.5		

Hydrodynamic Coil Volume

Dextran adopts a random coil conformation in water and in polar organic solvents such as formamide [Antoniou et al., 2007], and DMSO [Çatiker and Güner, 1998], as attested by analysis of viscosity [Güner, 1999; Antoniou et al., 2005] and small angle x-ray scattering data [Antoniou et al., 2007]. We recently investigated the conformation of dextran T500 in the single solvents water, formamide, DMSO, and ethanolamine [Antoniou et al., 2008(a)]. We found that the ability of a solvent to increase [η] and expand the dextran coils increases in the order: water < formamide < dimethylsulfoxide.

For the four binary solvent systems of water mixed with formamide, glycerol, DMSO, or ethanol considered here, we observe three trends in the coil volume: the dextran V_{coil} increase (positive slope), decrease (negative slope), or change non-linearly, depending on the organic solvent added in the aqueous solution. The dextran T500 V_{coil} are plotted against the organic solvent content (volume % or mole %) in the mixture in Figures 1 and 2. Higher V_{coil} values indicate more expanded coils.

More specifically, dextran T500 coils expand from 16.2×10^3 nm^3 in water to 22.1×10^3 and 18.1×10^3 nm^3 with addition of formamide (up to 100 v/v %) and glycerol (up to 25 v/v%), respectively. These are cases where the quality of the mixed solvent increases with cosolvent content. Addition of formamide expands the dextran coil linearly over the 0-100 v/v% formamide range. Glycerol is a rather viscous solvent (dextran dissolved very slowly in pure glycerol) and that is why we examined mixed solvents containing only up to 25 v/v% glycerol. An assessment of solvent quality by comparing the dextran dimensions at 25 v/v% cosolvent shows the aqueous glycerol mixture to be a better solvent for dextran compared to aqueous formamide mixture. The V_{coil} versus mole % content plot (Figure 2) shows steeper slopes than the V_{coil} versus v/v% plot (Figure 1). Glycerol as a cosolvent increases the solvent quality of water more than formamide.

A non-linear trend is observed in DMSO-water mixtures. The solvent power remains roughly constant up to approximately 50 v/v% DMSO in the mixture, as reflected in small variation in the dextran coil volume. Above 50 v/v% DMSO, the dextran T500 coils expand to V_{coil} values (26.5×10^3 nm^3) that are much higher than those in pure water or formamide, thus the solvent quality increases significantly.

The addition of ethanol (bad solvent for dextran according to $\chi_{12}>0.5$ and $\Delta G_{121}<0$ (Table 2)) had the opposite effect on V_{coil} from the cosolvents (good solvents) examined above. The dextran coils contract considerably when up to 30 v/v% ethanol is added to water: V_{coil} decreases from 16.2×10^3 to 9.4 nm^3. Ethanol decreases significantly the solvent power of water. Our experiments indicated that dextran did not dissolve in aqueous mixtures containing more than ~35 v/v% ethanol, presumably because the ethanol-water mixed solvent becomes a non-solvent for dextran at these conditions.

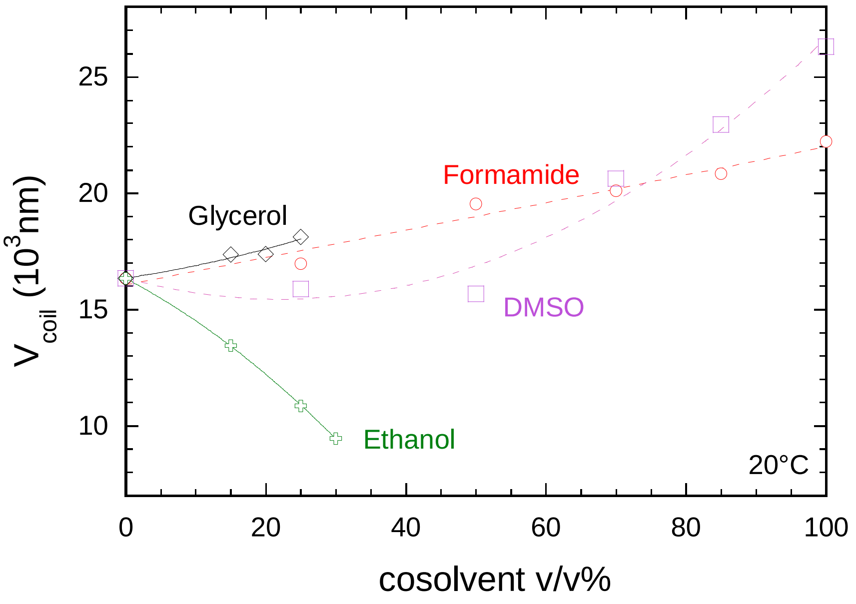

Figure 1: Dependence of dextran T500 coil radius, R_{coil}, on the content (v/v%) of polar organic solvent (glycerol, formamide, dimethylsulfoxide or ethanol) in aqueous solvent mixtures at 20^{o}C.

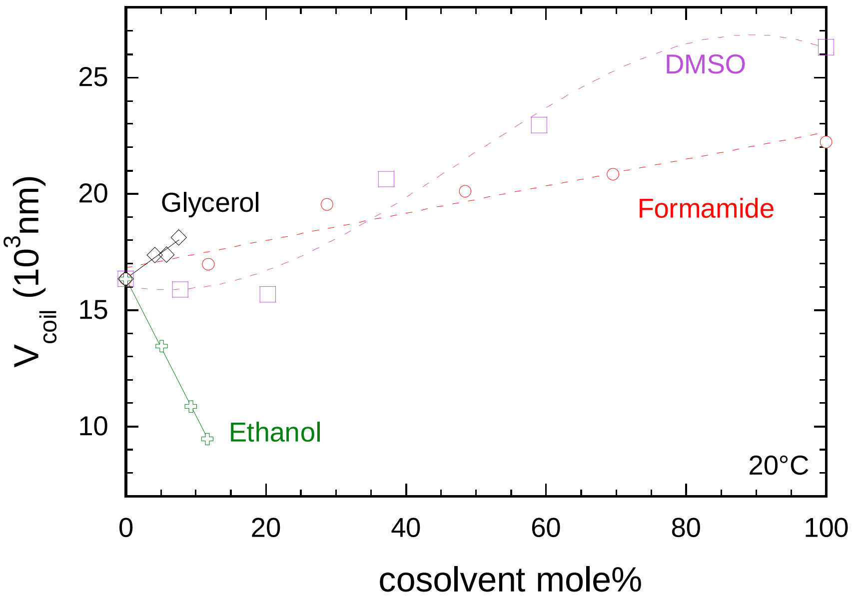

Figure 2: Dependence of dextran T500 coil radius, R_{coil}, on polar organic solvent (glycerol, formamide, dimethylsulfoxide or ethanol) content (mole %) in aqueous solvent mixtures at 20^{o}C.

Dextran structure - solvent property correlation

In order to rationalize the solubility of dextran in mixed solvents we used surface tension, σ, (estimated according to [Reichardt, 1990]) as the property to examine its relationship to the dextran V_{coil}.

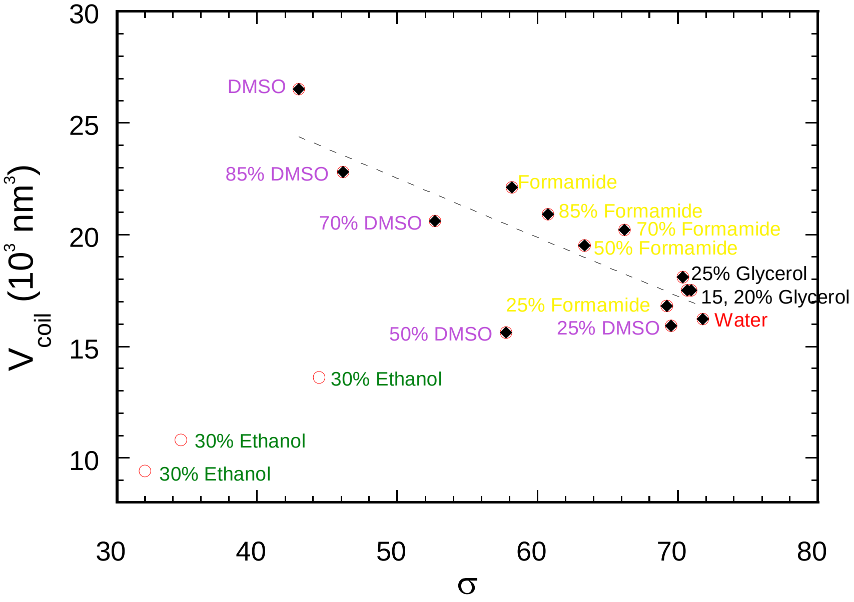

Figure 3: Coil volume, V_{coil}, of dextran T500 plotted vs. the surface tension, σ, of the mixed solvents at 20°C. The dotted lines are linear fits to the data indicated by the filled symbols.

In Figure 3 we observe that the V_{coil} of dextran increases while σ decreases for solvents like formamide, glycerol and DMSO that enhanced the solvent quality of water. In the case of ethanol addition where the solvent power of water decreased, V_{coil} decreased when surface tension decreased. Surface tension describes cohesive forces between the solvent molecules. For the first case it is possible that the weaker the cohesive forces between the solvent molecules, the easier for dextran to break them and form hydrogen bonds with the solvent. For the case of aqueous ethanol mixtures that do not follow the same monotonic relationship as the other solvent mixtures, dextran coils do not expand probably because the ethanol molecules enhance the water structure around the coil.

A correlation between dextran solution conformation and cohesive energies (manifested in σ) of the mixed solvents considered in this work has been established. A similar correlation between R_{coil} and the hydrogen bonding Hansen solubility parameter δ_H (which also expresses cohesive forces [Hansen, 2000]) was observed in our previous work done on dextran in single solvents (water, formamide, DMSO, and ethanolamine) [Antoniou et al., 2008(a)]. Cohesive forces emerge as important interactions between dextran and the solvent molecules.

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