Carbohydrate Polymers 74 (2008) 372–378

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis and characterization of blue dextrans

David Viet [b], Stephanie Beck-Candanedo [c], Derek G. Gray [a,*]

[a] Department of Chemistry, Pulp and Paper Research Centre, McGill University, Montréal, Qué., Canada H3A 2A7
[b] UMR 6517, CNRS et Universités Aix-Marseille I, II and III Avenue Escadrille Normandie Niemen, Case 542, 13397 Marseille Cedex 20, France
[c] Department of Chemical Pulping, FPInnovations Paprican, 570 Boulevard St.-Jean, Pointe-Claire, Qué., Canada H9R 3J9

ARTICLE INFO

Article history:
Received 14 December 2007
Received in revised form 4 March 2008
Accepted 5 March 2008
Available online 12 March 2008

Keywords:
Dextran
Cibacron blue dye
Polyelectrolyte
Physicochemical properties
Intrinsic viscosity
Molecular weight

ABSTRACT

Blue dextrans of varying molecular weights and dye ligand densities have been synthesized and characterized. Reaction conditions, particularly time and dye and base concentrations, have a significant effect on the reaction products. Gel permeation chromatography (GPC) confirms the purity and narrow molecular weight distribution of the products. Blue dextrans are polyelectrolytes due to the ionic dye ligands, and this strongly influences their properties in aqueous solution. The intrinsic viscosity decreases with increasing solvent ionic strength.

1. Introduction

Dextran is a polymer of α-D-(1,6)-linked anhydroglucose containing ~5% α-(1,3) branching, produced for commercial use by strains of *Leuconostoc mesenteroides* bacteria (de Belder, 2003; Evans & Hibbert, 1946). Blue dextran is a soluble dextran derivative consisting of the triazinyl dye Cibacron blue F3GA covalently bound to high molecular weight (2000 kDa) dextran (Sigma-Aldrich, 1997). It was developed for use as a visible marker for void volume in gel filtration chromatography columns (Dean & Watson, 1979; Subramanian, 1984). Other reactive dyes have also been used to derivatize polysaccharides, including the triazine dyes of the Procion series (Lowe & Pearson, 1984). Blue dextran and other dextran derivatives such as FITC-dextran have recently been used as easily-visualized macromolecules in studies of demixing (phase separation) and partitioning phenomena for systems of rodlike colloidal particles (Beck-Candanedo, Viet, & Gray, 2006; Beck-Candanedo, Viet, & Gray, 2007; Dogic, Purdy, Grelet, Adams, & Fraden, 2004; Edgar & Gray, 2002).

Blue dextran preparation methods are adapted from those used to couple dyes to support matrices such as cross-linked dextran and agarose (Böhme, Kopperschläger, Schulz, & Hofmann, 1972; Johansson & Andersson, 1984; Lowe & Pearson, 1984). The dye–dextran coupling occurs as a nucleophilic substitution reaction between the chlorine moiety of the triazine dye and the hydroxyl groups of the dextran substrate in basic aqueous media (Stead, 1982). Reaction conditions significantly affect the degree of dye substitution which may be obtained with both cross-linked dextran gels and dextran itself (Dean & Watson, 1979; Johansson & Andersson, 1984; Lowe & Pearson, 1984; Mayes, Eisenthal, & Hubble, 1992; Mayes, Moore, Eisenthal, & Hubble, 1990). Blue dextran is produced commercially, but the effect of altering the reaction conditions on the properties of the dextran–dye conjugate has not been assessed.

The physico-chemical properties of unmodified dextrans have been thoroughly studied. The molecular weight distribution of native and fractionated dextrans (Antonini et al., 1964; Senti et al., 1955), as well as the radius of gyration (Ioan, Aberle, & Burchard, 2001; Nordmeier, Xing, & Lechner, 1993) have been obtained by light scattering and other methods such as size exclusion chromatography (Granath, 1958; Granath & Flodin, 1961). Branching and dextran structure have been studied by several methods (Covacevich & Richards, 1977; Jeanes & Wilham, 1950; Rankin & Jeanes, 1954; Van Cleve, Schaefer, & Rist, 1956), yielding conflicting data which suggest branches shorter than three glucose units (Larm, Lindberg, & Svensson, 1971; Lindberg & Svensson, 1968) or as long as 50 units (Bovey, 1959; Senti et al., 1955). Viscosity behavior of dextrans in various solvents has been explored for a variety of molecular weight fractions (Antonini et al., 1964; Granath, 1958; Rotureau, Dellacherie, & Durand, 2006; Senti et al., 1955). Dextran derivatives such as dextran sulfates have also been characterized (Pasika & Cragg, 1962).

Perhaps not surprisingly given their principal use as bound ligands for affinity chromatography, blue dextrans have been characterized mainly with regard to degree of dye substitution

* Corresponding author. Tel.: +1 514 398 6182; fax: +1 514 398 8254.
E-mail address: derek.gray@mcgill.ca (D.G. Gray).

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.03.003

(Mayes et al., 1990). The optical properties of blue dextran have also been exploited in order to test an optical detection system for molecular weight measurement of dye-labeled polysaccharides by analytical ultracentrifugation, during which study the molecular weight of blue dextran 2000 was verified (Errington, Harding, & Rowe, 1992).

In-depth studies of the physico-chemical properties of blue dextran 2000 have not been performed (Ohta, Yamaguchi, Jikumaru, & Kawahara, 1985). While dextran properties such as branching and polydispersity remain unchanged upon binding the dye ligands to the dextran chain, it is reasonable to assume that the nature of the attached dye can significantly affect the physico-chemical properties of the polymer. The bound aromatic sulfonate dye introduces both hydrophobic and ionic elements into the dextran macromolecule, which will alter its properties and behavior in solution as compared to unmodified dextrans. This article presents a first step towards the optimization of the tailored synthesis of blue dextrans of different molecular weights and provides an initial look at their physico-chemical properties in solution. An understanding of the effect of dye ligands on the properties of blue dextrans may ultimately provide insight into their interactions (electrostatic, hydrophobic and steric) with other polyelectrolytes such as cellulose nanocrystals with surface sulfate groups. It is hoped that this data will be useful in elucidating the effects of charged and uncharged dextrans on the phase behavior of aqueous cellulose nanocrystal suspensions observed in the literature (Beck-Candanedo, Viet, Gray, in press; Beck-Candanedo et al., 2006, 2007; Edgar & Gray, 2002).

2. Methods and materials

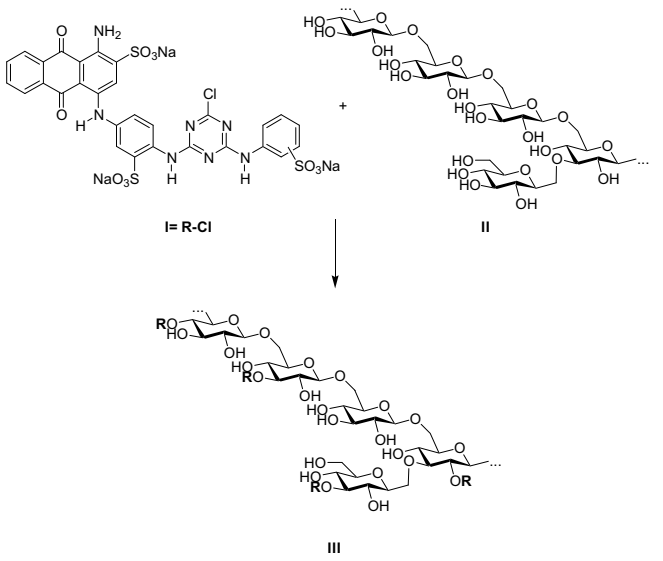
2.1. Chemicals

Dextrans T-70, T-110, T-500, T-2000 (nominal $\bar{M}_w$ = 70,000, 106,000, 532,000 and 2,000,000, respectively, manufacturer's data) were purchased from Pharmacia Fine Chemicals (Sweden). Blue dextran 2000 ($\bar{M}_w \approx$ 2,000,000 with 0.1 mmol Reactive blue 2 dye per gram of dextran bonded randomly via an ether linkage to the polymer backbone (Sigma-Aldrich, 1997) was purchased from Aldrich. Cibacron blue 3 G-A (CB, 55% dye content) was purchased from Aldrich and used without further purification; see Scheme 1 for the structure. Sodium chloride (ACS reagent grade) was purchased from Fisher Scientific. Sodium carbonate (ACS reagent grade) was purchased from Anachemia. All reactions were performed using water purified with a Millipore Milli-Q purification system.

2.2. Preparation of blue dextrans

Blue dextrans of various molecular weights and degree of dye substitution (Table 1) were prepared using methods adapted from Mayes et al. (1990) and Tang, Zhang, Bowyer, Eisenthal, and Hubble (2004) (Scheme 1). Blue dextran samples are denoted by their molecular weight followed by a subscript number indicating the degree of dye substitution per anhydroglucose repeat unit. However, according to convention, commercially available blue dextran 2000 purchased from Aldrich will be referred to as "blue dextran 2000" in the text, except where necessary for clarity.

A typical preparation is as follows: a solution of 4 g of dextran T-2000 in 120 mL of water was mixed with solutions of 0.8 g of Cibacron blue 3 G-A (55% dye content) in 40 mL of water and 0.2 g of Na_2CO_3 in 40 mL of water. The mixture was shaken in a water bath at 45 °C. After around 75 h, the free reactive dye was consumed (Mayes et al., 1990) and another 0.8 g of CB was added. To precipitate the dextran–dye conjugate, an equal volume of ethanol was added to the mixture, which was allowed to stand at



Scheme 1. Speculative coupling of Cibacron blue 3G-A (I = R–Cl) with dextran (II) to give blue dextran (III), showing examples of possible binding sites of the dye (Dean & Watson, 1979).

−20 °C for at least 60 min. The precipitate was then spun down and rinsed with ethanol. Following resuspension in 40 mL of water, the dextran was dialyzed (Spectrum Spectra/Por membrane, molecular weight cutoff 12,000–14,000) against distilled water to remove any traces of unreacted dye and salt. Cleavage of the chromophore from the dextran chain has not been found to occur in aqueous solution (Champ, Xue, & Huglin, 2000; Huglin & Yip, 1987); this was confirmed by gel filtration using Bio-Gel P-30 size exclusion polyacrylamide gel (nominal exclusion limit 40 kDa, Bio-Rad Laboratories), which indicated the absence of unreacted or free dye. Solutions of the blue dextrans were filtered through glass microfiber filters (Ahlstrom, 0.7 μm pore size). The final product was lyophilized and stored as a solid. Final yields were comparable to literature values (Mayes et al., 1990).

2.3. Thermogravimetric analysis (TGA)

Moisture contents of the dextrans and dextran–dye conjugates were determined by thermogravimetric analysis (TA Instruments TGA Q500). The dextrans contained 10.5–11.5% water by mass, and the blue dextrans contained 9–15% water by mass. All dextran concentrations were calculated taking moisture contents into account.

2.4. UV–visible spectroscopy

The dye content of the dextran–dye conjugates was determined from UV–visible spectra measured with a Varian Cary 300 Bio spectrophotometer. A calibration curve was obtained from solutions of commercially-available blue dextran 2000. Adherence to the Beer–Lambert's law was established. The molar extinction coefficient for blue dextran 2000 at 609 nm was found to be 8870 M^{-1} cm^{-1}. The extinction coefficient at 620 nm was 8970 M^{-1} cm^{-1} = 0.823 g^{-1} dm^3 cm^{-1}, which agrees well with the value of 0.812 g^{-1} dm^3 cm^{-1} found by Champ et al. (2000) for commercially available blue dextran 2000. The ligand density was estimated from absorbance measurements at 609 nm for solutions of the dextran–dye conjugates, and calculated in terms of the degree of substitution (DS) per anhydroglucose (AGU) repeat unit. Absorbance measurements in dilute aqueous solution and in 6 M HCl (aq) (Chambers, 1977; Mayes et al., 1990) have shown that negli-

Table 1
Preparation conditions and dye ligand densities for blue dextrans

Dextran	Reaction time (h)	Dye/Dx ratio (mol:mol)	$[Na_2CO_3]$ (M)	Dye ligand density (DS per AGU[a], 10^{-4})	Yield blue dextran (%)
Blue Dx 2000_{16}	21.5	300	0.010	16	46
Blue Dx 2000_{30}	48.0	300	0.010	30	60
Blue Dx 2000_{36}	96.5	300	0.010	36	46
Blue Dx 2000_{56}	144.5	300	0.010	56	48
Blue Dx 2000_{161}	n/a[b]	n/a[b]	n/a[b]	161	n/a[b]
Blue Dx 2000_{7}	48	150	0.018	7	22
Blue Dx 2000_{8}	48	300	0.018	8	40
Blue Dx 2000_{15}	48	600	0.018	15	19
Blue Dx 2000_{12}	48	300	0.036	12	72
Blue Dx 2000_{17}	48	300	0.072	17	87
Blue Dx 2000_{19}	48	300	0.126	19	84
Blue Dx 500_{11}	24.0	300	0.008	11	39
Blue Dx 110_{23}	24.0	300	0.040	23	28
Blue Dx 70_{66}	144.0	10	0.189	66	47

[a] Degree of substitution per anhydroglucose repeat unit.
[b] Commercially available blue dextran 2000 (Aldrich).

gible intramolecular dye stacking occurs at dye ligand densities comparable to those reported in this article.

2.5. Viscosity

Viscosity measurements were carried out in a capillary viscometer of Cannon–Fenske type at a temperature of 25 ± 0.01 °C. Solvent flow times were on the order of 10 s; the kinetic energy correction was performed. Each data point is the average of at least three trials. Samples contained from 3×10^{-4}–2×10^{-2} g/mL polymer and were filtered with a 0.45-μm membrane prior to analysis.

The data were fitted to the Huggins equation

$$\eta_{red} = \frac{\eta_{sp}}{c} = [\eta] + k_H \cdot [\eta]^2 \cdot c,$$

where η_{sp} is the specific viscosity, given by $(\eta - \eta_s)/\eta_s$ where η_s is the viscosity of the solvent and η is the viscosity of the solution, and c is the concentration of the polymer. Plotting the reduced viscosity, $\eta_{red} = \eta_{sp}/c$ against c and extrapolating to $c = 0$ allows us to calculate the intrinsic viscosity $[\eta]$, which gives an indication of the hydrodynamic volume of the polymer coils. The slope of the plot yields the empirical Huggins constant k_H, which is a measure of the intermolecular interactions for a given polymer–solvent system (Kulicke & Clasen, 2004).

2.6. Gel permeation chromatography (GPC)

Blue dextrans (1 mg/mL) were dissolved in the eluent (pH 7, 0.8 M $NaNO_3$ and 0.01 M NaH_2PO_4 containing 0.2% NaN_3) and filtered using 0.45-μm membranes. Samples (100 μL) were injected onto a GPC column (300 × 75 mm PL aquagel-OH mixed 8 μm, 100–1×10^7 Da separation, Polymer Laboratories) at a flow rate of 1.0 mL/min using a Merck Hitachi AS-2000A autosampler and Waters 510 HPLC pump. The column was coupled to a Wyatt Optilab rEX IR detector and Wyatt Technology Corporation DAWN EOS light scattering detector. Data were analyzed with Astra v5.3.0 18 software (Wyatt Technology Corporation).

2.7. Dynamic light scattering (DLS)

Hydrodynamic radii (R_H) were determined by dynamic light scattering. Solutions of dextrans and blue dextrans (0.2–1.0 g/L) were prepared in 0.200 M NaCl (aq). The solutions and solvents were clarified by filtration through a nylon filter of pore size 0.2-μm (Millipore); samples were filtered directly into the light scattering cells. Light scattering measurements were made at 21–22 °C on a Brookhaven Research BI-200SM laser light scattering goniometer and BI-2030AT digital correlator, using a polarized incident beam of wavelength 632.8 nm from a He–Ne laser (35 mW total power). DLS measurements were made at a 90° angle with sample times of 20 μs over a duration of 15–30 s. Results are the average of five or more measurements. The data were analyzed with BI-2030AT software.

2.8. Static light scattering (SLS)

Molecular weights ($\bar{M}_w$,$\bar{M}_n$), radii of gyration (R_g) and indices of polydispersity (I_p) were determined using a Wyatt Technology Corporation DAWN EOS light scattering detector (690 nm) coupled to the GPC column (Tables 6 and 7) Data were analyzed with Astra v5.3.0 18 software (Wyatt Technology Corporation). Specific refractive index increments, dn/dc, were measured at 690 nm by injecting 2-mL samples of blue dextrans (0.05–0.60 mg/mL) in 0.8 M $NaNO_3$ and 0.01 M NaH_2PO_4 solution containing 0.2% NaN_3 (pH 7) into a Wyatt Optilab rEX IR detector.

3. Results and discussion

3.1. Effect of reaction conditions on blue dextran properties

According to Mayes et al. (1990), for a given set of initial reaction conditions, increasing the reaction time increases the degree of dye substitution of the final product, despite the decrease in the rate of dye loading as the free dye reacts with both the dextran and the hydroxyl group of the solvent water. Not unexpectedly, increasing the initial dye-to-dextran molar ratio from 150:1 to 600:1 also increases the degree of dye substitution somewhat (Table 1). Higher base concentrations appear to significantly increase the yield of blue dextran obtained, perhaps by favoring the nucleophilic dye–dextran reaction over the dye–water reaction (Table 1).

3.2. Viscosity

In water, blue dextran 2000 shows typical polyelectrolyte behavior (Fig. 1). As polymer concentrations decrease from high to intermediate values (13–1 mg/mL), η_{red} levels off and then increases with further decreasing concentration. At very low polymer concentrations, η_{red} decreases once again. This rheological behavior has been seen for other polyelectrolytes (Cohen & Priel, 1989; Vink, 1992). The sharp increase in reduced viscosity is attributed to expansion of the polyelectrolyte coils caused by gradually

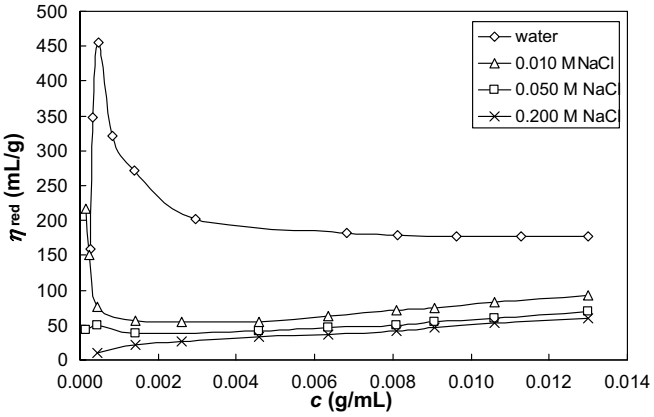


Fig. 1. Reduced viscosity η_{red} as a function of concentration c for blue dextran 2000 in solutions of increasing ionic strength.

increasing dissociation of the ionic groups (dye ligands). Increasing intramolecular coulomb repulsions between increasingly dissociated groups result in an expansion of the coil and increase its hydrodynamic volume, leading to the maximum in η_{red} observed at low polymer concentration. At concentrations below this maximum η_{red}, the polymer is in a dilute state even for nearly fully expanded polyelectrolyte coils, which reduces intermolecular interactions to such an extent that the reduced viscosity decreases again (Kulicke & Clasen, 2004). Although it is not shown, at high concentrations (e.g., greater than those shown in Fig. 1), the reduced viscosity η_{red} should again increase with increasing polymer concentration.

As the surrounding ionic strength increases, the shielding of inter- and intramolecular coulomb forces by the additional counterions attenuates the increase of η_{red}, until at 0.200 M sodium chloride the blue dextran behaves as a neutral polymer. Viscosity measurements were performed in 0.200 M NaCl (aq) solutions to allow the extrapolation of the reduced viscosity to $c \rightarrow 0$.

It is important to note that although the dye ligands contain ionic groups which tend to improve the polymer–solvent interactions, their aromatic rings also impart a hydrophobic character to the blue dextrans. Hydrophobic association in hydrophobically modified polyelectrolytes, such as anionic polyvinyl alcohol (PVA^-) or polymethacrylic acid/ethyl acrylate (MAA/EA), has been found to reduce the polyelectrolyte effect and lower the values of the reduced viscosity at low polymer concentrations, suggesting that the chains are less expanded (Ng, Tam, & Jenkins, 1999; Knudsen, Lauten, Kjøniksen, & Nyström, 2004). A similar phenomenon may occur in blue dextran, with the result that the overall structure may be more tightly coiled than an unmodified dextran of the same molecular weight. In addition, substitution may result in a decrease in solvation of the dextran hydroxyls, and steric hindrance may also affect chain conformation.

All viscometric measurements were performed in the dilute solution regime, such that polymer-solvent interactions determined the flow properties. Experiments were performed at relative viscosities $\eta_{rel} = \eta_s/\eta$ between 1.2 and 2.0 to ensure accurate data. Over the concentration range studied, both unmodified and blue dextrans showed decreasing η_{red} with decreasing polymer concentration (Fig. 2). This confirms that the 0.200-M NaCl was sufficient to suppress the chain expansion which would occur during dilution of the polymer. The slopes of the plots increased with dextran molecular weight; for a given dextran molecular weight, plots of η_{red} vs. c are steeper for blue dextrans than for unmodified dextrans. The increase in intrinsic viscosity $[\eta]$ with increasing dextran molecular weight (Table 2) is attributed to the larger coil size of the higher molecular weight polymers; dynamic light scattering data supports this conclusion.

The differences in the $[\eta]$ values shown in Table 2 indicate that blue dextrans experience poorer polymer–solvent interactions than their unmodified counterparts. This phenomenon can be explained by the presence of hydrophobic association of the aromatic dye ligands in the blue dextran structure. The effect is particularly noticeable in the most highly substituted blue dextran 2000, which contains the most aromatic rings and shows a large decrease in intrinsic viscosity $[\eta]$ as compared to dextran T-2000. For a given dextran molecular weight, the $[\eta]$ values are smaller for blue dextrans because they are more tightly coiled.

These results differ from those obtained for branched and linear dextran sulfates, where the intrinsic viscosity *increased* upon sulfa-

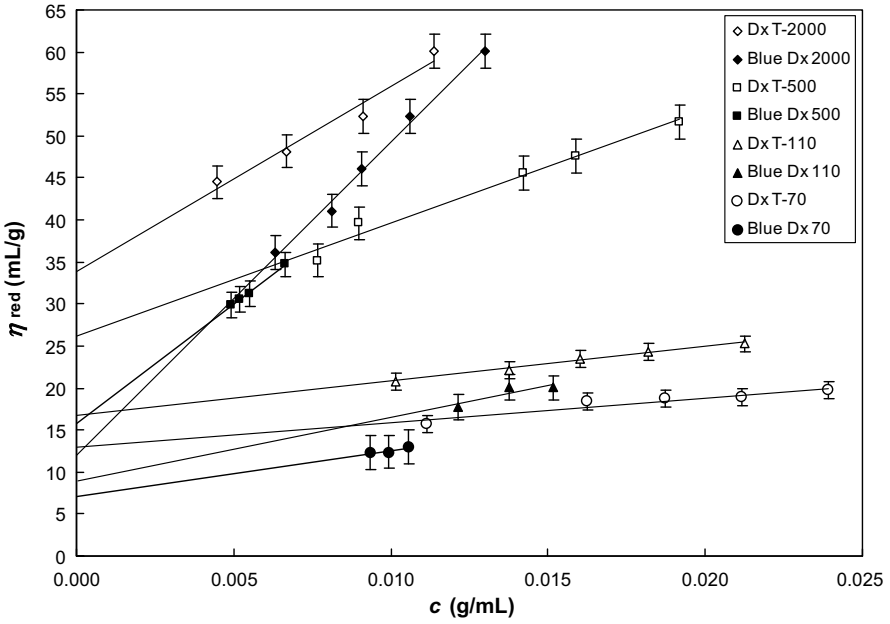


Fig. 2. Reduced viscosity η_{red} as a function of the concentration c for unmodified dextrans (open symbols) and blue dextrans (filled symbols) in 0.200 M NaCl (aq). Values were taken from the linear portion of the η_{red} vs. c curve.

Table 2
Intrinsic viscosity [η] for unmodified and blue dextrans in 0.200 M NaCl (aq)

Dextran	[η] (mL/g)
Dextran T-2000	34
Dextran T-500	26
Dextran T-110	17
Dextran T-70	13
Blue dextran 2000_{161}	12
Blue dextran 500_{11}	16
Blue dextran 110_{23}	9
Blue dextran 70_{66}	7

tion and with increasing sulfation (see below) (Pasika & Cragg, 1962). However, sulfate groups are not hydrophobic and the intrinsic viscosity behavior is likely governed by the coil expansion caused by repulsion of the charged sulfate groups.

Viscosity data taken in 0.200 M NaCl (aq) for blue dextrans 2000 with different degrees of substitution support this hypothesis (Table 3). Intrinsic viscosity values decrease with increasing degree of dye substitution, which would not be expected if only the ionic groups on the dye ligands affected the viscometric behavior. Blue dextran 2000_8, with the lowest DS and therefore the lowest degree of hydrophobicity, has the highest intrinsic viscosity. Blue Dx 2000_{16} has a similar [η] value to that of dextran T-2000, suggesting that at low degrees of substitution, the ionic character and coulombic repulsion of the dye ligands dominates over the effect of hydrophobicity and causes coil expansion (cf. blue dextran 2000_8), while at higher dye substitutions, the coils shrink due to the hydrophobic nature of the dye molecules.

That the ionic groups on the dye ligands do play a role in the viscosity behavior of blue dextran is shown by the effect of increasing the ionic strength of the solvent (Fig. 3). The hydrodynamic volume of each blue dextran coil depend on the electrostatic interactions (and hydrophobic associations) present, which are sensitive to the solvent ionic strength. The intrinsic viscosity of blue dextran 2000 decreases monotonically with increasing ionic strength (Table 4), indicating a decreasing coil expansion due to further shielding of the coulomb repulsion forces between the ionic sulfonate groups of the dye ligands (Goring & Rezanowich, 1960; Moan & Wolff, 1974). Ohta et al. (1985) also observed a decrease in the so-called "charge effect" upon increasing the solution ionic strength. Hydrodynamic radii of blue dextran 2000 in solutions of increasing ionic strength obtained by dynamic light scattering support these data.

It is interesting to note that increasing the degree of dye substitution of dextran blue 2000 in 0.200 M NaCl solution has an effect of similar magnitude on the intrinsic viscosity to that of increasing the ionic strength of solutions of blue dextran 2000_{161} (compare data in Tables 3 and 4). Specifically, increasing the dextran 2000 dye substitution from 16×10^{-4} to 56×10^{-4} in 0.200 M NaCl and increasing the ionic strength from 0.010 M to 0.025 M for blue dextran 2000_{161} both result in similar values of [η].

Table 3
Effect of increasing degree of dye substitution on intrinsic viscosity [η] for blue dextrans 2000 in 0.200 M NaCl (aq)

Dextran	Dye ligand density (DS per AGU, 10^{-4})	[η] (mL/g)
Dextran T-2000	0	34
Blue dextran 2000_8	8	36
Blue dextran 2000_{16}	16	34
Blue dextran 2000_{30}	30	33
Blue dextran 2000_{36}	36	28
Blue dextran 2000_{56}	56	26
Blue dextran 2000_{161}	161	12

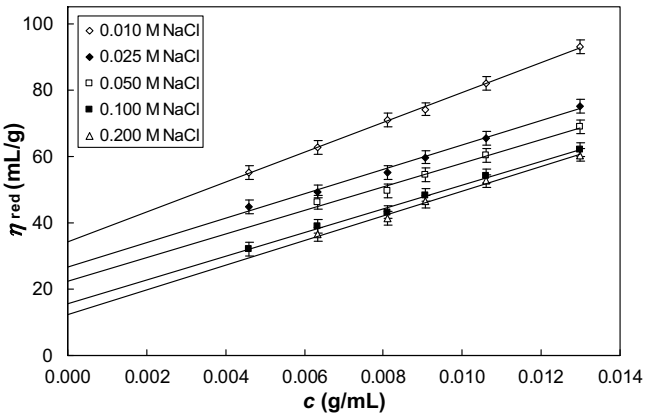


Fig. 3. Reduced viscosity η_{red} as a function of the concentration c for blue dextran 2000 in solutions of increasing ionic strength.

3.3. Refractive index increments

The refractive index increments dn/dc of the blue dextrans lie in the range 0.120–0.175 at 690 nm (Table 5). It has been found that the dn/dc of unmodified dextran increases slightly over a wide range of molecular weights (Basedow, Ebert, & Ruland, 1978; Nordmeier et al., 1993). For example, aqueous solutions of dextrans of molecular weight 80–7400 kDa have been found to have dn/dc values of 0.150–0.154 at 436 nm (Nordmeier et al., 1993). According to the Cauchy equation, the dn/dc for dextran at 690 nm should be slightly lower than these values (Huglin, 1972). The high salt content of the solvent will also tend to reduce the dn/dc for unmodified dextrans below the typical values of ~0.15, owing to the increased refractive index of the solvent (Vink & Dahlström, 1967). Table 5 shows that increasing dye substitution increases the refractive index increment, which may explain the anomalously high value obtained for blue dextran 70_{66}. The refractive index increments were used in the molecular weight calculations from the GPC data.

3.4. Gel permeation chromatography

Ethanol precipitation/centrifugation and exhaustive dialysis were used to purify blue dextrans and remove unreacted dye and

Table 4
Effect of solvent ionic strength, I, on intrinsic viscosity [η] and R_H values for blue dextran 2000_{161}

Ionic strength (M)	[η] (mL/g)	R_H (nm)
0.010	34	45.4 ± 0.9
0.025	27	43.7 ± 0.5
0.050	23	43.0 ± 1.0
0.100	16	41.7 ± 1.0
0.200	12	40.2 ± 0.8

Table 5
Refractive index increments dn/dc at 690 nm for blue dextrans of various molecular weights and dye loadings

Dextran	Dye ligand density (DS per AGU, 10^{-4})	dn/dc[a]
Blue dextran 2000_{12}	12	0.124 ± 0.004
Blue dextran 2000_{36}	36	0.138 ± 0.005
Blue dextran 2000_{56}	56	0.153 ± 0.005
Blue dextran 2000_{161}	161	0.157 ± 0.006
Blue dextran 110_{23}	23	0.118 ± 0.015
Blue dextran 70_{66}	66	0.173 ± 0.005

[a] In pH 7 0.80 M $NaNO_3$ and 0.01 M NaH_2PO_4 (aq) with 0.2% NaN_3.

Table 6
Molecular weights and indices of polydispersity of blue dextrans

Dextran	$\bar{M}_w^{calc}$ (kDa)	$\bar{M}_w^{GPC}$ (kDa)	$\bar{M}_n^{GPC}$ (kDa)	I_p
Blue dextran 2000_{56}	2060	2100 ± 20	1800 ± 90	1.16
Blue dextran 110_{23}	107	130 ± 5	130 ± 7	1.03
Blue dextran 70_{66}	72	65 ± 3	52 ± 3	1.25

Table 7
Experimental and literature values of R_H and R_g for unmodified and blue dextrans

Dextran	Dye ligand density (DS per AGU, 10^{-4})	R_H[a] (nm)	R_g (nm)
Blue dextran 2000_{161}	161	40.2 ± 0.8	–
Blue dextran 2000_{56}	56	36.9 ± 0.9	40.8 ± 2.0[b]
Blue dextran 2000_{36}	36	35.1 ± 1.1	–
Blue dextran 2000_{16}	16	37.9 ± 0.8	–
Blue dextran 2000_{12}	12	40.8 ± 1.0	–
Dextran T-2000	0	37.7 ± 0.8	34[c]
Blue dextran 500_{11}	11	18.4 ± 0.7	–
Dextran T-500	0	17.8 ± 0.3	19.8[c,d]
Blue dextran 110_{23}	23	30.7 ± 3.1	16.3 ± 1.6[b]
Dextran T-110	0	7.7 ± 1.3	9.7[c]
Blue dextran 70_{66}	66	<10[e]	<10[e]
Dextran T-70	0	<10[e]	8.3[c]

[a] Measured by DLS in 0.200 M NaCl (aq).
[b] Measured by GPC-SLS in 0.80 M $NaNO_3$ and 0.01 M NaH_2PO_4 (aq) with 0.2% NaN_3.
[c] Fishman, Damert, Phillips, and Barford (1987).
[d] Nordmeier et al. (1993).
[e] Isotropic scatterer; dimensions too small for measurement by light scattering.

residual sodium carbonate. GPC chromatograms confirm the purity of the samples; peaks are sharp, indicating the absence of any unreacted dextran (Fig. 4).

3.5. Molecular weights

Molecular weights were calculated for the blue dextrans using nominal weight-average molecular weights for unmodified dextrans and dye loadings from UV–visible spectroscopy (see Table 1):

$$\bar{M}_w^{calc} = \bar{M}_w^{Dx}[1 + M_{CB}(DS/M_{AGU})]$$

where $\bar{M}_w^{calc}$ is the calculated molecular weight, $\bar{M}_w^{Dx}$ is the nominal molecular weight for unmodified dextran (manufacturer's data), M_{CB} = 840.12 Da is the molecular weight of the Cibacron blue dye, DS is the degree of dye substitution per anhydroglucose unit of the blue dextran and M_{AGU} = 162.2 Da is the molecular weight of the anhydroglucose repeat unit.

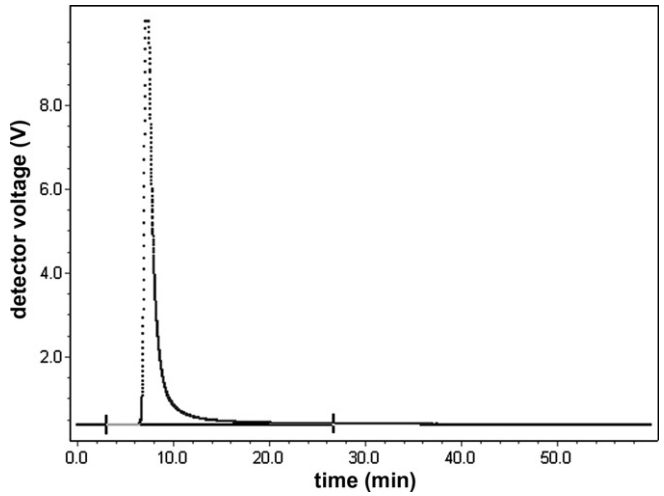


Fig. 4. Sample GPC chromatogram for blue dextran 2000_{12}.

The molecular weights and indices of polydispersity of the blue dextrans were determined by GPC. Table 6 presents the calculated molecular weights together with the weight- and number-average molecular weights and indices of polydispersity, Ip, of several blue dextrans. Masses calculated by GPC are in agreement with the calculated molecular weights. The molar mass distribution is very narrow, as indicated by the indices of polydispersity.

3.6. Hydrodynamic radii and radii of gyration

The hydrodynamic radii of unmodified and blue dextrans were determined by DLS in 0.010–0.200 M NaCl solutions. Blue dextrans were found to have larger hydrodynamic radii than the unmodified dextrans owing to coulomb repulsion between the ionic dye ligands; values decrease with decreasing dextran molecular weight (Table 7). Our R_H value for dextran T-2000 (37.7 nm) is somewhat larger than the value found by Nordmeier (1993) for dextran T-2000 in deionized water at 25 °C (27 nm); values for the smaller dextrans are comparable to literature values. Overall, the hydrodynamic radii of the blue dextrans and unmodified dextrans lie in the same range, except for blue dextran 110, which has a much larger radius at the given concentration. (Note that R_H values were measured at finite blue dextran concentrations, and not by extrapolation to zero concentration.)

Increasing the solution ionic strength from 0.010 to 0.200 M resulted in a decrease of about 5 nm of the hydrodynamic radius of blue dextran 2000 (Table 4). The increased shielding of electrostatic repulsions between the dye ligand sulfonate groups would contribute to the observed coil shrinkage. No specific trend in the hydrodynamic radius was observed among blue dextrans 2000 of increasing DS (Table 7).

The radii of gyration of the blue dextrans were obtained with a static light scattering detector in 0.8 M $NaNO_3$ and 0.01 M NaH_2PO_4 (aq). Blue dextrans exhibit larger values of R_g as compared to their unmodified counterparts. Values of R_g for the unmodified dextrans are comparable to literature values (Nordmeier et al., 1993; Ioan, Aberle, & Burchard, 2000).

4. Conclusions

Measurements on Cibacron blue-labeled dextrans of varying molecular weights and dye ligand densities indicate that the dye labels influence the solution properties of these widely used water-soluble polymers. In particular, viscosity measurements show the polyelectrolyte character of blue dextrans in aqueous media.

Acknowledgments

We thank the Natural Sciences and Engineering Council of Canada and Paprican for support and the Centre for Self-Assembled Chemical Structures (CSACS) at McGill University for the use of their TA Instruments TGA Q500 and Varian Cary 300 Bio UV-visible spectrophotometer. We also thank Julie Boivin at the Université de Montréal for technical assistance with GPC-LS and d*n*/d*c* measurements and for helpful discussions.

References

Antonini, E., Bellelli, L., Bruzzesi, M. R., Caputo, A., Chiancone, E., & Rossi-Fanelli, A. (1964). Studies on dextran and dextran derivatives. I. Properties of native dextran in different solvents. *Biopolymers, 2*, 27–34.
Basedow, A. M., Ebert, K. H., & Ruland, U. (1978). Specific refractive index increments of dextran fractions of different molecular weights. *Makromolekulare Chemie, 179*, 1351–1353.
Beck-Candanedo, S., Viet, D., & Gray, D. G. (2006). Induced phase separation in low-ionic-strength cellulose nanocrystal suspensions containing high-molecular-weight blue dextrans. *Langmuir, 22*, 8690–8695.

Beck-Candanedo, S., Viet, D., & Gray, D. G. (2007). Triphase equilibria in cellulose nanocrystal suspensions containing neutral and charged macromolecules. *Macromolecules, 40*, 3429–3436.

Beck-Candanedo, S., Viet, D., & Gray, D. G. (in press). Partitioning of charged and neutral dextran–dye derivatives in biphasic cellulose nanocrystal suspensions. *Canadian Journal of Chemistry*.

Böhme, H.-J., Kopperschläger, G., Schulz, J., & Hofmann, E. (1972). Affinity chromatography of phosphofructokinase using Cibacron blue FG3-A. *Journal of Chromatography A, 69*, 209–214.

Bovey, F. A. (1959). Enzymatic polymerization. I. Molecular weight and branching during the formation of dextran. *Journal of Polymer Science, 35*, 167–182.

Chambers, G. K. (1977). Determination of Cibacron blue F3GA substitution in blue Sephadex and blue dextran-Sepharose. *Analytical Biochemistry, 83*, 551–556.

Champ, S., Xue, W., & Huglin, M. B. (2000). Concentrating aqueous solutions of water soluble polymers by thermoreversible swelling of poly[(*N*-isopropylacrylamide)-co-(acrylic acid)] hydrogels. *Macromolecular Chemistry and Physics, 201*, 931–940.

Cohen, J., & Priel, Z. (1989). Viscosity of dilute polyelectrolyte solutions: concentration dependence on sodium chloride, magnesium sulfate and lanthanum nitrate. *Macromolecules, 22*, 2356–2358.

Covacevich, M. T., & Richards, G. N. (1977). Frequency and distribution of branching in a dextran: an enzymic method. *Carbohydrate Research, 54*, 311–315.

de Belder, A. N. (2003). *Dextran*. Piscataway, NJ: Amersham Biosciences.

Dean, P. D. G., & Watson, D. H. (1979). Protein purification using immobilised triazine dyes. *Journal of Chromatography – Chromatography Reviews, 165*, 301–319.

Dogic, Z., Purdy, K. R., Grelet, E., Adams, M., & Fraden, S. (2004). Isotropic-nematic phase transition in suspensions of filamentous virus and the neutral polymer Dextran. *Physical Review E, 69*, 051702-1–051702-9.

Edgar, C. D., & Gray, D. G. (2002). Influence of dextran on the phase behavior of suspensions of cellulose nanocrystals. *Macromolecules, 35*, 7400–7406.

Errington, N., Harding, S. E., & Rowe, A. J. (1992). Use of scanning absorption optics for sedimentation equilibrium analysis of labelled polysaccharides: Molecular weight of blue dextran. *Carbohydrate Polymers, 17*, 151–154.

Evans, T. H., & Hibbert, H. (1946). Bacterial polysaccharides. *Advances in carbohydrate chemistry* (Vol. 2, pp. 203–233). New York, NY: Academic Press.

Fishman, M. L., Damert, W. C., Phillips, J. G., & Barford, R. A. (1987). Evaluation of root-mean-square radius of gyration as a parameter for universal calibration of polysaccharides. *Carbohydrate Research, 160*, 215–225.

Goring, D. A. I., & Rezanowich, A. (1960). The Huggins viscosity coefficient for a polyelectrolyte microgel. *Journal of Colloid Science, 15*, 472–482.

Granath, K. A. (1958). Solution properties of branched dextrans. *Journal of Colloid Science, 13*, 308–328.

Granath, K. A., & Flodin, P. (1961). Fractionation of dextran by the gel filtration method. *Makromolekulare Chemie, 48*, 160–171.

Huglin, M. (1972). *Light scattering from polymer solutions*. New York: Academic Press.

Huglin, M. B., & Yip, D. C. F. (1987). An alternative method of determining the water content of hydrogels. *Die Makromolekulare Chemie, Rapid Communications, 8*, 237–242.

Ioan, C. E., Aberle, T., & Burchard, W. (2000). Structure properties of dextran. 2. Dilute solution. *Macromolecules, 33*, 5730–5739.

Ioan, C. E., Aberle, T., & Burchard, W. (2001). Light scattering and viscosity behavior of dextran in semidilute solution. *Macromolecules, 34*, 326–336.

Jeanes, A., & Wilham, C. A. (1950). Periodate oxidation of dextran. *Journal of the American Chemical Society, 72*, 2655–2657.

Johansson, G., & Andersson, M. (1984). Parameters determining affinity partitioning of yeast enzymes using polymer-bound triazine dye ligands. *Journal of Chromatography A, 303*, 39–51.

Knudsen, K. D., Lauten, R. A., Kjøniksen, A.-L., & Nyström, B. (2004). Rheological and structural properties of aqueous solutions of a hydrophobically modified polyelectrolyte and its unmodified analogue. *European Polymer Journal, 40*, 721–733.

Kulicke, W.-M., & Clasen, C. (2004). *Viscosimetry of polymers and polyelectrolytes*. Berlin: Springer.

Larm, O., Lindberg, B., & Svensson, S. (1971). Studies on the length of the side chains of the dextran elaborated by *Leuconostoc mesenteroides* NRRL B-512. *Carbohydrate Research, 20*, 39–48.

Lindberg, B., & Svensson, S. (1968). Structural studies on dextran from *Leuconostoc mesenteroides* NRRL B-512. *Acta Chemica Scandinavica, 22*, 1907–1912.

Lowe, C. R., & Pearson, J. C. (1984). Affinity chromatography on immobilized dyes. *Methods in Enzymology, 104*, 97–113.

Mayes, A. G., Eisenthal, R., & Hubble, J. (1992). Binding isotherms for soluble immobilized affinity ligands from spectral titration. *Biotechnology and Bioengineering, 40*, 1263–1270.

Mayes, A. G., Moore, J. D., Eisenthal, R., & Hubble, J. (1990). Investigation of binding site density: Effects on the interaction between Cibacron blue-dextran conjugates and lysozyme. *Biotechnology and Bioengineering, 36*, 1090–1096.

Moan, M., & Wolff, C. (1974). Viscometric study of polyelectrolyte solutions by isoionic dilution. Effect of the charge density on the polyion conformation. *Makromolekulare Chemie, 175*, 2881–2894.

Ng, W. K., Tam, K. C., & Jenkins, R. D. (1999). Evaluation of intrinsic viscosity measurements of hydrophobically modified polyelectrolyte solutions. *European Polymer Journal, 35*, 1245–1252.

Nordmeier, E. (1993). Static and dynamic light-scattering solution behavior of pullulan and dextran in comparison. *Journal of Physical Chemistry, 97*, 5770–5785.

Nordmeier, E., Xing, H., & Lechner, M.-D. (1993). Static and dynamic light scattering studies of dextran from *Leuconostoc mesenteroides* in the dilute region. *Makromolekulare Chemie, 194*, 2923–2937.

Ohta, K., Yamaguchi, K., Jikumaru, N., & Kawahara, K. (1985). Heterogeneities of blue dextrans and differences among samples. *Journal of Chromatography A, 350*, 292–295.

Pasika, W. M., & Cragg, L. H. (1962). The viscosity behavior of linear and branched dextran sulfates. *Journal of Polymer Science, 57*, 301–310.

Rankin, J. C., & Jeanes, A. (1954). Evaluation of the periodate oxidation method for structural analysis of dextrans. *Journal of the American Chemical Society, 76*, 4435–4441.

Rotureau, E., Dellacherie, E., & Durand, A. (2006). Viscosity of aqueous solutions of polysaccharides and hydrophobically modified polysaccharides: Application of Fedors equation. *European Polymer Journal, 42*, 1086–1092.

Senti, F. R., Hellman, N. N., Ludwig, N. H., Babcock, G. E., Tobin, R., Glass, C. A., et al. (1955). Viscosity, sedimentation, and light-scattering properties of fractions of an acid-hydrolyzed dextran. *Journal of Polymer Science, 27*, 527–546.

Sigma–Aldrich (1997). *Blue dextran molecular weight 2,000,000 product no. D5751. Product information*. Sigma–Aldrich.

Stead, C. V. (1982). Halogenated heterocycles in reactive dyes. *Dyes and Pigments, 3*, 161–171.

Subramanian, S. (1984). Dye–ligand affinity chromatography: The interaction of Cibacron Blue F3GA with proteins and enzymes. *CRC Critical Reviews in Biochemistry, 16*, 169–205.

Tang, M., Zhang, R., Bowyer, A., Eisenthal, R., & Hubble, J. (2004). NAD-sensitive hydrogel for the release of macromolecules. *Biotechnology and Bioengineering, 87*, 791–796.

Van Cleve, J. W., Schaefer, W. C., & Rist, C. E. (1956). The structure of NRRL B-512 dextran. Methylation studies. *Journal of the American Chemical Society, 78*, 4435–4438.

Vink, H. (1992). Rheology of dilute polyelectrolyte solutions. *Polymer, 33*, 3711–3716.

Vink, H., & Dahlström, G. (1967). Refractive index increments for polymers in solutions in multicomponent solvents. *Makromolekulare Chemie, 109*, 249–252.