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Size-exclusion chromatography and dynamic light scattering of dextrans in water: explanation of ion-exclusion behaviour

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Abstract

The size-exclusion chromatographic (SEC) and ion-exclusion chromatographic (IEC) behaviour of dextrans in water was studied in combination with dynamic light scattering (DLS) of their fractions obtained from micro-preparative SEC in water. Dextrans do not aggregate in dilute aqueous solution, but very low dust particle contamination, of particle size 110–170 nm, was revealed from DLS measurements after its separation and its concentration enhancement by SEC. The ion exclusion of charged dextran molecules is the only cause of two excluded peaks observed in the SEC of dextrans in pure water. Model dextran oxidation experiments were performed and mono- and dicarboxydextran were identified as forming these ion-excluded peaks. An IEC–SEC experiment is capable of detecting the presence of polysaccharide chains bearing one or two carboxyl groups only, and of resolving them from each other and from the uncharged chains of molecular masses up to 10^6 if pure water is used as the mobile phase. The presence of such low charge density macromolecules appears to be a general feature of polysaccharides.

1. Introduction

Dextrans are polysaccharides consisting essentially of α-1,6-linked D-glucose units with a few per cent of branching. Although already clinically used in the 1940s and more deeply studied since the 1950s [1–3], neither the detailed branching structure [4] nor the solution properties [5] are completely understood.

Well characterized dextrans are frequently used as calibration standards [6] in the size-exclusion chromatography (SEC) of polysaccharides. In the absence of salt in the mobile phase, the chromatograms showed an apparent high-molecular mass shoulder around the exclusion limit on a silica SEC packing [7] and on hydrophilic packings based on silica [8] or organic resins [9]. An explanation [10] was that dextrans tend to form high-molecular-mass associates and the addition of a small amount of salt breaks them down. Nowadays, a general belief [7–9] is that dextrans carry some minute negative charge and ion exclusion is responsible for the observed excluded peaks due to a negative charge present on the surface of the column packing. SEC of dextrans in water with on-line refractometric and low-angle light-scattering detection confirmed [11] this explanation, but

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nothing is known about the origin and amount of these charged groups.

Nevertheless, the possibility of dextran aggregation in water has been indicated by studies of solution properties [12] and dynamic light scattering (DLS) [13]. Owing to the extreme sensitivity of DLS experiments [14] to large particles (compared with SEC), the minor formation of aggregates cannot be fully rejected on the basis of an SEC experiment only.

The first aim of this study was to judge, using SEC on both analytical and micro-preparative scales with subsequent DLS measurements on the original sample and fractions, if some large particles are present and/or formed in dextran solutions in water in amounts below the SEC detection threshold. Some dextran model oxidation experiments were also performed and the origin of charged groups in dextrans was explained on the basis of their SEC and IEC behaviour.

2. Experimental

2.1. Materials

Well characterized dextran standards (Table 1, D1–D7) were kindly donated by Dr. Kirsti Granath (Kabi-Pharmacia, Uppsala, Sweden) and other commercial dextrans were obtained from Kabi-Pharmacia (D8, D9) and Sigma (St. Louis, MO, USA) (D10, D11). Pullulan standard ($M_r = 186\,000$) was purchased from Polymer Labs. (Church Stretton, Shropshire, UK). Hydroxyethylstarch ($M_r = 200\,000$) was a commercial product of Laevosan (Linz, Austria). Gluconic acid (50% aqueous solution) and saccharic acid (pure) were obtained from Fluka (Buchs, Switzerland).

All analytical-reagent grade inorganic chemicals were supplied by Lachema (Brno, Czech Republic). Water from a Millipore Milli-Q ultrapure water purification unit was used.

LiChrospher 300 and 1000 (mean particle diameter $d_p = 10$ μm) were purchased from Merck (Darmstadt, Germany).

2.2. DLS experiments

An Inova 70-series 4-W argon ion laser (Coherent Laser Division, Palo Alto, CA, USA) tuned to 514.5 nm was focused on to a precision scattering cell (Hellma, Mullheim Baden, Germany). The optical components were mounted on a massive steel bench (Newport Research, Fountain Valley, CA, USA). Light scattering fluctuations were detected at 90° using a commercially available Brookhaven photomultiplier tube and a 128-channel BI-8000 digital correlator (Brookhaven Instruments, Holtsville, NY, USA). The Brookhaven particle distribution software package contains five of the most common distribution analytical procedures [15]; the CONTIN procedure was used here.

The measured intensity autocorrelation function $G^{(2)}(\tau)$ is related to the electric field autocorrelation function $g^{(1)}(\tau)$ by [15]

$$G^{(2)}(\tau) = A[1 + \beta|g^{(1)}(\tau)|^2] \quad (1)$$

where A is the baseline constant, β is an equipment-related constant, τ is the correlation time and

$$g^{(1)}(\tau) = \int_0^\infty \psi(\Gamma) \exp(-\Gamma\tau)\, d\Gamma \quad (2)$$

for the case of continuous distribution of decay rates Γ. The decay rate $\Gamma = K^2D$, where D is the diffusion coefficient, is inversely proportional to the hydrodynamic diameter d_h through the Einstein–Stokes equation; the scattering vector K is defined as $K = (4\pi n/\lambda)\sin(\vartheta/2)$, where n is the refractive index of the medium, λ is the wavelength of the light used in vacuum and ϑ is the scattering angle. The normalized distribution $\psi(\Gamma)\, d\Gamma$ is defined [15] as the fraction of the total intensity scattered by molecules having Γ within the increment $d\Gamma$. In the case of a monodisperse solute, $g^{(1)}(\tau)$ reduces to a single exponential decaying asymptotically to the baseline. In the case of a polydisperse solute, the distribution of diffusion constants and, hence, the hydrodynamic diameter distribution function required is obtained from $g^{(1)}(\tau)$ by inverse Laplace transformation using the Brookhaven program CONTIN.

The BI-8000 correlator provides [16] measurements in linear (uniform channel spacing) or non-linear (non-uniform channel spacing, a sampling time ratio for consecutive channels selectable) mode; this feature substitutes the precedent multi-tau option and allows the use of the total correlator range to determine widely spaced correlations in one experiment. The correlator calculates [16] a statistical baseline from the average photon counting rate and determines the measured baseline from six successive channels delayed by 1024 sampling times; their difference was used as a useful criterion [17–19] of sample purity.

Dextran solutions (0.1%, w/w), prepared by weighing, were filtered through 0.8-μm Millex filters (Millipore, Bedford, MA, USA) directly into the scattering cells. When specified, Anotop 0.02-μm filters (Anotec Separations, Banbury, UK) were also used.

Possible particle contamination of the fractions by particle leakage from the SEC columns and from the mobile phase itself was carefully checked. The mobile phase purity before and after the column set was checked in both linear (optimum selected for dextran experiments) and non-linear (wide window) modes, the DLS equipment setting and experiment duration being identical with those in the dextran measurement. No correlations were observed, either in the mobile phase or in the effluent.

2.3. Chromatography

The SEC equipment consisted of a VCR 40 HPLC pump (Academy Development Works, Prague, Czech Republic), a Model 7125 injection valve (Rheodyne, Cotati, CA, USA) with a 100-μl loop and an R-401 differential refractometer (Waters, Milford, MA, USA) connected through a Black Star (Huntingdon, UK) 2308 A/D converter to an IBM-compatible computer with a printout facility. The software (© J. Horský, Institute of Macromolecular Chemistry) with overlay option uses a broad-standard calibration procedure and allows calculations of molecular mass distributions and averages. Its performance was tested on different (D8–D11) dextran samples (Table 1, calibration with D1–D7). Two stainless-steel columns in series (250 × 8 mm I.D.) (Tessek, Prague, Czech Republic) were packed with diol-modified LiChrospher 300 and 1000 packing, prepared according to a recently described procedure [20], by the slurry technique at 30 MPa with methanol–dioxane (1:1, v/v) as the slurry liquid. Concentrations of SEC sample solutions were 0.1% (w/w) unless stated otherwise.

Micro-preparative experiments were done with a D4 (Table 1) sample concentration of 3.8% (w/w); volumes of 100 μl were injected five times and the main (F2) and excluded fractions (F1) were collected in pure water. The total volumes of both fractions were 44 and 14 ml, respectively; their volume was reduced eightfold on a vacuum rotary evaporator (Büchi, Flawil, Switzerland) with the greatest care with respect to dust-free operation. The resulting solutions were then filtered through 0.8-μm Millex filters directly into the scattering cells.

2.4. Oxidation experiments

Ag_2O precipitated by 10 ml of 1.5% NaOH solution from 10 ml of 2.5% $AgNO_3$ solution (filtered and washed to neutrality) was added to 20 ml of a 2.5% aqueous solution of dextran D8 (Table 1) and boiled for 15 min while stirring magnetically.

A 0.05 *M* Sodium hypobromite solution was prepared by adding 1.3 ml of bromine dropwise to a solution of 1 g NaOH in 250 ml of water under ice cooling. A 0.5-g amount of dextran D8 was dissolved in 100 ml of NaOBr solution and left to react overnight. The oxidation product was dialysed against water through SPECTRA/POR membrane tubing having a molecular mass cut-off of 6000–8000 (Spectrum Medical Industries, Los Angeles, CA, USA) to remove salts.

3. Results and discussion

A typical example of the SEC behaviour of two different dextrans, D4 and D5 (Table 1), in pure water as a mobile phase is shown in Fig. 1a

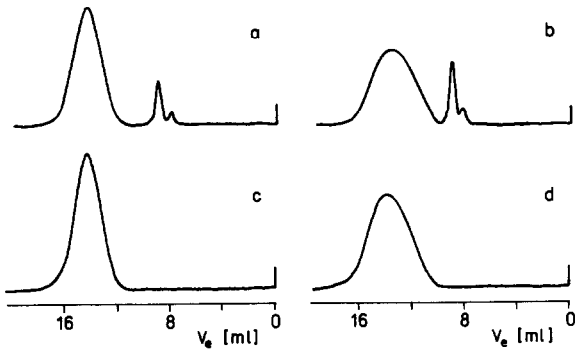

Fig. 1. SEC of dextrans in (a, b) pure water and (c, d) 0.01 *M* NaCl. Samples: (a, c) Dextran D4 (M_w = 99 600) and (b, d) dextran D5 (M_w = 240 570), 100 μl of 0.1% (w/w) solution in the mobile phase injected; flow rate, 1.92 ml/min; refractive index detection, attenuation 2×.

and b. Owing to a fairly high column resolution, the excluded components of the samples are clearly separated into two peaks near the column set exclusion limit. The relative content of this excluded fraction in other dextrans (Table 1) varies in batches with a comparable molecular mass and in different products and seems to increase with increase in molecular mass. When 0.01 *M* NaCl is used instead of pure water, the excluded peaks disappear completely, as illustrated in Fig. 1c and d. Hence, both explanations, *i.e.*, aggregate disruption by the salt addition [10] and ion exclusion of charged dextrane molecules [11], could apply. The amount of excluded species is roughly 10 mass-% of the sample D4 (Table 1) and its molecular mass can be calculated, assuming its approximate diameter to be about 80–100 nm (exclusion limit of the column set used) and using the relationship between the mass-average molecular mass, M_w, and the gyration radius, R_G, expressed in angströms:

$$M_w = 2.62\,R_G^{2.32} \quad (3)$$

valid for dextrans [21], as $M_w = 3 \cdot 10^6$–$5 \cdot 10^6$. Dextran D7, having DLS average d_h = 62 nm, exhibits partial exclusion on the column set used and native dextran with d_h = 195 nm is fully excluded. The estimate used of the value of the exclusion limit, in terms of diameter, for the sake of simplicity based on the manufacturer's nominal pore size, is probably underestimated and represents a minimum excluded size. Nevertheless, particles of this (and larger) size should clearly dominate [14] in a comparable DLS experiment if 10% of their mass were contained in the sample. The scattered intensity *I* scales as $c_i M_i$, c_i being the mass concentration; hence, up to 80% of the scattered intensity might originate from these particles if the Mie effect is neglected. The absence of large particles on the CONTIN plots of intensity-defined distributions

Table 1
Characterization of dextran samples D1–D9 (Pharmacia) and D10 and D11 (Sigma)

Sample	Supplier's data		Determined		Excluded part[a] (%)
	M_w	M_n	M_w	M_n	
D1	10 200	5900	9960	2600	1.6
D2	39 690	26 900	46 400	24 350	2.0
D3	70 600	40 850	70 370	42 470	3.2
D4	99 600	76 100	89 050	71 700	10.2
D5	240 570	113 970	208 200	105 300	12.6
D6	474 670	153 400	602 600	129 700	7.0
D7	2160 000	–	1827 000	235 000	43.2
D8	70 000	–	67 000	28 000	0.7
D9	500 000	–	713 000	172 000	15.3
D10	162 000	–	168 900	33 400	7.5
D11	298 000	–	276 000	42 600	18.8

[a] SEC in pure water.

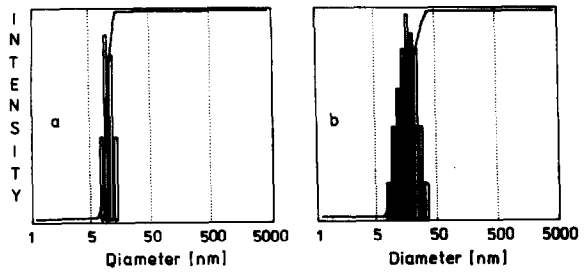

Fig. 2. CONTIN plots of particle size distributions of (a) dextran D4 ($M_w = 99\,600$) and (b) dextran D5 ($M_w = 240\,570$) as determined in pure water.

of particle sizes in Fig. 2a and b thus provides unambiguous evidence that this is not the case. DLS experiments were made in both mobile phases used in SEC experiments and no differences between comparable CONTIN plots corresponding to water (Fig. 2) and 0.01 *M* NaCl solutions were observed. Hence, the ion exclusion of charged molecules is the only possible explanation of the observed SEC excluded peaks. Mean hydrodynamic diameters, d_h, for dextrans D4 and D5 were obtained from CONTIN calculations as 15 and 27 nm, respectively. The corresponding experimentally determined values [21] of R_G are 9.6 and 15.5 nm, respectively. The ratio of hydrodynamic to gyration radii of polymer coils is known to be less than unity; experimentally, $R_h/R_G = 0.77$ was found [22]. A close comparable value of $R_h/R_G = 0.78$ was obtained for the D4 sample; the increase in this ratio to 0.87 for sample D5 might be due to its higher degree of branching [23].

The baseline difference in a DLS experiment is known to reflect the presence of dust; it is commonly agreed that a difference of not more than a few tenths of a per cent is required in a good experiment [17] and 0.01% or less is achieved [18] only with very clean solutions. In our experiments with samples D1–D7, the observed baseline differences were 0.1–0.3% in both water and 0.01 *M* NaCl, thus indicating the possible presence of some large particles in an amount too low to be detected by the CONTIN procedure. The 20 nm pore size of the Anotop filter should allow proper filtration of a solution of dextran D4 having $d_h = 15$ nm. The reliability of this filtration was checked by SEC experiments in both mobile phases (as in Fig. 1a and c) and no differences of the peak areas before and after the filtration were observed. Indeed, the DLS baseline differences found after the Anotop filtration of sample D4 were 0.01–0.02% and 0.04% in the worst case in repeated experiments and the same decrease of the baseline difference was observed with Anotop filtered samples D1–D3. This was the only detectable difference in experiments before and after the filtration; the CONTIN outputs were identical within the experimental error. The behaviour of these Anotop-filtered samples remained unchanged if stored in a refrigerator over 10 days, again confirming no formation of associates or aggregates.

Therefore, a micro-preparative SEC of sample D4 was performed in pure water. The aim was twofold: first, the separation of a sufficient concentration of dust particles (if any) for a successful DLS experiment should take place due to the size-exclusion effect. Second, the amount of collected fractions should be high enough to allow a determination of the molecular mass distribution of the excluded fraction in 0.01 *M* NaCl as a mobile phase. A micro-preparative chromatogram obtained in pure water is shown in Fig. 3a; a separation almost down to the baseline is observed. The same injection of the sample into 0.01 *M* NaCl as a mobile phase is shown in Fig. 3b. The refractometer sensitivity and all other SEC conditions were the same as in Fig. 1a. Any dust, if present in the sample, should be excluded in both mobile phases. Approximately 0.5% of the total sample mass is easily detectable under the conditions of Fig. 1a and c; in Fig. 3b the sample concentration was increased 38-fold and no minor excluded peak is observed in 0.01 *M* NaCl. A reliable conclusion [24] is that the impurity concentration must be less than 150 ppm if its refractive index response equals that of dextran. The DLS experiments on concentrated fractions fully confirmed the presence of such an impurity.

The CONTIN plot in Fig. 4a shows the presence of dextran with $d_h = 15$ nm (compare Fig. 2a) and particles with $d_h = 137$ nm in the concen-

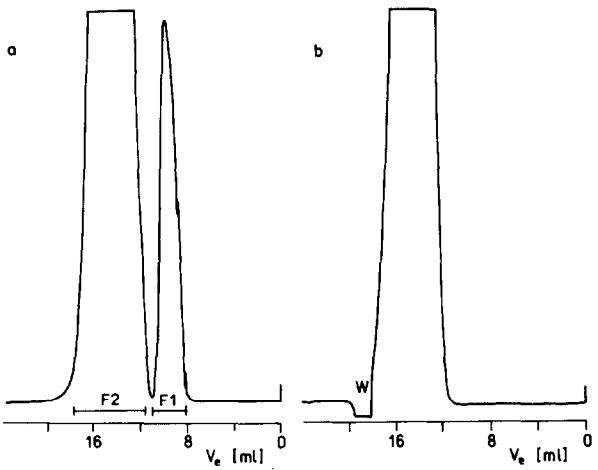

Fig. 3. Micro-preparative SEC of dextran D4 (M_w = 99 600) in (a) pure water and (b) 0.01 *M* NaCl. Sample concentration, 3.8% (w/w) in water, 100 μl injected; flow-rate, 1.92 ml/min; refractive index detection, attenuation as in Fig. 1. F1 = excluded fraction; F2 = main fraction; W = negative water peak.

trated excluded fraction and may be compared with the result of the CONTIN obtained for the main concentrated D4 fraction (Fig. 4b). The addition of NaCl to the excluded fraction had no influence on the DLS data and, of course, these particles could be completely removed with a 20-nm Anotop filter, again confirming foreign particles not detectable in an ordinary SEC and DLS experiment. DLS measurements of the unconcentrated excluded fraction behaved as typical [19] of a situation when there is too small a number of particles in the scattering volume; the baseline differences went up to 15%. Much better DLS behaviour was obtained when the concentration was increased. Hence these experiments show that dust can be separated by SEC, concentrated and its size successfully measured by DLS. The great similarity of DLS measurements of all D1–D7 samples concerning the baseline difference indicated that the particles found in sample D4 are present also in all other samples in similar amounts. It should be noted that these particles in the size range 110–170 nm would pass completely the most common filter size of 0.22 μm usually used in a DLS sample clean-up and no improvement of a DLS experiment can be achieved in this way.

An overlay of chromatograms of both main and excluded D4 fractions obtained in 0.01 *M* NaCl, *i.e.*, under ion-exclusion suppression, is shown in Fig. 5a and corresponding differential molecular mass distributions (MMD) in Fig. 5b. Both distributions are identical within the experimental error and the obtained M_w = 100 000 (M_n = 76 900) for the main fraction and M_w = 96 900 (M_n = 84 000) for the fraction excluded in water fit the D4 sample specification fairly well. This result confirms the uniform distribution of a minute charge over the whole molecular mass distribution of the original sample.

The last question to be solved concerns the origin and amount of charge responsible for the observed ion exclusion. A natural assumption is that partial oxidation of dextrans may form carboxyl groups; the single aldehyde end group of the dextran macromolecule should be the most easily oxidizable. Assuming a strictly linear dextran molecule, there should be just one C_6 hydroxy group on the opposite end as a candidate for the next oxidation step and there should

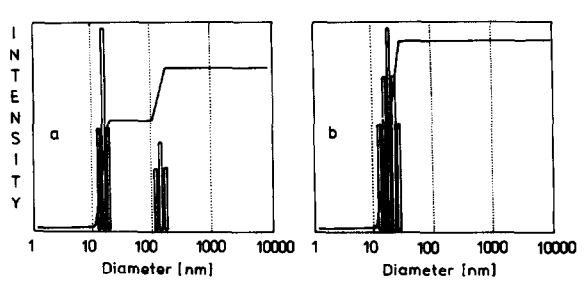

Fig. 4. CONTIN plots of particle size distributions of (a) concentrated excluded D4 fraction and (b) concentrated D4 main fraction.

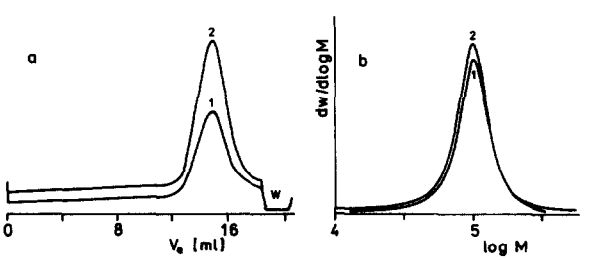

Fig. 5. Comparison of dextran D4, (1) excluded and (2) main fraction: (a) SEC in 0.01 *M* NaCl and (b) corresponding differential molecular mass distributions.

be some more C_6 hydroxy groups per molecule owing to the known dextran branching [4]. A mixture of gluconic acid and saccharic acid was selected as a low-molecular-mass model of charged dextran molecules (Fig. 6). Indeed, two partially separated peaks were obtained in an ion-exclusion chromatographic experiment in water. The third peak at the void volume must be uncharged and should be a mixture of 1,4- and 1,5-gluconolactone formed [25] from gluconic acid (supplied as a 50% aqueous solution, *i.e.*, in equilibrium). Fortunately, the gluconolactone formation in water is slow [25] and did not interfere with these experiments. IEC in pure water is known [26] to form strongly asymmetric peaks with elution volumes dependent on both the volume and/or concentration of the sample injected. By injecting different volumes of the sample with the same mass concentration, the leading edges of peaks are superimposed [26] (Fig. 6) but the peak locations and trailing edges differ. When the sample concentration is different, even the coincidence of leading edges disappears [26]. This must be remembered when IEC traces are compared. Another typical feature [26,27] of IEC is that the elution volume of ionized species may decrease on adding a low concentration of a strong acid (a suppression of solute ionization) or salt (a decrease in Debye screening length) to the mobile phase.

The dependence of the elution volumes of both dextran-excluded peaks on the NaCl concentration in the mobile phase is shown in Fig. 7. It is seen that IEC is the only mechanism governing the dextran ion exclusion; an identical behaviour of both excluded peaks is observed with two dextrans, D3 and D4 (Table 1), with different molecular masses. The elution volumes of the corresponding main peaks are different and constant, *i.e.*, a correct SEC behaviour is observed for uncharged parts of the samples.

Dextran D8 (Table 1), found to contain a very small amount of ion-excluded species, was selected for model oxidation experiments. Silver oxide is known to oxidize aldehyde groups selectively to carboxyls [28]. An IEC comparison of the parent D8 dextran with its silver oxide oxidation product is shown in Fig. 8a. One excluded peak is observed corresponding to a monocarboxydextran and the minor peak ahead of that one indicates a small amount of a higher oxidation product. The dotted part of this chromatogram, probably representing colloidal silver, was ob-

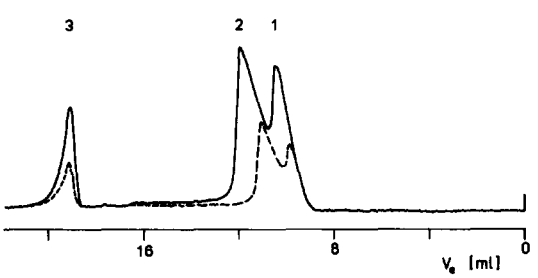

Fig. 6. Ion-exclusion chromatogram of a mixture of gluconic and saccharic acid in pure water. Injection, 0.05% (w/w) solution (by mixing 0.05% solutions 1:1, v/v), 100 μl (solid line) and 50 μl (dashed line); other conditions as in Fig. 1. 1 = Saccharic acid; 2 = gluconic acid; 3 = gluconolactones.

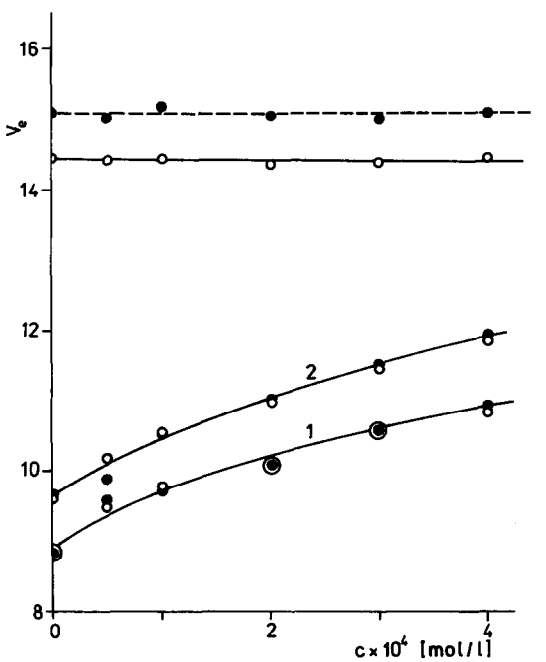

Fig. 7. Dependence of elution volumes of both excluded (1 and 2) and main peaks of dextrans (●) D3 and (○) D4 on the NaCl concentration in the mobile phase. Main peak D3 (M_w = 70 600, dashed line) and D4 (M_w = 99 600, solid line); other conditions as in Fig. 1.

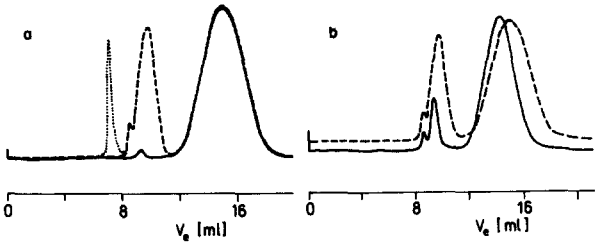

Fig. 8. Comparison of IEC–SEC traces of dextran D8 (M_w = 70 000) Ag_2O oxidation product (dashed line) with (a) parent dextran D8 (solid line) and (b) dextran D4 (solid line) in water. Sample injected, 100 μl of Ag_2O oxidation mixture diluted 1:25 (v/v); 0.1% (w/w) dextrans D4 and D8. Dotted peak of colloidal silver indicated; other conditions as in Fig. 1.

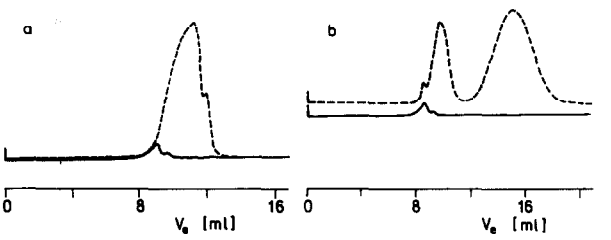

Fig. 9. Comparison of IEC–SEC traces of dextran D8 (M_w = 70 000) NaOBr oxidation product at (a) concentrations of 0.015% (solid line) and 0.5% (dashed line) and (b) a concentration of 0.005% (solid line) with Ag_2O oxidation product at a concentration of 0.125% (dashed line). Other conditions as in Fig. 1; different refractive index scales in (a) and (b).

served after filtration of the sample through 0.22-μm filter, but disappeared after filtration through a 20-nm Anotop filter. When dextran sulphate ($M_r \approx 70\,000$) with a degree of substitution of 0.07 (per glucose unit, *i.e.*, containing on an average of 28 sulphate groups) was injected, its peak coincided with the peak of the colloidal silver. The maximum ion-exclusion volume can thus be conveniently determined. Fig. 8b confirms the identification of the second excluded peak (with higher elution volume) from D4 as the monocarboxydextran.

Nitrogen dioxide [29] and dinitrogen trioxide [30] are known to oxidize almost selectively C_6 hydroxy groups on cellulose to carboxyls. An $NaNO_2$–H_3PO_4 oxidation mixture [30] was applied, but extreme degradation of dextran D8 was observed. To prevent degradation, sodium hypobromite oxidation [29] (believed to operate similarly) in neutral solution was used, and turned out to be successful. The resulting IEC trace (Fig. 9a) of the oxidized product after dialysis shows a typical elution volume shift (*cf.*, Fig. 6) at two different mass concentrations. All dextran molecules therefore carry at least one carboxyl group, the monocarboxy derivative content being low, and the comparison of the NaOBr and Ag_2O oxidation products (Fig. 9b) confirms that the first excluded peak (*cf.*, Fig. 8b) contains mainly dicarboxydextran. The different leading edge of the NaOBr oxidation product (Fig. 9b) might indicate more than two carboxyls, *i.e.*, oxidized C_6 hydroxy groups in dextran branches.

An IEC trace of the fresh, excluded fraction of D4 from the micropreparative experiment (the same day as isolated) in water is compared with the same injection of this fraction 1 week later in Fig. 10a. The formation of a neutral species identical with the parent dextran is observed and conveniently explained by lactone formation. The same behaviour of the fresh main (uncharged) D4 fraction (Fig. 10b) shows a small excluded peak after 1 week and indicates a slow formation of free acid. Hence, the ion exclusion normally observed with dextran solutions should show only free carboxyls and some amount of oxidized dextran may be "hidden" in the main peak owing to the possible lactone formation during the whole sample history.

Finally, the SEC trace of a pullulan standard

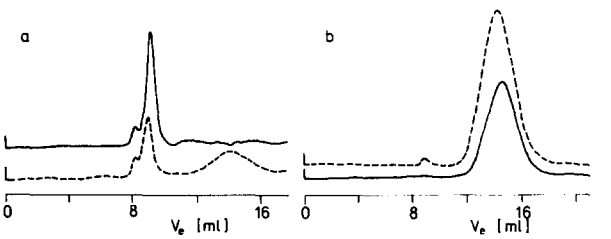

Fig. 10. Comparison of IEC–SEC traces of (a) dextran D4 fresh (solid line) and 7 days old (dashed line) excluded fraction and (b) dextran D4 fresh (solid line) and 7 days old (dashed line) main fraction in water. Other conditions as in Fig. 1.

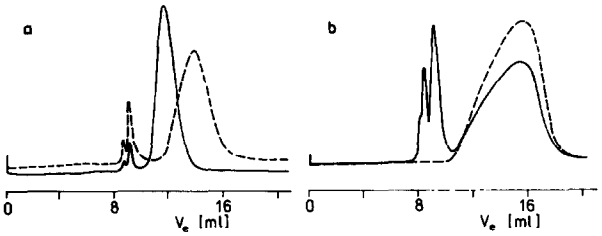

Fig. 11. Comparison of IEC–SEC traces of (a) pullulan standard (M_w = 186 000, solid line) with dextran D4 (M_w = 99 600, dashed line) in water and (b) of hydroxyethylstarch (M_w = 200 000) in water (solid line) and in 0.01 *M* NaCl (dashed line).

in pure water is compared with an injection of dextran D4 under the same conditions in Fig. 11a. The excluded peaks of both samples coincide and confirm the presence of identical charged groups in the pullulan sample. SEC traces of a hydroxyethylstarch sample in pure water and in 0.01 *M* NaCl are compared in Fig. 11b. The leading edge of the first excluded peak in water probably indicates more than two carboxyls per molecule. Otherwise, a behaviour completely identical with that of dextran D4 is again observed and indicates that this is probably a general effect typical of most polysaccharides. An IEC–SEC experiment in pure water is perhaps the only technique that is able to detect and resolve one and two carboxyl groups in a polysaccharide molecule with molecular mass up to 10^6.

4. Conclusions

Dextrans do not aggregate in dilute solutions. A very small amount of dust particles contained in dextran samples, having a particle size within the range 110–170 nm, was separated by SEC and successfully measured by DLS. Dextran excluded peaks observed in SEC in pure water originate purely from ion exclusion. Monocarboxy- and dicarboxydextran constitute the main charged species. The presence of such charged groups is independent of the chain length. IEC of dextrans may detect carboxyls only partially owing to the possible lactone formation. The carboxy groups are mainly the result of partial oxidation of aldehyde and C_6 hydroxy end-groups. An IEC–SEC experiment is capable of detecting the presence of polysaccharide chains bearing one or two carboxy groups only, and of resolving them from each other and from the uncharged chains for molecular masses up to 10^6. The presence of these charged groups seems to be a general feature of polysaccharides.

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6. References

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