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Liquid chromatography of dextrans on porous silica beds☆

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Abstract

Kinetics, equilibrium isotherms and chromatography retention times for sorption of dextrans T-10, T-20, T-40, T-70, T-110, T-161, T-250 and T-500 on porous silica were measured at 25 °C. The Henry constant and retention factors for the dextrans were obtained. The values of the partition coefficient for the distribution of the dextrans between the bulk solution and the pore space were calculated within the framework of a pore volume filling model with consideration given to the ratio between the sizes of the macromolecular coils and the pore inlet. The measurements showed that this parameter depends on the structure of the sorbent and the molecular mass distribution of the dextran. The interaction of aqueous dextran solution with porous silica is characterized by the sieve effect. Large macromolecular coils of dextran T-161 cannot penetrate into the pore space of the silica sorbent with pore diameter 14 nm. The difference in Henry law constants calculated from adsorption and chromatographic data for dextrans T-70 and T-110 can be explained by the slow diffusion of dextran macromolecules into silica pores under chromatographic conditions.

Keywords: Liquid chromatography; Carbohydrates; Water; Silicas

1. Introduction

In liquid chromatography, narrow fractions of dextrans serve as references in calibrating of HPLC columns to be used for the separation of water-soluble polymers and for the analysis of their nature, like molecular mass or polydispersity [1–7]. In many studies, the eluent was pure water. In recent years, however, water-methanol and water–acetonitrile mobile phases have gained a wide application in liquid column chromatography.

Dextrans, which belong to the class of polysaccharides, consist of residues of D-glucose monosaccharide linked with one another predominantly via glycoside a-1,6 bonds. One should note that a-1,4 and a-1,3 bonds can also serve as bridges between monosaccharide in the polysaccharide chain. The latter two types of bonds can impart a significant degree of branching to the polymer chain of the polysaccharide. The degree of branching depends on how and under what conditions the polysaccharide was synthesized. The dextrans entering into the composition of blood plasma have a molecular mass 40,000–70,000.

For chromatographic processes involving an aqueous mobile phase, the elution order of the analytes is determined by the cooperative effect of intermolecular forces of various natures [5,7]. To optimize a chromatographic process, it is necessary to understand on the molecular level how the chemical nature of the components influences their retention on the sorbent. In the case of sieve (size-exclusion) chromatography, it is necessary to elucidate the mechanism of action of the porous structure of the sorbent on the separation of polysaccharides.

In particular, it is interesting to establish how mixtures of water-soluble polysaccharides are separated on porous silica. Pursuing this aim, we studied the sorption of dextrans from aqueous solutions on porous silicas under static and dynamic conditions and compared the results.

2. Theoretical

Sieve liquid chromatography (SLC) is a version of negative molecular chromatography (NMC) and is based on molecular-sieve steric effects. More specifically, macromolecular coils whose size is larger than the size of a pore inlet cannot penetrate into the pore. As a result, large macromolecules are eluted before smaller macromolecules, and, hence, the excess adsorption (n) is negative, since the equilibrium concentration C of polymer macromolecules in bulk solution becomes higher than

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the initial concentration C_0:

$$n = \frac{(C_0 - C)V}{m} \quad (1)$$

where V is the volume of the polymer solution; m, the mass of adsorbent; and C_0 and C, the initial and equilibrium concentrations of polymer macromolecules (mg ml^{-1}), respectively.

The time of retention t_R of a polymer in an SLC experiment is shorter than the time of retention of an un-retained (low-molecular-weight) substance, and, therefore, the retention factor

$$K = \frac{t_R - t_0}{t_0} \quad (2)$$

or

$$K = \frac{V_R - V_0}{V_0} \quad (3)$$

is a negative quantity. Here, the subscript R is for the polymer and 0 for the un-retained substance.

In adsorption chromatography, the degree of retention can also be characterized by Henry law constant K_H (mg L^{-1}) [8–13]:

$$K_H = \frac{V_R - V_0}{m} \quad (4)$$

where m is the mass of the sorbent in the chromatographic column.

For NMC, K_H is also negative quantity. It was demonstrated in [7] that NMC may be based on the stronger interaction of the sorbent with the eluent compared to the sorbent–analyte interaction. Therefore, the separation of weakly adsorbed substance may occur in the NMC mode, i.e., at $t_R < t_0$.

In SLC, the accessibility of the sorbent pores to polymer macromolecules is characterized by the partition coefficient K_p. It was demonstrated in [14] that

$$K_p = \frac{V_i}{V_s} \quad (5)$$

where V_i is the volume of the pores accessible to polymer macromolecules and V_s is the total pore volume (the volume of the pores accessible to molecules of an un-retained (low-molecular-weight) substance and to eluent molecules). According to [14],

$$V_i = V_R - V_0 + V_s \quad (6)$$

Then

$$K_p = \frac{V_i - V_0}{V_s} + 1 \quad (7)$$

In case of SLC, Eq. (7) yields the value of $V_{R,0}$ at which $K_p = 0$:

$$V_{R,0} = V_0 - V_s \quad (8)$$

An analysis of the initial linear segments of negative (sieve) sorption isotherms yields the following expression for the partition coefficient $K_{p,a}$:

$$K_{p,a} = \frac{C_s}{C} = \frac{n + V_s C}{V_s C} \quad (9)$$

Table 1
Characteristics of silica sorbents: the pore diameter D_P, pore volume V_P and specific surface area S

Sorbent	D_P (nm)	V_P (cm^3/g)	S (m^2/g)
Silica gel KSM-5	3.2	0.6	720
Silica gel KSK-2	14	1.2	340
Silochrom S-80	55	1.5	105

where C_s and C are the equilibrium concentration of polymer macromolecules in the sorbent pores and in the bulk solution, respectively. Thus,

$$K_{p,a} = \frac{n}{V_s C} + 1 \quad (10)$$

For the initial linear segment of the sorption isotherm, $n \times C^{-1} = K_H$, where K_H is smaller than zero for negative sorption. In this case,

$$K_{p,a} = \frac{K_H}{V_s} + 1 \quad (11)$$

3. Experimental

The sorbents were KSK-2 and KSM-5 porous silica gels from Reakhim (Moscow, Russia) and porous silica Silochrom S-80 from Luminifor (Stavropol, Russia). The structural characteristics of the sorbents are listed in Table 1.

The specific surface area values for KSK-2 and S-80 were carried out on an Areatron installation (Paris, France), by determining the argon adsorption isotherm at −195.7 °C (77.3 K). The total pore volume and pore size distribution were assessed from the mercury adsorption isotherm performed on Micromeritics AutoPore II 9220 (Norcross, GA, USA). The structure characteristics of KSM-5 were taken from its certificate.

We used dextrans with weight-average molecular masses (M_W) of 9300; 22,300; 44,400; 69,500; 106,000; 154,000; 253,000 and 532,000 and lactose from Pharmacia (Uppsala, Sweden). In the adsorption and chromatographic experiments, we used thrice distilled water. When preparing the initial solutions, we added sodium azide in an amount of 0.08%. The characteristics of dextrans are summarized in Table 2.

The concentrations of the solutions for adsorption measurements were determined with LOMO a liquid interferometer

Table 2
Characteristics of the dextrans: the weight-average molecular mass M_W and polydispersity n ($n = M_W \times M_N^{-1}$, where M_N is the number-average molecular mass)

Dextrans	M_W	n
T-10	9300	1.63
T-20	22,300	1.49
T-40	44,400	1.54
T-70	69,500	1.76
T-110	106,000	1.40
T-161	154,000	1.79
T-250	253,000	2.25
T-500	532,000	2.81

LIR-2 (St. Petersburg, Russia). The interferometer was calibrated with special aqueous solutions of the dextrans at 25 °C. All solutions were prepared in glass flasks. The obtained interferometer signals of few solutions with different concentration were used as comparison values for determining the concentration of the dextrans in the external solutions during experiment.

In the adsorption experiments, a weighed portion of an adsorbent (0.1–0.3 g) was placed into a test tube with a ground-glass stopper and flushed with 2 and 6 ml of an aqueous solution of a dextran. The mixture was kept at 25 °C until a state of equilibrium was attained. The excess (Gibbs) adsorption (n) was calculated from the decrease in the concentration of the dextran in the external solution by Eq. (1).

In the chromatographic experiments, the retention times for the dextrans were measured with a chromatograph Microtechna LC-601 (Prague, Czech Republic) equipped with a refractometric detector RID K-101 having a measurement cell volume of 10 μL and sensitivity of 10-8 refractive index units. Glass columns, 150 mm × 3.3 mm in size, were packed with dry KSK-2 or S-80 sorbent (25 ± 5 μm fraction). The eluent was freshly distilled water. The volumetric flow rate of the eluent was 1 ml min^{-1}; it was measured with a calibrated micro-burette. A 20 μL probe of 1% solution of dextran in water was injected into the system using manual injector with a dosing loop Rheodyne 7206 (USA). Chromatographic peaks were recorded with a chart recorder Microtechna T-34620 (Prague, Czech Republic); the retention time was also measured with a stopwatch. The retention volume for an analyte was set equal to the average value of three measurements. The dead volume was calculated from the partition coefficient, which, in turn, was determined from the adsorption isotherm for lactose measured under static conditions and the retention volumes for the elution of lactose from each column.

4. Results and discussion

4.1. Kinetic's characteristics

Fig. 1A–D show the kinetic curves for the sorption of the dextrans from their aqueous solutions on S-80, KSK-2 and KSM-5. When an aqueous solution of dextran compound interacts with a silica sorbent, the pores accommodate both water molecules and, if the size of molecules permits, dextran molecules.

The minimum in kinetic curve for the sorption of dextran T-40 from aqueous solution on S-80 (Fig. 1A) (a macro-porous sorbent) suggests that dextran macromolecules rapidly (during first 5–15 min) penetrate into the pores of S-80 (for KSK-2, a sorbent with narrow pores, no such minimum is observed). At later stages of the sorption process, water molecules occupy the narrow pores and simultaneously remove dextran T-40 molecules from the wide pores. As a result the equilibrium macromolecules distribution of dextran T-40 between the pore space and bulk solution is attained. Dextran T-70 macromolecules cannot penetrate into the micro-pores of KSM-5 sorbent and therefore they accommodate only water molecules (Fig. 1B). At the initial stage water molecules are probably accumulated in the macropores as well (or in the space between sorbent grains). However, later these pores are gradually filled by dextran T-70 macromolecules. This mechanism explains why the maximum (at t = 40 min) appears in the kinetic curve for the sorption of dextran T-70.

Dextran T-500 is characterized by high polydispersity (Table 2); its molecules are too large to penetrate into the pore of KSK-2 silica gel (14 nm in diameter; Fig. 1D). At the same time the low-molecular-weight fraction of dextran T-500 can penetrate into some of the pores of Silochrom S-80, a macro-porous sorbent. This conclusion is supported by the observation that

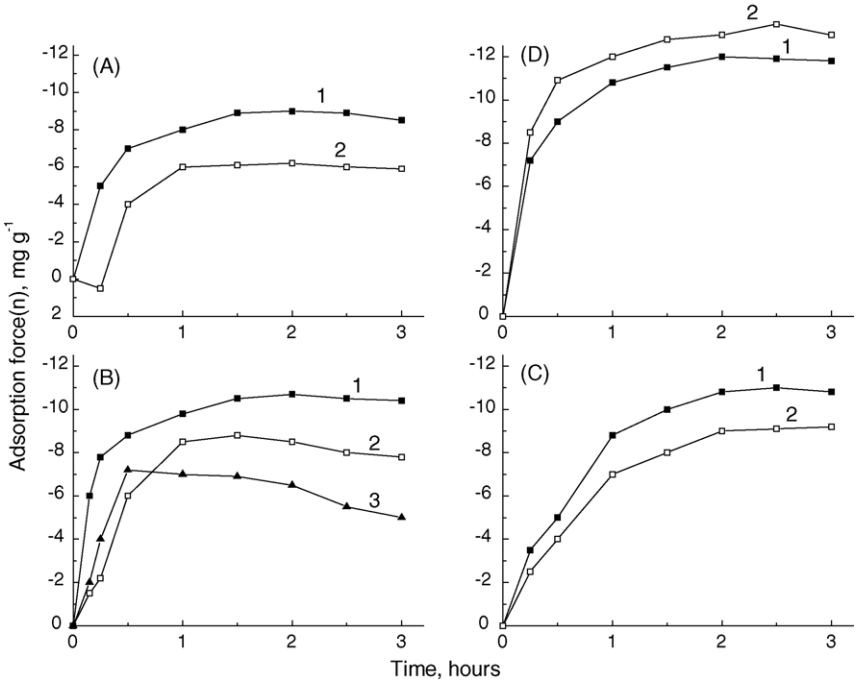

Fig. 1. Kinetic's curves for sorption of the T-40 (A); T-70 (B); T-110 (C) and T-500 (D) dextrans from aqueous solutions on KSK-2 (1), S-80 (2) and KSM-5 (3).

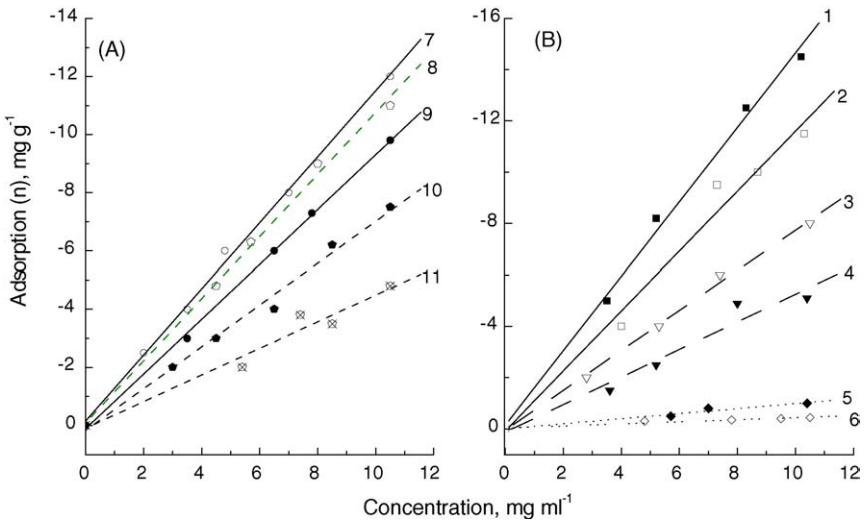

Fig. 2. Adsorption isotherms of dextrans T-500 (B-1, 2), T-40 (B-3, 4), T-110 (A-7, 9), T-70 (A-8, 10, 11) and lactose (B-5, 6) from aqueous solutions on S-80 (1, 4, 5, 9, 10), KSK-2 (2, 3, 6, 7, 8) and KSM-5 (11). Points: experimental data, lines: Henry low equation.

the values of the sorption of this dextran on KSK-2 and S-80 sorbents are similar.

4.2. Adsorption isotherms

Fig. 2A and B show typical isotherms of sorption of the dextrans from their aqueous solutions on the silicas. The position and shape of the negative sorption isotherms for these systems are determined by the molecular sizes of the polysaccharide molecules, the porous structure of the silicas, and the total energy of the interactions in the water–dextran–silica system. The characteristics of the negative sorption of the dextrans depend substantially on the size distribution of the macromolecular coil of the dextran and the pore inlet.

The molecules of the dextrans are highly branched. In aqueous solutions they form loosely hydrated coils. For this reason water molecules (which are more mobile than dextran molecules) interact with hydroxyl groups at the silica surface stronger and rapidly due to higher interaction potential. The obtained isotherms of sorption of the dextrans from their aqueous solution on the silica obey the Henry law over the entire equilibrium concentration range covered. Therefore, the slope of the isotherm at $C \rightarrow 0$ yields the Henry constant:

$$K_H = \frac{n}{C} \tag{12}$$

The partition coefficient K_p was calculated by Eq. (10) from the values of the specific pore volume for the sorbents from Table 1.

4.3. Retention characteristics

Table 3 summarizes the chromatographic characteristics for the elution of the dextrans and lactose from column packed with KSK-2 silica gel and Silichrom S-80. The retention factor K and the partition coefficient K_p were calculated by Eqs. (3) and (7), respectively. The volume of the mobile phase (the dead volume) was calculated from the retention time and the value of sorption of lactose from the aqueous solutions on silica by Eqs. (7) and (9). The order of elution of dextrans from the columns packed with KSK-2 and S-80 suggest that the chromatographic processes are led by the sieve mechanism: large dextran coils, which cannot penetrate into the pore space of the sorbent drift at a higher velocity than the macromolecules capable of completely or partially penetrating into the pore of the silica sorbent.

As can be seen from Table 3, the values of the partition coefficient (K_p) lie within 0–1, the range typical for the negative sorption and SLC. Only for lactose and the low-molecular-weight dextran (T-40) the value of the partition coefficient equals 1 (one), i.e., no selectivity.

Fig. 3 shows the partition coefficient K_p for the elution lactose and the dextrans from the columns packed with KSK-2 and S-80 as a function of the logarithm of the molecular mass of the polysaccharide. As can be seen the K_p values for these processes are positive and enclose in the range from 0 to 1. For lactose the values of K_p obtained on these two columns virtually coincide. For the dextrans the values obtained on the column with KSK-2 are lower than in the case of column packed with S-80. For

Table 3
Retention volume V_R, retention factor K and partition coefficient K_p for the elution of lactose and dextrans with water on KSK-2 and S-80 silicas

Saccharide	KSK-2, V_0 = 13.3 ml			S-80, V_0 = 15.6 ml		
	V_R (ml)	K	K_p	V_R (ml)	K	K_p
Lactose	13.2	−0.01	0.98	15.5	−0.01	0.99
T-10	10.0	−0.23	0.48	15.1	−0.03	0.93
T-20	9.0	−0.31	0.31	13.4	−0.14	0.71
T-40	7.8	−0.40	0.12	12.0	−0.23	0.52
T-70	7.2	−0.44	0.03	10.8	−0.31	0.37
T-110	7.1	−0.45	0.02	10.0	−0.36	0.26
T-161	7.0	−0.46	0	9.2	−0.41	0.16
T-500	7.0	−0.46	0	8.2	−0.47	0.03

The flow rate of mobile phase was 100 μL/min, temperature 25 °C, V_0 is the volume of the mobile phase.

Table 4
Values of Henry constant K_H for the sorption of lactose and dextrans from aqueous solutions on KSK-2 and S-80 sorbents calculated from batch sorption and chromatographic data

Saccharide	KSK-2		S-80	
	Chromatographic K_H (cm^3/g)	Batch K_H (cm^3/g)	Chromatographic K_H (cm^3/g)	Batch K_H (cm^3/g)
Lactose	−0.02	−0.02	−0.01	−0.01
T-40	−1.06	−0.82 ± 0.1	−0.69	−0.58 ± 0.1
T-70	−1.17	−1.08 ± 0.1	−0.94	−0.72 ± 0.1
T-110	−1.19	−1.15 ± 0.1	−1.1	−0.92 ± 0.1
T-500	−1.2	−1.19 ± 0.1	−1.42	−1.38 ± 0.1

dextrans T-10–T-110 the dependences displayed in Fig. 3 have nearly linear segments. The values K_p calculated from these segments sharply decrease with increasing molecular mass of the dextran.

4.4. A comparison of the static and dynamic characteristics of the sorption of the dextrans

Table 4 compares the values of K_H calculated by the corresponding equation from the chromatographic data and the isotherms of static sorption of the dextrans from aqueous solutions on KSK-2 and S-80. The accuracy of the static determination of the sorption values for the dextrans (from which K_H was calculated) was not higher than 10%, since the degree of extraction (the difference between C_0 and C) was = 3%. As can be seen from Table 4, the values of K_H calculated from the static sorption and SLC data virtually coincide for sorption of lactose (disaccharide) and T-500 (high-molecular-weight dextran) on KSK-2 and S-80. These systems represent the two limiting cases: lactose molecules freely penetrate into the pore space of the sorbent, while large dextran T-500 molecules can not. Dextrans T-40, T-70 and T-110 show differences within 25% between the values of K_H obtained from static and chromatographic measurements on KSK-2 and S-80. This result can be explained by slow diffusion of dextrans T-40, T-70 and T-110 into the pore of space of KSK-2 and S-80 under chromatographic conditions.

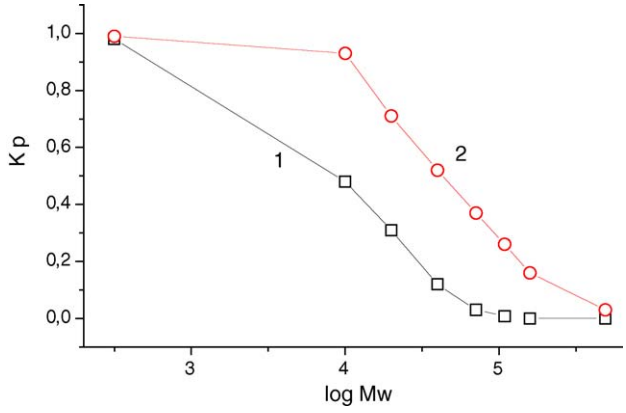

Fig. 3. Dependences of partition coefficient on molecular mass of dextrans on KSK-2 (1) and S-80 (2) columns. Eluent: water.

One should note that under negative chromatography conditions (in the absence of adsorption in the pore space of the sorbent) the separation of dextrans on porous silicas occurs through the sieve mechanism.

Thus obtained results show that the sorption of dextrans from aqueous solutions on mesoporous silica sorbents occurs by the sieve mechanism. The pore space of the sorbent is filled by water molecules; dextran macromolecules can also penetrate into the pores if their size is smaller than the pore inlets.

A comparison of the obtained static sorption and chromatographic data (the partition coefficients and Henry constants) for the sorption of the dextrans from aqueous solutions on hydroxylated silicas demonstrated that this process is characterized by the sieve (size-selective mechanism) and negative sorption effects.

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