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MEASUREMENT OF EXCLUSION VOLUMES OF PACKED COLUMNS BY MEANS OF ELECTROKINETIC DETECTION

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SUMMARY

A method is described for the measurement of the exclusion volumes of packed chromatographic columns by means of electrokinetic detection in liquid chromatography.

INTRODUCTION

Exclusion volume is a significant parameter in gel permeation chromatography. The exclusion effect arises when the solute molecules are too large to enter the pores of the gel[1]. However, exclusion also occurs with molecules of suitable dimensions but which possess the same charge as the groups on the gel surface. For instance, in a silica gel–water system, where the silica gel surface is believed to have a negative charge, the exclusion of sodium acetate[2] was observed. These papers suggest that the exclusion effect and the electrostatic characteristics of the chromatographic system are related.

The exclusion effect may even be applied in other types of column chromatography to estimate the efficiency of the packing procedure used. The use of sufficiently large molecules is, however, not suited for the determination of the exclusion volume owing to adsorption effects, and there are few systems with acceptable electrostatic properties.

The electrokinetic principle of detection has recently been described for liquid chromatography[3–5]. The streaming current can be measured either after the column[4] from capillaries or adsorption beds or directly in the chromatographic column[5]. In addition to solute peaks, a vacant peak[6] (in the case of a binary mobile phase) and a peak recorded before the peak due to the dead volume were found in the chromatogram if the streaming current was measured directly in the column. It was shown that the peak before the peak due to the dead volume corresponds to the exclusion volume. The response can be recorded for both solute and vacancy by either an electrokinetic detector or conventional detectors (refractive index or UV detectors), but the exclusion peak can only be recorded by electrokinetic detection.

It was also found that an exclusion peak is observed when a steel wire is

inserted into the column through an injector septum in such a way that it comes into contact with the sorbent. The origin of the exclusion peak in this case is not yet clear. However, the mixtures injected contained neither charged molecules nor molecules sufficiently bulky for exclusion. This phenomenon has therefore been utilized in the determination of the exclusion volume of adsorption columns in high-performance liquid chromatography (HPLC). The advantage of this procedure consists in the possibility of avoiding corrections for the detector and the injector volumes since the response is obtained directly from the chromatographic column, and, furthermore, of avoiding the introduction into the column of such substances that could be adsorbed irreversibly and as a result change the adsorption properties of the column.

EXPERIMENTAL

The measurements were carried out in a normal chromatographic arrangement using instruments of our own construction. One detector (UV detector or refractometer) was always connected after the element used for sensing the electrokinetic signal.

In order to verify that one of the electrokinetic responses registered after the passage of the mobile phase volume is equal to the exclusion volume, a stainless-steel column (500 × 2 mm I.D.) packed with Porasil B (d_p = 50–63 μm; Waters Assoc., Milford, Mass., U.S.A.) was used. Porasil was gradually replaced with glass beads (d_p = 50–63 μm; Kavalier, Sklárny, Czechoslovakia). The exclusion and dead volumes of the bed of Porasil A (d_p = 50–63 μm) were measured on a 200 × 2 mm I.D. column. The columns were dry packed. Other columns used were stainless-steel columns (200 × 4 mm I.D.), packed with microparticulate materials. The viscosity variant of the slurry packing technique was applied, cyclohexanol with 5% methanol serving as suspension medium. The silica gels used were mostly commercial products or materials developed by Lachema, Brno, Czechoslovakia, of Silasorb type with specific surface area 400–600 m^2/g and mean particle diameter 6–10 μm. Microparticulate silica gel prepared on a laboratory scale at our institute and the fraction of spherical silica gel Silpearl (d_p = 10–15 μm, Kavalier) for thin-layer chromatography, graded by the authors, were also applied. Microparticulate alumina Alusorb (d_p = 9–15 μm) was supplied by Lachema.

The streaming current was sensed from the capillaries and adsorption beds[4], but, most often from the column[5] that was screened and electrically isolated. In this arrangement, used also for the measurement of the exclusion volumes of the columns, the output stainless-steel capillary was earthed and electrically isolated from the column. A diagram of the electric circuits has been shown previously[5].

n-Propanol, isopropanol or butanol (analytical grade purity, Lachema) solutions in hexane (analytical grade; International Enzymes Ltd., Windsor, Great Britain) were used as mobile phases. Other components were sometimes also added in concentrations up to 1% (acetic, chloroacetic and perchloric acids).

Samples of various origins were applied in the highest available purity without any preliminary purification. They were usually dissolved in the mobile phase or injected in pure state. Solutions of butanol in mobile phase (1 : 10) were generally used for the measurements of exclusion volumes. Samples were injected by means of

syringes through a septum either into the mobile phase stream at various distances from the chromatographic bed or directly into the packing. Injection effects were generated either by the syringe needle or by a stainless-steel wire.

RESULTS

After injecting a nonsorbed solute (in the present case *n*-butanol), two chromatographic peaks appear in the chromatogram with the electrokinetic detection. The first peak, having a smaller elution volume, appears even when the needle of the injection syringe, or the wire, is only introduced on the beginning of the column packing. Neither refractometer nor UV detector, connected after the column, provide any measurable signal in this elution time.

The elution volume of the first peak was shown to correspond to the exclusion volume of the column. The porous packing of the column (Porasil B) was gradually replaced with glass beads and the change in the positions of both peaks in the chromatogram was investigated. The results are shown in Table I. Besides experimental values of the dead volume, V_M, of the column, the table also presents values calculated according to

$$V_M = V_e + V_p$$

where the exclusion volume, V_e, was determined experimentally by the described procedure. The volume of the pores of the packing, V_p, was calculated from the value of V_M, measured for the column packed with Porasil B only. The elution volume of the first peak obviously does not change with changing volume of the pores of the column packing and corresponds to the exclusion volume.

TABLE I

CHANGES IN EXCLUSION VOLUME AND DEAD VOLUME WITH CHANGING INTRAPARTICLE POROSITY OF THE COLUMN

Column: 500 × 2 mm I.D.; packing, Porasil B (d_p = 50–63 μm), glass beads (d_p = 50–63 μm). Mobile phase: 5% *n*-butanol in *n*-hexane. Sample: 5 μl of the solution of *n*-butnaol in the mobile phase.

Column packing	*Content of glass beads in the packing (%)*	V_e *(ml)*	V_M *(ml)*		V_e/V_M
			Exptl.	*Calc.*	
Porasil B (50 cm)	0	0.54	1.05	—	0.51
Porasil B (35 cm), glass beads (15 cm)	30	0.55	0.89	0.90	0.62
Porasil B (20 cm), glass beads (30 cm)	60	0.55	0.77	0.75	0.71
Glass beads (50 cm)	100	0.54	0.54	0.54	1.0

The elution of the exclusion peak was studied for various mobile phases, amounts and qualities of the samples of pure substances, their solutions in the mobile phase and other solutes. Table II summarizes typical results from one series of the measurements of this type. The exclusion responses were also recorded for columns packed with silica gel of various types and with alumina. The values are given in Table III. The results of the experiments can be summarized as follows.

TABLE II

VERIFICATION OF INDEPENDENCE OF MEASURED EXCLUSION VOLUMES OF THE MOBILE PHASE COMPOSITION AND INJECTED SOLUTE

Column: 200 × 4 mm I.D.; packing, Silasorb 600 ($d_p = 9.4 \pm 0.8\ \mu m$).

Mobile phase	*Solute*	V_e *(ml)*	V_M *(ml)*	k'
3% *n*-butanol in *n*-hexane	needle injection	1.30	—	—
	2-bromopyridine	1.27	2.03	2.00
	benzyl cyanide	1.29	2.03	2.00
	p-nitroaniline	1.29	2.03	2.95
4% *n*-butanol in *n*-hexane	needle injection	1.29	—	—
	nitrobenzene	1.29	2.03	0.26
	o-nitroaniline	1.27	2.00	2.48
	acetonitrile	1.27	2.03	7.3
5% *n*-butanol in *n*-hexane	*n*-butanol	1.29	2.00	0.0
	2-bromopyridine	1.27	1.95	1.44
	o-nitroaniline	1.29	2.06	2.05
	n-propanol	1.29	2.00	1.92
7% *n*-butanol in *n*-hexane	needle injection	1.27	—	—
	benzyl cyanide	1.29	2.00	0.65
	2-bromopyridine	1.27	2.03	1.18
	n-propanol	1.28	2.00	1.80
10% butanol in *n*-hexane	*n*-butanol	1.27	1.95	0.0
	m-dinitrobenzene	1.26	2.00	0.78
	2-bromopyridine	1.27	2.00	0.80
	m-nitroaniline	1.29	2.00	2.64

TABLE III

EXCLUSION VOLUMES AND DEAD VOLUMES OF VARIOUS CHROMATOGRAPHIC COLUMNS

Mobile phase: 5% *n*-butanol in *n*-hexane. Sample: 5 μl of the solution of *n*-butanol in the mobile phase.

Column		*Packing*	d_p *(μm)*	V_e *(ml)*	V_M *(ml)*	V_e/V_M
Length (cm)	*I.D. (mm)*					
20	4	Silasorb 600	6–10	1.34	1.92	0.70
20	4	Silasorb 600	6–10	1.40	2.10	0.67
20	4	Silasorb 600	6–10	1.40	2.02	0.69
50	2	Porasil B	50–63	0.54	1.05	0.51
50	2	Porasil B	50–63	0.61	1.15	0.53
20	2	Porasil A	37–75	0.32	0.50	0.64
20	4	Al_2O_3-160	9–15	1.59	1.99	0.80

The polarity of the exclusion signal changes for a given column with the mobile phase used, sometimes even with the volume of the mobile phase passed through. It is also affected by the amount of the injected solutes, linear velocity of the mobile phase in the bed and speed of the injection. The magnitude of the signal is dependent on the relative positions of the needle and the bed at the moment of injection and there after. The elution time (volume) of these signals does not, however, depend on any of the above mentioned parameters, except for the injection speed. If the sample is injected quickly, as is usual in chromatography,

the elution volume of the exclusion peak is constant for the given column, does not depend on the mobile phase used and on the method of generation of the exclusion signal (Table II). Its character thus only depends on the packing material and the procedure for placing it into the column (Tables I and II).

Compared with the existing procedure, realized only in gel permeation chromatography, the measurement of exclusion volumes described in this paper has several advantages:

It may be realized in any system that provides a suitable electrokinetic signal.

If the weight of the column packing is known, the volume of the pores of the column packing may easily be calculated from the dead volume of the column and the exclusion volume.

The interstitial velocity of the mobile phase, the determination of which has often been a problem, can be calculated directly from the elution time of the exclusion response and from the length of the bed.

The record of the exclusion volume, sometimes even of the dead volume in multicomponent phases, is obtained automatically after each injection. Therefore it may be used to advantage for checking the long-term stability of the flow-rate.

No substances are introduced into the column that might be sorbed irreversibly and thus cause a change in the properties of the column.

The results of the measurements need not be correlated for extra column volumes.

The mechanism of the origin of the exclusion response has not yet been explained. Despite this we consider it useful to draw attention to this phenomenon and to show the possibilities of its practical application.

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