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SURFACE EXCLUSION AND GEOMETRICAL EXCLUSION

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SUMMARY

The new concepts of surface exclusion and geometrical exclusion are clearly defined in this paper. These two fundamental exclusion mechanisms are successfully applied to open-capillary chromatography, hydrodynamic chromatography and size-exclusion chromatography. The experimental elution volumes are in excellent agreement with predicted data. An elegant iteration method is proposed to estimate the interstitial porosity and to correct the partition coefficient from experimental data in size-exclusion chromatography. The relationship between surface and geometrical exclusion and their contribution to the overall exclusion are quantitatively examined. The similarity and difference between hydrodynamic chromatography and size-exclusion chromatography are fully revealed.

INTRODUCTION

Hydrodynamic chromatography (HDC) and size-exclusion chromatography (SEC) have been the subjects of extensive study. Both separation methods are based upon the solute size difference; the major difference is that HDC makes use of non-porous packings and SEC makes use of porous packings. However, the separation mechanism of HDC is generally regarded as the flow velocity profile, and is often called separation by flow (SBF)[1], while that of SEC is considered to be distribution in the pore volume[2].

In the early 1960s, Pederson recognized the "external size exclusion" phenomenon in HDC[3]. At the beginning of the 1970s, Di Marzio and Guttman described the similarity between SBF and SEC[4]. Recently, Squire *et al.* proposed "external size exclusion" as the predominant mechanism for HDC and called SEC "internal size exclusion"[5].

However, internal and external size exclusion are only superficial differences and do not reflect the nature of the separation. The separation mechanisms for HDC and SEC are still somewhat vague.

In the present study, surface and geometrical exclusion concepts are clearly

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defined. The experimental results are in excellent agreement with theoretical prediction. The similarity and difference between HDC and SEC are quantitatively examined.

DESCRIPTION OF SURFACE AND GEOMETRICAL EXCLUSION

In the pure size-exclusion mode, in which there is no interaction between a stationary phase and the solute molecules (this is assumed in all texts), the measurable elution volume is not actually the accessible volume of the solute molecules. This concept can be demonstrated simply by a two-dimensional circle of radius R and an inner small circular probe of radius r (Fig. 1). Obviously, the accessible area for the circular probe is πR^2. However, if the circular probe is considered as a point at its center, just as the motion of the mass center in physics, the accessible area then becomes $\pi(R-r)^2$, which represents the maximum possible geometrical arrangement of the circular probe inside the larger circle. Thermodynamically, the accessible area of a solute volume center is the critical factor for determining solute entropy. Being well known, size exclusion is an entropy-controlled process[2], so that the measurable elution volume is virtually the accessible volume of a solute volume center rather than the physically accessible volume of the solute. Apparently, the volume center for a spherical solute is simply the center of a sphere; for a non-spherical solute, such as a random coil, rod and some other irregular shape, the volume center is defined as the center of a statistically equivalent sphere[5]. On the basis of the new concept, any surface would result in a difference between the accessible volume of a solute and that of the solute volume center, unless the solute is infinitesimal. This difference is defined as the surface-exclusion volume.

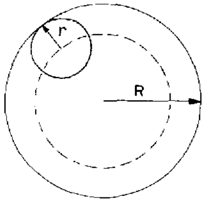

Fig. 1. Surface exclusion for a circular probe within a larger circle.

Explicitly, the surface-exclusion volume (SV) is a function of solute size and total accessible surface area, as well as surface geometry. For example, $SV = (a + 2r)^3 - a^3$ for the external surface of a cube with sides of length a.

The relative surface-exclusion volume (RSE) can be defined as the ratio of surface-exclusion volume to total accessible volume of a solute (AV) and can be expressed in the form:

$$RSE = SV/AV \tag{1}$$

It should be noted that the total accessible volume of a solute is not always equal to the total physical volume. However, the total physical volume is equal to the total accessible volume of the solute with infinitesimal size. This can be illustrated easily by a two-dimensional square with sides of length a and a small circular probe of radius, r (Fig. 2). Apparently, the accessible area of the solute is less than the physical area due to the inaccessibility of the four corners. This volume difference is quite visible and is defined as the geometrical-exclusion volume. Schou and Larsen pointed out this phenomenon as being the interstitial volume for spherical packings[6].

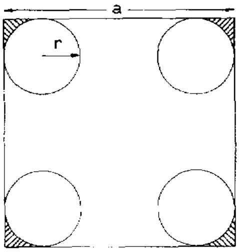

Fig. 2. Geometrical exclusion for a circular probe within a square.

Obviously, the geometrical-exclusion volume (GV) is determined by surface geometry and solute size. In the case of a three-dimensional cube, as in Fig. 2, $GV = 8(1 - \pi/6)r^3$.

Similarly, the relative geometrical-exclusion volume (RGE) is defined as the ratio of geometrical-exclusion volume to total physical volume (PV) and can be expressed in the form:

$$RGE = GV/PV \qquad (2)$$

SEC, regardless of "external or internal exclusion", should involve either surface exclusion, geometrical exclusion or both. In both HDC and SEC, the total column void-volume, *i.e.* V_t, is a characteristic parameter of the column and is a constant for a particular column. Hence, it is convenient to correlate RSE, RGE and the actual elution volume (EV) with reference to V_t. With some manipulations, the ratio of EV/V_t can be expressed by the formula:

$$EV/V_t = 1 - [(1 - RGE)RSE + RGE] \qquad (3)$$

The term $[(1 - RGE)RSE + RGE]$ is defined as the overall size-exclusion factor (OSE).

It is well known that resolution for a column, R_s, can be expressed by the formula:

$$R_s = 1/4\,\sqrt{N}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{k' + 1}\right) \qquad (4)$$

where N is the number of theoretical plates, α is the column selectivity, and k' is the capacity factor.

The last two terms in eqn. 3 can be reduced to:

$$\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{k' + 1}\right) = \frac{V_2 - V_1}{V_1} \qquad (5)$$

where V_1 and V_2 are elution volumes for solutes 1 and 2, respectively ($V_1 > V_2$).

Inserting eqn. 3 into eqn. 5, eqn. 4 can be transformed into:

$$R_s = C\,\frac{OSE_1 - OSE_2}{1 - \mathrm{OSE}_1} \tag{6}$$

where C is the column efficiency factor ($\sqrt{\mathrm{N}}/4$), and OSE_1 and OSE_2 are the corresponding factors for solutes 1 and 2, respectively ($OSE_2 < OSE_1$).

Eqn. 6 is a general formula to estimate the resolution between two solutes in a size-exclusion process.

The resolution is directly proportional to ΔOSE and inversely proportional to $(1 - OSE_1)$. Obviously, the resolution can be improved by increasing the OSE_1 value when ΔOSE has a fixed value.

In an open capillary column, RGE is zero and OSE can be expressed in the form:

$$OSE = 1 - (1 - r/R)^2 \tag{7}$$

where R is the radius of the capillary column and r is the equivalent radius of a solute.

For a HDC column packed with uniform, impervious spheres, both surface and geometrical exclusion are involved and OSE can be expressed by the formula (see Appendix 1):

$$OSE = \frac{1 - E}{E}\left[3\,\frac{r\,\overline{R^2}}{\overline{R^3}} + 3\,(1 - n/4)\,\frac{r^2\bar{R}}{\overline{R^3}} + (1 - n/2)\,\frac{r^3}{\overline{R^3}}\right] \tag{8}$$

where E is column porosity; n is sphere coordination number; $\bar{R}$ is mean sphere radius, defined as $1/m\Sigma R$; $\overline{R^2}$ is the square of the mean sphere radius, defined as $1/m\Sigma R^2$; and $\overline{R^3}$ is the cube of the mean sphere radius, defined as $1/m\Sigma R^3$ (m is the total number of the spheres in the column).

The RGE in HDC can be expressed by the formula (see Appendix 2):

$$RGE = \frac{3\,(1 - E)}{4\,E}\cdot\frac{n\,r^2}{\overline{R^3}}\left[\bar{R} - \sqrt{2\,\bar{R}\,r + r^2}\,\sin^{-1}\left(\frac{\bar{R}}{\bar{R} + r}\right)\right] \tag{9}$$

The corresponding RSE can be estimated by the formula:

$$OSE = (1 - RGE)RSE + RGE \tag{10}$$

EXPERIMENTAL

Stainless-steel columns I (250 mm × 2.0 mm I.D.) and II (75 mm × 4.6 mm I.D.) were packed dry under vibration with non-porous glass beads of diameter 16–20 μm and 1–4 μm, respectively (Polyscience, Warrington, PA, U.S.A.).

The mean sphere radii were determined microscopically by counting a sufficient number of the spheres.

The void-volumes of the columns were calculated from the volume difference between the empty column and the glass-bead volume. The empty column volume and specific volume of the glass bead were predetermined by the conventional water displacement method. The weight of packed glass beads was calculated from the weight difference before and after packing.

The retention times for benzene and linear polystyrene (molecular weight 950 000 daltons) (Waters Assoc., Milford, MA, U.S.A.) were determined on a Beckman Model 320 isocratic high-performance liquid chromatographic (HPLC) system equipped with a Beckman 450 data processor, using methylene chloride (UV grade) as eluent, a detection wavelength of 254 nm, and a flow-rate of 0.5 ml/min. The concentration of both solutes were controlled to obtain half-scale of the chart recorder (Beckman BD 41, dual channels) with a 10-mV input at 0.1 a.u.f.s. The elution was performed at ambient temperature and a thermal sensor (Omega K type) was fastened near the outlet of the column to monitor the temperature change, which was plotted on one channel of the chart recorder. The flow-rate was calibrated in a precise 2-ml pipet connected to the detector outlet, in which 0.2 ml of water was placed to minimize the evaporation of methylene chloride. The pipet was dried for each calibration to eliminate surface water.

To minimize the effect of pump flow-rate variation on the elution volume, the flow-rate was measured in the period from a few seconds before injection to a few seconds after complete elution of the solute.

A proprietary Basic program was utilized to compute the first-moment retention time for all solutes.

Stainless-steel column III (250 × 4.6 mm I.D.) was slurry-packed with 10-μm spherical silica of mean pore diamter 300 Å and specific pore volume 1.652 ml/g, determined by mercury porosimetry and the static exclusion method[7]. The empty column volume was determined as before. The total void-volume of the column and the weight of silica inside the column were determined by a differential density method similar to Berendsen's static method[8]. In this method the total column set, including two short stainless-steel tubes (one for connecting to the injection valve and another for simulating the detector inlet), was tared accurately before packing. The column was first equilibrated with methanol and weighed after packing. Then, the column was equilibrated with chloroform and weighed. The total void-volume (V_t) was calculated by the formula:

$$V_t = \frac{W_1 - W_2}{d_1 - d_2} \tag{11}$$

where W_1 and W_2 were the weights of the column with chloroform and methanol, respectively; d_1 and d_2 were the corresponding densities for the solvents.

The weight of silica inside the column (W_s) could be calculated by the formula:

$$W_s = W_1 - W_t - V_t\, d_1 \tag{12}$$

where W_t was the tare of the stainless-steel column set.

The column pore volume (V_p) can be calculated as

$$V_p = 1.652\ W_s \tag{13}$$

The elution volumes of benzene and polystyrene on this column were also determined under previously stated conditions.

RESULTS AND DISCUSSION

Owing to experimental inconvenience, the open microcapillary was not examined in the present study, although this is the simplest case. However, Tijssen *et al.* separated polystyrene polymers using 1.2- and 10-μm I.D. fused-silica capillaries as the columns and tetrahydrofuran as the mobile phase[9]. Hence, some of Tijssen's experimental data are employed to compare with the results calculated from eqn. 3 (Table I). The effective sphere radius r of polystyrene is estimated by the equation[10]:

$$r\ (\text{Å}) = 0.123\ \bar{M}_w^{0.588} \tag{14}$$

where $\bar{M}_w$ is the weight-average molecular weight.

TABLE I

COMPARISON OF TIJSSEN'S EXPERIMENTAL RESULTS AND THE CALCULATED VALUE FROM SURFACE EXCLUSION

Polystyrene molecular weight	*Equivalent radius (Å)*	*Tijssen's data** *EV/PV (R = 1.2 μm)*	*Calculated value* $(1 - r/R)^2$
Toluene	3.60	1.0000	0.9994
50 000	72.10	0.9874	0.9880
160 000	141.23	0.9649	0.9766
670 000	327.82	0.9474	0.9461

* Column length, 126 cm; mobile phase, tetrahydrofuran.

Evidently Tijssen's experimental data are in fairly good agreement with prediction from eqn. 3. The slight deviation might be attributed to peak maximum retention and accurate value of the capillary diameter, as well as some experimental error.

The present experimental data and the calculated results are summarized in Table II. The sphere coordination number is a function of packing porosity and is approximately 8 for porosity 0.395[6]. There is excellent agreement between HDC data and the results predicted from eqns. 3 and 8, which indicates that surface and geometrical exclusion are the actual mechanisms for HDC. If SBF is also a contribution to the separation mentioned by Squire *et al.*[4], the combined separation, which should be significantly greater than the calculated value, could be observed.

Squire *et al.* assumed that their "external size exclusion" model was based on the equilibrium conditions. DiMarzio and Guttman also admitted that SBF was not an important effect at low rate or high solute diffusivity[3]. In SEC, the flow-rate is generally controlled at a fairly low value and most solute diffusivities are sufficiently

TABLE II

COMPARISON BETWEEN EXPERIMENTAL HDC RESULTS AND CALCULATED DATA

Term	*Column I*	*Column II*	*Term*	*Column I*	*Column II*
$\bar{R}^3$ (μm^3)	5785.83	9.80	EV_s (benzene, ml)*	0.3061	0.4807
$\bar{R}^2$ (μm^2)	320.17	3.70	EV_{p1} (50 000)*	0.3060	0.4727
$\bar{R}$ (μm)	17.80	1.65	EV_{p2} (950 000)*	0.3030	0.4466
PV (ml)	0.3063	0.4811	EV_s/PV	0.9993	0.9990
E (%)	39.00	38.60	EV_{p1}/PV	0.9990	0.9825
r of benzene (Å)	3.60	3.60	EV_{p2}/PV	0.9892	0.9282
r of polystyrene 50 000	71.27	71.27	Calculated EV_s/PV	0.9999	0.9994
r of polystyrene 950 000	402.54	402.54	Calculated EV_{p1}/PV	0.9982	0.9872
			Calculated EV_{p2}/PV	0.9896	0.9288

* Average of three replications.

high[11]. Hence, equilibrium conditions are well established and non-equilibrium effects (*i.e.* equilibrium not being maintained at the surface of the stationary phase) have been shown both theoretically and experimentally to be of no significance in SEC[12]. Equilibrium conditions are definitely the case in HDC in that there is no pore effect, which minimizes the solute diffusion distance and the restricted diffusion.

The recently developing field flow fractionation (FFF) really makes use of the SBF mechanism[13]. It applies an external transversal field, such as a centrifugal force field (SFFF) or a thermal gradient field (TFFF), to force the larger or more dense solutes into a slow flow stream near the wall, so as to be eluted later than the smaller or less dense solutes. FFF is capable of separating macromolecules with a molecular weight range of 10^6–10^{13} (refs. 14 and 15). However, it appears unlikely to achieve natural SBF in HDC, in that the size difference itself is unable to overcome the Brownian motion and diffusion.

It can be visualized that a geometrical exclusion would inevitably result in a decrease in surface exclusion so as to form the complex term in eqn. 10.

Eqn. 9 indicates that RGE is directly proportional to the n value and decreases with an increase in E and R values. However, eqn. 8 indicates that the overall contribution of n to OSE is negative if E is a constant. This antagonistic mechanism between geometrical and surface exclusion is shown clearly in eqn. 10, which indicates that RGE is insensitive to OSE in comparison with RSE because RGE in HDC is considerably lower than RSE (see eqn. 9). Nevertheless, in a packed column, an increase in n will concomitantly cause a decrease in E and consequently result in a higher OSE value, for n has a negligible effect whereas E has a significant effect on OSE. For example, OSE would be 1.9 times that for $n = 8$ and $E = 0.395$ if n increases to 12, which corresponds to $E = 0.26$ (close hexagonal packing). This would explain mathematically what Schou and Larsen noted[6]. If n vanishes and the distance between spheres is greater than r, geometrical exclusion will not exist, so that HDC will be solely a surface exclusion mode.

The recently proposed static-exclusion method for determination of specific pore volume is another example[7]. It decreases n and increases E considerably, to achieve a very low OSE valve, thus enhancing the accuracy of the pore volume determination.

In fact, both surface and geometrical exclusion have already been implied in size-exclusion chromatography. The distribution coefficient in SEC, k, is $(1 - r/R')^2$ for a cylindrical pore with pore radius R' and a solute with equivalent radius r (pore-end effects are neglected)[16]. This situation is exactly the same as that for the open capillary column and is the sole surface-exclusion mode. However, when r is equal to or greater than R', k reduces to zero, which implies geometrical exclusion.

Since the exclusion conditions are quite complicated in SEC, for convenience, the total void-volume in a column is divided into two portions, *i.e.* interstitial volume V_1 and pore volume V_p. The $OSE_{R'}$ for the pore with radius R' is then

$$OSE_{R'} = (1 - RGE)\,[1 - (1 - r/R')^2] \tag{15}$$

where $RGE = 0$ for $r < R'$, and $RGE = 1$ for $r \geqslant R'$.

The OSE_p for the column pore volume should be the sum of all $OSE_{R'}$ and can be expressed as:

$$OSE_p = \sum_{R'=0}^{R'=\infty} V_{R'}/V_p\,(1 - RGE)\,[1 - (1 - r/R')^2] \tag{16}$$

where $V_{R'}$ is the pore volume for the pore with radius R'.

Similarly to HDC, the OSE_i for the column interstitial volume would be:

$$OSE_i = \frac{1 - E_i}{E_i}\left[3\frac{r\,\bar{R}^2}{\bar{R}^3} + 3\,(1 - n/4)\,\frac{r^2\,\bar{R}}{\bar{R}^3} + (1 - n/2)\,\frac{r^3}{\bar{R}^3}\right] \tag{17}$$

where E_i is the column interstitial porosity.

The RGE for pore volume (RGE_p) is the sum of the column pore volumes with $R' \leqslant r$

$$RGE_p = \sum_{R'=0}^{R'=r} V_{R'}/V_p \tag{18}$$

The RGE for the column interstitial volume (RGE_i) is the same as eqn. 9. The corresponding RSE_p and RSE_i can be estimated from eqn. 10.

The elution volume in SEC can be expressed by the formula:

$$V = (1 - OSE_p)\,V_p + (1 - OSE_i)\,V_i \tag{19}$$

Inserting eqn. 19 into eqn. 3, the overall OSE (OSE_t) for SEC can be obtained

$$OSE_t = \frac{V_p OSE_p + V_i OSE_i}{V_p + V_i} \tag{20}$$

For convenience, eqn. 17 can also be expressed in the form

$$OSE_t = \frac{\varepsilon(1 - E_i)\,OSE_p + E_i OSE_i}{E_t} \tag{21}$$

where ε is the porosity of a packing material and E_t is column total porosity defined as V_t/V_c (V_c is empty column volume).

The term $(1 - OSE_p)$ is the familiar partition coefficient in SEC, K, and can be expressed as

$$K = \frac{V - (1 - OSE_i)\, V_i}{V_p} \tag{22}$$

In practice, K is calculated from experimental elution volumes with the following formula:

$$K = \frac{V - V_b}{V_s - V_b} \tag{23}$$

where V is the elution volume for a solute; V_b is the elution volume for a sufficiently large solute totally excluded from the pore volume; and V_s is the elution volume for a very small solute such as benzene or toluene.

Both OSE_p and OSE_i can be considered to be zero for the very small solute and V_s is then equal to $(V_p + V_i)$. However, for a very large solute, OSE_p is unity but OSE_i would have a significant value other than zero. Thus V_b can be expressed by the form:

$$V_b = (1 - OSE_b)\, V_i \tag{24}$$

where OSE_b is the OSE_i for the large solute.

Eqn. 23 can be then expressed in the form:

$$K = \frac{V - (1 - OSE_b)\, V_i}{V_p + V_i - (1 - OSE_b)\, V_i} \tag{25}$$

There is an apparent discrepancy between eqns. 25 and 22. This discrepancy can easily be corrected as follows:

$$K = \frac{V - V_b\,(1 - OSE_i)/(1 - OSE_b)}{V_s - V_b/(1 - OSE_b)} \tag{26}$$

where OSE_i and OSE_b can be estimated by eqn. 17 if the equivalent sphere radii of the solutes are known.

In practice, E_i for a column with a porous packing is rather difficult to determine accurately, so it can be a problem in estimating the above two OSE values by eqn. 17. A simple iteration approach is suggested. Firstly, E_i^1 is simply approximated to be V_b/V_c and a sufficiently large solute can be arbitrarily chosen in order to obtain V_b. Secondly, the V_i^1 can be computed from $V_b/(1 - OSE_i^1)$, where OSE_i^1 is calculated from eqn. 17 with the E_i^1. Then the E_i^2 is estimated from V_i^1/V_c. The iteration is performed until V_i and E_i become constant. This method is applied successfully to column III and the results are summarized in Table III.

TABLE III

COMPARISON OF INTERSTITIAL VOLUME IN COLUMN III BETWEEN EXPERIMENTAL DATA AND CALCULATED VALUES

Parameter	*Value*	*Parameter*	*Value*
Empty column volume V_c (ml)	4.1527	V_s of benzene (ml)*	3.5979
Column total void-volume (ml)	3.5984	V_b of polystyrene 950 000 (ml)*	1.5928
Silica weight in column W_s (g)	1.1941	E_i^1 (%) (V_b/V_c)	38.3558
Specific pore volume (ml/g)	1.6520	First V_i^1 [$V_b/(1-OSE_i^1)$]	1.6273
Column pore volume V_p (ml)	1.9726	E_i^2 (%) (V_i^1/V_c)	39.1861
Column interstitial volume V_i (ml)	1.6258	Second V_i^2 [$V_b/(1-OSE_i^2)$]	1.6261
Interstitial porosity E_i (%)	39.1500	E_i^3 (%) (V_i^2/V_c)	39.1569
$\bar{R}^3$ (μm^3)	723.0323	Third V_i^3 [$V_b/(1-OSE_i^3)$]	1.6261
$\bar{R}^2$ (μm^2)	79.2903	E_i^4 (%) (V_i^3/V_c)	39.1579
$\bar{R}$ (μm)	8.8387	Fourth V_i^4 [$V_b/(1-OSE_i^4)$]	1.6261
		E_i^5 (%) (V_i^4/V_c)	39.1579

* Average of three replications.

Evidently, the calculated interstitial volume is in good agreement with experimental results, which proves the feasibility of the present method. Virtually, E_i and V_i converge very fast and iteration with three cycles appear to be sufficient to obtain the final value. Owing to a significant OSE_b value, a specific pore volume determined by SEC is always considerably greater than the true value and the retention volumes from the peak maximum further aggravate this effect because the peak of a large molecule always possesses a considerable tailing. Nevertheless, the present method enables SEC to obtain a specific pore volume equivalent to that from porosimetry and the static-exclusion method.

Strictly speaking, RSE_i in SEC is not exactly the same as that in HDC because of the pore openings on the surface of the packing. This effect is illustrated in Fig. 3. There are two causes for this effect. If $r < R'$, there is a decrease of the surface exclusion volume on the external surface of a sphere. If $r \geqslant R'$, there is a decrease in the surface-exclusion volume as well as a decrease of the geometrical-exclusion volume in the column pore volume.

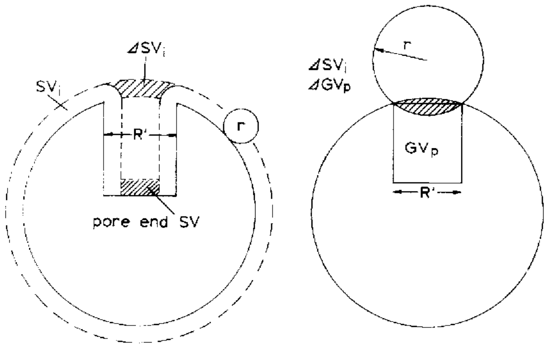

Fig. 3. Pore-opening effect on surface exclusion and geometrical exclusion.

The pore end can be envisaged as a flat circle inside a sphere[17]. Thus, the decrease of the surface-exclusion volume could be approximately compensated by the surface-exclusion volume on the pore end for the first case. In the second case, the decrease of the surface-exclusion volume is equal to the decrease of the geometrical-exclusion volume. The relative effect is defined as the ratio of the decrease to GV_p or SV_i (do not confuse them with RGE_p and RSE_i).

Based upon the newly developed intersecting cylindrical pore network model for spherical porous material[17], the maximum relative effect of the pore entrance of the typical commercial SEC column (pore diameter 300 Å, particle size 5 μm, specific pore volume 0.7 cm^3/g and specific surface area 90 m^2/g) is estimated to be $<0.32\%$ for GV_p and $<5.8\%$ for SV_i, respectively. The maximum values can be obtained while the probe solute has a radius of about the mean pore radius. This is quite understandable because the majority of pores is involved in this situation (the detailed estimate is available upon request).

Obviously, a large solute with r considerably greater than R'_b would minimize the pore-opening effect in the foregoing iteration to determine V_i. Nevertheless, the pore-opening effect is so small as to be virtually neglected in the practice.

CONCLUSION

Based upon the surface- and geometrical-exclusion mechanisms, open capillary chromatography is a simple and unique size-exclusion chromatography because surface exclusion is solely involved (column-end effects are neglected). HDC and SEC contain both surface and geometrical exclusion and literally belong to the same category.

Qualitatively speaking, a non-porous packing has a very low specific surface area, such as 2 m^2/g for a 5-μm glass sphere, while a porous packing has a specific surface area of 20–200 m^2/g for commercial chromatographic silica. Thus, the surface area per column volume for SEC is much greater than that for HDC. As a result, SEC could offer a much higher separation power. This point has been recognized by chromatographers for a long time.

Although OSE in HDC can be enhanced by increasing the ratio of r/R, 0.1 is considered as the theoretical upper limit and a solute of the size approaching this value might be trapped in the interstitial channels so as not to pass through the column[4].

Using $r/R = 0.1$ and $E = 0.4$, the theoretical maximum RGE in HDC is estimated to be 0.0429, the maximum OSE is 0.4035 and the corresponding RSE is 0.3768. This reveals that surface exclusion is the principal separation mechanism in HDC.

The estimate of RGE and RSE for SEC is fairly complicated. Similar to OSE_t, the overall RGE in SEC (RGE_t) can be expressed in the form:

$$RGE_t = [\varepsilon(1 - E_i)\, RGE_p + E_i\, RGE_i]/E_t \tag{27}$$

The overall RSE (RSE_t) can be expressed by the formula:

$$RSE_t = \frac{\varepsilon(1 - E_i)(OSE_p - RGE_p) + E_i\,(OSE_i - RGE_i)}{E_t - (1 - E_i)\,RGE_p - E_i\,RGE_i} \tag{28}$$

The porosity of commercial SEC packings is generally in the range of 0.5–0.7, and 0.6 is taken as the typical value. Inserting all numerical values into eqn. 28, one obtains

$$RSE_t = \frac{0.36\,(1 - K - RGE_p) + 0.1453}{0.76 - 0.36\,RGE_p - 0.0172} \tag{29}$$

Eqn. 29 would have the theoretical maximum value of 0.3796 if $K = 0$ and $RGE_p = 1$. It should be noted that the corresponding RGE_t has a very high value, 0.4822. As a consequence, RSE_t and RGE_t result in the OSE_t value as high as 0.6787, which is much greater than the maximum OSE in HDC, *i.e.* 0.4035. This discloses that surface and geometrical exclusion are comparable mechanisms in SEC.

This point can be further elucidated by eqn. 6. Assuming $r_2 = 2\,r_1$, $E_i = 0.4$, and $r_2/R = 0.1$, the maximum HDC resolution between these two solutes is estimated to be $0.238C$ (C is the efficiency factor).

Using eqns. 21 and 6, the corresponding SEC resolution can be expressed by the formula:

$$R_s = C\,\frac{\varepsilon\,(1 - E_i)(K_1 - K_2) + E_i\,(OSE_i^2 - OSE_i^1)}{(1 - E_i)\,K_1 + E_i\,(1 - OSE_i^1)} \tag{30}$$

Inserting the above numerical values into eqn. 30, one thus obtains

$$R_s = C\,\frac{0.36\,(K_1 - K_2) + 0.1873}{0.6\,K_1 + 0.7868} \tag{31}$$

R_s would have the theoretical maximum value of $0.395C$ if $K_1 = 1$ and $K_2 = 0$, and the theoretical minimum value of $0.238C$ if $K_1 = K_2 = 0$. The actually R_s value depends on the values of K_1 and K_2, which are governed by the absolute sizes of the two solutes and the pore size distribution of the column packing. Even if r/R has an extremely low value, which corresponds to $OSE_i^1 \approx OSE_i^2 - 0$, the theoretical maximum R_s would still have the high value, *i.e.* $0.26C$.

Based upon the above quantitative analysis, it can be concluded that surface exclusion is the predominant mechanism in HDC and both surface and geometrical exclusion play important roles in SEC. Since the overall RSE in SEC does not differ as much from that in HDC, the geometrical exclusion resulting from the pore effect is the crucial factor for enhancing overall separation power in SEC. Therefore, SEC can be regarded as a high-performance HDC.

Furthermore, SEC can be envisaged as the combination of HDC and a number of open capillary columns with various diameters, which represent the pores. High geometrical exclusion results from the portion of the pore volume totally excluded by a solute.

It should be noted that both surface and geometrical exclusion diminish and approach zero as solute size decreases.

In order to avoid column obstruction, HDC and open capillary chromatography are usually operated at a fairly low value of r/R so as to lose their separation power considerably. However, this situation does not affect SEC so much, because K values are independent of r/R values and can be optimized by choosing packings with the appropriate pore size distribution. Hence, SEC has great versatility for separating a wide range of solute sizes, with good resolving power.

APPENDIX 1

As shown in Fig. A1, the physical volume of a sphere, V_R, is

$$V_R = 4/3\ \pi\ R^3 \tag{A1}$$

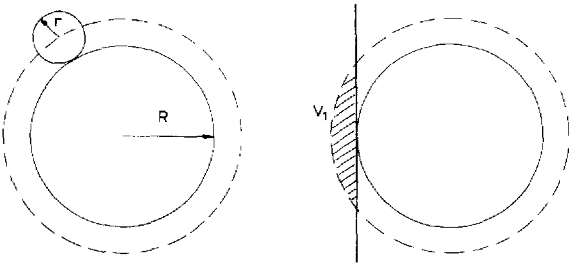

Fig. A1. Illustration for sphere volume, effective sphere volume and reduced volume due to one contact.

Considering the accessible volume of a solute volume center, the effective volume of a sphere, V_{R+r}, would be

$$V_{R+r} = 4/3\ \pi\ (R + r)^3 \tag{A2}$$

The volume reduced by one contact with another same sphere, V_1, would be

$$V_1 = \pi \int_R^{R+r} [(R + r)^2 - x^2]\, \mathrm{d}x = 4/3\ \pi\ (3/4\ R\ r^2 + 1/2\ r^3) \tag{A3}$$

If a sphere has n contacts with other spheres, its effective volume would be

$$V^n_{R+r} = 4/3\ \pi\ [R^3 + 3\ R^2\ r + 3\ (1 - n/4)\ R\ r^2 + (1 - n/2)\ r^3] \tag{A4}$$

In practice, high-performance liquid chromatographic packings are rarely spheres with the same radius, so that eqns. A1 and A4 should be

$$V_R = 4/3\ \pi\bar{R}^3 \tag{A5}$$

$$V^n_{R+r} = 4/3\ \pi\ [\bar{R}^3 + 3\ \bar{R}^2\ r + 3\ (1 - n/4)\ \bar{R}\ r^2 + (1 - n/2)\ r^3] \tag{A6}$$

The ratio of V^{n}_{R+r} to V_R is then

$$\frac{V^{n}_{R+r}}{V_R} = \left[1 + 3\,\frac{\bar{R}^2\, r}{\bar{R}^3} + 3\,(1 - n/4)\,\frac{\bar{R}\, r^2}{\bar{R}^3} + (1 - n/2)\,\frac{r^3}{\bar{R}^3}\right] \tag{A7}$$

A column interstitial volume is the difference between the empty column volume (V_c) and the total sphere volume (V_s)

$$PV = V_c - V_s \tag{A8}$$

Similarly, the elution volume of a solute, EV, would be the difference between the column empty volume and the total effective sphere volume (V^{e}_{s})

$$EV = V_c - V^{e}_{s} \tag{A9}$$

Thus, one can obtain

$$EV/PV = \frac{1 - (1 - E)\, V^{e}_{s}/V_s}{E} \tag{A10}$$

One should notice that V^{e}_{s}/V_s is equal to V^{n}_{R+r}/V_R if column radius is considerably greater than that of the sphere, so that the wall effect is negligible. Hence, eqn. A10 can be expressed as

$$\frac{EV}{PV} = 1/E - (1/E - 1)\left[1 + 3\,\frac{\bar{R}^2 r}{\bar{R}^3} + 3\,(1 - n/4)\,\frac{\bar{R}\, r^2}{\bar{R}^3} + (1 - n/2)\,\frac{r^3}{\bar{R}^3}\right] \tag{A11}$$

Noting $OSE = 1 - EV/PV$, one then obtains

$$OSE = \frac{1 - E}{E}\left[3\,\frac{\bar{R}^2\, r}{\bar{R}^3} + 3\,(1 - n/4)\,\frac{\bar{R}\, r^2}{\bar{R}^3} + (1 - n/2)\,\frac{r^3}{\bar{R}^3}\right] \tag{A12}$$

APPENDIX 2

The geometrical-exclusion volume of a spherical solute on a packing sphere due to one contact is the shaded volume shown in Fig. A2.

The point 0 is considered as the origin of the Cartesian coordinates. x_1 and x_2 are the domains for the geometrical volumes. One can obtain y as the function for the sphere and solute within each domain, respectively

$$y = \sqrt{R^2 - x^2} \tag{A13}$$

$$y = \sqrt{2Rr + r^2} - \sqrt{r^2 - (x - R)^2} \tag{A14}$$

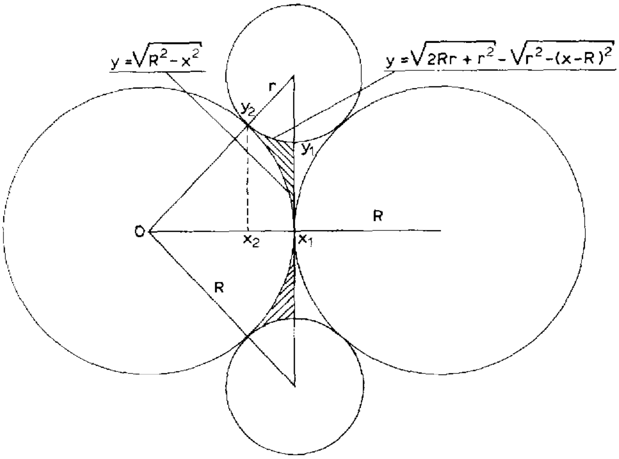

Fig. A2. Geometrical-exclusion volume of a spherical solute on a packing sphere due to one contact.

One can easily find $x_1 = R$ and $x_2 = R^2/(R + r)$. The geometrical-exclusion volume (V_g) can be generated by rotating the shaded area about the x-axis. This is equivalent to the volume difference between the rotating area $x_1x_2y_2y_1$ (V_1) and the rotating area $x_1x_2y_2$ (V_2). Hence, one obtains

$$V_g = V_1 - V_2 \tag{A15}$$

$$V_1 = \int_{x_2}^{x_1} \pi \, [\sqrt{2Rr + r^2} - \sqrt{r^2 - (x - R)^2}]^2 \, dx \tag{A16}$$

$$V_2 = \int_{x_2}^{x_1} \pi \, (R^2 - x^2) \, dx \tag{A17}$$

After integration, one obtains

$$V_1 = \pi \, \{(2Rr + 2r^2 - R^2)\, x + R\, x^2 - x^3/3 - \tag{A18}$$

$$\sqrt{2Rr + r^2}\, [(x - R)\sqrt{r^2 - (x - R)^2} + r^2 \sin^{-1} - (x - R)/r]\} \Big|_{x_2}^{x_1}$$

$$V_2 = \pi \, (R^2\, x - x^3/3) \Big|_{x_2}^{x_1} \tag{A19}$$

Inserting eqns. A18 and A19 into eqn. A15 and then performing some reduction, one finally obtains

$$V_g = \pi r^2[R - \sqrt{2Rr + r^2}\sin^{-1}R/(R + r)] \tag{A20}$$

The total geometrical-exclusion volume (GV) should be a product of n, V_g, and the number of packing spheres in a column, m.

$$GV = m\, n\, V_g \tag{A21}$$

It is noted that the total solid volume in the column is the product of m and the average sphere volume

$$V_c\,(1 - E) = m\, 4\pi\bar{R}^3/3 \tag{A22}$$

Combining eqns. A22 and A21, one then obtains

$$GV = \frac{3\, V_c\,(1 - E)\, n\, V_g}{4\pi\, \bar{R}^3} \tag{A23}$$

Recalling $RGE = GV/PV$ and $PV = V_cE$, one finally obtains

$$RGE = \frac{1 - E}{E} \cdot \frac{3n\, r^2}{4\, \bar{R}^3}\,[\bar{R} - \sqrt{2\bar{R}\, r + r^2}\sin^{-1}\bar{R}/(\bar{R} + r)] \tag{A24}$$

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