Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1037 (2004) 273–282
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Review

Determination of pore size distributions of porous chromatographic adsorbents by inverse size-exclusion chromatography

Yan Yao, Abraham M. Lenhoff*

Department of Chemical Engineering, University of Delaware, Newark, DE 19716, USA

Abstract

The macroscopic properties of porous chromatographic adsorbents are directly influenced by the pore structure, with the pore size distribution (PSD) playing a major role beyond simply the mean pore size. Inverse size-exclusion chromatography (ISEC), a widely used chromatographic method for determining the PSD of porous media, provides more relevant information on liquid chromatographic materials in situ than traditional methods, such as gas sorption and mercury intrusion. The fundamentals and applications of ISEC in the characterization of the pore structure are reviewed. The description of the probe solutes and the pore space, as well as theoretical models for deriving the PSD from solute partitioning behavior, are discussed. Precautions to ensure integrity of the experiments are also outlined, including accounting for probe polydispersity and minimization of solute–adsorbent interactions. The results that emerge are necessarily model-dependent, but ISEC nonetheless represents a powerful and non-destructive source of quantitative pore structure information that can help to elucidate chromatographic performance observations covering both retention and rate aspects.

Keywords: Reviews; Inverse size-exclusion chromatography; Pore size distribution; Adsorbents

Contents

1. Introduction

The porous nature of chromatographic adsorbents provides properties that are exploited in chromatographic practice, such as passages for transport of materials, increased surface area for adsorption, and voids that serve as molecular sieves to differentiate particles based on relative dimensions. The structure of the pore space is of principal significance in determining the functional properties. Most natural and synthetic porous media contain pores with irregular geometry and heterogeneous sizes. To describe the complicated pore structure, a number of structural parameters are

* Corresponding author. Tel.: +1-302-831-8989; fax: +1-302-831-4466.
E-mail address: lenhoff@che.udel.edu (A.M. Lenhoff).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.02.054

relevant, among which the pore size distribution (PSD) represents the distribution density of pores within a certain range of dimensions and serves as a statistical descriptor of the diverse size features.

A number of methods for determining the PSD of porous materials have been discussed elsewhere [1–3]. The commonly used techniques can be categorized into four main classes: gas sorption [4], mercury intrusion [5], microscopy [3,6] and solute exclusion [7], among which solute exclusion is the most suitable for investigating structures under similar conditions to those in chromatographic applications. Solute exclusion is routinely applied in size-exclusion chromatography (SEC) [8] (gel permeation chromatography, GPC [9]) to determine solute size based on known pore dimensions of the adsorbents or empirical retention information relative to that of standards. The inverse application of the SEC concept, inverse size-exclusion chromatography (ISEC) [10], utilizes a set of molecular probes with defined sizes to determine pore dimensions, and is also referred to as chromatographic/macromolecular porosimetry [11,12]. The ISEC principle, first applied in determining pore distributions in cellulose fibers [10], was introduced by Halász and Martin for characterization of chromatographic stationary phases [13] and has been further refined and extended [14–16].

ISEC has a number of advantages over alternative methods. Column experiments with intact samples packed in a bed can conserve sample integrity and are easy to carry out, as opposed to the special sample preparation procedures in electron microscopy. No additional equipment other than a chromatography system is necessary for ISEC, so it is relatively inexpensive and convenient. Operating conditions such as high pressure, low temperature and drying conditions, which are involved in gas sorption or mercury intrusion, are not imposed in ISEC. Experimental conditions similar to those in normal operations result in less significant morphological changes, thus assuring structural information that is relevant to properties of functional interest. This is especially important for swellable gels, the structure of which is greatly affected by the liquid content of the material. One specific benefit of using ISEC to characterize adsorbents is that changes in the PSD, e.g., as a result of polymer grafting [17], salt concentration [18] and hydrothermal treatment [19], can be captured. Comparison of pore accessibility in aqueous versus organic solutions can provide some hints on the hydrophilic/hydrophobic characteristics of the adsorbent surface [17]. Controlled investigation of the effects of salt concentration or hydrothermal treatment on pore morphological changes have been facilitated by ISEC [18,19]. Multiple molecular probes are utilized to explore the pore space, therefore more extensive information on the macromolecular dependence of adsorbent accessibility is provided. In the extraction of the PSD from experimental measurements, fewer assumptions are built into ISEC than into mercury porosimetry [15]. The working pore dimension range of 1–400 nm attainable by ISEC [13], which includes resolution not achievable by mercury porosimetry or gas sorption [13,20–22], is of major interest in studies of microporous materials for liquid chromatography.

Because of its extensive applicability and advantages, ISEC has been widely used in obtaining pore size information for various porous media. The pore structure of chromatographic adsorbents has direct effects on efficiency in preparative bioseparations as well as in analytical chromatography, but a thorough characterization of the PSD of most commercial stationary phases is still lacking. To date ISEC characterization of pore statistics covers a range of chromatographic stationary phases, including silica [12,14,15,20], modified silica [17,19,23–27], carbohydrate gels and synthetic polymer-based adsorbents [18,28–34]. The non-destructive nature of ISEC is an advantage also in structural characterization of monolithic columns [35]. Apart from the characterization of chromatographic adsorbents, the technique has also been extensively implemented in obtaining pore size information on materials such as membranes [36], cellulose [37–41], activated carbons [42] and coals [43].

The theoretical and practical aspects of early implementations of ISEC were extensively reviewed by Gorbunov et al. [11]. A more recent review that focused on structural characterization of chromatographic media presented a simple iterative fitting approach for estimating the PSD [16]. Both papers discuss the representation of solute and pore characteristics, correlation of the distribution coefficient and the dimensions of the probe and pore, and the derivation of the PSD. With the continuing application of ISEC and the increasing number of porous materials with diverse structural characteristics, it is helpful to update these reviews to aid in effective utilization of the technique. In the following, therefore, we re-examine the fundamentals and applications of ISEC. The consequences of simplifications and assumptions in the PSD derivation are examined accordingly. Common misconceptions and contributions from non-standard ISEC features in practical experiments are emphasized.

2. Inverse size-exclusion chromatography experiments

SEC separates macromolecules based mainly on the size and shape of the molecules relative to those of the voids in the adsorbent. The underlying mechanism in this separation technique has been discussed extensively [44–47]. Steric exclusion is considered the major contributor to the varying permeation extents of polymers in the porous matrix [48], with the determining factor primarily related to the solute and pore dimensions [8,9,28,49,50]. The distribution coefficient of a solute between the intra- and extra-particle volumes [51], K_d, also referred to as the partition coefficient [52,53] or the exclusion coefficient [14], is represented by:

$$K_d = \frac{c_p}{c_i} \quad (1)$$

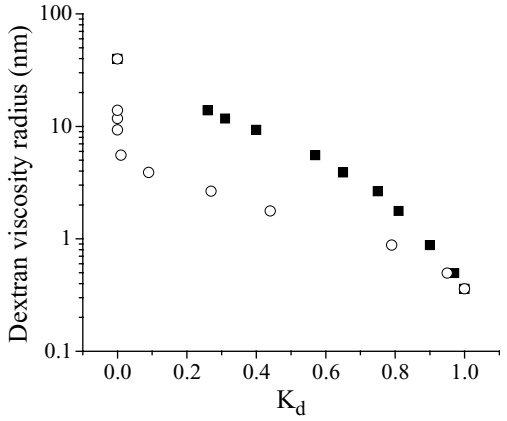

Fig. 1. Dextran calibration curves for Amersham Biosciences Source 30Q (■) and Q Sepharose XL (○) anion exchangers [34].

in which c_p and c_i are the equilibrium solute concentrations in the pore and the bulk, respectively. K_d is measured chromatographically according to:

$$V_e = V_0 + V_p K_d \quad (2)$$

where V_e is the solute residence time in the column, V_0 the interstitial void volume, and V_p the total pore volume inside the stationary phase. Ideally an infinitesimally small solute that does not interact with either other solutes or the adsorbent is used to access the complete pore space, and the interstitial space is measured by a large probe that is excluded from all pores but explores the total interparticle void. In practice, the elution volume of a small non-adsorbing probe, such as acetone or sodium chloride, is taken as the sum of total pore volume and interstitial volume, and a solute with a high molecular weight that can be reasonably regarded as completely excluded is used to determine the column interstitial volume.

K_d can be considered a permeation extent that represents the fraction of pore space accessible to a certain probe. A number of ISEC probes covering a range of dimensions are used. The probe size versus K_d plot, the calibration curve or the selectivity curve [16] (Fig. 1), shows the accessibility of a porous material to molecular probes and contains information that can be further used to derive characteristic pore size parameters. The philosophy for choosing ISEC probes is that homologous macromolecules that are close in chemical characteristics to the solutes used in specific applications are optimal for constructing the corresponding selectivity curves. Various standard solute series have been used in calibrating the pore space in different solvent environments, including compact globular molecules as well as flexible random coil polymers. Polystyrene has been widely used in ISEC in organic solvents [13,20,54,55], while dextran, polyethylene glycol and polyethylene oxide are commonly utilized in aqueous solutions [7,16,18]. Other polymers/biopolymers, such as pullulan, ficoll, proteins and DNA, have also been studied in the calibration of SEC [56–58] and can be regarded as actual or potential ISEC probes [18,59].

Peak detection of polymers is commonly realized by a refractive index detector, which is applicable to the isocratic elution that is typically used in ISEC. Stable temperature control and minimal salt concentration differences between the solute and the elution buffer are critical to preventing baseline drift and ghost peaks, especially when the refractive index detector is set at a high sensitivity for monitoring trace samples. The column should be long enough to yield appreciable differences in the elution of different probes. Hindered intraparticle diffusion is usually considered to be the limiting step in SEC [60], and the combination of a long column and the detector can result in high pressure buildup. These factors both militate in favor of a lower flow rate, but this must be traded off against the longer experimental times that would then be required.

There are a number of precautions necessary for realizing effective ISEC procedures. As noted above, retention differences are considered to result purely from steric interactions, so solute standards with low polydispersity, i.e., that are well-defined in size and shape, should be used for PSD determination. Dilute standard solutions are typically used to reduce solute–solute interactions, especially aggregation. Appropriate ISEC probes and solvent conditions should be chosen to minimize solute–adsorbent binding and to avoid aggregation. If these prerequisites for standard ISEC are not satisfied, alternative treatments of non-standard ISEC must be used to extract the PSD. Potential anomalies include solute adsorption that cannot be eliminated by manipulating solvent conditions, and the use of polydisperse standards when monodisperse solutes are not available. Some adsorbents also contain large pores that are accessible to even the largest polymer standards typically used. Consequently, the macropore volume cannot be quantitatively differentiated by ISEC, and it is difficult to determine accurately the interstitial volume in a column containing such macroporous media. Micrometer-size latex particles can be used as large probes for quantifying the compositions of macropores [34], in which case extra care is needed in choosing the size of the filters and frits in the chromatography system.

3. Analysis of inverse size-exclusion chromatography data

3.1. Description of macromolecules and stationary phase

ISEC analysis of the PSD depends on an a priori physical model to describe the relative partitioning of solutes in the adsorbent voids. Such a system description, including the properties of the probe solutes, the structural characteristics of the pores, and practical means to describe their respective features for ISEC purposes, must be specified before ISEC measurements of permeation extents and pore size composition can be reconciled.

3.1.1. Properties of macromolecules with inverse size-exclusion chromatography applications

Different approaches have been used to define the molecular size of chromatographic relevance, such as average end-to-end distance, radius of gyration, equivalent radius of spherical counterpart, Stokes radius and viscosity radius [2,61–64]. The Stokes radius R_s [65] can be measured by dynamic light scattering based on the Stokes–Einstein equation:

$$D_T = \frac{kT}{6\pi\eta R_s} \quad (3)$$

where D_T is the translational diffusion coefficient and η is the solvent viscosity. The viscosity radius R_h has been treated as a general size parameter in SEC applications [62], with the hydrodynamic volume expressed as [61]:

$$V_h = \frac{|\eta| M}{\nu N} \quad (4)$$

where $|\eta|$ is the intrinsic viscosity of the polymer, ν Simha's factor characterizing the molecular shape and N is Avogadro's number. Explicit empirical relations between the viscosity radius and molecular weight for different solutes have been obtained [2,66], such as $R_h = 0.271M^{0.498}$ for dextrans based on a spherical molecular shape, which has been used in ISEC calibration [16,18]. The Stokes radius R_s and viscosity radius R_h are close for native, globular proteins, but may differ by about 15–25% for random coil or extended polymers [56,67]. Deen and Smith observed that predicted diffusivities of dextrans described as spheres deviate from experimental values, suggesting that such partially flexible polymers do not behave as hard spheres [68]. That flexible macromolecules can partition into pores smaller than the equivalent molecular size is another indication of this, as it can be attributed to conformational change of the solutes [69], which depends on shear rate and ionic strength [68,69]. Giddings et al. proposed the mean external length as a shape-independent parameter better suited to describing solute partitioning [52]. The accepted universality of these SEC radii was questioned by Dubin and Principi in the study of macromolecules of different conformational types [57], which they attributed to changes in the behavior of macromolecules between the bulk and a confined region. However, it was suggested that although not universal, these size parameters can serve as characteristic terms for a range of molecular types, which are proportional to the radius of gyration and scaled by a factor depending on the solute geometry [11].

One distinctive property of polymers that differs from that of proteins is that the molecular weight of polymers is typically characterized as a distribution, the width of which varies depending on the polymerization or fractionation process used in sample preparation. Parameters characterizing the molecular mass distribution (MWD) include the weight-average molecular mass M_w, the number-average molecular mass M_n and the polydispersity PDI $= M_w/M_n$.

Table 1
Polydispersities of dextrans from Polymer Standards Service–USA Inc. (Silver Spring, MD, USA)

M_p	M_w	M_n	PDI
180	180	180	1.00
342	342	342	1.00
1,080	1,350	1,160	1.16
4,400	5,200	3,300	1.60
9,900	11,600	8,100	1.43
21,400	23,800	18,300	1.30
43,500	48,600	35,600	1.36
69,000	66,700	37,900	1.76
124,000	148,000	100,000	1.47
196,000	273,000	164,000	1.66
277,000	410,000	236,000	1.73
2,285,000	3,800,000	1,500,000	2.53

M_p is the molecular mass associated with the peak, M_w and M_n are the mass-average and number-average molecular masses, respectively, and PDI is the polydispersity.

Typical PDI values of polymer standards are below 1.10 for polystyrene and polyethylene glycol, and <1.25 for polyethylene oxide [70–72]. The branching in dextran molecules leads to comparatively large polydispersities, especially for dextrans of high molecular mass, including those commercially available dextran standards intended for GPC calibration. The PDI values of some dextran standards (Polymer Standards Service–USA Inc., Silver Spring, MD, USA) are significantly greater than 1.1 (Table 1). These not only complicate determination of the effective molecular mass [58], but also cause additional peak broadening [71,73–75]. If the molecular mass corresponding to the peak maximum, M_p, is not available from the supplier, the relation of the MWD to the chromatogram must be analyzed. The first moment of the elution data, corresponding to the weight-average molecular mass of a polydisperse solute, is a more accurate representation of the retention extent than the peak maximum, except when the peak is symmetric [55]. For ISEC with broad standards it has been observed that the peak maxima correspond to average molecular masses defined as $(M_w M_n)^{0.5}$ [56,73,76]. Rigorous analysis of elution bands by Kubín [75] shows that the molecular mass associated with the first moment of the chromatogram, M^*, corresponds to $(M_w M_n)^{0.5}$ for the logarithmic normal MWD, or M_w for the Schulz–Zimm distribution. The advantage of using M* is that it is less sensitive to the exact form of the MWD, and thus can be approximated as a simple expression as a function of polydispersity, which is applicable for a number of molecular mass distributions, including the logarithmic normal, Schulz–Zimm and Rosin–Rammler–Tung distributions [75]:

$$\ln\left(\frac{M^*}{M_n}\right) = 0.5(\mathrm{PDI}-1) + 0.0157(\mathrm{PDI}-1)^2 - 0.1438(\mathrm{PDI}-1)^3 \quad (5)$$

Discrepancies are also seen when pore information derived from dextrans is applied to proteins [56,77,78], which

is not surprising considering the appreciable property differences between these two classes of molecules. Despite these complications, dextrans are widely used in aqueous ISEC, because they are well-characterized and considered capable of providing size information relevant for globular proteins [57].

Besides dextrans, proteins have been proposed as standards, with the advantage of being well-defined monodisperse biopolymers. However, difficulties in representing the shape and the effective size of protein molecules arise from their irregular geometry, potential conformational distortions, including via orientation-specific interactions with the adsorbent, and hydration that influences the effective size [61,79,80]. Unfolded proteins may be an alternative to resolve the difficulties related to protein shape, but uncertainties regarding the changes in pore morphology induced by denaturing solvents become additional concerns [2]. The electrostatic component of protein-adsorbent interactions due to ionizable residues on the protein surface can be minimized using high salt concentrations, but under such conditions hydration effects in the system may be enhanced. Additional effects may arise from the distortion of the electrical double layer at the surface of the solute by the presence of the pore wall [81]. Thus ISEC using protein standards requires evaluation of the significance of non-steric interactions and decoupling of exclusion effects from the measured retention volumes.

3.1.2. Pore geometry and pore size distribution

Chromatographic supports with various structures have been used in SEC [2], and the materials of interest for ISEC investigations span the full range of chromatographic adsorbents. Rigid materials such as silica have the virtue of easy pore size control and more robust structural stability [82]. On the other hand, gel-type media based on crosslinked carbohydrates or polymers are widely used in chromatography and play important roles in SEC. Typical gel-type materials consisting of a 3D network of the base polymer are relatively flexible and their structures depend strongly on solvent content and composition. Electron micrographs that reveal the surface and the inner pore structure of some chromatographic supports [2,83] illustrate the heterogeneity of pore geometry and dimensions. Simplified model descriptions of the pore space, such as cylinders or slit-like pores, are generally considered to be reasonable representations of certain materials and have been employed in pore characterization by ISEC as well as gas sorption methodologies [12,84]. On the other hand, gel materials such as Sepharose adsorbents are better envisioned as randomly intertwining fibers as depicted in the Ogston model [85]. Despite these diverse models for describing pore geometries, ISEC has been found to be fairly insensitive to the descriptions of pore geometry [14,86], so the cylindrical model has been used almost universally in pore size characterization of different materials [18]. Random networks have also been implemented in providing a more realistic description of the pore space [29,52].

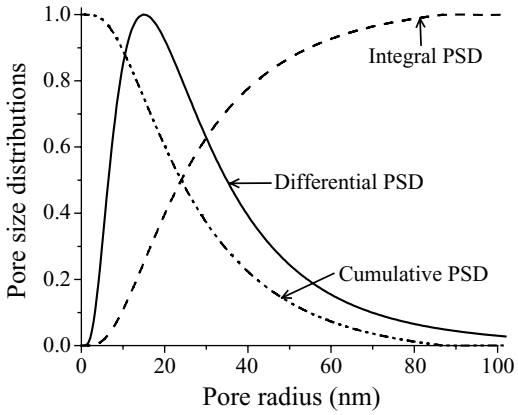

Fig. 2. Fitted differential, integral and cumulative pore size distributions for Amersham Biosciences Source 30Q from ISEC [34].

The collection of pore dimensions is described using a distribution function. There is sometimes confusion concerning the pore size distribution determined experimentally, which is essentially an accessible pore volume distribution, i.e., distribution of pore volume versus some length scale [87]. Different bases for the distribution have been used, including pore volume/surface/size distributions, and differential/integral/cumulative pore size distributions [2,11,13,14,87,88]. Special attention is needed to determine the actual meanings and to differentiate among the different types of distributions in a given treatment.

The definitions of these distribution functions are recounted briefly here and illustrated in Fig. 2 for clarification [2,14]. If $f(r)$ is defined as the differential pore size distribution as a function of radius r, so that $f(r)\,\mathrm{d}r$ corresponds to the fraction of pore volume within the range from r to $r + \mathrm{d}r$, the cumulative pore size distribution $g(r_0)$ and integral pore size distribution $F(r_0)$ are calculated as:

$$g(r_0) = 1 - \int_0^{r_0} f(r)\,\mathrm{d}r \tag{6}$$

$$F(r_0) = \int_0^{r_0} f(r)\,\mathrm{d}r \tag{7}$$

where the cumulative pore size distribution is the volume fraction of pores with radii greater than r_0, which corresponds to the distribution coefficient of a probe of radius r_0 if the wall effect is ignored. The integral pore size distribution corresponds to the volume fraction of pores with radii smaller than r_0.

The distributions discussed above are actually pore volume descriptions, but they have been customarily defined as size distributions and infrequently referred to explicitly as volume distributions [87,89]. The actual pore size distribution $D(r)$ is defined as the fraction of *pores* with radii within a certain range [87], which is less commonly used

in structural characterization. It can be calculated from the pore volume distribution [87]:

$$D(r) = \frac{r^{-(n+1)} f(r)}{\int_0^\infty r^{-(n+1)} f(r)\,\mathrm{d}r} \tag{8}$$

in which n is a shape index characterizing the pore geometry, and can be evaluated via an iterative procedure [87]. The pore volume distribution is usually used for characterization purposes but often referred to as a pore size distribution, and that practice is followed below as well.

The form used for the distribution $f(r)$ can follow any of a number of standard functions. A Gaussian distribution, $f(r) = A\exp[-(1/2)((r - r_p)/s_p)^2]$, has usually been used in describing pore size statistics [16], with r_p, the mean pore radius, and s_p the standard deviation, the two parameters to be determined and A a normalization constant. A disadvantage of the Gaussian distribution is that it must be truncated to eliminate negative pore dimensions. This problem is obviated if an asymmetric function such as the log normal distribution, $f(r) = (A/r)\exp[-(1/2)(\ln(r/r_p)/s_p)^2]$, is used [18]. The mode, r_p, is now no longer the same as the mean in the untruncated Gaussian distribution, and the s_p provides a more indirect measure of distribution width than for the Gaussian distribution case.

Multimodal pore size distributions have been suggested to represent particular collections of pores more accurately [87,90]. For example, so-called perfusion chromatography media such as Poros [91] are designed to contain macropores and micropores, giving rise to a structure that deviates significantly from the monodisperse PSD, which is thus inadequate in providing meaningful PSD parameters for this type of material [34]. Rather, a bimodal distribution is intuitively a better representation of the pore structure, in the form:

$$f(r) = \sum_i A_i f_i(r) \tag{9}$$

in which $f_i(r)$ is the unimodal distribution. Multimodal Gaussian and log normal distributions have been used in such formulations in deriving pore size information as well as modeling transport behavior [34,92,93]. Harlan et al. showed that a double Gaussian PSD was able to describe the ISEC permeation behavior of a number of adsorbents, including Sephacryl, Superose and TSK SW materials [94]. However, the drawback of a multimodal pore distribution is the larger number of fitting parameters: even for the simplest bimodal distribution, there are five independent parameters. Thus it becomes more difficult to determine the distribution parameters reliably. ISEC is not always capable of accurately describing pore space with combined distribution functions, as has also been suggested from the observation that a bimodal PSD did not improve ISEC data fitting for Sephacryl S-300 HR [16].

3.2. Inverse size-exclusion chromatography theoretical analysis

3.2.1. Distribution coefficient versus solute size relative to pore size

Equilibrium models [52,95], which consider the partitioning of solutes between the pore space and the bulk, are the main basis for describing the relation between the selectivity and the geometry and size of the pores and probes. Various representations of the solute and the pore have been used in the extraction of pore structural parameters from distribution coefficients within the equilibrium framework. The first rigorous theoretical work relating partitioning to the probe and pore sizes used simple geometrical representations, e.g., spherical solutes in cylindrical pores [96,97]. Probe size effects are accounted for via the relation of the local partition coefficient, K, to probe size R_m and pore size r by:

$$K = \left(\frac{1 - R_m}{r}\right)^q \tag{10}$$

in which q equals 1, 2 or 3 for slit-like, cylindrical and spherical pores, respectively [52]. A more specific treatment of wedge-shaped pores in the slit pore case was discussed by Vilenchik et al. [12].

The distinctive structure of gel materials was accounted for in the study of solute partitioning using the Ogston model [85], which describes the structure using a random rod network [28]. The partition coefficient of a probe of radius R_m between a matrix of long cylindrical fibers of radius r_f and the bulk can be expressed as [98]

$$K = \exp\left[-\phi\left(\frac{1 + R_m}{r_f}\right)^2\right] \tag{11}$$

in which ϕ is the volume fraction of the gel network. Isotropic random planes were also employed in a statistical approach to treat non-spherical molecules [52]. Here the partition coefficient K was related to s, the pore surface area per unit free volume, and the mean external length $\bar{L}$:

$$K = \exp\left(\frac{-s\bar{L}}{2}\right) \tag{12}$$

Random-flight analysis of partitioning of flexible polymers between the pore and the outer phase yields analytical expressions for the distribution coefficient in terms of the molecular size relative to the pore size for a spherical, cylindrical or slit-like pore space [95]:

$$K_{\text{sphere}} = \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left[-n^2\pi^2\left(\frac{R_m}{r}\right)^2\right] \tag{13}$$

$$K_{\text{cylinder}} = 4\sum_{n=1}^{\infty}\frac{1}{\beta_n^2}\exp\left[-\beta_n^2\left(\frac{R_m}{r}\right)^2\right] \tag{14}$$

$$K_{\text{slit}} = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-\frac{(2n+1)^2\pi^2}{4}\left(\frac{R_\text{m}}{r}\right)^2\right] \quad (15)$$

where the β_n are the roots of a Bessel function $J_0(\beta) = 0$. Doi used statistical thermodynamics to obtain simple formulas for the partitioning of both rigid and flexible macromolecules based on different random networks, i.e., composed of spheres, rods and flexible chains [53].

The random plane model was considered inadequate to describe partitioning of large solutes, for which the curvature of the pore walls becomes important [99]. This underscores the complicated nature of events involving large solutes in restricted spaces. The penetration of large molecules into narrow pores in GPC was first recognized by Casassa [95], and the importance of conformational changes of flexible macromolecules can be inferred from the value of the distribution coefficient K as a function of R_m/r when the solute is much larger than the pore [11]:

$$K \approx \frac{8}{\pi^2}\exp\left[-\left(\frac{\pi R_\text{m}}{2r}\right)^2\right], \quad R_\text{m} >> r \quad (16)$$

Although the differences in pore structure parameters generated by ISEC using pore models of cylinders and random spheres are generally small [14], it is important to keep the description of PSD as realistic as possible. Unfortunately definitive experiments to confirm the validity of one or other specific model are not always possible.

Ideally only steric exclusion plays a significant role in determining the elution volume in SEC [100], but there are also situations in which non-steric interactions cannot be completely neglected [11]. Activated carbon has been characterized by an approach similar to ISEC using a simple mathematical inversion procedure that accounted for adsorption of tracers via Henry's law using calibrated adsorption equilibrium constants [42]. More general treatments of probe-adsorbent interactions have also been proposed. Short-range interactions were incorporated in the random flight treatment of SEC by introducing a correlation length |H| [101,102], so that Eq. (16) becomes:

$$K \approx \frac{8}{\pi^2}\exp\left[-\left(\frac{\pi R_\text{m}}{2(r+|H|)}\right)\right]^2, \quad R_\text{m} >> r \quad (17)$$

Other studies have suggested that simple relations can be used to separate the individual contributions of exclusion and adsorption to the capacity factor, expressed as $K = K_\text{e}K_\text{a}$ [103] or $K = K_\text{e} + K_\text{a} + K_\text{p}$ [104], where K_e is the distribution coefficient in standard GPC, and K_a and K_p correspond to the contributions from adsorption and additional partitioning separately. More controlled experimental investigations on the validity of such relations are needed [102].

3.2.2. Determination of pore size distribution

A suitable relation between the local distribution coefficient K and pore dimensions allows the overall distribution coefficient K_d to be derived from the cumulative effects of a polydisperse collection of pores. de Vries et al. correlated the elution volume to the pore size distribution based on the concept that the pore volume accessible to a solute is the volume of pores larger than that solute [20]. Discrepancies were noted between calculated pore dimensions and those found from mercury porosimetry [20], which can be explained by the neglect of the probe size effect on pore accessibility [2]. Halász and Martin used a similar approach but introduced an adjustable parameter to fit the pore size distribution to the porosimetry data to compensate for the omission of wall effects [13], which had been recognized earlier [52].

These approximate approaches were superseded by analyses that included an explicit PSD function in the correlation of permeation extents with the probe dimensions [14,89]. Knox and Scott also incorporated wall effects and derived an explicit formula for obtaining the PSD from calibration curves for rigid spherical molecules in cylindrical pores [14],

$$K_\text{d} = \int_{R_\text{m}}^{\infty} f(r)K\,\text{d}r \quad (18)$$

$$f(r)_{r=r_0} = -\frac{1}{2r_0}\left[\left(\frac{\text{d}^3K_\text{d}}{\text{d}(\ln R_\text{m})^3} - 3\frac{\text{d}^2K_\text{d}}{\text{d}(\ln R_\text{m})^2} + 2\frac{\text{d}K_\text{d}}{\text{d}(\ln R_\text{m})}\right)\right]_{R_\text{m}=r_0} \quad (19)$$

where K_d is the overall distribution coefficient, K the local distribution coefficient, i.e., the fraction of a pore of radius r accessible to a probe of radius R_m, given by Eq. (10) with $q = 2$, and $f(r)$ is the differential PSD. This approach requires a continuous function $K(\ln R_\text{m})$ as well as its derivatives. An alternative approach to relate distribution coefficients to the PSD is to use an iterative routine to fit the experimental and calculated K by adjusting the distribution parameters. This approach makes use of integrated rather than differentiated functions and is thus generally robust; it can be realized by different data fitting routines, such as the Excel Solver function [16], IMSL routines [18] and programs tailored to specific needs [12]. Care is necessary in several respects, though, especially in ensuring that integration is carried out sufficiently accurately and to a sufficiently high upper limit (∞ in analytical form), and that the best fit is globally rather than just locally optimal.

Although standards with narrow molecular mass distributions are usually used in measuring accessible pore volumes, strictly monodisperse standards are usually not available. Polydisperse solutes are easier to obtain and less expensive, and can be used for ISEC measurements with the contribution of polydispersity considered. The correlation of peak profile and molecular mass distributions has been investigated, with the surprisingly sparsely cited analysis of Kubín [75] especially thorough. Mathematical models to describe SEC behavior considering the polydispersity of solutes have also been used to obtain PSD information from the chromatographic response by deconvolution [22,105].

3.2.3. Pore dimension parameters

With the pore size distribution determined, additional pore structural parameters can be calculated. Indeed, several parameters of functional interest can be determined directly from SEC data without the actual PSD information. For instance, the critical size, defined as the size of the largest solute that can permeate into the pore space [106], is one characteristic parameter in describing pore size. It can be determined by extrapolating the generally linear plot of $\ln(1 - K_d)$ versus $\ln M$ to $K_d = 0$, but is applicable only for rigid solutes. The physical significance of this critical size is that the connectivity of the pores accessible to probes of this size gives rise to the percolation threshold of the pore network, below which the pore structure is not interconnected well enough to be open to solute transport [107]. Another parameter in representing the pore dimension is the median pore size, viz. the probe radius at $K_d = 0.5$ [25]. The inflexion point of the SEC calibration curve can be used to estimate an average pore size of the adsorbent [108], which is another representative size parameter not necessarily corresponding to the mean pore size in the PSD calculated from the first moment. The selectivity, evaluated as the local slope of the calibration curve, $dK_d/d\log R_m$, was introduced to describe the inherent ability of the adsorbent to separate solutes sterically [2].

The parameters discussed above are based mainly on the measured permeation extents without the need for information on pore size composition such as that obtained from ISEC. With the PSD determined for an adsorbent, a number of parameters not directly accessible from the calibration curve can be further calculated. The mean pore size $\bar{r}$ is found from the first moment:

$$\bar{r} = \int_0^\infty r f(r)\,dr \tag{20}$$

The accessible surface area A and phase ratio ϕ are defined as the accessible surface area per unit pore volume or per unit mobile phase volume, respectively [18], and are directly related to the adsorption capacity as well as the retention factor k' manifested in the relation $k' = K_{eq}\phi$, in which K_{eq} is the solute adsorption equilibrium constant. Based on a simplified pore shape, such as a cylindrical pore, the accessible surface area per unit pore volume of an adsorbent with a continuous PSD can be calculated as

$$A = \int_{R_m}^\infty \frac{2(r - R_m)}{r^2} f(r)\,dr \tag{21}$$

where R_m is the solute radius. The phase ratio can then easily be found by multiplying the surface area by the total accessible pore space/void volume. Narrower-pore materials typically provide higher surface areas and phase ratios for solutes accessible to the pore space [18]. For example, Fig. 3 shows that the phase ratios for small dextrans in Source 30Q (mean pore radius 32.4 nm) are significantly lower than those in Q Sepharose XL (mean pore radius 5.9 nm).

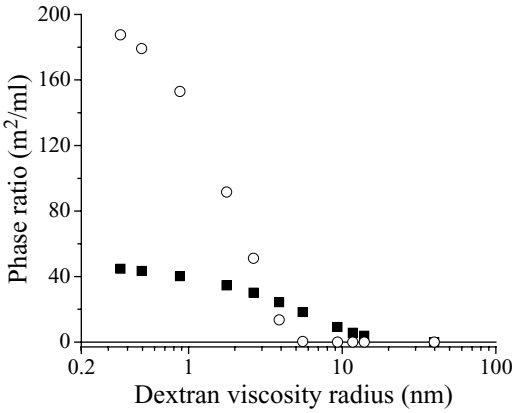

Fig. 3. Calculated phase ratios as a function of the dextran viscosity radius for Amersham Biosciences Source 30Q (■) and Q Sepharose XL (○) [34].

4. Limitations of inverse size-exclusion chromatography

Being based on a simplified pore model, ISEC is capable of providing comparative descriptions of pore statistics, which can be used for understanding macromolecular retention and transport behavior in chromatographic processes [34,109]. However, no information on the pore geometry can be deduced from ISEC, which has been found to be insensitive to the pore model assumed [14]. Similarly, the connectivity of the pore space in the adsorbent, an important structural parameter governing solute transport [110], cannot be probed directly by ISEC. In such cases, it may be useful to combine ISEC with other PSD characterization techniques such as microscopy to obtain better knowledge of the pore structure.

Additional precautions are appropriate in several practical aspects of SEC. As long columns are needed for attaining appreciable resolution among different sized probes, pressure buildup and ensuing compression of the column become one concern, especially for some softer materials. In such cases batch uptake can work as an alternative [2]. Appropriate solvents relevant to practical usage should be chosen, with additional considerations such as to minimize adsorption effects and favor optimal solute conformations. The total pore volume and interstitial space are typically measured by solutes at opposite ends of the size spectrum of the standards. Considering the rigidity of the solutes, wall effects can affect precise representation of the exact values [111], with the significance depending on the relative abundance of pores. Macropores larger than available standards with high molecular mass in SEC cannot be quantitatively characterized.

5. Conclusions

ISEC is a non-destructive technique for functional characterization of chromatographic adsorbents under

conditions of practical interest. As a chromatographic method, it is applicable to most adsorbents, and requires only a standard experimental chromatographic set up operated under conditions similar to those in SEC, in which appropriate column dimensions and flow rates are utilized for solute partitioning, differentiation and pressure limits. Well-defined standards that span the dimensions of the pore space are needed to probe the accessibility of the pore structure. ISEC provides a comparative statistical representation of the pore space that is open to solute transport, but no information on pore geometry and topology. Since real porous materials are always more complex than any idealized model implies, ISEC results must be regarded as being model-dependent, and it is essential to bear this in mind in using the results, especially in comparing materials of different types rather than simply different versions of the same base material. Nevertheless, the information obtainable from ISEC allows quantification, to much better than an order of magnitude, of the underlying effects driving partitioning and adsorptive behavior, and its ease of implementation can make it a valuable chromatographic science tool to aid in interpretation of empirical results.

Acknowledgements

This work was supported by the National Science Foundation under grant No. CTS-9977120.

References

[1] F.A.L. Dullien, Porous Media, Fluid Transport and Pore Structure, Academic Press, New York, 1992.
[2] L. Hagel, in: P.L. Dubin (Ed.), Aqueous Size-Exclusion Chromatography, Elsevier, Amsterdam, 1988, p. 119.
[3] A. Hernández, J.I. Calvo, P. Prádanos, L. Palacio, in: T.S. Sørensen (Ed.), Surface Chemistry and Electrochemistry of Membranes, 1999.
[4] S. Brunauer, P.H. Emmett, E. Teller, J. Am. Chem. Soc. 60 (1938) 309.
[5] H.L. Ritter, L.C. Drake, Ind. Eng. Chem. 17 (1945) 782.
[6] F.A.L. Dullien, G.K. Dhawan, J. Colloid Interface Sci. 47 (1974) 337.
[7] S. Kuga, J. Chromatogr. 206 (1981) 449.
[8] W.W. Yau, J.J. Kirkland, D.D. Bly, Modern Size-Exclusion Chromatography, Wiley, New York, 1979.
[9] J. Porath, P. Flodin, Nature 183 (1959) 1657.
[10] L.G. Aggebrandt, O. Samuelson, J. Appl. Polym. Sci. 8 (1964) 2801.
[11] A.A. Gorbunov, L.Y. Solovyova, V.A. Pasechnik, J. Chromatogr. 448 (1988) 307.
[12] L.Z. Vilenchik, J. Asrar, R.C. Ayotte, L. Ternorutsky, C.J. Hardiman, J. Chromatogr. 648 (1993) 9.
[13] I. Halász, K. Martin, Angew. Chem., Int. Ed. Engl. 17 (1978) 901.
[14] J.H. Knox, H.P. Scott, J. Chromatogr. 316 (1984) 311.
[15] J.H. Knox, H.J. Ritchie, J. Chromatogr. 387 (1987) 65.
[16] L. Hagel, M. Östberg, T. Andersson, J. Chromatogr. A 743 (1996) 33.
[17] K. Jerabek, A. Revillon, E. Puccilli, Chromatographia 36 (1993) 259.
[18] P. DePhillips, A.M. Lenhoff, J. Chromatogr. A 883 (2000) 39.
[19] O. Schou, L. Gotfred, P. Larsen, J. Chromatogr. 254 (1983) 289.
[20] A.J. de Vries, M. Lepage, R. Beau, C.I. Guillemi, Anal. Chem. 39 (1967) 935.
[21] C. Vilklund, F. Svec, M.J. Fréchet, Chem. Mater. 8 (1996) 744.
[22] N.V. Saritha, G. Madras, Chem. Eng. Sci. 56 (2001) 6511.
[23] R. Nikolov, W. Werner, I. Halász, J. Chromatogr. Sci. 18 (1980) 207.
[24] W. Werner, I. Halász, J. Chromatogr. Sci. 18 (1980) 277.
[25] S.E. Cook, T.C. Pinkerton, J. Chromatogr. 368 (1986) 233.
[26] I. Mazsaroff, F.E. Regnier, J. Chromatogr. 442 (1988) 15.
[27] H. Guan, G. Guiochon, J. Chromatogr. A 731 (1996) 27.
[28] T.C. Laurent, J. Killander, J. Chromatogr. 14 (1964) 317.
[29] T. Laurent, Biochim. Biophys. Acta 136 (1967) 199.
[30] W.W. Yau, C.P. Malone, S.W. Fleming, J. Polym. Sci. Pol. Lett. 6 (1968) 803.
[31] I. Halász, P. Vogtel, Angew. Chem., Int. Ed. Engl. 19 (1980) 24.
[32] M. Kremer, E. Pothmann, T. Rössler, J. Baker, A. Yee, H. Blanch, J.M. Prausnitz, Macromolecules 27 (1994) 2965.
[33] M. Ousalem, X.X. Zhu, J. Hradil, J. Chromatogr. A 903 (2000) 13.
[34] Y. Yao, A.M. Lenhoff, in preparation.
[35] M. Al-Bokari, D. Cherrak, G. Guiochon, J. Chromatogr. A 975 (2002) 275.
[36] A.P. Broek, H.A. Teunis, D. Bargeman, E.D. Sprengers, H. Strathmann, C.A. Smolders, J. Membr. Sci. 99 (1995) 217.
[37] L.F. Martin, S.P. Rowland, J. Chromatogr. 28 (1967) 139.
[38] J.E. Stone, A.M. Scallan, Cell. Chem. Technol. 2 (1968) 343.
[39] S.P. Rowland, C.P. Wade, N.R. Bertoniere, J. Appl. Polym. Sci. 29 (1984) 3349.
[40] M. Grünwald, E. Burtscher, O. Bobleter, J. Appl. Polym. Sci. 39 (1990) 301.
[41] J. Berthold, L. Salmén, Holzforschung 51 (1997) 361.
[42] G. Bunke, D. Gelbin, Chem. Eng. Sci. 40 (1985) 2079.
[43] M. Morino, H. Kaneko, T. Takanohashi, M. Iino, Energy Fuels 10 (1996) 1012.
[44] J.C. Giddings, in: J.C. Giddings, P.R. Brown, E. Grushka (Eds.), Advances in Chromatography, Marcel Dekker, New York, 1982, p. 217.
[45] J. Janca, in: J. Janca (Ed.), Steric Exclusion Liquid Chromatography of Polymers, Marcel Dekker, New York, 1984.
[46] G. Glöckner, Polymer Characterization by Liquid Chromatography (J. Chromatogr. Library 34), Elsevier, Amsterdam, 1987.
[47] R. Tijssen, J. Bos, in: F. Dondi, G. Guiochon (Eds.), Theoretical Advancement in Chromatography and Related Separation Techniques, Kluwer, Dordrecht, 1992, p. 397.
[48] J.C. Giddings, Unified Separation Science, Wiley, New York, 1991.
[49] G.H. Lathe, C.R.J. Ruthven, Biochem. J. 62 (1956) 665.
[50] J. Porath, Pure Appl. Chem. 6 (1963) 233.
[51] J. Ståhlberg, B. Jönsson, Cs. Horváth, Anal. Chem. 63 (1991) 1867.
[52] J.C. Giddings, E. Kucera, C.P. Russell, M.N. Myers, J. Phys. Chem. 72 (1968) 4397.
[53] M. Doi, J. Chem. Soc., Faraday Trans. II 71 (1975) 1720.
[54] D. Berek, I. Novák, Z. Grubisic-Gallot, H. Benoit, J. Chromatogr. 53 (1970) 55.
[55] M.E. Van Kreveld, N. Van Den Hoed, J. Chromatogr. 83 (1973) 111.
[56] R.P. Frigon, J.K. Leypoldt, S. Uyeji, L.W. Henderson, Anal. Chem. 55 (1983) 1349.
[57] P.L. Dubin, J.M. Principi, Macromolecules 22 (1989) 1891.
[58] S. Hussain, M.S. Mehta, J.I. Kaplan, P.L. Dubin, Anal. Chem. 63 (1991) 1132.
[59] S. Kuga, in: P.L. Dubin (Ed.), Aqueous Size-Exclusion Chromatography, Elsevier, Amsterdam, 1988, p. 157.
[60] W.W. Yau, C.P. Malone, J. Polym. Sci. Part B 5 (1967) 663.
[61] C. Tanford, Physical Chemistry of Macromolecules, Wiley, New York, 1961.

[62] Z. Grubisic, P. Rempp, H. Benoit, J. Polym. Sci. Pol. Lett. 5 (1967) 753.
[63] E.F. Casassa, Y. Tagami, Macromolecules 2 (1969) 14.
[64] G.K. Ackers, Adv. Protein Chem. 24 (1970) 343.
[65] P. Andrews, Methods Biochem. Anal. 18 (1970) 1.
[66] D.J. Harmon, in: R. Epton (Ed.), Chromatography of Synthetic and Biological Polymers, vol. 1, Column Packings, GPC, GF and Gradient Elution, Ellis Horwood, New York, 1978.
[67] C. Tanford, Physical Chemistry of Macromolecules, Wiley, New York, 1961, p. 395.
[68] W.M. Deen, F.G. Smith III, J. Membr. Sci. 12 (1982) 217.
[69] T.D. Long, D.L. Jacobs, J.L. Anderson, J. Membr. Sci. 9 (1981) 13.
[70] J.V. Dawkins, M. Hemming, Makromolekul. Chem. 155 (1972) 75.
[71] J.H. Knox, F. McLennan, J. Chromatogr. 185 (1979) 289.
[72] PSS Polymer Standards Data Sheet, Polymer Standards Service, GmbH, 2001.
[73] H.L. Berger, A.R. Schultz, J. Polym. Sci. A-2 (1965) 3643.
[74] J.H. Knox, F. McLennan, Chromatographia 10 (1977) 75.
[75] M. Kubín, J. Appl. Polym. Sci. 30 (1985) 2237.
[76] Y. Kato, K. Komiya, H. Sasaki, T. Hashimoto, J. Chromatogr. 190 (1980) 297.
[77] K.E. Jørgenson, J.V. Møller, Am. J. Physiol. 5 (1979) F103.
[78] P.G. Squire, J. Chromatogr. 210 (1981) 433.
[79] J.T. Edsall, in: H. Neurath, K. Balley (Eds.), The Proteins, Academic Press, New York, 1953, p. 549.
[80] Y. Nozaki, N.M. Schechter, J.A. Reynolds, C. Tanford, Biochemistry-US 15 (1976) 3884.
[81] F.G. Smith III, W.M. Deen, J. Colloid Interface Sci. 91 (1983) 571.
[82] W. Haller, Nature 206 (1965) 693.
[83] A.K. Hunter, G. Carta, J. Chromatogr. A 897 (2000) 65.
[84] S. Figueroa-Gerstenmaier, J.B. Avalos, L.D. Gelb, K.E. Gubbins, L.F. Vega, Langmuir 19 (2003) 8592.
[85] A.G. Ogston, Trans. Faraday Soc. 54 (1958) 1754.
[86] W.W. Yau, J.J. Kirkland, D.D. Bly, H.J. Stoklosa, J. Chromatogr. 125 (1976) 219.
[87] R.N. Nikolov, J. Chromatogr. 364 (1986) 163.
[88] T. Crispin, I. Halász, J. Chromatogr. 239 (1982) 351.
[89] A.A. Gorbunov, L.Y. Solovyova, V.A. Pasechnik, Vysokomol. Soedin. Ser. A 26 (1984) 967.
[90] M. Le Maire, A. Ghazi, J.V. Moller, L.P. Aggerbeck, Biochem. J. 243 (1987) 399.
[91] N.B. Afeyan, N.F. Gordon, I. Mazsaroff, L. Varady, S.P. Fulton, Y.B. Yang, F.E. Regnier, J. Chromatogr. 519 (1990) 1.
[92] K. Loh, D.I.C. Wang, J. Chromatogr. A 718 (1995) 239.
[93] J.J. Meyers, A.I. Liapis, J. Chromatogr. A 852 (1999) 3.
[94] J.E. Harlan, D. Picot, P.J. Loll, R.M. Garavito, Anal. Biochem. 224 (1995) 557.
[95] E.F. Casassa, J. Polym. Sci. Pol. Lett. 5 (1967) 773.
[96] J.R. Pappenheimer, E.M. Renkin, L.M. Borrero, Am. J. Physiol. 167 (1951) 13.
[97] E.M. Renkin, J. Gen. Physiol. 38 (1954) 225.
[98] J. Tong, J.L. Anderson, Biophys. J. 70 (1996) 1505.
[99] O. Chiantore, M. Guaita, J. Chromatogr. 260 (1983) 41.
[100] W.W. Yau, J.J. Kirkland, D.D. Bly, Modern Size-Exclusion Chromatography, Wiley, New York, 1979, Chapter 2.
[101] P.G. DeGennes, Rep. Prog. Phys. 32 (1969) 187.
[102] A.M. Skvortsov, A.A. Gorbunov, J. Chromatogr. 358 (1986) 77.
[103] J.V. Dawkins, M. Hemming, Makromol. Chem. 176 (1975) 1795.
[104] D. Bakoš, T. Bleha, A. Ozima, D. Berek, J. Appl. Polym. Sci. 23 (1979) 2233.
[105] M. Goto, B.J. McCoy, Chem. Eng. Sci. 55 (2000) 723.
[106] A.M. Basedow, K.H. Ebert, H.J. Ederer, E. Fosshag, J. Chromatogr. 192 (1980) 259.
[107] M. Sahimi, Applications of Percolation Theory, Taylor & Francis, Bristol, PA, 1994.
[108] D.H. Freeman, I.C. Poinescu, Anal. Chem. 49 (1977) 1183.
[109] P. DePhillips, A.M. Lenhoff, J. Chromatogr. A 933 (2001) 57.
[110] J.J. Meyers, A.I. Liapis, J. Chromatogr. A 827 (1998) 197.
[111] H. Dai, P.L. Dubin, T. Andersson, Anal. Chem. 70 (1998) 1576.