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Stochastic theory of size exclusion chromatography by the characteristic function approach

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Abstract

A general stochastic theory of size exclusion chromatography (SEC) able to account for size dependence on both pore ingress and egress processes, moving zone dispersion and pore size distribution, was developed. The relationship between stochastic-chromatographic and batch equilibrium conditions are discussed and the fundamental role of the 'ergodic' hypothesis in establishing a link between them is emphasized. SEC models are solved by means of the characteristic function method and chromatographic parameters like plate height, peak skewness and excess are derived. The peak shapes are obtained by numerical inversion of the characteristic function under the most general conditions of the exploited models. Separate size effects on pore ingress and pore egress processes are investigated and their effects on both retention selectivity and efficiency are clearly shown. The peak splitting phenomenon and peak tailing due to incomplete sample sorption near to the exclusion limit is discussed. An SEC model for columns with two types of pores is discussed and several effects on retention selectivity and efficiency coming from pore size differences and their relative abundance are singled out. The relevance of moving zone dispersion on separation is investigated. The present approach proves to be general and able to account for more complex SEC conditions such as continuous pore size distributions and mixed retention mechanism.

Keywords: Stochastic theory; Size-exclusion chromatography; Pore size distribution; Retention mechanisms

1. Introduction

Size exclusion chromatography (SEC) is the most widespread technique for polymer molecular mass determination [1]. As is well known, this determination relies on a calibration step based on the retention of well-characterized monodispersed samples [2]. Because of the limited resolution and peak capacity of SEC, the use of peak deconvolution procedures able to correctly account for the peak shape features is equally important for the precision. These procedures are all based on some theoretical assumptions about the peak shape of individual macromolecular components of the sample and thus any advance in theoretical modelling of these aspects will introduce a benefit for a good SEC practice.

Most theoretical investigations of SEC (see the

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reviews of Refs. [1,3–5]) have been intended to throw light on the dependence between retention and dimensions and shape of the separated species. Various possible retention mechanisms were identified [3–6] such as hydrodynamically and stress-induced diffusion, the polarization effect, multipath, enthalpic and soft body interactions [7]. However the size exclusion effect of macromolecules within the stagnant zone entrapped in the porous or gel column packing material is today recognized as the most important one [8]. In this instance, separation is accounted for by a pure entropic mechanism, i.e. by the relative loss of the number of configurations, or accessible volume, of a partitioning species in the stagnant zone [9–11]. These size exclusion models are also called 'equilibrium' or 'thermodynamic' models since they correctly account for the differential partition both in static experiments and in dynamical chromatographic separations [3,12,13]. None of these models, however, gives insight into peak shape and peak broadening in SEC.

The *stochastic theory of chromatography*, originally conceived by Giddings and Eyring in 1955 [14], further elaborated by Giddings [15] and by McQuarrie [16] to account for adsorption chromatography, was recast by Carmichael to represent SEC processes [17–20]. In fact the stochastic theory of chromatography, by representing the migration of a molecule along the chromatographic bed as a random chain of ingress–egress steps on identical binding sites of the stationary phase is completely independent of the specific physicochemical mechanism responsible for retention and can represent all types of chromatography. As remarked by Casassa in the case of SEC [13], 'all we then have to do is to replace the word 'adsorption' by the phrase 'entrapment in micropores' and recognize that in SEC, unlike adsorption chromatography, the solvent — consisting of small molecules and, therefore, most easily trapped in voids — is retarded in the column relative to macromolecular solutes'. The superiority of the stochastic theory with respect to other theories lies in its ability both to account for the dynamical character of the chromatographic process and to determine the exact peak shape, i.e. it is at the same time able to explain and represent the chromatographic process. Carmichael, however, did not achieve complete success in mastering the complexity of SEC. In fact, his treatment of the effect of packing pore size distribution [18] was new and mainly intuitive, but not completely rigorous since he considered only the mean residence time dependence in pores. Moreover the hypothesis that macromolecules of different sizes spend the same time within pores is limiting, if not unacceptable.

The partial failure of Carmichael's work on SEC was dependent on the fact that he only translated the original mathematical handling of Giddings and Eyring into the SEC context without making further advances in handling the complexity of the SEC process. This can be understood and explained with just the following thoughts of Giddings, the father of the stochastic theory: 'one aspect of the stochastic theory which has been pursued from the beginning is the effect of a nonuniform surface with different kinds of adsorption sites. The mathematics rapidly becomes intractable, however, as we pass from the sheltered simplicity of one site theory' [21]. Thus the limit was not in the stochastic approach, but in the mathematical tools employed at that time.

Several important contributions to stochastic theory of chromatography appeared after the original Carmichael's work on SEC. Most important were the Weiss contributions [22,23] accounting for mobile phase dispersion and column heterogeneity. However this advancement leads to complex mathematics and was not systematically applied to study the many open points in chromatography, including the SEC problem. Moreover the determination of the exact peak shape has not been solved. With the introduction of the characteristic function (CF) method in stochastic theory of chromatography, the mathematical intractability was completely overcome [24,25]. For example, the problem of stationary phase heterogeneity proves to be fully tractable [26,27]; moving zone dispersion effects can be accounted for [28]; mixed retention mechanisms can be considered as well [26]. Thus there is no a priori limit in handling the complexity of the SEC problem, including the effect of the pore heterogeneity and different size separation mechanisms, e.g. separation by flow [29]. By the CF approach the exact peak shape can be obtained by CF numerical inversion [27,30]. Furthermore the peak shape can be fully analyzed by determining its statistical moments from the derivatives of the CF [25].

Here the stochastic theory of SEC is reconsidered in the light of the characteristic function approach. A general model for a single pore type ('monopore' model), a model for two pore types ('two-pore' model), and a model accounting for dispersion within the moving zone will be successively developed and exploited. Among the different band broadening phenomena, attention will be focused mainly on those determining size exclusion effects, i.e. the pore ingress and egress processes. It must be underlined that many of the models exploited have potential impact for both column packing design and SEC practice. However emphasis will be devoted mostly to theoretical aspects of the CF approach and to its potential results in SEC research. Exhaustive handling of all the topics raised lies beyond the aims of the present investigation, but they can be dealt with separately.

2. Theory

2.1. Size exclusion chromatographic quantities and size exclusion thermodynamic quantities

The chromatographic parameters used to characterize retention and separation in SEC and the relationships between them are well known. However, in order to better appreciate the features of the stochastic approach, it is useful to briefly recall them and to discuss the sometimes overlooked hypotheses which underlie these parameters and relationships.

In the following, we consider a well-defined chemical species i of the sample, which is thus assumed monodispersed. This species is retained in the SEC column. Although the stochastic approach does not imply a given retention mechanism and can be adapted to any individual or mixed mechanism, we consider here that this species is essentially retained by a size exclusion mechanism. Retention is thus based on partition of the sample species between two regions occupied by the carrier liquid in the column: the interstitial space, where the carrier is effectively flowing between the solid particles making up the column and which is therefore called the *moving zone* (labelled 0), and the space occupied by the carrier in the porous structure inside the packing particles. As the permeability of this porous structure [4] is generally much lower than that of the interstitial space, the carrier is assumed to be essentially stagnant inside this intraparticle space which therefore is called the *stagnant zone* (labelled *p*, to recall the pore structure concept). The volumes occupied by the carrier in the moving and stagnant zones within the column are denoted V_0 and V_p, respectively.

The accurate determination of these two volumes is not obvious [5]. It is generally made by measuring the retention times of two species, a totally permeable species, supposed to have access to the whole volume $(V_0 + V_p)$ occupied by the carrier in the column and a species which is totally excluded from the intraparticle porous structure. These species are referred to by subscripts *perm* and *excl*, respectively. It is reasonable to suppose that a small solute of a molecular size similar to that of the carrier can effectively act as a totally permeable species which can sample the whole $V_0 + V_p$ volume. However, the selection of a totally excluded species is not trivial. Indeed, a macromolecule large enough to be sterically excluded from the intraparticle volume is also likely to be sterically excluded from that part of the interstitial volume in the immediate vicinity of the outside surface of the solid particles. The retention time of such a species is then more or less affected by mechanisms involved in hydrodynamic chromatography [4], and may differ somewhat from the mean elution time $\bar{t}_0$ of a hypothetical species which would sample the whole volume V_0.

Two basic parameters are employed to experimentally measure the retention of species i:

$$K_{\mathrm{SEC},i} = \frac{\bar{t}_{c,i} - \bar{t}_{c,excl}}{\bar{t}_{c,perm} - \bar{t}_{c,excl}} \quad (1)$$

$$k_i'' = \frac{\bar{t}_{c,i} - \bar{t}_{c,excl}}{\bar{t}_{c,excl}} \quad (2)$$

where the various $\bar{t}_c$s are the mean residence times of the corresponding species in the column. These times correspond to the first moments of the peaks. In the following, the bars over the quantities indicate mean quantities, whereas quantities without the bar indicate random variables. The use of this notation is dictated by the sake of precision since here a molecular stochastic approach is developed. We note

that a $\bar{t}_c$ quantity corresponds to the classical definition of retention time (t_R) of the corresponding species. It is assumed that elution is carried out under the condition of infinite dilution (linearity condition). It is thus apparent that K_{SEC} is evaluated by using three species (*i*, *excl*, *perm*) instead of two as in other chromatographic techniques.

In order to derive the link between chromatographic quantities and equilibrium or kinetic quantities two groups of hypotheses must be assumed: the first group is essentially chromatographic, the second one relates to the statistical thermodynamics (ergodic hypothesis).

$\bar{t}_{c,excl}$ is first assumed to be the measure of the mean time spent by an inert tracer in the moving zone, $\bar{t}_0$:

$$\text{HYP 1} \quad \bar{t}_{c,excl} = \bar{t}_0 \tag{3}$$

Thus, no hydrodynamic chromatography effect is acting on *excl* species transport, as mentioned above. Likewise, all the species *i*, *excl* and *perm* are assumed to spend the same mean time $\bar{t}_0$ in the moving zone:

$$\text{HYP 2} \quad \bar{t}_{0,i} = \bar{t}_{0,perm} = \bar{t}_0 \tag{4}$$

The mean times spent by the species *i* and *perm* in the stagnant zone, $\bar{t}_{p,i}$ and $\bar{t}_{p,perm}$, respectively, are then defined as:

$$\bar{t}_{p,i} = \bar{t}_{c,i} - \bar{t}_{c,excl} \tag{5}$$

and

$$\bar{t}_{p,perm} = \bar{t}_{c,perm} - \bar{t}_{c,excl} \tag{6}$$

If all the above mentioned hypotheses hold true, the experimental measurements of either K_{SEC} or k'' can be expressed as:

$$K_{SEC} = \frac{\bar{t}_{p,i}}{\bar{t}_{p,perm}} = \frac{\bar{t}_{p,i}}{\bar{t}_{0,i}} \frac{\bar{t}_{0,perm}}{\bar{t}_{p,perm}} \tag{7}$$

and

$$k_i'' = \frac{\bar{t}_{p,i}}{\bar{t}_{0,i}} \tag{8}$$

The total times spent by one molecule of the different species in the moving or stagnant zones, t_0 or t_p are expressed by a sum of a large number, n_0 or n_p of small time contributions, τ_0 or τ_p which are the times spent in that zone between two subsequent zone changes. Because of the random character of the chromatographic process, both the total time, *t*, and the two individual quantities, *n* and τ, are random quantities. However it can be intuitively assumed that the mean total time $\bar{t}$ is equal to the product of the mean values of the two individual random *n* and τ quantities, i.e. equal to $\bar{n} \times \bar{\tau}$. This assumption is reasonable but its proof is not trivial: it will be one of the results of the CF method (see the following section). Therefore for the different species and zones the following equations must hold true:

$$\left.\begin{array}{ll} \text{(a)} & \bar{t}_{p,i} = \bar{n}_{p,i}\bar{\tau}_{p,i} \\ \text{(b)} & \bar{t}_{0,i} = \bar{n}_{0,i}\bar{\tau}_{0,i} \\ \text{(c)} & \bar{t}_{p,perm} = \bar{n}_{p,perm}\bar{\tau}_{p,perm} \\ \text{(d)} & \bar{t}_{0,perm} = \bar{n}_{0,perm}\bar{\tau}_{0,perm} \end{array}\right\} \text{HYP 3} \tag{9}$$

By introducing Eqs. (3), (4), (9a)–(9d) in Eqs. (7) and (8), one has:

$$K_{SEC,i} = \frac{\bar{n}_{p,i}\bar{\tau}_{p,i}}{\bar{n}_{0,i}\bar{\tau}_{0,i}} \frac{\bar{n}_{0,perm}\bar{\tau}_{0,perm}}{\bar{n}_{p,perm}\bar{\tau}_{p,perm}} \tag{10}$$

$$k_i'' = \frac{\bar{n}_{p,i}\bar{\tau}_{p,i}}{\bar{n}_{0,i}\bar{\tau}_{0,i}} \tag{11}$$

If the number of ingress steps is equal to the number of egress steps from the stagnant zone, one has, for both the species *i* and *perm*:

$$\left.\begin{array}{ll} \text{(a)} & \bar{n}_{0,i} = \bar{n}_{p,i} \\ \text{(b)} & \bar{n}_{0,perm} = \bar{n}_{p,perm} \end{array}\right\} \text{HYP 4} \tag{12}$$

By combining Eqs. (12a), (12b), (10) and (11), one obtains:

$$K_{SEC,i} = \frac{\left(\frac{\bar{\tau}_{p,i}}{\bar{\tau}_{0,i}}\right)}{\left(\frac{\bar{\tau}_{p,perm}}{\bar{\tau}_{0,perm}}\right)} \tag{13}$$

$$k_i'' = \frac{\bar{\tau}_{p,i}}{\bar{\tau}_{0,i}} \tag{14}$$

and, by combining Eqs. (13) and (14):

$$K_{SEC,i} = k_i'' \frac{\bar{\tau}_{0,perm}}{\bar{\tau}_{p,perm}} \tag{15}$$

In order that HYP 4 (Eqs. (12a) and (12b)), holds true, it is required that no molecule of a given species leaves the column without having 'visited' the stagnant zone. Moreover it is assumed that the sample is introduced in the column in the moving zone and the elution process starts immediately before allowing the species to reach partition equilibrium between the two zones. If such a relaxation process is not made — as is usual in chromatography — HYP 4 is met only when the $\bar{n}$ quantities are large enough and consequently there are no differences between $\bar{n}_0$ and $\bar{n}_p$. The problem will be handled later on in the context of the stochastic theory.

Eqs. (13) and (14) establish a link between chromatographic quantities, K_{SEC} and k'', and microscopic kinetic quantities $\bar{\tau}$, provided that the hypotheses 1–4 hold true. In order to establish a link between chromatographic quantities and equilibrium quantities, the ergodic hypothesis must be assumed [31]. In the present case, this can be formulated by saying that, in a phase exchange at equilibrium, the mean number of molecules $N^{eq}_{\beta,i}$ in a given phase domain V_β is proportional to the mean time $\bar{t}_{\beta,i}$ spent in that phase domain by a single species:

$$\text{HYP 5} \quad N^{eq}_{\beta,i} = c^{eq}_{\beta,i} V_\beta \propto \bar{t}_{\beta,i} \tag{16}$$

where the superscript 'eq' indicates the 'equilibrium' condition, i.e. that condition reached in a batch process after a long time. Moreover it is assumed that both the moving zone and the stagnant zone are homogeneous from a thermodynamic point of view. In practice, no mixed retention mechanisms are considered here. Under these conditions, by introducing Eq. (16) into Eq. (7), the following equation is derived:

$$K_{SEC,i} = \frac{\dfrac{c^{eq}_{p,i}}{c^{eq}_{0,i}}}{\dfrac{c^{eq}_{p,perm}}{c^{eq}_{0,perm}}} \tag{17}$$

The classical expression:

$$K_{SEC,i} = \frac{c^{eq}_{p,i}}{c^{eq}_{0,i}} \tag{18}$$

is obtained by assuming:

$$K_{SEC,perm} = \frac{c^{eq}_{p,perm}}{c^{eq}_{0,perm}} = 1 \tag{19}$$

which specifies the properties of the *perm* species.

Likewise, by applying the ergodic hypothesis (HYP 5, Eq. (16)) to an equilibrium condition over the whole column volumes V_0 and V_p in Eq. (8), one derives:

$$k''_i = \frac{c^{eq}_{p,i}}{c^{eq}_{0,i}} \frac{V_p}{V_0} \tag{20}$$

and thus:

$$K_{SEC,i} = k''_i \frac{V_0}{V_p} \tag{21}$$

Eqs. (13) and (18) give, respectively, the kinetic interpretation and the equilibrium–thermodynamic interpretation of K_{SEC}, the ergodic hypothesis of Eq. (16) being the bridge between them. It can be seen that K_{SEC}, even if it cannot furnish estimates of the separate kinetic quantities concerning the pore ingress and egress processes, nonetheless depends on their ratio. Moreover it is allowed to translate the various equilibrium thermodynamic models [3,8–12] into the kinetic analogue. This correspondence can indeed be found in theoretical handling of hindered transport of large molecules in liquid-filled pores [32]: both the methods employed and the derived expressions accounting for the exclusion effects are similar to those derived for SEC either by using a pure equilibrium approach [9] or by solving the diffusion equation [9,10].

The number of theoretical plates and the effective number of theoretical plates are, respectively:

$$N = \left(\frac{\bar{t}_c}{\sigma_t}\right)^2 \tag{22}$$

$$N' = \left(\frac{\bar{t}'_c}{\sigma_t}\right)^2 \tag{23}$$

where σ_t is the standard deviation of the peak and:

$$\bar{t}'_c = \bar{t}_p = \bar{t}_c - \bar{t}_0 \tag{24}$$

is the mean time spent in the stagnant zone. The plate height is:

$$H = \frac{L}{N} \tag{25}$$

L being the column length. The mean moving zone velocity $\bar{v}_0$ is related to $\bar{t}_0$ by:

$$L = \bar{t}_0 \bar{v}_0 \tag{26}$$

The skewness and the excess are, respectively:

$$S = \frac{\kappa_3}{\sigma_t^3} \tag{27}$$

$$E = \frac{\kappa_4}{\sigma_t^4} \tag{28}$$

where κ_3 and κ_4 are the time-based third and fourth cumulants of the chromatographic peak [33].

2.2. The stochastic description of the chromatographic process

From a stochastic point of view, the chromatographic process can be described as a chain of ingress–egress random processes, i.e. state exchanges of the sample molecules between the moving zone and the stagnant zone occupied by the carrier liquid.

Fig. 1 describes progress of single molecules in the column by a trajectory in the (l, t) plane, l being the position of the molecule along the column length at the time t. Each molecular trajectory is composed of a succession of slanting segments with slope equal to the moving zone velocity v_0 and of horizontal segments corresponding to the time τ_p spent during each step in the stagnant zone. An elementary displacement step dl along the column axis corresponds to $v_0\tau_0$. In Fig. 1, both τ_p and τ_0 are random variables characterized by their frequency functions, $f(\tau_p)$ and $f(\tau_0)$, respectively. The chromatographic history and the corresponding trajectory will end when the position $l = L$, where L is the column length, is reached. Three types of molecules are represented in Fig. 1: totally excluded molecules always staying in the moving zone and two other types of molecules which are retained by the porous structure. In Fig. 1, v_0 is assumed both constant and independent of the species. The random character of τ_p has the consequence that the column residence time is a random quantity, characterized by a given distribution, i.e. the chromatographic peak. This, however, holds true only for retained species and not for the totally excluded ones, since moving zone dispersion factors are here neglected. According to the picture of Fig. 1, the resultant residence time is a sum of the random quantities τ_p, the number of terms of the sum, n, also being a random variable. In fact n is a random variable, with the specific distribution function $P(n)$, because of the random character of the τ_0 quantity. The task of the theory will be to compute the result, i.e. the residence time distribution, under the most general conditions. One general feature must however be pointed out: the stochastic theory of chromatography is a 'pure' chromatographic theory, describing the dynamical features of the band migration [34–36]. Its strength lies in the ability to master the 'chromatographic complexity' such as the combined effects of the stationary phase heterogeneity, nonconstant moving zone velocity, etc. This approach requires only the knowledge of the statistical properties of the times spent by the molecule in the different steps (rate constants for the zone exchange, time distributions). The specific physicochemical aspects of the various zones (moving, stagnant) are not explicitly considered.

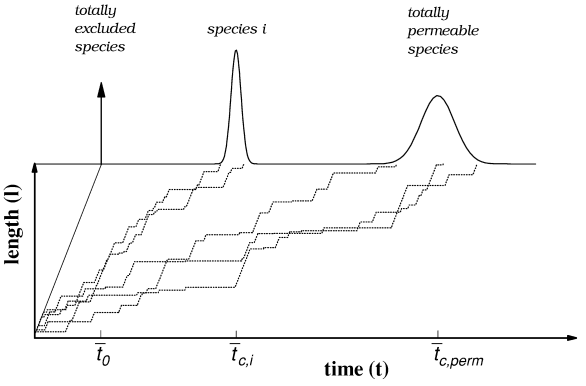

Fig. 1. Chromatographic process represented as a stochastic process. Each molecule history is represented as a random trajectory in the l, t plane. Constant moving zone velocity case.

2.3. Classical stochastic model of SEC

Carmichael's work was based on the Giddings–Eyring treatment which corresponds to well-defined hypotheses about the statistical characteristics of the column processes, simply considered as first-order processes with an exponential time distribution function. This SEC model will be here referred to as the Giddings–Eyring–Carmichael (GEC) model.

The fact that the time spent in the moving zone

between two consecutive ingress steps has an exponential distribution function, has the important consequence that $P(n)$ is Poissonian. However it must be pointed out that the property that the ingress number is Poissonian does not mean that we can identify a single ingress mechanism. The Poisson distribution is, in fact, the 'limit' distribution for 'point processes', i.e. for an event series represented as points over a time axis, here corresponding to ingress events. This limit condition represents well the case of superimposition of a great number of point processes, corresponding to different ingress mechanisms. However, even if strongly founded, the Poisson distribution cannot be the only one driving the pore ingress process. Pore access is more precisely driven by Brownian movement: when a molecule has arrived near a pore, it is likely that it visits this pore more than once [36], before wandering to a new pore. This matter has up to now been largely unexplored in its dependency on both molecule type and column structure. A general form for $P(n)$ with proper dependencies on the molecular size r and pore size d, i.e. $P(n; r, d)$ and for a given type of column, must be allowed.

The second hypothesis of the GEC model is that the time spent in a single pore is exponential and independent of molecule size [17] although the relevance of this last constraint was discussed by Carmichael [19]. Both of these hypotheses are too restrictive and a general expression for the pore residence time distribution $f(\tau_p; r, d)$ must likewise be allowed. Under these conditions, the general SEC model has only the constraints of constancy of the moving zone velocity and independence of the ingress and egress processes. This general SEC model belongs to a broad class of stochastic processes, the general composite processes or 'mixture processes', extensively described in probability theory. It can be shown [24,25] that the expression of the stagnant zone retention time distribution has the following form:

$$f(t_p; r, d) = \sum_{n=0}^{n=\infty} P\{n; r, d\} f(t_p|n; r, d) = \sum_{n=0}^{n=\infty} P\{n; r, d\} f^{*n}(\tau_p; r, d) \quad (29)$$

where:

$$f(t_p|n; r, d) = f^{*n}(\tau_p; r, d) \quad (30)$$

represents the distribution of the time spent in the stagnant zone by a molecule performing exactly n ingress steps. The symbol $*n$ means an n-fold convolution. The distribution of the column residence time t_c is simply the t_p distribution scaled by $\bar{t}_0$, since the hypothesis of constant moving zone velocity was put forward:

$$f(t_c; r, d) = f\left[(t_p + \bar{t}_0); r, d\right] \quad (31)$$

It must be observed that, in Eq. (29), $P\{n; r, d\}$ may be significant for $n = 0$ under certain conditions. This means that the probability of performing zero entrapment steps is significant and the relative number of molecules travelling in the column without visiting the stagnant zone will be high in these cases. This will produce the so-called 'peak splitting' at the time scale origin. This point will be explained in the Discussion.

2.4. The characteristic function method

The CF is defined as the Fourier transform of the probability density function [33,37,38]:

$$\Phi(\omega) = \int e^{i\omega t} f(t_c)\, dt_c \quad (32)$$

where i is the imaginary unit and ω an auxiliary real variable (frequency). $f(t_c)\, dt_c$ represents the infinitesimal probability that a molecule leaves the column after a column residence time between t_c and $t_c + dt_c$.

The two CF expressions for the entry number, n, and for the pore residence time, τ_p, are, respectively:

$$\Phi_n(\omega; r, d) = \sum_n e^{i\omega n} P(n; r, d) \quad (33)$$

and

$$\Phi_{\tau_p}(\omega; r, d) = \int e^{i\omega \tau_p} f(\tau_p; r, d)\, d\tau_p \quad (34)$$

The CF of the general model described by Eq. (29) can be obtained by using a log-exp transformation [24,25]:

$$\Phi_{t_p}(\omega; r, d) = \Phi_n\left\{\left[\frac{\ln \Phi_{\tau_p}(\omega; r, d)}{i}\right]; r, d\right\} \quad (35)$$

where $\Phi_{t_p}(\omega; r, d)$ is the CF for the time t_p spent in the stagnant zone by a molecule of dimension r, in a column having pore size d:

$$\Phi_{t_p}(\omega; r, d) = \int e^{i\omega t_p} f(t_p; r, d)\, dt_p \quad (36)$$

Note the simplicity of the expression (35) stating that, under general conditions, the CF of the stagnant zone residence time is equal to that of the stagnant zone entry number with the substitution of the auxiliary variable ω by the natural logarithm of the pore residence time CF, divided by the imaginary unit i.

The CF of the column residence time (i.e. including the moving zone time $\bar{t}_0$), is obtained by applying the shift properties in the Fourier domain [38] to Eq. (35):

$$\Phi_{t_c}(\omega; r, d) = \Phi_{t_p}(\omega; r, d)\, e^{i\omega \bar{t}_0} \quad (37)$$

Any type of model, meeting the above mentioned hypothesis, of constant moving zone velocity can be worked out and the pertinent CF obtained, by simply specifying both $\Phi_n(\omega; r, d)$ and $\Phi_{\tau_p}(\omega; r, d)$ [38,39]. Once $\Phi_{t_c}(\omega; r, d)$ is obtained, the statistical moments of the peak profile are calculated from the derivatives evaluated at $\omega = 0$. From these quantities all the chromatographic quantities $\bar{t}_c$ (i.e. t_R), N, H, S, E can be computed [24,25]. The shape of the column residence time distribution (i.e. the chromatographic peak) can be obtained in all cases by numerical inversion [26,28,30].

2.5. Exploitation of general monopore models based on constant moving zone velocity

The general monopore SEC model derived in the previous section is too general and consequently uninteresting in practice. However, from it, interesting features are derived that hold true for all models belonging to this class. More precisely, the features singled out in the following will be common not only to the GEC model for which the ingress process is Poissonian and the pore egress process is exponential, but to any other case with the only constraint that the ingress and egress processes be independent of each other. The first moment is [24,25]:

$$\bar{t}_p = \bar{n}_p(r, d)\, \bar{\tau}_p(r, d) \quad (38)$$

where $\bar{n}_p(r, d)$ and $\bar{\tau}_p(r, d)$ are, respectively, the mean values of the ingress step number and of the time spent in a single pore. One can thus see that Eqs. (9a)–(9c) hold true under these most general conditions. Likewise one has the following equation for the peak variance:

$$\sigma_{t_p}(r, d)^2 = \left[\sigma_{n_p}(r, d)\right]^2 \left[\bar{\tau}_p(r, d)\right]^2 + \bar{n}_p(r, d)\left[\sigma_{\tau_p}(r, d)\right]^2 \quad (39)$$

where the σ^2 are the variances of the quantities specified in the suffixes.

The HETP value corresponds, in this case, to the C term of the Deemter equation since all moving zone dispersive effects were neglected. It is [24]:

$$H_C = \left\{ \left(\frac{\sigma_{\tau_p}(r, d)}{\bar{\tau}_p(r, d)} \right)^2 + \frac{\left[\sigma_{n_p}(r, d)\right]^2}{\bar{n}_p(r, d)} \right\} \frac{k''}{(k'' + 1)^2}\, v_0\, \bar{\tau}_p(r, d) \quad (40)$$

where v_0 is the constant moving zone velocity.

From Eqs. (38) and (40), one can see that the chromatographic quantities $\bar{t}_p$ and H_C are affected by size effects both through the ingress process and residence in the pore, i.e. by the quantities $\bar{n}_p(r, d)$, $\sigma_{n_p}(r, d)$, $\bar{\tau}_p(r, d)$ and $\sigma_{\tau_p}(r, d)$.

2.6. Monopore GEC model

The pore egress time distribution for this model is exponential:

$$f[\tau_p; r, d] = \frac{1}{\bar{\tau}_p(r, d)} \exp\left[-\frac{\tau_p(r, d)}{\bar{\tau}_p(r, d)} \right] \quad (41)$$

where $\bar{\tau}_p(r, d)$ is the mean time spent by the molecule for each visit in the pores. The number of ingress steps in pores is Poissonian:

$$P[n; r, d] = \frac{\exp[-\bar{n}(r, d)] \times [\bar{n}(r, d)]^n}{n!} \quad (42)$$

where $\bar{n}(r, d)$ is the mean ingress number for the particular species.

It can be proved [25] for this model that the CF is:

$$\Phi_{t_p}(\omega; r, d) = \exp\left\{\bar{n}_p(r, d)\left[\frac{1}{1 - i\omega\bar{\tau}_p(r, d)} - 1\right]\right\} \quad (43)$$

The expression for the stagnant zone residence time is, from Eq. (38):

$$\bar{t}_p = \bar{n}_p(r, d)\,\bar{\tau}_p(r, d) \quad (44)$$

and from Eq. (39) the peak variance is:

$$\sigma^2_{t_p} = 2\bar{n}_p(r, d)\,\bar{\tau}^2_p(r, d) \quad (45)$$

since

$$\bar{\tau}_p(r, d) = \sigma_{\tau_p}(r, d) \quad (46)$$

$$\bar{n}_p(r, d) = \sigma^2_{n_p}(r, d) \quad (47)$$

hold true because the time spent in the pore was assumed to be exponentially distributed and the ingress process was assumed to be Poissonian. The H_C term is [35]:

$$H_C = 2\frac{k''}{(1 + k'')^2} v_0 \bar{\tau}_p(r, d) \quad (48)$$

The molecule size selectivity of the SEC process is reflected by the dependence of K_{SEC} on molecular size r and on pore size d. In various geometrical–equilibrium models of SEC, K_{SEC} is expressed in terms of a unique size parameter ρ defined as:

$$\rho = \frac{r}{d} \quad (49)$$

by the following relation [1,3–6]:

$$K_{SEC,i} = (1 - \rho)^m \quad (50)$$

in which m is a parameter which depends on pore shape. Hence, m is equal to 1, 2 or 3, respectively, for slit shaped pores, cylindrical pores of height much greater than their diameter, and spherical or conical pores [5,11]. It is also possible for m to assume noninteger values for more complex pore shapes.

As mentioned above, the ergodic hypothesis allows one to establish the correspondence between the kinetic and equilibrium approaches of the SEC process. Accordingly, as the total residence time in the pore space is controlled by both the ingress process (through $\bar{n}_p$) and the egress process (through $\bar{\tau}_p$), as can be seen from Eq. (9a), the SEC selectivity can be understood as arising in part from each of these processes. Thus one can write:

$$\bar{\tau}_{p,i}(\rho) = \bar{\tau}_{p,perm}(1 - \rho)^{m_p} \quad (51)$$

and

$$\bar{n}_{p,i}(\rho) = \bar{n}_{p,perm}(1 - \rho)^{m_e} \quad (52)$$

since, by combining Eqs. (7), (9a), (9c), (51) and (52):

$$K_{SEC} = (1 - \rho)^{m_e + m_p} \quad (53)$$

and, by comparing with Eq. (50), one has:

$$m = m_e + m_p \quad (54)$$

Obviously, for $\rho = 0$, the species i becomes the totally permeable species, hence the subscript *perm* appearing in the RHS terms of Eqs. (51) and (52). According to Eqs. (12a) and (12b) (HYP 4), one gets from Eq. (52):

$$\bar{n}_{0,i}(\rho) = \bar{n}_{0,perm}(1 - \rho)^{m_e} \quad (55)$$

which together with HYP2, and Eqs. (9b) and (9d), gives:

$$\bar{\tau}_{0,i}(\rho) = \bar{\tau}_{0,perm}(1 - \rho)^{-m_e} \quad (56)$$

The effective number of theoretical plates for this type of model is obtained from Eqs. (23), (44) and (45):

$$N'_i = \frac{\bar{n}_{p,i}(\rho)}{2} \quad (57)$$

and thus combining Eq. (57) with Eq. (52) one has:

$$N'_i = N'_{perm}(1 - \rho)^{m_e} \quad (58)$$

where

$$N'_{perm} = \frac{\bar{n}_{p,perm}}{2} \quad (59)$$

is the effective number of theoretical plates for the totally permeable species.

By combining Eqs. (50) and (58), one obtains:

$$N'_i = N'_{perm} K_{SEC}^{1-\alpha} \quad (60)$$

where the parameter

$$\alpha = \frac{m_p}{m_e + m_p} = \frac{m_p}{m} \tag{61}$$

expresses the relative magnitude of the size exclusion effect for the egress process (m_p) with respect to the total size exclusion effect (m). The domain here considered is: $0 \leq \alpha \leq 1$. When $\alpha = 1$, m_e is zero, i.e. there is no size exclusion effect coming from the pore ingress and the whole molecular size selectivity arises from the inside of the pore. In the opposite case, when $\alpha = 0$, the size exclusion determining factor arises solely from the pore ingress process. This last condition corresponds to the original GEC model of SEC [17]. In addition to the possibility of varying α, it must be underlined that the present model can be exploited also for different α ranges outside the (0, 1) domain. This however corresponds to different retention mechanisms, which are not considered in the present study.

The skewness and the excess are:

$$S_i = \frac{3}{2} \frac{1}{\sqrt{K_{\mathrm{SEC},i}^{1-\alpha} N'_{perm}}} \tag{62}$$

$$E_i = \frac{3}{K_{\mathrm{SEC},i}^{1-\alpha} N'_{perm}} \tag{63}$$

2.7. Two-pore GEC model

The previous GEC monopore model of SEC can be extended to include the case of columns with two types of pores of sizes d_A and d_B ($d_B > d_A$) and volume fractions:

$$p_A = \frac{V_{p,A}}{V_{p,A} + V_{p,B}} \tag{64a}$$

and

$$p_B = 1 - p_A \tag{64b}$$

where $V_{p,A}$ and $V_{p,B}$ are the total pore volumes of types A and B, respectively. A procedure similar to that previously developed and applied to the case of multiple-site adsorption chromatography [27,28] is followed here. In Appendix A an extended discussion of the two-pore model is presented. A simplified model is here considered and discussed with the aim of focusing on the relevant aspects of the problem. In this section the index p indicating the pore is omitted in the quantities n and τ since they always refer to the pore space.

The SEC mechanisms for pores of types A and B are assumed to be the same with the same values of m_e and m_p. The expressions for the size effects of the molecule i are:

$$\bar{n}_{A,i}(\rho_A) = \bar{n}_{perm} p_A (1 - \rho_A)^{m_e} \tag{65a}$$

$$\bar{n}_{B,i}(\rho_B) = \bar{n}_{perm} p_B (1 - \rho_B)^{m_e} \tag{65b}$$

$$\bar{\tau}_{A,i}(\rho_A) = \bar{\tau}_{perm} (1 - \rho_A)^{m_p} \tag{66a}$$

$$\bar{\tau}_{B,i}(\rho_B) = \bar{\tau}_{perm} (1 - \rho_B)^{m_p} \tag{66b}$$

where:

$$\rho_A = \frac{r}{d_A} \tag{67a}$$

and:

$$\rho_B = \frac{r}{d_B} \tag{67b}$$

are the pore size parameters of the molecule i referred to the d_A and d_B pore types.

The pertinent CF corresponding to Eq. (43) is:

$$\Phi_{t_p}(\omega; \rho_A, \rho_B) = \exp\left\{ \bar{n}_A(\rho_A) \left[\frac{1}{1 - \mathrm{i}\omega \bar{\tau}_A(\rho_A)} - 1 \right] + \bar{n}_B(\rho_B) \left[\frac{1}{1 - \mathrm{i}\omega \bar{\tau}_B(\rho_B)} - 1 \right] \right\} \tag{68}$$

Note that, for the sake of simplicity, in this model the pore differences are only accounted for by the size parameters ρ_A and ρ_B, in addition to the total pore volume fractions, p_A and p_B. These simplified assumptions are made in order to have a two-pore model easy to exploit and to gain an initial insight into the two-pore effect. Equations for the different chromatographic quantities obtained under the above referred hypotheses are given.

For $r < d_A < d_B$ one has:

$$K_{\mathrm{SEC},AB} = p_A (1 - \rho_A)^m + p_B (1 - \rho_B)^m = p_A K_{\mathrm{SEC},A} + p_B K_{\mathrm{SEC},B} \tag{69a}$$

For $d_A < r < d_B$:

$$K_{\mathrm{SEC},AB} = p_B K_{\mathrm{SEC},B} \tag{69b}$$

and for $r > d_B$:

$$K_{SEC,AB} = 0 \tag{69c}$$

The expression of the effective plate number is simplified if, as assumed by Carmichael [18], the mean egress times of the permeable species in pores *A* and *B* are equal (see Appendix A). In this instance, for $r < d_A < d_B$, one gets:

$$\frac{N'_{AB}}{N'_H} = \frac{K_{AB,SEC}^{\alpha+1}}{p_A(1-\rho_A)^{m(\alpha+1)} + p_B(1-\rho_B)^{m(\alpha+1)}} = \frac{K_{AB,SEC}^{\alpha+1}}{p_A K_{SEC,A}^{\alpha+1} + p_B K_{SEC,B}^{\alpha+1}} \tag{70a}$$

where N'_H is the efficiency of a homogeneous column which would provide the same K_{SEC} value for species *i*, and the same effective plate number for a permeable species, as does the two-pore column.

For $d_A < r < d_B$, one gets:

$$\frac{N'_{AB}}{N'_H} = p_B^{1-\alpha} \tag{70b}$$

The skewness for $r < d_A < d_B$ is:

$$S = \frac{3}{\sqrt{4N'_{perm}}} \frac{p_A(1-\rho_A)^{m+2m_p} + p_B(1-\rho_B)^{m+2m_p}}{\left[p_A(1-\rho_A)^{m+m_p} + p_B(1-\rho_B)^{m+m_p}\right]^{2/3}} \tag{71}$$

A corresponding expression is obtained for $d_A < r < d_B$ by noting that in this instance $K_{SEC,A} = 0$.

In order to exploit the two-pore GEC model, the following average size parameter will be employed:

$$\rho' = \frac{r}{p_A d_A + p_B d_B} \tag{72a}$$

or:

$$\frac{1}{\rho'} = \frac{p_A}{\rho_A} + \frac{p_B}{\rho_B} \tag{72b}$$

ρ' is equal to ρ when $d_A = d_B$ (see Eq. (49)).

2.8. Stochastic dispersive models of SEC

When the molecule is in the moving zone of a real SEC column, it does not travel with a constant velocity as previously assumed. Indeed, because of various physical processes, such as molecular diffusion, viscous flow nonuniformities and streamline splitting around packing particles (a process sometimes called anastomosis or eddy diffusion), the time t_0 spent by a molecule in the moving zone is a random quantity. The contribution to the total band broadening from this source is not negligible. It was reported that it can contribute up to 50% of the total band dispersion [1] and this must be accounted for. The problem of non-constant moving zone velocity was solved by the CF [25]. This last method is followed here.

The distribution of residence time t_0 in the moving zone is assumed to be Gaussian [28]:

$$f(t_0) = \frac{1}{\sigma_0\sqrt{2\pi}} \exp\left[-\frac{(t_0 - \bar{t}_0)^2}{2\sigma_0^2} \right] \tag{73}$$

where $\bar{t}_0$ and σ_0 are the mean and the standard deviation, respectively. For the sake of simplicity σ_0 is derived from the hypothetical expression of the number of theoretical plates for a species visiting only the moving zone:

$$N_0 = \left(\frac{\bar{t}_0}{\sigma_0}\right)^2 \tag{74}$$

The handling of nonconstant moving zone velocity in stochastic modelling of chromatography was discussed in Ref. [23] and it was recently applied by some of the present authors to develop the stochastic dispersive model of adsorption chromatography [28]. A similar approach is here followed and the details are reported in Appendix B.

The resulting CF of the total residence time in the column, including the moving zone dispersive effect is:

$$\Phi_{t_c}(\omega) = \exp\left\{ \bar{n}_p(\rho)\left[\frac{1}{1 - i\omega\bar{\tau}_p(\rho)} - 1 \right] + i\omega\bar{t}_0 + \frac{1}{2N_0}\left(\bar{n}_p(\rho)\left[\frac{1}{1 - i\omega\bar{\tau}_p(\rho)} - 1 \right] + i\omega\bar{t}_0 \right)^2 \right\} \tag{75}$$

Peak moments are obtained by differentiation [25]:

$$\bar{t}_c = \bar{n}_p(\rho) \times \bar{\tau}_p(\rho) + \bar{t}_0 \tag{76}$$

and

$$\sigma^2_{t_c}(\rho) = 2\bar{n}_p(\rho) \times \bar{\tau}^2_p(\rho) + \frac{\bar{t}^2_c}{N_0} \quad (77)$$

Eq. (75) represents the solution for the monopore GEC model of SEC including the dispersive effect. By following the same approach, a two-pore model or multiple-pore model including the dispersion effect can be derived. Exploiting such complex cases lies beyond the aim of the present work.

3. Discussion

3.1. Monopore model

By using the CF approach and by assuming simple expressions for the size exclusion effects (see Eqs. (51) and (52)) the GEC model of SEC has been fully developed (see Eqs. (44)–(49), (53), (57)–(63)). The general power law form of the size effects employed here is based on the geometrical–equilibrium concept of SEC [3,9,11] making the comparison of the results straightforward. With respect to the original treatment of Carmichael, a size effect was allowed for both the ingress and egress processes. Importantly, one notes that the factor 2 appearing in several variance, plate height and plate number expressions (see Eqs. (45), (48) and (57)) was missing in the Carmichael results [17]. This factor 2 is, on the other hand, correctly reported by Casassa and Tagami [10,13]. It is, in fact, the result of two independent band broadening processes, one coming from the ingress process and the other from the egress process. When the former follows a Poisson distribution and the latter an exponential one (as in the Giddings–Eyring model), each contributes a value of unity for the two terms in brackets in Eq. (40) and their sum is 2 [24].

The main result is given by Eqs. (53) and (54) where it is shown that retention is affected by both the ingress and egress processes through parameter m, whereas efficiency is only affected by the ingress process through parameter m_e (see Eq. (58)). Eq. (60) establishes the link between retention and efficiency through the critical parameter α, measuring the relative relevance of the size effect inside the pore with respect to the total size effect (see Eq. (61)). Fig. 2 reports the dependence of K_{SEC} on the size parameter ρ' for different selected values of m. The main effect of parameter m is on selectivity $|dK_{SEC}/d\rho|$: it is constant through the whole size range for $m=1$, but decreases with increasing species size for $m>1$. On the same figure we compare the present monopore model (for which K_{SEC} becomes zero at $\rho'=1$) with other expressions obtained for nonconstant pore size [1,3]. Another interesting aspect to underline is that K_{SEC} for the stochastic model is the same as that in the geometric–equilibrium model [11]. This is not surprising and it can be understood under the light of the above discussion on the key role of the ergodic hypothesis and of the expressions employed for the size effects.

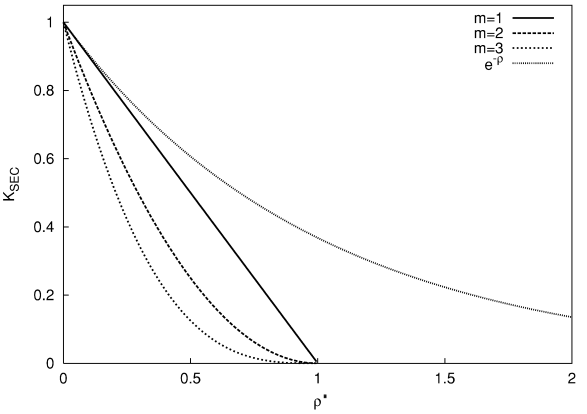

Fig. 2. K_{SEC} values vs. average size parameter value, ρ'. Comparison of monopore models at different m values with multiple-pore models, $e^{-\rho}$ [3,11].

Fig. 3 demonstrates the dependence of the effective number of theoretical plates on K_{SEC} on changing parameter α. Only positive values of this parameter are considered because this assumption corresponds to a pure exclusion mechanism. We can see that the higher the parameter α, the greater is the column efficiency. Practically, if the total size exclusion effect is dominated by the egress process, i.e. by the sizing effect inside the pore ($\alpha=1$), separation is performed over the whole K_{SEC} domain with minimum loss of efficiency, under the condition of $\rho<1$. Thus when the pore ingress process contributes to the size separation effect, this is accompanied by an efficiency loss and should be avoided if possible.

Series of chromatograms calculated with the mono-pore model are presented in Figs. 4 and 5,

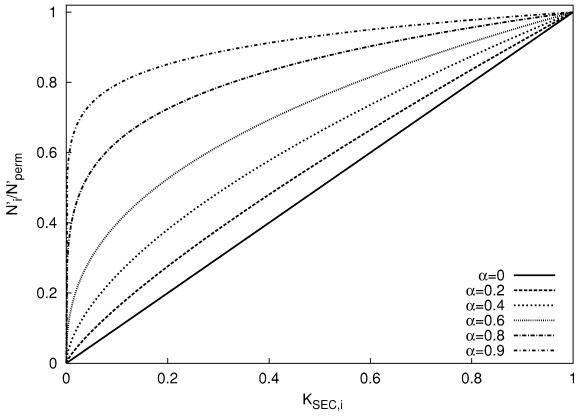

Fig. 3. Monopore model: dependence of efficiency vs. K_{SEC} for different α values.

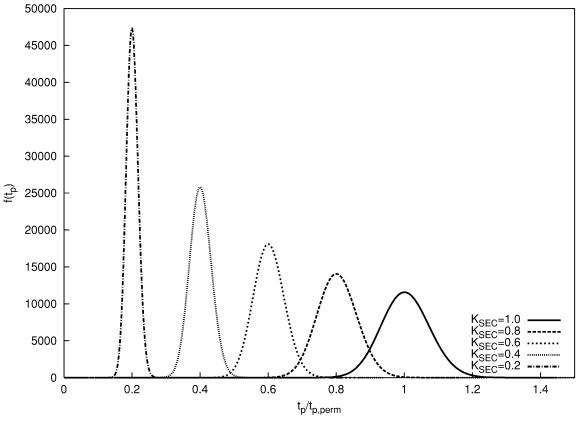

Fig. 5. Monopore model: theoretical chromatograms for different K_{SEC} values for $\alpha = 0.8$.

where the above mentioned effects can be detected. Fig. 4 corresponds to the case of $\alpha = 0$ where the rate of efficiency loss with decreasing K_{SEC} is constant (see Fig. 3). One can see in the insert of Fig. 4 that, near the exclusion limit, the peak shape becomes tailing and that peak splitting occurs just at the origin. Peak tailing is characterized by the skewness as expressed by Eq. (62), which also predicts significant tailing when K_{SEC} is close to zero. The peak splitting effect is due to the fact that some molecules leave the column without ever entering a pore. This happens when K_{SEC} is very small. It was shown that the relative amount of molecules that do not enter a pore is proportional to $e^{-2N_i'}$ (see Refs. [27,28]).

A comparison of Figs. 4 and 5 shows the effect of increasing α on column efficiency. It can be seen that a significant improvement is achieved especially for slightly retained species when α is increased from 0 to 0.8. Fig. 6 illustrates the dramatic effect of the change in α on the peak shape, for a constant K_{SEC}, near the exclusion limit. Peak splitting disappears and the peak becomes narrower higher and more symmetrical when α is increased. All these findings are particularly relevant for calibration (where usually the peak maximum rather than the

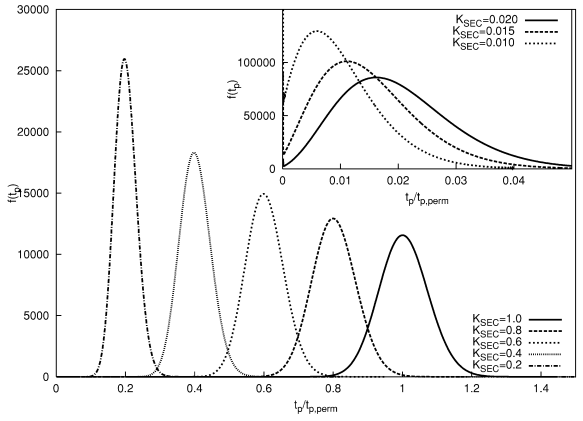

Fig. 4. Monopore model: theoretical chromatograms for different K_{SEC} values for $\alpha = 0$.

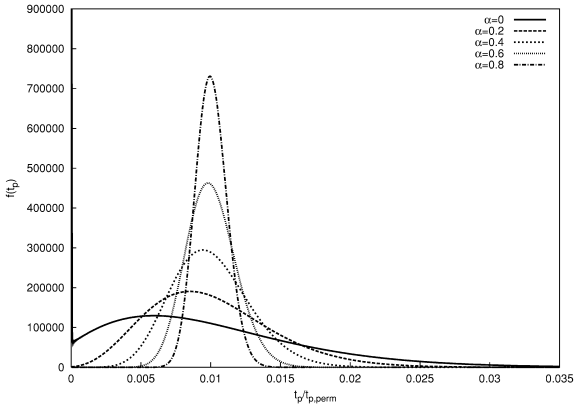

Fig. 6. Monopore model: effect of α change near to exclusion limit.

first moment is used) and in establishing a proper deconvolution method for determining the molecular mass distribution. One can see that it should be relevant to know for the different columns, not only the calibration plot of log M vs. K_{SEC}, but also N_i' and the skewness vs. K_{SEC}.

All the chromatograms reported in Figs. 4–6 as well as the other chromatograms presented in the following are obtained by numerical inversion of the CF [30]. Chromatograms of Figs. 4–6 corresponding to the GEC model could be also obtained by using the well known expression based on the Bessel Function [14]. This would require in any case special software to compute it. Numerical inversion now available from many mathematical packages is general for any CF expression and is thus the standard method for building peak shape.

Development of the GEC model is relatively easy and provides many useful insights. Now the question is how general these results are and how dependent they are on model hypotheses on the distributions of the egress time and on the number of entries. It is thus necessary to make a comparison between this model and any other general monopore model. If the same size parameter dependence is assumed for the ingress and egress pore processes, K_{SEC} will not be affected, because this quantity, for both the general model and the GEC model, derives from the same Eq. (38). A difference will instead appear in the efficiency (as well as in any higher-order peak shape parameter like S and E) if pore ingress and egress processes are different from the Poisson and exponential one, respectively. It must be observed that these hypotheses make the factors inside the brackets of Eq. (40) both equal to unity and their sum equal to 2 (see Eq. (48)), because of equalities (46) and (47). This factor appears also in Eqs. (57) and (59). Consequently a deviation from unity in either of the two terms inside the brackets will cause a difference between the GEC model and any other.

Let us first assume that the ratios inside the brackets of Eq. (40) are both different from unity and independent of size parameter. In this case, the values of each of these two terms are likely to be greater than unity since it is hard to imagine a pore ingress process less dispersed than the Poisson one or an egress process more regular than first order kinetics, corresponding to the exponential. In this instance, values of both N_i' and N_{perm}' will be affected in the same way (see Eqs. (57) and (59)). However, the ratio N_i'/N_{perm}' (see Eq. (60)) will not be affected and Fig. 3 is thus valid for any general monopore model. If, on the contrary, the terms in brackets in Eq. (40) are size dependent, these effects will appear in the column efficiency expressions. However it is a difficult task to derive the pertinent expressions by following the procedure presented here. Still, exploration of the simple GEC model will facilitate the understanding of these additional effects. Likewise the peak shape can be obtained by numerical inversion of Eq. (43) under the sole condition that the ingress and egress CF expression be known in closed form.

3.2. Two-pore model

SEC packings with bimodal pore size distributions have been proposed for their expanded calibration range and optimum performances in characterizing polydisperse polymer samples [40]. A full investigation of peak shape features by the CF approach is thus of practical relevance.

For the two-pore model, Figs. 7 and 8 illustrate the dependence of K_{SEC} on the average size parameter ρ' with $m=1$ and $m=3$, respectively. For small species, for which $r<d_A<d_B$, both pores are effective (this corresponds to the interval before the break points in Fig. 7). In this domain the absolute value of the slope is greater than for larger species that can

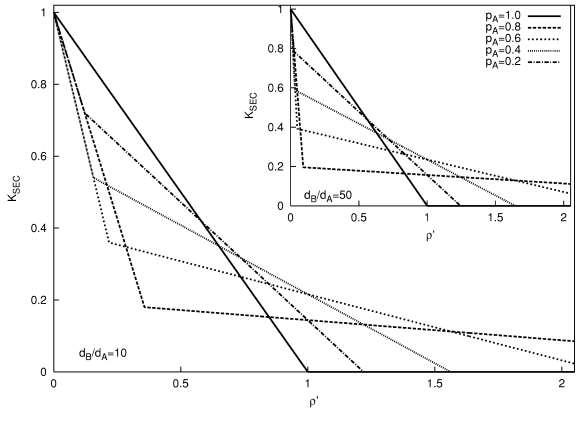

Fig. 7. Two-pore model. K_{SEC} values vs. average size parameter value, ρ', for $m=1$.

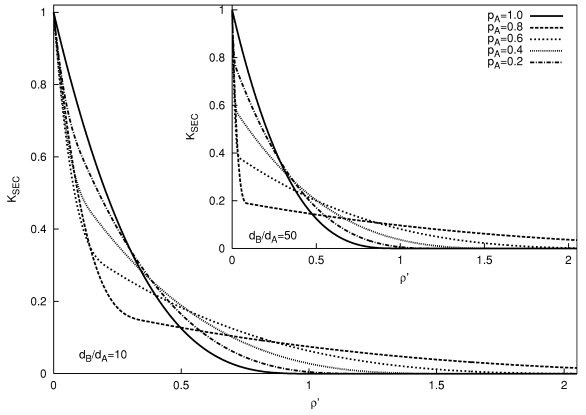

Fig. 8. Two-pore model. K_{SEC} values vs. average size parameter value, ρ', for $m=3$.

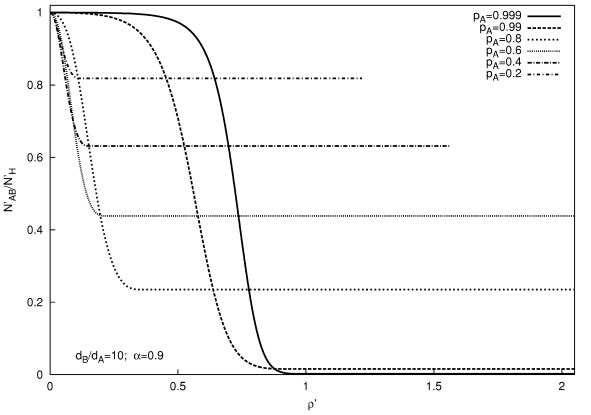

Fig. 9. Two-pore model: dependence of efficiency on ρ for $\alpha = 0.9$.

enter only the wider pores. When ρ_A is unity ($r = d_A$), there is no discontinuity in the slopes of the curves, except for $m = 1$. In this case the plots are bilinear (see Fig. 7) with a sharp slope change when $\rho_A = 1$. This can be understood with reference to Eq. (69a) and (69b) which then show a linear dependence of K_{SEC} on ρ_A and ρ_B and consequently on ρ'. The selectivity properties of the system are thus strongly affected by the pore heterogeneity, as one can see by comparing the main part and the insert in both Figs. 7 and 8 where two different pore size ratios are considered. The effective number of theoretical plates can be calculated with Eq. (70) for the two-pore model. One of the consequences of pore size heterogeneity is that efficiency is reduced compared to the monopore model when cases for the same size factor ρ are considered.

Figs. 9 and 10 report the efficiency of the two-pore column relative to that of a homogeneous monopore column providing the same K_{SEC} value for the species i and the same plate number for the permeable species, for $\alpha = 0.9$ and $\alpha = 0.1$, respectively. As the size of the species increases fewer pores are penetrated, therefore the column efficiency is reduced. This explains the drop of efficiency at the beginning of the plots. The magnitude of the drop is obviously increased when the proportion of the narrow pores increases. The plateau before the drop corresponds to a single-pore dominated mechanism (due to the small pores) whereas, on the plateau after the transition, species are totally excluded from the small pores and the mechanism becomes a single-pore one governed by the wide pores. This region is described by Eq. (70b). In addition to the influence of the relative amount of narrow pores, we can observe, by comparing Figs. 9 and 10, that the relative loss of efficiency is more significant when α is larger. However, it must be kept in mind that Figs. 9 and 10 illustrate the relative effect of the two-pore column with respect to the monopore case. If one refers to Fig. 3, one can see that the efficiency of a monopore column increases with increasing α, whatever the value of K_{SEC}, the effect being larger for larger species (lower K_{SEC} values). This tempers the influence of α on the efficiency loss noted above for

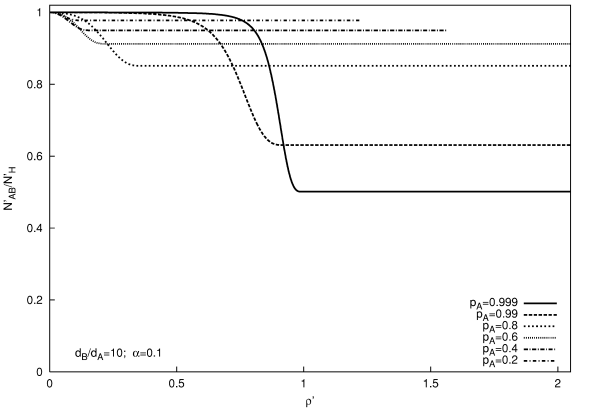

Fig. 10. Two-pore model: dependence of efficiency on ρ for $\alpha = 0.1$.

the two-pore column. In fact, it can be shown that, keeping constant the values of $K_{SEC,i}$ and N'_{perm} values for the two individual pore types (A and B), the plate number of a species i in the two-pore column increases with increasing α, i.e. by increasing the relative contribution of the egress process to the size selectivity in SEC (see Eq. (A.23) of Appendix A).

For large species both selectivity and efficiency are small, therefore a two-pore separation media will not be useful. This is illustrated in Fig. 11 where the peak splitting effect is distinct. If one compares Figs. 11 and 6, the adverse effect of pore size heterogeneity on species near the exclusion limit can be evaluated. One can see that in the homogeneous case peak splitting completely disappears at $K_{SEC} = 0.01$ and $\alpha \geq 0.2$, whereas peak splitting persists in a two-pore system even at $\alpha = 1$ for similar K_{SEC} values. Homogeneous packings are thus recommended when working near the exclusion limit.

In Fig. 12, the mechanism generating such a splitting effect is detailed. The broken lines show the hypothetical peak shapes one would obtain on homogeneous columns made of either pores *A* or pores *B*. The solid line is the peak observed with a two-pore system. The resulting peak shape is the convolution of the two former peak shapes. It must be underlined that in a two-pore column a specific peak-splitting effect appears, different from that observed in a homogeneous column. In the latter case peak splitting is related to those molecules not

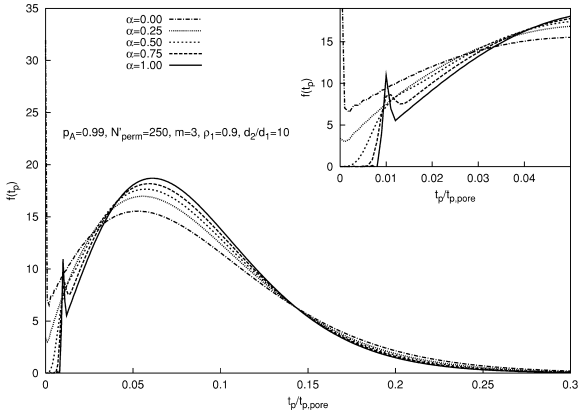

Fig. 11. Two-pore model. Theoretical chromatograms of large species illustrating the peak splitting effect at $p_A = 0.99$.

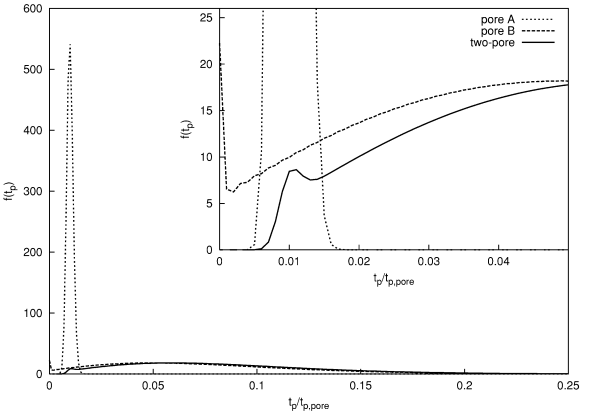

Fig. 12. Two-pore model. Theoretical chromatograms illustrating the peak splitting. The total peak in the two-pore column (full line) is the convolution of two peaks (dashed lines) from separate monopore columns. $\alpha = 0.75$.

visiting the stagnant zone. In the former case peak splitting appears as a memory effect. The ripples in Figs. 11 and 12 are the results of the numerical inverse Fourier transform. Smoothing over the numerically computed band profiles was not applied in order to avoid altering the spikes that appear because of the peak splitting effect.

Up to now hypothetical columns with two unique and distinct pore sizes have been considered. Real columns present, instead, continuous bimodal distributions of the pore dimensions [40]. This problem can be fully handled by the CF approach, as shown elsewhere in the case of the adsorption chromatography [27]; however exhaustive treatment of this problem lies beyond the aims of the present study.

3.3. Stochastic-dispersive model

Eq. (75) is the CF of the peak profile of a GEC type model of SEC including the effect of non-constant moving zone velocity. The dispersion of the time t_0 spent in the moving zone leads to a rather complex function which cannot be represented as a simple convolution of the distribution function of t_0 and t_p, as postulated by Carmichael [41]. Some molecules of a given species stay for a longer time in the moving zone, thus having the chance of performing a greater number of pore ingresses. As we can see from Eq. (76), the dispersion has no influence on

retention time but Eq. (77) shows that the variance is the sum of the variance contributions of the kinetic component and the moving-zone dispersion. In Ref. [26], a general discussion of the stochastic-dispersive model of chromatography can be found. In order to apply Eq. (75) to the SEC case, an assumption about the N_0 value appearing in the various expressions must be made. According to Giddings [21], the N_0 expression is:

$$\frac{1}{N_0} = \frac{d_{\text{part}}}{L}\left(\frac{2\gamma}{v_0} + \frac{1}{\frac{1}{a} + \frac{1}{Cv_0}}\right) \tag{78}$$

where a and C are constants, d_{part} is the packing particle diameter, γ the obstructive factor and v_0 the reduced velocity:

$$v_0 = \frac{Ld_{\text{part}}}{\bar{t}_0 D_0} \tag{79}$$

where D_0 is the diffusion coefficient of the analyte in the carrier liquid. It should be noted that Eq. (78) accounts for the contributions of both axial diffusion and eddy diffusion (also called hydrodynamic dispersion) to the moving zone dispersion. The influence of this dispersion on peak shape is specially significant in the case of low efficiencies and asymmetric peaks, as shown in Fig. 13: the peak due to peak splitting around $\bar{t}_0$ becomes more and more diffuse as the relative contribution of the moving zone to the overall band broadening increases (i.e. as N_0/N' decreases). It must be underlined that, in spite of the complexity of the CF expression (see Eq. (75)), obtaining the peak shape by numerical inversion is as straightforward as in other more simple cases.

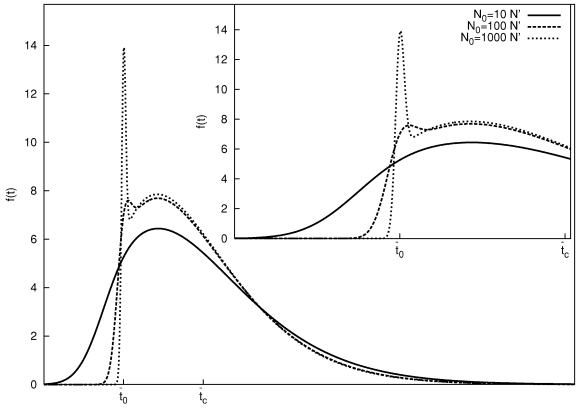

Fig. 13. Stochastic-dispersive two-pore model: theoretical chromatograms illustrating the influence of N_0 over peak splitting.

4. Conclusion

Monopore and two-pore GEC models, including the dispersive effect, are successfully solved by means of the CF. The main question is the physical foundation of the model. Comments about the Poisson character of the pore ingress process appear under the Theory section. The most important questions are about the exponential character of the egress time distribution and about the dependence of the mean egress times $\bar{\tau}_{p,i}$ or $\bar{\tau}_{p,perm}$ on size parameter ρ. One must keep in mind that the pore egress by the analyte molecules is governed by their Brownian movement and thus one must refer to this for in-depth information. The references in this field are so abundant that it is hard to grasp a synthetic overview [42]. However there is agreement that the pore residence time distribution shows tailed forms, similar to the exponential one (see the case of Ref. [37], Vol. 1, page 368). Thus, choosing such a hypothesis as the first one to analyze seems logical. To exploit different pore ingress and egress kinetics requires a separate handling. It is worthwhile to observe that Brownian processes are very often represented in either the Laplace or Fourier domain and thus that they can be directly integrated in the present CF approach.

In this study the major aspects of SEC have been considered. Several other size effects producing separations [3,4,12] are left out. However they can be handled within the stochastic description presented here by following the same procedure, either in extending the monopore model to the two-pore model or in handling the moving zone dispersive effect. Moreover, still other aspects are left out, such as the intraparticle diffusion giving rise to a H_C term contribution of type $Cd_{\text{part}}^2\bar{v}_0/D_p$ where D_p is the stagnant-phase diffusion coefficient of the species. This term is dependent on the support structure and it can be equally well handled by the CF approach [28].

5. Nomenclature

a	constant
C	constant
c	concentration
CF	characteristic function
d	pore size
d_{part}	support particle diameter
D_0	diffusion coefficient of the species in the carrier liquid
D_P	diffusion coefficient of the species in the stagnant zone
E	excess
$f()$	frequency function
GEC	Giddings–Eyring–Carmichael (model)
H	plate height
i	imaginary unit
k''	zone capacity ratio
K_{SEC}	distribution coefficient in SEC
l	length
L	column length
m_p	size factor exponent for the egress process
m_e	size factor exponent for the ingress process
m	total size factor exponent
n	number of steps
N	number of species
N	number of theoretical plates
N'	effective number of theoretical plates
p	total pore volume fraction
$P()$ or $P\{\}$	probability
r	size of species
S	skewness
SEC	size exclusion chromatography
t	residence time as stochastic variable
$\bar{t}$	mean residence time
t_R	retention time
v_0	moving zone velocity
v_{rd}	reduced velocity
V	Volume

Symbol

$\vert$	condition

Superscripts

–	mean quantity
eq	equilibrium quantity
*	convolution

Subscripts

A	A pore
AB	$A+B$ pores
B	B pore
0	moving zone
C	C term of the van Deemter Equation
c	quantity referred to the whole column (stagnant and moving zones)
excl	totally excluded species
H	homogeneous column
i	species i
p	stagnant zone
part	packing particle
perm	totally permeable species
R	retention quantity

Greek letters

α	size factor ratio
β	phase domain
γ	obstructive factor
κ_i	cumulant of order i
ρ	size factor
ρ'	average size factor
σ_t	peak standard deviation
τ_0	ingress time
τ_p	pore egress time
ω	auxiliary variable (frequency)
Φ	characteristic function

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Appendix A. Two-pore model of SEC

One considers a column made of two kinds of pores A and B, of sizes d_A and d_B. The volumes of

the moving zone and of the stagnant zone in pores *A* and *B* are V_0, $V_{p,A}$ and $V_{p,B}$, respectively. According to the ergodic hypothesis applied to a permeable species *perm* which can access the whole mobile phase volume, both in the moving zone and in the stagnant zone, one has:

$$N^{eq}_{0,perm} = c^{eq}_{0,perm} V_0 \propto \bar{t}_{0,perm} \quad (A.1)$$

$$N^{eq}_{p,A,perm} = c^{eq}_{p,A,perm} V_{p,A} \propto \bar{t}_{p,A,perm} \quad (A.2)$$

$$N^{eq}_{p,B,perm} = c^{eq}_{p,B,perm} V_{p,B} \propto \bar{t}_{p,B,perm} \quad (A.3)$$

where the subscripts 0; *p*, *A*; and *p*, *B*; refer to the moving zone, the stagnant zone of pores *A*, and the stagnant zone of pores *B*, respectively. N^{eq}_{perm}, c^{eq}_{perm}, and $\bar{t}_{perm}$ are the mean number of molecules of the permeable species at equilibrium, the equilibrium molar concentration of this species, and the mean time spent by this species in a zone, respectively. Since at equilibrium the molar concentrations of a permeable species in the various zones are equal, one obtains from Eqs. (A.1)–(A.3):

$$\frac{\bar{t}_{0,perm}}{V_0} = \frac{\bar{t}_{p,A,perm}}{V_{p,A}} = \frac{\bar{t}_{p,B,perm}}{V_{p,B}} \quad (A.4)$$

The K_{SEC} values, $K_{SEC,A,i}$ and $K_{SEC,B,i}$, of species *i* in homogeneous columns of pores *A* and *B*, are defined by (see Eq. (7)):

$$K_{SEC,A,i} = \frac{\bar{t}_{p,A,i}}{\bar{t}_{p,A,perm}} \quad (A.5)$$

and

$$K_{SEC,B,i} = \frac{\bar{t}_{p,B,i}}{\bar{t}_{p,B,perm}} \quad (A.6)$$

where $\bar{t}_{p,A,i}$ and $\bar{t}_{p,B,i}$ are the mean times spent by species *i* in stagnant zones *A* and *B*, respectively.

Defining the apparent K_{SEC} value of species *i* in the mixed column containing pores *A* and *B*, $K_{SEC,AB,i}$, as:

$$K_{SEC,AB,i} = \frac{\bar{t}_{p,AB,i}}{\bar{t}_{p,AB,perm}} \quad (A.7)$$

where $\bar{t}_{p,AB,i}$ and $\bar{t}_{p,AB,perm}$ are the mean times spent in the stagnant zones by species *i* and *perm*, respectively, one gets:

$$K_{SEC,AB,i} = \frac{\bar{t}_{p,A,i} + \bar{t}_{p,B,i}}{\bar{t}_{p,A,perm} + \bar{t}_{p,B,perm}} \quad (A.8)$$

By combining Eqs. (A.6)–(A.8), one gets:

$$K_{SEC,AB,i} = p_A K_{SEC,A,i} + p_B K_{SEC,B,i} \quad (A.9)$$

with

$$p_A = \frac{\bar{t}_{p,A,perm}}{\bar{t}_{p,A,perm} + \bar{t}_{p,B,perm}} \quad (A.10)$$

and

$$p_B = 1 - p_A \quad (A.11)$$

Using Eq (A.4) to account for the ergodic hypothesis, the weighting factor p_A can be expressed in terms of the pore volumes as:

$$p_A = \frac{V_{p,A}}{V_{p,A} + V_{p,B}} \quad (A.12)$$

The mean time spent by species *i* in one stagnant zone of a given type (*A* or *B*) is expressed in terms of its mean number of pore egresses, $\bar{n}_{p,i}$, and of its mean egress time, $\bar{\tau}_{p,i}$:

$$\bar{t}_{p,i} = \bar{n}_{p,i} \bar{\tau}_{p,i} \quad (A.13)$$

As in the monopore case, one assumes that these quantities are related to the corresponding ones for the permeable species by similar relationships (see Eqs. (51) and (52)). By employing Eqs. (50)–(52) and (61) one gets:

$$\bar{n}_{p,A,i} = \bar{n}_{p,A,perm}(1-\rho_A)^{m_e} = \bar{n}_{p,A,perm} K^{1-\alpha}_{SEC,A,i} \quad (A.14)$$

$$\bar{n}_{p,B,i} = \bar{n}_{p,B,perm}(1-\rho_B)^{m_e} = \bar{n}_{p,B,perm} K^{1-\alpha}_{SEC,i,B} \quad (A.15)$$

$$\bar{\tau}_{p,A,i} = \bar{\tau}_{p,A,perm}(1-\rho_A)^{m_p} = \bar{\tau}_{p,A,perm} K^{\alpha}_{SEC,A,i} \quad (A.16)$$

$$\bar{\tau}_{p,B,i} = \bar{\tau}_{p,B,perm}(1-\rho_B)^{m_p} = \bar{\tau}_{p,B,perm} K^{\alpha}_{SEC,B,i} \quad (A.17)$$

where ρ_A and ρ_B are the ratios of molecular size to sizes of pores *A* and *B*, respectively. α, defined by Eq. (61) reflects the relative contribution of the

egress process to the size selectivity and is assumed to be the same for pores *A* and *B* since the SEC mechanism is assumed to be the same for these two kinds of pores.

The variance arising from the stagnant zones is:

$$\sigma^2_{p,AB,i} = \sigma^2_{p,A,i} + \sigma^2_{p,B,i} = 2\left(\bar{n}_{p,A,i}\bar{\tau}^2_{p,A,i} + \bar{n}_{p,B,i}\bar{\tau}^2_{p,B,i}\right) \quad \text{(A.18)}$$

The effective plate number for species *i* is defined as:

$$N'_{AB,i} = \frac{\bar{t}^2_{p,AB}}{\sigma^2_{i,p,AB}} = \frac{\left(\bar{n}_{p,A,i}\bar{\tau}_{p,A,i} + \bar{n}_{p,B,i}\bar{\tau}_{p,B,i}\right)^2}{2\left(\bar{n}_{p,A,i}\bar{\tau}^2_{i,p,A} + \bar{n}_{p,B,i}\bar{\tau}^2_{p,B,i}\right)} \quad \text{(A.19)}$$

Combining this expression with Eqs. (A.3), (A.10), (A.11)–(A.18), one gets:

$$N'_{AB,i} = \frac{\left[p_A(1-\rho_A)^m + p_B(1-\rho_B)^m\right]^2}{2\left[\dfrac{p_A^2}{\bar{n}_{p,A,perm}}(1-\rho_A)^m + \dfrac{p_B^2}{\bar{n}_{p,B,perm}}(1-\rho_B)^m\right]} \quad \text{(A.20a)}$$

or

$$N'_{AB,i} = \frac{\left(p_A K_{\mathrm{SEC},A,i} + p_B K_{\mathrm{SEC},B,i}\right)^2}{2\left(\dfrac{p_A^2}{\bar{n}_{p,A,perm}} K^{1+\alpha}_{\mathrm{SEC},A,i} + \dfrac{p_B^2}{\bar{n}_{p,B,perm}} K^{1+\alpha}_{\mathrm{SEC},B,i}\right)} \quad \text{(A.20b)}$$

Writing

$$N'_{A,perm} = \frac{\bar{n}_{p,A,perm}}{2} \quad \text{(A.21)}$$

$$N'_{B,perm} = \frac{\bar{n}_{p,B,perm}}{2} \quad \text{(A.22)}$$

this gives:

$$N'_{AB,i} = \frac{\left(p_A K_{\mathrm{SEC},A,i} + p_B K_{\mathrm{SEC},B,i}\right)^2}{\dfrac{p_A^2}{N'_{A,perm}} K^{1+\alpha}_{\mathrm{SEC},A,i} + \dfrac{p_B^2}{N'_{B,perm}} K^{1+\alpha}_{\mathrm{SEC},B,i}} \quad \text{(A.23)}$$

One notes that, since the *AB* column is equivalent to the *A* and *B* columns connected in series, $N'_{A,perm}$ and $N'_{B,perm}$ are the effective plate numbers of the *perm* species in each of these individual columns, respectively.

Of course, when the two kinds of pores become identical ($p_B = 0$, $p_A = 1$), one retrieves the expression for the mono-pore case:

$$N'_i = N'_{perm} K^{1-\alpha}_{\mathrm{SEC},i} \quad \text{(A.24)}$$

as found in Eq. (60).

Writing $K_{\mathrm{SEC},A,i} = K_{\mathrm{SEC},B,i} = 1$ in Eq. (A.23), one obtains the effective plate number for the permeable species:

$$N'_{AB,perm} = \frac{1}{\dfrac{p_A^2}{N'_{A,perm}} + \dfrac{p_B^2}{N'_{B,perm}}} \quad \text{(A.25)}$$

When the pores *A* and *B* are identical, the ratio of the pore volume fractions, p_A and p_B becomes equal to the ratio of the lengths of columns *A* and *B* connected in series, and, hence to the ratio of their effective plate numbers for a given species:

$$\frac{p_A}{1-p_A} = \frac{N'_{A,perm}}{N'_{B,perm}} \quad (\text{pores } A = \text{pores } B) \quad \text{(A.26)}$$

It can then be verified, by combining Eqs. (A.25) and (A.26), that:

$$N'_{AB,perm} = \frac{N'_{A,perm}}{p_A} = N'_{A,perm} + N'_{B,perm} \quad (\text{pores } A = \text{pores } B) \quad \text{(A.27)}$$

as one expects for columns packed with a given material and connected in series.

One can consider a homogeneous column which would provide the same K_{SEC} value for species *i*, given by Eq. (A.9), and the same effective plate number for a permeable species, given by Eq. (A.25), as does the two-pore column. By combining Eqs. (A.24) and (A.25), the effective plate number for species *i* in the homogeneous column, $N'_{H,i}$ is given by:

$$N'_{H,i} = \frac{K^{1-\alpha}_{\mathrm{SEC},AB,i}}{\dfrac{p_A^2}{N'_{A,perm}} + \dfrac{p_B^2}{N'_{B,perm}}} \quad \text{(A.28)}$$

and, with Eq. (A.23), one gets:

$$\frac{N'_{AB,i}}{N'_{H,i}} = \frac{\dfrac{p_A^2}{N'_{A,perm}} K_{SEC,AB,i}^{1+\alpha} + \dfrac{p_B^2}{N'_{B,perm}} K_{SEC,AB,i}^{1+\alpha}}{\dfrac{p_A^2}{N'_{A,perm}} K_{SEC,A,i}^{1+\alpha} + \dfrac{p_B^2}{N'_{B,perm}} K_{SEC,B,i}^{1+\alpha}} \tag{A.29a}$$

or

$$\frac{N'_{AB,i}}{N'_{H,i}} = \frac{\left(\dfrac{p_A^2}{N'_{A,perm}} + \dfrac{p_B^2}{N'_{B,perm}}\right)(p_A K_{SEC,A,i} + p_B K_{SEC,B,i})^{1+\alpha}}{\dfrac{p_A^2}{N'_{perm,A}} K_{SEC,A,i}^{1+\alpha} + \dfrac{p_B^2}{N'_{perm,B}} K_{SEC,B,i}^{1+\alpha}} \tag{A.29b}$$

This equation is quite general for a GEC two-pore model. It shows that the ratio of the effective plate number for the two-pore column to that for the monopore column depends on the individual K_{SEC} values and on the pore volume fractions, and also on the ratio of the effective plate numbers of the permeable species for the two individual parts of the two-pore column. If further assumptions are made, the above expression can be simplified. For instance, if the mean egress times of the permeable species in pores A and B are equal ($\bar{\tau}_{p,A,perm} = \bar{\tau}_{p,B,perm}$), as assumed by Carmichael, then, from (A.21) and (A.22), one has:

$$\frac{N'_{B,perm}}{N'_{A,perm}} = \frac{\bar{n}_{p,B,perm}}{\bar{n}_{p,A,perm}} = \frac{\bar{t}_{p,B,perm}}{\bar{t}_{p,A,perm}} \quad \text{(constant } perm \text{ egress time)} \tag{A.30}$$

According to the ergodic hypothesis and Eqs. (A.4), (A.11) and (A.12), this becomes:

$$\frac{N'_{B,perm}}{N'_{A,perm}} = \frac{p_B}{p_A} \quad \text{(constant } perm \text{ egress time)} \tag{A.31}$$

Then, the effective plate number ratio can be expressed as:

$$\frac{N'_{AB,i}}{N'_{H,i}} = \frac{K_{SEC,AB,i}^{1+\alpha}}{p_A K_{SEC,A,i}^{1+\alpha} + p_B K_{SEC,B,i}^{1+\alpha}}$$

(constant *perm* egress time) (A.32)

This is Eq. (70a) of the main text.

Appendix B. Stochastic-dispersive models of SEC

The distribution of residence time in the moving zone, t_0 is assumed to be Gaussian:

$$f(t_0) = \frac{1}{\sigma_0\sqrt{2\pi}} \exp\left[-\frac{(t_0 - \bar{t}_0)^2}{2\sigma_0^2} \right] \tag{B.1}$$

where $\bar{t}_0$ and σ_0 are the mean and the standard deviation, respectively. For the sake of simplicity σ_0 is derived from the hypothetical expression of the number of theoretical plates for a species visiting only the moving zone:

$$N_0 = \left(\frac{\bar{t}_0}{\sigma_0}\right)^2 \tag{B.2}$$

It can be proved that the CF for the total residence time in the column (including thus the moving zone time) has the following general expression [21]:

$$\Phi_{t_c}(\omega) = \Phi_M\left\{\frac{\ln[\Phi_r(\omega)]}{\mathrm{i}}\right\} \tag{B.3}$$

where Φ_M is the characteristic function of the time spent in the moving zone and Φ_r is the CF of the residence time in the column on the t_0 normalized time scale, i.e. of the variable t_c/t_0. The solution of the dispersive model for the GEC case can be obtained under the hypothesis that there is a unique value of average time $\bar{\tau}_0$ spent in the moving zone between an egress step and the subsequent ingress step.

By applying CF shift and scaling transformations [33,34], one has for the GEC monopore model:

$$\Phi_r(\omega;\rho)=\exp\left\{\frac{\bar{n}_p(\rho;t_0)}{t_0}\left[\frac{1}{1-i\omega\bar{\tau}_p(\rho)}-1\right]+i\omega\right\} \quad (B.4)$$

where $\bar{n}_p(\rho;t_0)$ is the average number of egress steps for a given t_0 value. This quantity depends on t_0, but, taking into account Eqs. (9b) and (12a), its ratio with respect to t_0 can be assumed to be constant and equal to $1/\bar{\tau}_0$:

$$\Phi_r(\omega;\rho)=\exp\left\{\frac{1}{\bar{\tau}_0}\left[\frac{1}{1-i\omega\bar{\tau}_p(\rho)}-1\right]+i\omega\right\} \quad (B.5)$$

The CF of Eq. (B.1) is [34]:

$$\Phi_M(\omega)=\exp\left[i\omega\bar{t}_0-\frac{\omega^2\bar{t}_0^2}{2N_0}\right] \quad (B.6)$$

By combining Eqs. (B.3), (B.5) and (B.6) one obtains the CF of the column residence time distribution (i.e. the peak shape), including the dispersive effect:

$$\Phi_{t_c}(\omega;\rho)=\exp\left\{\bar{n}_p(\rho)\left[\frac{1}{1-i\omega\bar{\tau}_p(\rho)}-1\right]+i\omega\bar{t}_0+\frac{1}{2N_0}\left(\bar{n}_p(\rho)\left[\frac{1}{1-i\omega\bar{\tau}_p(\rho)}-1\right]+i\omega\bar{t}_0\right)^2\right\} \quad (B.7)$$

where, by considering Eqs. (9b) and (12a), the average number of sorption steps was defined as:

$$\bar{n}_p(\rho)=\frac{\bar{t}_0}{\bar{\tau}_0} \quad (B.8)$$

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