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# Theories of chromatographic efficiency applied to expanded beds☆

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**Abstract**

Various quantities such as plate height (HETP), number of plates ($N$), axial dispersion coefficient ($D_{ax}$) and Bodenstein number (Bo) are used to describe the efficiency of, and dispersion in chromatographic columns. Different quantities highlight different aspects of the performance. Due to the expansion of expanded-bed columns, the information contained in some of these quantities is not the same for expanded beds as for packed beds. In this article the mentioned quantities are described and discussed both theoretically and related to experimental data. It is concluded that they are often used in a confusing way. Quantities modified to be more informative when comparing beds of different expansions are developed ($N_{EB}=N/\text{expansion}^2$ and $\text{HETP}_{EB}=\text{HETP}\cdot\text{bed expansion}$) and recommendations of which quantity to use in what situation are given. © 2001 Published by Elsevier Science B.V.

*Keywords:* Expanded beds; Efficiency; Dispersion

## 1. Introduction

Packed beds of porous spherical beads have proven to be a very successful format for chromatography. In some applications, where the sample contains particulate material, a higher porosity of the bed is desired. A format that has been considered and often rejected is liquid–solid fluidised beds. Such contactors are frequently used in the chemical industry for various purposes, e.g., for catalysis and adsorption. Fluidised beds were considered to be unsuitable for chromatography, because of the large dispersion in the bulk liquid in the space between the matrix particles and furthermore because of the dispersion of the particles themselves.

The introduction of the expanded bed utilising beads of different sizes and densities showed that the dispersion could be kept much lower than expected [1]. As compared to packed beds the dispersion is still high, but chromatography methods employing an adsorption/desorption procedure (e.g., ion-exchange and affinity chromatography) are not that sensitive to the dispersion and have been used with excellent results [2]. This was a breakthrough in the possibilities of using chromatography, without pretreatment of samples, for primary recovery where cell debris and particles contaminate the feed.

When introducing the expanded bed technique, it is of course interesting to compare the efficiency with the conventional packed bed. It is also important to have reliable methods for the evaluation of

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the equipment and day-to-day variations in performance.

In packed bed chromatography, well-established quantities exist. The question is whether these quantities can be used for an expanded bed in the same way as for a packed bed. The problems arise when the quantities are linked to porosity, interstitial velocity, bed height or other parameters that are related to the expansion of the bed.

In this present work common quantities used to describe the chromatography process are discussed, i.e., plate number ($N$), plate height (HETP), resolution ($R_s$), Bodenstein number (Bo), particle Peclet number ($Pe_p$) and axial dispersion coefficient ($D_{ax}$). Our discussion will be based on the often-cited works of Levenspiel [3] and Giddings [4]. The quantities are applied to packed beds as well as expanded beds. To clarify the concepts, a comparison is made first on a theoretical basis and then on an experimental basis.

## 2. Theory

Important information about the performance of a column can be obtained using pulse experiments under non-binding conditions. In this article we will discuss the quantities used to describe the result of such measurements.

We use the term dispersion defined according to the chemical engineering literature, describing only the band-broadening that is due to shuffling and redistribution of the liquid in the inter-spaces between the beads. ''Band broadening'' is used more generally and could include mass transfer hindrance between the flowing mobile phase and the stagnant liquid in the pores of the matrix, and adsorption/desorption at the solid surface.

### *2.1. Band broadening in packed beds*

#### *2.1.1. The tanks in series model*

In the tanks in series model [5] the actual column is represented by a number of fictive ideally mixed tanks ($N$). Many small tanks would represent a system in which the pulse is only spread to a low degree, and a few larger tanks, a system with more spreading. In an experimental system the number of tanks is calculated from the standard deviation of the pulse as:

$$N = (t_r/\sigma_t)^2 \quad (1)$$

or

$$N = (L/\sigma_L)^2 \quad (2)$$

where $\sigma_t$ is the standard deviation of the pulse in time units, $\sigma_L$ in length units, $L$ the column height and $t_r$ the residence time.

The concentration profile derived from this model will appear symmetrical (i.e., Gaussian) if $N$ is high ($>50$), but have a more pronounced tailing at lower numbers of $N$.

For a Gaussian pulse the standard deviation can be found as, for example, the width of the pulse at half of its height, divided by 2.36. Whatever the shape is of the experimental pulse, the mean and standard deviation can be calculated from the profile using the retention-time distribution (RTD) method [3]. Other estimations, and discussions of which method is the most suitable for various purposes, are given in Refs. [6–8].

#### *2.1.2. The dispersion model*

The dispersion model has an alternative phenomenological depiction of the chromatography column. The shuffling and redistribution in the inter-spaces are described. When the concentration front moves through the column tiny fluctuations around the mean concentration arise in the front. Although this deviation is difficult to describe mechanistically it is realised that the fluctuations are statistical in nature. Therefore it is reasonable to apply the same approach as for molecular diffusion although on a macroscopic scale instead of on a molecular scale. By using an approach similar to Fick's law of diffusion, exchanging the diffusion coefficient to a dispersion coefficient ($D_{ax}$), a concentration profile can be determined [3].

To solve the relation between standard deviation and $D_{ax}$ different solutions are found for different cases. When the dispersion is low, the following apply [3]:

$$\sigma_t^2/t_r^2 = 2D_{ax}/(U_i L) \quad (3)$$

were $U_i$ is the interstitial flow-rate and $L$ the column

length. The quantity on the right-hand side is often referred to as the vessel dispersion number or the inverse of the Bodenstein number, Bo:

$$\mathrm{Bo} = U_i L / D_{ax} \quad (4)$$

The case usually applied to chromatography systems with higher dispersion (i.e., closed vessels with intermediate deviation from plug flow [3]) gives rise to the numerically acquired equation:

$$(\sigma_t / t_r)^2 = 2/\mathrm{Bo} - 2/\mathrm{Bo}^2 \cdot (1 - e^{-\mathrm{Bo}}) \quad (5)$$

However, the use of Eq. (3) would be quite acceptable in most cases. (Other approximations are given in Refs. [9,10]). For small Bo numbers the dispersion model loses its physical relevance and a few tanks in a tanks-in-series-model more easily describe the contactor (reactor, column).

*2.1.3. The interpretation of Bodenstein numbers*

The dispersion model develops into an ideal plug flow model at high Bo numbers. This means that the dispersion in the direction of flow is negligible compared to the length of the column. It also demonstrates the advantage of designing the chromatography column with a high length/diameter ratio, increasing the residence time.

When the dispersion in an expanded bed is described in terms of Bodenstein numbers, it causes some confusion due to that the increase in column length/residence time due to the expansion is not accompanied by an increase in the amount of packing. This will be discussed in Section 2.2.

*2.1.4. Chromatographic efficiency*

The ''plate theory'' as described by Martin and Synge [11] provides a way of viewing the whole band broadening and its effect on the separation power of a chromatography column. They define the height of a plate in the column as ''the thickness of the layer such that the solution issuing from it is in equilibrium with the mean concentration of solute in the non-mobile phase through out the layer''. It is very similar to the tanks in series model, but was originally only used to describe the band broadening due to slow equilibration between the moving and stagnant phases in a column. The plate number is usually denoted $N$ and calculated from the variance in the same way as the number of tanks in series (see Section 2.1.1 above). In the following we will not differentiate between the two models. In some cases we will write $N_{\mathrm{dispersion}}$ emphasising that dispersion is the only band-broadening process considered in that case.

In the ''rate theory'' the total band broadening is viewed in similar terms as the dispersion in the dispersion model. In 1956, van Deemter et al. [12] critically reviewed the theories at hand describing band broadening and the mechanisms behind it. They summarised the work in the van Deemter equation. It treats the different band broadening processes as separate additive terms, which are added to give the height of a theoretical plate (HETP = $L/N$) [12,13].

*2.1.5. Estimations*

Numerous estimations of the dispersion in chromatography columns are available. In engineering literature, the values are often presented as particle Peclet numbers ($\mathrm{Pe}_p$):

$$\mathrm{Pe}_p = U_i \cdot d_p / D_{ax} \quad (6)$$

where $d_p$ is the particle diameter.

All correlations show that the $\mathrm{Pe}_p$ number is fairly constant around 0.5–2 for a wide range of conditions in reasonably well packed columns [14,15]. Therefore rather accurate estimations can be calculated from a $\mathrm{Pe}_p = 1$. This is also in correspondence with estimations using the van Deemter equation [13,16,17].

*2.2. Band broadening in expanded beds*

*2.2.1. N and HETP as applied to expanded beds – $N_{EB}$ and $HETP_{EB}$*

In the evaluation of expanded beds the quantities developed for packed beds, described in Section 2.1, are regularly used. Thus, the plate number is calculated from the variance and column length for a pulse or step input (Eqs. (1), (2)). The variance represents the mixing in the column and the column length represents the distance in which separation can occur.

However, no separation occurs in the liquid alone. Only the interaction between sample molecules and matrix can achieve separation. In packed beds this is not a problem because the void fraction (~40%) is

normally independent of running conditions. In expanded beds the void fraction changes significantly over the range of running conditions applied. At an expansion of two to three times, as normally used, the void would be 70% and 80%, respectively.

Hence, the bed height and residence time, used as a measure of the separation distance in packed beds, are not suitable for this purpose in expanded beds. The bed height before expansion ($L_0$) would, on the other hand, be a better alternative. It is a measure of the amount of packing encountered by the analytes passing the column, regardless of the degree of expansion.

To distinguish between the plate number calculated in the conventional way from our modified expanded bed plate number we will denote the latter $N_{EB}$ as expressed in the following equation:

$$N_{EB} = (L_0/\sigma_L)^2 = (L/\sigma_L)^2/\exp^2 = N/\exp^2 \quad (7)$$

The plate height equivalent for expanded beds would in analogy be:

$$HETP_{EB} = L_0/N_{EB} = HETP \cdot \exp \quad (8)$$

where exp is the degree of expansion ($L/L_0$).

When the resolution ($R_s$) of two components, A and B, is determined this correction for ''useless void volume'' is done automatically as the difference in residence time for the two components is used instead of the absolute residence times ($R_s = [t_{rB} - t_{rA}]/2[\sigma_{tA} + \sigma_{tB}]$).

$N_{EB}$ can also be compared with the ''effective plate number'' [13,18,10] developed to compensate for a high void fraction found in some capillary columns.

### *2.2.2. Use and misuse of the plate concept*

In practice, both the zone width and separation distance is often measured in time, volume or recorder paper length units. The conversion between units is complicated by the fact that not all molecules have access to the same volume in the column, and therefore does not move with the same speed either. $N$ can be calculated using various units as long as the same unit is used for the standard deviation as for the separation distance, but the correction for bed expansion carried out in the calculation of $N_{EB}$ is not that straightforward unless the column length is used (as opposed to residence time, etc.). To avoid mistakes it is recommended to start by calculating the traditional $N$ or HETP and then correct for the expansion, or recalculate the $\sigma_t$ into $\sigma_L$ using Eqs. (1) and (2).

To describe the properties of new chromatographic packing materials, the number of plates per meter, under optimum conditions, is often given ($N/L$). Occasionally this practice has been adopted also for expanded beds, using $N/L_0$ [$= \exp \cdot N/L = L^2/(L_0\sigma^2)$] as the characteristic quantity [19–22]. This practice will certainly exaggerate the performance of the described material in a deceptive way. The ''plates'' found in the empty space between the matrix beads are counted and then assigned to the matrix. A better description is made by using $N_{EB}$, i.e., $N_{EB}/L_0$ ($= L_0/\sigma^2$).

If the degree of expansion is not taken into account in the calculation of theoretical plates, it could be considered analogous to include extensive tubing attached to a column when evaluating the performance of the column. If the tubing has a high plate number, the separation that occurs in the column will not be counteracted during transport to and from the column, but these plates do not add to the separation power.

### *2.2.3. The information communicated by the Bodenstein number for an expanded bed*

It is common to use Bo (sometimes also referred to as the column Peclet number or the axial Peclet number $Pe_{ax}$) to describe the column performance in expanded beds. The Bo is, as discussed in Sections 2.1.2 and 2.1.3, a measure of the extent of mixing, taking place in a flow system, normalised by the length of the system. As long as nothing more is read into the Bo number it applies to expanded beds as well as packed beds or any other flow system. However, the close resemblance between $N_{(disp)}$ and Bo [$N = (t_r/\sigma_t)^2 \approx 2Bo$ Eqs. (1), (3), (4) and (5)] could promote the idea to use Bo when comparing the chromatographic efficiency of different columns. As discussed above (Sections 2.2.1 and 2.2.2) this is not possible for expanded beds due to the variable degree of expansion resulting in different ratios of matrix to void volume in the column.

### 2.2.4. Axial dispersion coefficients in expanded beds

The axial dispersion coefficient ($D_{ax}$) is a measure of the mixing speed in the column (Section 2.1.2) relating the dispersion of a pulse to its residence time ($2D_{ax} = \sigma_L^2 / t_r$ [4]). The rather simple correlations used to predict $D_{ax}$ in packed beds do unfortunately not apply to expanded beds.

A number of correlations developed for fluidised beds can be found in the literature [23–25]. These correlations differ quite a lot. The experimental $Pe_p$ for fluidised beds also varies greatly with the conditions used [26].

The evaluation of how different parameters influence the dispersion in an expanded bed is complicated by the fact that flow-rate, degree of expansion, particle size, etc., are interdependent. One parameter cannot easily be changed without affecting the others. The confusion is increased by the lack of a unified strategy in the evaluation. Sometimes pore penetrating tracers are used in the evaluation of flow-properties (e.g., Refs. [27,20]) without an appropriate discussion of the influence of pore penetration on the results. When the dispersion is discussed in general terms referring to results described using different quantities or the same quantity calculated in different ways, the confusion is further increased.

In spite of the problems mentioned above, a lot of valuable information can be found in various articles dealing with the dispersion and efficiency of expanded beds. Recent work on aspects like the influence of flow distributor characteristics [28–30], the degree of bed expansion and linear velocity [27,31], particle size distribution [32], the vertical alignment of the column [33] interactions between matrix and biomass [34] and the effect of the sample load [35] are some examples.

## 3. Theoretical parameter study comparing the discussed quantities (Table 1)

To clarify how different operating conditions influence the behaviour of the quantities discussed, a theoretically based case is shown in Table 1 and illustrated in Fig. 1. One parameter is changed at a time to demonstrate its influence on each quantity. In Section 4, the numbers and conclusions drawn here will be compared with experimental results.

Table 1
Theoretically derived values of the dispersion in a packed and expanded bed column containing matrix particles of 50 μm diameter

| | $L_0$ (cm) | $L$ (cm) | $U_s$ (cm min$^{-1}$) | $D_{ax}$ (cm$^2$ min$^{-1}$) | $\epsilon$ | $t_r$ (min) | Bo | $N$ | $\sigma_L/L = \sigma_t/t_r$ | $\sigma_t$ (min) | $\sigma_L$ (cm) | $4\sigma_L\epsilon$ (cm) | HETP (cm) | $N_{EB}$ | $HETP_{EB}$ (cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *a Packed bed (increased dispersion and bed length)* | | | | | | | | | | | | | | | |
| 1 | 5 | 5 | 2 | 0.025 | 0.4 | 1.0 | 1000 | 500 | 0.04 | 0.04 | 0.22 | 0.36 | 0.01 | (500) | (0.01) |
| 2 | 5 | 5 | 2 | 1 | 0.4 | 1.0 | 25 | 13 | 0.28 | 0.28 | 1.4 | 2.2 | 0.39 | (13) | (0.40) |
| 3 | 10 | 10 | 2 | 1 | 0.4 | 2.0 | 50 | 25 | 0.20 | 0.40 | 2.0 | 3.2 | 0.39 | (25) | (0.40) |
| *a:1 Expanded bed (moderate dispersion)* | | | | | | | | | | | | | | | |
| 4:1 | 5 | 10 | 3.5 | 1 | 0.7 | 2.0 | 50 | 25 | 0.20 | 0.40 | 2.0 | 5.5 | 0.39 | 6 | 0.78 |
| 5:1 | 5 | 20 | 8.5 | 2 | 0.85 | 2.0 | 100 | 50 | 0.14 | 0.28 | 2.8 | 9.6 | 0.40 | 3 | 1.6 |
| *a:2 Expanded bed (higher dispersion)* | | | | | | | | | | | | | | | |
| 4:2 | 5 | 10 | 3.5 | 1.2 | 0.7 | 2.0 | 42 | 21 | 0.22 | 0.44 | 2.2 | 6.1 | 0.47 | 5 | 1.0 |
| 5:2 | 5 | 20 | 8.5 | 3 | 0.85 | 2.0 | 67 | 34 | 0.18 | 0.35 | 3.4 | 11.7 | 0.60 | 2 | 2.4 |
| *a:3 Expanded bed (lower dispersion)* | | | | | | | | | | | | | | | |
| 4:3 | 5 | 10 | 3.5 | 0.8 | 0.7 | 2.0 | 63 | 32 | 0.18 | 0.36 | 1.8 | 5.0 | 0.32 | 8 | 0.64 |
| 5:3 | 5 | 20 | 8.5 | 1.4 | 0.85 | 2.0 | 143 | 72 | 0.12 | 0.24 | 2.4 | 8.0 | 0.28 | 4 | 1.1 |

In the first row values that could be expected to be found for a well-packed bed are used. In each of the following rows one parameter is changed in order to demonstrate its effect this on the column performance. For further details see Appendix A.1.1 and the discussion in Section 3.

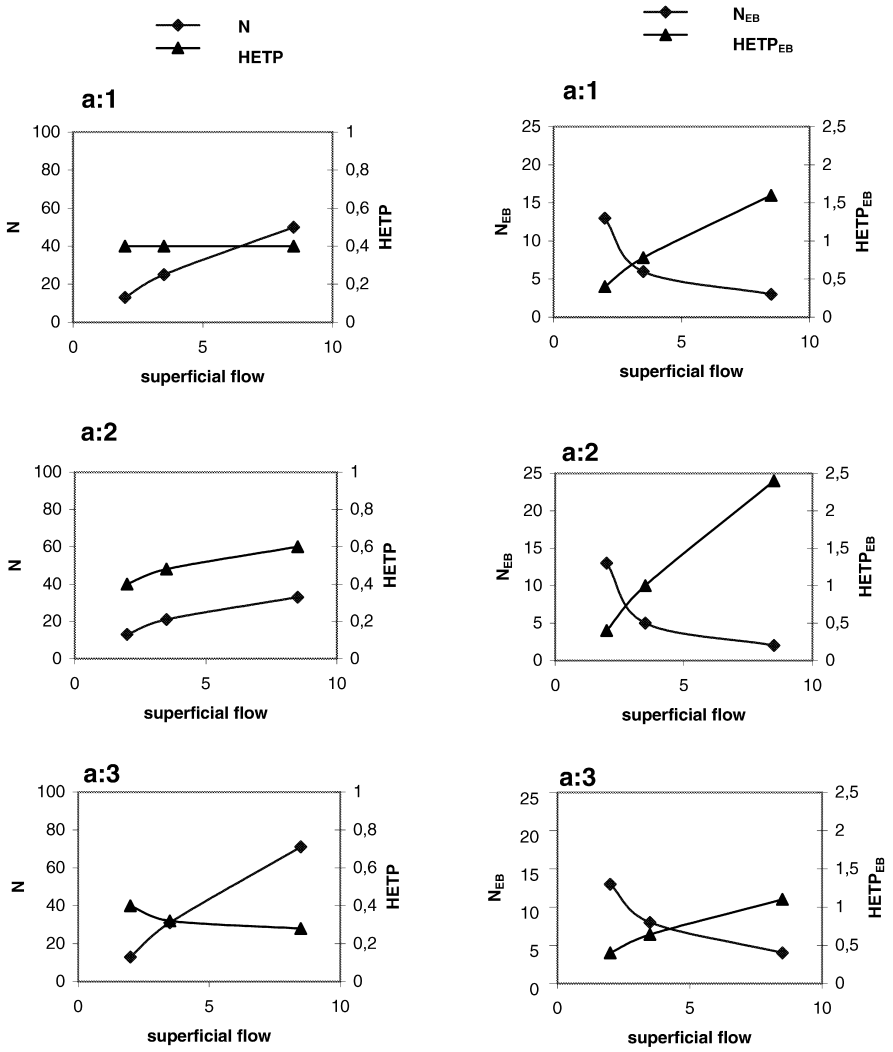


Fig. 1. *N*, HETP, $N_{EB}$ and $HETP_{EB}$ as a function of flow when $D_{ax}$ increases proportionally to the flow (a:1), less than proportionally (a:2), and more than proportionally (a:3). All data refer to the theoretical column a in Table 1. All cases starts with a packed bed mode with a high dispersion (a2, flow = 2 cm $min^{-1}$) followed by expanded bed mode at higher flow-rates. An excluded tracer is assumed. HETP values are given in cm and flow-rates in cm $min^{-1}$. For more details of how the values are derived see Appendix A.1.1.

### *3.1. Packed bed with increasing dispersion – theoretical (lines a1, a2)*

As a starting point (Table 1, line a1) for the comparison we use a packed bed with 50 μm Ø matrix particles and a height of 5 cm with a reasonable flow velocity of 2 cm $min^{-1}$ containing a non-adsorbing solute which does not penetrate into the gel particles. From Eq. (6) we can estimate the dispersion coefficient for the solute to be 0.025 $cm^2$ $min^{-1}$ (for $Pe_p = 1$). All the other parameters are also calculated as described in Appendix A.1.1.

The very high Bo number indicates a very small dispersion in terms of relative standard deviation in the column, which is to be expected.

To see the influence of a dispersive flow, the dispersion coefficient in line a1 is increased 40 times (line a2). Though totally unrealistic for a well-packed bed, this gives a reasonable value for the dispersion coefficient in an expanded bed, which are

regularly found to be in the range 10–100 times larger than in packed beds. Not to confuse the effect of the increased dispersion the bed is still considered not to be expanded.

As expected, the Bo and $N$ values decreases and the resulting peak-width [$4\sigma_{(L)}\epsilon$ = the volumetric peak width normalised by the cross-section area of the column] and HETP increases, as a result of the increased (relative and absolute) dispersion.

When $D_{ax}$, as in line a1, is 0.025 (Bo = 1000) the axial dispersion is negligible, whereas a Bo of 25 as in line a2, indicates a strong dispersion. The HETP should ideally be only a few particle diameters in length for packed beds at the optimal flow-rate, which can be compared to 2 and 80 particle diameters, respectively here.

### *3.2. Packed bed with increased length – theoretical (lines a2, a3)*

Here we compare two packed beds (with a high dispersion coefficient) lines a2 and a3, where the second bed has a doubled length. The calculation of the parameters is done as before. When the bed length is increased it results in an increased Bo number though the dispersion coefficient is kept the same. The increased Bo number reflects the change in the column proportions – a higher ratio of height to width – indicating that the volume contained in the column is localised in a more plug-flow promoting way. Thus, the *relative* standard deviation decreases although the *absolute* standard deviation increases.

The elongation of the packed bed does not affect the HETP, but $N$ is doubled. Thus, it is more efficient with a longer column. The resulting peak-width [i.e., $4\sigma_{(L)}\epsilon$] increases showing that even if the column is more efficient, the total dilution of the pulse always increases when the column is lengthened.

### *3.3. Bed expansion – theoretical (lines a2, a4, a5)*

In this case we again use the packed bed with a high dispersion coefficient (line a2) as the base case. This bed is expanded at two levels: to a double height (line a4) and to a height four times that of the packed base case bed (line a5) and all the time keeping the amount of packing material constant. The degree of expansion is dependent on the flow-rate. Let us assume that the density of the matrix is such that the first expansion (two times the packed bed height) takes place when the flow adapter is raised and the flow-rate increased enough to keep the interstitial flow-rate the same as in the packed bed. According to the Richardsson and Zaki equation [36], it is reasonable to assume that the interstitial flow-rate have to be approximately doubled in order to expand the bed further to four times the packed bed height, i.e., $U_i$ is increased from 5 to 10 cm $min^{-1}$.

The experimental data, discussed later in this article (Table 2), show that $D_{ax}$ increases approximately proportional to the interstitial velocity. We will therefore give three examples, where the dispersion is increased proportionally, less than proportionally and more than proportionally to the interstitial velocity.

If $D_{ax}$ increases exactly proportional to the interstitial velocity (lines a4:1 and a5:1), all the traditional quantities describing the dispersion behave just as if it was a packed bed (line a3). Significantly, only the resulting peak-width [$4\sigma_{(L)}\epsilon$] and the quantities specially adopted for expanded beds, $N_{EB}$ and $HETP_{EB}$, indicates that the performance of this bed is quite different and inferior to that of the packed bed.

When $D_{ax}$ increases somewhat more than $U_i$ (lines a4:2 and a5:2), $HETP_{dispersion}$ increases, giving the message that the packing becomes less efficient. The Bo and $N_{dispersion}$ also increases giving the impression that the column became more efficient, which is unreasonable bearing in mind that both the dispersion coefficient and the resulting peak-width increased and the amount of matrix encountered by the analytes on their way through the column was unchanged. This contradiction points out that the contribution from pure dispersion to the (in)efficiency of the column is not described satisfactorily by these numbers.

The new parameters $N_{EB}$ and $HETP_{EB}$, on the other hand, are both conveying that the column and packing is less efficient for separations when the expansion is increased ($N_{EB}$ decreases and $HETP_{EB}$ decreases).

In lines a4:3 and a5:3, the dispersion coefficient was assumed to increase less than the interstitial flow-rate. Here the HETP is reduced with increasing

Table 2
Experimentally determined dispersion data from Refs. [37,38] using three different columns in packed (row b1 and c1) or expanded mode (all the others)

| | $L_0$ (cm) | $L$ (cm) | $U_s$ (cm min$^{-1}$) | $D_{ax}$ (cm$^2$ min$^{-1}$) | $\epsilon$ | $t_r$ (min) | Bo | $N$ | $\sigma_t$ (min) | $\sigma_L$ (cm) | $4\sigma_L\epsilon$ (cm) | HETP (cm) | $N_{EB}$ | $HETP_{EB}$ (cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *b Column: 1.0 cm diameter, pellicular beads 50 μm diameter, density 4.4 g ml$^{-1}$, Ref. [37]* | | | | | | | | | | | | | | |
| 1 | 6.1 | 5.8 | 0.64 | 0.06 | 0.34 | 3.1 | 182 | 91 | 0.32 | 0.61 | 0.82 | 0.06 | (91) | (0.06) |
| 2 | 6.1 | 12.2 | 4.1 | 0.97 | 0.68 | 2.0 | 77 | 39 | 0.32 | 2.0 | 5.3 | 0.31 | 9.7 | 0.63 |
| 3 | 6.1 | 15.2 | 6.4 | 1.3 | 0.75 | 1.8 | 102 | 52 | 0.25 | 2.1 | 6.3 | 0.29 | 8.3 | 0.73 |
| 4 | 6.1 | 25.0 | 10.2 | 1.4 | 0.84 | 2.1 | 220 | 110 | 0.20 | 2.4 | 8.0 | 0.23 | 6.6 | 0.93 |
| *c Column: 1.0 cm diameter, Streamline 130 μm diameter, density 1.3 g ml$^{-1}$, Ref. [37]* | | | | | | | | | | | | | | |
| 1 | 5.9 | 5.6 | 0.64 | 0.08 | 0.40 | 3.5 | 109 | 55 | 0.47 | 0.75 | 1.2 | 0.10 | (55) | (0.10) |
| 2 | 5.9 | 11.8 | 4.4 | 1.2 | 0.71 | 1.9 | 60 | 30 | 0.35 | 2.1 | 6.1 | 0.39 | 7.6 | 0.77 |
| 3 | 5.9 | 14.5 | 6.4 | 2.2 | 0.78 | 1.8 | 53 | 27 | 0.34 | 2.8 | 8.7 | 0.53 | 4.5 | 1.3 |
| *d Column: 1.6 cm diameter, Streamline 130 μm diameter, density 1.3 g ml$^{-1}$, Ref. [38]* | | | | | | | | | | | | | | |
| *1* | *6.0* | *9.7* | *3.4* | *7.0* | *0.63* | *1.8* | *11* | *6* | *0.73* | *3.9* | *9.9* | *1.6* | *2.9* | *2.0* |
| *2* | *6.0* | *11.7* | *4.7* | *8.0* | *0.69* | *1.7* | *15* | *8* | *0.60* | *4.1* | *11.4* | *1.5* | *2.1* | *2.8* |
| *3* | *6.0* | *14.4* | *6.2* | *5.1* | *0.75* | *1.7* | *33* | *17* | *0.42* | *3.5* | *10.5* | *0.85* | *2.3* | *2.6* |

*Latex particles were used as tracers in columns b and c, and blue dextrane in column d for pulse determinations. For further details see Appendices A.1.2 and A.1.3 and the discussion in Section 4.*

bed expansion, giving the impression that the column packing is improved. Thus, comparing line a5:3 with line a4:3 both the dispersion coefficient and the resulting peak-width are increased, but the HETP value, Bo and $N$ all falsely indicate improvements.

In contrast, $N_{EB}$ and $HETP_{EB}$ both show that the efficiency in all the above cases is reduced when the column is expanded (Fig. 1). They also catch the small changes in efficiency found between the columns with the same degree of expansion, but somewhat different dispersion coefficients.

Thus, the new quantities developed for the evaluation of expanded bed columns are describing the column in a clear and simple way, whereas the traditional quantities give a confusing message much more difficult to interpret.

## 4. Comparison with experimental dispersion data (in Table 2)

In Table 2 experimental data from Refs. [37,38] are used for calculations of the quantities discussed above. Thus, all the parameters were obtained from pulse experiments and no theoretical estimations are used. Sections b and c are based on the raw data from experiments presented in Ref. [37]. In section d, the data is calculated from the information given in Ref. [38]. Standard columns (Amersham Pharmacia Biotech, Uppsala, Sweden) with modified flow distributors were used. They had inner diameters of 1.0 cm in b and c, and 1.6 cm in d. A new ''laboratory-made'' pellicular matrix with a density of approximately 4.4 g ml$^{-1}$ and an average particle diameter of 50 μm was used in column b, and Streamline protein A (Amersham Pharmacia Biotech) with an average density of 1.3 and diameter of 130 μm in columns c and d. As tracers were used 310 nm hydroxyl-modified latex particles (Seradyn, Indianapolis, IN, USA) in sections b and c and Blue Dextran 2000 (Amersham Pharmacia Biotech) in section d. The tracers were assumed not to penetrate the pores of the matrix.

The calculations were carried out in a similar way as before, starting from the experimentally determined parameters. For further details see Appendices A.1.2 and A.1.3.

### 4.1. Packed bed – experimental (lines a1, b1, c1)

In the experimental cases, b1 and c1, $D_{ax}$ was low as expected for packed beds. This was also demonstrated by the high values of Bo and $N$, and a low HETP. When $D_{ax}$, Bo, $N$, HETP and $4\sigma_L\epsilon$ for the

theoretical and real columns are compared, they all give a clear message concerning the performances of the columns, i.e., a1 had the best performance followed by b1 and finally c1.

### 4.2. Bed expansion – experimental

The behaviour of the three EB-columns studied differed in the degree of expansion and the dispersion behaviour at increased flow-rates. Some of the quantities discussed changed in the same direction (increase or decrease) for all the EB-columns with increased flow, whereas others behaved more irregularly. The performance of each EB-column is discussed separately below, and then summarised to give a general picture (Fig. 2).

#### 4.2.1. EB-column b (lines b1–b4)

When the packed bed (b1) was expanded two times (b2) the $D_{ax}$ increased considerably, more than 10 times. At further expansion $D_{ax}$ was kept fairly constant increasing just about 40% when expanded up to 4.1 times the initial height. The simultaneous increase in interstitial flow-rate was three times from packed to expanded and two times for two to four times expansion. Thus, $D_{ax}$ increased less than $U_i$ after the initial expansion (as in the theoretical case lines a4:2 and a5:2 discussed in Section 3.3).

Again, Bo and $N$ increased at increased expansion as discussed earlier (a2, a4, a5) in response to the changed column proportions. HETP decreased due to the almost constant dispersion coefficient, and the resulting peak-width ($4\sigma_L\epsilon$) increased, as always when the bed length is increased. Thus, the traditional quantities falsely convey the message that the performance improved with flow.

In contrast, $N_{EB}$ and $HETP_{EB}$ tells us that the efficiency of the bed for separation purposes decreased markedly when the packed bed was expanded and then slightly more as the expansion was increased.

#### 4.2.2. EB-column c (lines c1–c3)

The larger particle size used in this column resulted in an increased band broadening for the packed bed. When the degree of expansion was increased from 2.0 to 2.5 times, the dispersion coefficient increased more than the $U_i$. Thus, HETP increased with increased expansion (as in the theoretical case lines a4:3 and a5:3 discussed in Section 3.3). The increase in $D_{ax}$ was so large that in spite of the longer column, $N$ and Bo were reduced. Thus, in this case the traditional quantities give at least a correct trend of the performance at increased expansion. $N_{EB}$ and $HETP_{EB}$ decreased markedly and much more clearly inform about the lowered separating ability of the more expanded column.

#### 4.2.3. EB-column d (lines d1–d3)

In the experimental case d, the dispersion was higher than in the previous cases and had a maximum at a void of 0.7 (two times expansion). This is a phenomenon reported for some fluidised beds as well as for some (classified) expanded beds [25,26]. Thus, the dispersion coefficient and peak-width first increased and then decreased as the expansion was increased. The simultaneous lengthening of the column resulted in increased values of Bo an $N$ indicating a reduced relative dispersion. The increase in $D_{ax}$ from line 1 to 2 was smaller than the increase in $U_i$ resulting in a reduced HETP throughout the expansion, implying an improved ‘‘packing’’. Thus, most of these parameters falsely indicate improvements at increased degree of expansion.

If we turn to $N_{EB}$ and $HETP_{EB}$ they are giving a very different message, i.e., that the efficiency of the column was, as in the other cases, highest when the smallest expansion (two times) was applied, but the column was more efficient at four times expansion than at 2.5.

#### 4.2.4. Summarising the results on bed expansion

The behaviour of an expanded bed, in regards to dispersion, is much more complex than the behaviour of a packed bed. The quantities describing the relative dispersion normalised using the expanded column height vary in relation to each other in a way that is not possible for packed beds (Fig. 1, 2), i.e., both N and HETP can increase simultaneously.

When an EB-chromatography column with high separation efficiency is sought, the modified quantities $N_{EB}$ and $HETP_{EB}$ can be used to identify the best choice of column and flow-rate. None of the traditional quantities $D_{ax}$, $N$, HETP and Bo give this information.

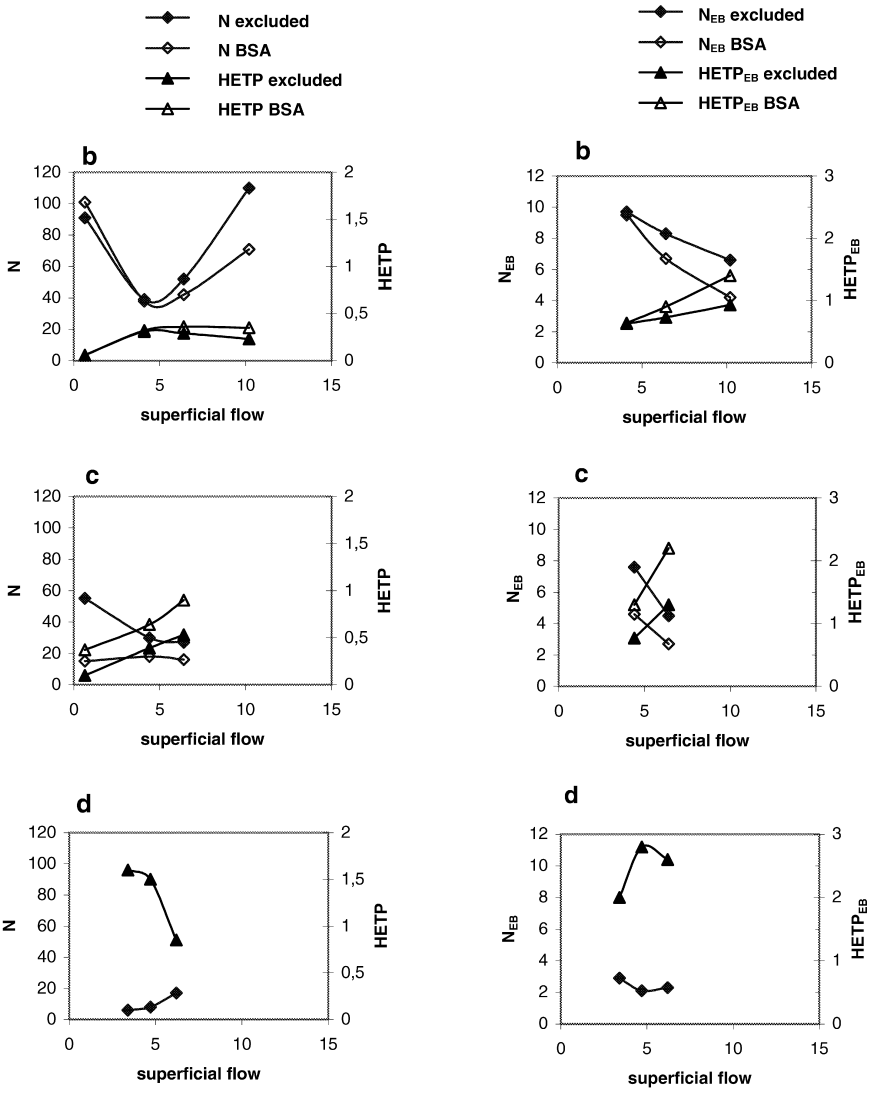


Fig. 2. $N$, HETP, $N_{EB}$ and $HETP_{EB}$ for the real columns b, c and d as a function of flow using the values given in Table 2, derived from pulse experiments with excluded tracers (latex particles or blue dextrane) and the pore penetrating tracer BSA. With columns b and c the first values were obtained with the column in a packed bed mode (flow = 0.64 cm $min^{-1}$). These values are not included in the $N_{EB}$ and $HETP_{EB}$ diagrams. HETP values are given in cm and flow-rates in cm $min^{-1}$. The columns and matrices are further described in Section 4 and in Appendices A.1.2 and A.1.3.

$D_{ax}$ gives a clear message all through the study, informing about the flow properties in the columns.

## 5. The band-broadening of pore-penetrating solutes (Table 3)

The band broadening of a pore-penetration solute is of course greater than for the excluded molecules discussed above, due to slow diffusion in the matrix pores. This additional band broadening increases proportionally to the interstitial velocity, according to the van Deemter equation, but is not affected when the bed is expanded (if the interstitial velocity is not changed). There is an additional mass transfer hindrance in the stagnant film surrounding the beads.

The film mass transfer resistance decreases when the interstitial velocity increases.

According to engineering literature, $D_{ax}$ and Bo should only be used to describe the dispersion, not the total band broadening of pore-penetrating solutes. Sometimes, small molecules, like acetone, are used in dispersion measurements though. Even if such molecules diffuse fast in most matrices, it will effect the result of the measurement (giving a longer residence time and a higher volumetric variance). A clear distinction of what tracer substance has been used should thus be made.

The efficiency of a column will also differ depending on which substances measured on. In Table 3 bovine serum albumin (BSA) was used as tracer molecule for measurements carried out on the columns denoted b and c above. As the substances to be purified often are rather large, but with access to most of the porous part of the column, a tracer of a similar size will give valuable additional information compared to the measurements using excluded tracers.

### 5.1. Comparison of the band-broadening of BSA and excluded latex particles (Table 3)

By comparing the dispersion of an excluded tracer with that of a pore-penetrating molecule of the same size as the substance to be adsorbed, the relative importance of the dispersion as compared to the pore diffusion was determined. In the columns b and c, the dispersion was not negligible in comparison with the pore diffusion. A reduced dispersion would thus improve the efficiency of the column.

#### 5.1.1. Column b

This column contained a new pellicular matrix with very short diffusion distances. Thus, at low flow-rates (lines b1 and b2 in Table 3 and Fig. 2), the additional band-broadening due to slow pore-diffusion was very small as seen in the rather unchanged values of HETP and $HETP_{EB}$ for BSA as compared to latex particles. The higher values of $N$ and $N_{EB}$ in the packed bed (line b1) for BSA than latex are theoretically impossible. At the low flow-rate used with the packed beds the peaks were rather asymmetric, increasing the error in the measurement. (Already a rather small error in the standard deviation will be noticeable in the plate number). As the flow-rate was increased and the bed expanded further the band broadening obtained for BSA increased more than for the latex particles, due to the increasing effect of the slow pore diffusion.

#### 5.1.2. Column c

The Streamline particles used in this column were larger than the particles of the pellicular matrix above, and also porous all through the bead. The diffusion distance was therefore much longer in this case, which showed up as an increasing band

Table 3
Experimentally determined values for band broadening in packed and expanded beds from Ref. [37] using BSA as tracer in pulse determinations

| | $L_0$ (cm) | $L$ (cm) | $U_s$ (cm min$^{-1}$) | $\epsilon$ | BSA | | | | | | | | Latex | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $t_r$ (min) | $\sigma_t$ (min) | $\sigma_L$ (cm) | $N$ | $4\sigma_L\epsilon_{v+p}$ (cm) | HETP (cm) | $N_{EB}$ | $HETP_{EB}$ (cm) | $N$ | HETP (cm) | $N_{EB}$ | $HETP_{EB}$ (cm) |
| *b Pellicular beads 50 μm diameter, density 4.4 g ml$^{-1}$, Ref. [37]* | | | | | | | | | | | | | | | | |
| 1 | 6.1 | 5.8 | 0.64 | 0.34 | 5.3 | 0.53 | 0.58 | 101 | 1.4 | 0.06 | (101) | (0.06) | 91 | 0.06 | (91) | (0.06) |
| 2 | 6.1 | 12.2 | 4.1 | 0.68 | 2.3 | 0.37 | 2.0 | 38 | 6.2 | 0.32 | 9.5 | 0.64 | 39 | 0.31 | 9.7 | 0.63 |
| 3 | 6.1 | 15.2 | 6.4 | 0.75 | 2.1 | 0.32 | 2.3 | 42 | 8.1 | 0.36 | 6.7 | 0.90 | 52 | 0.29 | 8.3 | 0.73 |
| 4 | 6.1 | 25.0 | 10.2 | 0.84 | 2.2 | 0.26 | 3.0 | 71 | 10.5 | 0.35 | 4.2 | 1.4 | 110 | 0.23 | 6.6 | 0.93 |
| *c Streamline 130 μm diameter, density 1.3 g ml$^{-1}$, Ref. [37]* | | | | | | | | | | | | | | | | |
| *1* | *5.9* | *5.6* | *0.64* | *0.40* | *7.6* | *1.9* | *1.4* | *15* | *4.9* | *0.37* | *(15)* | *(0.37)* | *55* | *0.10* | *(55)* | *(0.10)* |
| *2* | *5.9* | *11.8* | *4.4* | *0.71* | *2.3* | *0.54* | *2.7* | *18* | *9.5* | *0.64* | *4.6* | *1.3* | *30* | *0.39* | *7.6* | *0.77* |
| *3* | *5.9* | *14.5* | *6.4* | *0.78* | *2.1* | *0.52* | *3.6* | *16* | *13.1* | *0.90* | *2.7* | *2.2* | *27* | *0.53* | *4.5* | *1.3* |

*$\epsilon_{v+p}$ is the cross section area accessible to the tracer. Some dispersion data from Table 2 are included for comparisons. For further details see Appendix A.1.4 and the discussion in Section 5.*

broadening for BSA already at the low flow-rate used with the packed bed. This higher porosity also resulted in more retention of the tracer.

## 6. Conclusion

Expanded beds behave differently from packed beds in that the column void changes when the flow-rate, viscosity or density of the mobile phase is changed. This variation in column void (and volume) affects the dispersion and residence time in the column.

The intensity of local mixing can be expressed as an axial dispersion coefficient, $D_{ax}$, i.e., this tells something about the flow quality. When $D_{ax}$ is measured over the whole column, it gives an average of the flow quality throughout the column.

In the design of new flow distributors or matrices $D_{ax}$ gives the very basic information whether this design promotes a low-dispersion liquid-flow through the bed, compared to existing solutions, or not.

When a chromatographic system for expanded bed isolation is set up, the overall mixing in the system could be measured and stated as the Bodenstein number of the system. Doing this, it will be revealed whether the flow through the system can be viewed as having a plug flow as compared to being a mixed vessel. It does not give any precise information about the efficiency of the column.

The efficiency of a chromatography column is often expressed through the plate number, *N*, or plate height, HETP. These two numbers are obviously closely related when the total volume in the column is constant. For expanded beds the degree of bed expansion, increasing the volume contained in the column, has to be compensated for to preserve the meaning of these quantities, i.e., it is suggested that N should be substituted for $N_{EB}$ ($N_{EB} = N/\exp^2$) and HETP substituted for $HETP_{EB}$ ($HETP_{EB} = HETP \cdot \exp$). Only then they will continue to be related to each other and to the dilution of the sample in a meaningful way.

Thus, $N_{EB}$ and $HETP_{EB}$ are suitable quantities to be used to state the efficiency of expanded bed chromatography columns.

## 7. Nomenclature

| | |
|---|---|
| Bo | Bodenstein number |
| $D_{ax}$ | Axial dispersion coefficient |
| $d_p$ | Particle diameter |
| exp | Bed expansion ($L/L_0$) |
| HETP | Height equivalent to a theoretical plate |
| $HETP_{EB}$ | HETP modified for expanded beds |
| $L_0$ | Column height before expansion |
| $L$ | Expanded column height |
| $N$ | Plate number, number of tanks |
| $N_{EB}$ | Plate number modified for expanded beds |
| $Pe_p$ | Particle Peclet number |
| $R_s$ | Resolution |
| $t_r$ | Residence time |
| $U_i$ | Interstitial liner flow-rate |
| $U_s$ | Superficial linear flow-rate |
| $\epsilon$ | Column void |
| $\epsilon_0$ | Column void before expansion |
| $\sigma_L$ | Standard deviation in length units as measured in the column |
| $\sigma_t$ | Standard deviation in time units |

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## Appendix A

### *A.1. The derivation of the values given in Tables 1–3*

#### *A.1.1. Table 1, theoretical values*

The values contained in Table 1 were derived as follows:

$L_0$, $L$ and $U_s$ were chosen in order to demonstrate the changes occurring in the quantities often used for evaluating chromatography columns. The imagined matrix has a bead size of 50 μm in diameter and a void of 0.4 when packed in the column.

The $D_{ax}$ in line a1 was calculated using the equation:

$$D_{ax} = U_s \cdot d_p / \epsilon \cdot Pe_p \quad (A.1)$$

assuming a $Pe_p$ of 1. From line 2 and onwards higher values were used. They were chosen to be in the range often found for expanded beds at the flow-rates in question. In two cases the higher value is thus applied in the packed bed. This was done to simplify the comparison with expanded beds, showing the effect of an increased $D_{ax}$, column height and $\epsilon$ one by one.

The remaining quantities were calculated using the equations given below:

$$\epsilon = 1 - [L_0(1 - \epsilon_0)/L] \quad \text{(A.2)}$$

the residence time:

$$t_r = L \cdot \epsilon / U_s \quad \text{(A.3)}$$

Bo is given as:

$$\text{Bo} = L \cdot U_s / \epsilon \cdot D_{ax} \quad \text{(A.4)}$$

$$N = (\text{Bo} + 1)/2 \quad \text{(A.5)}$$

From $N$ and $t_r$, $\sigma_t$ and $\sigma_L$ are calculated as:

$$\sigma_t = t_r/(N) \times {}^{1/2} \quad \text{(A.6)}$$

$$\sigma_L = \sigma_t \cdot U_s / \epsilon \quad \text{(A.7)}$$

The HETP is then:

$$\text{HETP} = L/N \quad \text{(A.8)}$$

The new quantities adjusted for bed expansion were calculated as follows:

$$N_{EB} = (t_r/(\sigma_t \cdot \exp))^2 \quad \text{(A.9)}$$

$$\text{HETP}_{EB} = L_0/N_{EB} \quad \text{(A.10)}$$

*A.1.2. Table 2, columns b and c*

In Table 2 examples are given of experimental results obtained in expanded beds. Sections b and c contains values from [37] (raw data not included in the article was used for the calculations). They were derived as follows:

$L_0$ and $L$ were measured with a ruler, $U_s$ was calculated from the volumetric flow given on the pump. (The column was 1.0 cm ∅):

$$U = U_{vol}/\text{cross section area of column} \quad \text{(A.11)}$$

$\sigma_t$ and $t_r$ were retrieved from RTD calculations with corrections for extra-column effects.

The porosity $\epsilon$ was calculated as:

$$\epsilon = t_r \cdot U_s / L \quad \text{(A.12)}$$

$N$ and Bo were calculated as:

$$N = (t_r/\sigma_t)^2 \quad \text{(A.13)}$$

$$\text{Bo} = 2N - 1 \quad \text{(A.14)}$$

$D_{ax}$ was then calculated as:

$$D_{ax} = L \cdot U_s/(\epsilon \text{Bo}) \quad \text{(A.15)}$$

the standard deviation and resulting peak-width were calculated as:

$$\sigma_L = \sigma_t \cdot U_s / \epsilon \quad \text{(A.16)}$$

$$4\sigma_L \epsilon = 4\sigma_t U_s \quad \text{(A.17)}$$

HETP, $N_{EB}$ and $\text{HETP}_{EB}$ were calculated as above using Eqs. (A.8), (A.9) and (A.10).

*A.1.3. Table 2, column d*

The values in section d of Table 2 are from Ref. [38]. (In this case only information presented in the article was used). The column diameter was 1.6 cm and the matrix volume 12 ml. Blue Dextrane 2000 was used as tracer when the dispersion was measured.

$L_0$ was calculated as:

$$L_0 = \text{matrix volume}/\text{column cross section area} \quad \text{(A.18)}$$

Bo and $\epsilon_0$ (0.4) were given in the article, $\epsilon$ was calculated from:

$$U = U_t \epsilon^n \quad \text{(A.19)}$$

where $U_t$ is the terminal fluidisation velocity given as 0.165 m $\text{min}^{-1}$ and $n$, the Richardson–Zaki exponent given as 3.4 for a column expanded in water.

$D_{ax}$ was calculated using the superficial flow in Ref. [38] and was thus recalculated by division with $\epsilon$ here, to obtain values comparable with the others in the table.

$L$ was calculated from Eq. (A.2)

$U_s$ was given, $N$ was calculated from Bo using Eq. (A.14), $\sigma_L$ and $\sigma_t$ from:

$$\sigma_L = L/N^{1/2} \quad \text{(A.20)}$$

$$\sigma_t = \sigma_L \cdot \epsilon/U_s \quad \text{(A.21)}$$

$t_r$ was calculated from:

$$t_r = \sigma_t N^{1/2} \quad \text{(A.22)}$$

HETP, $N_{EB}$ and $HETP_{EB}$ were calculated from Eqs. (A.8), (A.9) and (A.10).

*A.1.4. Table 3, columns b and c*

The values in Table 3 using BSA as a tracer were obtained using the same columns as in sections b and c in Table 2. The $\epsilon$ values given in this table was obtained from the latex measurements. $t_r$ and $\sigma_t$ were retrieved from RTD measurements and $4\sigma_L\epsilon_{v+p}$, the resulting peak width, as:

$$4\sigma_L\epsilon_{v+p} = 4\sigma_t U_s \quad \text{(A.23)}$$

$\epsilon_{v+p}$ being the column fraction (between and inside the beads) accessible to BSA.

$N$, HETP, $N_{EB}$ and $HETP_{EB}$ were calculated using Eqs. (A.13), (A.8), (A.9) and (A.10).

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