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Review

Some reflections on speed and efficiency of modern chromatographic methods

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Abstract

The development of high-performance liquid chromatography in the years 1965–1985 was undeniably a success story. Nevertheless, during those years a painstaking limitation of the technique, noticed by some and ignored by other workers in the field, remained: the resolving power accessible in a reasonable analysis time is poor, at least in comparison to capillary gas chromatography. The latter technique went through a delayed, but comparable success story during the same years. The limitation is well described in terms of the Knox concept of separation impedance; the available pressure limitation determines that the analysis time increases in proportion to the square of the plate number, i.e., to the fourth power of the resolution aimed at. By the introduction of a range of miniaturized liquid phase fractionation techniques, exploiting either classical pressure or electrical propulsion, a new era appears to have begun. Since then efficiencies similar to those in gas chromatography are accessible. The price to pay is the miniaturization needed. This leads to an aggravation of the detection problems. In this contribution an attempt is made to compare various techniques in terms of attainable efficiency and speed as well as of the demands on the detectors. Some speculations on future proliferation will be given. © 1997 Elsevier Science B.V.

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1. Introduction

Resolving power is what it is all about in analytical separation science. Traditionally, it is measured in terms of the theoretical plate number, N, defined as:

$$N = \left(\frac{t_R}{\sigma_t}\right)^2 \quad (1)$$

where t_R is the residence time in the column and σ_t is the standard deviation of the peak observed. We note that from the operational point of view, this is a somewhat awkward measure, as the resolving power is only proportional to the square root of the plate number. This holds for two sensible ways to define "resolving power": either as the maximum number of well separated peaks (peak capacity) within a time frame, or as the minimum distance between two consecutive peaks to be separated (resolution). Thus, a twice as good column in terms of plate number gives us only $\sqrt{2}$ times more peak capacity or resolution, or $\sqrt{2}$ times more information.

The definition of N as it is stems of course from the theoretical and experimental observation that, keeping everything else constant (if we can!), N increases with the length of the column used.

We note in passing that the second half of the column contributes less to the analytical performance (peak capacity, resolution) than the first one. However, it is not uncommon that more effort results in a less than proportional increase in performance: diminishing returns: driving twice as fast on the Autobahn does not bring us home in half the time; repeating analytical determinations and taking the average outcome as the end result improves the precision, but the last few determinations out of, say, twenty appear to make only little difference in what is reported.

The incentive in the development of high-performance liquid chromatography (HPLC) was to make N as large as possible while keeping the analysis time reasonable. This was done by decreasing the particle size of the particles holding some form of stationary phase. The theoretical framework within which this was done by i.a. the workers recently honoured in a special issue of this Journal [1] was variable and often not so transparent. However, in a very sketchy, incomplete and rather unconventional manner the idea can be described as follows.

Chromatography is a process of repeated equilibrations. This is most manifest when we consider the well known analogy of the chromatographic column with the Craig repeated-liquid–liquid- distribution machine. The number of equilibrations in the time the (retained) component spends in the column, t_R, in this model determines the plate number N:

$$N \approx \frac{t_R}{\tau_{eq}} \quad (2)$$

Aiming at many plates within a given time is therefore tantamount to decreasing the equilibration time, τ_{eq}, of the distribution process. The value of τ_{eq} depends of course on many factors, such as the distribution ratio, the geometry of the phases, etc. However, most important are the diffusion coefficients and the length, l, of the diffusion path. Thus, for an equilibration towards a spherical particle, of diameter l, it holds:

$$\tau_{eq} \approx \frac{1}{30}\frac{l^2}{D_p} \quad (3)$$

This expression only takes the resistance to mass transfer within the particle into account; in practice and more refined theories many more terms have to be considered. However, for the sake of illustration of the concept this suffices.

Eq. (3) (or more refined versions) could be said to describe the "TETP", the time equivalent of a theoretical plate. Indeed, when multiplied with the linear velocity of the component, u_i, one obtains (apart from a numerical factor depending on the retention) the plate height, H:

$$H \approx u_i \cdot \tau_{eq} \quad (4)$$

However, of more direct importance in the discussion of resolution versus analysis time is the fact that the plate number accessible in a time t_R is found by substitution of Eq. (3) in Eq. (1) as:

$$N \approx 30\frac{D_p}{l^2} t_R \quad (5)$$

This inaccurate but instructive equation explains a few very fundamental facts in the development of

chromatographic separations: (1) when comparing GC with LC, the vast difference in the diffusion coefficients in the two types of mobile phases (hexane in He: $3 \cdot 10^{-5}$ m^2/s, hexanol in water: $1 \cdot 10^{-9}$ m^2/s) has momentous consequences. For the same particle size in LC and GC (the situation before 1965) the speed of LC is orders of magnitude ($3 \cdot 10^4$ with the figures given) smaller than GC. (2) With given solutes and phases improvement can only be obtained by decreasing the diffusion path, i.e., the particle diameter. That, of course, was the pertinent aspect in the development of HPLC from TLC and classical glass column LC. (3) When accepting larger analysis times, more resolving power can be produced.

Indeed, parallel trends could be observed in various forms of chromatography. Simultaneously with the decrease in particle size in HPLC development one has seen a trend towards smaller particles in thin-layer chromatography (TLC), and towards smaller column diameters in capillary gas chromatography (cCG) [2,3]. Attempts to exploit the advantages of smaller particles in packed column GC have been made [4–6], however, such column could eventually not compete with the open tubular, cGC, types.

In the instrumental versions, HPLC and cGC, the decrease of particle size is ultimately limited by the pressure drop in the column. The resulting compromise between speed, efficiency and indeed pressure drop has been treated as early as 1969 in a landmark paper by Knox and Saleem [7]. The elegance of their approach has never been beaten, despite many later treatments of the subject. For the sake of clarity the approach will be briefly recapitulated in the following. Unfortunately, this is to be done in terms of plate height, rather than in terms of the equilibration time, one reason being that connection with existing literature has to maintained, another, more fundamental, that the equilibrium time approach would need more refinement (i.e., the longitudinal diffusion has to be included) before it can be applied in this matter.

The plate height plot, or "Van Deemter curve", plays a central role in the argument. Taking most of the significant contributions to peak broadening into account, the plate height curve for a packed column can be described well by an equation:

$$H = \frac{2D_m}{\gamma u_0} + d_p\, F(u_0 d_p / D_p) + d_p^2\, F'(u_0 d_p / D_p) \frac{k''}{(1+k'')^2} \quad (6)$$

In this, k'' is the retention ("capacity") factor, defined while considering the whole particle, its mobile-phase filled pores included, as the stationary "zone". This k'' value is different (by about 1 or by a factor of 2, whichever is greater) from the experimentally observed k' value, that describes the distribution between the mobile (pores included) and stationary phase (see Knox and Scott [8]). However, as we are interested at this moment only in a very broad characterization, this difference will be neglected; the influence of the retention factor k' is not so large in HPLC, so we introduce only a moderate distortion of the overall picture.

Expressions such as Eq. (6) describe a plate height curve; some examples are shown in Fig. 1. The height is of course the decisive property: the smaller the plate height, the better the resolving power at a given column length. The figure clearly shows the dramatic influence of the particle size; that can also be inferred from Eq. (6), where the second and third terms are in proportion to d_p and d_p^2, respectively.

A key element in the treatment is that the curves can be transformed into one universal curve for all particle sizes (and for all diffusion coefficients). This is done by introducing the reduced plate height, h, and the reduced velocity, ν:

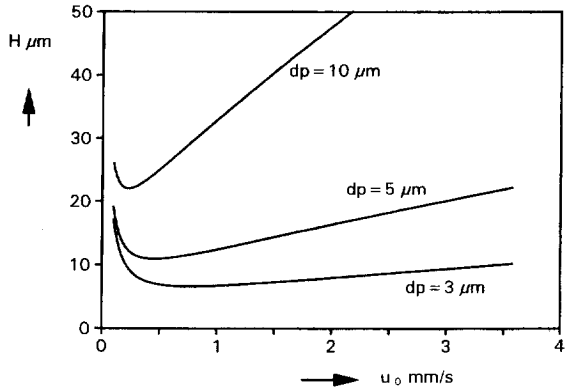

Fig. 1. Plate height curve, H vs. u_0 for different particle sizes. Curves were calculated according to Eq. (9) with $A = 1.0$, $B = 1.5$ and $C = 0.05$. Further data as in Fig. 3.

$$h = \frac{H}{d_p} \tag{7a}$$

$$\nu = \frac{u\, d_p}{D_m} \tag{7b}$$

Insertion of H and u_0 expressed in h and ν in Eq. (6) indeed produces a result that is independent of both d_p and D_m:

$$h = \frac{2}{\gamma\, \nu} + \mathrm{F}(\nu) + \mathrm{F}'(\nu)\frac{k''}{(1+k'')^2} \tag{8}$$

This equation is often summarized with numerical values for the constants γ, the function $\mathrm{F}(\nu)$ and $\mathrm{F}'(\nu)$ and the factor containing the retention factor (say for $k''=3$) as:

$$h = \frac{B}{\nu} + A\nu^{1/3} + C\nu \tag{9}$$

with $B \approx 1.5$; $A \approx 0.8$; $C \approx 0.02$–0.05.

Fig. 2 shows the h–ν plot for these values.

The validity of this simplification requires some remarks. One is that the cancellation of diffusion coefficients is contingent on the equality of the values in the particle (D_p) and in the mobile phase (D_m). That is the case for many HPLC systems; the pores of the applied particles are nearly completely filled with mobile phase and the resistance to mass transfer indeed resides mainly within this phase.

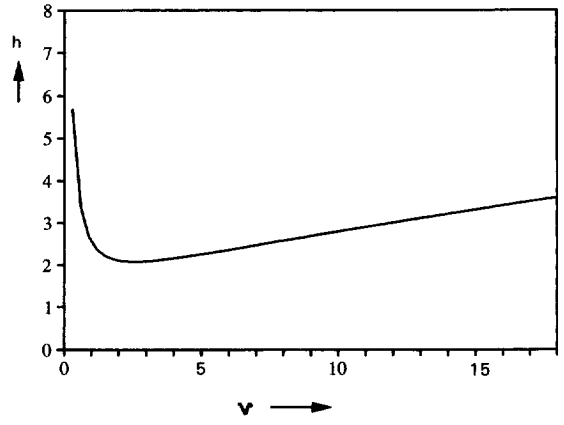

Fig. 2. Reduced plate height curve, h vs. reduced velocity ν. Curve was calculated according to Eq. (9) with $A=1.0$, $B=1.5$ and $C=0.05$.

Another point is the dependence of the geometric factors A, B and C and the shape of the two F-functions: These may be different for different porosities, particle shapes, packing geometries, etc. Again, such objections do not apply strongly for customary well-packed HPLC columns; these can be described adequately by a unified set of constants.

As indicated above the desirable reduction in particle size is limited by the pressure drop over the column. The latter is described by the equation:

$$\Delta P = \phi \cdot \frac{u_0 \cdot \eta \cdot L}{d_p^2} \tag{10}$$

where ϕ is the column resistance factor, which is in the range 500–1000 (we take 1000), and η is the viscosity of the mobile phase.

From this equation we can derive the maximum velocity u_0 of the mobile phase that is accessible with the pressure capability of the equipment, provided we know the L and d_p. However, L follows from the requirement that a given number of plates, N_r, is required. Expressing everything in terms of h:

$$L_r = N_r \cdot h \cdot d_p \tag{11}$$

This allows us to calculate the velocity, and also the retention time t_0. The result is rather simple as, surprisingly, d_p as well as ν cancel in the calculation:

$$t_0 = h^2 \phi \cdot N^2 \cdot \eta / \Delta P$$

$$t_0 = E \cdot N^2 \cdot \eta / \Delta P \tag{12}$$

where E, by definition equal to $h^2\phi$, is called the "separation impedance" [9]. It is a kind of ultimate figure-of-merit for a HPLC column, describing how favourable the compromise speed–efficiency–pressure is. For conventional HPLC columns, with $h=2.0$ in the minimum and $\phi \approx 700$–1000, the smallest value for E is 3000–4000.

This opportunity is taken to make a historical remark about the separation impedance: it is entirely analogous to a concept developed for cGC by Golay [10] in 1958, shortly after his invention of cGC, the "Column Performance Factor". Also, Endele et al. [11], discussing HPLC, must have considered the speed–efficiency–pressure trade-off when they proposed to derive the particle size from the observed

pressure drop (i.e., assume a pre-determined value for ϕ throughout) and using this value as the denominator in Eq. (7a). In this way h in itself becomes the figure-of-merit for the column.

Eq. (12), also known as the Knox equation, says that the shortest retention time, with a given backpressure, and a required plate number is obtained with the smallest E. As ϕ is constant and h variable, this is accomplished by working in the minimum of the h-curve.

It should be noted that this optimum cannot be approached with a given column, nor even with a given particle size. We need a set of (imaginary) columns which have N_r plates at a velocity where the backpressure is maximum, equal to ΔP. In this set the best column is the one that works near the minimum in h and "uses" the pressure at the same time.

That column must have a specified particle size. This is the optimal particle size, given by:

$$d_p(\text{opt}) = \left(\frac{\phi \cdot \eta \cdot D_{i,m} \cdot \nu_{min} \cdot h_{min} \cdot N_r}{\Delta P} \right)^{1/2} \qquad (13)$$

Note that the optimal particle size increases with the required plate number N_r; difficult, long lasting separations require bigger particles.

Table 1 translates these equations in practical terms. The retention times, particle size and column length are given as a function of the required plate number N_r. A minimum value of 2 for h was assumed at a reduced velocity of 3, back pressure is 200 bar, viscosity 0.001 Pa s and ϕ is 1000.

A few conclusions can be drawn from Table 1: (a) present particle technology, and problems with external peak broadening in standard HPLC equipment hardly allows work with particles of 1.7 μm or smaller in columns of a few cm to obtain 10 000 plates in 20 s. However, we are quite close to that. We are halfway between the pressure limited and particle size limited situation. The state-of-the-art HPLC column is not that far from the best packed pressure driven column possible. (b) For high plate numbers we are entirely in the pressure-limited situation, particle sizes of 5–20 μm are readily available. Indeed experiments by Scott and Kucera [12,13] and Ishii and coworkers [14–16] have shown the agreement with theory convincingly. However, the analytical prospects are not very bright, as Table 1 shows that physical laws do not allow us to use high-resolution chromatography, with 100 000 plates or more, in a reasonable time. (c) Very fast chromatography (low resolving power in a very short time) would require a new generation of particles and equipment.

In the above, reference was only made to packed, HPLC-like columns, in order to keep the text readable. However, we can apply the same equations and reasoning to other types of columns and to gas- and supercritical liquid chromatography (SFC). In open tubular chromatography, the particle size, d_p is replaced by the column diameter d_c.

In GC and SFC some complications arise as a result of the compressibility of the mobile phase. In GC this can be easily handled, by taking values for the velocity and the diffusion coefficient at the average pressure in the column (not that ν does not change over the column length, as u_0 and D_m change in the same proportion). Only slight inaccuracies are introduced in this way [5].

Thus, for an inlet pressure of 10 times the atmospheric pressure, 10^6 Pa, and atmospheric outlet pressure, the average pressure is about $7 \cdot 10^5$ Pa. The diffusion coefficient in He at this pressure and 80°C is $0.37/7 \cdot 10^{-5}$ m^2/s. For a N_r value of 30 000 then a optimum particle size of 100 μm is predicted. This indeed is a common value for GC, in practice somewhat larger particles were used to obtain a

Table 1
Unretained retention times, optimal particle size and column length in HPLC in pressure limited situation

Required plate number, N_r	t_0	d_p	L
1000	0.2 s	0.5 μm	1.1 mm
10 000	20 s	1.7 μm	35 mm
100 000	2000 s	5 μm	1100 mm
1 000 000	2.3 days	17 μm	35 m

smaller number of plates at a much smaller pressure drop. The issue has been forgotten since open tubular geometry is so vastly superior in GC. What does happen in that field is the exploitation of higher inlet pressures for obtaining faster analysis in narrower (100 down to 25 μm) capillaries [3].

Similarly, it has been noticed that the supercritical phase puts its own demands on the diameter of the capillary column or particle size. The maximum pressure drop here may be smaller than is instrumentally possible: because of the strong dependence of the distribution equilibrium on the pressure (via the density), too large a pressure drop may result in a too large variation across the column, and even in precipitation of solutes. Apart from this other value for the maximum pressure, the considerations on the optimal dimensions are quite analogous.

As diffusion coefficients in the supercritical phase are intermediate between those in the gases and liquids, one would expect that these optimal dimensions would differ from both. Indeed, for the packed column version the particles should be somewhat larger than they should be in HPLC. Fortunately, for the SFC devotees, the customary HPLC column packings are still a bit on the large side for HPLC, making them a very reasonable choice for SFC.

In capillary SFC, to the contrary, the reference point and experimental starting point was cGC, where column diameters are in the range of 200–400 μm. For SFC this is much too big. Eq. (1), with the smaller diffusion coefficient inserted, predicts slow separations, indeed an ailment in many early SFC separations. Schoenmakers [17] pointed out that a suitable column diameter for SFC is in the range of 25 μm.

2. Plot of plate times vs. plate number

Coming back to LC, it follows from Section 1 that apart from pressure, the experimentally accessible range of particle or column diameter may also be the limiting factor. In modern electrodrive method the pressure even does not play a role. Therefore, the comparison of type of chromatography and column just on the basis of the separation impedance, E, (with the implicit assumption that the particle/column size can be adapted to the situation) becomes more or less unsatisfactory. In this paper another method for comparison is used, the plot of the plate time (or time equivalent to a theoretical plate; TETP) vs. the plate number.

When a particle size, or column diameter is (or has to be, for experimental reasons) decided upon, it is still possible to optimize the column length and the linear velocity, in order to obtain the required plate number in the shortest possible time. Obviously the length of the column must equal N_r times the plate height, H; therefore it depends via H on u_0. The unretained time, t_0 equals L/u, and thus $N_r \cdot H/u$. The factor H/u is equivalent to the equilibrium time τ_{eq} used in Section 1. As H/u differs from τ_{eq} due to the presence of other contributions than non-equilibrium, is better indicated as "plate time". It describes the speed of the separation. H/u becomes smaller (more favourable) at higher values for u_0, because the contributions to H are at most proportional to u_0, never to a higher power of u_0. The maximum feasible value of u_0, in combination with the correspondingly increased length of the column, is therefore the most favourable.

Ultimately, of course, there is some limit to u_0. In the pressure driven case this is the maximum pressure, in the case of electrodrive, this is the maximum voltage available. The maximum value will depend strongly on the required plate number, as that dictates the column length for each individual thought experiment.

The idea is illustrated in Fig. 3 for conventional HPLC. The data were calculated as follows: for each chosen N_r value the maximum value of u_0 was found. This could have be done by successively increasing u_0 marginally, calculating H, the column length L and the resulting pressure from the known values of u_0, L and d_p, and ending the iteration when the pressure was exceeded. In fact, the calculations were done in a more efficient manner, but that technical detail is not important here. The resulting lines, one for each particle size, demonstrate the maximum speed obtainable with such particles at a given required plate number, N_r.

The duration of the chromatogram (in terms of the unretained time t_0 is to be found by multiplying N_r by H/u_0. As the plots are logarithmic, this comes down to straight lines, of which two (of a array of

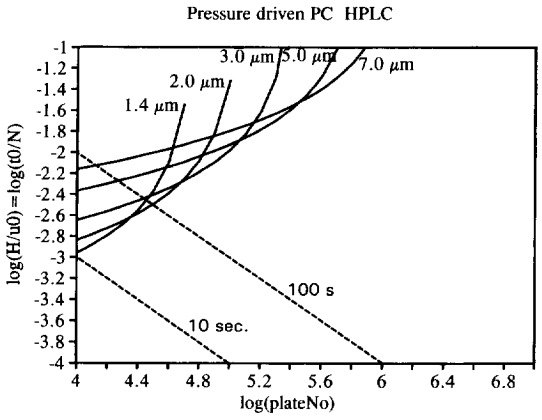

Fig. 3. Plot of plate time, H/u_0 vs. required plate number in conventional HPLC (PD-PCLC) with various particle sizes. Assumed parameters: maximum pressure $\Delta P = 4 \cdot 10^7$ Pa, viscosity $\eta = 0.001$ Pa/s, flow resistance factor $\phi = 1000$, diffusion coefficient $D = 1 \cdot 10^{-9}$ m^2/s, reduced plate height expression Eq. (9) with $A = 1.0$, $B = 1.5$ and $C = 0.05$.

parallel ones) have been drawn for $t_0 = 10$ and $t_0 = 100$.

2.1. Asymptotes

The hyperbola-shaped curve illustrates two concepts from classical chromatographic optimization theories. In the first place, when going to higher plate number N_r, it is observed that the curves go up steeply, apparently approaching some vertical asymptote with a plate number N_{crit} (not indicated in Fig. 3). What happens physically is that the N_r value is so large that the velocity u_0 is forced back into the diffusion region of the plate height curve (Fig. 1). The plate height goes up, and the column length has to be increased even further.

The position of the asymptote corresponds to the maximum plate number achievable with the given particle size at the given pressure. In older literature this is known as the "critical pressure": a reversed concept, the pressure required to achieve a given number of plates at a given particle size. It can be calculated easily: under these conditions the diffusion (B) term in the H expression predominates, in the first instance we can neglect the other ones. That is:

$$H = \frac{2 D_m}{\gamma u_0}$$

$$L = N_r \frac{2 D_m}{\gamma u_0}$$

$$\Delta P_{crit} = N_r \frac{2 D_m}{\gamma} \frac{\phi \eta}{\gamma d_p^2} \quad (u \text{ cancels!}) \tag{14}$$

$$N_{crit} = \Delta P_{max} \frac{\gamma}{2 D_m} \frac{d_p^2}{\phi \eta} \tag{15}$$

The smaller the particles, the smaller the plate number that can be achieved with them. In a few cases in chromatography this has been an issue, the most prominent being the case of packed column GC. However, also in HPLC this can become important; when using for example 1.4 μm particles, $N_r = > 30\,000$ become problematic, as can be seen in the Fig. 3.

Another asymptotic behaviour occurs at the low N_r end (in this case less clearly visible in the Fig. 3). Here the velocity, u_0, can be given extremely large values, because the L needed is so small. Consequently, the predominant term in the plate height is the last (C) term, the only one increasing in direct proportion to u_0. That case brings us back to the τ_{eq} of Section 1. The value of H/u_0 in this asymptote equals:

$$H/u_0 = C d_p^2 / D_m \approx 1/(1 + k'')\tau_{eq} \tag{16}$$

Particle size limits the achievable speed under such conditions.

When the results of this exercise for a continuity of particle/column diameter are combined, ultimately, the same result must be obtained as with the Knox and Saleem equations. This is indeed the case, the latter limit is described by the envelop at the low side of all the curves. For each plate number there is an optimal particle size, for which the curve in Fig. 3 would be lower than any other one.

Higher pressures, up to $5 \cdot 10^8$ Pa [18] give of course, more room for improvement but it is doubtful whether this approach can develop into a practical tool.

Fig. 3, made for $4 \cdot 10^7$ Pa, just above the limit of most conventional HPLC systems, is important as a reference for other such plots to be shown in the

sequel. It also demonstrates the effect of a choice of particle size, that, for one reason or the other, deviates from the optimum of Eq. (13). As can be seen, e.g., by comparing the graphs for $d_p = 2$ μm and for $d_p = 3$ μm, the effect is not dramatic, amounting to about a ≈ 0.2 in the log, i.e., a factor of ≈ 1.6 in the speed. That results from the fact that e.g., a too large particle can be compensated by a larger velocity, so that the speed loss is not that great.

3. New versions of liquid chromatography

Although earlier attempts to get around the Knox-Saleem limitation described above have been made before 1980, only since then have such efforts became a main stream in chromatographic research. The approaches can be categorized as follows: (i) "micro-capillary" liquid chromatography, i.e., packed beds with small column diameters, mostly fabricated using fused-silica tubes. (ii) Open tubular liquid chromatography with pressure drive (PD-OT-LC). (iii) Electrically driven liquid chromatography, in open tubes (ED-OT-LC) but also in packed beds (ED-PC-LC, also indicated as CEC, capillary electrochromatography). (iv) Micellar electrokinetic chromatography (MEKC).

3.1. Detection aspects

In this section some remarks will be made on the way the improvement in performance (compared to classical versions of HPLC) is accomplished. Before doing so, however, we note that none of these four methods can be realized on the volume scale of classical HPLC, that is with columns having a volume of one to a few ml and inner diameters of 3–5 mm. In all four methods a sufficiently fast radial mass or heat transport can only be obtained with column diameters below a given value (depending on the limiting process and the properties of the phases).

This results in serious detection problems. In conventional HPLC suitable detection can be accomplished with devices that average out the concentration over some 10 μl (corresponding to a cell volume of that size, or a contribution to peak broadening of that order of magnitude). In contrast, the techniques mentioned above can only work provided the detection manages to give averages over only a few nl or even a few pl. It is to be borne in mind here that in a suitable comparison of separation techniques it is best assumed, in the first instance, that the concentration range is the same in both methods. Therefore, considering the detection from the measurement point of view and considering a given concentration close to what is to be expected in the sample, a classical HPLC detector has to yield a sufficient *S/N* ratio from the observation of about 10 μl of a solution with that concentration. However, in a miniaturized system, where only, say, 1 nl averaging is allowed, the task is much more difficult. Phrased another way: in the second instance there are simply 10^4 less molecules available that can contribute to the signal. This difficulty is often seen as one of technical (optical) nature ("path length disadvantage"), but viewed as above it is of much more general character. Indeed, one of the conclusions of this work will be that the relative prospects of these four techniques are partly determined by the degree of detector miniaturization they require.

The issue of detection limits and the above-mentioned assumption of similar concentration scales has been the terrain of considerable confusion. Workers expected better "detectability" in miniaturized systems, because of less dilution in a smaller column; a given amount emerges at the end in a smaller volume, and a larger concentration is presented to the detector. Indeed, when the amount of sample is limited, as may occur in forensic applications, paediatric clinical work, single cell biological research, etc., the advantages of miniaturized chromatography are quite obvious. The smaller dilution more than compensates for the fact that the detector may need higher concentrations (e.g., path length disadvantage) for a suitable *S/N* ratio.

However, in the majority of HPLC applications, the amount of sample is, practically speaking, unlimited. This holds for environmental analysis, production control, regular clinical analysis and many types of biological research. In such cases the dilution within the chromatographic system is independent of the volume scale applied; in a miniaturized system on can simply inject proportionally less volume. Also when more sophisticated pre-column operations, such as on-line enrichment, are consid-

ered, the final conclusion is the same. One exception may be that with extremely large concentration factors in these operations (e.g., enrichment of organics on a non-polar adsorbent from many litres of aqueous sample), one may run into difficulties with the classical HPLC system, because of sample availability or just logistic reasons.

Thus, in general, the gains in speed and efficiency obtained in the above-mentioned new techniques, are obtained for a large part at the expense of the detectability and the dynamic range of the system. To what extent this happens in the four techniques is an important issue in the following sections.

3.2. Microcapillary liquid chromatography

Many workers have explored the possibilities of obtaining better speed by employing narrow bore column. Pioneering experimental work was e.g., done by Scott and Kucera [12,13], using a 1 mm column. A point of culmination was the work by Menet [19], in which one million theoretical plates were achieved in a 1 mm I.D. packed column system, using 800 bar inlet pressure. Analysis, though, of the speed obtained demonstrates that such columns behave essentially in the same manner as their wider bore counter parts, E being in the order of 3000. Thus, essentially similar results can be obtained with 4.6 mm HPLC columns. Packing of these in sufficient length is, to say the least, impractical, but shorter columns can be readily coupled. Such experiments have indeed been carried out [20], they now appear rather impractical since there is a waste of packing material and mobile phase, with a peak volume much larger than required for a UV detector.

Things took a different turn when fused-silica as a column material was introduced. The experimental technique for packing such columns with substantially smaller diameters (down to 100 μm) columns was readily developed e.g., [14,21]. It was nearly invariably found that reduced plate heights were smaller and column resistance factors were also better when compared to columns of conventional diameters. One rather comprehensive study of the improvement in separation impedance obtained in this way was reported by Kennedy and Jorgenson [22]. Their work clearly shows that the magnitude of the improvement is strongly dependent on the ratio of column to particle diameter, the aspect ratio. In the extreme case (5 μm particles in a 22 μm tube) the improvement in E amounted to a factor of 3.

The issue of degree of miniaturization now comes into focus. These results indicate that substantial improvement with close to optimal particle sizes is reached only with very small column diameters. The volume scale of these experiments is extremely small. It is even comparable to that of PD-OT-LC (see Section 3.3 for a comparison). As also the improvement in E by a factor of 3 is not so large (it means a factor 3 in the required analysis time, but a factor of less than 2 in plate number at a given analysis time, which then translates in only a factor 1.3 in the resolution) there is reason to doubt whether this approach will proliferate widely. One exception to that should be mentioned: in sample-amount limited cases, and especially when a detection method favourable for miniaturization (electrochemical, fluorescence) is applied, the microcapillary LC column is of utmost usefulness. This has been demonstrated i.a. in studies of neurotransmitter metabolism in vivo [23,24].

3.3. Open tubular liquid chromatography (PD-OT-LC)

The great performance of cGC and its widespread proliferation has inspired HPLC workers to try out a similar technique in the liquid phase. It is useful to discuss OT-LC from the outset in terms of the Knox equation.

The plate height curve is determined by the extended Golay equation [25]. For reasonable conditions (e.g., $k'=3$, thin stationary phase layer) the plate height amount to roughly the column diameter, i.e., $h\approx 1$, as compared to 2 for packed column. An even bigger advantage resides in the column resistance factor, which is only 32. The resulting E value, ≈ 32, is about a factor of 100 better than that of packed column, promising a gain in analysis time by this factor, or alternatively an increase in plate number of a factor of 10; resolution 3.2 times better in the same time.

Unfortunately, this concept fails here especially because of the detection aspect. The design of an open tubular column that would work at $h=1$, would require us to obtain an E value of ≈ 30, for a

pressure drop of $2 \cdot 10^7$ Pa, for 100 000 plates, would lead to an column diameter [as in pressure driven packed column chromatography (PD-PC-LC), the d_c (d_p) value must be adapted to the desired N and ΔP values, cf., Eq. (13)] of less than a micrometer. Such a column would have a cross-sectional area of less than 1 μm^2, a plate height H of 1 μm; the volume standard deviation of the peak, for a 1 m column ($1 \cdot 10^6$ plates) would amount to 10^{-15} m^3 or 1 pl. Detection in such volumes can be accomplished, but is in practice possible only with laser induced fluorescence detection. The difficulty is manifest when considering the concentration levels in regular HPLC experiments. These are quite often in the range of 10^{-8} mol/l, even with standard UV-absorption detection. The 1 pl emerging from the 1 μm OT-LC columns then contains only $10^{-8} \cdot 10^{-12} = 10^{-20}$ mol, i.e., some 6000 molecules.

Knox and Gilbert [25] analyzed this situation, taking the peak volume as another constraint to the optimization, i.e., they calculated which columns would deliver peak volumes larger than or equal to a given value and still give an improvement in comparison with their packed versions. The result was that only when peak volumes can be as small as 0.1 nl a substantial improvement can be expected. This corresponds to column diameters of 5–10 μm for plate numbers in excess of 100 000. Prospects for lower plate number experiments appeared to be dim; the feasible diameter is then too much different from the optimum one, while also the performance of packed columns is much better.

Apart from the detection problem another hurdle has to be taken in OT-LC. It is not trivial to prepare columns with a suitable stationary layer. Early attempts were made by modifying the wall of the column material to form e.g., a reversed-phase type of structure. However, in such systems the loadability (in terms of concentration) can only be extremely small, as a result of the quite unfavourable phase ratio (in HPLC one commonly has more than 100 m^2 surface per ml of mobile phase; in such OT-LC systems, even with a 5 μm diameter, there is only $4/d_c = = 8 \cdot 10^5$ $m^2/m^3 = = 0.8$ m^2 per ml). Thus, the concentration in the stationary phase must be very high for obtaining reasonable retention ratios, and overloading in the absorbing layer is likely. On the other hand, high concentration loadabilities are absolutely necessary in order to at least ameliorate the detection problems and to allow for a reasonable dynamic range.

A quick solution [26] is to adapt the silicone chemistry, universally used to prepare thick-layered cGC columns. However, the layer cannot be made very thick. Other, successful approaches involve the deposition of porous silica [27,28] and the use of acrylate chemistry [29].

Fig. 4 illustrates the performance of OT-LC columns of practical dimensions. Although most experimental work has been carried out with inner diameters, d_c, of 4 μm or more [30,28], a curve of 3 μm has been included also, as Tijssen and coworkers [31,32] demonstrated (be it for a non-interactive form of chromatography) that one can work in such narrow columns, even with UV detection.

When comparing the currently reasonably practical diameter of 5 μm with the set of curves in Fig. 3, one sees that the advantages of OT-LC are noteworthy only for large plate numbers, above 30 000. For smaller plate numbers the 5 μm OT-LC column diameter is much too far off the kinetic optimum.

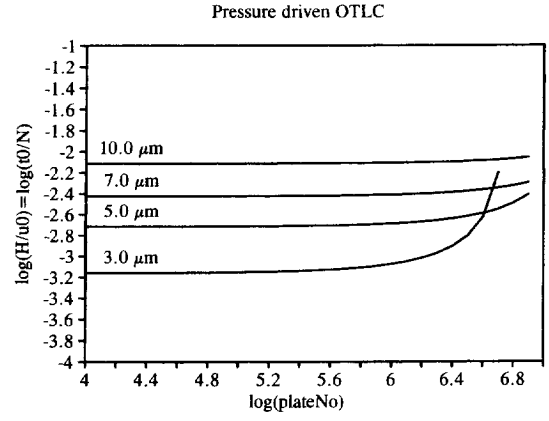

Fig. 4. Plot of plate time, H/u_0 vs. required plate number in pressure driven open tubular liquid chromatography (PD-OT-LC) with various column diameters. Assumed parameters: maximum pressure $\Delta P = 4 \cdot 10^7$ Pa, viscosity $\eta = 0.001$ Pa/s, flow resistance factor $\phi = 32$, diffusion coefficient $D_m = 1 \cdot 10^{-9}$ m^2/s, reduced plate height expression $h = 2/\nu + C\nu$ with $C = 1/96$ $(1 + 6k' + 11k'^2)/(1 + k')^2$, $k' = 3$.

3.4. Electrodrive open tubular liquid chromatography (ED-OT-LC)

The advantages of electrodrive in analytical separations encompass two aspects, as precisely put forward in the papers by Knox and Grant [33,34]. In the first place the electroosmotic and electromigration transport has a uniform profile, i.e., the velocities do not depend on the lateral position in the system (at least provided the flow channel diameters are chosen such that heat transport through the liquid is fast enough, usually the case when diameter are smaller than some 75 μm, and avoiding "double layer overlap" [34]). In the second place one can choose the particle size much smaller than is possible in a pressure-limited situation, since the velocity acquired by the mobile phase and solutes is to a large degree (again avoiding "overlap") independent of the channel diameter.

In capillary zone electrophoresis (CZE) the first advantage can be exploited to the full extent. As there is no phase equilibrium, selectivity being inherent in the different mobilities of ions, and no irregular bed of particles, the only source of band broadening is longitudinal diffusion. To the surprise of many chromatographers, brought up with the notion that resolution has its price in time, this means that a faster separation, brought about by using a higher voltage on the same capillary, can bring higher resolving power. Also speed can be improved by taking shorter tubes with the same voltage, while the resolving power is unaffected. The only limiting factor seems to formed by the dissipation of the Joule heat. Again miniaturization of the system is needed for improving the speed, although now the reason is in the radial thermal uniformity rather than in the uniformity of concentrations. This point will be discussed again in Section 3.7.

The predominant role of longitudinal diffusion suggests that in CE something similar to the critical pressure in PD chromatography must exist. Indeed, as was already clear from Jorgenson and Lukacs' landmark publication [35] on the subject, the maximum voltage available is decisive. With a ion migration velocity equal to the product of field strength and its mobility, μ_i, it holds:

$$u_i = E\ \mu_i = V/L\ \mu_i$$

and the plate height becomes

$$H = \frac{2D_m}{V/L\ \mu_i}$$

with a remarkably simple expression for the resulting plate number:

$$N = \frac{V\ \mu_i}{2D_m} \tag{17}$$

Kenndler and Schwer [36] made the important remark that the ratio μ_i/D_m is determined, via the Einstein equation, by the charge of the ion i, in fact:

$$\frac{\mu_i}{D_m} = \frac{z_i\ e/\text{Friction factor}}{k\ T/\text{Friction factor}} = \frac{z_i\ e}{k\ T} \approx \frac{z_i}{0.025\ \text{Volt}} \tag{18}$$

where z_i is the valency of the ion, e is the elementary charge, k is the Boltzmann constant and T is the temperature in Kelvin. "Friction factor" describes the proportionality between frictional force and velocity, for a spherical ion equal to $6\pi\eta R$, R being the radius. Thus, for the most common case of singly charged ions one arrives at:

$$N = \frac{V}{0.05\ \text{Volt}} \tag{19}$$

There seems to be general agreement that voltages higher than about 40 kV are impractical, because of sparking, etc. Taking this value as the maximum, one finds at room temperature:

$$N_{max} = \frac{40000}{0.050} = 800000 \tag{20}$$

It is interesting to note that the plate number equals half the energy loss for the ion on travel through the system when the latter is expressed in the thermal energy, kT. Similar reasonings can be found in the marvellous book by Giddings [37].

This paper focuses on interactive chromatography, in which neutral analytes can be separated. This requires the presence of electroosmotic flow. The advantages of this type of flow are the same as previously mentioned for general electrophoretic transport: plug profile and independence of the channel diameters.

A second requirement is of course to build in some interaction with another "phase" (quoted

because it is meant in a very broad sense). Various approaches are possible. The first (but by no means the most important, see the following sections) is to apply a stationary phase layer on the wall of a narrow tube. The resulting technique can be called electro-drive open tubular liquid chromatography, ED-OT-LC. This has been explored experimentally by Tsuda et al. [38] and by our own group [39].

The chromatographic distribution process leads again to an additional mass transfer (C) term in the plate height. Although somewhat smaller than in the pressure driven case (PD-OT-LC), for well retained compounds it is of the same order of magnitude (note: for unretained compounds, the term is of course zero, and for weakly retained ones it is small. This may be quite useful under some conditions, but in our opinion it is not much of a very general advantage, as one normally needs appreciable retention to obtain good separations). It follows that the column diameter chosen should be small (3–10 μm), much smaller than is required to keep the thermal dissipation under control (≈75 μm). With that the technique has characteristics as PD-OT-LC; good kinetics but a rather extreme form of detection miniaturization is required.

For this technique a plot of plate time vs. required plate number has been constructed, analogous to Figs. 3 and 4 (Fig. 5). The scheme was essentially the same: For each plate number a low value for the velocity, u_{EO}, was chosen as a starting point. This choice leads to a plate height via the plate height equation, this in turn leads to a length and a required voltage. The velocity u_{EO} was subsequently increased until the maximum voltage of 40 kV was exceeded.

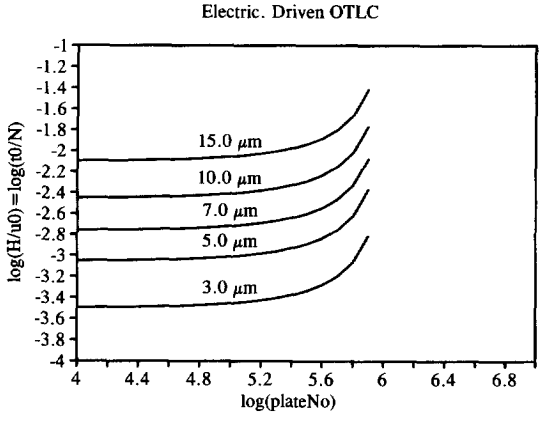

Fig. 5. Plot of plate time, H/u_0 vs. required plate number in electrically driven open tubular liquid chromatography (ED-OT-LC) with various column diameters. Assumed parameters: maximum voltage $V=40$ kV, electroosmotic mobility, $\mu_{EO}=50\cdot10^{-9}$ $m^2/V/s$, reduced plate height expression $h=2/\nu+C\nu$ with $C=1/16\ [(k'/(1+k')]^2$; $k'=3$.

The resulting curves as shown in Fig. 5 are quite similar to the ones for PD-OT-LC in Fig. 4. There are, however, two important differences.

In the first place the curves are in the intermediate N_r range lower by about 0.3 log units, a reflection of the smaller C term (cf. Ref. [39]) in this system.

In the second place the upward asymptotic behaviour occurs for all column diameter at the same N_r value, about $1\cdot10^6$. That is in line with the observation made above on the maximum plate number of the CE-technique; a derived technique where also other sources of peak broadening are present cannot be any better than CE itself.

3.5. Electrodrive packed column liquid chromatography (ED-PC-LC) or capillary electrochromatography (CEC)

Retention in ED-PC-LC or CEC, is obtained by using particles as a sorbent, rather than by preparation of the tube wall. The columns used are similar to the ones of "microcapillary LC"; they are commonly slurry-packed with particles of μm size. Transport is uniform over the cross-section (as it is in common HPLC), which means that the column diameter can be large enough to meet the requirement of effective dissipation of the Joule heat generated, about 75 μm. Therefore this variety requires substantially less miniaturization than the OT-LC techniques.

Jorgenson and Lukacs [35] already reported on this method in their pioneering paper on CE. Earlier, in 1974, Pretorius et al. [40] considered it for improving HPLC, but at those times the techniques available did not allow any useful exploitation of the principle. Also, Jorgenson and Lukacs still voiced concerns about the practical applicability: "..the method is a bit difficult and inconvenient to work with", a phrase seldomly used by proposers of new techniques.

At the end of the eighties Knox and coworkers

[33,34,41] examined the issue again, theoretically as well as experimentally. They reiterated the advantage of better plate height, less critically dependent on uniformity of packing structure and particle size uniformity than it is in PD-PC-LC. However, they also drew attention to the fact that particle size can be miniaturized much further in ED-PC-LC, due to the fact that the flow velocity is to a high degree independent of the channel diameter. This would open up the possibility to reconcile speed and efficiency to a much higher degree than will ever be possible with pressure driven packed column systems.

Since the first ED-PC-LC chromatogram was published by Knox and Grant [34] the activity in development of this technique has been seen to increase steadily; see e.g., work by Yan et al. [42], Unger and Eimer [43], Tsuda [44], Smith and Evans [45], Boughtflower et al. [46], Behnke and Bayer [47], Dittmann and coworkers [48,49], Choudhary and Horváth [50] and van den Bosch et al. [51,52].

The improvement in h values has meanwhile been substantiated clearly. However, the possibility of using significantly smaller particles thus far have escaped experimental proof. Nevertheless, very impressive separations have been demonstrated, especially by Smith and Evans [45] and by Dittmann and Rozing [48]. For instance, the latter authors obtained a 100 000 plates chromatogram in less then 20 min, something quite impossible in pressure driven systems.

The work on this topic comes in three experimental varieties:

(a) In the simplest one, the CE technique is used with little modification, except that the open tube is replaced by one that is partially slurry-packed with particles. There are two interrelated experimental problems that have to be solved before the method can work at all. In the first place one needs non-conducting packing retainers, in order to keep the packing in position. Their pore size should be carefully controlled, in order to on the one hand avoid leakage of the (preferably very small) packing particles, on the other hand avoid flow obstruction. Numerous unsuccessful attempts have been made (cf. Ref [52]). That proposed by Smith and Evans [45] has been adopted by many other workers. Here the retainer is formed by heating a small portion of a packed bed, usually consisting of the same particles as the chromatographic bed, while slurry-packing pressure and flow are still on. Some sort of sintering of the particles then occurs.

In the second place it turns out that often the electrical current, and with that the flow and the chromatographic process is interrupted by the formation of "air" bubbles (there is no practical way of finding out what the bubbles really consist of, therefore "air" is put between quotes). This also can occur when conditions are such that Joule heating is small, and when solution are helium sparged beforehand, so there is no reason to believe that the bubble formation is caused by thermal degassing or even boiling of the liquid.

A reasonable explanation has been given in Ref. [52]. Longitudinal variations in the electroosmotic mobility μ_{EO} (i.e., in the zeta-potential of the packing or tube material) can generate pressures below (absolute) zero at some positions in the tube. This is the case as the cubic flow-rate has to be uniform across the length. If e.g., in the first part of a packed bed the μ_{EO} value is smaller than in the second part, pressure differences will develop in order to keep the cubic flow-rate uniform. It can be derived [52] that pressure as a function of position, $p(z)$, measured relative to the injection point $z=0$, can be described by:

$$p(z) = E/k_0 \int_0^z (\mu_{EO}(z) - \bar{\mu}_{EO})\, dz \qquad (21)$$

where E is the electric field, k_0 is the permeability (linear velocity divided by pressure gradient), $\mu_{eo}(z)$ is the electroosmotic mobility and $\bar{\mu}_{eo}$ is that mobility averaged over the length.

When k_0 is small (smaller for smaller particles!) this can easily lead to a substantial negative pressure drop; when the inlet pressure is atmospheric, the pressure may well reach absolute zero, where either degassing or formation of a solvent vapour bubble will occur.

This may explain why Smith's technique for obtaining retainers is the most successful; the frit consists of the same material as the chromatographic packing and variations in μ_{EO} are minimized.

(b) Because of the occurrence of bubbles, many workers have chosen for the application of external

pressure. In this way the technique looses one attractive practical feature: the elegance of the injection-start sequence of CE is lost; although the sample introduction can be done in the regular CE way, after that the system has to be pressurized, requiring programmable pumps, valves, pressure-resistance buffer vials, etc. When one sticks to pure electrodrive operation the pressure has to be applied to both ends of the tube.

Nevertheless, the most impressive result with ED-PC-LC have indeed been obtained with this method. Some groups [51,42] still try to avoid this complication as much as possible. One good reason for trying to master this technique is that future use of smaller particles will make the formation of bubbles more likely. The above-mentioned negative pressure excursions can be expected (smaller k_0) to be much stronger, and without suitable uniformity of the μ_{EO} value, moderate pressures may not be sufficient. In other words, if we are unable to work with 5 μm at 1 bar starting pressure, we will also be unable to work with 1 μm particles at 25 bar (k_0 decreases with d_p^2!).

(c) When pressure is applied only at the inlet buffer vial, [43,47,53] the resulting hybrid between pressure and electrodrive LC is sometimes called electroosmotically assisted LC. In it a substantial part of the improvement in h will be lost. Also, the prospects for smaller particles are now less convincing. The speed of analysis can be improved, of course. Another positive point is that for mixtures of ions and neutrals an additional tool for selectivity manipulation becomes available: by changing the ratio of voltage and pressure one can control the relative positions. However, we believe that in general in LC systems there is a sufficient number of degrees of freedom for the manipulation of selectivity available (e.g., for ions and neutrals a ion pair mechanisms make it very easy to move the one group with respect to the other). Therefore it does not seem wise to compromise the kinetic performance in order to obtain yet another handle on relative retention.

The future of this technique, in our opinion, will depend to a large extent on the future experimental results, answering the following questions: (i) is it experimentally possible to make the EO-flow as stable as is required to obtain reasonably reproducible chromatograms, also when samples contain non-polar c.q. high molecular mass materials that tend to adsorb irreversibly onto the packing. (ii) Is the road to particles smaller than, say, 3 μm indeed open, also experimentally? It appears to us that the improvement, compared to PD-PC-LC, obtained with 3 μm, although significant, is not large enough to justify in the long term the development of quite a new generation of LC-instruments.

Fig. 6 shows the curve of log plate time vs. required plate number for this technique. The curves have been constructed in the same manner as Figs. 3–5. The maximum voltage was 40 000 V, the assumed electroosmotic mobility μ_{EO} was $50 \cdot 10^{-9}$ $m^2/V/s$. The B and C factors in the Knox reduced plate height equation were the same as in Fig. 4, 1.5 and 0.05, respectively. The A factor was chosen to be 0.75 (rather than 1.5), which corresponds to a minimum h value of 1.67, reflecting the better performance of electrodrive in this respect.

As can be seen, even with 3 μm particles, the speed of the electrodrive is much larger than with pressure drive. For instance, comparing the time required to generate 100 000 plates (log $N_r = 5$) in Figs. 3 and 6 shows an improvement by a factor of about 10. This is more than can be expected on the basis of the improved h value, according to Eq. (12). Another aspect of the improvement is indeed in the much larger ν value that can be obtained in electrod-

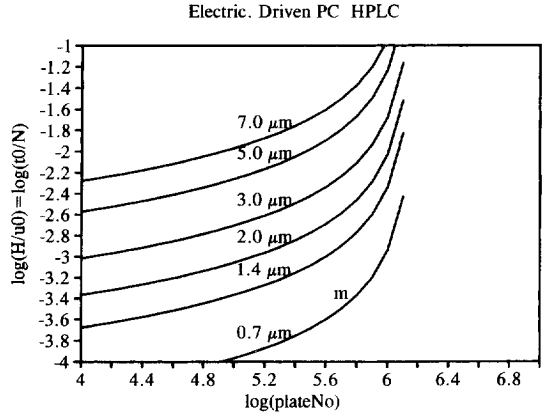

Fig. 6. Plot of plate time, H/u_0 vs. required plate number in electrically driven packed column liquid chromatography (PD-OT-LC) with various particle sizes. Assumed parameters: maximum voltage $V = 40$ kV, electroosmotic mobility $\mu_{EO} = 50 \cdot 10^{-9}$ $m^2/V/s$, reduced plate height expression Eq. (9) with $A = 0.75$, $B = 1.5$ and $C = 0.05$; $k' = 3$.

rive with this value for N_r. As can be seen in Fig. 6, the advantage of electrodrive becomes less important at higher plate numbers.

3.6. Micellar electrokinetic chromatography (MEKC)

Micellar electrokinetic chromatography (MEKC; other acronyms are in use) was introduced in a series of innovative papers by Terabe and coworkers [54–56]. The "pseudo" stationary phase is here formed by micelles, that are formed spontaneously in a solution of certain surfactants when their concentration exceeds a certain value, the critical micelle concentration, CMC. In this way any "construction" of a chromatographic column, i.e., packing particles and coating of a tube wall or particle surface with a suitable sorbent, is circumvented. The technique is therefore very easy to implement in a standard CE-instrument, in all likelihood the reason why this high resolution technique was so swiftly adapted in numerous useful applications (cf. Ref. [57].

The micelle-forming surfactants have to be cationic or anionic, as they have to acquire a velocity relative to the aqueous phase. The charged micelles have the ability to sorb foreign species, including neutral ones. Thus, injected analytes can distribute themselves between the bulk part of the carrier and the micelles. Micelles from sodium dodecyl sulphate (SDS) are most frequently used and in the following this will assumed; the behaviour in other case can be derived once the principle is clear.

The aqueous phase moves with the electroosmotic velocity, the SDS micelles move "upstream", but their velocity is normally smaller than μ_{EO}, so that they undergo a net transport towards the cathode.

Analytes with high "retention", i.e., with a strong distribution towards the micellar pseudo-phase, emerge at the position corresponding to the net velocity of the micelles; the position on the mobility scale is at $\mu_{EO} - \mu_{micel}$. Analytes that do not distribute themselves at all into the micelles ("unretained") will emerge at the position corresponding to the electroosmotic flow, μ_{EO}. The net migration velocity of most neutral analytes will lie between these limits.

An advantage of MEKC is that all analytes (with the possible exception of fast ions with the "wrong" charge, electrophoretically moving against the electroosmotic flow) are eluted. As in TLC, the "general elution problem" is nonexistent. The full k' range, from zero to infinity, is projected onto the elution time scale. By suitable manipulation of the values of μ_{EO} and μ_{micel} it is possible to give the "window" between these values a reasonable size on the time scale of the chromatogram, so that good separations are obtained.

The efficiency of MEKC is determined in the first place by the effects that occur also in CE: longitudinal diffusion is inevitable; thermal non-uniformity and other undesirable effects can be minimized by keeping the tube diameter below about 75 μm. In addition, as in the electrodrive techniques discussed above, there are contributions connected with the repeated transfer of analytes molecules toward and from the micelles. Apparently [55], the mass transfer term described as adsorption–desorption rate constant is the most important. However, in the case studied it is much smaller than anything observed as mass transfer terms in conventional chromatographic systems. Also, the widespread observation of high efficiency in numerous implementations of the technique suggests that the equilibrium is very fast. It is logical to assume that this is because the micellar phase is much more dispersed then traditional HPLC packings (Eq. (1)). Micelles are huge agglomerates on the molecular scale (40–100 surfactant ions), but they are still very small compared to even 2 μm particles.

The uncertainty about the rate of mass transfer makes it difficult to construct a curve such as those illustrated in Figs. 3–6 for MEKC. When the observed resistance to mass transfer is really a proper adsorption–desorption rate constant, it is not likely that the value can be used for other chemical implementations of the system. However, it is likely that the curves would be similar to the ones presented in Fig. 6 for the smallest particles. Therefore, MEKC is probably one of the fastest techniques for plate numbers in the range of 100 000–300 000.

3.7. Comparison of the five mentioned techniques

Although comparison of techniques was the very purpose of this contribution, the sequential treatment of the technical aspects of each of these has more or

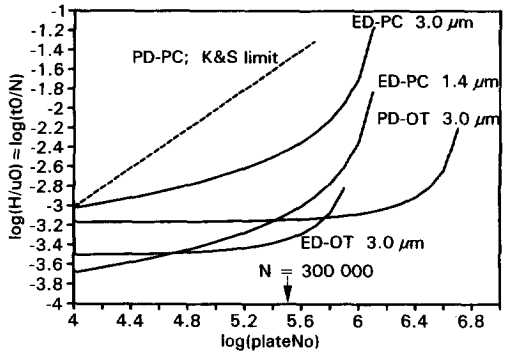

Fig. 7. Comparison of some lines from Figs. 3–6. Broken line is calculated according to Knox and Saleem approach (particle size optimized, Eq. (13)), with data as given in legend of Fig. 3.

less put the comparison out of focus. It is useful therefore to try and discuss the techniques in relation to each other, taking into account also other aspects than just speed of separation.

When aiming at a comparison of reasonable simplicity, it is necessary in the first place to decide on assumed practical limits set on certain values, in particular the particle and column diameters.

Fig. 7 was constructed with the following assumptions: particle size can be diminished to 1.4 μm; column diameters in open tubular can be 4 μm. Both, arbitrary, choices reflect roughly the state-of-the-art in research work; packed columns of 1–2 μm particle size as well as open tubular chromatography in 2–5 μm diameter have been demonstrated experimentally.

In Fig. 6, for packed column pressure drive, as a sort of reference point, the Knox and Saleem limit (particle size adapted to the required plate number according to Eq. (13)) is included. As can be seen in the Figure, all new techniques perform significantly better than conventional HPLC, the more so when the plate numbers are well above 10 000.

Making a comparison at 300 000 plates, the best improvement is obtained with ED-OT-LC, whereas ED-PC and PD-OT-LC still gives an improvement of a factor of 1.6 log units (factor 40) in time. It is also clear from the Figure that the improvement gained with plate numbers in the range 10 000 to 100 000 is the greatest for ED-PC. However, for excessively high plate numbers the technique of choice would be the open tubular pressure drive; the electrodrive techniques have a too low value for N_{crit}.

In such a comparison it is useful also to consider the volume scale of separation that is enforced by the chosen column designs. This has been done in Table 2. It states the peak volumes, expressed in volume standard deviations, for $k'=3$, for plate numbers of 100 000 and 300 000, respectively.

As can be seen, there is a considerable wide range in the volume scale on which separations takes place. In conventional HPLC (first line in Table 2), the scale is essentially free; it is now well documented that dispersion can be virtually independent of the chosen column diameter.

The second line of Table 2 give values estimated for the extreme form of "microcapillary" PD-PC-LC investigated by Kennedy and Jorgenson [22], a column of 22 μm I.D. packed with 5 μm particles. A plot of plate time vs. plate number was not included in this paper; the corresponding line in Fig. 3 would be about 0.5 log units lower. As can be seen this variety requires a substantial miniaturization, enforced by the requirement to choose column diame-

Table 2
Values of peak volumes, expressed in volume standard deviation, for components with $k'=3$

Technique	σ_V (nl)		
	$N=10^5$	$N=3\cdot10^5$	$N=10^6$
PD-PC (5 μm)	Free	Free	Free
Micro-PD-PC (Ref. [22]) (22/5 μm)	1.0	1.0	1.0
PD-OT-LC (3 μm)	0.080	0.081	0.087
ED-OT-LC (3 μm)	0.024	0.027	0.050
ED-PC (1.4 μm)	4.3	6.3	24
MEKC (n.a)	≈5	≈5	–

Assumed parameters: column and particle diameters as in Fig. 7 and indicated in parentheses under 'technique'. Column diameter for electrodrive methods 75 μm.

ters only 4–5 times larger than the particle diameter. The 1 nl is comparable to what would be needed for 10 μm columns (cf. Ref [25]), that would outperform HPLC columns in the same or a better way.

On the extreme end of miniaturization is, according to Table 2, ED-OT-LC in 3 μm columns, requiring detectors capable of a contribution to peak width of less then 0.02 nl. It is even more unfavourable than what is needed in PD-OT-LC; where the limit lies at roughly 0.1 nl. The latter value was already mentioned long ago by Knox and Gilbert [25] as a breakpoint between PC- and OT-LC. The more stringent demand of electrodrive OT-LC stem from two facts. In the first place, the better h values increase the speed, but also decrease the peak width. In the second place it is impossible in the ED-variety to work at extremely high reduced velocities (the analysis of Knox and Gilbert was based on that), because with a fixed diameter of 3 μm the efficacy of mobile phase propulsion with electrodrive is smaller than that with pressure.

It may appear surprising that in the two OT-LC varieties the peak volume hardly increases with the required plate number. This fact also was already noted by Knox and Gilbert; their simple conclusion was that each assumed peak volume (as a minimum dictated by the detector used) corresponds to a certain column diameter, irrespective of the plate number aimed at. Here a column diameter was assumed; logically that leads to roughly similar peak volumes.

Much larger peak volumes are obtained with MEKC and ED-PC-LC, the difference being in the order of a factor of 50. These techniques, though, only perform well at plate numbers of 300 000 or less. When the latter is the target, these techniques are significantly more attractive from the experimental point of view. However, two points have to be bore in mind when dealing with this conclusion.

In the first place, as mentioned above, it is still to be verified that, with real-world applications, sufficient reproducibility and stability can be obtained in the EOF-dependent ED-PC-LC variety. The coming years will certainly provide an answer to that, in view of the large activity in this field.

In the second place, there is the question of the mobile phase composition in relation to detection and spectrometric identification. In the complicated sample mixtures that one can expect to be analyzed with these high-resolution methods, there will be a strong need for identification tools, mass spectrometry or other spectrometric techniques. The electrodrive and MEKC methods require that the mobile phases contain ions, in MEKC even substantial concentrations of relatively high molecular mass detergents are needed. Unless methods and tools are developed in order to avoid interference of these mobile phase additives, a more straightforward form of chromatography such as PD-OT-LC appears to have an important advantage in this respect.

4. Conclusions

The relative performance of various new chromatographic techniques have been discussed in comparison to conventional packed column HPLC. All new methods put severe demands on the detection/identification systems. Which method is to be preferred in terms of immediate application and in terms of fundamental development work appears to depend strongly on the acceptable degree of miniaturization in the detection.

Open tubular varieties of LC open a window to separations with plate numbers in the range of $1 \cdot 10^6$ and higher. This capability, however, is only accessible when detection/identification methods are available that work with 100 pl or smaller volumes of solution. When such methods are not available, MEKC and ED-PC-LC methods, requiring a miniaturization that is 50 times less severe, are to be preferred. Their performance, however, degrades sharply when then a limit of $1 \cdot 10^6$ plates is approached.

Appendix 1

Symbols used

Symbol	Description	Value (c.q. for HPLC)	Unit
A	Numerical factor for convection term in reduced plate height equation	1.0	–

B	Numerical factor for diffusion term in reduced plate height equation	1.5	–
C	Numerical factor for mass transfer term in reduced plate height equation	0.05	–
D_m	Diffusion coefficient in mobile zone (i.e., inter-particle space)10^{-9}	$m^2 s^{-1}$	
d_p	Particle size	1–10	μm
D_p	Diffusion coefficient in stationary zone (i.e., particle or stationary layer)	10^{-10}	$m^2 s^{-1}$
e	Elementary charge (of electron)	$1.6 \cdot 10^{-19}$	C
E	Separation impedance, $h^2\phi$	3000	–
$F'(\nu)$	Dimensionless function describing resistance to mass transfer in stationary zone		
$F(\nu)$	Dimensionless function describing resistance to mass transfer in mobile zone		
h	Reduced plate height, H/d_p, or H/d_c	2	–
k	Boltzmann constant	$1.36 \cdot 10^{-23}$	J
k''	Retention factor (capacity), mass in stationary zone over that in mobile zone	5	–
k'	Retention factor (capacity), mass in stationary phase over that in mobile phase	3	–
l	Characteristic length, here either particle, size, d_p, or column diameter, d_c	1–10	μm
N	Theoretical plate number	10^3–10^6	–
N_{max}	Maximum attainable plate number	10^5	–
N_r	Required plate number 10^3–10^6	–	
T	Temperature	300	K
t_0	Retention time of unretained component	10–1000	s
t_R	Retention time	10^2–10^4	s
u_i	Velocity of analyte zone	1	mm s^{-1}
u_0	Velocity of mobile phase and unretained component	3	mm s^{-1}
V	Applied voltage in electrophoretic techniques	40	kV
z_i	Charge of ion ("valency")	1	–
γ	Obstruction factor for diffusion in packed bed	1.5	–
ΔP	Pressure drop over column	$2 \cdot 10^7$	Pa
η	Viscosity of the mobile phase	0.001	Pa s
μ_{EO}	Electroosmotic mobility	$70 \cdot 10^{-9}$	$m^2 s^{-1} V^{-1}$
μ_i	Eelectrophoretic mobility		$m^2 s^{-1} V^{-1}$
μ_{micel}	Electrophoretic mobility of micelles	$-30 \cdot 10^{-9}$	$m^2 s^{-1} V^{-1}$
ν	Reduced mobile phase velocity, $u_0 d_p/D_m$, or $u_0 d_c/D_m$	20	–
σ_t	Standard deviation in elution profile	1–300	s
τ_{eq}	Equilibration time in phase equilibrium	0.003	s
ϕ	Column resistance factor, numerical factor in expression for pressure drop	750	–

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