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Prospects of miniaturized separation systems

J.C.Kraak

Laboratory for Analytical Chemistry University of Amsterdam
Nieuwe Achtergracht 166, 1018 WV Amsterdam The Netherlands

Abstract: The prospects of using extremely small particles in liquid chromatography and of open tubular liquid chromatography will be discussed. The application of 1 µm non-porous particles for the separation of macromolecules by hydrodynamic chromatography will be shown. The practical realisation of open tubular liquid chromatography in 5-10 µm i.d. fused silica capillaries using polyacrylates as retentive layer and on-column UV detection will be demonstrated. The features of electrically driven liquid chromatography will be indicated.

INTRODUCTION

Since the first developments of gas and liquid chromatography, there is a contineous urge to reduce the dimensions of these separation techniques. Initially the diminution was necessary because the systems, in their first design, were largely oversized; technical limitations restricted the choice of small diameters for particles or columns. The efforts in the last three decades have the result that nowadays GC is performed exclusively in capillaries and that LC has become a popular high performance separation technique by the application of 3-10 μm particles. According to the theory of chromatography the ultimate performance of GC has been now closely realised but this is not the case yet for LC. In the latter technique significant improvements in performance can be expected by further decreasing the particle size down to 1 μm or performing LC in miniaturized open geometries e.g. a channel or capillary column. The prospects of these extreme miniaturization of the LC system will be discussed and the progress in the practical realisation will be demonstrated.

SEPARATION SPEED VERSUS EFFICIENCY

The dead time, t_o, of a chromatographic system is given by the following equation:

$$t_o = L/u = N.H/u = N^2.h^2.\phi.\eta/\Delta P \qquad (1)$$

where: L is the column length; u is the linear velocity; N is the plate number; H is the plate height; ΔP is the pressure drop; η the viscosity of the mobile phase; ϕ the permeability factor; h is the reduced plate height = H/d_p or H/d_c , in which d_p and d_c are the diameter of the particles and open column respectively.

The relationship between the separation speed and required plate number (the resolution) at a given maximum pressure drop can be conveniently visualized by plotting log H/u versus log N. Fig.1 shows such a plot for packed bed and open tubular columns. All plots show an asymptotic behaviour: N becomes constant at infinite H/u and H/u becomes constant at infinite small N.

At infinite H/u (separation speed) the plate height is predominated by the axial molecular diffusion. Under these conditions the ultimate plate

number is given by:

$$N = (\Delta P/B.\phi.\eta).d^2/D_{i_m} \qquad (2)$$

where: d is d_p or d_c ; $B = \Delta P/2.\phi.\eta$; D_{i_m} is the diffusion coeffient of the solute in the mobile phase.

At infinite small N the whole efficiency is sacrified for speed and the plate height is predominated by the slow mass transfer in stationary phase. Under these conditions the ultimate separation speed is given by:

$$H/u = C.d^2/D_{i_s} \qquad (3)$$

where: d is d_p or d_f (the thickness of the stationary layer)
C contains some geometrical factors and a k′ factor.
D_{i_s} is the diffusion coefficient of the solute in the stationary phase.

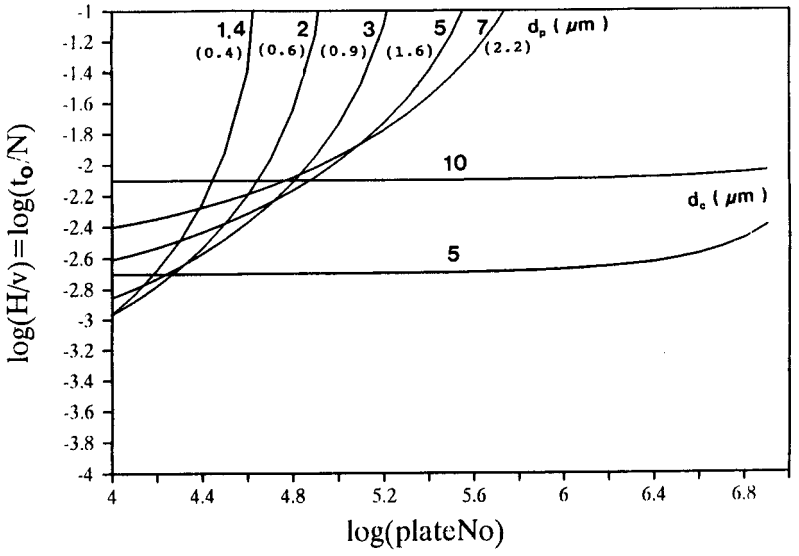

Fig.1 Plot of log H/u versus log N for pressure driven packed column (PC) and open tubular (OT) liquid chromatography. The figures in the parentheses are the d_p values for a larger solute with a ten times smaller D_{i_m}.
Plate height equation: $h = B/v + A.v^{0.33} + C.v$
Packed column: A = 1.5; B = 1.5; C = 0.05 ; k′=3; ΔP=400 bar
Open tubular column: A = 1.5; B = 1.5; C = 0.08; ΔP=400 bar

From the log H/u versus log N plot some important conclusions can be drawn:

- with packed bed LC very large plate numbers can only be realized with larger particles but at the cost of the separation speed.
- the ultimate plate number increases with decreasing diffusion coefficient of the solute in the mobile phase e.g.increases with increasing size of the solutes.
- the use of very small particles in LC is mainly attractive to speed up the separation of small solutes or to generate large plate numbers with macromolecules in a reasonable time.
- open tubular liquid chromatography (OTLC) in 5-10 μm i.d. is the method to generate very large plate numbers in an acceptable time.
- for non-interactive separation systems, such as hydrodynamic chromatography (HDC), the use of very small particles to separate macromolecules looks profitable.

It can be concluded that in pressure driven packed bed HDC and OTLC the largest improvements in performance can be expected when further miniaturizing the separation system i.e the particle size and column diameter. Therefore the prospects in these fields will be discussed in the next sections including the features of electrically driven liquid chromatography.

PROSPECTS OF OPEN TUBULAR LIQUID CHROMATOGRAPHY

In the development of OTLC in 5-10 μm i.d. fused silica capillaries one is faced with some serious practical problems connected with the extreme small dimensions of the column (1). The main bottle necks are the lack of sensitive detectors capable to detect pg amounts of solutes in a cell volume < nl and the fabrication of a uniform retentive layer with sufficient sample capacity on the inside surface of the capillary. The extreme small peak volumes necessitates the application of on-column detection such as laser induced fluorescence (LIF) and UV. LIF is very sensitive but is limited to fluorogenic compounds. The more universal UV should be the detection method of choice but the concentration sensitivity is poor because of the very small optical path in the 5-10 μm i.d. capillaries. This means that relatively large solute concentrations have to be injected with the risk of overloading the column and thus destroying the efficiency. Therefore it is very important to realize a thick retentive layer with sufficient sample capacity to preserve the efficiency. Two ways of preparing a retentive layer in fused silica capillaries have been tested: i) the fabrication of a porous silica layer on the wall followed by chemical modification of the layer. ii) polymeric layers such as silicones and acrylates. The sample capacity of the porous silica layers appears to be too small to apply UV detection. The most promising retentive layers are presently the polyacrylate films.

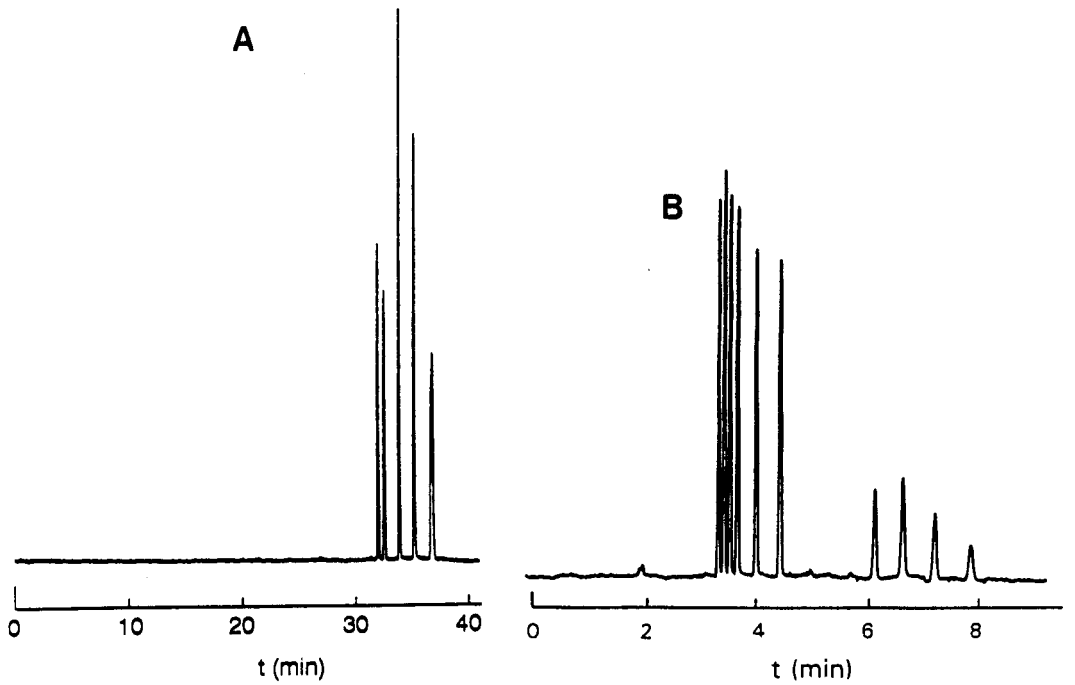

Fig.2 Separation of anthracene derivatives (A) and OPA derivatized alkylamines (B) by OTLC using LIF detection.
A) 5m x 10 μm i.d. coated with ethoxyethylacrylate, d_f = 1,5 μm
B) 1.2m x 5 μm i.d. coated with ethoxyethylacrylate, d_f = 0,75 μm

These films can be fabricated by in situ photopolymerisation of a mixture of siliconeacrylate and alkylacrylates (2,3). Uniform films with a thickness upto 1.5 μm can be realized resulting in a phase ratio of about 1. The layers are very stable, can be used in the reversed phase as well as in the normal phase mode and show a very large sample capacity allowing UV detection. The performance of the acrylate coated columns is demonstrated in Fig.2 showing the separation of test mixtures on a 5m x 10 μm i.d. column (A) and on a 1.2m x 5 μm i.d. column (B) using LIF detection. On the long column more than one million plates are generated.

PROSPECTS OF PACKED BED HYDRODYNAMIC CHROMATOGRAPHY

HDC, also named separation by flow, is a one phase separation method (4,5). In this technique use is made of the parabolic flow profile, as occuring in open tubes and in the interstitial space between particles, to separate macromolecules according to their size. The separation is basically due to exclusion of the solutes from the velocity flow regions near the wall (see Fig.3).

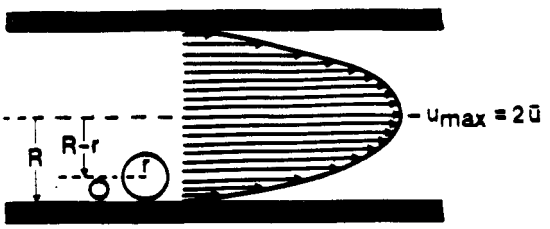

Fig.3 Schematic representation of the separation mechanisme in hydrodynamic chromatography

As large molecules are more excluded from the low velocity flow regions near the wall than smaller ones, they experience a higher mean velocity and thus will elute earlier than smaller molecules.

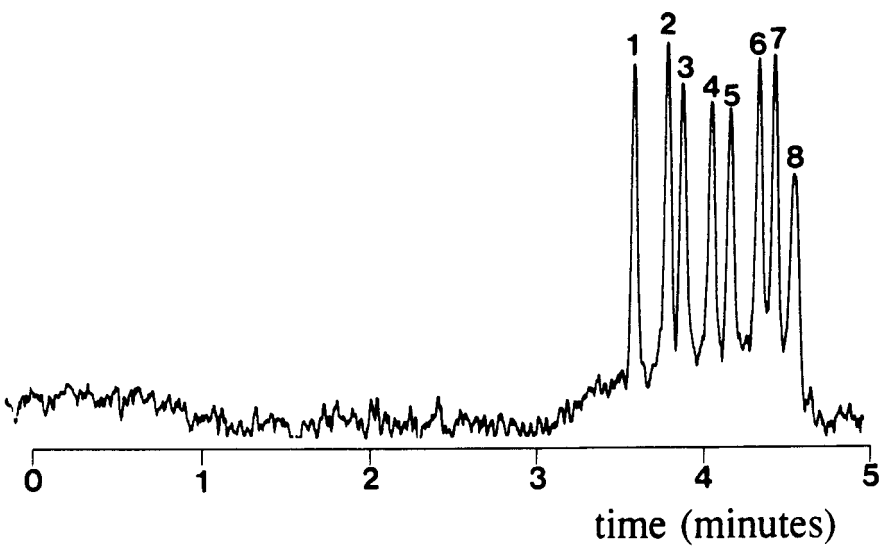

Fig.4 Separation of a mixture of polystyrenes (Mw 5.10^2 - 7.10^5) by HDC using 1 μm non-porous silica particles and THF as mobile phase. 1) PS 775000; 2) PS 435000; 3) PS 336000; 4) PS 160000; 5) PS 97000; 6) PS 34500; 7) PS 12000; 8) PS 550

In order to realize sufficient differences in migration rate between solutes, the ratio of the solute radius to that of the flow channels (the aspect ratio) should be sufficiently large (> 0.01). However, for too large aspects the solutes cannot migrate anymore through the flow channels. Therefore in practice the working range is limited to an elution window of 3/4 - 1 of the retention time of a small molecule. This means that the peak-capacity is limited and depends on the column efficiency. HDC in packed columns, preferable from the point of view of detection and sample capacity, is done with non-porous particles as packing material. When porous particles are used, the migration of macromolecules can be the result of a combined HDC and size exclusion chromatography (SEC) effect (6). Sofar it appears to be difficult to produce very small porous silica particles. However, non-porous silica particles can easily be produced in the range of 0.5 - 3 μm.
Since there is no mass transfer between two phases, the plate height in HDC is gouverned by longitudinal diffusion and Eddy diffusion:

$$H = D_{i_m}/u + 2\lambda d_p \qquad (4)$$

For macromolecules D_{i_m} is small and the second term dominates largely the plate height. This means that large plate numbers can be generated in an acceptable time because all solutes elute in front of the t_o. An example of a fast separation of a mixture of polystyrenes Mw range 5.10^2 - 7.10^5 on a 150 x 3 mm column filled with 1 μm non-porous particles can be seen in Fig.4. On this column 120.000 plates are generated. Such a fast separation in this molecular mass range is not possible with size exclusion chromatography.

ELECTRICALLY DRIVEN LIQUID CHROMATOGRAPHY

In this technique a potential gradient is applied across both ends of a packed or a coated open tubular capillary (7,8). Due to this potential gradient an electro-osmotic flow is generated towards one of the electrodes depending on the sign of the charge on the surface of the packing or layer.ghts with coresponding higher separation speed.

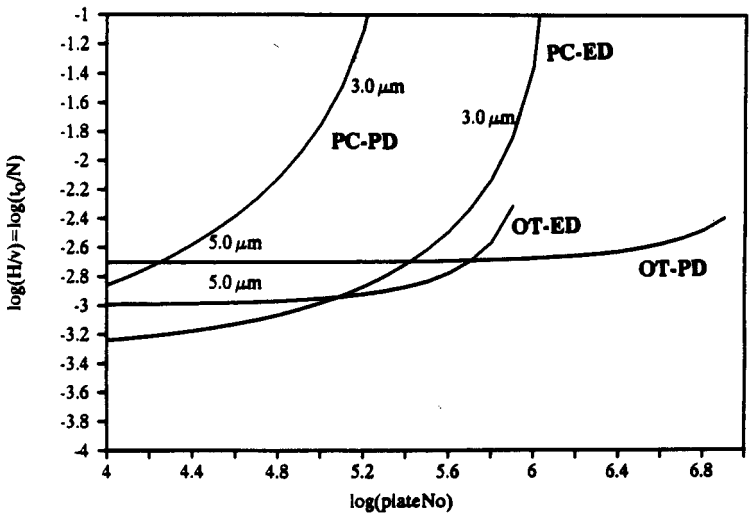

Fig.5 Plot of log H/u versus log N of pressure (PD) and electrically driven (ED) packed column (PC) and open tubular (OT) liquid chromatography.
Plate heigth equation: $h = B/\upsilon + A.\upsilon^{0.33} + C.\upsilon$
PC-PD: A = 1,5 ; B = 1.5 ; C = 0.05 ; ΔP = 400 bar
PC-ED: A = 0.75; B = 1.5; C = 0.05; V = 40.000; $\mu_{eo}=50.10^{-9}$ (m^2/Vs)
OT-PD: A = 1.5 ; B = 1.5 ; C = 0.08 ; ΔP = 400 bar
OT-ED: A = 1.5; B = 1.5; C = 0.04; V = 40.000; $\mu_{eo}=50.10^{-9}$ (m^2/Vs)

The use of endoosmosis as driving force to transport the mobile phase in liquid chromatography has some attractive features compared to pressure driven systems. With electrically driven systems the flow profile closely approaches that of plug flow and the electro-osmotic flow is independent of the channel width. Compared to pressure driven systems, where the flow profile is parabolic, endoosmotic flow results in smaller plate height with corresponding higher separation speed. This can be seen in Fig.5 showing the log H/u versus log N plot for pressure and electrically driven LC. The gain in efficiency and speed is impressive for packed capillaries and less for open tubular columns. Since the electro-osmotic flow is independent of the channel width, in principle capillaries filled with very small particles (< 1 μm) can be applied.

Initially the exploitation of electro-chromatography has been delayed because of bubble formation in the column. However, this problem is now satisfactorily solved by applying a small pressure on both electrode reservoirs (9).

CONCLUSIONS

The further miniaturization of the particle size in pressure driven liquid chromatography down to 1 μm or smaller is valuable when it is necessary to speed up the separation of a simple mixture of small solutes. For large molecules small particles are required to realize large plate numbers in an acceptable time.

The application of very small particles for the separation of macromolecules by hydrodynamic chromatography looks very promising as an alternative to size exclusion chromatography.

In theory open tubular liquid chromatography is the best geometry to apply liquid chromatography. Promising results are now realized on 5-10 μm capillaries using polyacrylate films as retentive layer. In order to approach the ultimate performance of liquid chromatography the application of this method to smaller diameters and mass spectrometric detection is one of the challenges in the coming years.

The use of endoosmosis to transport the mobile phase in packed capillaries opens exciting possibilities and research in that field should be given a high priority.

The advantages of electrically driven OTLC are small. Attractive is the simplicity of injection of ultra small samples compared to pressure driven OTLC were split injection has to be applied.

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