Short communication

Relationship between retention, linear velocity and flow for counter-current chromatography

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Abstract

A recent paper by Du et al. [*J. Chromatogr. A*, 835 (1999) 231] showed a very good correlation between the retention of stationary phase and the square root of mobile phase flow ($F^{1/2}$) for 12 different phase systems in counter-current chromatography. This paper shows there is a relationship between the above retention and the linear velocity of the mobile phase. In this way, Du et al.'s results can be related to the kinematics of the mobile phase flow in the tubing. This will open the door for further engineering analysis of this fluid dynamic phenomenon. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Counter-current chromatography (CCC) is becoming widely used as a versatile method of purifying a variety of materials [1]. Its liquid–liquid nature makes it particularly suitable for new drug discovery where 100% sample recovery is important and its ability to be easily scaled up is a distinct advantage. The stationary phase in CCC is liquid and is only retained against the flow of mobile phase due to the Archimedean action from multilayer helical coils of tubing constrained to rotate in planetary motion. Clearly retention of the stationary phase is essential if liquid–liquid chromatography is to take place. The importance of the paper by Du et al. [2] is that by defining a linear relationship between retention and the square root of flow, only two retention tests are required at different flow-rates to predict the optimum flow to achieve maximum throughput for a given retention. This is particularly important for scale up when each test uses very large volumes of solvents.

This paper builds on the results of Du et al.'s work and uses their results to show that not only is there a linear relationship between retention and the square root of flow, but there is a linear relationship between the square of the mobile phase linear velocity and flow. This discovery opens the door for a fluid mechanics approach to the analysis of mobile phase flow.

2. Theory

Du et al.'s linear regressions [2] approximate to:

$$S_f = 1 - CF^{1/2} \tag{1}$$

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where F is the volume flow in ml/min, C is a constant and S_f is the retention expressed as the volume of the stationary phase over the total coil volume (V_s/V_c) and not as a percentage (i.e., $S_f = 1$ is equivalent to 100% retention). Rearranging Eq. (1):

$$CF^{1/2} = (1 - S_f) = (1 - V_s/V_c) = V_m/V_c \qquad (2)$$

where V_c is the sum of the stationary (V_s) and mobile phase (V_m) volumes.

The mean linear velocity of the mobile phase through the system would be F/A if there was no retention, where A is the cross sectional area of the tubing. Assuming the volume retained (V_s) remains constant, then the mean linear velocity will be increased by the ratio of the total coil volume to the volume of the mobile phase (V_m) as follows:

$$v = F/[60A(1 - S_f)] = F/60A \cdot V_c/V_m \qquad (3)$$

substituting for V_m/V_c from Eq. (2) gives:

$$CF^{1/2} = F/60Av \qquad (4)$$

Dividing through by $F^{1/2}$ (assuming $F \neq 0$) and rearranging then:

$$v = F^{1/2}/60CA$$

or

$$v^2 = BF \qquad (5)$$

where $B = (1/60CA)^2 = $ constant.

Another relationship that emerges from this is that linear velocity is proportional to mobile phase volume (eliminating $F^{1/2}$ between Eqs. (2) and (5)) giving:

$$v = (1 - S_f)/(60C^2A) = V_m/(60V_cC^2A)$$

or

$$v = DV_m \qquad (6)$$

where $D = 1/(60V_cC^2A) = $ constant.

The importance of Eq. (5) is that it relates to the kinetic energy term in Bernoulli's equation for inviscid and non-compressible flow where:

$$p + \rho v^2/2 + \rho gh = \text{constant along a streamline} \qquad (7)$$

where p is the pressure term, $\rho v^2/2$ the kinetic energy term and ρgh the potential energy term.

3. The variation of mobile phase linear velocity with flow

The mean linear velocity (v) for a given retention from Du et al.'s results [2] was calculated using Eq. (3). Using the measured densities of Du et al.'s lower mobile phase from Table 1, the Bernoulli kinetic energy (KE) term ($1/2\rho_l v^2$) was calculated for the hexane–ethyl acetate–methanol–water phase systems from Du et al.'s flow and retention values [2]. This Bernoulli KE term was then plotted against flow (F) in Fig. 1. It can be seen that in all cases there is a linear relationship with the coefficient of linear regression (R^2) generally greater than 0.97. The linear regression equations are listed in Table 1. Fig. 2 plots the relationship between linear velocity (v) and the volume of mobile phase (V_m) after Eq. (6) for Du et al.'s hexane–ethyl acetate–methanol–water phase systems. Similar high correlation linear regressions are shown (Table 2). Similar linear relationships have been found for Du et al.'s other phase systems and independently using different

Table 1
Regression analysis between Bernoulli's kinetic energy term ($\rho_l v^2/2$) and flow (ml/min)

| Solvent phase system	Density of lower phase (ρ_l)	Bernoulli's KE term ($\rho_l v^2/2$)	Correlation coefficient (R^2)
Hexane–ethyl acetate–methanol–water (1:9:1:9)	0.980	$= 0.548F + 0.0982$	0.999
Hexane–ethyl acetate–methanol–water (2:8:2:8)	0.968	$= 0.504F - 0.0613$	0.999
Hexane–ethyl acetate–methanol–water (4:6:4:6)	0.932	$= 0.590F + 0.0625$	0.995
Hexane–ethyl acetate–methanol–water (5:5:5:5)	0.919	$= 0.784F - 0.181$	0.991
Hexane–ethyl acetate–methanol–water (6:4:6:4)	0.897	$= 1.157F + 0.319$	0.995
Hexane–ethyl acetate–methanol–water (8:2:8:2)	0.848	$= 2.384F - 0.994$	0.999
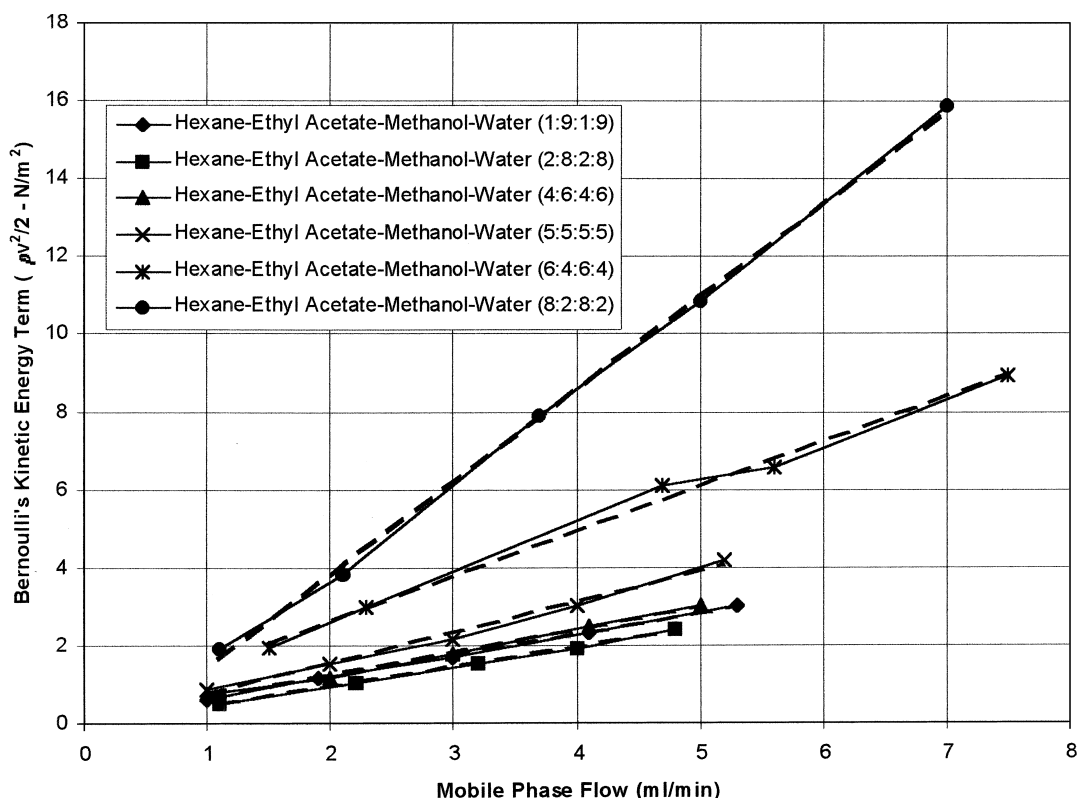
Fig. 1. The variation of Bernoulli's kinetic energy term ($\rho_l v^2/2$) versus flow for hexane–ethyl acetate–methanol–water phase systems calculated from the results of Du et al. [2].

equipment and different phase systems in CCC phase distribution studies at Brunel.

4. Conclusions

It is important to take the above results into account when performing any new work on CCC. Experiment time can be greatly reduced by knowing that there is a linear relationship between retention (S_f) and the square root of flow ($F^{1/2}$). Only two retention tests are needed for retention at other flow-rates to be calculated.

The fact that there is a well-correlated relationship between the Bernoulli kinetic energy term and flow supports the theory proposed in Eq. (5). The theory so far assumes that Du et al.'s linear regressions pass through 100% at zero flow and that the Bernoulli

term passes through the origin at zero flow. The constant terms in the regressions largely support this, but with some phase systems (not presented) it has been noticed they do not and that there would need to be some non-linearity at low flow for this to occur.

In future it would be worth everyone using CCC plotting a Du et al. retention against flow regression for an agreed phase system as a performance standard. There is a simple relationship [$B = (1/60CA)^2$] between the slope of Du et al.'s retention–flow regression [the constant C in Eq. (1)] and the slope of the $1/2\rho_l v^2$ verses flow regression [the constant B in Eq. (5)]. The slopes of these retention–flow or Bernoulli's kinetic energy–flow regressions will give a good index for comparison between different CCC instruments and may be used to compare their relative performance.
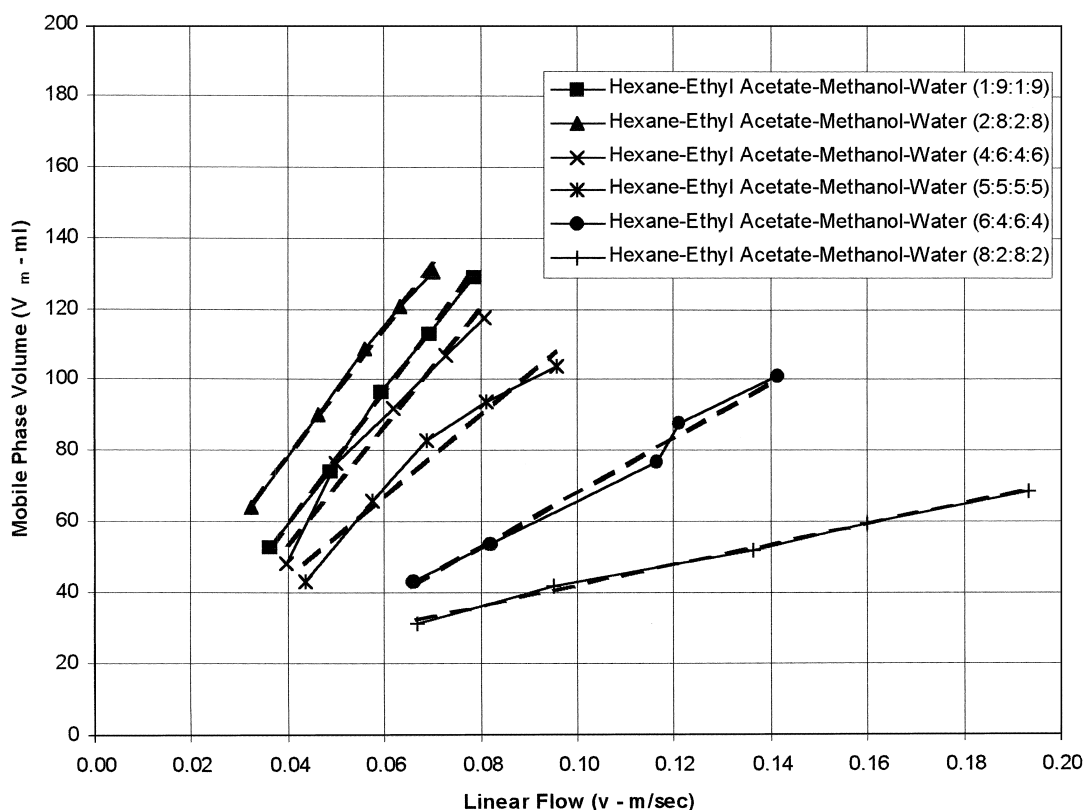
Fig. 2. The variation of mobile phase volume (V_m) versus linear flow (v) for hexane–ethyl acetate–methanol–water phase systems calculated from the results of Du et al. [2].

5. Nomenclature

"Head"   The end of the tubing to which a bubble or bead would move under the action of Archimedean screw action

"Tail"   The opposite end of the tubing to the "Head"

Symbols

A	Tubing cross-sectional area (mm^2)
B, C, D	Constants defined in text
d	Internal diameter of tubing (mm)
F	Flow-rate (ml/min)
h	Hydrostatic pressure head (m)
l	Length of tubing (m)

Table 2
Regression analysis between mobile phase volume (V_m, ml) and linear velocity (v, m/s)

Solvent phase system	Mobile phase volume (V_m, ml)	Correlation coefficient (R^2)
Hexane–ethyl acetate–methanol–water (1:9:1:9)	$=1824v - 13.35$	0.998
Hexane–ethyl acetate–methanol–water (2:8:2:8)	$=1770v + 7.86$	0.996
Hexane–ethyl acetate–methanol–water (4:6:4:6)	$=1615v - 10.36$	0.973
Hexane–ethyl acetate–methanol–water (5:5:5:5)	$=1165v - 2.92$	0.963
Hexane–ethyl acetate–methanol–water (6:4:6:4)	$=770.8v - 8.57$	0.985
Hexane–ethyl acetate–methanol–water (8:2:8:2)	$=291.5v + 12.73$	0.995

ρ	Density
ρ_l	Density of mobile lower phase (g/ml)
μ	Viscosity
S_f	Retention of stationary phase (%)
v	Linear velocity of mobile phase (m/s)
V_c	Coil system volume (ml)
V_m	Volume of mobile phase in coil system (ml)
V_s	Volume of stationary phase in coil system (ml)

References

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