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COATED-OPEN-TUBULAR CHROMATOGRAPHY WITH FLOW SEGMENTATION

I. THEORY

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SUMMARY

A new separation technique is described: capillary liquid chromatography with segmentation of the mobile phase by air-bubbles. A theory is developed for the dependence of separation efficiency (plate height H) of this new procedure on different experimental variables. Calculations based on this theory suggest that segmented-flow liquid chromatography (LC) is potentially much more efficient than other capillary LC systems, when experimental conditions are the same. Compared to present packed columns, segmented-flow LC is relatively inefficient. As discussed in the following paper, its main application should be for the pretreatment of samples prior to conventional LC analysis.

INTRODUCTION

Liquid chromatography (LC) in packed columns is now reasonably well understood, and it is possible to predict the performance of "good" columns within narrow limits, as a function of particle size, mobile phase velocity, column length, sample type, etc.[1]. During the past few years, the use of LC with coated open-tubular columns ("capillary LC"), has received increasing attention. Possible objectives of capillary LC include: (1) increased column efficiency or separation speed *versus* packed-column LC; (2) ability to handle very small samples, for the case where sample size is limited, or where small separated fractions are desired for subsequent on-line analysis by mass spectrometry; (3) as a pretreatment procedure for the partial separation of complex samples, prior to subsequent final separation and analysis of selected sample fractions by conventional LC (or other means).

The continuing hope that capillary LC might provide higher column plate numbers than for packed-column LC is based to some extent on the performance of capillary gas chromatography (GC). Following earlier applications of capillary LC[2–4], however, it was soon widely appreciated that mobile phase mass transfer is much more limiting in LC than in GC, due to the 10^4–10^5 slower diffusion of solute molecules in liquids *versus* gases. This effect in capillary LC can in principle be overcome by reducing the internal diameter d_t of the capillary, so as to reduce the diffusion distance

between mobile and stationary phases. Hibi *et al.*[5] have demonstrated a resulting improvement in separation efficiency by use of capillaries with internal diameters of 50 μm and smaller. However, the experimental difficulty in working with such fine capillaries is pronounced: they are easily plugged, they are difficult to construct and to coat, and the remaining LC instrumentation must be miniaturized so as to reduce sample size, mobile phase flow-rate and detector flow-cell volumes by orders of magnitude (*versus* conventional LC equipment). Finally, reported separations of retained compounds ($k' \geqslant 2$) are thus far inferior to those obtainable with good packed columns.

An alternative approach to improving the efficiency of capillary LC columns is by breaking up the laminar flow pattern that prevails under normal LC conditions in straight capillaries. Coiling of the capillary provides secondary flow which serves to reduce significantly column plate heights H[6–9]. However, the resulting column plate numbers are still modest when compared to conventional packed LC columns, and the advantage of coiling the column decreases with decrease in column diameter. An increase in mobile phase velocity eventually results in turbulent flow, with a dramatic lowering of plate height; however, such an approach to capillary LC would involve prohibitive pressure drops across the column[10]. Crimping or other distortion of the capillary cross-section allows a significant decrease in column plate height[11], but the ability of such capillary LC systems to provide improved plate numbers N has not yet been demonstrated.

In this paper we will explore still another means for disrupting the laminar flow pattern in capillary LC, and thereby improve separation efficiency. Our approach is segmented-flow capillary LC (SF-LC), which was first described a few years ago[12]. It has been known for 20 years that sample dispersion during flow of a liquid stream through an open tube can be greatly reduced by air-segmentation, and thisconcept forms the basis of the widely used continuous-flow-analysis AutoAnalyzer™ systems[13,14]. It can therefore be expected that capillary LC with air-segmentation of the moving liquid will be more efficient than in the absence of air-segmentation. Here, we will develop a preliminary theory for this new separation technique, and draw a few comparisons with capillary LC (without segmentation), and with conventional packed-column LC. In following papers we will compare the results of experimental studies with the theory of SF-LC[15], and we will demonstrate the applicability of SF-LC as a general technique for sample pretreatment, prior to conventional LC analysis or other handling of the sample.

THEORETICAL

Retention in SF-LC

We will assume a length L (cm) of tubing of internal diameter d_t (cm), coated with a uniform layer of stationary phase of thickness d_f. The latter may be variously:

(1) a film of liquid that is immiscible with the mobile phase and wets the walls of the capillary;

(2) a polymeric or gel layer;

(3) a layer of compacted, porous particles.

In Part II of this series[15], we report data on capillaries for size-exclusion chromatography, where the coating is a gel layer of type 2 above. In the following discus-

sion we treat the specific characteristics of this type of stationary phase, and will assume (which is not strictly true) that the total volume of the gel is permeable by small molecules. That is, the gel volume is regarded as equivalent to an identical volume of mobile phase which is contained within the gel.

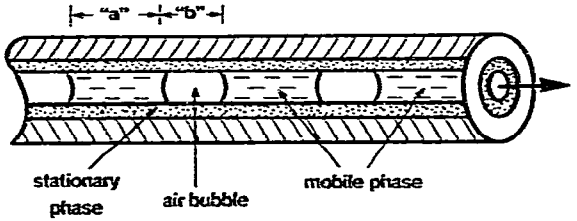

Fig. 1. Visualization of SF-LC.

The capacity factor k' in SF-LC will be defined so as to equal the k' value measured in an identical unsegmented (us) capillary LC system; *i.e.*,

$$k' = (m_s/m_m)_{us} \tag{1}$$

Here, m_s refers to the total mass of solute in the stationary phase and m_m is the corresponding mass of solute in the mobile phase. The quantity m_m in eqn. 1 is also equal to $V_m C_m$, where V_m is the volume of mobile phase within the column, and C_m is the average concentration of solute in the mobile phase. In SF-LC, the volume V_m is composed both of mobile phase and air-bubbles, as illustrated in Fig. 1 for a section of the capillary. Non-volatile solutes will not be distributed into the air-space of the bubbles, so the effective volume of liquid within the column is not V_m, but $V_m[F_m/(F_m + F_a)]$. Here, F_m and F_a refer to the flow-rates (ml/sec) of mobile phase and of air entering the column. The apparent capacity factor k_{ap} can be defined for SF-LC, just as for conventional LC[1]:

$$k_{ap} = (t_R - t_0)/t_0 \tag{2}$$

Here, t_R is a measured retention time for the band of interest, and t_0 is the column dead-time, which can be measured in SF-LC by determining the time required for a given air-bubble to pass through the column. Since air-bubbles are ineffective in effecting the migration of solute molecules down the column, the apparent capacity factor k_{ap} is related to k' as:

$$k_{ap} = k'(F_m + F_a)/F_m \tag{3}$$

This increase of k_{ap} over k' can be better visualized from the example of Fig. 1. Where stationary and mobile phase elements are adjacent (*e.g.*, "a" in Fig. 1), the local capacity factor for this part of the column will be equal to k', just as in an unsegmented system. Where stationary phase and an air-bubble are contiguous (as in "b" of Fig. 1), the solute will be almost totally in the stationary phase*, and the

* Since a thin film of mobile phase separates the air-bubbles from the inside surface of the stationary-phase film[16], a small amount of solute will be in the mobile phase in region "b" of Fig. 1.

local capacity factor will be quite large. Therefore the average capacity factor (or k_{ap} in eqn. 2) will be greater than k'.

The column dead-time t_0 in SF-LC can also be defined as

$$t_0 = V_m/F \tag{4}$$

where F is the total flow (ml/sec) of air plus liquid through the capillary ($F = F_m + F_a$). V_m is here defined as the volume of the mobile phase plus air within the capillary, *exclusive* of any mobile phase contained within the stationary phase layer (*e.g.*, as in gel or compacted-particle layers). The volume V_m is given by

$$V_m = (\pi/4)\, d_t^2 L \tag{5}$$

For the case where the film thickness d_f is significant in comparison with d_t, it is useful to take d_t as the diameter of the mobile phase stream. Thus, if d_t^0 refers to the diameter of the uncoated capillary, then

$$d_t^0 = d_t + 2d_f \tag{5a}$$

The quantity t_0 is also the retention time of a solute that does not penetrate the stationary-phase layer. If such a solute is injected into a single liquid segment that then enters the column, the total mass of solute will remain in that segment during its passage through the capillary*.

The retention time t_R of a retained band can be obtained from eqn. 2:

$$t_R = t_0(1 + k_{ap}) \tag{6}$$

Note that it is k_{ap}, and not k', that directly determines retention in SF-LC. The retention volume V_R is similarly derivable as

$$V_R = V_m(1 + k_{ap}) \tag{6a}$$

Here, V_R refers to the volume of air plus liquid required to elute the band.

In a following paper[15], values of k' are used to estimate the film thickness d_f in agarose-coated capillaries. If the film volume (for the entire capillary) is V_f, then it is assumed for a molecule that totally permeates the agarose film that

$$k' = V_f/V_m \tag{7}$$

We have previously noted this approximation and its significance above. Furthermore,

$$V_f/V_m = (\pi d_t d_f L)/(\pi/4)\,(d_t^2 L) \tag{7a}$$

so that from eqns. 7 and 7a we have

$$d_f = k' d_t/4 \tag{8}$$

* This overlooks (again) the thin film of mobile phase that separates the stationary phase from the air-bubbles; however, its effect on solute retention can normally be ignored in SF-LC (see ref. 6).

Since we know d_t^0, rather than d_t, combination of eqn. 8 with eqn. 5a gives a more useful relationship for d_f:

$$d_f = k' d_t^0/(4 + 2k') \tag{9}$$

Finally, in the following section the retention parameter R is used:

$$R = 1/(1 + k') \tag{10}$$

or

$$(1 - R) = k'/(1 + k') \tag{10a}$$

Column efficiency in SF-LC

We will argue that the dispersion of sample bands during SF-LC is essentially similar to the dispersion of bands during flow of segmented liquid through an *uncoated* tube. The latter phenomenon has been treated previously[16–18], and a semi-rigorous model for this process has been derived. Application of that model to experimental data for a wide range in experimental conditions (uncoated tubes) predicts bandwidths within $\pm 10\%$ (1 S.D.), suggesting a reliability for calculation of H values (plate heights, cm) in SF-LC within $\pm 20\%$ (since $H = \sigma^2/L$).

For the uncoated tube case, retention of sample was shown to be related to the volume of liquid film V_f laid down on the inside of the tube wall, as a result of passing a single liquid segment through the tube (with conditions otherwise equivalent to the passage of segmented liquid through the tube). Net retention of sample (measured in number of liquid segments) q was derived as

$$q = V_f/V_s \tag{11}$$

where V_s is the volume of a single liquid segment. Net retention in time units is then simply the retention in volume units (mobile phase only), divided by the flow-rate of mobile phase F_m:

$$t_R - t_0 = qV_s/F_m \tag{12}$$

eqns. 2 and 12 then yield

$$k_{ap} = qV_s/F_m t_0 \tag{12a}$$

and eqns. 3 and 12a give

$$\begin{aligned} k' &= qV_s/t_0(F_m + F_a) \\ &= qV_s/t_0 F \end{aligned} \tag{12b}$$

Finally, from eqns. 4 and 12b,

$$k' = qV_s/V_m \tag{12c}$$

and eqns. 12c and 11 give

$$k' = V_f/V_m \tag{13}$$

In flow through uncoated tubes, as described in refs. 15, 16, it does not matter whether the film of volume V_f is mixed with adjacent liquid segments as these segments overtake the film during passage through the tube, or whether the film is considered to remain intact (*i.e.*, to maintain its identity, distinct from the adjacent liquid segment). What is important is that in either case the solute initially dissolved in the film is rapidly distributed into the adjacent (moving) liquid. Mixing of film and segment corresponds more closely to the case of flow through uncoated tubes, while non-mixing resembles the case of SF-LC. In either process, retention and k' are defined by eqn. 13, providing that (1) the liquid in the coating or film is the same as the moving liquid phase; (2) V_f refers in each case to the volume of this liquid in the film or coating and (3) no other retentive phases are contained within the coating. All of these conditions are met, for example, if the stationary phase in SF-LC is a size-exclusion material (as discussed further in ref. 15).

The above analogy between uncoated and coated tubes breaks down for (1) positive retention within the stationary phase film (*i.e.*, q is larger than given by eqn. 11) and/or (2) large values of k'. Positive retention applies when the stationary phase consists of a sorbing material; *e.g.*, adsorbent, partitioning (different) liquid, ion exchanger, etc. In this case, k' is larger than given by eqn. 11, which is mathematically equivalent to a larger value of V_f. The mathematical comparability of coated and uncoated tubes is maintained, however, if the quantity V_f/V_m from the uncoated-tube model is simply replaced by the experimental value of k', wherever V_f/V_m appears in the original derivation of refs. 16–18.

The derivation of ref. 16 also assumes that the film thickness in flow through uncoated tubes is small, and therefore $k' \ll 1$. We will see below, that for the case of larger values of k' (in SF-LC), the quantity k' is then replaced by $k'/(1 + k')$.

Dispersion in uncoated tubes was shown earlier to be defined by a total variance σ^2 which is the sum of two parts:

$$\sigma^2 = \sigma_t^2 + \sigma_r^2 \tag{14}$$

The terms σ_t^2 and σ_r^2 are due to so-called "ideal" and "slow-mixing" contributions, with σ, σ_t and σ_r all measured in units of segment-number. For the more common measure of variance σ_x in units of distance along the tube, we can write (from eqn. 14)

$$\sigma_x^2 L_s^2 = \sigma_{x,t}^2 L_s^2 + \sigma_{x,r}^2 L_s^2 \tag{14a}$$

Here, L_s refers to the length of a single liquid segment, and $\sigma_{x,t}$ and $\sigma_{x,r}$ are equal to $\sigma_t L_s$ and $\sigma_r L_s$, respectively.

If the height equivalent of a theoretical plate is defined[19],

$$H = \sigma_x^2/L \tag{14b}$$

similar expressions can be written for ideal and "slow-mixing" contributions to H (H_t and H_r, respectively), so that

$$H = H_t + H_r \tag{14c}$$

In ref. 15 the quantity σ_t^2 was derived as

$$\sigma_t^2 = q \tag{15}$$

which when combined with eqn. 12c and the definition of H_t ($\sigma_{x,t}/L$) and $\sigma_{x,t}$ ($\sigma_t L_s$) gives

$$H_t = L_s k' \tag{15a}$$

Eqn. 15a is approximate, because we have assumed that k' is small. For the general case, which yields eqn. 15a as a limiting expression for small k', we can use the usual model for dispersion during Craig counter-current distribution. The latter model is equivalent to the case of SF-LC, when motion of the liquid segments down the tube proceeds by rapid "jumps", as illustrated in Fig. 2. For the Craig model, it has been shown[20] that

$$\begin{aligned} N &= (V_R/\sigma_v)^2 \\ &= (n + 1)(k' + 1)/k' \end{aligned} \tag{15b}$$

Here, V_R is the retention volume, σ_v^2 is the band variance in volume units, and n is the number of equilibrium stages in the distribution. Since rapid mass transfer is assumed between "jumps", we can equate H_t with L/N as defined in eqn. 15b. Further assuming that n is large (so that $n + 1 \approx n$), we then have

$$H_t = (L/n)\, k'/(1 + k') \tag{15c}$$

In the Craig model there are no air-bubbles, just mathematical boundaries between contigous liquid segments, so that $L_s = L/n$. With this, eqn. 15c then becomes

$$H_t = L_s k'/(1 + k') = L_s(1 - R) \tag{16}$$

Eqn. 16 is our final expression for H_t, which for small k' becomes equal to eqn. 15a.

The liquid-segment length L_s can be calculated from the residence time t_0 of

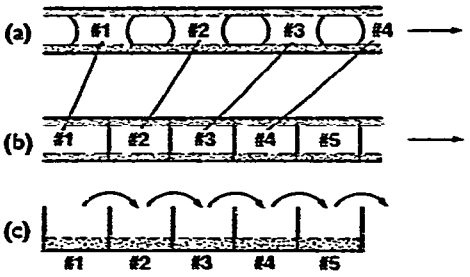

Fig. 2. Similarities in the Craig distribution process and SF-LC and derivation of the segment-length term H_t. (a) SF-LC, (b) equivalent description of SF-LC (since air-bubbles do not take part in separation), (c) Craig distribution.

bubbles in the column, and the bubble-segmentation rate n (sec^{-1}). Thus, the total number of air-bubbles in the column will be nt_0, and the combined length of an air-bubble plus liquid segment will then be L/nt_0. The fraction of this length due to the liquid segment is then $F_m/(F_m + F_a)$, or

$$L_s = (L/nt_0)\, F_m/(F_m + F_a) \tag{16a}$$

Consider next the mobile-phase mass-transfer term H_r. It was shown[17] that this is related to the mobile-phase mass-transfer H value for slug flow as derived by Giddings (eqns. 4.5–18 of ref. 19):

$$H_{slug} = (1 - R)^2\, d^2u/16\, D_m \tag{17}$$

However, the tube diameter d_c in eqn. 17 must be replaced by $2\,d_t/3$ for segmented-flow, and the sample diffusion coefficient D_m must be replaced by a mass transfer coefficient D'_m. With these substitutions for SF-LC, we then have

$$H_r = (1 - R)^2\, d_t^2u/36\, D'_m \tag{18}$$

The quantity u is the mobile phase linear velocity (cm/sec), equal to L/t_0. Values of D'_m are compared with D_m in Table I.

TABLE I

DEPENDENCE OF D_m AND D'_m ON SAMPLE MOLECULAR WEIGHT

Sample mol.wt.	D_m*	D'_m**
200	$0.88 \cdot 10^{-5}$	$4.9 \cdot 10^{-5}$
2000	0.23	2.5
20,000	0.06	2.1

* From Wilke–Chang equation[1].
** From ref. 17.

Eqn. 14 assumes rapid mass transfer in and out of the stationary phase film. In the general case, where this may not be true, eqn. 14c is expanded to

$$H = H_l + H_r + H_s \tag{19}$$

where H_s has been given by Giddings[19] as

$$H_s = (2/3)\, R(1 - R)\, d_f^2u/\gamma D_s \tag{20}$$

The quantity γ is an obstruction factor, which can be assumed equal to one for liquid or gel stationary phases, and to 0.6 for compacted particles. D_s is the diffusion coefficient of solute molecules within the stationary phase.

Eqns. 16, 18–20 define column efficiency for SF-LC systems, when no further complications exist. Values of H predicted from these relationships therefore re-

present limiting column performance in SF-LC. In a following paper[15], we will see that preliminary examples of SF-LC show lower values of N than predicted in this fashion, and the reasons for these derivations from simple theory will be further explored there.

Longitudinal diffusion of sample along the capillary is unimportant as a contribution to H in SF-LC. First, this effect is normally unimportant in capillary LC without segmentation, because of the high mobile phase velocities normally used, and the relatively large values of H. Second, the air bubbles largely block any longitudinal diffusion in the mobile phase, limiting such diffusion to the stationary phase.

Pressure drop in SF-LC systems

For flow of an unsegmented liquid through an open tube, the pressure drop P across the tube is given by the Hagen–Poiseuille equation:

$$P = (128/\pi)\,\eta LF/d_t^2 \tag{21}$$

Here, η is the viscosity (Poise) of the mobile phase, and F is the liquid flow-rate (ml/sec). A similar equation for P in SF-LC systems does not exist. Various workers have examined pressure drop for segmented flow through tubes of relatively large diameter[21,22], and have found a rather complex relationship for P as a function of experimental variables. Generally, the value of P found in segmented-flow systems is larger than that given by eqn. 21, due to the additional work involved in bolus *versus* laminar flow.

We have examined the dependence of P in SF-LC over a limited range in experimental conditions. Data for a 206 × 0.095 cm tube ($d_t^0 = 0.10$, $d_f = 0.0025$ cm) are given in Fig. 3. Values of n' are shown for each curve; n' is the air-segmentation rate (bubbles/cm). As n' increases, it is seen that P increases also. While the dependence of P on u (and F) is linear for unsegmented flow ($n' = 0$ in Fig. 3), as

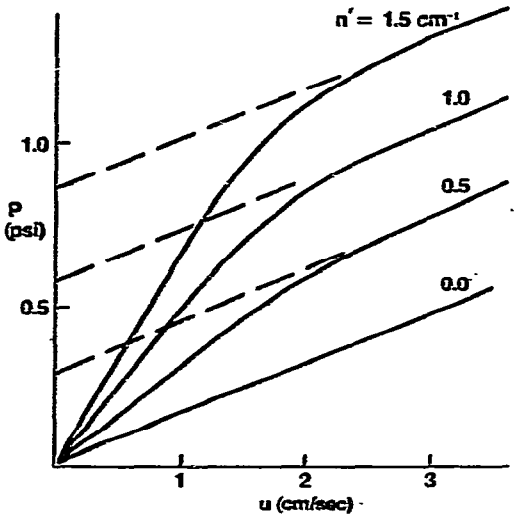

Fig. 3. Pressure drop in segmented flow. A 206 × 0.1 cm coated tube was used, with indicated segmentation rates n' (per cm, rather than per sec).

predicted by eqn. 21, the *P vs. u* curves for segmented flow tend to bend at values of u greater than 1–1.5 cm/sec in Fig. 3, and to approach limiting slopes at higher u values which are the same as for $n' = 0$. Extrapolation of the latter limiting slopes (dashed lines in Fig. 3) give pressure increments at $u = 0$ which are approximately proportional to n'.

The form of the *P versus u* plots in Fig. 3 for higher segmentation rates n' appears to contribute to flow instability for segmented systems. The higher pressure drops combined with the greater compressibility of segmented liquids with large n' leads to the storage of significant energy within the flowing stream, energy that can be disippated by surging of the stream at any given time. Furthermore, the rapid drop in the *P vs. u* curves for large n' (see Fig. 3) means that there is less resistance to sudden increases in flow-rate at higher values of u, thus providing no natural barrier to surging of the liquid flow.

Other variables in SF-LC

Pulsatile flow is a characteristic feature of SF-LC systems. If a peristaltic pump is used for pumping liquid and air through the column, there is a temporary reduction in flow from the pump, each time the roller lifts off the pump tube. There is an additional tendency to variation in flow through the column, as a result of surging effects (see previous section). Normally the addition of surfactant to the mobile phase reduces the tendency for such surging, but it is still found to some degree, particularly at low flow-rates.

What is the effect of these variations in F with time, during an SF-LC separation? Generally, the major effect will be some increase in H and resulting decrease in column efficiency N. Thus, the form of eqn. 19 leads to the following dependence of H on u:

$$H = A + Cu \tag{22}$$

In the extreme case, imagine that the flow varies between two extremes: half the time liquid flows at a velocity of $2u$, while the remainder of the time u equals zero. The average flow velocity is then u. However, nothing happens while the flow of liquid is at rest, and this period can effectively be ignored. During the time liquid flows through the column, the value of u is doubled with respect to the average flow velocity, leading to a corresponding increase in H as given by eqn. 22.

A second consequence of such variations in F with time (surging) is an irregular injection of air-bubbles into the flowing stream, if the pump is not equipped with an air-bar, a device that provides for the regular insertion of air-bubbles (commonly at 2-sec intervals). With sporadic variation in the frequency of air-bubble injection, variation in L_s values for individual liquid segments results. This again causes an increase in H, although a theoretical analysis of this effect is somewhat complex.

Since surging, pulsing and irregular bubble-injection occur mainly at low flow-rates, the resulting increase in H over that predicted by eqn. 19 will be found mainly for small values of u. The consequences of these effects are discussed in the following paper, as well as means for their mitigation. Table II summarizes some characteristics of these three hydraulic effects.

TABLE II

CONTRIBUTIONS TO HYDRAULIC INSTABILITY

Effect	*Description*	*Cure*
Pulsing	A regular oscillation in the flow velocity leaving a peristaltic pump; in severe cases, the flow velocity can actually reverse during each oscillation; caused by roller lift-off, so that the oscillations coincide with the frequency of roller lift-off	Use several small-diameter pump-tubes; pulse-dampers can also be effective; run pump at higher rotation rates
Surging	A sporadic phenomenon in which the flow velocity through a coated tube suddenly increases by several-fold, for a few seconds; the frequency of surging is much lower than for pulsing	Use air-bar; lower surface tension of liquid as much as possible; use lower ratio of air to liquid; avoid very long tubes
Irregular bubble-pattern	Bubbles are not injected into liquid stream at precise intervals; therefore liquid segment length varies randomly; bubble breakup may also occur	Use air-bar; eliminate irregular surfaces (butt joints) in system; use surfactant; use proper injection fitting for insertion of air-bubbles

COMPARISONS OF SF-LC WITH OTHER FORMS OF LIQUID CHROMATOGRAPHY

It is useful at this point to compare the theoretical potential of SF-LC with what can be expected of other forms of LC. While we will see in later papers (*e.g.*, ref. 15) that the theoretical performance of SF-LC is not easily attained in practice, at least such comparisons can indicate whether the eventual optimization of SF-LC is a worthwhile endeavor, and for what possible applications SF-LC might be most appropriate.

SF-LC versus capillary (unsegmented) LC

For unsegmented capillary LC, the plate height H_{us} can be expressed as the sum of mobile-phase and stationary-phase terms, H_m and H_s, respectively:

$$H_{us} = H_m + H_s \tag{23}$$

The mobile-phase term is given by the Golay equation[19]:

$$H_m = [(6R^2 - 16R + 11)/96]\,(1/D_m)\,d_t^2 u$$
$$= f(R)\,f(D_m)\,d_t^2 u \tag{24}$$

The stationary phase term H_s is given by eqn. 20.

Optimization of both SF-LC and capillary LC will involve minimization of the H_s term, as by the use of small values of d_f. Generally it is possible to make H_s small compared to either H_m or H_r. Similarly, optimization of SF-LC will involve the minimization of H_t, as by making L_s small (eqn. 16). Under these conditions, we have $H_{us} \approx H_m$ and $H_{sf} \approx H_r$. Thus the ratio of N values for segmented-flow (sf) *versus*

unsegmented (us) flow will be equal to $H_{us}/H_{sf} \approx H_m/H_r$. The larger is the latter ratio, the more favorable is SF-LC *versus* capillary LC without segmentation.

Eqn. 18 for H_r can be rearranged to give

$$H_r = [(1 - R)^2/36]\,(1/D'_m)\,d_t^2 u \tag{25}$$

Since D'_m is a function of D_m (Table I), eqn. 25 can be restated as

$$H_r = g(R)\,g(D_m)\,d_t^2 u \tag{25a}$$

The latter is seen to be of the same form as eqn. 24. The ratio H_m/H_r is now given as

$$H_m/H_r = [f(R)/g(R)]\,[f(D_m)/g(D_m)] \tag{26}$$

According to eqn. 26, relative column efficiency in SF-LC *versus* unsegmented capillary LC varies with both R (or k') and with sample diffusion coefficient (which varies with sample molecular weight). The quantity H_m/H_r from eqn. 26 is plotted *versus* k' in Fig. 4 for different sample molecular weights (or different values of D_m and D'_m).

It is seen in Fig. 4 that SF-LC promises a substantial advantage over unsegmented capillary LC, one that grows for smaller k' values (as in SEC separations) and for higher molecular weight samples. Generally, it is predicted that plate numbers N in SF-LC will be at least 50 times greater than for capillary LC, and for some cases over 500 times greater. Of course, other techniques which break up laminar flow in capillary LC (coiling the column, crimping the tube) will serve to reduce this advantage of SF-LC. However, it is questionable that 50- to 100-fold reductions of H in capillary LC are possible by these means (see discussion of ref. 9). Therefore a preliminary conclusion would be that SF-LC offers the potential for improved separations *versus* other forms of capillary LC. Whether this potential can be achieved in actual practice can only be known after further work is carried out.

SF-LC versus packed-bed LC

At present it is not known if column diameter in SF-LC can be reduced much below 0.5 mm, or if pressure drop along the capillary can much exceed a few p.s.i.

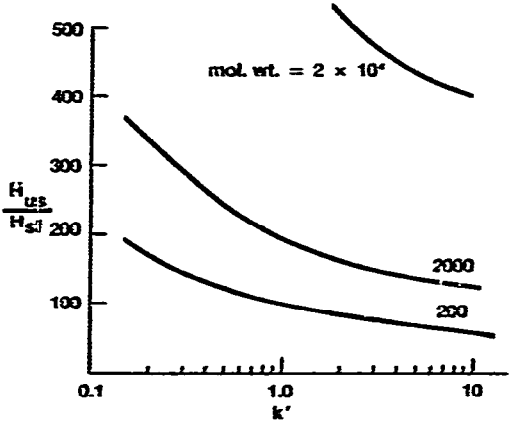

Fig. 4. Relative efficiency of segmented *versus* unsegmented capillary LC. Values from eqn. 26.

As long as these restrictions are assumed, the possible column efficiency (value of N) for SF-LC *versus* current packed-column LC is low. It does not appear that the initial hope of higher N values from SF-LC is at all realizable. This can be illustrated with a model calculation. Assume that the allowable pressure drop is 2 p.s.i., a 400 × 0.1 cm capillary is assumed, the liquid segment length is 0.6 cm, and sample molecular weight is 200 (from which $D_m = 4.9 \cdot 10^{-5}$; Table I). From Fig. 3 we calculate u equal 4.2 cm/sec. Further assume that stationary phase-mass-transfer can be made small, so H_s equal zero. From eqn. 19 we then calculate the following values of N *versus* k':

k'	N	N_{eff}
0.1	1600	13
1.0	64	16
10	20	17

The value of t_c calculated for the above separation is 105 sec, so the separation time varies from about 2 to 20 min.

Values of the effective plates N_{eff} are also shown above:

$$N_{eff} = N(1 - R)^2 \tag{27}$$

N_{eff} is a more useful measure of column performance, since it takes into account the effect of k' on the separation[1]. From the definition of N_{eff} and $H = L/N$, as well as the form of eqn. 25 ,it is seen that N_{eff} in SP-LC becomes independent of k' at higher values of k'. While this makes SF-LC appear to advantage at low values of k' (*versus* other LC methods), low k' values are normally avoided for the other LC procedures because N_{eff} is maximized at higher k' values. The value of N_{eff} for SF-LC is estimated above at about 16 plates, or 0.01–0.1 plates/sec. This is a much lower figure than can be achieved in packed-column LC with small-particle columns, particularly at higher values of P[1]. The advantage of packed-columns in this respect is lower for larger solute molecules, and particularly for particles.

CONCLUSIONS

The theory of separation efficiency (N and H values) in SF-LC has been derived in straightforward fashion from previous treatments. From prior work with segmented-flow in uncoated tubes, an accuracy of these N and H values of roughly ±20% would be expected, assuming the ideal conditions assumed in the derivation are met. In the following paper we will examine further some effects that result in deviation of experimental H values from theory, but it will be argued that these effects can be suppressed by optimizing the SF-LC system.

The application of this theory for SF-LC allows a paper-calculation of relative column efficiency *versus* unsegmented capillary LC and present packed-column LC. The results of this comparison show that segmentation leads to a 50-fold or greater reduction in H *versus* unsegmented capillary LC, when laminar flow persists in the latter. Disruption of laminar flow in unsegmented capillary LC would reduce this

advantage. The absolute column efficiency of SF-LC (better the effective plate number N_{eff}) is much less than can be achieved in present packed-column LC with small particles.

SF-LC shows to greatest advantage at small k' values and large solute molecular weights. For the separation of particles (from soluble solutes), band spreading can be less in SF-LC than in packed-column LC with good, small-particle columns. These characteristics of SF-LC are complementary to certain other features of this technique: ability to separate particulate-containing samples because open tubes tend not to become plugged by particles, the need for very simple, low-pressure equipment and the ability to combine SF-LC operations with other continuous-flow (AutoAnalyzer™) procedures for chemical processing of the sample. It is possible to combine all of these advantages into a particular application of SF-LC: its use in an AutoAnalyzer™ system for the pretreatment of samples prior to their injection and analysis by a conventional packed-column LC system. This possibility is further explored in the following paper.

SYMBOLS

A list of symbols used in Parts I and II is included at the end of Part II[15].

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