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Dennis C. Shelly^a; Vincent L. Antonucci^a

^a Department of Chemistry and Chemical Engineering, Stevens Institute of Technology Hoboken, New Jersey

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EFFECTS OF SOLUTE FOCUSING ON ZONE SHAPE IN COLUMN - PLANAR SEPARATIONS

DENNIS C. SHELLY* AND
VINCENT L. ANTONUCCI

*Department of Chemistry and Chemical Engineering
Stevens Institute of Technology
Hoboken, New Jersey 07030*

ABSTRACT

Solute transfer at the interface in column - planar separations is characterized by non-selective bulk transport forces such as elution, solvent evaporation, and capillarity as well as the selective chromatographic force of classic thin-layer development. Ultimate zone shape will be dictated by a combination of these forces. Band broadening in the planar medium can be suppressed using solute focusing, a technique whereby a strong solvent is used to force all solutes to migrate together. During an analysis of focused and nonfocused zones we observed apparent "orthogonality" in the capacity ratio vs. efficiency relationships for the two treatments. This suggests that the extent of zone dispersion during solute transfer arises from the actual differences and similarities of the two displacement modes.

* Author to whom correspondence should be addressed.

INTRODUCTION

Column - planar separations have undergone periodic development for nearly two decades, but practically no effort has been devoted to characterize or quantitate solute transfer phenomena at the interface of the two dimensions. Recently several reports of advances in instrumentation and new applications for the technique have appeared (1-4). Of particular significance is the most recent work of Hofstraat et al (5), who have discussed the unique orientation of zones deposited on a moving layer and attempted to characterize these zones via densitometry. The spatial integrity of the chromatographic zones was attributed to the effects of elution and there was a weak dependence on elution time noted.

The relationship of both displacements in a two-dimensional separation has been treated extensively. Giddings has illustrated the differences between separations where one and both of the displacement mechanisms are independent (6). For two independent displacements the overall peak capacity is the product of individual peak capacities for each displacement dimension. When both displacement modes carry some degree of similarity the overall peak capacity is approximately equal to that from either separation. The term "orthogonality" has been used to refer to the requisite differences in displacement modes in order to achieve the maximum theoretical peak capacity. Freeman has introduced the term "nonredundancy" (7) to refer to the relationship between modes in a

mode sequencing experiment. A test of redundancy was proposed in which capacity ratio, k' , and efficiency, N , were related as

$$\Phi_r = 1 + \frac{(N)^{0.5}}{4} \ln(1+k')$$

Here, Φ_r is the overall peak capacity. Sagliano et. al. have amplified this treatment somewhat (8), still there are no acceptable quantitative methods for demonstrating mode redundancy.

The goal of our work in this area was to develop a better understanding of solute transfer in column - planar separations through a rigorous evaluation of the interface. Solute focusing was used in an attempt to recover "lost" efficiency during solute transfer. During analysis of efficiencies, pre and post focusing, we observed anomalous relationships between efficiency and capacity ratio for two different separation mode combinations, partition X adsorption and adsorption X adsorption.

MATERIALS AND METHODS

Instrumentation

A column - planar chromatographic apparatus was constructed in our laboratory. Details of its operation have been described in several presentations (9-11). Our instrument is similar to that of Hofstraat et al (1) and Jinno (4). The column chromatography

portion of the instrument consisted of a microinjector valve with a 500 nL internal loop (Modle 7520, Rheodyne, Cotati, CA) and a Varian 8500 (Varian Assoc., Sunnyvale, CA) high pressure syringe pump. A moving loop injection technique was used to minimize band broadening due to injection (12). A fused silica microcolumn (0.25 mm I.D.) was packed with 5 μ m Spherisorb ODS-2 (Alltech Assoc., Waukegan, IL) reversed phase material using a computer-controlled packing apparatus (13). Glass-lined stainless steel microbore columns (1.0 mm I.D.) were also prepared in similar fashion, one packed with 5 μ m Spherisorb ODS-2 and the other packed with 3 μ m Spherisorb NH₂ material.

Scanning densitometry was performed on a Camag Scanner II (Camag, Wrightsville Beach, NC) in the fluorescence mode (366 nm excitation and $>$ 400 nm emission). Output signals were recorded on a chromatographic integrator (Model 4290, Spectra Physics, San Jose, CA). Preliminary evaluation of the microcolumn partition separation was accomplished using a modified variable wavelength UV detector (Model 87.00, Knauer, Woodcliff Lake, NJ) (17).

Chemicals and Reagents

High performance liquid chromatography-grade solvents (Burdick & Jackson, Muskegon, MI, USA) were used for micro HPLC and solute focusing. All chromatographic plates were 10 x 10 cm non-F254 Silica Gel 60 HPTLC plates (E. Merck, Darmstadt, FRG). The polynuclear aromatic hydrocarbon mixture was obtained from Chem.

Service (P/N PPH-10M, Chem. Service, West Chester, PA). The model solutes naphthalene, anthracene and perylene were also obtained from Chem. Service.

Microcolumn Chromatography

Partition micro HPLC was performed using a 98% acetonitrile/8% water mobile phase on a 530 mm long microcolumn and a 94.7% acetonitrile/4.1% methanol/1.2% water mobile phase on a 180 mm long microbore column. A 250 mm long microbore column was used with a mobile phase that consisted of 8% heptane in cyclohexane for an adsorption separation. All column separations were performed at their appropriate optimum linear velocities. Efficiency calculations were performed manually using careful measurements of peak width at half height from a reticle. Capacity ratio calculations were based on t_0 values, estimated by the appearance of system peaks rather than a void volume marker compound. Trend-line plots of N vs $\ln k'$ were constructed with Harvard Graphics (Software Publishing, Mountain View, CA).

Solute Focusing

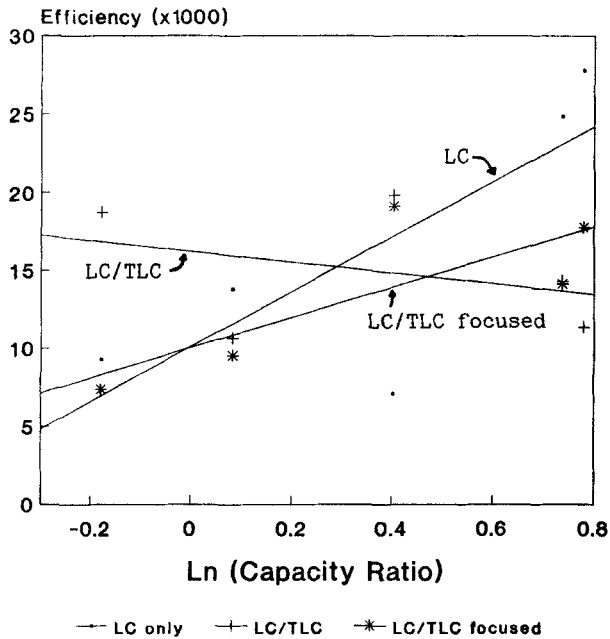
After deposition of the column effluent onto HPTLC plates and measurement by scanning densitometry each "track" was displaced 5 mm with 0.5% acetic acid in methanol (on silica gel plates) and with tetrahydrofuran/carbon tetrachloride 1:1 (on reversed phase

plates). A Camag twin trough chamber was used. Each "track" was again measured by scanning densitometry, making sure that the beam adequately followed the entire track.

RESULTS AND DISCUSSION

Partition X Adsorption Combination

A 16 component mixture of polynuclear aromatic hydrocarbons was separated using a C18 microcolumn and a 92/8 acetonitrile/water mobile phase. Five peaks were used in the calculations, peaks 5, 7, 10, 12 and 13. All components were resolved with baseline resolution. Figure 1 (\rightarrow) shows the relationship between N and $\ln k'$ for this column separation. Upon transfer to a silica gel HPTLC plate the solute bands are broadened as shown in Figure 1 (\rightarrow). Elution conditions, characteristic of adsorption chromatography, would imply that non-polar solutes (strongly retained in partition) migrate extensively with a polar mobile phase. This explains the negative slope to the LC/TLC plot. However, after focusing the trend in N vs $\ln k'$ (\rightarrow) returns to a nearly equivalent relationship to that of partition only. Zone shape of transferred solutes was influenced by the column mobile phase, solute polarity and stationary phase of the planar medium. Since densitometric scanning was performed in the column separation axis only, the effects of focusing were recorded as enhanced efficiencies in this dimension. A complete description of what

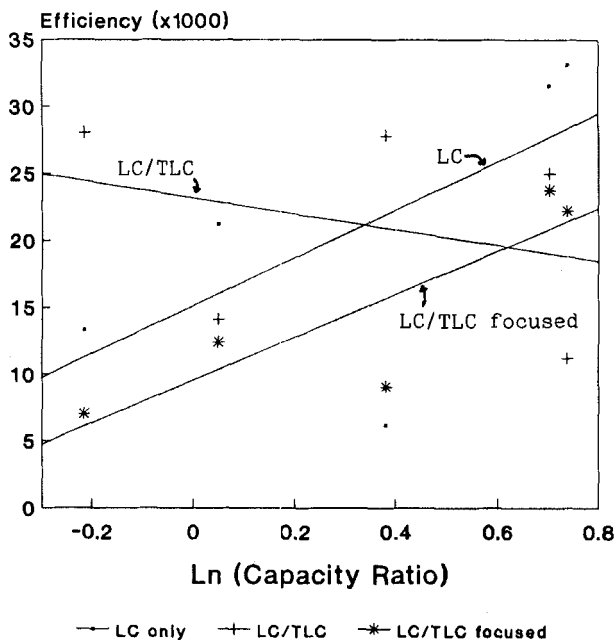


16 comp PAH mix

Figure 1. Partition X Adsorption Experiment 1

happened to the solute, upon transfer, is not addressed. This will be the subject of two future papers.

This experiment was repeated under identical conditions and the results are shown in Figure 2. Though the precision of N values is not particularly good, the trend-line analyses show very similar results. In both these cases, the maximum capacity ratio was approximately 2, implying that all solutes spent less than twice the amount of time in the stationary phase as compared to the mobile phase.



16 comp PAH mix

Figure 2. Partition X Adsorption Experiment 2

We evaluated the effect of magnitude of capacity ratio using the model solutes naphthalene, anthracene and perylene. Using the same type of mobile phase, identical column packing and planar stationary phase, the results are shown in Figure 3. Lower efficiencies are due to a larger I.D., shorter column (See Materials and Methods section.). Here, the maximum capacity ratio is nearly 8 and the unfocused trend-line does not have a negative slope. However, the points fit both lines better and the focused slope is more positive than the unfocused one. Figure 3 is

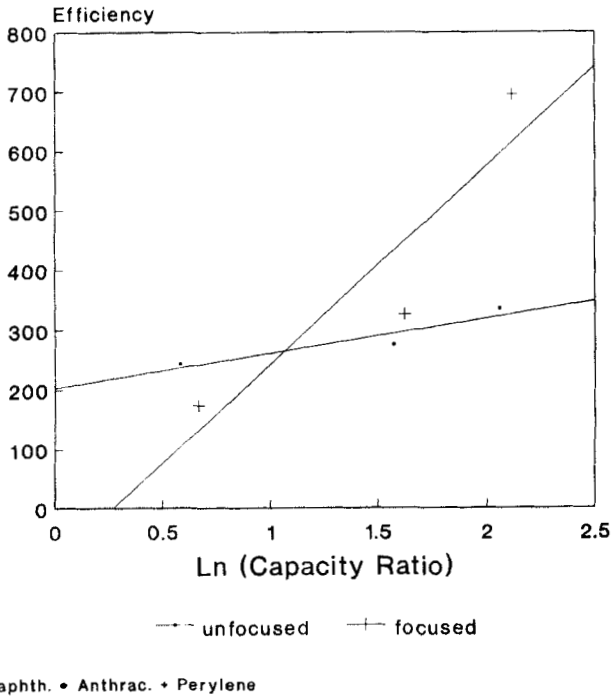
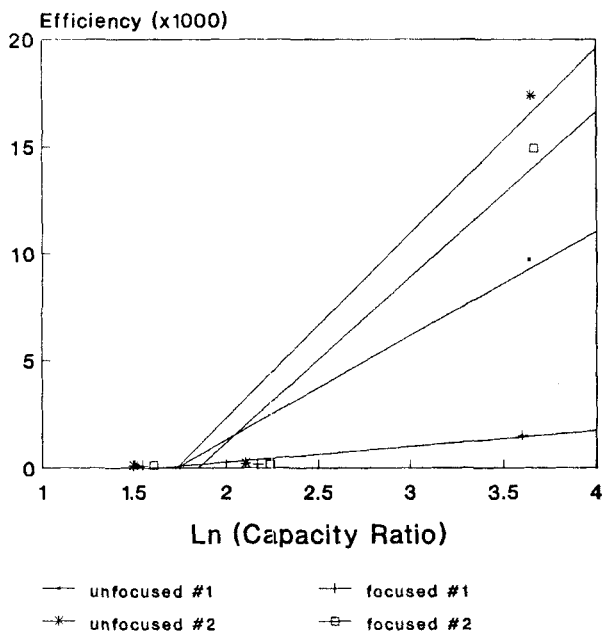


Figure 3. Partition X Adsorption Experiment 3

significant to the extent that it suggests intracolumn and chromatographic broadening can dominate over extracolumn effects. This implies that perhaps a significant amount of efficiency loss during solute transfer is non-chromatographic; effects more visible at low k' values.

Adsorption X Adsorption Combination

The model compounds naphthalene, anthracene and perylene were separated on an amino microbore column interfaced to a silica gel



Naphth. • Anthrac. + Perylene

Figure 4. Adsorption ($-\text{NH}_2$) X Adsorption (SiOH) Experiment

HPTLC plate. The column mobile phase permitted a "ring class separation" analogous to that described by Wise and co-workers (14). Here, the capacity ratios ranged to nearly 40 and the efficiencies were quite good. The results of duplicate experiments are shown in Figure 4. Notice that the experiments do not agree very well, but that in both cases the trend-lines for the focused solutes have lower slopes than those for unfocused solutes. This is opposite to that observed in Figures 1, 2 and 3. This implies

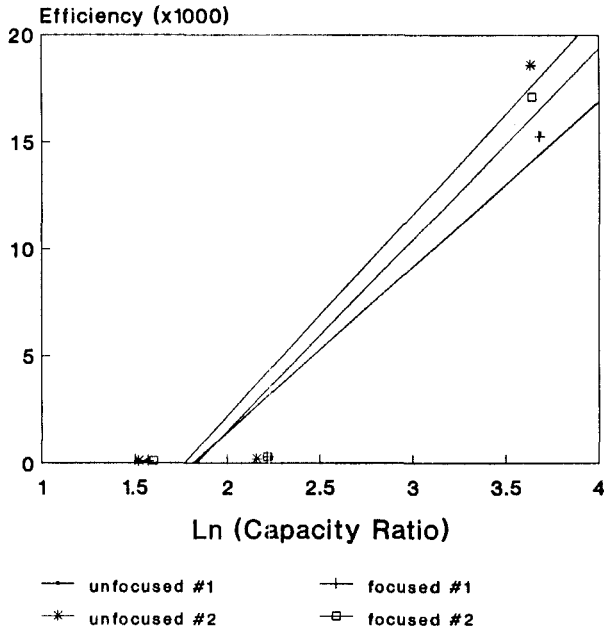
that focusing decreased efficiency, or perhaps that efficiency increased during solute transfer. It is probably more accurate to say that minimal solute migration occurred during focusing for this combination, implying that the separation modes were similar.

Adsorption X Partition Combination

The same amino column separation, as above, was interfaced to a partition planar medium (RP18) in this experiment. Ethylacetate/methylene chloride (1/1) was used as the focusing solvent. The results of this experiment are shown in Figure 5. Notice that similar N and k' ranges were seen. Notice, also, that focused slopes are lower than unfocused slopes, as above. However, the differences in the slopes are much lower, with experiment 1 having overlapping trend-lines. Obviously, even less solute movement was produced by focusing, compared to the experiment reflected in Figure 4.

Partition X Partition Combination

A reversed phase column separation (identical to that described above) was interfaced to a reversed phase HPTLC plate. In this experiment we observed complete loss of resolution for the solutes naphthalene, anthracene and perylene. Though we did not pursue optimization or diagnosis of this combination, we believe that naphthalene and anthracene migrated into one peak and perylene was pushed off the plate. This suggests extensive migration,



Naphth. • Anthrac. • Perylene

Figure 5. Adsorption ($-\text{NH}_2$) X Partition Experiment

probably nonselective elution. Eventhough the separation mechanisms were presumably the same, the selectivities of the column and planar media were very different.

Summary of N vs ln k' Slope Data

Much can be drawn from an analysis of the calculated slopes from the above figures. Table 1 shows a list of these data. All partition X adsorption experiments resulted in focused zones with

Table 1. N vs Ln (k') Slope Data

| | | |
|---------------------|--------------------|-------|
| C18 vs SiOH* | | |
| unfocused | -3181 | -7091 |
| focused | 9090 | 15818 |
| C18 vs SiOH | | |
| unfocused | 50 | |
| focused | 320 | |
| C18 vs RP18 | | |
| unfocused | LOSS OF RESOLUTION | |
| focused | LOSS OF RESOLUTION | |
| NH2 vs SiOH | | |
| unfocused | 5100 | 8700 |
| focused | 1000 | 8000 |
| NH2 vs RP18 | | |
| unfocused | 7967 | 9684 |
| focused | 7960 | 8867 |

* 16 Component data, all other data were 3 component

much greater efficiency than unfocused ones. In fact, initial experiments generated nearly orthogonal relationships. Both adsorption X adsorption and adsorption X partition experiments produced focused zones with less efficiency than unfocused zones. The focused and unfocused solute zones were fairly similar in efficiency, however.

These differences and similarities are attributable to thermodynamic aspects of the various displacement mechanisms. In the partition X adsorption experiments we observe that the displacements are very, very different (nearly orthogonal). What we see, actually, is that trends in selectivity follow predictable,

opposite courses. Therefore, increasing magnitudes of partition coefficients are matched by decreasing values in sticking coefficients for the respective adsorption isotherms. The more different these trends the more independent the displacements. The similarities of the adsorption \times adsorption and adsorption \times partition experiments indicate that selectivity trends are very similar; hence, independence is not apparent. The amino column stationary phase is unique in that it can display partition and adsorption behavior for the same solutes (15). These two observations would seem to indicate that "true" independence of displacement modes is only a mathematical concept, because the separation "chemistry" (in practice) produces a continuum between two theoretical extremes. Therefore, the chromatographic migration during solute transfer in a column - planar separation can be used to indicate independence of displacement modes because the decrease in efficiency is due to selectivity differences (which have a thermodynamic origin) between the two modes.

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