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ARTICLE *in* ANALYTICAL CHEMISTRY · APRIL 1986

Impact Factor: 5.64 · DOI: 10.1021/ac00295a054

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Whole Column Detection Chromatography: Computer Simulations

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The potential advantages of simultaneous detection along the entire length of a chromatography column have been explored by using a computer model. In the technique of whole column detection (WCD) chromatography, the distribution of peaks as a function of column position can be obtained at any time. The variation of this distribution with time is utilized as an additional dimension for quantitation and identification. In contrast to conventional chromatography where resolution is limited by the degree of separation at only one point on the column (i.e., the exit), WCD allows quantitation of any pair of peaks at the position on the column where maximum resolution occurs. Results from modeling chromatographic elution demonstrate that simple solvent programs may be used to selectively separate a chosen set of peaks. Once these peaks are separated to the desired degree of resolution, the solvent program may be altered to separate additional sets of peaks without concern for any degradation of resolution of the earlier set. An appealing feature is a significant reduction in the analysis time required to achieve a predetermined separation goal. It is anticipated that an expert system monitoring WCD data from an ongoing separation could manipulate the solvent program to provide a real-time optimization of the separation process.

In recent years liquid chromatography has progressed from vertical glass tubes allowing visual detection (Tswett, 1906) to high-pressure columns with extremely sensitive postcolumn detection. However, the well-known advantages of high-performance liquid chromatography (HPLC) have been achieved at the sacrifice of the continuous visual information once useful in monitoring the elution process. Detection problems associated with the use of microcolumns prompted recent investigators (*1*, *2*) to develop on-column detection methods. The advantages of on-column detection over postcolumn detection include (1) elimination of band broadening associated with the detector dead volume, (2) elimination of sensitivity loss resulting from postcolumn dilution, and (3) elimination of the need to make difficult postcolumn connections. The success of on-column detection and the potential advantages of the additional data have prompted us to consider the extension of this concept to a technique we will term "whole column detection" (WCD) chromatography. In this method the column is continuously monitored at multiple sites along its length, and as a result the advantages of visual observation are recovered.

In order to evaluate the potential advantages of whole column detection, a computer model of chromatographic elution and band broadening was constructed and applied to a separation problem previously optimized for postcolumn detection by Glajch, Kirkland, Squire, and Minor (*3*). This model produces a "movie" of the elution process, monitoring the distribution of solutes along the column as a function of time for any chosen solvent program.

Table I. k' Data for Computer Simulation

		mobile phase		
no.	solute	50:50 methanol/ water	40:60 acetonitrile/ water	37:63 THF/ water
1	sodium benzenesulfonate	0.0	0.0	0.0
2	benzoic acid	0.5	0.3	0.4
3	1-naphthanoic acid	0.7	0.4	0.8
4	benzamide	0.9	0.7	0.7
5	aniline	1.3	1.5	1.7
6	phenol	1.6	1.4	2.3
7	benzaldehyde	2.2	2.2	1.9
8	methyl *p*-hydroxybenzoate	2.3	1.5	2.0
9	cyanobenzene	2.3	2.7	2.3
10	acetophenone	2.7	2.0	1.9
11	nitrobenzene	3.4	3.6	3.4
12	1-aminonaphthalene	3.7	3.5	3.5
13	ethyl *p*-hydroxybenzoate	3.8	2.2	2.8
14	anisole	4.5	4.3	3.9
15	benzene (ref)	4.7	4.7	4.7

COMPUTER MODEL

The computer simulation was executed on an IBM PC microcomputer using BASIC version 1.10. Following the initiative of Glajch et al. (*3*), we used the capacity factor data provided by Bakalyar et al. (*4*) to simulate a separation of 15 substituted benzenes and naphthalenes. The capacity factor, k', data for the three solvent mixtures studied by Bakalyar et al. are provided in Table I. The percentage of water in each of the three solvents was chosen to produce equal strengths of elution for benzene, the most strongly retained component. While capable of simulating chromatograms similar to those of Glajch et al. (*3*), our model focused on producing "snapshots" of the distribution of the solutes along the entire column as a function of time.

For modeling WCD chromatography, it is useful to consider the velocity of a peak through the column when in the presence of a given solvent. The peak velocity of component a in solution i is related to the capacity factor, $k'_{a,i}$, and the solvent velocity, v_0, by

$$v_{a,i} = v_0/(k'_{a,i} + 1) \quad (1)$$

Cumulative peak positions, x_a, may be calculated from the times, t_i, spent in different solvents as follows:

$$x_a = \sum_i v_{a,i} t_i = v_0 \sum_i [t_i/(k'_{a,i} + 1)] \quad (2)$$

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Peak shapes were assumed to be Gaussian with standard deviations, σ_x, given by

$$\sigma_x = (xH)^{1/2} \tag{3}$$

where x is the column position and H is the height equivalent to a theoretical plate (5). It is interesting to note that for simple chromatography theory in which it is assumed that one value of H applies to all components, the width of any peak is a function only of the column position and is independent of the time spent on the column. This result is familiar to many chromatographers, but counterintuitive to others. For the latter, we note that the broader peaks (in exit chromatograms) observed for longer retained components are due to slower velocities of the exiting peaks. The standard deviation in the time domain, σ_t, is obtained by dividing σ_x by the velocity, $v_{a,i}$, of that component in the eluting solvent

$$\sigma_t = \sigma_x / v_{a,i} = (xH)^{1/2}(k'_{a,i} + 1)/v_0 \tag{4}$$

For exit chromatography, x is equal to the column length, L, the number of theoretical plates, N, is equal to L/H, and we have the familar result

$$\sigma_t = L(k' + 1)/(v_0 N^{1/2}) = t_0(k' + 1)/N^{1/2} = t_R/N^{1/2} \tag{5}$$

where t_0 is the retention of an unretained peak and t_R is the retention time of the component.

A simple formula may be derived for calculating the on-column resolution of a pair of peaks from the peak positions

$$R = \Delta x/[4(\bar{x}H)^{1/2}] \tag{6}$$

In order to compare the WCD simulations with the optimized isocratic exit chromatogram of Glajch et al. (3), N was set equal to 16 000 plates, and the solvent velocity was chosen such that an unretained peak exited the column after 180 s. Initial peak heights were assigned arbitrarily except for exit chromatograms. For exit chromatograms, the peak heights were set approximately equal to those Glajch et al. (3) for comparison purposes.

The simulation of WCD chromatography reported here was the result of trying a wide range of solvent programs consisting of sequential applications of plugs of the three solvents for varying lengths of times. The experience gained by one of us (D.G.G.) with the solvent effects demonstrated by the computer model led to the simulation reported here. It is not necessarily the optimum separation, but it does illustrate many of the features and potential advantages of WCD chromatography. The problem of optimizing WCD chromatography will be dealt with in future papers.

RESULTS AND DISCUSSION

As a first test of the computer model and for comparison with the WCD simulations, we calculated the exit chromatogram, as optimized by Glajch et al. (3), of 15 substituted benzenes and naphthalenes using the isocratic solvent composition, 35% methanol/water (MeOH), 34% tetrahydrofuran/water (THF), and 37% acetonitrile/water (ACN). The fraction of water in each solvent is given in Table I. The results, shown in Figure 1, is identical to that of Figure 13 of Glajch et al. (3). The optimized exit chromatogram required 18 min, and two pairs of peaks remained unresolved. In particular, peaks 11 and 12 were separated with a resolution of only 0.8.

Model results for the simulated WCD separation of these 15 components are shown in Figure 2. Each figure represents a "snapshot" of solute distribution along the column at a specific time.

The first frame of Figure 2 shows the initial separation after the application of ACN for 90 s. Peak 1, sodium benzene sulfonate, is unretained and moves with the solvent front. Peaks 4 and 9 have already achieved base line resolution (here defined as $R > 1.5$) with respect to their nearest neighbors. During an actual experiment, k' values could be obtained for these and other peaks within this period of time. In the simulation, the eluting solvent is changed from ACN to THF after 90 s. The second frame (t = 144 s) shows the solute distribution while both ACN and THF are on the column. Peaks 2 and 3 are completely resolved at this time.

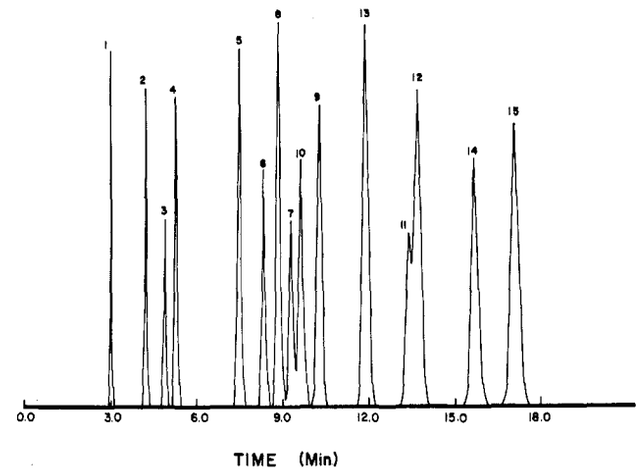

Figure 1. Computer simulation of the optimized exit chromatogram. All conditions are identical to those of Glajch et al. (3).

The third frame (t = 234 s) shows all three solvents on the column. Peak 9, previously resolved, has merged with peak 13. Peak 5 is beginning to separate from peaks 6 and 8. Peaks 14 and 15 are newly resolved.

The fourth frame (t = 342 s) shows peaks 5 and 13 fully resolved from their neighbors for the first time. Peaks 6 and 8 are completely overlapped, as are peaks 7 and 10. Furthermore, peak 9 has crossed over peak 13 and is fully resolved once again since frame 2. Peak 9 has a higher velocity in MeOH than peak 10. The purpose of the entry of the small plug of THF shown in frame 4 is to move the (7, 10) pair (equal $k's$ in THF) a sufficient distance away from peak 9 to prevent the eventual merging of peak 9 with peak 10. Following the THF plug, MeOH is applied for the remainder of the separation, because the yet unresolved pairs (7, 10) and (11, 12) are both best separated wth this solvent.

Peaks 6 and 8 have become resolved by frame 5 (t = 450 s). Frame 6 shows the maximum resolution achieved for peaks 7 and 10. The calculated value of R is 1.3. The final frame (t = 774 s) shows the maximum resolution obtained for peaks 11 and 12, R = 1.4.

Figure 3 is an overview of the separation process. Peak positions are plotted vs. time in this diagram. To indicate band width, the peak positions are plotted with a spread equal to 4σ. Thus, isolated bands are separated from their neighbors with a resolution greater than 1. This figure contains all of the information of the frames of Figure 2 and more, with the exception of peak height.

A vertical cross section through Figure 3 is the distribution of components along the column at a particular time. A horizontal cross section shows the distribution of times of arrival of components at a given point in the column. If this point corresponds to the column length, then this cross section is the exit chromatogram. All of the peak crossovers discussed above may be seen in this figure (e.g., note both the crossing of peaks 9 and 13 and the resolution of peak 9 in two distinct time regimes).

In whole column detection chromatography, column position is utilized as an additional dimension for the separation. We may compare the WCD results, Figure 2, with the exit

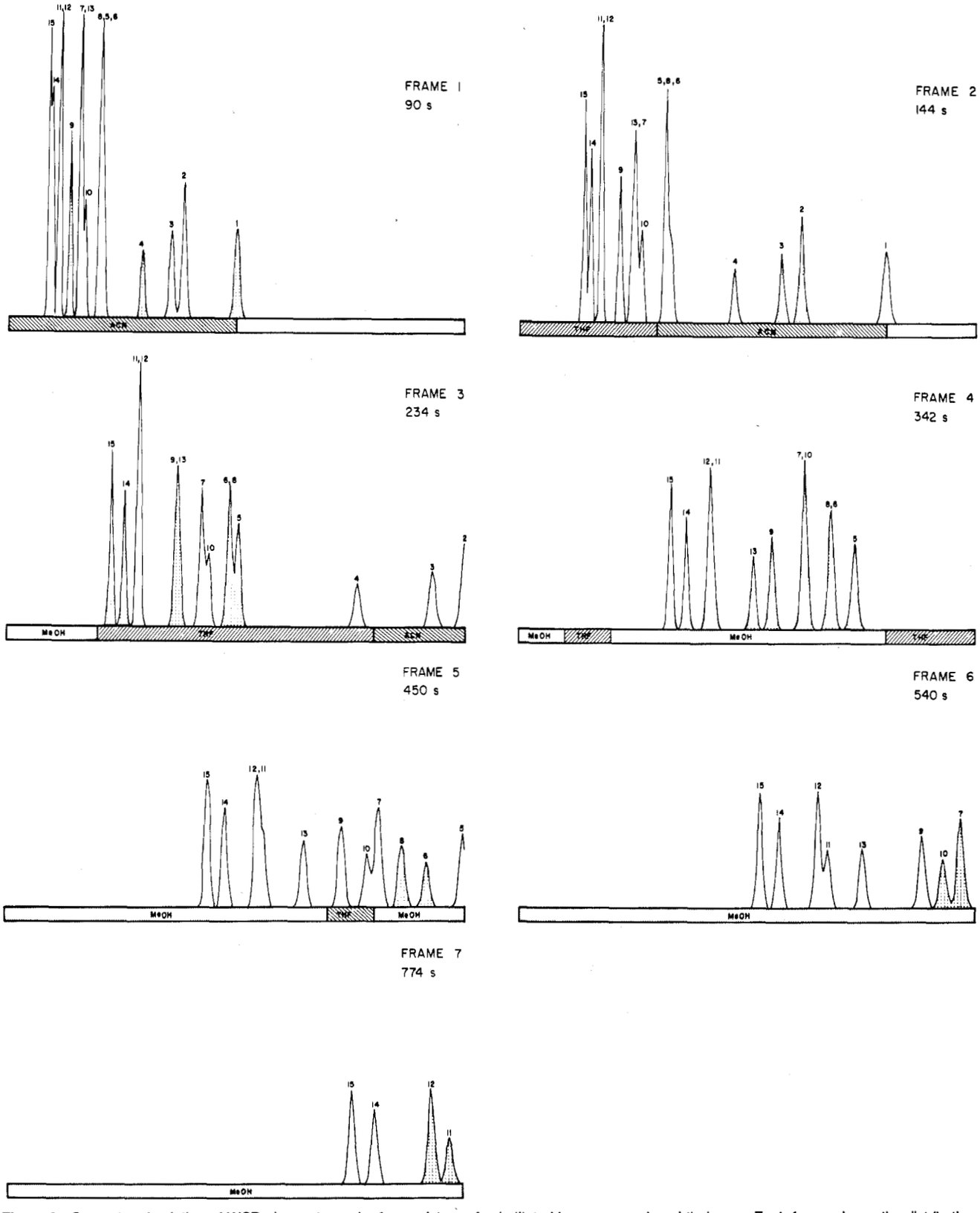

Figure 2. Computer simulation of WCD chromatography for a mixture of substituted benzenes and naphthalenes. Each frame shows the distribution of components along the column at a particular time following injection. The solvent distribution along the column is indicated. Peaks discussed in the text are crosshatched.

chromatogram produced by optimizing a single isocratic solvent composition prior to the separation, Figure 1. Equivalent or superior resolution is achieved for every peak in a much shorter analysis through the use of a sequence of simple solvent plugs in combination with whole column detection. For the WCD simulation, all peaks were resolved with a resolution better than 1.3 in less than 13 min while the optimized exit chromatogram required 18 min to achieve a minimum resolution of only 0.8.

A possible advantage of WCD chromatography as implemented here is the ability to predict the elution behavior without making any assumptions about the linear averaging of k' values in mixed solvents. This is because the elution is brought about by use of plugs of isocratic solvents *for which*

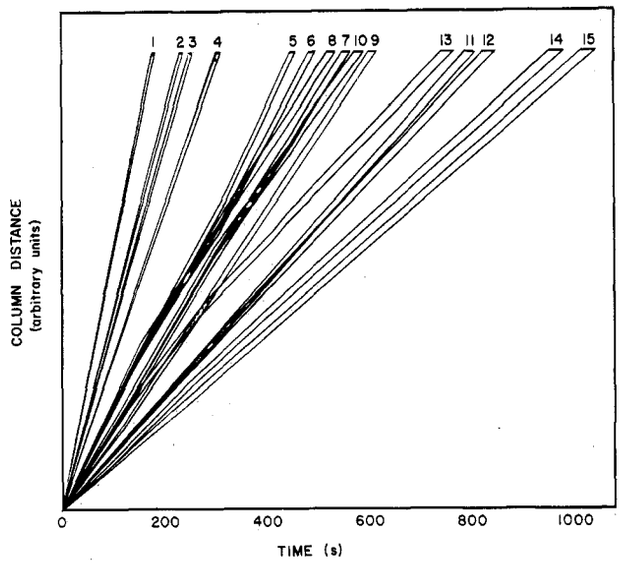

Figure 3. Plot of peak position vs. elution time for the simulation of WCD chromatography. The shaded areas represent peak widths of 4σ.

all k′ data are known. The ability to accurately predict peak movements is essential to any optimization procedure. The overlapping resolution method (ORM) used by Glajch et al. (3) for optimizing exit chromatograms requires either a very large k' data base or an assumption concerning the dependence of k' on solvent composition. We also note that our assumption that k' values change instantaneously at the solvent interface is probably unrealistic for most types of chromatography. In reality, some equilibration time is required, which in turn compromises our ability to predict accurately the peak velocities. However, experimental studies using WCD should provide the information necessary to include solvent equilibrium in models of WCD chromatography.

Recognizing that k' is given by the following simple function of peak velocity within the column (from eq 1)

$$k'_{a,i} = (v_0/v_{a,i}) - 1 \qquad (7)$$

WCD in conjunction with its data analysis system could be used to generate a large data base of capacity factors in a relatively short period of time by using a number of plugs of varying solvent composition. The solute need move through only a small portion of the column (sufficient to reach equilibrium) in order for its velocity and therefore its capacity factor in a particular solvent to be measured. Thus, WCD would have the advantage of short (cartridge) columns in that k' information could be obtained in a minimal amount of time, while retaining the ability to separate more complex mixtures.

The difficulties in peak assignment associated with peak crossovers in complex mixtures are eliminated by WCD in that a map of peak movement can be generated by the data system. For example, the peak crossovers occurring throughout the WCD simulation of Figure 2 were easily traced. By contrast, in conventional exit chromatography, crossovers of peaks having comparable amplitudes cannot be documented without auxiliary means of qualitative peak analysis.

Another unique feature of WCD chromatography is the real-time adjustment capability. The effect of a change made in solvent composition designed to bring about a desired separation can be observed directly through real-time feedback of data from the column. This suggests the possibility of on-column optimization and selective separation, which would result in another notable simplification for complex mixture analysis.

An expert system (6) incorporated into the feedback loop could make decisions directed to achieve a chosen separation goal, execute the adjustments, and monitor the results of those adjustments. A data base of k' values for solutes in a variety of solvents could be used by the expert system to either identify a compound or provide a short list of possibilities. Such information could be used by the expert system to optimize a post-column detector such as a mass spectrometer on a peak by peak basis. The ability of a WCD chromatograph to anticipate the exact arrival time of a particular peak (or pair of overlapping peaks) at the exit detector or column switching valve would also be extremely useful. The combination of WCD chromatography with column switching (sometimes referred to as "multidimensional chromatography") would further augment this already powerful separation technique.

Although not utilized here, WCD may also employ solvent strength programming as a form of gradient elution. As has been established for conventional chromatography, this technique could greatly enhance the potential for separating complex mixtures in WCD as well.

In summary, computer simulations of whole column detection chromatography have demonstrated potential improvements in the ability to separate complex mixtures by HPLC. These improvements result from a previously ignored dimension of the separation process, the distribution of solutes along the column length. This added dimension relaxes the constraint of conventional column chromatography that all peaks be separated at one position on the column, i.e., the exit. In WCD chromatography, a peak may be quantitated at whatever position(s) (and therefore time(s)) on the column where it is isolated from other components. Some other advantages of continuously monitoring the chromatographic process include (1) rapid generation of k' data useful for present and future separations and for identification purposes, (2) the possibility of real-time control and optimization of the separation process, and (3) generation of data for optimizing and coordinating postcolumn detectors.

The use of multiple detection sites along a chromatography column is at this point hypothetical, although true on-column detection at single points already has been demonstrated (*1*, *2*). In our laboratories, we currently are evaluating the practical possibilities of the use of fluorescence, photoacoustic spectroscopy, and electrochemical techniques as means of implementing whole column detection chromatography.

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RECEIVED for review August 15, 1985. Accepted December 2, 1985. This work was partially supported by a grant from the Environmental Protection Agency (Project R-810717-01-0). CGE thanks the Cooperative Institute for Research in Environmental Sciences (CIRES) for a Visiting Fellowship.