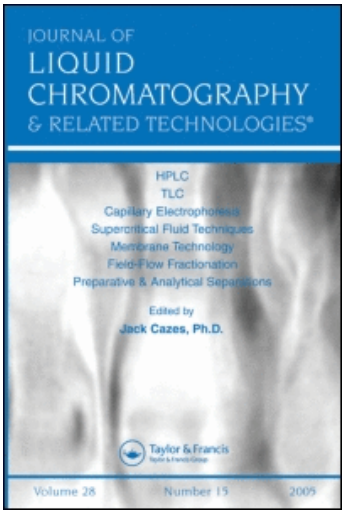

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S. T. Balke[a]; R. D. Patel[a]
[a] Xerox Research Centre of Canada, Ontario, Canada

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GRADIENT ELUTION ANALYSIS AND PREDICTION IN HPLC

S.T. Balke and R.D. Patel
Xerox Research Centre of Canada
2480 Dunwin Drive
Mississauga, Ontario L5L 1J9
Canada

ABSTRACT

Gradient Elution (programming of the carrier solvent composition with time) is increasingly used in HPLC to improve resolution. Mixing effects in the chromatographic system however, cause the actual gradient to be different at the columns than that programmed.

This paper shows how the concepts of residence time distribution and superposition were employed to characterize and predict actual gradients in a Spectra Physics SP8000 HPLC.

INTRODUCTION

In order to utilize gradient elution in HPLC the gradients should be both reproducible and predictable. The degree to which these qualities are required depends upon the final analysis requirements and is a function of the components analyzed as well as the chromatographic system employed.

The gradient system for the Spectra Physics SP8000 HPLC is shown in Figure 1. The gradient is formulated at the mixing valve but is altered by mixing effects in other parts of the system, such as pump, mixer, etc., on its way to the column(s).

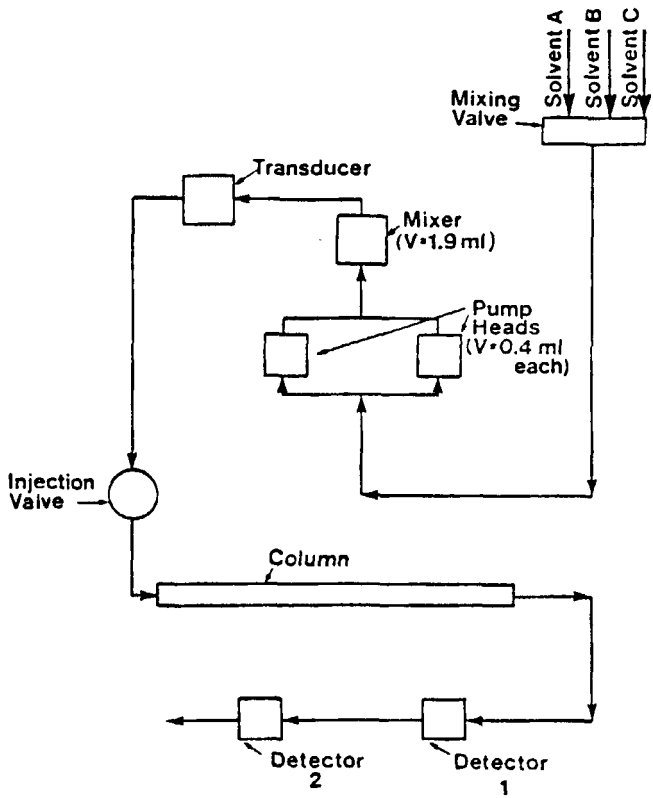

FIGURE 1: GRADIENT SYSTEM FOR THE SP8000 HPLC

The two practical aspects of utilizing gradient elution dealt with in this report are:

(1) "Universal" Characterization of Gradients:

(a) discerning where the actual mixing characteristics of the system have changed;

(b) identifying delay times and "time constants".

(2) Prediction of the actual gradient reaching the columns as opposed to that programmed at the solvent reservoirs' exits.

These aspects are not well examined the current HPLC literature. The usual approach is to assume that a particular type of mixing is present (Refs. 1 and 2).

THEORY

To characterize gradients, residence time distribution techniques can be applied.

If mixing is unchanged, the response to a step input of concentration of a tracer (which does not affect fluid mixing properties) can be superimposed for different flow rates and fluids (Ref. 3).

For a unit step, the response is plotted versus tv/V where t = time from application of the step; v = volumetric flow rate and V is volume of the system. For a fixed system of unknown volume a plot versus "tv" will suffice. Then the abscissa is interpreted as a "retention volume" rather than as a dimensionless time.

Mixing may be unchanged despite variation in fluid properties because of the compensation for such variations which is incorporated into the control software of the chromatograph's pump. If variations are observed, mixing model development could be undertaken to account for them (Ref. 3).

In attempting to predict the output (actual) gradient given the input (programmed) gradient, the principle of superposition can be employed. By this principle, the response [R(t)] to programmed input e(t) up to time t can be considered as the sum of the responses to a series of unit step inputs up to that time.

That is (Ref. 4):

$$R(t) = e(0)W_u(t) + \lim_{\Delta\tau \to 0} \sum_{\tau=0}^{t} \frac{\Delta e(\tau)}{\Delta\tau} \Delta\tau W_u(t-\tau) \qquad \text{Eqn. (1)}$$

where:

$W_u(t)$ = response to a unit step

$e(0)$ = initial value of $e(t)$

In the case of HPLC, the programmed gradient can be considered to have a zero concentration value at time zero. That is, concentration values are considered zero at the baseline and only deviations from the baseline are considered. Then Eqn. (1) becomes:

$$R(t) = \sum_{\tau=0}^{t} \Delta e(\tau) W_u(t-\tau) \qquad \text{Eqn. (2)}$$

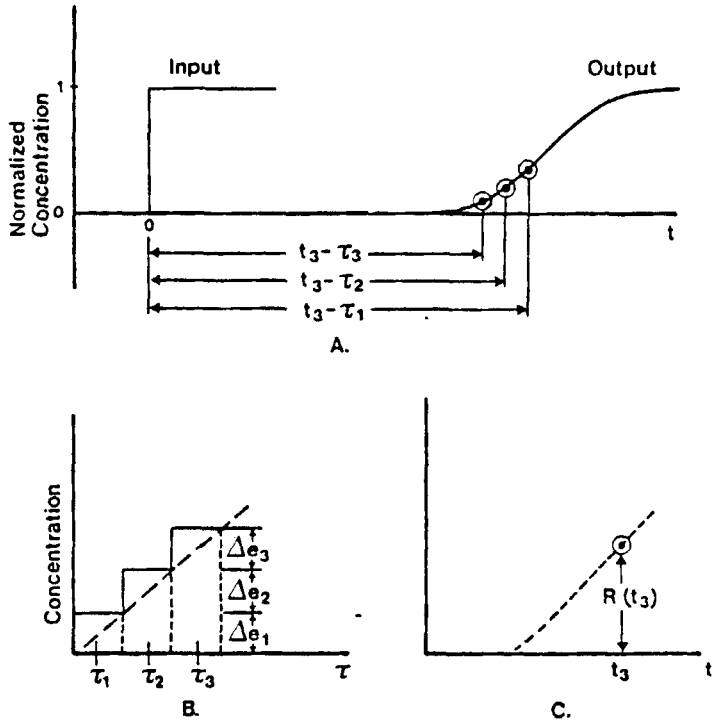

FIGURE 2: CALCULATION OF THE PREDICTED GRADIENT: A) INTERPOLATION OF POINTS ON THE STEP RESPONSE; B) DECOMPOSITION OF THE PROGRAMMED GRADIENT AND C) PREDICTION $(R(t_3) = \Delta e(T_1)W_u(t_3-T_1) + \Delta e(T_2)W_u(t_3-T_2) + \Delta e(T_3)W_u(t_3-T_3)$

Now, to use this equation, the instrument's mixing is first characterized by determining experimentally what the actual response is to a step change in concentration. (In practice, the response is converted to that from a "unit" step by normalizing each concentration point detected by dividing each by the height of the concentration step used as input.. Knowledge of this "unit step response" provides the $W_u(t-\tau)$ in Eqn. (2). The $\Delta e(\tau)$ is the height of each small concentration step into which the desired (programmed) gradient is decomposed. Figure 2 illustrates the calculation.

EXPERIMENTAL

Four step responses and three test gradients were run (Table 1). Only binary gradients were examined. Acetone was used as a UV absorbing dopant in one of the mobile phases. Use of a

TABLE 1: GRADIENT EXPERIMENTS

Gradient #	Mobile A	Phase B	Dopant Concentration (in Phase B)	Type of Gradient	Mobile Phase Flow Rate (cc/min)
1	Water	Water	1%	Step Input of Phase A (0 to 100% A)	1.0
2					2.0
3					3.0
4	Methanol	Water	1%	Step Input of Phase A (0 to 5%)	3.0
5	Methanol	Water	0.5%	Various Gradients of Phase B (Fig. 4)	2.0
6	Methanol	Water	0.5%	Various Gradients of Phase B (Fig. 5)	2.0
7	Methanol	Water	0.5%	Various Gradients of Phase B (Fig. 6)	2.0

variable wavelength detector set at a value below the UV cutoff of one of the mobile phases without using a dopant was briefly explored. Use of the dopant and the fixed wavelength (254nm) SP8310 detector was inconvenient but was found to provide a more stable baseline.

A 1.5 cc column filled with glass beads was used in the system to raise pressure drop across the pump to $\simeq$ 225 psi at 1 cc/min. solvent flow.

Thus the results are likely typical of what an analytical column would see part way down its length or after a guard column.

Temperature of the oven was set at $40^{o}C \pm 0.1^{o}C$. Outputs from the printer plotter were digitized manually.

RESULTS AND DISCUSSION

Figure 3 shows that all four step responses superimposed when plotted versus retention volume. No significant variation with flow rate or fluid properties was evident. The response is not that of a first order system. That is, mixing is not the same as that of an ideal stirred tank. This was

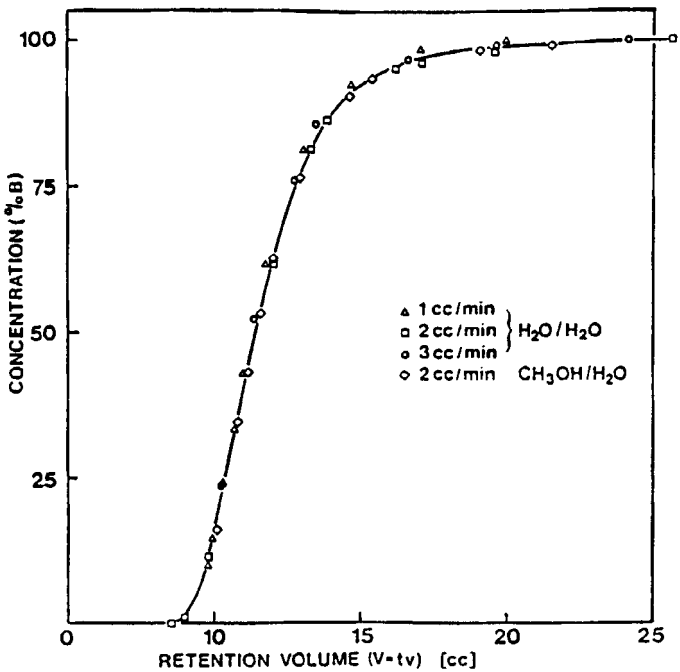

FIGURE 3: SUPERPOSITION OF STEP RESPONSES

understandable since mixing occurred in many and varied parts of the gradient system (Figure 1). For a first order system, the time required for the response to progress from 0 to 50% would equal the time from 50 to 75%. In this system, even after subtracting out a pure retention volume delay of 3.5 cc, these values are 2.9 cc and 1.4 cc, respectively. The lagging response and the spread residence time distribution of the system does place constraints upon what gradients are attainable. If the programmed gradient demands that the system change concentration faster than its response will allow, a distortion will result. Also, the time required to actually achieve programmed concentrations will have to be considered when stipulating premin delay times, run times and reequilibration times.

Figures 4, 5 and 6 show programmed gradients along with the predicted and actual gradient obtained. The predicted values were obtained through a computer program implementing Eqn. (2).

As expected from Figure 3, with pure time delay and mixing effects a time delay of > 1.7 minutes (1.7 to 4 minutes) between programmed and actual gradient is evident at 2 cc/min. In addition, however, time duration at plateau concentrations are sometimes considerably shorter than

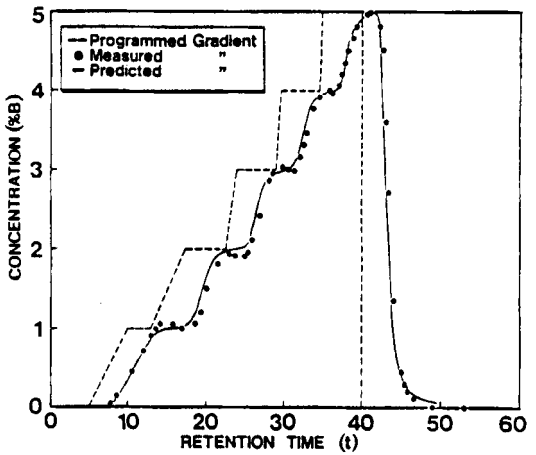

FIGURE 4: GRADIENT #5 - PROGRAMMED EXPERIMENTAL AND PREDICTED

that programmed. In Figure 4 for example, the response barely had time to reach 3% concentration, when it was called upon to proceed to 4 and then 5% concentration. Finally, at the 5% level, it spent only about 1 minute rather than the programmed 5 minutes. Figure 5 shows a similar result. However, in this latter case the gradient surprisingly appears to level off briefly at

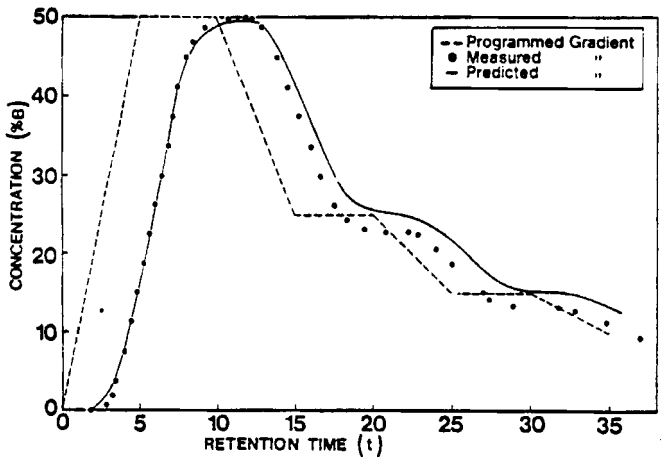

FIGURE 5: GRADIENT #6 - PROGRAMMED EXPERIMENTAL AND PREDICTED

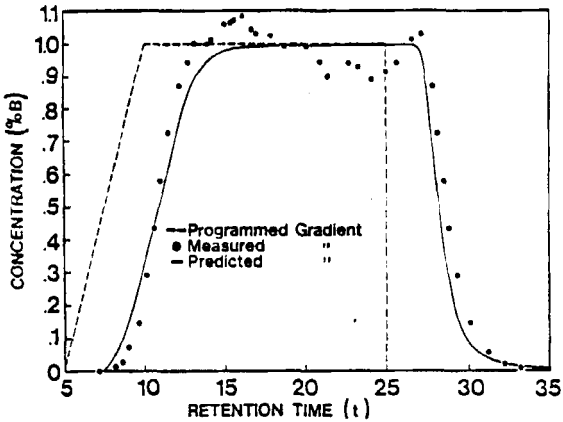

FIGURE 6: GRADIENT #7 - PROGRAMMED EXPERIMENTAL AND PREDICTED

23% rather than 25% concentration. Figures 4, 5 and 6 show that gradients are generally very well predicted by Eqn. (2). The step response at 2 cc/min and the programmed gradient were the only data required for prediction of any gradient. This step response could have been calculated from any of the other flow rates' step responses in this system (Fig. 3). Discrepancies between predicted and measured gradients are likely due most to detector measurement error. Figure 6 particularly shows what is likely baseline noise on the plateau level of 1% concentration. It is worth noting that such "noise" could also be due to the cycling action of the programmed mixing valve as it switches from one solvent reservoir to the other. Other similar valves have been reported to exhibit such characteristics (Ref. 5). The programmed gradient shown in this Figure is sufficiently slow to allow the plateau value to be obtained for ~12 of the required 15 minutes.

CONCLUSIONS

The concepts of residence time distribution and the superposition integral for a response to a step input were used to characterize and predict actual gradient elution profiles in High Performance Liquid Chromatography. Experimental verification of the theory was obtained using a Spectra Physics 8000 HPLC. No assumptions regarding the ideality of the mixing other than the validity of the superposition principle are involved. An experimentally obtained response to a step input of tracer concentration for the system involved, provides the basis for the analysis.

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