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Advances in process chromatography gradient elution using binary linear gradients

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Abstract

A number of chromatography techniques utilize mobile phase gradients with changing buffer concentration to effect separation. At process scale the majority of gradients are stepwise or isocratic. There are a number of instances where process performance or economics indicate that linear gradients would deliver better theoretical performance. However, to date this process advantage has not been implemented due to concerns about the accuracy, reliability or reproducibility of equipment used to produce linear changes in gradient composition. In this manuscript, recent developments in simple feedforward control and data from the resultant process-scale performance are presented. Information is provided on the practical heuristics underlying the control strategy. The data presented in this paper illustrate how the feedforward control strategy has been implemented on a commercial system to achieve better than $\pm 2\%$ accuracy of process-scale linear gradients. © 2001 Published by Elsevier Science B.V.

Keywords: Preparative chromatography; Gradient elution

1. Introduction

Liquid chromatography is a widely employed purification technique in the biotechnology industry. At process scale, gradient elution is the most utilized method of chromatographic desorption. To develop a robust and validatable chromatographic process, it is imperative that the gradient profiles can be accurately and reproducibly generated.

Ion-exchange chromatography is one of the most commonly employed chromatographic separations in process-scale chromatography. Elution of bound solutes is achieved by an appropriate change of the salt composition in the mobile phase. The salt concentration can be changed either in a step-wise (step gradient) or continuous (linear) fashion. Thus, for such an operation to be used for a licensed

product, it is imperative that the gradients can be formed accurately and reproducibly. For process-scale chromatography, gradient formation is either pump-based or valve-based. On the one hand, in pump-based systems, flow-rates from two or more pumps, each delivering a specific solvent, are proportioned to form the gradient. On the other hand, in valve-based systems, a proportioning valve toggles between the solvents generating the required gradient. Thus, in valve-based systems, only a single pump is required resulting in lower costs. In addition, the mixing range in valve-based systems is relatively independent of the flow-rate as opposed to the mixing range in pump-based systems. This is due to the limitation of pump turn down: with two pumps, in order to form very high or very low mixing ratios one pump must operate at a very low flow-rate. The accuracy of controlling the pump is therefore limited.

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For accurate and reproducible gradient formation at process scale, it is essential to have a good control strategy. In pump-based systems, the formation of gradients is typically achieved by controlling the flow-rates delivered by the pumps. Bellafiore et al. [1] discussed the factors affecting the accuracy and reproducibility of gradient formation and developed a feedback control strategy based on the on-line mobile phase analysis using an near IR detector. They considered ethanol gradients and their control strategy involved taking the column off-line when the solvent composition fell outside a preset range. Kowalczyk et al. [2] developed an adaptive algorithm to optimize the proportioning time in valve-based gradient formation. However, their work was entirely confined to simulations. In this manuscript, the formation of accurate and reproducible valve-based gradients is achieved using feedforward and feedback strategies.

2. Experimental

2.1. Materials

Sodium chloride (crystalline) was purchased from Fisher Scientific (NJ, USA).

2.2. Equipment

All experiments were performed using a K-Prime 40-III process-scale chromatography skid (Millipore, USA) with a proprietary Common Control Platform (CCP) software. The K-Prime 40-III system uses a GE-FANUC Programmable Logic Controller (PLC). The PLC logic was written using Logicmaster 90 TCP. The valve-based gradient formation was implemented by the K-Prime 40-III system using high-speed three-way radial diaphragm valves. The conductivity monitoring on the skid comprised a Wedgewood Model 722 conductivity/temperature monitor and a BT-607 in-line sensor.

3. Results and discussion

Throughout this manuscript, solvent A refers to pure water and solvent B to the salt solution. In

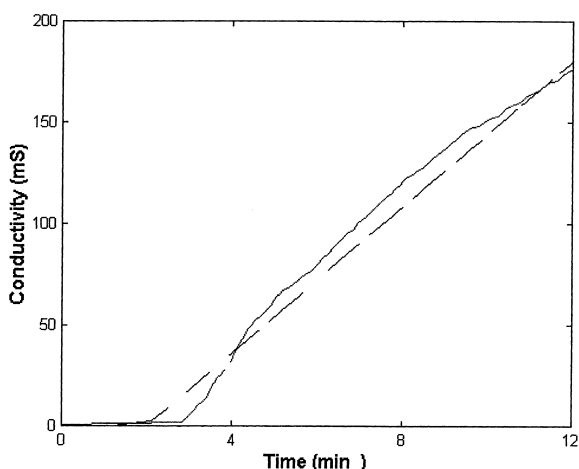


Fig. 1. Uncontrolled linear gradient from 0 to 100% B (180 mS) over 10 min. Flow-rate = 2 l/min. The linear gradient was initiated at time = 2 min. The dotted line is the setpoint and the solid line is the actual response.

addition, the mixing ratio is denoted by % B. Fig. 1 shows a gradient from 0 to 100% B over 10 min.

The formation of the gradient was monitored by measuring the conductivity of the effluent solution. As can be seen in the figure, the shape of the resultant gradient was nonlinear. The reason for this nonlinearity arises from both the intrinsic valve dynamics and the nonlinearity of the mixing of solutions of different densities. In order to correct for these effects, it is necessary to employ an adapted control system. In this manuscript a feedforward control strategy based on a mathematical description of the dependence of the effluent conductivity on the mixing ratio was considered.

A feedforward control approach was preferred to a feedback control approach due to the following reasons: (1) the system dead volume and flow-rate introduced a response lag that evoked an oscillatory response from the feedback loop. (2) There is a continuous change in the set point in linear gradient formation that renders the traditional tools for feedback controller tuning irrelevant.

The feedforward control strategy involved developing a quadratic fit to the variation of the effluent conductivity (mS) at various mixing ratios of the two solvents A and B (% B). This quadratic fit was then employed within the PLC to compute the

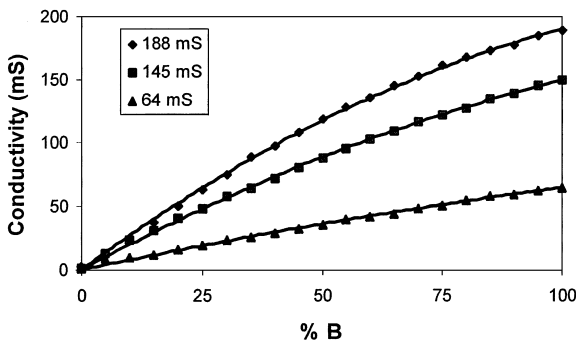


Fig. 2. Variation of the effluent conductivity as a function of the mixing ratio. Experiment 1: 0–100% B (188 mS) over 20 min; flow-rate=2 l/min; The symbols denote the experimental data and the solid line is the best fit ($\alpha = -0.009$, $\beta = 2.79$, $R^2 = 0.99$). Experiment 2: 0–100% B (145 mS) over 20 min; flow-rate=2 l/min; The symbols denote the experimental data and the solid line is the best fit ($\alpha = -0.006$, $\beta = 2.04$, $R^2 = 0.99$). Experiment 3: 0–100% B (64 mS) over 20 min; flow-rate=2 l/min; The symbols denote the experimental data and the solid line is the best fit ($\alpha = -0.002$, $\beta = 0.8$, $R^2 = 0.99$).

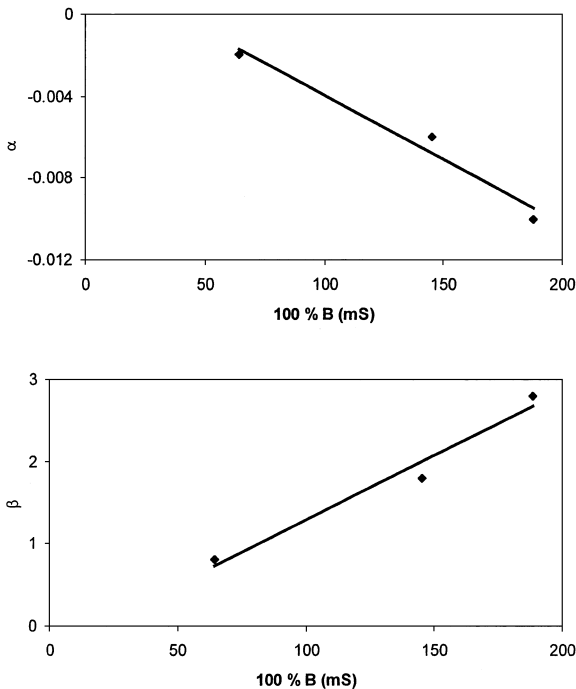


Fig. 3. Dependence of the quadratic fit parameters, α (top) and β (bottom), on the conductivity of solvent B. The symbols are data points and the solid line is the linear fit in both figures. The correlation for α : $-6 \cdot 10^{-5} \cdot (100\% \text{ B mS}) + 0.002$, $R^2 = 0.97$; the correlation for β : $0.016 \cdot (100\% \text{ B}) - 0.27$, $R^2 = 0.97$.

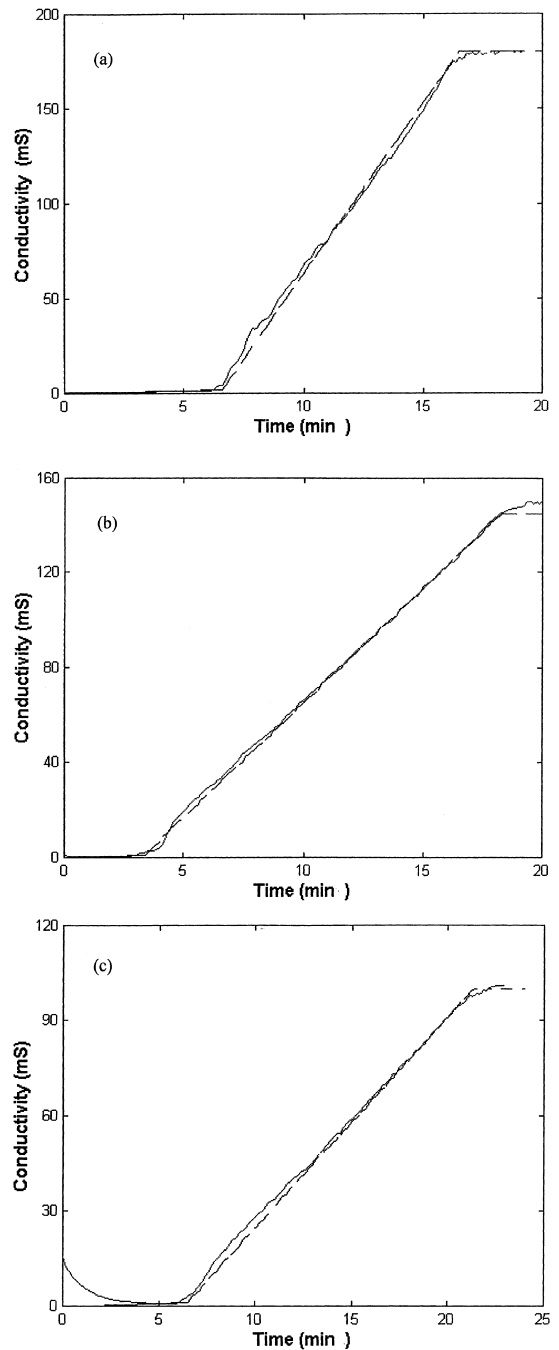


Fig. 4. Results of the implementation of the feedforward control strategy. In all cases, the dotted line is the setpoint and the solid line is the actual trace. (a) Top: 0–100% B over 10 min; 100% B = 180 mS; flow-rate=2 l/min. (b) Middle: 0–100% B over 15 min; 100% B = 145 mS; flow-rate=2 l/min. (c) Top: 0–100% B over 15 min; 100% B = 100 mS; flow-rate=2 l/min.

appropriate mixing ratio for a given conductivity setpoint:

$$mS = \alpha(\% B)^2 + \beta(\% B) \quad (1)$$

$$(\% B)_{\text{reqd}} = \frac{-\beta \pm \sqrt{\beta^2 + 4(mS)_{\text{sp}}\alpha}}{2\alpha} \quad (2)$$

It was determined that due to the non-linearity of mixing solutions of differing densities, the parameters of the quadratic fit, α and β are dependent on the conductivity of solution B. An illustration of the

variation of the effluent conductivity as a function of the mixing ratio (% B) for different salt solutions is shown in Fig. 2. The mixing ratio (% B) in Fig. 2 was computed from the programmed gradient after accounting for the system dead volume. As can be seen in all the figures, the data is well described by a quadratic equation. The signs of the two parameters in addition to the requirement that % B not exceed 100 cause the solution of Eq. (2) based on the negative square root value to be neglected.

The parameters, α and β of the various fits were plotted as a function of the conductivity of solvent B

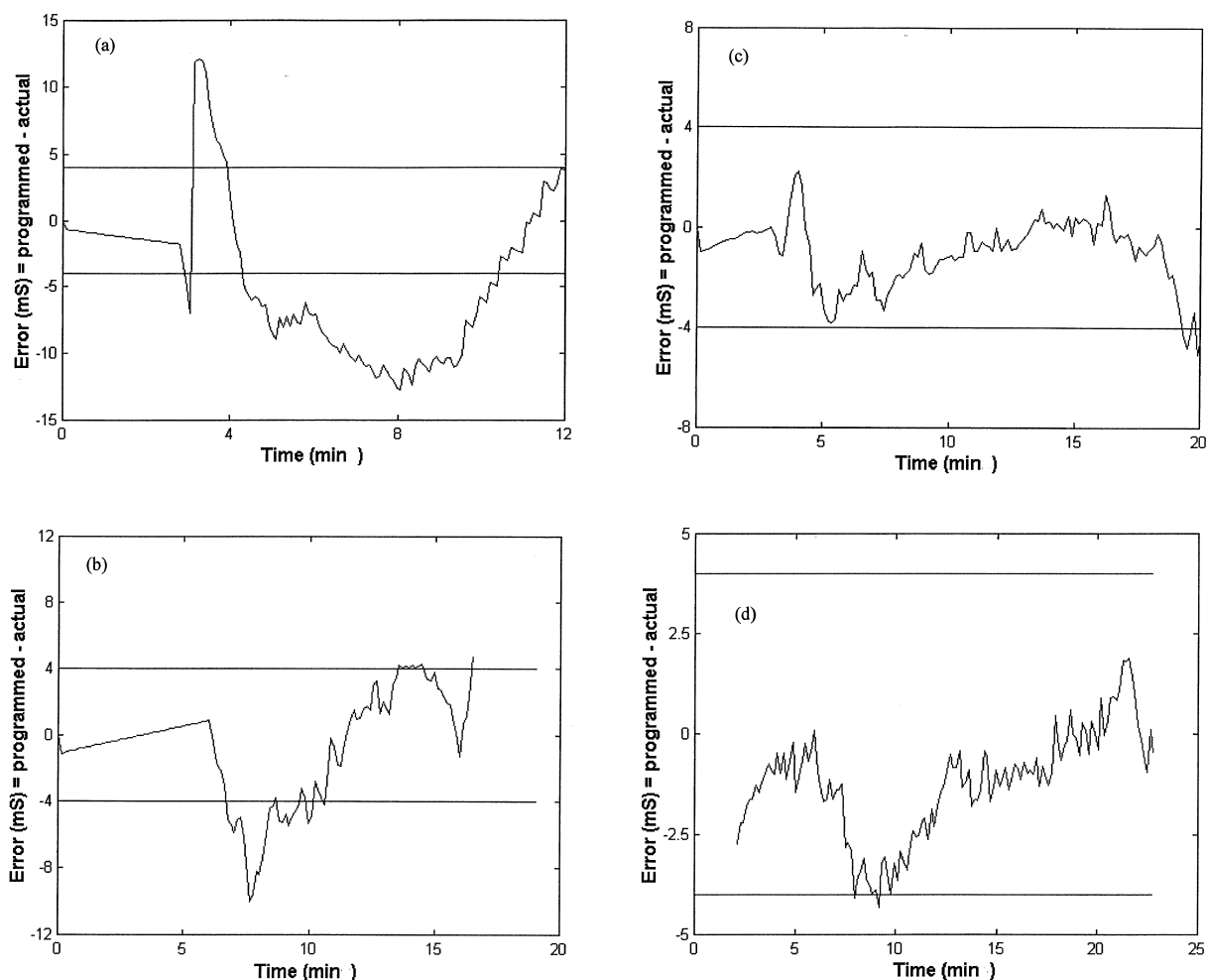


Fig. 5. Evaluation of the accuracy of the gradients formed by the K-Prime 40-III system. The errors were evaluated by comparing the actual gradient to the programmed gradient corrected for dead time. (a) Errors for the uncontrolled gradient (Fig. 1). (b) Errors for the gradient shown in Fig. 4a. (c) Errors for the gradient shown in Fig. 4b. (d) Errors for the gradient shown in Fig. 4c.

in Fig. 3a and b. The resulting correlation between the parameters and the conductivity of the solvent B was well described by a linear function.

Subsequently, the feedforward control algorithm described above was coded in the PLC. The results of the implementation of this control strategy are shown in Fig. 4a–c.

In each of the cases, the appropriate α and β values were computed using the correlations in Fig. 3 and updated in the PLC. The PLC logic employed these values along with the desired conductivity at a given time (from the programmed gradient) to evaluate the necessary mixing ratio using Eq. (2). As can be seen from Fig. 4a–c, such an approach results in a marked improvement in the gradient shape. To monitor the accuracy of the control, the actual gradient was compared with the programmed gradient corrected for the dead time. Fig. 5a–d illustrate the errors associated with the gradient formation with (Fig. 5b–d) and without (Fig. 5a) the feedforward control strategy. As is evident from the figures, there is a marked improvement in the accuracy of the gradient with the feedforward control strategy. For the uncontrolled gradient (Fig. 1), the errors are significantly greater than the preset limits of ± 4 mS [based on the accuracy of 2% full scale deflection (FSD) of the Wedgewood sensor] over the course of the gradient (Fig. 5a). On the other hand, for the controlled gradients, the corresponding errors are within the preset limits over the most part of the gradient (Fig. 5b–d).

It should be noted here that one would expect a similar dependence of the conductivity on the mixing ratio (Fig. 2) for different salt types such as KCl.

However, the relationship will vary with the salt type and hence, the parameters obtained with one salt type may not be valid with another salt type. In such a case, the parameters need to be re-evaluated for that particular salt type.

4. Conclusions

A relatively straightforward feedforward control strategy has been implemented to accurately form gradients suitable for process scale chromatography. Minimal experimental data is necessary to calculate the required parameters. These parameters are not expected to change with time provided the instruments are regularly calibrated. The implementation of such a control approach will enable very simple generation of accurate and reproducible conductivity-based gradients. The current standard method relies on mixing known percentages of buffer solutions in a linear fashion, but as can be seen from the data presented in this manuscript, this can result in significant deviations from the desired setpoints.

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