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Some pH control problems in gradient elution ion-exchange chromatography*

Buffered eluent solutions are often used in ion-exchange chromatography to minimize pH instabilities and to avoid adverse effects on the liquid–resin equilibria or on the chemical forms of the species being separated. However, in gradient elution chromatography, where the eluent changes from a dilute buffer solution to a highly concentrated buffer as the run progresses, the initial buffering capacity may be insufficient to maintain the system pH within desired limits. As a result, the column and eluate pH may vary with time although the eluent pH is constant.

This problem of pH control during the early stages of gradient elution chromatography has been examined on a high-pressure anion-exchange chromatograph developed at the Oak Ridge National Laboratory for the determination of ultraviolet (UV)-absorbing constituent of body fluids[1–3].

This report describes the results of tests designed to define the mechanisms relating operating parameters to eluate pH and discusses methods for minimizing the adverse effects of insufficient pH buffering.

Experimental

A Mark II model of the UV-analyzer previously described[3] was adapted with a 0.5 cc flow cell for continuous monitoring of the eluate pH. With that analyzer the sample is introduced into a flowing stream of 0.015 *N* acetate buffer (ammonium acetate–acetic acid) at pH 4.4 and pumped at 100–200 atmospheres pressure through a 150 cm × 0.62 cm anion-exchange column. During the course of elution the concentration of acetate buffer is gradually increased from 0.015 *N* to 6.0 *N*, with no change in pH. The eluate pH was recorded from normal runs with and without a urine sample and also with different operating conditions to demonstrate the effects of sample constituents and abrupt buffer concentration changes.

Normal analytical runs. During the course of normal analyses the buffer concentration in the liquid phase entering the column is changed from 0.015 *N* to 6.0 *N* as shown in Fig. 1. Over 100 chromatographic peaks are produced from a urine sample. During such an analysis the eluate pH has significant variations as shown in Fig. 2. A comparison of pH and chromatographic recordings from this and repeated analyses shows that two undesirable features of the chromatograms were found to coincide with pH excursions. The first undesirable chromatographic feature was the variability in size and position of peaks, including that for creatinine, which elute in the first hour (Fig. 1). The second was the variability in position and relative broadness of as many as 15 peaks, including hypoxanthine, xanthine, and tryptophan peaks, eluting in the 3 to 11 h region.

Run without sample. In the absence of a sample, the eluate pH (Fig. 2) shows only two major pH excursions. The first excursion is a broad rise beginning at 2 h where the eluent buffer concentration is about 0.3 *N*; it reaches a maximum at about 11 h where the buffer concentration is about 1.0 *N*; and it ends near 22 h with the

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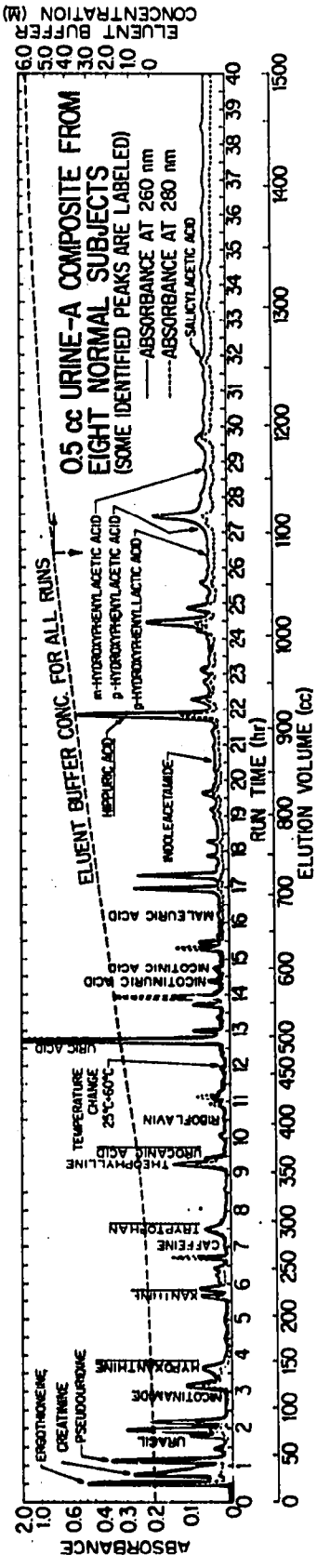

Fig. 1. The eluent buffer gradient and a representative chromatogram.

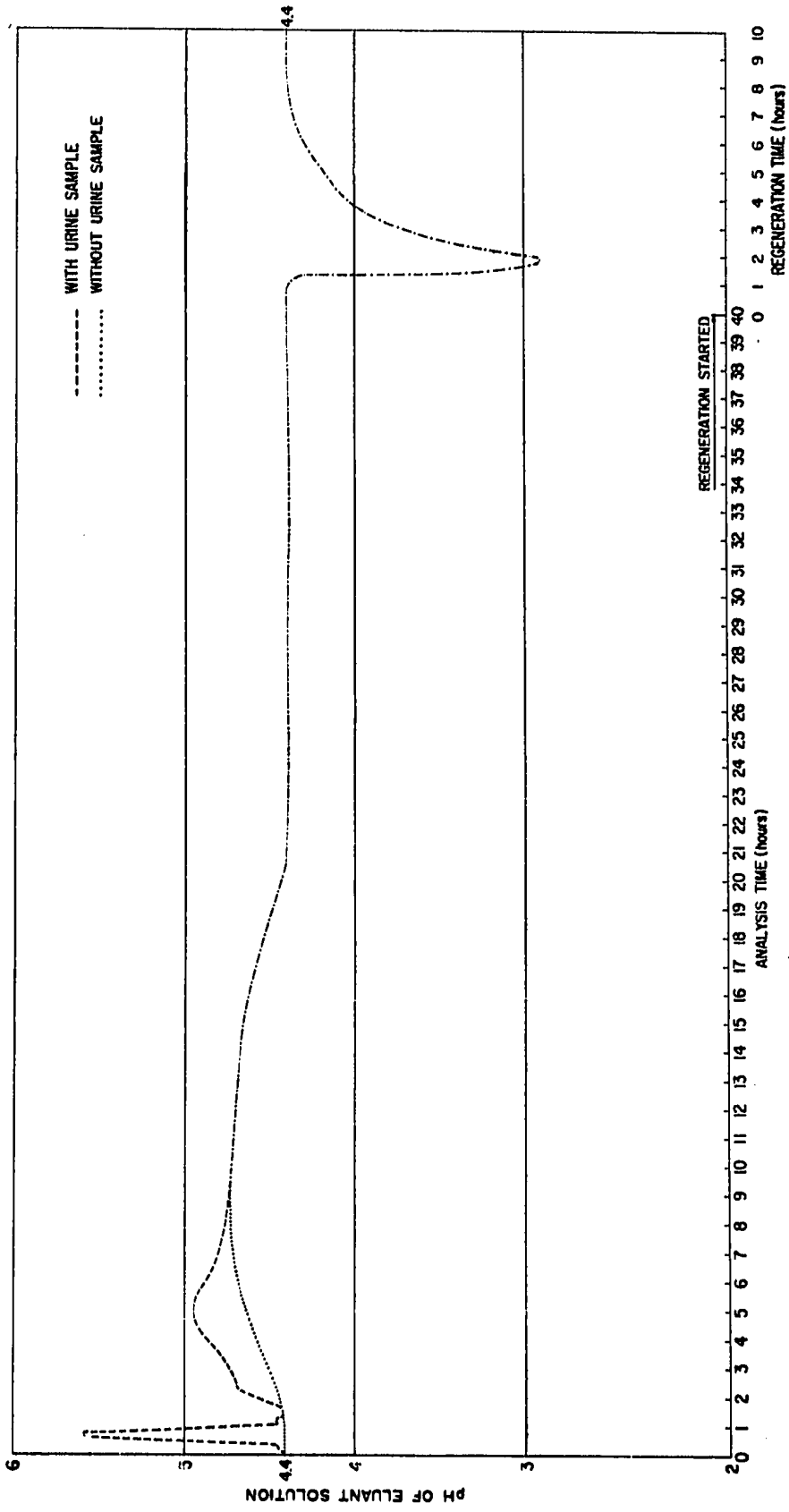

Fig. 2. A comparison of the eluate pH recordings with and without a sample.

buffer concentration about 2.5 *N*. The second pH excursion is the large dip immediately following the change from a feed concentration of 6.0 *N* to 0.015 *N* at the end of the run where regeneration is begun.

An abrupt increase in buffer concentration. Operating the system with step changes in eluent buffer concentration shows that eluate pH excursions occur after such changes. For example, after an abrupt change in eluent buffer concentration from 0.015 *N* to 6.0 *N* a rapid increase in eluate pH from 4.40 to 5.65 occurs.

Discussion

Buffer concentration changes. The experimental eluate pH excursions associated with changes in eluent buffer concentration appear to be a sharp decrease following an abrupt drop in buffer concentration, a sharp rise following an abrupt increase in buffer concentration, and a broad peak following an initial gradual increase in concentration (Fig. 2). The direction of these changes are consistent with current ideas of the nature of the chemical equilibria[4,5]. That is, at each point along the anion-exchange column an equilibrium exists between the acetate and hydroxyl ions in the liquid and at the active sites of the resin. To satisfy the anion-exchange resin equilibrium with the acetate ion, a net transfer of acetate ion from the liquid to resin phase occurs with a corresponding transfer of hydroxyl ion to the liquid phase when the concentration of buffer is increased in the liquid phase. This results in the acid present in the buffer being neutralized. Conversely the opposite exchange occurs when the buffer concentration is decreased. Thus, in this anion-exchange system it is to be expected that an increase in eluent buffer concentration will cause a temporary increase in eluate pH and a decrease in eluent buffer concentration will cause a temporary decrease in eluate pH.

The broad pH peak starting at 2 h in a normal analysis is therefore most important since it is caused by an increase in buffer concentration from about 0.3 to 1.0 *N* and is associated with erratic chromatographic peak positions and broadening of peaks. While this pH variation could be reduced in magnitude by starting the elution at a higher buffer concentration, such a change would be counterproductive because of undesirable crowding of peaks from basic and neutral compounds which elute early. Another possible solution would be to impose a declining pH gradient upon the increasing concentration gradient; this, however, greatly alters the order of peak elution and appears to cause broadening of peaks in the latter half of the chromatogram. Some improvement appears possible through reducing the slope of the concentration gradient.

Sample constituents. The eluate pH peaks that occurred with the urine sample but were absent in the run without sample (Fig. 2) appear related to eluted sample constituents. Where the acetate concentration of the sample differs from the 0.015 *N* buffer the effect will be as discussed above. In the area of the chromatogram where the dilute 0.015 *N* buffer was used, ions more strongly retained by the resin, like chloride ions, will displace the acetate ion and thereby affect the liquid–resin equilibria. Other constituents of body fluid samples are pH buffers and affect the pH of the eluate as they elute. The first large pH peak in the runs with a urine sample, rising from pH 4.4 to 5.9, appears due to the additive effect of each of these mechanisms. The smaller pH peaks which follow are associated with the arrival in the eluate of buffering compounds such as urea and amino acids.

The best way to reduce the magnitude of the pH excursions due to sample constituents is by using smaller samples. That may be possible with the development of more sensitive photometric systems.

Conclusions

The excursions in pH caused by concentration gradient elution of a buffer and by eluted sample constituents have been demonstrated for anion-exchange chromatography and have been shown to be predictable in direction. Consideration of the mechanisms affecting the eluate pH are helpful in making system and operational changes designed to mitigate the adverse effects of pH excursions.

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