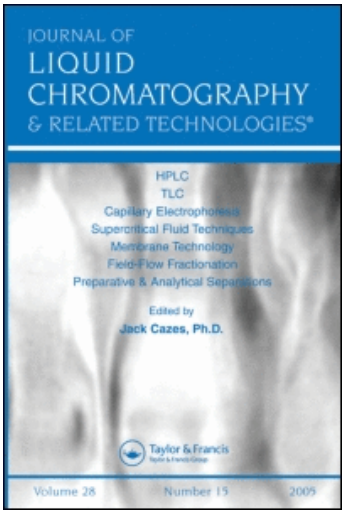
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Coulometric Generation of Gradient Elution Programs: Separation of Amino Acids Without Buffer Mixing

Jeffery C. Wright[a]; Ronald F. Evilia[a]
[a] Department of Chemistry, University of New Orleans, Lake Front New Orleans, LA.

To cite this Article Wright, Jeffery C. and Evilia, Ronald F.(1979) 'Coulometric Generation of Gradient Elution Programs: Separation of Amino Acids Without Buffer Mixing', Journal of Liquid Chromatography & Related Technologies, 2: 5, 719 — 724
To link to this Article: DOI: 10.1080/01483917908060098
URL: http://dx.doi.org/10.1080/01483917908060098

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 2(5), 719-724 (1979)

COULOMETRIC GENERATION OF GRADIENT ELUTION PROGRAMS: SEPARATION OF AMINO ACIDS WITHOUT BUFFER MIXING

Jeffery C. Wright and Ronald F. Evilia
Department of Chemistry
University of New Orleans
Lake Front
New Orleans, LA. 70122

Downloaded At: 19:31 24 January 2011

ABSTRACT

A coulometric cell and buffer system is reported which allows control of the pH of the mobile phase by control of the electrolysis current passed through the cell. The electrolysis cell is capable of varying the pH over the range of 2.5 - 12 without gas evolution at flow rates up to 1 ml/min. The pH is flow rate dependent in the configuration reported, but appropriate pH monitoring and electronic feedback can eliminate that problem. The use of coulometric pH control for the separation of amino acids without any buffer mixing is illustrated.

INTRODUCTION

The liquid chromatographic separation of amino acid mixtures is an old and time tested procedure (1). The basic idea of exploiting pKa differences by gradually changing the eluant pH has found such wide utility and has performed so well that a variety of commercial equipment capable of automatically carrying out assigned elution programs is available. In spite of the availability of sophisticated instruments, there is room for improvement.

One area which has received little attention is the means by which pH programs are generated. The basic technique of mixing buffers of different pH's and ionic strengths in varying proportions to generate the desired pH versus time profile appears to have universal acceptance and has changed little over the years (2).

Although the mixing of buffer solutions can produce the desired pH variation the problems associated with mechanically mixing reagents under high pressures can lead to many practical problems. The most obvious prob-

Copyright © 1979 by Marcel Dekker, Inc. All Rights Reserved. Neither this work nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming, and recording, or by any information storage and retrieval system, without permission in writing from the publisher.

lems are associated with high pressure leaks and excessive down time for routine and preventive maintenance. There is the practical problem of the need for expensive and sophisticated devices for monitoring and mixing the reagents in the proper proportions which greatly increases the cost of instrumentation and discourages small users from considering the purchase of an amino acid analyzer.

One approach to the elimination of reagent mixing is the coulometric generation of reagents. The coulometric generation of hydroxide and hydrogen ions has been known for a long time. For example, recently the coulometric generation of hydroxide controlled by a pH stat circuit was utilized for the analysis of acids (3). The electrolysis of aqueous buffer solutions to produce H+ (and O_2) or OH^- (and H_2), however, is not appropriate for liquid chromatographic systems because of the generation of gases and the probable loss of 100% current efficiency at the high current densities needed for largh pH variation. A buffer system suitable for coulometric pH variation in liquid chromatography must satisfy the following requirements: It must be electrochemically active with 100% current efficiency over a wide range of current densities; either H^+ or OH^- must be produced in the electrochemical reaction but no gases evolved; neither the buffer nor any of the buffer's electrolysis products should react with amino acids (except OH^-, of course) or ninhydrin; neither the buffer nor its electrolysis products should absorb at the wavelengths commonly used for detection; the buffer should be readily available in reasonable purity and at a reasonable price. The buffer system and electrolysis cell design reported in this paper satisfies all of the above requirements.

Downloaded At: 19:31 24 January 2011

EXPERIMENTAL

Electrolysis cell: A flow through mercury electrode electrolysis cell similar to one reported by Parsons and Seaman was utilized (4). The cell was modified by incorporation of an agar salt bridge to prevent mixing of the copper ion produced at the anode with the mobile phase. The cell design is shown in Figure 1. The agar salt bridge contained 1.7 M KNO_3 for good electrical conduction and a small amount of Na_2H_2 EDTA to repress electrical migration of Cu^{2+}

Constant Current Source: Current through the cell was controlled by a modified version of the pH stat reported by Adams, Betso and Carr (3). The pH stat was modified by removal of the pH measuring and comparison circuit and operation of the circuit as a variable output galvanostat. In operation the current through the cell (and, hence, the mobile phase pH) are set by adjustment of two variable resistors.

pH Measurements: A Corning 110 Digital pH meter and a Sargent-Welch micro combination pH electrode were used to obtain pH measurements throughout

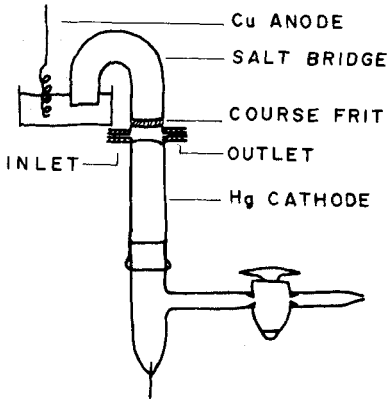


FIGURE 1 - Diagram of flow through Electrolysis Cell.

Downloaded At: 19:31 24 January 2011

the course of the experiments and were standardized against standard buffer solutions of 4.01, 7.00, and 9.15 before use. Measurements were made on 1 ml volume increments obtained by a Buchler Instruments Inc. model 57769 fraction collector.

Column: The column used in this experiment was constructed from a 17 cm section of 10 mm OD glass tubing and packed with 3.015 g of Dowex 50 x 8 cation exchange resin in the sodium form and equilibrated with the reservoir buffer solution of pH 3.25. A Scientific Industries model 403 paristaltic pump was used to pull the resevoir solution through the electrolysis cell column. The effluent from the column was collected in a Buchler Instruments, Inc. model 57769 fraction collector set to approximately 1 ml in the drop counting mode. A flow rate of approximately 0.8 ml/min was used for all experiments reported here.

Buffer solution and chemicals: The buffer solution which was placed in the 1ℓ resevoir of the system consisted of a .02 M trichloroacetic acid (reagent grade), .004 M phosphoric acid (reagent grade) and 0.1 M potassium nitrate (reagent grade) and was adjusted to an initial pH of 3.25 with sodium hydroxide. Amino acids were obtained from Sigma Chemical (Sigma Grade) and were used without further purification. All other reagents were of highest commercial purity and used without further purification.

RESULTS AND DISCUSSION

The buffer system chosen for this work had to meet the restrictions given in the introduction. The most serious restriction is the requirement that gases not be generated and that 100% current efficiency be maintained over a large range of currents and pH's. Examination of the literature indicated that the

electrochemical reduction of trichloroacetate $Cl_3CCO_2^- + 2e^- + H_2O \longrightarrow Cl_2CHCO_2^- + Cl^- + OH^-$ occurs with 100% current efficiency at high pH (5). This appeared to be a good possibility for the electroactive species, phosphoric acid was added to the system to provide buffering capacity. Potassium nitrate was used as supporting electrolyte to reduce electrical resistance.

In order to characterize the electrolysis cell and to provide as severe a test of the coulometric apparatus as possible, a buffer system of low buffer capacity was used so that the pH range up to 12 could be investigated. No evidence for gas evolution or deviation from 100% current efficiency was observed even at the highest current densities utilized in this work. Because of the resistance of the salt bridge, the maximum current which could be passed through the cell was approximately 60 ma.

Figure 2 shows the output pH of the coulometric cell as a function of the current flowing through the cell. The points in the figure are the measured data points. An approximate theoretical pH versus current curve generated neglecting activity coefficients agrees well with the experimental curve within the experimental error of the flow rate. (± 0.1 ml/min.) In the separation reported below the pH was determined by setting the current to the value determined by figure 2 for the pH desired. No problems were encountered by this procedure.

One problem associated with this method of pH variation is that the pH is flow rate dependent. Figure 3 shows the typical flow rate dependence of the pH. Obviously this difficulty can be easily overcome by incorporation of pH measurement and feedback into the galvanostat circuit. Since it was not the purpose of this study to refine all the details but rather to demonstrate the basic operating feasibility, this aspect was not pursued further. It should be noted, however, that the simple paristaltic pump used in this study provided very acceptable reproducibility and constancy of flow. Incorporation of this coulometric device into a liquid chromatograph containing a modern high performance pump should make this flow rate dependence a negligable concern.

Once the pH versus current dependence was determined the successful operation of the coulometric pH variation was insured. In order to demonstrate the utilization of this type of elution programing for amino acid analysis a mixture containing 3 amino acids was placed on a column and eluted with the buffer solution and pH change as shown in figure 4. The identity of the amino acids was confirmed by elution of the pure acids. No attempt was made to identify the impurities. The amino acids were collected in 1 ml increments by the fraction collector, treated with ninhydrin in the standard way (6) and read at 570 nm in a Spectronic 20 colorimeter. While figure 4

Downloaded At: 19:31 24 January 2011

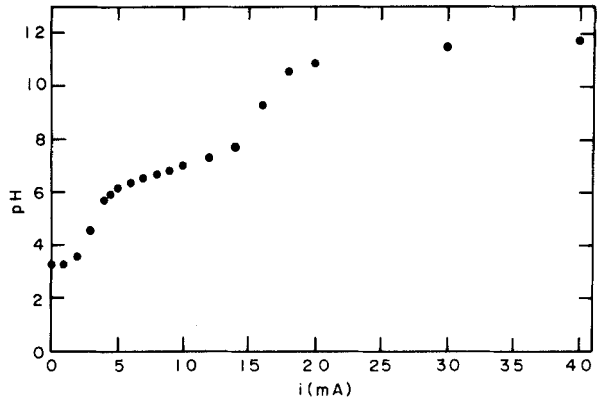


FIGURE 2 - pH of eluant versus current passed through electrolysis cell. Flow rate = 0.8 ml/min.

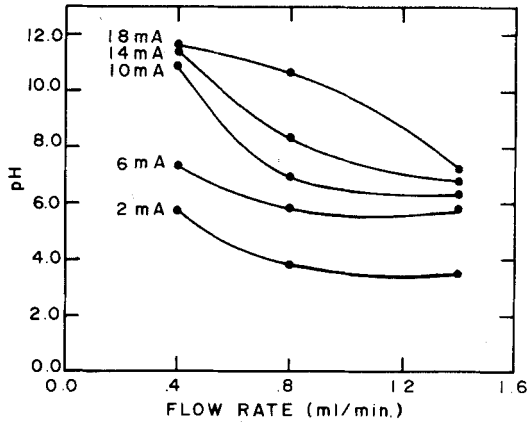


FIGURE 3 - Variation of eluant pH with flow rate at constant current.

does not show great resolution it should be recalled that these data were not obtained from a high resolution system and no attempt was made to optimize resolution. The purpose of these experiments was to demonstrate the feasibility of using coulometrically generated reagents for elution programming in liquid chromatography. The results reported here show clearly that this technique is feasible.

Several refinements are presently being examined in order to improve the system. One refinement involves the pH measurement and feedback referred to above. Another refinement which will be made is the replacement of the salt bridge by a low resistance ion exchange membrane to increase the maximum current. Studies are currently underway with higher buffer-capacity buffers

Downloaded At: 19:31 24 January 2011

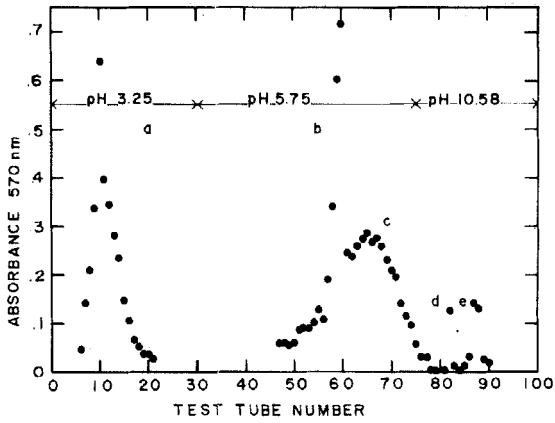


FIGURE 4 - Separation of Aspartic Acid, Leucine, and Lysine. Peak a 1.0μ mole Aspartic Acid, peak b 1.0μ mole Leucine, peak c 1.0μ mole Lysine, Peaks d and e unidentified impurities.

and the cell is being interfaced with HPLC so that high resolution separations can be performed.

ACKNOWLEDGEMENT

This work was supported in part by the UNO research council award.

References

(1) Moore, S., Spackman, D.H.and Stein, W. H., Anal. Chem., 30, 1185, (1958).

(2) Welch, R. W., J. Chromatogr., 152, 528, (1978).

(3) Adams, R. E., Betso, S. R., and Carr, P. W., Anal. Chem., 48, 1989, (1976).

(4) Parsons, J. S. and Seaman, W., Anal. Chem., 27, 210, (1955).

(5) Meites, T. and Meites, L., Anal. Chem., 27, 1531, (1955).

(6) Blackburn, S., "Amino Acid Determination," Marcel Dekker, Inc., New York, 1968, pp.77-78.

Downloaded At: 19:31 24 January 2011