

# Potentiometric Measurements in Air-Segmented Streams without Debubbling

Theodore K. Christopoulos

Laboratory of Analytical Chemistry, University of Athens, 104 Solonos Street, Athens 106 80, Greece

Eleftherios P. Diamandis\*

CyberFluor Inc., 179 John Street, Suite 400, Toronto, Ontario, M5T 1X4 Canada, and Department of Clinical Biochemistry, University of Toronto, 100 College Street, Toronto, Ontario, M5G 1L5 Canada

## INTRODUCTION

The concept of air-segmented continuous-flow analysis (CFA) was introduced by Skeggs in 1957 (1) and has been used extensively since then (2, 3). Although newer approaches like flow injection analysis are emerging (4), CFA is still used, especially in selected areas of industrial and pharmaceutical applications such as quality control.

Two approaches have been generally used to solve the problems related to the presence of bubbles in the measuring stream: (a) aspirate the bubbles with a device called a "debubbler" used in the Technicon Autoanalyzer system or recently published in a new configuration (5) or (b) use "bubble gating" techniques to electronically remove signal noise during the passage of the bubbles through the detector (6, 7).

Ion-selective electrodes (ISE) are now widely used as detectors in continuous-flow automated systems (8). Technicon succeeded in eliminating the noise generated by air bubbles in their sequential multiple analyzer with computer (SMAC) system, employing ISE for  $\text{Na}^+$  and  $\text{K}^+$ , by electronic means (9). In most other applications, best results are obtained when the bubbles are removed before reaching the potentiometric detector.

In this report, we propose a simple and inexpensive way of carrying out continuous-flow potentiometric measurements in the presence of air bubbles. This is accomplished by measuring the emf of the electrochemical cell simultaneously and continuously at two points of the flowing stream. The performance of the proposed bubble-through electrode unit was evaluated for iodide (solid-state) and propantheline (PVC-matrix) sensitive membranes. The observed potential oscillations in the presence of bubbles were  $\leq 0.2$  mV in all experiments conducted.

## EXPERIMENTAL SECTION

**Reagents.** All chemicals used were of analytical-reagent grade. Standard solutions were prepared with deionized, distilled water. Propantheline bromide was obtained from Sigma Chemical Co. (St. Louis, MO), poly(vinyl chloride) (PVC) of very high relative molecular mass from Aldrich Chemical Co. (Milwaukee, WI), and *o*-nitrophenyl octyl ether (*o*-NPOE) and sodium tetraphenylborate from Fluka (Buchs, Switzerland).

**Apparatus.** A schematic diagram of the automated analysis flow system used is shown in Figure 1. A Gilson Minipuls variable-speed peristaltic pump facilitated continuous pumping of solutions through the electrode assembly. All flow rates refer to liquid flow only (air flow is not included). The sampler was an A40 Sampler II form Hook and Tucker (Surrey, U.K.). The

external reference saturated calomel electrode was obtained from Radiometer (Copenhagen, Denmark). All potentiometric measurements were carried out at ambient temperature with a Corning Model 12 Research pH/mV meter and recorded on a Radiometer strip chart recorder (REC 61) through a high-sensitivity unit (REA 112) interface.

**Construction of the Salt Bridge Electrolyte-Sample Solution Contact Zone.** A cylindrical piece of porous ceramic, 6 mm long and 1 mm in diameter, was constructed from a standard porous ceramic plate as described previously (10). This was used in all flow-through cells described below to facilitate contact between the measuring solution and the salt bridge of the external reference electrode. Before use, the ceramic plug was placed for 5 min in the appropriate salt-bridge solution (see below) to wet, and any excess was then removed with absorbent paper.

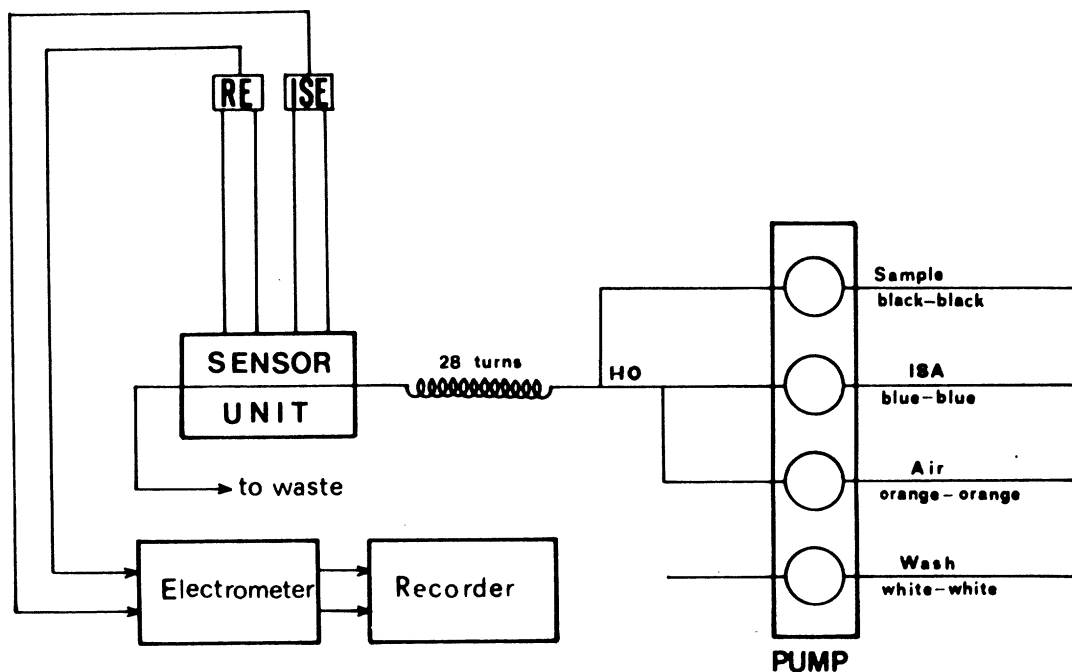
**Construction of Bubble-Through Flow Cell for Solid-State Membrane, ISE.** The cell configuration is shown in Figure 2a. An AgI-Ag<sub>2</sub>S coprecipitate pellet-sensing membrane was employed, prepared as described elsewhere (11). After construction, the pellet was ground with emery paper to obtain a semicircular shape (Figure 2a). The solid membrane and the ceramic plug were then embedded in the epoxy resin (Epofix resin; M. Struers Scientific Instruments, Copenhagen, Denmark) by using a plastic mold (dimensions  $4.5 \times 4 \times 1.5$  cm) as reported earlier (10). Holes of appropriate diameter were then made on the upper surface of the resulting orthogonal cast block as shown in Figure 2a, with slow-speed drilling, using regular drill bits. Two flow-through channels, 1.5 mm in diameter and at a 5-mm distance between each other, were also drilled vertically to the solid membrane and the ceramic plug, so that each channel contacted both of these components, leaving the ion-sensitive membrane opposite the ceramic junction. Finally, a pump tube (2 cm long and 1 mm in internal diameter) was used to join the two channels together by means of two stainless steel nipples.

**Cell Organization.** The internal reference solution ( $10^{-2}$  M  $\text{AgNO}_3$ ) and the salt bridge ( $100 \text{ g L}^{-1}$   $\text{NaNO}_3$ ) were added in the appropriate holes of the flow-through unit. A silver wire and a saturated calomel electrode were used as internal and external reference electrodes, respectively. The electrochemical cell was mounted in the continuous-flow system by using two stainless steel nipples.

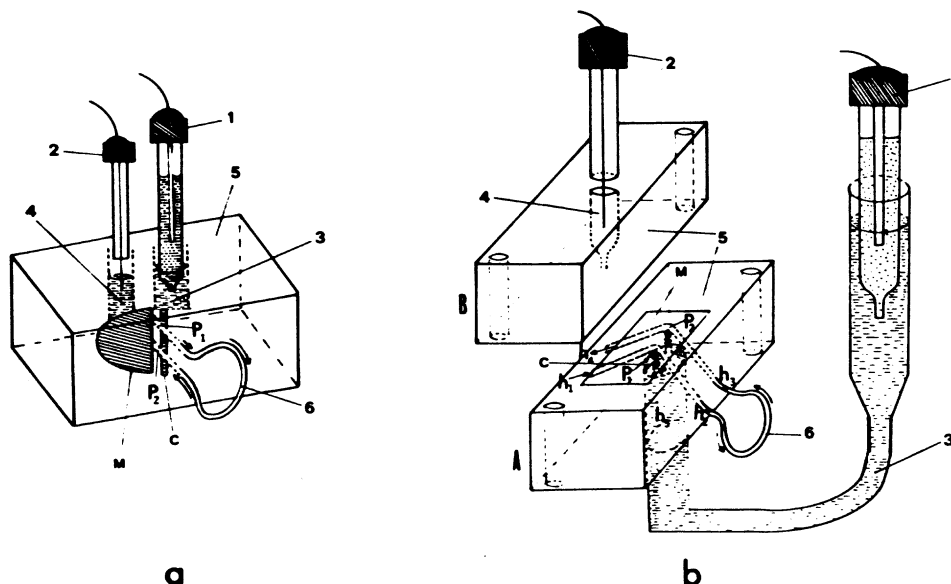
**Preparation of a PVC Membrane Sensitive to Propantheline.** The construction and characteristics of a propantheline liquid-membrane ISE and its application to pharmaceutical analysis were described previously (12, 13). The ion exchanger was the tetraphenylborate salt of propantheline dissolved in *o*-nitrotoluene. The liquid ion exchanger for the PVC membrane used in this work was prepared in the same manner except that *o*-nitrotoluene was replaced by *o*-NPOE. The liquid ion exchanger was then entrapped in PVC by the procedure described elsewhere (14).

**Construction of Bubble-Through Flow Cell for PVC-Matrix Membrane ISE.** The configuration of the constructed cell is shown in Figure 2b. The unit consists of two orthogonal Plexiglas pieces, A and B, with dimensions of  $3 \times 1.7 \times 1.4$  cm. The pieces were assembled as follows:

\* To whom correspondence should be addressed at CyberFluor Inc.



**Figure 1.** Schematic diagram of the continuous-flow system without debubbler: ISE, ion-selective electrode; RE, reference electrode; HO, "cactus"; ISA, ionic strength adjusting solution. The color code of the pump tubes is that given by Technicon.



**Figure 2.** (a) Bubble-through electrode unit for solid-state membrane ISE: 1, external reference electrode; 2, internal reference electrode; 3, salt bridge; 4, internal reference solution; 5, electrode body; 6, plastic loop connecting the inlet and outlet channels; C, ceramic plug; M, electrode membrane;  $P_1$ ,  $P_2$ , points of sample contact with the membrane. (b) Bubble-through electrode unit for PVC-matrix membrane ISE: A, B, orthogonal pieces of Plexiglas; 1-6, C, M,  $P_1$ ,  $P_2$  as in (a); holes  $h_1$ -5 are described in detail in the text.

*Piece A.* The sample stream segmented by air bubbles enters the cell from hole  $h_1$  (inlet), leaves piece A from hole  $h_2$ , travels over the loop 6, and then enters again in piece A (but in opposite direction) from hole  $h_3$ . Finally, it leaves the cell from hole  $h_4$  (outlet). All holes ( $h_1$ - $h_4$ ) have a diameter of 1.5 mm. Holes  $h_1$  and  $h_2$  coincide at point  $P_1$  and holes  $h_3$  and  $h_4$  at point  $P_2$  in a distance of 5 mm from  $P_1$ .

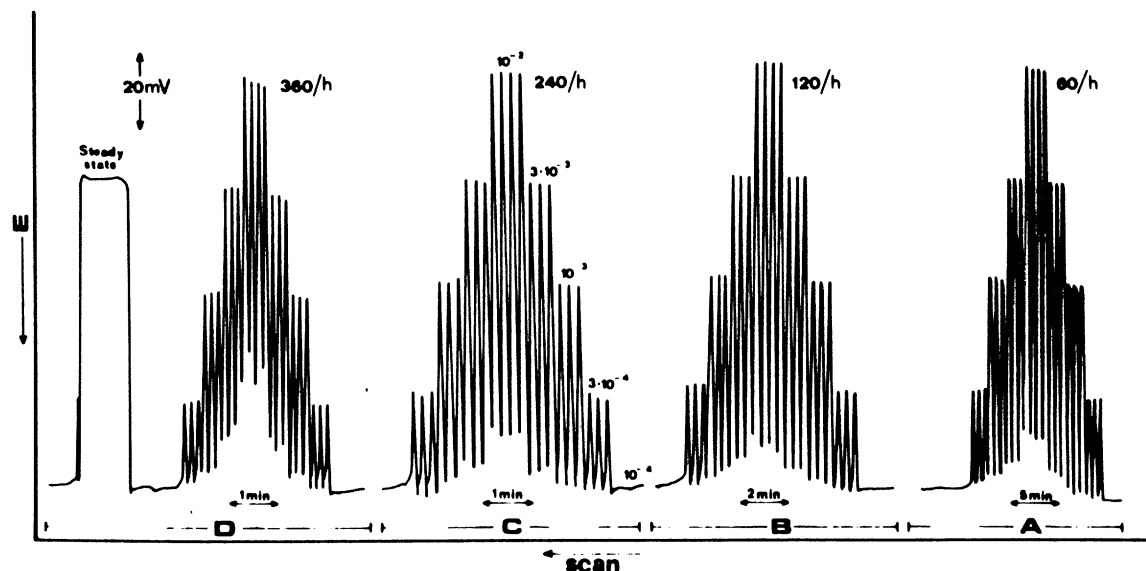
In the center of the lower surface of piece A another hole is drilled ( $h_5$ ) 12 mm in diameter and 9 mm deep. Two holes are further drilled from the bottom of hole  $h_5$ , with a 1.2-mm bit and vertically to the upper surface, in such a way that they encounter holes  $h_2$  and  $h_4$  at points approximately 0.5 mm from points  $P_1$  and  $P_2$ , respectively. In each narrow hole a ceramic junction plug is inserted so that the one end is positioned approximately in the middle of hole  $h_2$  (or  $h_4$ ) and the other lies in  $h_5$ . Each junction is secured in place with Araldite epoxy adhesive, which is added from points  $P_1$  or  $P_2$  and from the bottom of hole  $h_5$ . The adhesive fills the space between the plugs and the Plexiglas and is left for

2 h to harden. The excess of the adhesive is removed by using a 1.5-mm bit in holes  $h_2$  and  $h_4$  and a 2-mm bit in  $h_5$ . It is essential that the two ends of each ceramic plug are free from an excess of adhesive; otherwise electrolytic contact may not be established. A 4-cm-long glass tube with approximate internal and external diameters of 5 and 7.5 mm, respectively, is inserted in  $h_5$  and stuck with Araldite adhesive as shown in Figure 2b. This glass tube is connected with a wider "reservoir" tube via PVC tubing (approximately 10 cm long).

*Piece B.* In the center of the upper surface of this piece, hole 4 is drilled, 7 mm in diameter and 8 mm deep. The hole is further drilled with a 2-mm-diameter bit until it reaches the lower surface of the piece.

The two pieces can be stacked together by means of two screws that are positioned in holes at the two corners of the pieces.

*Cell Organization.* A 10 × 5 mm piece of PVC membrane is cut and placed on the upper surface of Plexiglas piece A so as to cover the small holes that are created at points  $P_1$  and  $P_2$  by



**Figure 3.** Typical recordings of continuous-flow measurements of discrete samples with the iodide bubble-through electrode at flow rates of (A) 1.74, (B) 3.64, and (C) and (D) 6.70 mL min<sup>-1</sup>. The final concentration of the standards and the sampling rates are shown. Sample to wash ratio was 1:1.

the coincidence of hole  $h_1$  with  $h_2$  and of  $h_3$  with  $h_4$ . Piece B is then placed over piece A and the screws are fastened. Hole  $h_5$ , the PVC tube, and the reservoir tube are filled with 3 M KCl solution (salt bridge).

The level of fluid in the reservoir tube must be at least 5 cm higher than the level of the ceramic junction so that a positive hydrostatic pressure difference exists. Hole 4 of piece B is filled with internal reference solution ( $10^{-2}$  M propantheline bromide-0.1 M NaCl solution, saturated with AgCl). An Ag-AgCl wire is dipped in the internal reference solution as shown in Figure 2b. A standard calomel electrode dipped in the salt bridge serves as an external reference electrode.

## RESULTS AND DISCUSSION

A typical measurement cycle, in the bubble-through units described, consists of four different events:

(a) A bubble may be present at point  $P_1$  (Figure 2), thus opening the circuit at this point. However, electrode potential noise is not produced because there is liquid filling loop 6 and electrical contact through  $P_2$ , which also contains measuring solution.

(b) The bubble moves entirely in loop 6. In this case, both points  $P_1$  and  $P_2$  are free from any bubble effects. The electrode potential is measured through electrical contact at both points.

(c) The bubble moves to point  $P_2$ . This situation is identical with (a), but electrical contact is facilitated through point  $P_1$ .

(d) The bubble leaves point  $P_2$  going to waste. A new bubble has not yet reached  $P_1$  so that electrical contact is facilitated at both points without any bubble effects. These events are repeated in an identical fashion throughout the measurements. In this system, noise-free measurements are successful if the length of liquid between two successive bubbles is greater than the length of one bubble, so that at least one of the points  $P_1$ ,  $P_2$  is not in contact with a bubble, throughout. The  $P_1P_2$  distance can be easily adjusted by changing the length of the plastic tube in loop 6 (Figure 2).

The proposed bubble-through electrodes for iodide anion and propantheline cation exhibited characteristics similar to those of the corresponding conventional dip-type electrodes. Measurements were carried out with the continuous-flow system shown in Figure 1 without the use of the sampler. Solutions of the measured ions were aspirated continuously into the system via the sampling tube, from each stock solution bottle. The bottles were interchanged manually every few minutes. With this method, the steady-state response of the

**Table I.** Response Times of the Proposed Bubble-Through Electrodes at Various Flow Rates and Sampling Rates

| flow rate,<br>mL min <sup>-1</sup> | sampling<br>rate, h <sup>-1</sup> | percentage of steady state |                 |                |                 |
|------------------------------------|-----------------------------------|----------------------------|-----------------|----------------|-----------------|
|                                    |                                   | propantheline              |                 | iodide         |                 |
|                                    |                                   | I <sup>a</sup>             | II <sup>b</sup> | I <sup>a</sup> | II <sup>b</sup> |
| 1.74                               | 60                                | 98                         | 99              | 100            | 100             |
| 3.64                               | 120                               | 98                         | 99              | 100            | 99              |
| 6.70                               | 240                               | 95                         | 96              | 99             | 99              |
| 6.70                               | 360                               |                            |                 | 97             |                 |

<sup>a</sup>The concentration changes were from  $3 \times 10^{-6}$  to  $10^{-3}$  M (propantheline) and from  $10^{-4}$  to  $3 \times 10^{-3}$  M (iodide). <sup>b</sup>The concentration changes for both electrodes were from  $3 \times 10^{-3}$  to  $10^{-3}$  M.

electrodes is recorded. Na<sub>2</sub>SO<sub>4</sub> solutions of 0.050 and 0.025 M were used as wash solutions for the iodide and propantheline ISE, respectively. Calibration graphs based on these recordings gave slopes of 59.5 mV (linear range:  $1.5 \times 10^{-6}$  to  $10^{-1}$  M;  $r = 0.9999$ ) and 57.1 mV (linear range:  $2.5 \times 10^{-6}$  to  $8 \times 10^{-3}$  M;  $r = 0.9995$ ) for the iodide and propantheline bubble-through electrodes, respectively.

The proposed electrodes were also tested in continuous-flow measurements on discrete samples where each sample was separated from the next one by wash solution. These measurements were performed at three flow rates, viz., 1.74, 3.64, and 6.70 mL min<sup>-1</sup>, and at sampling rates of 60, 120, and 240 (and 360 for the iodide electrode) determinations per hour, respectively. The wash solutions used contained either constant dilute (Figures 3 and 4) or concentrated (Figure 5) background levels of the measured ions. With this method, the response of the electrodes from dilute to concentrated solutions and vice versa can be evaluated under continuous-flow conditions. The sample to wash ratio was 1:1 or 2:1 when dilute and concentrated background wash solutions were used, respectively. With increasing flow rate, the response time of the electrodes decreases, as has been shown previously (10, 15). With the iodide solid-state ISE as sensor, sampling rates of up to 360 per hour have been obtained with flow rates as high as 9.2 mL min<sup>-1</sup> with the use of a debubbler (16). Data obtained here with the proposed electrode units for response time studies are given in Table I. The response in all instances is expressed as the percentage of the peak height observed with respect to the peak height of the steady-state

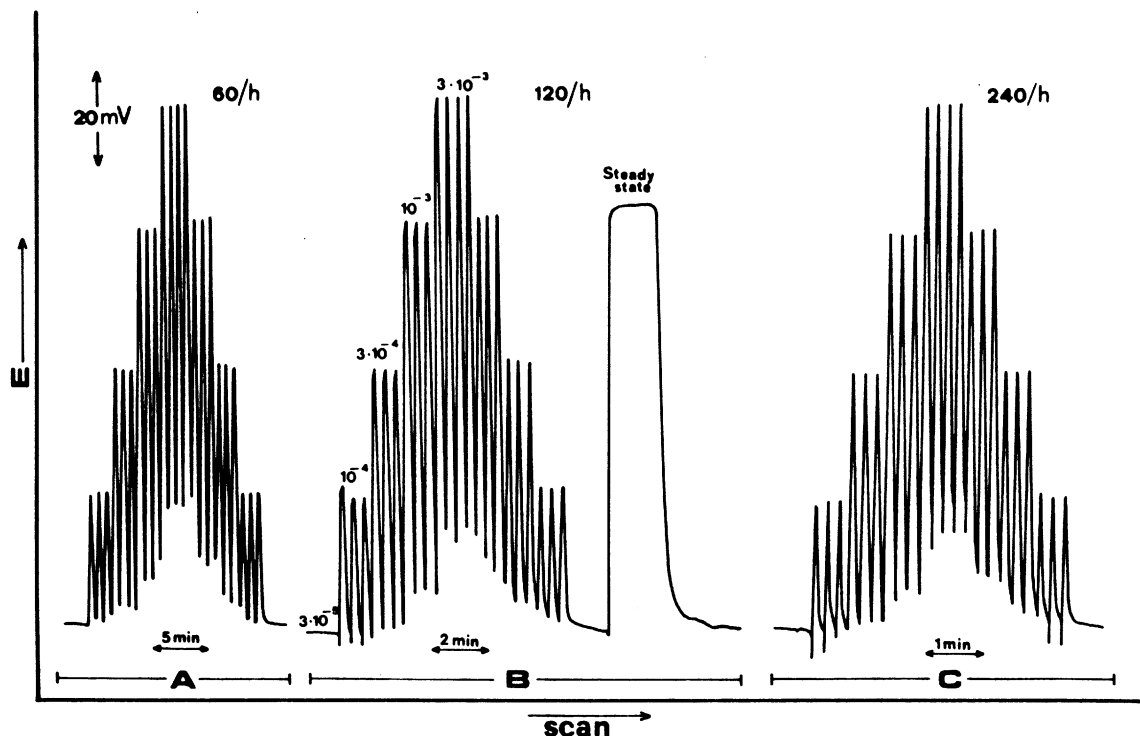


Figure 4. Typical recordings of continuous-flow measurements of discrete samples with the propantheline bubble-through electrode. Flow rates are as in Figure 3. The final concentrations of the standards and the sampling rates are shown. Sample to wash ratio was 1:1.

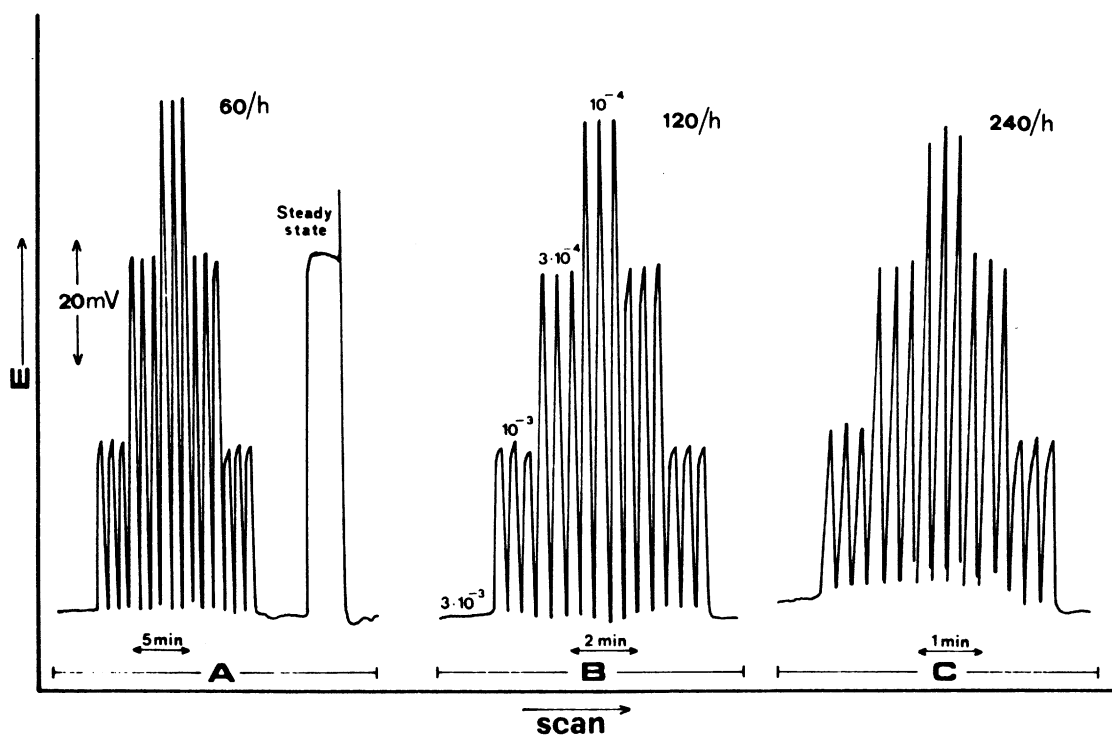


Figure 5. Typical recordings of continuous-flow measurements with the iodide bubble-through ISE using concentrated wash solutions. Flow rates are as in Figure 3. Sample to wash ratio was 2:1.

response. The precision of the measurements can be appreciated by examining the peaks in Figures 3–5. The average coefficient of variation in all instances was better than 2%.

In conclusion, we have described new flow-through units for solid-state and PVC-matrix membrane ISE's. Instead of the segmenting bubbles being debubbled before entering the electrode, they are passed directly through it along with the liquid, without any additional disturbance of the signal measured. The units described are complete electrochemical cells. There are numerous advantages in performing noise-free potentiometric measurements in air-segmented streams

without debubbling: First, all the liquid sample goes through the detector. This is desirable, especially in the case of electrodes with large response times. Thus, increasing the sample volume that passes through the electrode may result in improvements in sensitivity. Secondly, the wash of the electrode membrane is improved. Furthermore, the additional sample contamination occurring after removing the bubbles in the CFA systems that make use of a debubbler is avoided. Finally, another advantage is the ability to perform multipoint analysis by using several bubble-through detectors in series, separated by incubation coils.

## LITERATURE CITED

- (1) Skeggs, L. T., Jr. *Am. J. Clin. Pathol.* **1957**, *28*, 311-322.
- (2) Snyder, L. R. *Anal. Chim. Acta* **1980**, *114*, 3-18.
- (3) Horvai, G. Pungor, E. *CRC Crit. Rev. Anal. Chem.* **1987**, *17*(3), 231-264.
- (4) Ruzicka, J.; Hansen, E. H. *Flow Injection Analysis*; Wiley: New York, 1981.
- (5) Martin, G. B.; Cho, K. H.; Meyerhoff, M. E. *Anal. Chem.* **1984**, *56*, 2612-2613.
- (6) Neeley, W. E.; Wardlaw, S. C.; Yates, T.; Hollingsworth, W. G.; Swinnen, M. E. T. *Clin. Chem.* **1976**, *22*, 227-231.
- (7) Patton, C. I.; Rabb, M.; Crouch, S. R. *Anal. Chem.* **1982**, *54*, 1113-1118.
- (8) HadjiIoannou, T. P.; Papastathopoulos, D. S.; Diamandis, E. P. In *Computerization and Automation in Healthy Facilities*; Rubin, M., Ed.; CRC Press: Boca Raton, FL, 1984; pp 169-196.
- (9) Rao, K. J. M.; Pelavin, M. H.; Morgenstern, S. *Adv. Autom. Anal. Technicon. Int. Cong.* **1972-3**; *1*, 33-36.
- (10) Christopoulos, T. K.; Diamandis, E. P. *Analyst* **1987**, *112*, 1293-1298.
- (11) Papastathopoulos, D. S.; Karayannis, M. I. *J. Chem. Educ.* **1980**, *57*, 904-906.
- (12) Mitsana-Papazoglou, A.; Christopoulos, T. K.; Diamandis, E. P.; HadjiIoannou, T. P. *Analyst* **1985**, *110*, 1091-1094.
- (13) Mitsana-Papazoglou, A.; Christopoulos, T. K.; Diamandis, E. P.; Koupparis, M. A. *J. Pharm. Sci.* **1987**, *76*, 724-730.
- (14) Griggs, A.; Moody, G. J.; Thomas, J. D. R. *J. Chem. Educ.* **1974**, *51*, 541-544.
- (15) Lindner, E.; Toth, K.; Pungor, E. *Anal. Chem.* **1976**, *48*, 1071-88.
- (16) Alexander, P. W.; Seegopaul, P. *Anal. Chem.* **1980**, *52*, 2403-2406.

RECEIVED for review July 18, 1988. Accepted November 21, 1988.