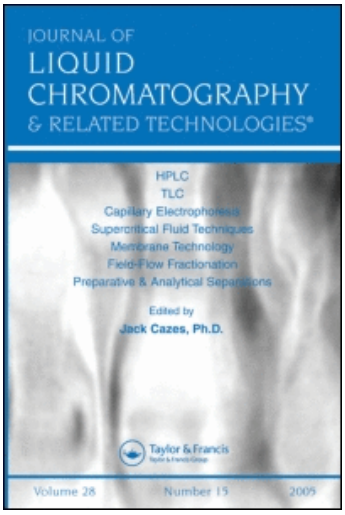

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Direct Electrochemical Detection of Amino Acids at a Platinum Electrode in an Alkaline Chromatographic Effluent

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To cite this Article Polta, J. A. and Johnson, D. C.(1983) 'The Direct Electrochemical Detection of Amino Acids at a Platinum Electrode in an Alkaline Chromatographic Effluent', Journal of Liquid Chromatography & Related Technologies, 6: 10, 1727 — 1743
To link to this Article: DOI: 10.1080/01483918308064887
URL: http://dx.doi.org/10.1080/01483918308064887

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(10), 1727-1743 (1983)

THE DIRECT ELECTROCHEMICAL DETECTION OF AMINO ACIDS AT A PLATINUM ELECTRODE IN AN ALKALINE CHROMATOGRAPHIC EFFLUENT

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ABSTRACT

It is the general experience that most organic compounds including amino acids do not produce reversible or even quasi-reversible anodic waves at a Pt electrode under conditions of conventional cyclic voltammetry. Furthermore, amperometric detection of these compounds at a constant electrode potential is not successful because of the accumulation of adsorbed reaction products and/or an oxide film at the electrode surface. However, it is observed that a Pt electrode surface is cleaned quite effectively of adsorbed organic molecles and radicals simultanaeously with the anodic formation of the oxide layer. This oxidation of adsorbed organic species is concluded to be electrocatalyzed by PtOH formed as the first step in the production of the oxide layer (PtO). A pulsed-potential waveform applied at a frequency of ca. 1 Hz is demonstrated to provide direct amperometric detection of adsorbed amino acids at a Pt electrode. Satisfactory analytical precision (i.e., < 3% rel. std. dev.) results because the waveform reproducibly generates the catalytically active surface state at the Pt electrode. Both primary and secondary amino acids are determined with satisfactory detection limits: e.g., ca. 13 ng for glycine, 7 ng for phenylamine and 23 ng for hydroxyproline in 50-μL samples. Analytical response is concluded to depend on the adsorption isotherm of the amino acid being detected. Hence, the calibration plot of $1/I_{peak}$ vs. $1/C^{b}$ is linear for low surface coverages. Results are shown for amperometric detection of a synthetic mixture of amino acids by anion-exchange chromatography using NaOH as the eluent and supporting electrolyte.

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INTRODUCTION

An extensive literature (1-5) has accumulated during the last three decades as a result of studies of the electrochemical oxidations of organic compounds at noble-metal electrodes, in general, and Pt electrodes, in particular. Nevertheless, comparatively few electroanalytical procedures have received wide acceptance for the anodic detection of organic compounds at noble-metal electrodes in aqueous solvents. The reasons are easily understood by observing that the anodic reactions generally yield voltammetric responses (e.g., I-E curves) characterized as being "surface-controlled". Surface-controlled reactions are those in which the total faradaic charge passed is controlled by the surface area of the electrode. Strong chemical interaction of the surface with the reactants and/or reaction products usually is concluded to exist for surface-controlled reactions. In the case of the anodic detection of simple alcohols at a Pt electrode, for example, a surface-catalyzed dehydrogenation occurs for the adsorbed molecules with the concomitant oxidation of the adsorbed hydrogen atoms, i.e. $(H)_{ads} \rightarrow H^+ + e$ (6,7). The remaining carbonaceous products of the dehydrogenation reaction are strongly adsorbed at the electrode, thereby blocking effectively those adsorption sites from further participation in the anodic dehydrogenation of unreacted molecules from the solution phase. Hence, the electrode current decays quickly to zero for the case of a constant applied potential. Surface-controlled anodic reactions of organic analyte previously have not found significant applications for detection in liquid chromatography.

It has long been the general experince in conventional voltammetric studies of inorganic systems that the presence of even trace levels of organic compounds can alter significantly the I-E response of electroactive species. As a result, it has become standard practice to prescribe certain rites of surface pretreatment for noble-metal electrodes to insure the

reproducibility of voltammetric data (8). Pretreatment inevitably includes the alternate anodic and cathodic polarizations of the electrode at potential values near the limits for decomposition of the aqueous solvent to bring about the rapid, repeated formation and dissolution, respectively, of an oxide layer at the electrode surface. It is our premise that surface-controlled anodic reactions of organic compounds at Pt and other noble-metal electrodes can be applied for amperometric detection with satisfactory sensitivity and precision if the active surface-state of the electrode can be reproduced exactly prior to each measurement of the faradaic signal. We conclude further that the oxide-catalyzed oxygenation reactions responsible for the anodic cleaning of Pt electrodes can be used as the basis for amperometric detection of those compounds in aqueous chromatographic effluents.

Traditional procedures for voltammetric analysis of large volumes of solution in conventional cells have required potential waveforms which were designed to provide resolution of the separate I-E responses for each component of a mixture of electroactive species. Hence, the various waveforms (e.g., linear sweep, normal pulse, differential pulse, etc.) were required to produce a plot of the electrode current over a substantial portion of the available potential range for the particular electrode material in use. In applications of amperometric detection to LC, we develop our waveforms on the premise that the chromatographic system is responsible for resolution of mixtures. Furthermore, the background signal is easily determined in LC/EC from the detector response between elution peaks. Consequently, extensive freedom is allowed in the design of the waveforms to maximize the beneficial electrocatalytic properties of the electrode surface.

There have been a few observations of greater stability in the electrochemical response of solid electrodes resulting from application of pulsed-potential waveforms. Brown [9] expressed

the observation that for anodic organic electrolysis, the "coating of the anode with insoluble, insulating, polymeric films is a common hazard but it can be alleviated by use of periodic polarity reversal techniques." Clark, et al., [10] applied pulsed voltammetry for oxidation of propylene at a Pt electrode to maintain electrode surface activity. MacDonald and Duke [11] observed an improvement in stability for the anodic detection of p-aminophenol at a Pt flow-through electrode when normal pulse amperometry was used instead of DC amperometry. Stulik and Hora [12] applied periodic potential pulses during the cathodic detecton of Fe^{3+} and Cu^{+2} at a Pt electrode and reported improved stability of the cathodic signal when the pulse amplitude was sufficiently large to result in formation and subsequent dissolution of the oxide film on the electrode surface. In previous work from our laboratory (13-16), a triple-step potential waveform was applied successfully for the anodic detection of the C-OH moiety of alcohols and carbohydrates at a Pt electrode in alkaline solutions. According to this waveform, see Fig. 1 of (15), the faradaic current for oxidation of adsorbed molecules is measured using an analog sample-hold circuit in the last few milliseconds of the detection period at potential E_1. The potential then is stepped to a value E_2 near the limit for anodic breakdown of the aqueous solvent which causes the formation of an oxide layer at the electrode surface with simultaneous oxidative removal of the adsorbed organic radicals which had been produced during the detection period. Further anodic detection of molecules from the solution does not occur at the oxide-covered surface and the subsequent step of potential to E_3 is necessary to bring about cathodic reduction of the oxide layer. Molecules of unreacted analyte from the solution phase are adsorbed at E_3 which, in turn, are detected following the subsequent potential step to E_1. The electrical current corresponding to the "residual" surface processes following the step to E_1 (i.e., double-layer

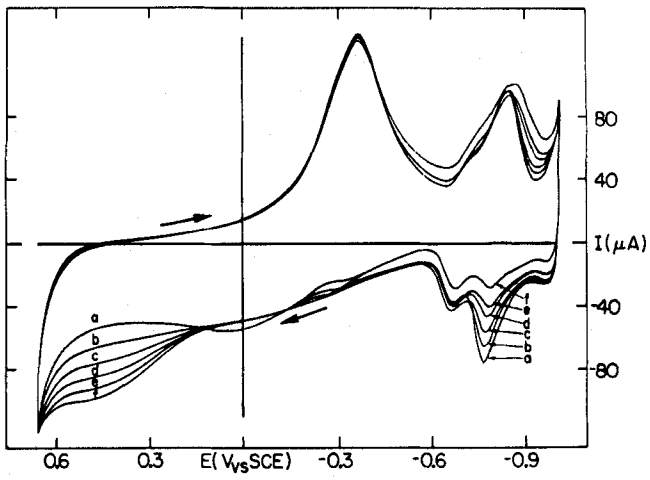

FIGURE 1.
Current-potential curves for glycine by cyclic linear scan voltammetry at a Pt RDE.

Conditions: 0.25 M NaOH, $\emptyset$ = 7.2 V min^{-1}, ω = 168 rad sec^{-1}.
Concentrations(mM): a - 0.00, b - 0.050, c - 0.15, d - 0.35, e - 0.70, f - 1.20.

charging, anodic dissolution of adsorbed hydrogen atoms produced at E_3, and the formation of a small amount of PtOH) decays more quickly than the current for oxidation of the adsorbed alcohols and carbohydrates. Hence, measurement of the desired analytical signal can be made accurately after a delay of only a few milliseconds. The use of a pulsed-potential waveform for detection of alcohols and carbohydrates at Au electrodes has also been demonstrated recently for detection in LC (17).

The general experience among electroanalytical chemists that the quantitative determination of aliphatic amines and amino acids in aqueous solutions cannot readily be achieved by conventional voltammetry and amperometry is illustrated here by selected quotations. Adams (18) stated: "...aliphatic amines are difficult to anodically oxidize in any quantitative

fashion." Malfoy and Reynaud (19) claimed: "Among the 20 amino acids present in the proteins only tryptophan and tyrosine are selectively oxidized at a gold, platinum or carbon electrode. Histidine is oxidizable only at a carbon electrode." Joseph and Davies (20) reported: "Most amino acids are not electroactive..." They proceeded to describe the a priori derivitization of amino acids to enable their electrochemical detection in LC.

The direct anodic detection of amino acids at a constant electrode potential has been reported recently by Huber et al. (21,22) at an oxide-covered Ni electrode in alkaline solutions. The detection reaction has been diagnosed by Fleischman et al. (23) to occur with direct involvement of the oxide. The amino acids reduce $NiO(OH)_2$ to $Ni(OH)_2$ with subsequent anodic oxidation of $Ni(OH)_2$ back to $NiO(OH)_2$. Disadvantages of using the Ni electrode result from 1) a long start-up time, during which the thickness of the oxide layer is stabilized and the background current decays to a steady value; and 2) the finite solubility of the oxides in the alkaline electrolyte solutions.

Here we report on the successful testing of triple-step potential waveforms for the direct anodic detection of primary and secondary amino acids at a Pt electrode in 0.25 M NaOH.

MATERIALS

Current-potential curves (I-E) were obtained by cyclic, linear scan voltammetry at a Pt rotated disk electrode (RDE, 0.460 cm^2; Pine Instrument Co., Grove City, PA) using a model PIR rotator and a model RDE3 potentiostat (Pine Instrument Co.). The I-E curves were recorded by an X-Y recorder (model RE0074, EG&G Princeton Applied Research). The chromatographic system consisted of a CMA-1 chromatographic module and a PMA-1 pumping module (Dionex Corp., Sunnyvale, CA). Separations were achieved with an anion-exchange column (model 48F, Dionex Corp.;

10-μm, 1 cm i.d. x 25 cm) at 40°C using 0.25 M NaOH as the eluent at a flow rate of 0.6 mL min^{-1}. Sample volumes were 50 μL. Flow-injection detections were performed with the chromatographic instrumentation after removal of the anion-exchange column from the fluid stream.

The amperometric detector was constructed from 22-ga. Pt wire which was heat-sealed into a 100-μL disposable glass pipet. The flow-through detector cell was constructed in the Iowa State University Chemistry Shop according to a previous design (16). Glass-filled Teflon (Crown Plastic, Inc., St. Paul, MN) was used for the major portion of the detector body with PTX plastic (Mitusi Petrochemical Ind., Ltd., Tokyo, Japan) for the inlet system. Back pressure was applied to the solution in the detector cell by a needle-valve connected into the outlet tubing to eliminate eluent degasing and accumulation of bubbles at the detector electrode. The triple-step waveform was generated automatically by a microprocessor- controlled potentiostat (model UEM, Dionex Corp.). All electrode potentials are reported in volts (V) vs. the saturated calomel electrode (SCE) as a reference.

CHEMICALS

All chemicals were reagent grade. Water was distilled, demineralized, and passed through an activated carbon column prior to use. All eluent solutions of NaOH were prepared from a saturated stock solution (18.0 M) using freshly boiled water to minimize carbonate contamination. All eluents were passed through a 0.45-μm filter prior to use.

RESULTS AND DISCUSSION

The voltammetric responses of amino acids at Pt electrodes in 0.25 M NaOH are illustrated adequately by the I-E curves for glycine obtained for a cyclic, linear scan of potential as shown in Fig. 1 for the Pt RDE. The residual response of the

electrode (curve a) obtained in the absence of the amino acid is characterized by an anodic wave during the positive scan for E > -0.3 V which corresponds to the formation of the oxide layer (PtOH and PtO). Rapid evolution of $O_2(g)$ occurs for E > 0.6 V. The oxide layer is cathodically reduced on the negative potential scan to produce the peak at -0.3 V. The cathodic and anodic peaks in the region -0.6 to -0.9 V correspond to the electrochemical production and dissolution, respectively, of adsorbed atomic hydrogen. Rapid evolution of $H_2(g)$ occurs for E < -0.9 V. Additions of the amino acid result in a decrease in the quantity of adsorbed atomic hydrogen which can be produced during the negative potential scan. This is explained if the amino acid is adsorbed at the electrode surface thereby depleting the number of available Pt sites. Oxidation of the adsorbed amino acid produces an anodic wave on the positive potential scan in the region E = 0.3 - 0.6 V, with the current increasing as a nonlinear function of the bulk concentration of the amino acid (C^b). There is virtually no evidence for oxidation of the amino acid on the subsequent negative potential scan in the region E = 0.6 - 0.3 V.

The anodic wave for the amino acid obtained on the positive potential scan was determined to vary in height as a linear function of the rate of potential scan and to be virtually independent of the rotational velocity of the RDE. Such behavior is consistent with the conclusion that the oxidation is a surface-controlled reaction. Furthermore, the oxide film produced on the positive potential scan to 0.65 V prevents further detection of the amino acid on the negative potential scan.

Various triple-step potential waveforms were developed on the basis of the I-E curves in Fig. 1. Three such waveforms are described in Table 1. Anodic detection of the amino acids occurs at potential E_1. The anodic signal is measured using an analog sample-hold circuit after the delay period t_d; ca. 50

TABLE 1. Description of three triple-step potential waveforms for detection of amino acids at Pt electrodes in 0.25 M NaOH.

Waveform	Step	Potential (V)	Period (msec)	Function
A.	1	E_1 = 0.50	t_1 = 580 (t_d = 530)	anodic detection (delay before sampling)
	2	E_2 = -0.89	t_2 = 750	reduction/adsorption
	3	E_3 = 0.78	t_3 = 50	anodic activation
B.	1	E_1 = 0.50	t_1 = 250 (t_d = 200)	-as above-
	2	E_2 = -0.89	t_2 = 650	
	3	E_3 = 0.78	t_3 = 50	
C.	1	E_1 = 0.50	t_1 = 510 (t_d = 460)	-as above-
	2	E_2 = -0.89	t_2 = 360	
	3	E_3 = 0.70	t_3 = 50	

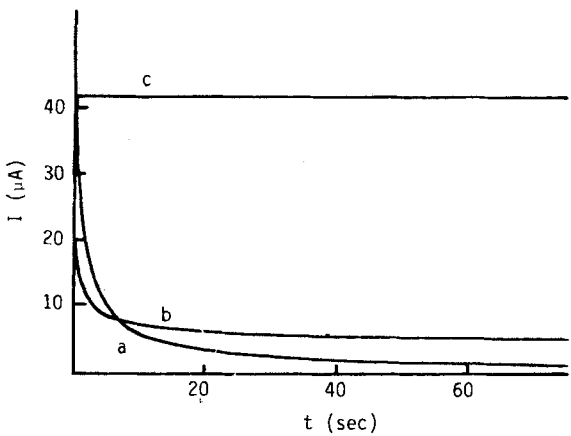

FIGURE 2.

Current-time curves for glycine at a Pt RDE.

Conditions: 0.25 M NaOH; ω = 41.9 rad sec^{-1}.
Curves: a - 0.00 mM glycine, E = 0.50 V; b - 0.50 mM glycine, E = 0.50 V; C - 0.50 mM glycine, waveform A.

msec was allowed for the sampling operation. The potential required to oxidize the amino acids is significantly more positive than required for alcohols and carbohydrates in the same alkaline medium. Hence, the coverage of the electrode surface by oxide at the respective detection potentials (E_1) is substantially greater for amino acids than for carbohydrates, and the corresponding residual current is slow to decay to a negligible value. The expected need for a long delay period (t_d) before the analytical signal sampled is obviated in the case of the amino acids by use of the large value for E_3 with the step back to the detection potential E_1. For $E_3 > E_1$, the oxide coverage produced during the short period t_3 is greater than the equilibrium coverage for potential E_1. Hence, the step from E_3 to E_1 results in the immediate cessation of oxide growth. Electrochemical reduction of PtOH and PtO does not occur at E_1 and, therefore, the residual current is negligible after several milliseconds. The potential step from E_1 to E_2 does result in the rapid reduction of the oxide layer followed by the adsorption of unreacted amino acids from the bulk solution. Clearly, the surface-controlled oxidation of amino acids commences immediately upon the potential step from E_2 to E_3. The analytical success obtained for these waveforms results because the period for decay of the faradaic current for the amino acids is slow relative to the combined time periods $t_3 + t_d$. The waveforms are applied at a frequency of ca. 1 Hz which is sufficient for the "continuous" monitoring of a chromatographic effluent stream. Because the values of E_1 and E_3 are near the limit for anodic breakdown of the alkaline medium, retention on the electrode surface of free-radical products generated during the detection period does not persist substantially beyond the next repetition of the waveform.

The difficulty associated with application of surface controlled reactions for amperometric detection at a constant applied potential is illustrated for glycine in Fig. 2. Prior to recording the current-time (I-t) data, the potential of the

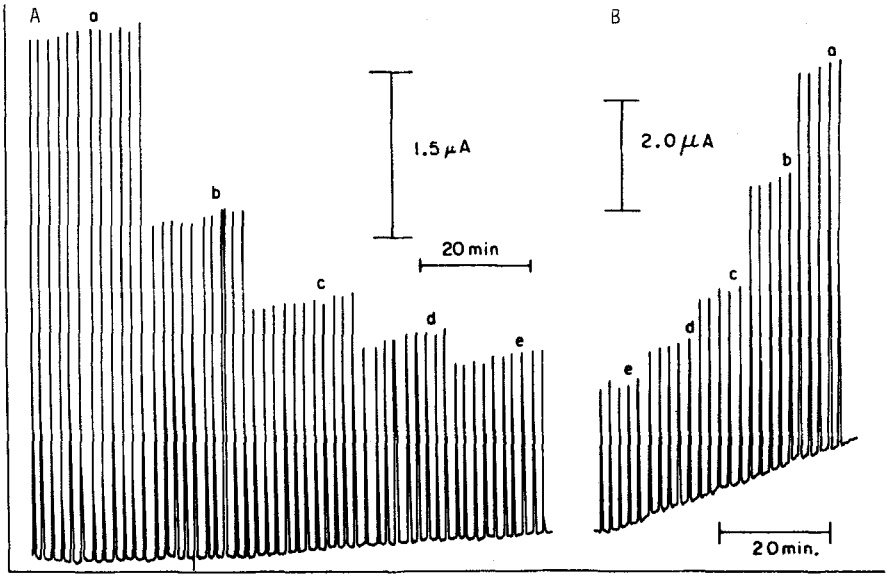

FIGURE 3.

Flow-injection detection peaks for glycine.

Conditions: 50-μL injections; 0.25 M NaOH flowing at 0.80 mL min^{-1} ; waveforms as indicated.
Concentrations(mM): a - 1.00, b - 0.50, c - 0.33, d - 0.25, e - 0.20.

Pt RDE was cycled repeatedly in the manner used for obtaining Fig. 1. The final scan was terminated at the negative limit, the potential was stepped to 0.50 V and the I-t curve recorded. The anodic signal in the presence of the amino acid (curve b) decreased rapidly. The residual response in the absence of the amino acid (curve a) is shown for comparison. The I-t curve for glycine is also shown in Fig. 2 which was obtained using the triple-step waveform A (curve c); no pretreatment of the electrode was needed in conjunction with curve c. No decrease of the signal was observed over a 10-min period.

Waveforms A and B in Table 1 were applied for the detection of various amino acids in the flow-through detector under conditions of flow-injection detection with repeated injections of 50-μL samples of the amino acids. The concentration of NaOH in the samples and the carrier stream was 0.25 M. Representative results are shown in Fig. 3 for glycine. Baseline drift is less for waveform A, whereas sensitivity is greatest for waveform B. For the purpose of comparing the sensitivity of detection for various amino acids by the waveforms in Table 1, the average peak currents obtained by flow injection detection are given to Table 2 for 21 amino acids at a concentration of 5.0×10^{-4} M. Precision is satisfactory for all amino acids by the flow-injection technique with a relative standard deviation < 3%.

Calibration plots for glycine (I_{peak} vs. C^b), prepared from data obtained by the flow-injection technique, approached linearity at low concentrations (i.e., C^b < 0.6 mM) but deviated significantly from linearity at higher concentrations. This behavior is the same as that observed for detection of alcohols and carbohydrates (13-16) by the triple-step technique and is concluded to be the consequence of a reaction mechanism in which only adsorbed species are detected. Hence, the anodic signal is proportional to the surface coverage (θ) by the adsorbed analyte. Based on the Langmuir isotherm, which is expected to be valid for $\theta \ll 1$ (i.e., small C^b), plots of $1/I_{peak}$ vs. $1/C^b$ are predicted to be linear. This prediction is verified by the data in Fig. 4 for glycine.

Detection limits (signal:noise = 2) for several amino acids using waveform B determined by flow-injection detection are given in Table 3.

The applicability of the triple-step waveform for amperometric detction of amino acids in the flow-through detector is further demonstrated in Fig. 5 for the chromatgraphic separation of a synthetic mixture of amino acids. The potential waveform was C (Table 1). Response for

TABLE 2. Average peak current (μA) obtained by flow-injection detection for 21 amino acids.

Electrolyte: 0.25 M NaOH at 0.50 mL min^{-1}
Amino Acids: 50 μL, 5.0 x 10^{-4}M in 0.25 M NaOH

Amino Acid	Waveform A	Waveform B	Amino Acid	Waveform A	Waveform B
alanine	6.44	13.9	leucine	4.32	8.13
β-alanine	10.46	17.8	lycine	18.6	22.7
arginine	26.9	32.0	methionine	23.7	26.5
asparagine	11.8	20.6	phenylalanine	23.5	30.3
cysteine	20.6	21.4	proline	2.19	5.19
cystine	13.6	16.7	serine	9.40	18.3
glutamic acid	3.92	7.19	threonine	10.38	18.3
glycine	8.58	16.4	tryptophan	25.9	31.6
Histadine	21.6	29.6	tyrosine	20.3	23.7
hydroxyproline	5.30	9.50	valine	3.56	6.53
isoleucine	3.86	7.68			

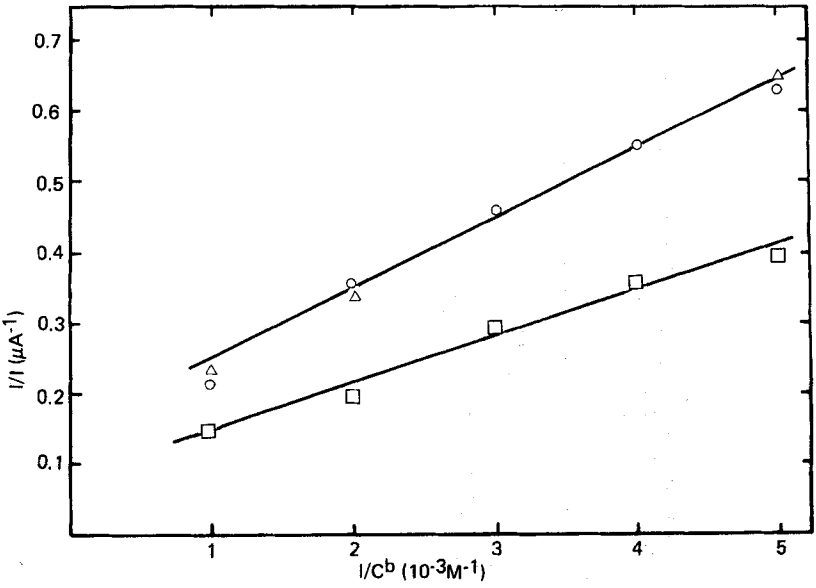

FIGURE 4.

Calibration curves ($1/I_{peak}$ vs. $1/C^b$) for glycine by flow-injection detection.

Conditions: 50-μL injections; 0.25 M NaOH flowing at 0.80 mL min^{-1}.
Curves: a - waveform A; b,c - waveform B.

TABLE 3. Detection limits for several amino acids using waveform B.

amino acid	ppm ($\mu g\ mL^{-1}$)	ng (50 μL sample)
phenylalanine	0.12	7
methionine	0.12	7
glycine	0.23	13
hydroxyproline	0.45	23
proline	0.86	43

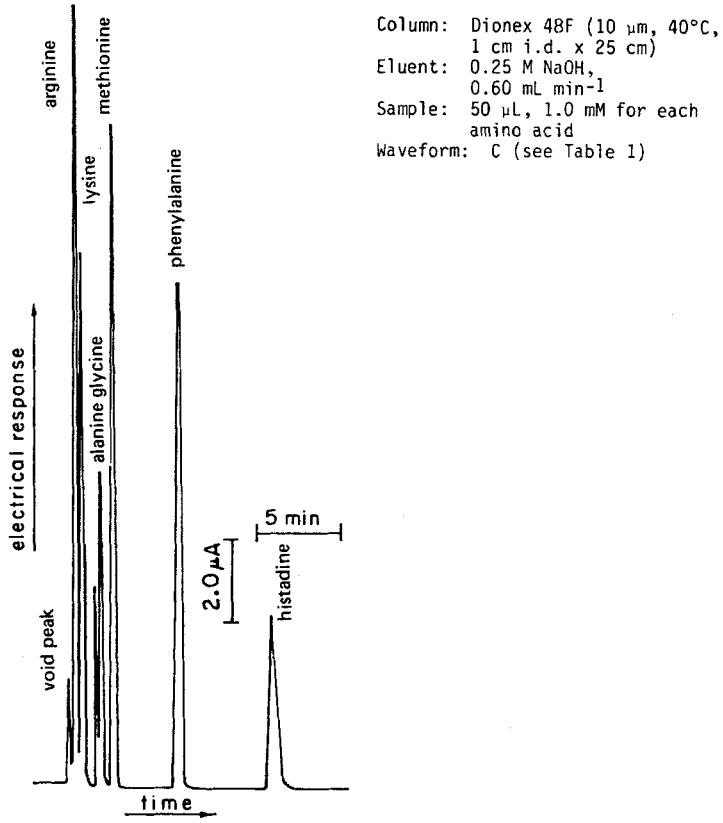

FIGURE 5.

Chromatogram of Selected Amino Acids Using Triple-Step Pulsed-Potential Amperometric Detection.

Peaks, in order of appearance: void, arginine, lysine, alanine, glycine, methionine, phenylalanine, histadine.

waveform C is similar to that for waveform A (i.e., low baseline drift); however, the frequency is somewhat larger (i.e., 1.1 Hz vs. 0.6 Hz for waveform A). The improvement in frequency came by a substantial decrease of period t_2. Further decrease of t_2 resulted in a serious sacrifice of sensitivity, presumably because adsorption of the amino acids did not reach the equilibrium value of surface coverage. We hasten to emphasize with regard to Fig. 5 that we are stressing the feasibility of the amperometric detection system and not the quality of the separation. The Dionex 48F anion-exchange column was used for separations of carbohydrate mixtures under elution with dilute solution of NaOH and conditions were not optimized for separations of amino acids. The freedom to use alkaline solutions for elution is certainly advantageous, although use of post-column addition of the electrolyte can be successful (15,16).

We predict, on the basis of work with amino acids and carbohydrates, that triple-step potential waveforms will make available many additional surface-controlled anodic reactions of organic functional groups at noble-metal electrodes for amperometric detection in liquid chromatography.

ACKNOWLEDGEMENTS

The support of this research by Dionex Corp., Sunnyvale, CA, is gratefully acknowledged.

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