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Study of Pulse Generation Technique for Serial dual Electrode Detection of Amino Acids and Proteins in Flow Injection Analysis

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ABSTRACT :A new analytical procedure using a serial dual electrode detector was developed for the analysis of amino acids and proteins. Bromine was generated at the upstream electrode and detected by the downstream electrode. The presence of amino acids and proteins was shown to lower the downstream current but with no apparent effect on the upstream current. This indirect mode of detection can be applied to the determination of amino acids and proteins which are electrochemically inactive or too large to be accessible to the electrode surface for electron exchange. The method is shown capable to determine various amino acids (cystine, tyrosine, lysine, tryptophan, glycine, methionine and arginine) and proteins (cytochrome c, hemoglobin, HAS, a-Amylase, Conalbumin I, Catalase and Myglobin) with linear working range for amino acids between 10^{-6} to 10^{-3} M and total proteins between 10^{-7} to 10^{-3} M. The method has been applied for the analysis of amino acids and total protein in food using Flow Injection Analysis with results obtained comparable to those using the traditional analytical procedure. Use of pulse generation technique was shown to produce a more stable flow injection analysis peaks for repetitive determination than the use of conventional constant current method which showed increase of the background current after determination over 200 minutes. The pulse method was found to give stable baseline even after 400 minutes. Thus, the method is shown able to provide a suitable analytical procedure for automatic analysis of amino acids and proteins in food by flow injection analysis.

Keywords : Serial Dual Pulse Electrode, Pulse Generation Technique, Amino acids and Proteins Analysis, Flow Injection Analysis.

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1. Introduction

The recent rapid advance in genetic engineering and biotechnology, and the need of nutritional assessment necessitate the development of methods for amino acids and proteins analysis in food and biological material, in particular in the areas of automatic analysis and the determination of amino acids and proteins in a complex medium [1-3].

Due to its simplicity and sensitivity the electrochemical detection provides a suitable analytical method for amino acids and proteins determination in mobile electrolyte after HPLC separation and for direct detection in the simple automatic Flow Injection Analysis [4-6]. The problem facing the application of electrochemical method for amino acid and protein analysis is the lack of electrochemical active groups in most of these compounds. Thus, it necessitates the derivatization procedure prior to determination. Although many derivatizing reagents were developed covering a large number of amino acids with suitable sensitivity and selectivity, the procedure suffers inherent difficulties, as it either requires additional sample handling procedures, extra instrumentation and additional connections for on-line, pre- or post-column reaction which led to problem of peak broadening and the unavoidable dilutions and possible interference due to the addition of the derivatizating reagents.

A better approach to tackle the above problem is generating *in situ* chemical reactions at electrode surface to produce an electrochemical active product for detection. Serial Dual Electrode method was developed using reactive intermediate generated directly at the electrode surface of the upstream electrode prior to detection by the downstream electrode [7] which extends the scope for the detection of amino acids and proteins which do not react at the electrode surface.

The work reported in this paper is a further extension of the work using pulse current instead of direct current, which is shown to improve the background current and produce stable baseline up to more than 400 minutes of operation. Thus, the method is shown to provide a suitable analytical procedure for automatic analysis of amino acids and proteins.

2. Experimental

2.1 Apparatus

FIA-ECD. The flow injection system electrochemical detector system consists of a syringe pump (Sage Instruments model 352), a sample injection value 4-way rotary valve (Rheodyne), connection and dispersing tubing (teflon tubing 1.5 mm internal diameter and length 5 cm) and a self-constructed thin layer dual electrode cell (Fig. 1) with two platinum planar working electrodes (4 mm x 3 mm) connected in series along the flow path separated by a distance of 1 mm. Ag/AgCl is used as the reference electrode and the counter electrode is a stainless steel block facing the two working electrodes of the dual electrode cell. The potential of the working

electrodes is controlled by a self constructed bi-potentiostat with a common reference electrode. The currents are sampled by a Cromenco system III microcomputer and converted into suitable form for recording using the Houston Hiplot X-Y digital plotter.

2.2 Reagents. The 0.25 M KBr mobile electrolyte for FIA studies is prepared by dissolving 15 gram KBr in 0.25 M sodium phosphate buffer solution. All amino acids and protein standard solutions, (1.0 x 10^{-2} M) are prepared by dissolving suitable amounts of the standard in the 0.25 M KBr mobile electrolyte and dilutes to specified concentrations with the mobile electrolyte immediately before use.

2.3 Procedure. For the FIA-ECD method, the mobile electrolyte is maintained at a flowrate of 0.5 mL/min and 100 μL injection volume is used. Samples are filtered prior to injection via the sample loops. Background current is measured prior to injection of analytes and the stability of the peak is checked by repetitive injection up to 150 to within 5% variation.

3. Results and Discussion

3.1 General Characterisation of the Dual Electrode Detector

The efficiency of the Dual Electrode Detector is highly dependent on the generation efficiency of the upstream electrode and the collection efficiency of the downstream electrode. The anodic current for the generation of bromine at the upstream electrode was found to increase with more anodic potential up to a flat plateau at 1.5V. It is flowrate dependent with larger current at higher flowrate. The cathodic collection current of the downstream electrode fixed at +0.6V follows the same trend as the upstream electrode with reduced effect on flowrate and upstream electrode potential. In general, the effective current collection efficiency is fairly constant at 0.25 with slight flowrate dependent.

With the upstream electrode potential controlled at 1.0V, the downstream current-voltage curve shows a flat plateau from -0.6 to +0.6V. The increase in current more negative than -0.6V is due to the liberation of hydrogen whereas the decrease in current at potential anodic to +0.6V is due to incomplete oxidation of the bromine collected. In order to reduce the liberation of hydrogen and the oxidation of other impurities, the downstream electrode potential is fixed at +0.6V.

With the introduction of amino acid to the mobile electrolyte, they are shown not affecting the generation efficiency of the upper electrode but led to a flow-rate dependent depression of the current of the downstream electrode. In general, the lower the flowrate the higher the difference between the curves at a given potential at the plateau region. The variation of the upstream electrode potential would led to the generation of different amounts of bromine and hence affecting the working range of the method which indicate the expected results of the higher the potential the larger the linear range.

There are practical consideration in choosing suitable flowrate and upstream electrode potential. Lower flowrate, though increase the sensitivity, would affect the dispersion of the Flow Injection System and reduce the number of samples analyzed per hour. Higher potential though extends the working range would lead to the complication of the reduction of unwanted impurities and larger flowrate could affect the dispersion of the flow injection system sion. Thus, the upstream electrode potential is selected at 1.0V at a flowrate of 0.5 mL/min with an injection volume of 100 uL and the downstream electrode potential at 0.6V.

The method is shown capable to determine various amino acids (cystine, tyrosine, lysine, tryptophan, glycine, methionine and arginine) and proteins (cytochrome c, hemoglobin, HAS, a-Amylase, Conalbumin I, Catalase and Myglobin) with linear working range for amino acids between 10^{-6} to 10^{-3} M and total proteins between 10^{-7} to 10^{-3} M. The method has been applied for the analysis of amino acids and total protein in food using Flow Injection Analysis with results obtained comparable to those using the traditional analytical procedure [8] (Table 1).

Table 1 Application of Dual Electrode FIA method for the Determination of Total Protein in Food

Food	Milk	Cereal Sample	Tonic Drink
FIA-ECD			
Mean	25	9.5	20
RSD	0.15	0.37	0.14
REF. METHOD			
Mean	24	9.1	21
RSD	0.40	0.42	0.23

N.B. 1) Concentration given in percentage
2) n = 3

3.2 Effect of Pulse vs Constant Current for Bromine Generation

To reduce the problem of fouling of electrode surface upon continuous use of the dual electrode detector in Flow Injection Analysis, a pulse method was used to generate bromine for detection. The waveform is shown in Figure 1, which indicates the sampling of current at downstream electrode with a delayed time of 1 second after the imposition of potential at the upstream electrode. The use of a delay time is due to the time needed to establish the diffusion layer at the upstream electrode and this is shown clearly with the decrease in upstream current after imposition of a potenial step at the upstream electrode. The increase of the sampling current with time at the downstream electrode is due to the time needed for physical transport of bromine generated at the upstream electrode.

The effect of the pulse potential of the upstream electrode on the FIA peak current detected at the downstream electrode is shown in Figure 2. The FIA peak current is shown to rise rapidly from 0.7 to 0.9 V and then kept constant till 1.1 V before falling rapidly at rising potential. Thus, the pulse potential should be kept at a potential range from 0.9

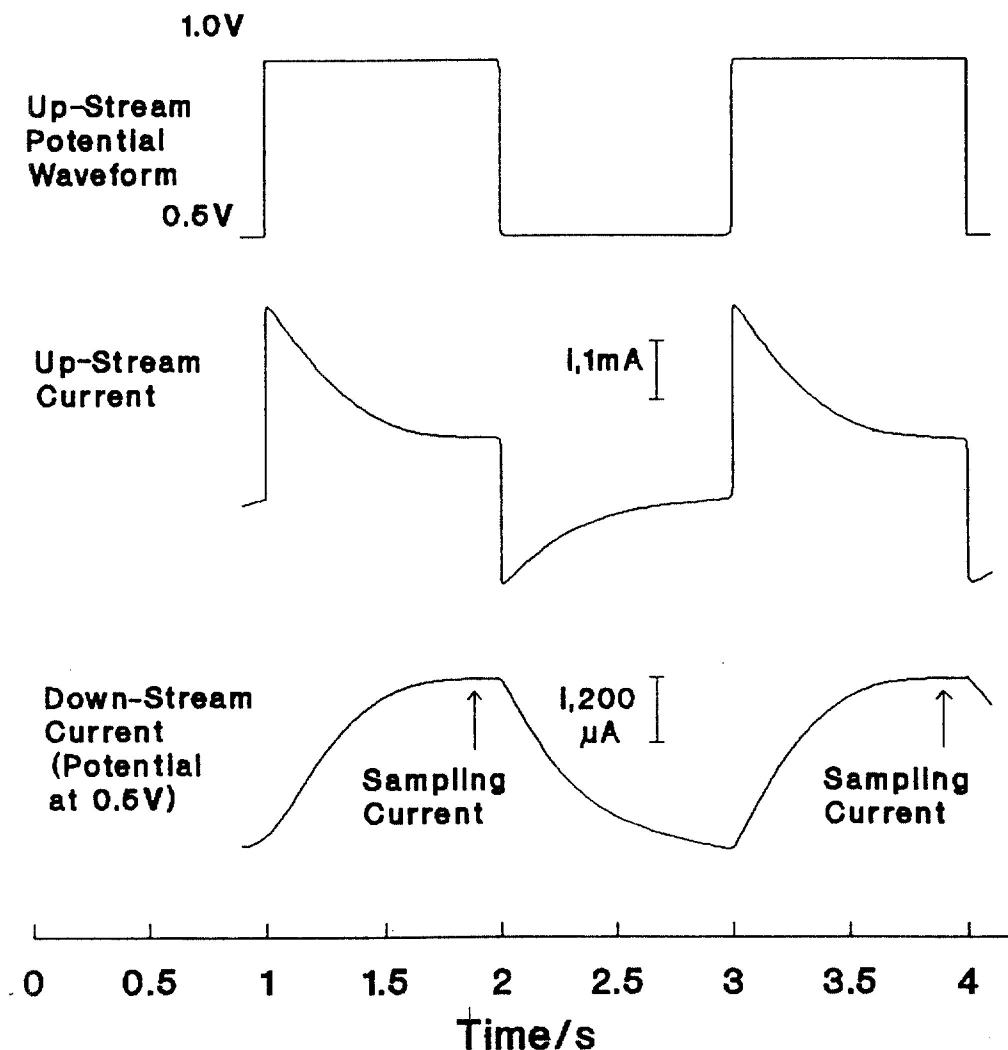

Fig. 1 Current Response of Up-Stream Electrode and Down-Stream Electrode with Pulse Generation.(Flow Rate,0.5ml/min.)

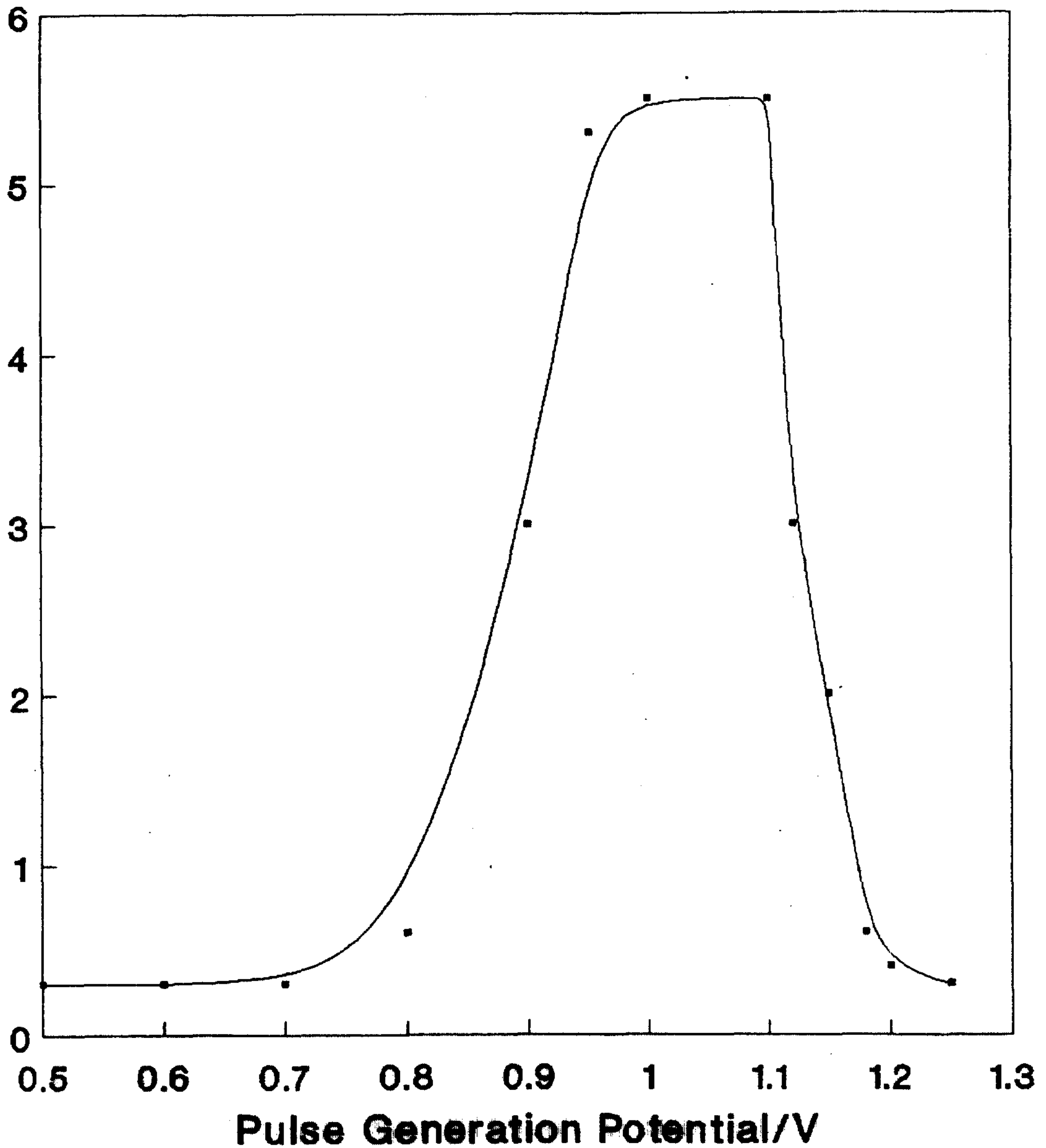

Fig. 2 Effect of Pulse Potential on FIA Peak Current (L-cystine 1×10^{-5}M in 0.5M KBr, Down-Stream Potential,0.5V)

FIA Peak Current/uA x10

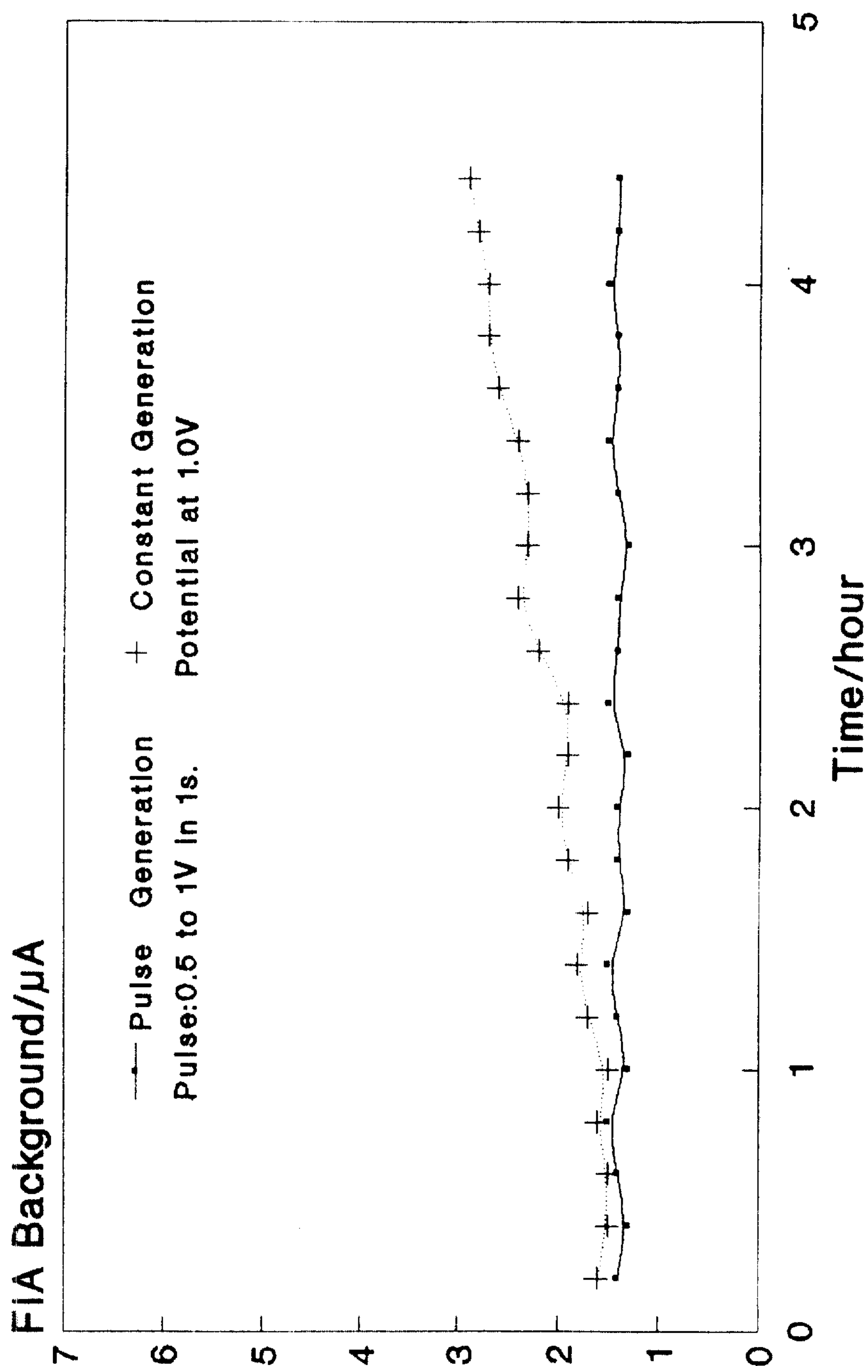

Fig. 3 Background FIA Signal Obtained in Pulsed and Constant Potential Generation.(Down Stream Potential,0.5V)

to 1.1 V during operation in order to obtain a constant output of bromine. The rise in current efficiency from 0.7 to 0.9 V is due to the overpotential of bromine generation and the rapid fall after 1.1 V is due to the occurrence of side reaction at highly positive potentials.

The effect of using pulse potential vs constant potential on the background current is shown in Figure 3. Obvious increase in the background current was observed using constant potential generation as compared to the pulse method after 4 hours of operation. The pulse method was found to produce constant background current after successive determination for 400 minutes, whereas the constant potential method can only produce a stable current during the initial 200 minutes and the FIA peak current was found to decrease up to about 0.7 uA after 400 minutes' determination.

In summary, the series dual electrode detection with *in situ* generation and detection of bromine is shown to provide a simple sensitive and selective method for the determination of amino acids and proteins. It could be used directly as detector in Flow Injection Analysis for the analysis of total protein or after HPLC separations for the analysis of amino acids. The use of potential pulse method was shown to produce a more stable flow injection analysis peaks for repetitive determination than the use of conventional constant current method which showed increase of the background current after determination over 200 minutes. The pulse method was found to give stable baseline even after 400 minutes. Thus, the method is shown able to provide a suitable analytical procedure for automatic analysis of amino acids and proteins in food by Flow Injection Analysis.

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