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Monitoring of Methyldopa by Fast Fourier Transform Continuous Cyclic Voltammetry at Gold Microelectrode

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A continuous cyclic voltammetric study of methyldopa at gold micro electrode was carried out. The drug in phosphate buffer (pH 2.0) is adsorpted at 400 mV, giving rise to change in the current of well-defined oxidation peak of gold in the flow injection system. The proposed detection method has some of advantages, the greatest one of which are as follows: first, it is no more necessary to remove oxygen from the analyte solution and second, this is a very fast and appropriate technique for determination of the drug compound in a wide variety of chromatographic analysis methods. Signal-to-noise ratio has significantly increased by application of discrete Fast Fourier transform (FFT) method, background subtraction and two-dimensional integration of the electrode response over a selected potential range and time window. Also in this work some parameters such as sweep rate, eluent pH, and accumulation time and potential were optimized. The linear concentration range was of 1.0×10^{-7}—1.0×10^{-11} mol•L^{-1} (r= 0.9975) with a limit of detection and quantitation 0.004 nmol•L^{-1} and 0.03 nmol•L^{-1}, respectively. The method has the requisite accuracy, sensitivity, precision and selectivity to assay methyldopa in tablets. The influences of pH of eluent, accumulation potential, sweep rate, and accumulation time on the determination of the methyldopa were considered.

Keywords methyldopa, continuous cyclic voltammetry, Fourier transform, micro-electrode

Introduction

Methyl-dopa [α-methyl-β-(3,4-dihydroxyphenylalanine)] (Figure 1) is a catecholamine widely used to treat high blood pressure. It is converted to α-methyl norepinephrine in adrenergic nerve terminal and its antihypertensive action appears to be due to stimulation of central α-adrenoreceptors by this agent.[1] Many analytical methods including spectrophotometric,[2,3] chromatographic,[4-8] titrimetry[9-11] have been employed for analysis and determination of catecolamine drugs (methyldopa) in biological fluids. Some of techniques described above are not simple for direct application to a large scale routine determination and require expensive instruments.

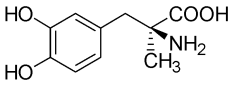

Figure 1 Structure of methyldopa.

As far as voltammetric techniques are considered, they are generally rapid and economical in the determination of some organic and inorganic compounds in aqueous system with a sensitivity range of part-per-billion. Indeed, because of the selective detector, voltammetric techniques are useful for the samples. The use of voltammetric techniques has been further stimulated by the advent of micro-electrode (ME), due to their steady state currents, higher sensitivity, increased mass transport and their ability use in electroanalysis in solution with high resistance.[12,13] ME, for instance, has been applied as sensors to various techniques such as flow injection analysis,[14,15] cardiovascular monitoring and organic compound analysis.[16,17] Now, our work describes a new electrochemical method based on flow injection analysis (FIA) and FFT cyclic voltammetry for determination of methyldopa.

Experimental

Reagents and materials

Double-distilled deionized water was used for preparation of samples by using analytical grade reagents (Merck Chemicals). The reagents used for preparation of the running buffer or background elec-

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trolyte (BGE) solution for flow-injection analysis (0.05 mol•L^{-1} H_3PO_4 and 1 mol•L^{-1} NaOH used for adjusting pH of the eluent), were obtained from Merck Chemicals. Methyldopa standard powder was a gift from the quality control center (Tehran, Iran). In all experiments, solutions were made up in the background electrolyte solution, and were used without removal of dissolved oxygen. All the experiments have been done at laboratory temperature 25 ℃.

Background electrolyte

The background electrolyte was made by addition of 8.7 mL of phosphoric acid (0.85 g•L^{-1}) into a 1000 mL volumetric flask and dilution to a constant volume with distilled water. The pH was adjusted to 2 with sodium hydroxide and all solutions were freshly prepared and filtered using a Millipore filter (0.45 μm) each day.

Standards and sample solutions

Standard stock solutions A standard stock solution of methyldopa (1 mg•mL^{-1}) was prepared in the distilled water which was protected from light using foil and stored at 4 ℃ would be stable during this period.

Standard solutions for FIA Aliquots of standard stock solution of methyldopa were dispensed into 10 mL volumetric flasks and the flasks made up to volume with the running buffer to give final concentrations ranging 1.0×10^{-6}—1.0×10^{-11} mol•L^{-1}.

Sample preparation of human urine and plasma Plasma was obtained from Tehran University Hospital, Tehran, Iran and kept frozen until use after gentle thawing. Urine was also collected from healthy volunteers (males, around 30-years-old).

One mL of untreated urine containing 10 μg•mL^{-1} methyldopa was placed into a 50 mL volumetric flask and diluted with water to the mark. 1 mL of this solution was diluted with pH 2 buffer solution to 20 mL into a volumetric flask. Then a 20 μL aliquot was injected into the FIA system.

For the determination of methyldopa in plasma, 100 μL of aqueous methyldopa solutions (100 ng•mL^{-1}) were added to 1 mL of untreated plasma. The mixture was vortexed for 30 s. In order to precipitate the plasma proteins, the plasma samples were treated with 20 μL of perchloric acid $HClO_4$ 20%. After that, the mixture was vortexed for a further 30 s and then centrifuged at 6000 r/min for 5 min. Then a 20 μL of aliquot of the obtained supernatant was injected into the FIA system.

Electrode preparation A gold ME (with a 25 μm in diameter) was prepared as described in our previously papers.[14-18] Before each experiment the electrode surface was polished for 1 min using extra fine carborundum paper and then for 10 min with 0.3 μm alumina. Prior to being placed in the cell the electrode was washed with water. In all measurements, one Ag (s)|AgCl (s)|KCl (aq, 1 mol•L^{-1}) reference electrode was used. The auxiliary electrode was made of a Pt wire, 1 cm in length and 0.5 mm in diameter.

Flow injection setup The equipment for flow injection analysis included a 10 roller peristaltic pump (UltrateckLabs Co., Iran) and a four way injection valve (Supelco Rheodyne Model 5020) with a 50 μL sample injection loop. Solutions were introduced into the sample loop by means of a plastic syringe. The volume of the cell was 100 μL. In all experiments described in this paper, the flow rate of eluent solution was 3 mL/min.

Data acquisition and processing All of the electrochemical experiments were done using a setup comprised of a PC PIV Pentium 900 MHz microcomputer, equipped with a data acquisition board (PCL-818HG, Advantech. Co.), and a custom made potentiostat. All data acquisition and data processing programs were developed in Delphi 6 ® program environment.

Results and discussion

In Figure 2 the diagram of applied waveform potential during cyclic voltammetric measurements is shown. The potential waveform consists of three parts: (a) potential steps, E_{C1} and E_{C2} (used for oxidizing and reduction of the electrode surface, respectively), by which electrochemical cleaning of the electrode surface takes place, (b) E_s, where accumulation of analyte takes place, and (c) the final, part potential ramp, in which current measurements are performed.

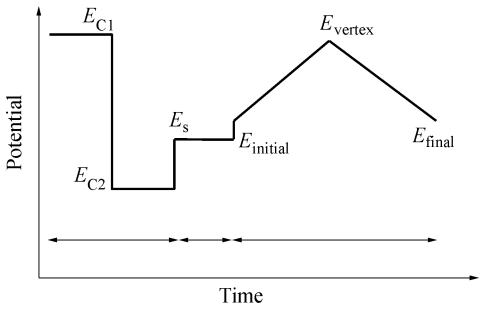

Figure 2 Diagram of the applied potential waveform.

Signal calculation in this method was established based on the integration of net current changes over the scanned potential range. It must be noted that in this case, the current changes (result of injected analyte) at the voltammograms can be caused by various processes, which take place on the electrode surface. Those processes include (a) oxidation and reduction of adsorbed analyte, and (b) inhibition of oxidation and reduction of the electrode surface by the adsorbed analyte. Indeed, in order to see the influence of the adsorbed analyte on the oxidation and reduction peaks of the gold surface, the scan rate must be set at very high rates (*e.g.*＞20 V/s).

However, during the scan, some of the adsorbed analyte molecules are desorbed. Depending on the rate of those processes and scan rate, the amount of the desorption analyte molecule (during the scan) can be changed.[18-30] The important point here is that part of the adsorbed analyte molecules still remain on the electrode surface which can inhibit the red/ox process of the elec-

trode surface. In this method, ΔQ was calculated based on the all current changes at the cyclic voltammetry (CV). However, the selectivity and sensitivity of the analyte response expressed in terms of ΔQ strongly depends on the selection of the integration limits. One of the important aspects of this method is application of a special digital filtration, which is applied during the measurement. In this method at the first, a CV of the electrode was recorded and then by applying FFT on the collected data, the existing high frequency noises were indicated. Finally, by using this information, the cutoff frequency of the analog filter was set at a certain value (where the noises were removed from the CV).

Since the crystal structure of a polycrystalline gold electrode strongly depends on the condition of applied potential waveform,[13] various potential waveforms were examined in order to obtain a reproducible electrode surface (or a stable background signal). In fact, application of cyclic voltammetry for determination of electroactive compound mainly faces to low stability of the background signal, due to changes occurring in the surface crystal structure during oxidation, and reduction of the electrode in each potential cycle. In this work, after examination of various potential wave forms, the best potential waveform for obtaining a stable background during the measurement was the waveform shown in Figure 2. As mentioned above, in this work, the potential waveform was continuously applied during an experiment run where the collected data were filtered by an FFT method before using them in the signal calculation.

The electrochemical oxidation process of gold surface started with adsorption of hydroxyl ion which at more positive potentials of gold oxide formed and went structural rearrangement.[23,24] The surface oxidation can be initiated by adsorption of water molecule and then at more positive potential AuOH forms leading to the formation of a two-dimensional phase of gold oxide;

$$2Au + 3H_2O \longrightarrow Au_2O_3 + 6e + 6H^+ \qquad (1)$$

An example of recorded CV is shown in Figure 3 (a, b).

Figure 3a shows a sequence of CV recorded during the flow analysis for determination of the drug. The volume of the injection was 50 μL of 3.0×10^{-6} mol•L^{-1} methyldopa (in 0.05 mol•L^{-1} H_3PO_4) into the eluent solution containing 0.05 mol•L^{-1} H_3PO_4. The time axis of the graph represents the time of the flow injection experiment. In the absence of methyldopa, the shape of the CV curves is typical for a polycrystalline gold electrode in acidic media.[24] Figure 3b shows the absolute current changes in the CV curves after subtracting the average background 4 CVs (in the absence of the analyte). As can be seen, this way of presentation of the electrode response gives more details about the effect of adsorbed ion on currents of the CV. The curves show that current changes mainly take place at the potential regions of the oxidation and reduction of gold. When

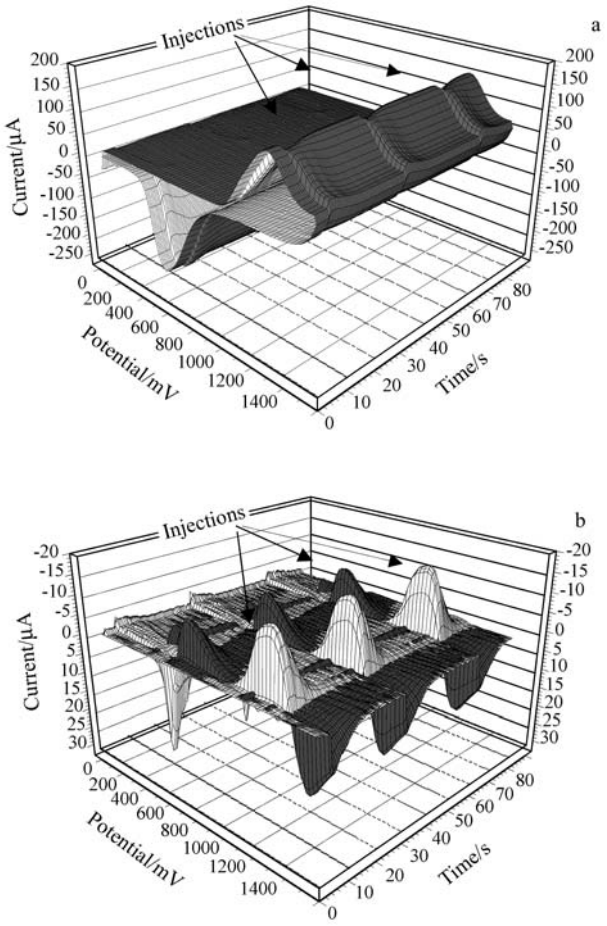

Figure 3 (a) Cyclic voltammograms at Au ultra-microelectrode recorded during the flow injection of 50 μL of 3.0×10^{-6} mol•L^{-1} of methyldopa in the optimum conditions. The eluent was 0.05 mol•L^{-1} H_3PO_4 and the flow rate was 3 mL/min. (b) Curves resulting from subtracting an average CV (in the absence of analyte) from test of the CV in (a).

the electrode-solution interface is exposed to methyldopa, which can be adsorbed on the electrode, the oxide formation process becomes strongly inhibited. In fact, the inhibition of the surface process causes significant change in the currents in the potential region, and as a consequence the profound changes in the shape of CV take place. Universality of the detector in this mode is very advantageous for chromatographic analysis, where a mixture of compounds is present in samples.

It must be noted that, theoretically, in this method, the analyte response can be affected by the thermodynamic and kinetic parameters of adsorption such as the rate of mass transport and electrochemical behavior of the adsorbed species. The free energy and the rate of adsorption depend on the electrode potential, the electrode material, and to some extent, on the choice of the concentration and type of supporting electrolyte. By taking these points into consideration, in order to achieve maximum performance of the detector, the effect of experimental parameters (such as pH of the supporting electrolyte, potential and time of the accumulation and potential scan rate) must be examined and op-

timized.[31-41]

Optimizing the experimental parameters

The effect of eluent pH on performance of the detector was examined and the results are shown in Table 1. As shown, the best ΔQ was obtained between pH 2—3. In addition, the results show that at pH values higher than 9 noise level in the baseline (ΔQ vs. time), is higher up to 12% compared to acidic solution.

Table 1 pH effect on the microelectrode response

pH	2.1	4	6	8	10	12
ΔQ/μC	300	220	200	195	190	180

Also, in order to investigate the influence of scan rate and the eluent flow rate on the sensitivity of the detector response, of methyldopa solutions at concentration of 1.0×10^{-7} mol•L^{-1} were injected. At different scan rates (from 10 to 150 V/s) and the eluent flow, the responses of the detector to the injected sample were recorded. The results are presented in Figure 4. As it is clear from the Figure 4, the detector exhibits the maximum sensitivity at 50 V/s of scan rate and 3 mL/min of the flow rate. The effects of the sweep rate on the detection performance can be taken into consideration from three different aspects: first, speed in data acquisition, second, kinetic factors of adsorption of the methyldopa, and finally the flow rate of the eluent that controls the time window of the solution zone in the detector. The main reason for application of high scan rates, is prevention from desorption of the adsorbed methyldopa during the potential scanning, because under this condition, the inhibition outcome of the adsorbed methyldopa on the oxidation process can take place.

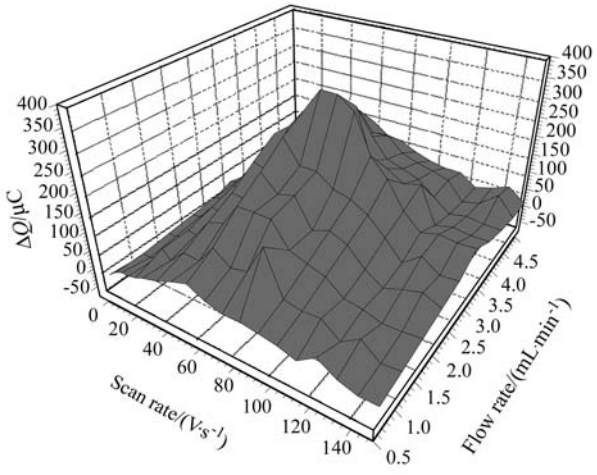

Figure 4 Effect of the sweep rate on the response of the Au microelectrode to injections of 1.0×10^{-7} mol•L^{-1} methyldopa in 0.05 mol•L^{-1} H_3PO_4.

Indeed, the use of this detection method in conjunction with fast separation techniques such as capillary electrophoresis also requires the employment of high scan rates. From this point of view, checking how the sensitivity of the method is affected by the sweep rate is necessary. To detect the amount of the adsorbed analyte on the electrode surface high sweep rates must be employed, so that the potential scanning step is short in comparison with the accumulation period. Notably, when the accumulation of methyldopa occurs at a potential that is very larger or smaller than E_i, this is very significant in this detection method. However, sensitivity of the detection system mainly depends on the potential sweep rate mainly due to kinetic factors in adsorption, and instrumental limitations.

Due to this fact any changes in the parameters related to adsorption process show a strong dependence upon the applied potential, and the time and the potential of accumulation strongly affect the sensitivity of the measurement. Therefore, the influence of the accumulation potential and time on the response of the method for the injection of a solution of 1.0×10^{-7} mol•L^{-1} methyldopa, in 0.05 mol•L^{-1} H_3PO_4, was studied. Figure 5 shows the detector response over the accumulation potential range from −1000 to 1800 mV and accumulation time range from 0.1 to 1.0 s. Based on the figure accumulation potential of 400 mV at time 500 ms was chosen as the optimum condition because the surface of the electrode became saturated by methyldopa within 200 s time window.

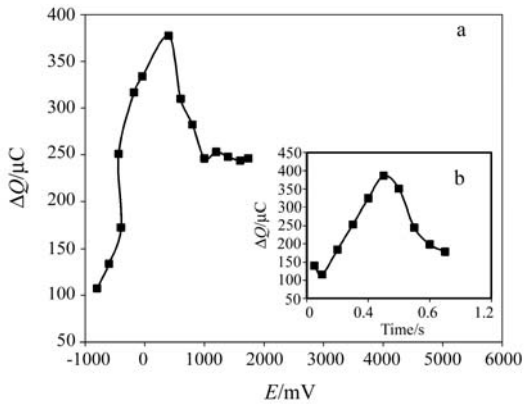

Figure 5 Effect of accumulation potential (a) and the effect of accumulation time (b) on the electrode response to injections of 1.0×10^{-7} mol•L^{-1} methyldopa in 0.05 mol•L^{-1} H_3PO_4.

On the electrode, the accumulation of methyldopa took place during the accumulation step (assuming that an appropriate potential was selected). In fact, the difference in the time of saturation of the various compounds can be related to the existing differences in their kinetics of the electron transfer and mass transport. As mentioned above, the surface of the gold microelectrode is very small, and in a very short time the surface of the electrode can be saturated.

Validation

The investigation of validity was performed with respect to linearity, limit of detection (LOD), precision,

accuracy, ruggedness/robustness, recovery and selectivity.[42-44]

Linearity

Linear regression analysis of a least square method was used to evaluate the linearity.[45,46] The linear range of 0.1—0.00001 mol•L^{-1} was conspicuous in constructed calibration curve. Peak areas of methyldopa were plotted versus its concentration and linear regression analysis was performed on the resultant curve. A correlation coefficient of R=0.9972 with RSD values ranging from 0.15%—3.85% across the concentration range studied was obtained following the linear regression analysis. Typically, the regression equation for the calibration curve was found to be y=3522x+33.875. Figure 6 shows the calibration graph obtained for the monitoring of methyldopa in a 0.05 mol•L^{-1} H_3PO_4.

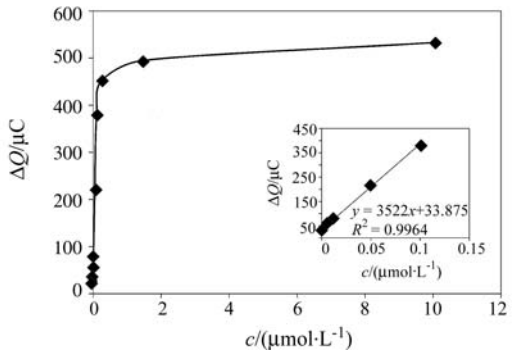

Figure 6 Calibration curves obtained for methyldopa on the Au microelectrode in 0.05 mol•L^{-1} H_3PO_4.

LOD

The lowest amount of the analyte that may be detected to produce a response is defined as LOD. Based on the calculation of standard deviation of the response (δ) and the slope (S) of the calibration curve at the levels approaching the limits according to equation LOD= 3.3δ/S,[47] the limit of detection that was found to be 0.004 nmol•L^{-1}, was approved.

Precision

Precision was investigated by injecting nine replicate samples of each of the 0.02, 0.008 and 0.0008 μmol•L^{-1} standards. The final mean concentrations were found to be 0.019, 0.007 and 0.00088 μmol•L^{-1} with associated RSD of 0.5%, 1.0% and 1.1%, respectively. The inter-day precision was assessed by injecting the same three concentrations for 3 consecutive days, resulting in mean methyldopa concentrations of 0.02, 0.0078 and 0.0008 μmol•L^{-1} with associated RSD values of 0.6%, 0.8% and 1.0%, respectively.

Accuracy

Interpolating replicate (n=6) peak areas of three accuracy standards (0.02, 0.008 and 0.0008 μmol•L^{-1}), the accuracy of the method was assessed by a calibration curve prepared as previously described. In each case, the percent relevant error and accuracy were calculated. The resultant concentrations were (0.02 ± 0.001), (0.008±0.002) and (0.0007±0.0003) μmol•L^{-1} with relevant error percentage of 0.67%, 0.9% and 0.85%, respectively.

Ruggedness

Comparing the intra- and inter-day assay results for tow methyldopa analytes was used to check the ruggedness of the method. The RSD values for intra- and inter-day assays of methyldopa in the cited formulations performed in the same laboratory by the two analysts did not exceed 4.5%, this way the ruggedness of the method is illustrated. The robustness was also examined while the parameter values (the pH of the eluent, the flow rate, the buffer composition and the laboratory temperature) were slightly changed.[48] According to Table 2, the methyldopa recovery percentages were satisfactory in most cases, without presenting any important changes during the alteration of the critical parameters.

Table 2 Influence of the changes in the experimental conditions on the performance of the FIA system

Parameter	Modification	Methyldopa/%
pH	1.8	100.3
	2	101.1
	2.3	99.9
	3.0	99.8
Flow rate/(mL•min^{-1})	2.8	100.6
	3.0	101.3
	3.2	99.9
Buffer composition/(mol•L^{-1})	0.04	97.9
	0.05	101.6
	0.06	100.4
Lab. temperature/℃	20	101.3
	25	99.9
	30	100.8

Recovery

In order to perform the recovery test, methyldopa standard powder at a concentration of 0.5 ng•mL^{-1} was added to samples of known amounts at 0.02, 0.008 and 0.0008 μmol•L^{-1} and then the voltamograms were recorded. The assay was repeated (n=9) over 3 consecutive days to obtain intermediate precision data. The resultant RSD for this study was found to be 0.9% with a corresponding percentage recovery value of 99.95%.

Selectivity

Standard solutions of methyldopa, were exploited to determine the sensitivity of the method in the presence of formulation components. As expected, the responses were not different from that obtained in the calibration.

We found that the formulation compounds had no interference to the determination of methyldopa due to the well fixed optimized parameters.

Determination of methyldopa in real samples

The voltamograms were recorded according to the above recommended procedure. Those of samples without methyldopa do not show any signal that can interfere with the direct determination, so external calibration can be used. The result has been shown in Table 3. The major advantage of the method as applied to plasma and urine is that no prior extraction step is required.

Table 3 Application of the proposed method to the determination of methyldopa in spiked Human plasma and urine[a]

Added/ ($ng \cdot mL^{-1}$)	Interpolated concentration/ ($ng \cdot mL^{-1}$)	RSD/%	RE/%
10 (plasma)	9.94 ± 0.2	1.5	1.05
100 (urine)	101.2 ± 0.5	1.0	1.6

[a] Data obtained from five replicates at each concentration. Interpolated concentration data expressed as mean $\pm$ SD.

Comparison of the sensitivity of the method with other previously reported methods

The detection limit of the proposed method was compared with the other reported methods. The results are shown in Table 4. In comparison to other reported methods, the sensitivity of this method is considerably higher than those of previously reported methods. As can be seen in Table 4, the detection limit of the method is 10000 times lower than the most sensitive reported methods.

Table 4 Comparison between the detection limit of the proposed method with those of other reported methods

Method	LOD/($ng \cdot mL^{-1}$)	Ref.
Flow-injection spectrophotometric	2100	49
Spectrophotometric determination	7174	50
A rapid HPLC with fluorescence detection and alumina extraction	8	51
FFTCCV	0.0008	This work

Conclusion

This report described a novel, sensitive, and widely applicable FFTCCV in FIA detection method using gold of ME. FFTCCV was demonstrated to provide sensitive detection of a wide range of analytes based on oxidation of the electrode surface. Currently, work is progressing in the enhancement of the detection electronics and FIA cell to allow incorporation of a second sensing electrode positioned away from the detection zone which will enable automatic, analog subtraction of the background response. It is hoped that this will make FFTCCV easier to use as well as providing enhanced sensitivity. Also, application of FFTCCV to high-performance liquid chromatography is being considered.

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