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Voltammetric and Flow Injection Amperometric Determination of Cysteine at a Glassy Carbon Electrode in the Presence of Copper(II) Ions

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The voltammetric behaviour of cysteine was investigated in the presence of Cu(II) ions at a rotating glassy carbon electrode (RGCE). Two reduction peaks related to the successive reduction of the cysteine complexes of Cu(II) and Cu(I) ions were observed at –0.30 and –0.60 V vs. SCE respectively. These peaks were increased upon the addition of cysteine into a pH 9.20 borate buffer solution containing 0.1 M KNO_3 and 1.0 x 10^{-5} M Cu(II) ions. The calibration curve was linear in the concentration range of 5.0 x 10^{-10}- 2.0 x 10^{-8} M for cysteine by utilising the peak current at –0.6 V vs. SCE.

In the flow technique, 50 μL portions of off-line prepared mixtures of 1.0 x 10^{-5} M Cu(II) and varying concentrations of cysteine were injected into the carrier solution of pH 9.20 borate buffer containing 0.1 M KNO_3 flowing through 25 cm transmission tubing (i.d. 0.75 mm) at a rate of 1.5 mL/min. The signals were monitored at – 0.60 V vs. SCE. The calibration graph was found to be linear in the concentration range 1.0 x 10^{-9} – 1.0 x 10^{-7} M with a relative standard deviation of 1.0% (n = 5) for 5.0 x 10^{-8} M cysteine. The detection limit was calculated as 5 x 10^{-10} M for S/N = 3 ratio.

Introduction

L-Cysteine is of great importance in biological and biomedical fields. Being an important amino acid in protein structures, cysteine participates in a number of biochemical processes which depend directly on the particular reactivity of thiols. Its oxidised derivatives have additional metabolic functions. Many methods for its determination have been reported including spectrophotometry[1–4], fluorimetry[5–7], electrophoresis[8], high performance liquid chromotography[9], and electrochemical methods[10–15]. Of these, voltammetric techniques offer high sensitivity and selectivity for cysteine determination. It is known that cystine (RSSR) is reduced at a dropping mercury electrode (DME) to produce cysteine (RSH)[10]. It was observed that if after the first scan the electrode was poised at 0 V for a short time prior to the second scan, a peak at more anodic potentials appeared. This peak was attributed to the reduction of mercurous cysteinate (RSHg).

More sensitive techniques utilise the hanging mercury drop electrode (HMDE); the cathodic stripping voltammetric (CSV) determination of cysteine depends on the reduction of the mercury complex of the

cysteine at –0.59 V vs. SCE[11]. The peak current-concentration curve for cysteine was reported to be linear from 2 x 10^{-8} – 5 x 10^{-7} M for 3 min deposition. However, the CSV behaviour of cysteine was found to be more complex and somewhat less reproducible than the other organic sulphur compounds. It was also noted that traces of oxygen seriously affect the results. This interaction was studied at an HMDE by monitoring the first reduction peak of dissolved oxygen[12] and it was concluded that metallic mercury has a profound effect on this interaction.

A substantial improvement in the detection limit occurs if a catalytic electrode process is coupled with a stripping method involving an HMDE. The catalytic electrode process has been used for the determination of cysteine and related compounds in the presence of nickel ions[13]. Cysteine and cystine can be detected by this means at nanomolar levels for 3 min accumulation. Chemically modified electrodes have also been used for the electrocatalytic determination of cysteine using some mediators in carbon paste such as copper (I) oxide or copper (II) phthalocyanine[14].

Several biosensors based on the inhibition of enzyme activity have also been proposed[15–17]. An FIA method based on the polyphenol oxidase enzyme which catalyses the oxidation of catechol to o-quinone with a strong absorption at 410 nm was reported[15]. When L-cysteine solution was injected into the flow system, its inhibitory effect on the polyphenol oxidase activity was found to be proportional to the L-cysteine concentration. Dynamic range was reported as 6.0 x 10^{-5} – 8.0 x 10^{-4} M and sample frequency was 26 samplings per hour.

Recently, an on-line determination method for cysteine was proposed[18]. The method is based on the reaction of the amino acid with chloramine-T and the subsequent reaction of excess of this reagent with an acidic solution of iodide. The resulting tri-iodide was determined biamperometrically using two platinum electrodes polarised at 100 mV. Cysteine can be determined by this means at a sampling frequency of 220 per hour and the detection limit was given as 1.1 μg/mL.

Forsmann et al. have studied the cathodic stripping voltammetric determination of traces of peptides and proteins containing disulphide linkages at an HMDE[19]. It was shown that in the presence of an excess of Cu(II) ions the resulting peaks were related to the redox reactions of copper complexes formed with the cysteine parts of the molecules studied. Therefore, the sensitivity of the method was markedly improved when the Cu(I) complex is accumulated rather than the mercury complex. This complex yields a cathodic stripping peak about 100 mV more negative than the mercury complex. Only the complexes and not the free thiol are strongly adsorbed. It was also noted that the cathodic stripping peak of cysteine becomes narrower and higher with increasing excesses of the Cu(II) ion, though its area remains constant. This effect was interpreted as a change in the kinetics of the reduction process as more copper amalgam is formed during the accumulation.

A former study in this lab[20] revealed the mercury-free voltammetric behaviour of cysteine at a glassy carbon electrode (GCE) in the presence of Cu(II) ions. The optimum pH was maintained by using pH 9.2 borate buffer. The reaction mixture was allowed to stand for 20 min in order to complete the reaction. By utilising the reduction peak at –0.59 V cysteine was shown to be determined at the nanomolar level. From this point of view the present study describes the voltammetric behaviour of cysteine in the presence of Cu(II) ions at a bare GCE and the optimal conditions for cysteine determination. The applicability of the method to amperometric flow injection analysis (FIA) was also studied in order to enhance the sensitivity and sampling rate.

Experimental

Apparatus

Voltammetric measurements were carried out on a Tacussel PRT 500 LC Potentiostat in conjunction with a GRSO Tacussel recorder. The three-electrode system consisted of a Metrohm GCE (i.d.: 3 mm), an auxiliary platinum electrode and a saturated calomel reference electrode (SCE) as the reference electrode. A Metrohm GCY 82 x 4 rotator maintained a rotation speed of 750 rpm. The pH of the solution was adjusted by means of a Metrohm E 510 pH meter with a combined glass electrode.

The proposed FIA manifold is depicted in Figure 1. An eight-channel Micro tube MP-3 Model (Eyela, Tokyo Rikakikai Co. LTD) peristaltic pump supplied with 25 cm polyethylene tubing (0.75 mm i.d.) was used for the propulsion of the fluids. The flow injection determination of cysteine was studied using a GCE in a wall-jet configuration. The flow cell was laboratory built from two teflon blocks as described elsewhere[21]. The sample was introduced into the system by means of a Rheodyne 7125 sample injection valve with a 50 μL loop volume.

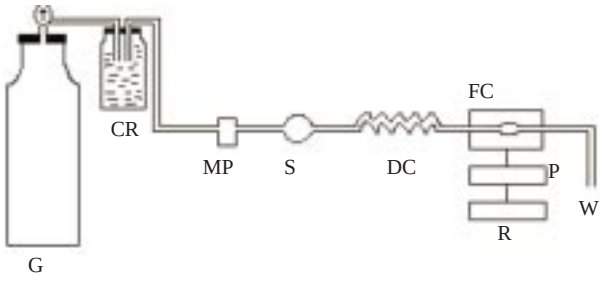

Figure 1. Flow injection manifold used for the determination of cysteine. G: nitrogen gas, CR: carrier stream reservoir, MP: mini peristaltic pump, S: sample injection valve, DC: delay coil, FC: flow cell, P: potentiostat, R: recorder, W: waste.

Reagents and solution

All the reagents used were of analytical-reagent grade and the solutions were prepared with water from an Elga ultra pure water system including reverse osmosis system. Cysteine was obtained from Sigma and a stock solution of cysteine (5.0 x 10^{-3} M) was daily prepared in deoxygenated distilled water. Borate buffer solution (pH 9.20) was prepared from a 0.01 M solution of $Na_2B_4O_7$ in 0.1 M KNO_3.

Procedures

Voltammetric studies

The GCE was polished with alumina slurry on a synthetic cloth, rinsed with distilled water and then sonicated for 5 min. The surface of the electrode was also activated electrochemically by applying a potential of + 2.0 V for 10 min and then -1.9 V for 2 min. A 25 mL aliquot of borate buffer solution was placed in a voltammetric cell and purged with nitrogen for 15 min. The reaction mixture of cysteine and Cu(II) was prepared in a separate container and kept under nitrogen atmosphere for 20 min. After a steady background current was obtained a necessary amount of reaction mixture was added onto the supporting electrolyte and then the voltammogram was recorded by rotating GCE at a speed of 750 rpm.

Flow injection studies

The buffer solution was propelled by a mini peristaltic pump with a flow rate of 1.5 mL/min as the carrier solution. Amperometric responses of the complex between cysteine and Cu(II) ions were investigated by two methods namely, on-line and off-line flow systems. On-line studies were carried out by injecting 50 μL of cysteine solution into the buffer solution containing Cu(II) ions flowing through the transmission tubing. The potential was adjusted at –0.60 V and the resulting peak currents were measured.

The off-line method, as in the stopped flow technique, includes the injection of a series of mixtures of Cu(II) and cysteine which were prepared in buffer solution at different concentration ratios in which the Cu(II) concentration was kept constant at 1 x 10^{-5} M. This mixture was then allowed to stand for 20 min in order to complete the complexation. All the reagents and carrier streams were purged with nitrogen gas due to the severe effect of dissolved oxygen on the signal.

Results and Discussion

Voltammetric studies

Initial studies were carried out voltammetrically to investigate the complexation reaction. As shown in Figure 2, 1.0 x 10^{-5} M Cu(II) ions give a small reduction peak at –0.3 V in pH 9.2 borate buffer solution containing 0.1 M KNO_3. This peak demonstrated an increase upon the addition of cysteine solution of 5.0 x 10^{-7} M in the cell (Figure 2c). Another peak at around –0.6 V also appeared and both peaks have built up with cysteine concentration (Figure 2d). It was also noted that the first peak potential gave a shift in the presence of cysteine indicating a complexation reaction.

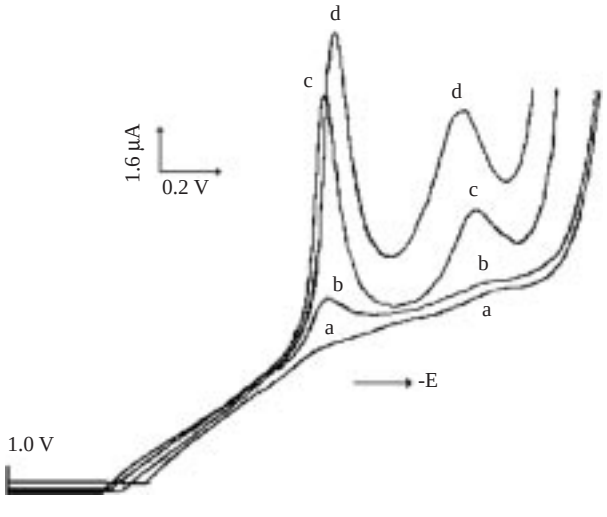

Figure 2. Voltammograms of a) background current at pH 9.20 borate buffer solution containing 0.1 M KNO_3, b) in the presence of 1.0 x 10^{-5}M Cu(II) solution. Cysteine solution was added into b giving c) 5.0 x 10^{-7} M and d) 1.0 x 10^{-6} M in the final solution.

These peaks were considered to be consecutive reduction of the complex. The remarkable increase in the second peak height was attributed to the enhanced stability of the Cu(I) complex of cysteine by the adsorption process during the scan. This behaviour agrees well with the results obtained at an HMDE by

using the CSV technique[15]. In the light of the previous works performed with mercury electrodes[10,19,20], our results obtained at a GCE indicate the reduction mechanism given below:

$Cu(II) + 2\ RS^- \rightleftharpoons Cu(II)(RS)_2$

$Cu(II)(RS)_2 + e^- \rightleftharpoons Cu(I)RS + RS^-$

$Cu(I)RS\ (soln) \rightleftharpoons Cu(I)RS\ (ads)$

$Cu(I)RS\ (ads) + e^- \rightleftharpoons Cu + RS^-$

On the other hand, in the presence of Cu(II) ions cyteine also reacts via a redox process to form Cu(I)-cysteine complex and cystine. This mechanism was formerly proposed by Forsmann[22] from the results of a CSV study with a HMDE. It was also reported that the anodic formation of mercuric cysteinate was effectively inhibited and instead a cathodic wave corresponding to the reduction of Cu(I) complex appeared.

$Cu(II) + 2RS^- \rightarrow Cu(I)RS + {}^1\!/_2\ RSSR$

The influence of Cu(II) concentration on the complex peak of 1.0 x 10^{-8} M cysteine was studied by monitoring the second peak at –0.6 V upon the addition of Cu(II) ions into the cell. As shown in Figure 3, the optimum results were obtained with 1.0 x 10^{-5} M in the Cu(II) concentration range studied. Under these conditions the calibration graph was obtained and it was shown to be rectilinear over the range 0.5 x 10^{-9} – 2.0 x 10^{-8} M (Figure 4). The RSD value was calculated to be 2% at the 1.0 x 10^{-9} M level for five determinations.

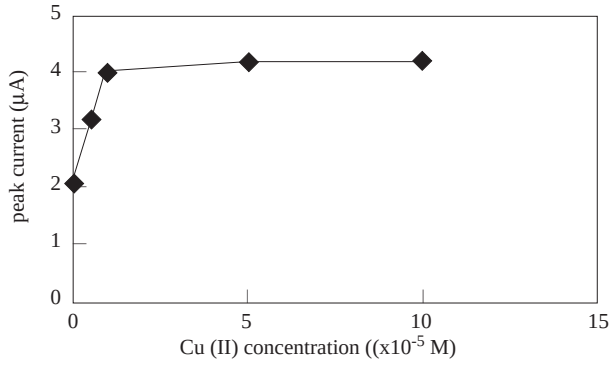

Figure 3. Effect of Cu(II) concentration on the peak currents at –0.60 V in the presence of 1.0 x 10^{-8} M cysteine at pH 9.20 buffer solution.

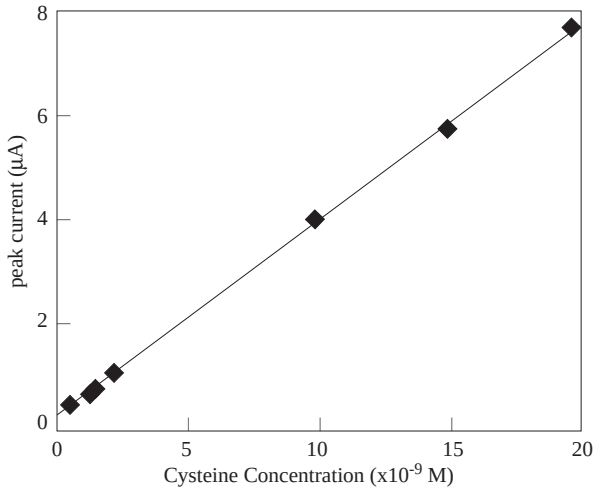

Figure 4. The calibration curve for cysteine obtained by measuring the peak currents at –0.60 V at a GCE in the presence of 1.0 x 10^{-5} M Cu(II) solution prepared in pH 9.20 borate buffer solution containing 0.1 M KNO_3.

Flow Injection Amperometric Studies

The method given above was exploited for the on-line amperometric determination of cysteine. On-line measurents of cysteine include the injection of a 50 μL portion of cysteine solution into the carrier stream of buffer solution containing 1.0 x 10^{-5} M Cu(II) ions flowing through transmission tubing. The peak heights at –0.60 V were monitored against the cysteine concentration. However, the sensitivity was poor indicating a slow complex formation on the way to the detector. The use of longer transmission tubing made for a slight improvement on the signal.

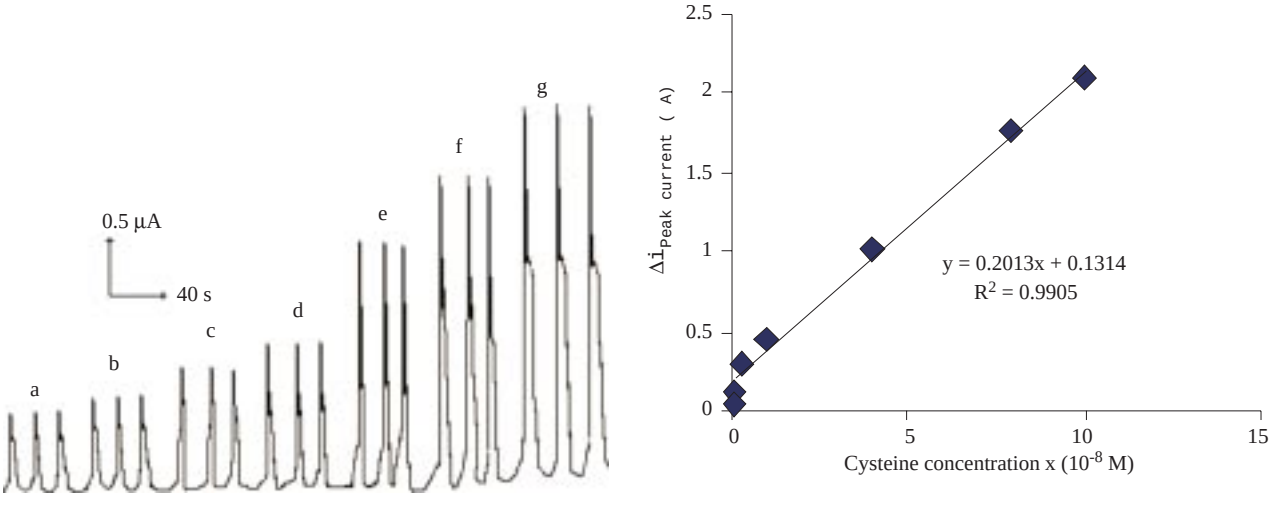

Figure 5A. Typical FIA signals obtained for 50 μL injection of a) 1.0 x 10^{-5} M Cu(II) alone, and the solution of (a) in the presence of b) 0.1, c) 0.4, d) 1.0, e) 4.0, f) 8.0 and g) 10 x 10^{-8} M cysteine into carrier stream of pH 9.20 borate buffer solution containing 0.1 M KNO_3. The current-time peaks were recorded at –0.6 V vs. SCE. Figure 5B. Resulting calibration curve.

Alternatively, the off-line method was studied and a series of mixtures of the complex was prepared as given in the experimental section. A 50 μL portion of these mixtures was injected into the carrier stream of buffer solution. The FIA signals are shown in Figure 5A. The reduction peak current differences (Δi_{peak}) were plotted against cysteine concentration and the calibration curve was found to be linear over the concentration range 1.0 x $10^{-9} - 1.0$ x 10^{-7} M (Figure 5B). In comparison with the on-line measurement, this method was found to be more sensitive giving a detection limit of 5.0 x 10^{-10} M calculated to be S/N = 3.

Conclusion

In this study, an FIA amperometric procedure for cysteine determination is proposed. The method is based on complex formation with Cu(II) ions and on reduction of the complex at a GCE held at –0.6 V. Initial voltammetric studies revealed that the complex undergoes a subsequent reduction process and gives two well formed peaks at –0.3 and –0.6 V respectively. The remarkable increase in the second peaks height with cysteine concentration was attributed to the enhanced stability of the Cu(I) complex of cysteine by the adsorption process during the scan. Cysteine can be determined by these means at nanomolar levels. This method utilises a mercury-free determination of cysteine without any accumulation step, which constitutes an advantage over other voltammetric methods reported[13,19].

In the second part of the study the method was exploited for FIA amperometric determinations of cysteine by using a GCE in a wall-jet configuration held at –0.60 V vs. SCE. The signal of Cu(I)-cysteine complex was monitored against cysteine determination by injecting standard cysteine solution into the carrier stream of buffer solution containing Cu(II) ions. However, the sensitivity was poor, indicating a slow complex formation on the way to the detector. As an alternative, the off-line method was studied. The previously prepared complex mixtures were injected into the carrier stream of buffer solution and more sensitive results were obtained. The detection limit was calculated to be 5 x 10^{-10} M. This improvement in

sensitivity may be attributed to the completeness of the complexation in the mixture. However, the time for the Cu(I) complex formed at the electrode surface to be adsorbed is very limited. This can be explained by the chemical formation of Cu(I) complex in the solution phase as proposed by Forsman[22]. Therefore, the waiting time of the off-line prepared mixtures is of importance in terms of sensitivity.

The sampling frequency of the FIA system was more than 100 per hour. In comparison to previous FIA studies[15,18], besides its simplicity, this method more sensitively determines cysteine.

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