

Potentiometric determination of cysteine with thiol sensitive silver-mercury electrode[★]

Ryszard Drożdż, Jerzy Naskalski[✉] and Anna Ząbek-Adamska

Department of Diagnostics, Chair of Clinical Biochemistry, Collegium Medicum, Jagiellonian University, Kraków, Poland

Received: 06 November, 2006; revised: 02 February, 2006; accepted: 16 February, 2007
available on-line: 09 March, 2007

A potentiometric procedure for cysteine thiol group concentration monitoring in media generating free radicals was developed using a thiol specific silver-mercury electrode. Electrolytic deposition of mercury on a silver wire and treatment with 20 mM cysteine in 0.5 M NaOH were used to produce the electrode. A silver-chloride electrode in saturated KCl was the reference. A glass capillary with 1 M KNO₃ in 1% agarose gel was the liquid junction. The electrode responded to cysteine concentration in the range from 0.01 to 20 mM yielding a perfect linear relationship for the dependence of log [cysteine] versus electrode potential [mV], with b_0 (constant) = -373.43 [mV], b_1 (slope) = -53.82 and correlation coefficient $r^2 = 0.97$. The electrode potential change per decade of cysteine concentration was 57 mV. The minimal measurable signal response was at a cysteine concentration of 0.01 mM. The signal CV amounted to 4–6% for cysteine concentrations of 0.01 to 0.05 mM and to less than 1% for cysteine concentrations of 0.5 to 20 mM. The response time ranged from about 100 s for cysteine concentrations of 0.01 to 0.1 mM to 30 s at higher cysteine concentrations. The standard curve reproducibility was the best at cysteine concentrations from 0.1 to 20 mM. In a reaction medium containing cysteine and copper(II)-histidine complex ([His-Cu]²⁺) solution in 55 mM phosphate buffer pH 7.4 the electrode adequately responded to changes in cysteine concentration. Beside cysteine, the silver-mercury electrode responded also to thiol groups of homocysteine and glutathione, however, the Nernst equation slope was about half of that for cysteine.

Keywords: cysteine, glutathione, homocysteine, potentiometry, real time assay

INTRODUCTION

The thiol residues of cysteine, glutathione and other low molecular mass peptides are readily oxidized in electrochemical reactions on the surface of various thiol sensitive electrodes. These reactions have been employed in a number of methods for electrochemical determination of thiol compounds. In the vast literature concerning electrochemical determination of thiol compounds, various types of electrodes including: mercury (Heyrovsky *et al.*, 1997; Lawrence *et al.*, 2001), silver (Yosipchuk & Novotny, 2002; Kolar *et al.*, 2002), gold (Yang *et al.*, 2001; Ozomena *et al.*, 2001; Kolar *et al.*, 2002), platinum (Zhao

et al., 2001), tungsten (Hidayat *et al.*, 1997), copper (Yang *et al.*, 2001; Yosipchuk & Novotny, 2002) and cobalt (Sugawara *et al.*, 1996) have been described. These electrodes, predominantly used in potentiometric measurement, have been designed for various purposes, such as determination of thiol compounds in pharmaceutical products (Kolar *et al.*, 2000; 2002; Kolar & Dobcnik, 2003) or specific biological samples (Jin & Wang, 1997; Calvo-Marzal *et al.*, 2006). In some studies free radical formation in the presence of cysteine in the reaction medium was described (Kachur *et al.*, 1999). We attempted to study cysteine involvement in the process, but most of the cysteine assay methods used to date, including HPLC, con-

[★]Results of the experiments described in this paper were in part presented on the 41st Meeting of the Polish Biochemical Society, Białystok, Poland; September 12–15, 2006.

[✉]Corresponding author: Jerzy W. Naskalski, Zakład Diagnostyki, Katedra Biochemii Klinicznej CM UJ, Kopernika 15B, 31-501 Kraków, Poland; tel./fax (48 12) 424 8361; e-mail mbnaskal@cyf-kr.edu.pl

sidered as cysteine assay gold standard, were unsuitable for this purpose. In this paper a method of real time monitoring of cysteine concentration changes in the reaction medium, employing a cysteine-sensitive silver-mercury electrode is described.

MATERIALS AND METHODS

Working solutions and dilutions of the reagents were prepared using glass-distilled water (with resistivity of 18 M Ω /cm, Millipore-Q). Stock solution of DL-cysteine (20 mM) was prepared in 55 mM sodium phosphate buffer pH 7.4. Dilutions of DL-cysteine solution with this buffer were prepared directly prior to use. The final pH of the employed DL-cysteine solutions was adjusted to 7.4 by adding 0.5 M NaOH. Oxidation of cysteine was carried out in a solution of a histidine-copper complex [His-Cu]²⁺ obtained by mixing equal volumes of CuCl₂ and histidine.

All reagents were obtained from Sigma and were of analytical grade.

The electrochemical measurements were carried out using an Elmetron CP-401 millivoltmeter. Measurement results were recorded on-line on a computer and final data were calculated using SigmaPlot v.7.0 program. The electrochemical potential of the electrodes studied was measured *versus* a reference Ag/AgCl electrode in saturated KCl solution.

Working electrodes tested. Selection of a working cysteine sensitive electrode was carried out basing on a comparison of the analytical performance of eight different electrodes potentially useful for real time monitoring of cysteine concentration in the studied samples. The assessment of the electrode's properties included: electrode sensitivity, signal response rate, concordance of response with the Nernst equation, variation of the electrode response and specificity *versus* other thiol compounds. Platinum, copper, graphite, graphite-silver, graphite-silver-cysteine, silver, silver-iodide and silver-mercury electrodes were characterized.

Preparation of working electrodes. To prepare cysteine sensitive graphite, platinum, copper, and silver electrodes the electrode base material (25 mm long graphite rod or metal wire 0.7 mm in diameter) was cleaned mechanically with a low mesh sand paper, connected to a voltmeter by an insulated copper lead and fixed in a glass tube housing, using epoxy resin. Then the electrode was placed in 20 mM cysteine in 0.5 M NaOH solution for 24 h.

The graphite-silver electrode was prepared by electrolytic deposition of silver on a graphite rod and further treatment as described above for metal wire electrodes. The graphite-silver-cysteine elec-

trode was the graphite-silver electrode which, after deposition of silver on the electrode surface, was subjected to cathode reduction in 20 mM cysteine solution in 1 M NaOH for 2 min employing 2 V potential. Then the electrode was washed with water and placed in 20 mM cysteine in 0.5 M NaOH solution.

The silver-mercury electrode was prepared using a silver wire inserted in a glass tube handle. The electrode surface was activated first for 5–10 s treatment with 0.5 M NH₃ water solution, the electrode then was washed with distilled water, placed for 2 min in 0.1 M HgCl₂ solution and mercury was deposited by electrolysis on the electrode surface. Then, the wire was again washed with distilled water and placed in 20 mM cysteine in 0.5 M NaOH.

The silver-iodide electrode was prepared as described by Kolar and coworkers (2002). Mechanically cleaned silver wire was placed for 5 min in 0.1 M HgCl₂ solution, then washed with water and placed for 24 h in 0.1 M KI. The electrode was stored in 0.1 M KI solution.

The prepared working electrodes were stable and suitable for use for at least 10 days if stored in 20 mM cysteine in 0.5 M NaOH. Prior to each series of measurements the electrodes were washed with 55 mM phosphate buffer, pH 7.4, and the electrode initial potential was measured *versus* reference silver-chloride (Ag/AgCl) electrode in 1 M KNO₃ solution. Measurements were carried out after stabilization of the electrode initial potential (from 20 s to 30 min for different electrodes tested). After measurements, the electrodes were washed with 55 mM phosphate buffer, pH 7.4, and stored again in 20 mM cysteine in 0.5 M NaOH.

Reference silver-chloride (Ag/AgCl) electrode. The reference electrode was a silver-chloride electrode in saturated KCl. The reference electrode cell was a 2.5 ml microvial with 1 M KNO₃. Fresh, saturated KCl solution in the silver-chloride reference electrode and fresh 1 M KNO₃ solution in the reference electrode chamber were used at each new measurement series.

Preparation of the liquid junction. A U-shaped glass capillary with 1 M KNO₃ in 1% agarose gel was used as the liquid junction. The measurement of the electrode potential was initiated by connecting the measuring cell with the reference electrode cell by placing the liquid junction into the workcell and the reference electrode cell. Each measurement series was carried out employing a new liquid junction capillary with a fresh agarose/KNO₃ solution.

Cysteine concentration measurement. The working electrodes tested were placed in the working cell (2.5 ml) with a magnetic micro stirrer inside containing the 1.5 ml of 55 mM phosphate buffer,

pH 7.4. After several minutes of incubation with the working buffer to obtain electrochemical equilibrium between the buffer and the electrode, an electro-voltaic cell was ensemble by inserting the liquid junction connecting the working cell with the reference electrode cell. The baseline electric potential (in mV) was measured for 3 to 30 min, using an Elmetron CP-401 millivoltmeter with an internal resistance of 20 M Ω . Once the baseline potential had been measured, 20 mM cysteine solution in 55 mM phosphate buffer, pH 7.4, was added to obtain a final cysteine concentration in the range of 0.01 to 20 mM. The measured electrochemical potentials were referred to the reference cysteine concentrations when the electrode signal was stable within of ± 2.0 mV.

Construction of standard curves of cysteine concentration versus millivolts. The cysteine sensitive electrode was inserted to the measuring cell placed on a magnetic stirrer and 2.5 ml solution of 0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM, 5 mM, 10 mM or 20 mM cysteine in 55 mM phosphate buffer, pH 7.4, was added. Then, the working electrode cell was connected with the reference electrode cell by inserting the liquid junction. Measurement of the electrode potential was started after 30 s of equilibration. The electrode potential was then continuously measured for 3 to 30 min. The electrode electromotoric potential, response rate and potential stability *versus* the given cysteine concentration were assessed. Diluted cysteine samples were prepared immediately before measurements to diminish the effect of spontaneous cysteine oxidation with atmospheric oxygen.

The obtained results were transformed to a logarithmic relationship according to the Nernst equation:

$$E[\text{mV}] = a + b \log [\text{cysteine concentration in mM}]$$

The transformed data were then presented in the graphic form using SigmaPlot v.7.0 program. A triplicate measurement of electrode potential for each cysteine concentration was carried out. Mean and

standard deviation (\pm S.D.) was then calculated and referred to the given cysteine concentration in the standard solution. The obtained values were used for the assessment of the correlation (r^2) and linear regression equation.

Measuring electrode response time to various cysteine concentrations. To the working cell containing 1.2 ml of 0.01 mM cysteine solution in 55 mM phosphate buffer, pH 7.4, consecutive portions of 0.05 ml, 0.1 ml, 0.2 ml, 0.4 ml, 0.8 ml or 1 ml of buffered 20 mM cysteine solution were added, and the electrode response time and values of the electrode signal were measured. The electrode response to decreasing cysteine concentrations was assessed by measuring the cysteine concentration in a reaction medium containing cysteine and [His-Cu]²⁺ complex in 55 mM phosphate buffer pH 7.4. This measurement was carried out by adding 0.06 ml of [His-Cu]²⁺ complex solution to the working cell with 1.5 ml of 0.6 mM cysteine solution, to obtain a final concentration 0.1 mM Cu²⁺ ions. The electrode potential was followed for 7 min. The results of the measurements were transformed into cysteine concentration (mM) and expressed as a function of cysteine concentration *versus* reaction time.

The electrode response time was also assessed when various cysteine concentrations were added to a reaction medium containing [His-Cu]²⁺ complex in 55 mM phosphate buffer. This measurements were carried out by adding of 0.005 ml cysteine solution to the working cell containing 1.5 ml of [His-Cu]²⁺ complex at 0.1 mM Cu²⁺ to obtain a final concentration 0.6 mM cysteine. The electrode potential was followed every 1 s up to termination of the reaction. The measurement results were transformed on-line into cysteine concentration (mM) and expressed as the function of cysteine concentration *versus* reaction time.

RESULTS

The analytical performance of the silver-mercury electrode in comparison to other electrodes tested is present in Table 1. The sil-

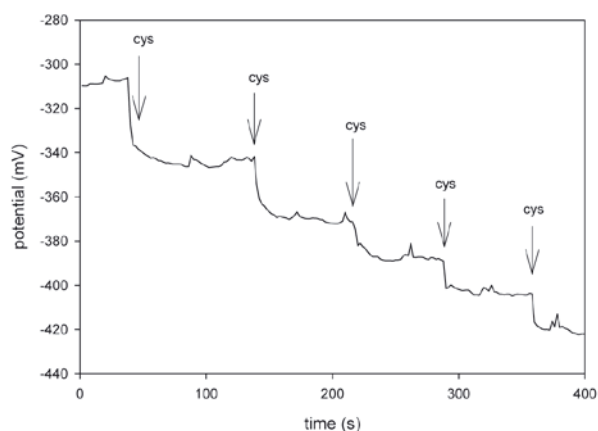
Table 1. Properties of electrodes tested

Electrode	Slope (mV)	Intercept (mV)	Response range (mM)	CV (mean, %)	Correlation coeff. (r^2)
Platinum	-26.5	141.5	0.01-10	17.6	0.91
Copper	-99.7	-212.1	0.10-20	4.6	0.94
Graphite	-72.6	-23.8	0.01-5	33.2	0.78
Graphite-silver	-115.7	124.1	0.1-20	43.2	0.84
Graphite-silver-cysteine	-150.6	41.5	0.01-20	41.2	0.98
Silver	-86.0	-175.3	0.01-20	14.9	0.97
Silver-iodide	-53.7	-180.7	0.01-20	2.51	0.96
Silver-mercury	-53.8	-373.4	0.01-20	1.82	0.97

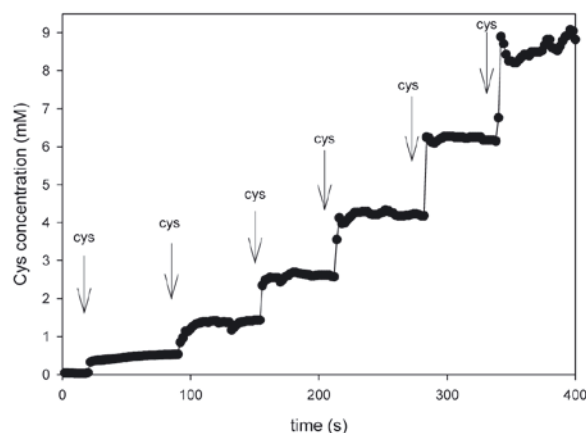
Table 2. Silver–mercury electrode response to cysteine concentration (0.01–20.0 mM)

Cysteine concentration (mM)	Serie I (mV)	Serie 2 (mV)	Serie 3 (mV)	Mean \pm S.D. (CV%)
0.01	-270	-273	-283	-275 \pm 7 (2.5)
0.05	-297	-281	-265	-281 \pm 16 (5.7)
0.10	-310	-302	-296	-303 \pm 7 (2.3)
0.50	-339	-344	-344	-342 \pm 3 (0.8)
1.00	-356	-361	-362	-360 \pm 3 (0.9)
5.00	-391	-398	-399	-396 \pm 4 (1.1)
10.0	-414	-419	-420	-418 \pm 3 (0.8)
20.0	-443	-448	-450	-447 \pm 4 (0.8)

ver–mercury electrode has shown perfect linearity, variation of 1.82% (CV) and the slope value in accordance with that predicted by the Nernst equation. The silver–mercury electrode was superior to other electrodes in respect to CV at various cysteine concentrations (Table 2). The lowest cysteine concentration yielding an electrode signal was 0.01 mM. The electrode response to various cysteine concentrations is shown in Figs. 1 and 2. The silver–mercury electrode standard curve is shown in Fig. 3. The obtained relationship expressed as linear regression equation parameters for \log [cysteine] versus mV yielded: regression constant = -373.43 [mV] and slope (b1) = -53.82 . The correlation coefficient square $r^2 = 0.97$ (for cysteine concentration in mM). The electrode signals versus cysteine concentration satisfactorily conform to the Nernst equation, yielding the molar potential coefficient (slope) of about 54 mV/p per decade of cysteine concentration (Figs. 1–3). The reproducibility of the standard curve is highest in the cysteine concentration range from 0.1 to 20 mM. Removing the results obtained for the two

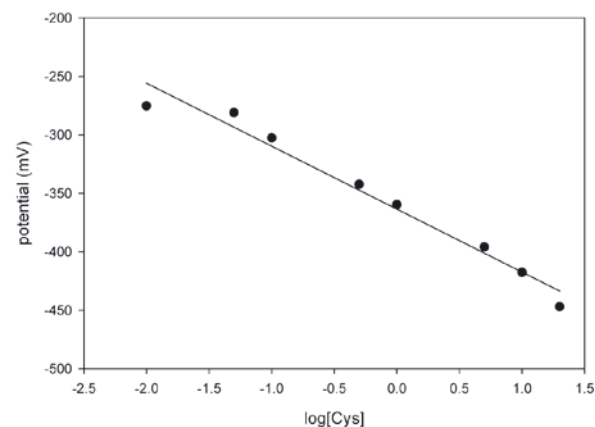
**Figure 1. Silver–mercury electrode response to increasing cysteine concentrations.**

To 0.01 mM cysteine solution aliquots of 20 mM cysteine were added to obtain final concentrations of 0.01, 0.05, 1.0, 5.0, 10.0 or 20.0 mM.

**Figure 2. Silver–mercury electrode response expressed in cysteine concentration (mM) occurring at consecutive cysteine additions at 30 s intervals.**

Increasing aliquots of cysteine stock solution (20 mM) were added to the measured sample to give final cysteine concentration of 0.01, 0.05, 1.0, 5.0, 10.0 mM.

lowest cysteine concentrations (0.01 and 0.05 mM) from the data did not meaningfully influence the slope value. The electrode response time ranged

**Figure 3. Standard curve for silver–mercury electrode.**

Electrode response was measured for cysteine concentration range of 0.01 to 20.0 mM; $mV = -373.49 - 53.8 \log$ [cysteine mM].

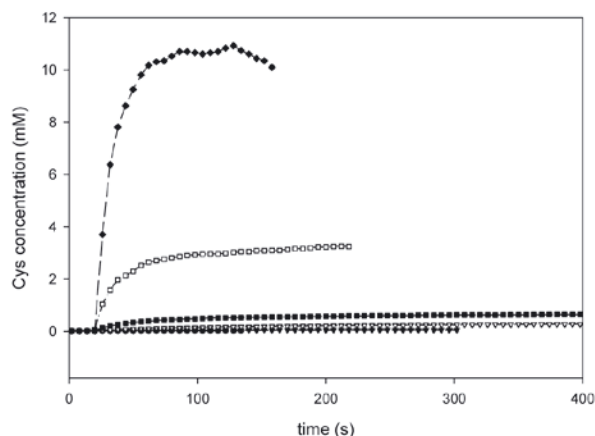


Figure 4. Silver-mercury electrode response time to increasing cysteine concentration.

The response time was measured upon addition of 0.01 mM (black stars), 0.05 mM (triangles), 1.0 mM (black squares), 5.0 mM (light squares) or 10.0 mM (black diamonds), cysteine solution.

from about 90 s for cysteine concentrations of 0.01 to 0.1 mM to 20 seconds at higher cysteine concentrations (Fig. 4). The electrode response to consecutive additions of cysteine to the sample is shown on the Figs. 5 and 6. Each addition of cysteine to $[\text{His-Cu}]^{2+}$ complex solution yields the same electrode response (Fig. 5). The electrode responded to each cysteine addition to the reaction medium (Fig. 6), reacting in the range of 0.05 to 20 mM.

The silver-mercury electrode showed certain specificity toward cysteine thiol groups. The electrode responded to the presence of thiol groups of homocysteine and glutathione, however, the mo-

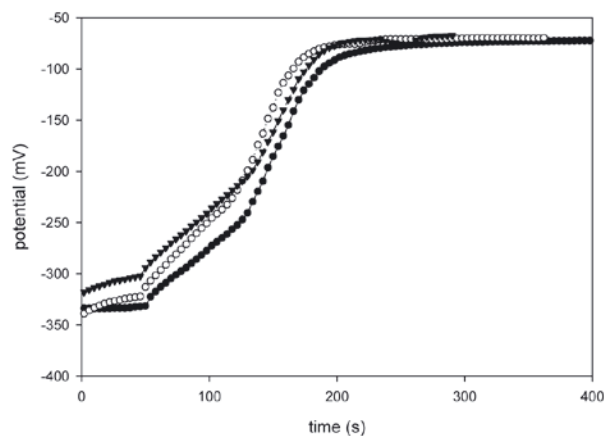


Figure 5. Silver-mercury electrode response to decreasing cysteine concentration.

To 0.6 ml $[\text{His-Cu}]^{2+}$ complex in 55 mM Na-phosphate buffer pH 7.4, 0.05 ml of 6 mM cysteine solution in three repetitive experiments was added. Final concentration of Cu^{2+} ions in solution was 0.1 mM.

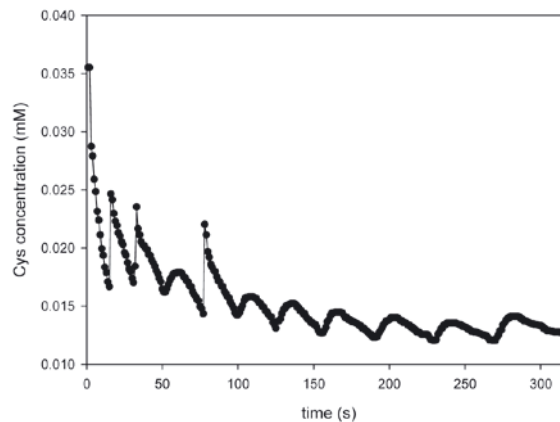


Figure 6. Silver-mercury electrode response to consecutive additions of cysteine.

Electrode response was measured to consecutive additions of cysteine (0.005 ml of 18 mM) at 30 s intervals to the $[\text{His-Cu}]^{2+}$ complex solution (final concentration of Cu^{2+} = 0.1 mM). At each cysteine addition the electrode monitored increase of the cysteine concentration, then followed by a decrease, due to consumption of cysteine in the reaction medium.

lar potential coefficients were about half of that for cysteine (Table 3).

DISCUSSION

Monitoring of thiol concentration changes is difficult due to its reactivity with oxygen present in the reaction medium, as well as with various components of the sample. The time consuming multi-step sample handling procedures of thiol assays are not suitable for following rapid changes in the concentration of thiols in various reactions. Electrode procedures reduce both sample handling and the duration of the thiol assay procedure. Jin and Wang (1997) used a mercury-gold amalgam electrode for amperometric determination of cysteine in human plasma, blood and urine. The employed electrochemical system showed a very high analytical sensitivity enabling detection of cysteine in non-deoxygenated solutions, in concentration as low as 5.8×10^{-8} M. However, the amperometric assay system with three electrodes is complicated and inconvenient for use in open measurements. Potentiometric determination of cysteine and glutathione was employed earlier in various flow analytical systems, HPLC (Kolar *et al.*, 2000), capillary electrophoresis (Lawrence *et al.*, 2001) or specific flow injection biochemical analyzers (Kolar *et al.*, 2000; 2002) used a potentiometric cysteine and glutathione assay in a flow injection analyzer using silver tubular electrode pretreated with mercuric (II) chloride solution and iodide solution. The sensitivity of such electrode was 10 mM at a slope of 55.2 ± 1.0 mV/p. In recent papers Kolar

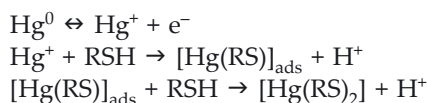
Table 3. Reactivity of silver–mercury electrode with homocysteine and glutathione

Homocysteine			Glutathione		
Slope (mV/log C) = -28.3 Intercept: (mV) = -272.4 r ² = 0.90			Slope (mV/log C) = -18.8 Intercept: (mV) = -232.8 r ² = 0.80		
Concentration (mM)	Mean electrode response ±S.D. (mV)	CV (%)	Concentration (mM)	Mean electrode response ±S.D. (mV)	CV (%)
0.01	-229 ± 2	0.7			
0.05	-232 ± 7	2.9	-	-	-
0.1	-239 ± 8	3.5	-	-	-
0.5	-252 ± 12	5.0	0.5	-216 ± 6	2.9
1	-265 ± 14	5.3	1	-246 ± 18	7.4
5	-291 ± 20	7.0	5	-265 ± 31	11.7
10	-315 ± 18	5.7	10	-297 ± 4	1.4
-	-	-	20	-353 ± 21	6.5

et al. (2002) and Kolar & Dobcnik (2000) employed chemically modified silver electrodes designed for batch analysis or flow analysis of samples containing cysteine and *N*-acetylcysteine. The electrode was prepared by pretreatment of silver wire or silver tube with mercuric (II) chloride and potassium iodide. The electrodes had a constant linear response within a concentration range of 4 µM to 1 mM at a slope of 60.6 ± 1 mV/p for *N*-acetylcysteine and sensitivity of 5 mM at a slope of 53.4 ± 1 mV/p for cysteine. These electrodes, however, were dedicated to drug analysis in a batch or a special flow analyzer and were unsuitable for our purpose of monitoring cysteine concentration in the small samples used in our studies. However, the results presented by those authors indicated potential favorable analytical properties of the silver–mercury electrode for thiol concentration monitoring.

Our present studies showed that a simple silver–mercury electrode activated with cysteine offered the best analytical performance of the eight electrodes tested. The thiol measurement system employed in our studies, composed of a silver–mercury working electrode, a silver–chloride reference electrode and a liquid junction with 1 M KNO₃ in 1% agarose was the most suitable one for monitoring changing cysteine concentrations (Table 2). The electrode had a perfect reproducibility and was easy to handle, thanks to the silver wire shock resistance. The molar potential coefficient (*S*-slope) amounting to 53.8 mV/p is in accord with that predicted by the Nernst equation. The electrode employed in our studies appeared to perform properly in the phosphate buffer solution, pH 7.4, and in the presence of the [His–Cu]²⁺ complex. The mechanism of the reaction occurring on the surface of the mercury or silver–mercury electrodes is highly complex and depends on

the pH of the reaction medium. Heyrovsky *et al.* (1997) indicated that on the surface of a mercury electrode cysteine is transformed to mercuric thiolate, which produces a secondary complex with the electrode surface, additionally employing the cysteine nitrogen atom:



The mechanism of action of the silver–mercury electrode is probably similar to that of the mercury electrode, but due to the complexity of reactions occurring on the electrode surface, prediction of the electrode properties was not possible.

Thiol compounds represent an important part of the oxidation–reduction balance of organisms. The cysteine–glutathione system is considered to be involved in the reduction of lipid and protein peroxides, and participates in the defense against reactive oxygen species (ROS). Thiol groups of free and protein-bound cysteine residues play a protective role for the active centers of many enzymes and ascertain the stability of various structural proteins. However, cysteine can also participate in reactive oxygen species production, thus enhancing the damaging effects of ROS on the biological structures (Kachur *et al.*, 1999), and the use of the silver–mercury electrode described in our study may facilitate the investigation of cysteine reactions in various biological systems where free radical formation may potentially occur.

Acknowledgement

Studies supported by Collegium Medicum grant No. BBN/CM – 4100/547/2006/501/P/222/L.

REFERENCES

- Calvo-Marzal P, Chumbimuni-Torres KY, Hoehr NF, Kubota LT (2006) Determination of glutathione in hemolysed erythrocyte with amperometric sensor based on TTF-TCNQ. *Clin Chim Acta* **371**: 152–158.
- Heyrovsky M, Mader P, Vavricka S, Vesela V, Fedurco M (1997) The anodic reactions at mercury electrode due to cysteine. *J Electroanal Chem* **430**: 103–117.
- Hidayat A, Hibbert DB, Alexander PW (1997) Amperometric detection of organic thiols at a tungsten wire electrode following their separation by liquid chromatography. *J Chromatogr B* **693**: 139–146.
- Jin W, Wang J (1997) Determination of cysteine by capillary zone electrophoresis with end-column amperometric detection at a gold/mercury amalgam microelectrode without deoxygenation. *J Chromatogr A* **769**: 307–314.
- Kachur AV, Koch CJ, Biaglow JE (1999) Mechanism of copper-catalyzed autoxidation of cysteine. *Free Radic Res* **31**: 23–34.
- Kolar M, Dobcnik D (2003) Chemically prepared silver electrode for determination of *N*-acetyl-L-cysteine by flow-injection potentiometry. *Pharmazie* **58**: 25–28.
- Kolar M, Dobcnik D, Radic N (2000) Potentiometric flow injection determination of vitamin C and glutathione with a chemically prepared silver tubular electrode. *Pharmazie* **55**: 913–917.
- Kolar M, Dobcnik D, Radic N (2002) Chemically treated silver electrodes for determination of cysteine. *Microchem Acta* **138**: 23–27.
- Lawrence NS, Davis J, Compton RG (2001) Electrochemical detection of thiols in biological media. *Talanta* **53**: 1089–1094.
- Ozoemena K, Westbroek P, Nyokong T (2001) Long-term stability of a gold electrode modified with a self assembled monolayer of octabutylthiophtalocyaninocobalt(II) towards L-cysteine detection. *J Electrochem Commun* **3**: 529–534.
- Sugawara K, Hoshi S, Akatsuka K, Shimazu K (1996) Electrochemical behaviour of cysteine at a Nafion^R/cobalt(II) modified electrode. *J Electroanal Chem* **414**: 253–256.
- Yang W, Gooding JJ, Hibbert BB (2001) Characterization of gold electrodes modified with self-assembled monolayers of L-cysteine for the adsorptive stripping analysis of copper. *J Electroanal Chem* **516**: 10–16.
- Yosypchuk B, Novotny L (2002) Cathodic stripping voltametry of cysteine using silver and copper solid amalgam electrodes. *Talanta* **56**: 971–976.
- Zhao C, Zhang J, Song J (2001) Determination of L-cysteine in amino-acid mixture and human urine by flow-injection analysis with a biamperometric detector. *Anal Biochem* **297**: 170–176.