

Direct Potentiometric Measurements of Cysteine by Using Iodide-Selective Electrode

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Potentiometric determination of cysteine (Cys) in aqueous solution by using iodide-selective electrode with ($\text{Ag}_2\text{S} + \text{AgI}$) membrane is described. The analytical behaviour of this electrode is discussed in terms of the potential *vs.* concentration curve, potential *vs.* pH curve, and potential-time response. The equilibrium potentials observed during serial dilution or standard addition, under constant pH and ionic strength, are plotted against $\log c(\text{Cys})$. The linear response with a slope 58 mV $\{\text{p}(\text{Cys})\}^{-1}$ has been obtained in the concentration range from 3×10^{-3} to under 10^{-5} M. The change in potential in the tested concentration range of Cys (also designated as RSH) indicates RSAg coating formation at the exposed membrane surface. On the basis of potentiometric experiments, the solubility product of RSAg precipitate has been determined. The calculated mean value was $\text{p}\bar{K}_{\text{sp}}(\text{RSAg}) = 18.66 \pm 0.08$.

INTRODUCTION

Many scientific papers have been published in the field of analysis of drug-type substances with potentiometric membrane electrodes. Cysteine (Cys) is the amino acid whose side chain contains the thiol group. Several potentiometric methods, mostly based on the reaction with the thiol group, have been proposed for the determination of cysteine. In the proposed potentiometric methods, different electrodes have been applied: Mercury(2+),¹ Ag_2S ,² Ag,³ Sn-SnO.⁴ Also, a dichromate-selective liquid-membrane electrode has been used for end-point indication in the titration of cysteine with $\text{Cr}_2\text{O}_7^{2-}$ solution.⁵

A number of catalytic and kinetic methods for the determination of cysteine have also been reported.⁶⁻⁸ For practical measurements, the use of commercially available electrodes: silver sulphide (direct potentiometry) and $\text{AgX}/\text{Ag}_2\text{S}$ (potentiometric titration) has been recommended.⁹

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Gruen and Harrap¹⁰ used a solid-state silver sulphide electrode and silver nitrate for the potentiometric titration of L-cysteine in an aqueous medium at pH 7. They noted a slow response of the electrode in the vicinity of the end-point. When points in the immediate vicinity of the end-point were ignored, the reliable end-point was determined as the intersection of the baseline with the curve of the potential *vs.* excess silver ions. Since silver ions may form complexes with thiols, leading to high results, Selig¹¹ used mercuric perchlorate as titrant. Titration of cysteine with this titrant was also slow.

There are some doubts about the specificity and stoichiometry of the reaction between cysteine and silver ions. They are mainly based on the variable stoichiometry observed between cysteine and silver ions before and after the equivalence point.^{12,13}

Using a Ag₂S membrane electrode, cysteine can be measured by the direct potentiometric method in strong basic aqueous media (0.1 M NaOH), where the stable complex between silver ions and cysteine is formed.² As has been observed,¹² cysteine is oxidized relatively rapidly in alkaline solutions.

In view of the above, our aim was to develop a direct potentiometric method for determination of cysteine in acid or moderately acid media. In this paper, work is reported wherein cysteine in buffered aqueous solution (pH 5) has been measured by the direct potentiometric method using a commercial iodide-selective electrode. The investigated electrode showed a Nernstian slope for cysteine down to near 10⁻⁶ M concentration.

EXPERIMENTAL

Apparatus

Potentiometric measurements were carried out with an Orion Autochemistry System using a double-walled, thermostated reaction vessel, maintained at 298 ± 0.5 K. Cell potential was measured with an Orion 94-53 iodide ISE *versus* an Orion 90-02 double-junction reference Ag/AgCl electrode (DJRE), with 10% potassium nitrate solution as the outer filling solution. The pH values were checked with an Orion 91-02 glass electrode and Microprocessor Ionalyser (Orion 901). In one part of the experiment, a »home made« iodide electrode was tested. The iodide-selective electrode with a membrane consisting of Ag₂S *w* = 25%, AgI *w* = 25% and PTFE *w* = 50% was prepared as already described.¹⁴ This electrode showed approximately the same slope, detection limit and potential-time response, but the apparent standard potential was different. The solution was stirred by means of a PTFE-coated magnetic stirring bar. Both the stirring rate and electrode distance were kept constant throughout all measurements. The selective electrodes were dry-stored between measurements and overnight.

Reagents

All chemicals were of analytical-reagent grade and were used without further purification. All solutions were prepared with water that was doubly distilled in glass.

Silver nitrate solutions. The standard silver nitrate solution was 0.1 M. The concentration of solutions was determined by potentiometric titration with 0.1 M sodium chloride using an Orion silver/sulphide - DJRE electrode pair.

Cysteine solutions. Aqueous solutions (0.1 M) of pure L-cysteine (Merck) were prepared weekly and stored in a refrigerator.

Buffer solution. Acetate buffer (pH 5) was made by diluting glacial acetic acid (60 cm³) containing sodium acetate (16.3 g) to 1 dm³.

Measurements Procedure

The potential response of the indicator electrode to cysteine was made by standard addition. Before addition of cysteine, 40.0 cm³ 0.25 M KNO₃ and 10.0 cm³ acetate buffer were added, as a background solution, into the thermostated reaction vessel.

The potential–time response of the electrode was measured in a regular analytical setup. The background solution, 0.2 M KNO₃ with acetate buffer was stirred and monitored under successive additions of known quantities of cysteine. The time response was recorded by redirecting normal Orion 960 printing to an external PC.

RESULTS AND DISCUSSION

Response of the electrode with the membrane consisting of pure silver halides AgX or AgX mixed with Ag₂S to anions Y^{z-} which form sparingly soluble salts Ag₂Y with Ag⁺, has been discussed by Morf *et al.*¹⁵ Determination of anions Y^{z-} is possible only if there is an equilibrium between the boundary of the sample solution and Ag₂Y deposited on the membrane. Studying the silver response of silver iodide based ion-selective electrode, Harsányi *et al.*¹⁶ found that the processes of adsorption and desorption take place at the surface of the membrane.

In our experiments, the iodide electrode with (Ag₂S + AgI) membrane in connection with a suitable reference electrode responds primarily to the activity of the silver ion at the sample solution – electrode membrane interface, according to the Nernstian equation

$$E = E' + \frac{RT}{F} \ln a_{\text{Ag}^+} \quad (1)$$

In the absence of complexing agents, this activity is described mainly as the sum of: (i) the silver ion activity in the sample solution, and (ii) the silver ion activity due to the dissolution of the electrode membrane.

All ions that form sparingly soluble silver salts or stable silver complexes may be considered as species taking part in the forming of the cell potential. When the utilized electrode is immersed in a solution containing cysteine (designated also as RSH), the following reaction may be expected to occur at the phase boundary



where (aq) denotes a dissolved undissociated molecule in equilibrium with the corresponding solid. The concentration of the undissociated form in solution is said to be approximately 0.1 – 1% of that corresponding to the total solubility of the parent compound in water.¹⁷ Consequently, the activity of the silver ion in the phase boundary can be altered through the first part of reaction (2) or by formation of a thin layer of RAg(s) at the electrode surface.

If the cysteine concentration in the solution is sufficient for reaction (2) to proceed to the right, the RAg coating may be expected to form mostly at AgI sites of the membrane, because AgI will be much more soluble than Ag₂S. As long as a precipitate RAg is present, whether as a uniform coating or an incomplete coating, the activity of the silver ions is determined by the concentration (activity) of cysteine in solution and the solubility of RAg (K_{sp}).

Thus, the potential of the electrode is given by the simplified equation at 298 K

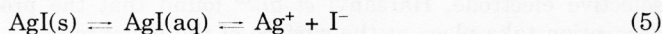
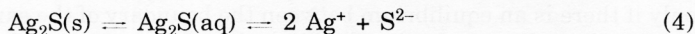
$$E = E'' - 0.059 \log [\text{Cys}] \quad (3)$$

where

$$E'' = E' + 0.059 \log K_{\text{sp}}^{\text{RSAg}}$$

Potential-pH Response

The membrane of the electrode used contains both Ag_2S and AgI . When the electrode is immersed in a solution without ions which can react with the membrane material, a change in S^{2-} activity at the surface of the membrane affects the Ag^+ activity and, hence, the electrode potential according to the equilibria



As it has been shown,¹⁸ the potential of the electrode with ($\text{Ag}_2\text{S} + \text{AgI}$) membrane is dependent on the pH of the solution. However, the recorded potential change was decreased when a sufficient concentration of ions which do not go into protonation equilibrium as Eq. (6), but react with silver ions at the phase boundary, was added into the solution.

In this work, the influence of pH on the potential response of the iodide electrode to cysteine has been examined. At constant cysteine concentration, $c(\text{Cys}) = 1 \times 10^{-4}$ M, and constant ionic strength, the potential dependence was measured as a function of pH. The pH was changed from 2 to 12 by mixing 4×10^{-2} M of acetic, boric and phosphoric acids with the necessary volume of 2 M sodium hydroxide. The pH response of the utilized electrode is shown in Figure 1. According to the experimental, the stability of RSAG coating depends on the pH of the solution.

Since the pH range over which the electrode potential is virtually pH-independent is very narrow (pH: 4–5 and 9–11), the electrode response to cysteine should be examined in a buffered solution. The use of a buffer of pH 5 to perform the calibration curve has been chosen for the following reason. In a moderately acid solution, the formation of silver-cysteine precipitate with 1 : 1 stoichiometry has been observed.^{10,13} When the $\text{Ag}_2\text{S}/\text{AgI}$ membrane electrode is immersed in a cysteine solution at this pH, a thin layer of RSAG can be formed on the electrode surface, and after this process the electrode is reversible to the cysteine, as well as to silver ions. However, at higher pH values, hydrolysis of RSAG coating or hydrolysis with dimerization have been postulated.¹⁹ The direction of the potential change after pH 8 in our experiment (Figure 1) may be attributed to this process.

Potential-Time Response

The potential-time response of the electrode was measured in buffered acetic acid solution. Typical results are shown in Figure 2. For the lowest concentration of cysteine (2.0×10^{-6} M), the cell potential showed a monotonic and asymptotic ap-

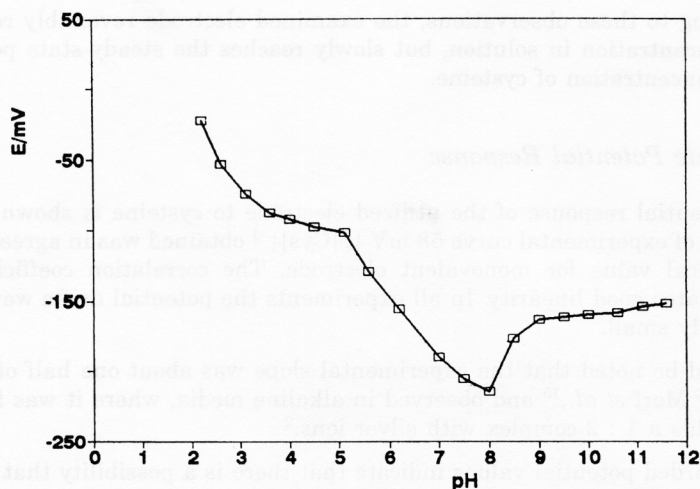


Figure 1. The potential-pH response for the cell with iodide-ISE in solution with cysteine. The concentration of cysteine was 1×10^{-4} M.

proximation to the final steady state, and no stable potential was recorded within 10 min. This behaviour at lower concentrations is probably due to the rate of RSAg formation at the exposed surface of ($\text{Ag}_2\text{S} + \text{AgI}$) membrane. When the concentration was 3×10^{-3} M, the steady-state potential of the cell was achieved within 1 min. After addition of silver ions into the reaction solution with cysteine, the cell potential was altered and the steady-state value was reached within the experiment time.

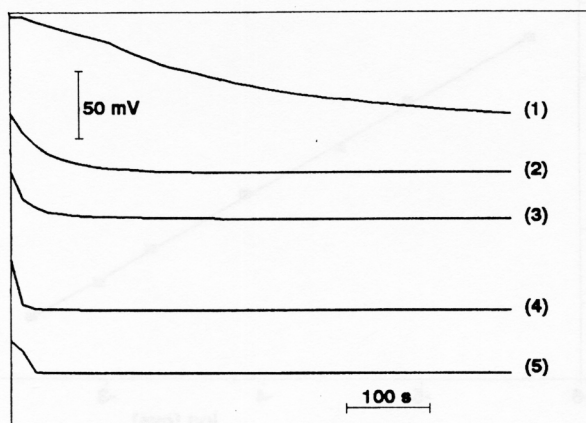


Figure 2. The potential-time response of the cell with iodide-ISE ($\text{pH} = 5$; $\mu = 0.20$ M). Cysteine concentrations are: (1) 2.0×10^{-6} ; (2) 1.2×10^{-5} ; (3) 3.2×10^{-5} ; (4) 5.3×10^{-4} ; (5) 3.0×10^{-3} M.

According to these observations, the examined electrode reversibly responds to cysteine concentration in solution, but slowly reaches the steady-state potential at the lower concentration of cysteine.

Steady-State Potential Response

The potential response of the utilized electrode to cysteine is shown in Figure 3. The slope of experimental curve $58 \text{ mV } \{p[\text{Cys}]\}^{-1}$ obtained was in agreement with the theoretical value for monovalent electrode. The correlation coefficient (r) of 0.9985 indicates good linearity. In all experiments the potential drifts were parallel and relatively small.

It should be noted that the experimental slope was about one half of the slope predicted by Morf *et al.*,¹⁵ and observed in alkaline media, where it was found that cysteine yields a 1 : 2 complex with silver ions.²

The recorded potential values indicate that there is a possibility that the cysteine, in reaction with the membrane, forms RSAg at the exposed membrane surface. However, the adsorbed thin layer of RSAg is presumably unstable in solution without cysteine, and the membrane responds to the I^- ion without polishing, after experiments with cysteine. In addition, the stoichiometry of this coating is in good agreement with those calculated from the average ratio between silver and cysteine at the equivalence point in potentiometric titration experiments. For practical analytical measurements, the estimated limit of cysteine detection is 10^{-5} M because, under this concentration, the steady-state potential of the cell may be achieved only after prolonged time.

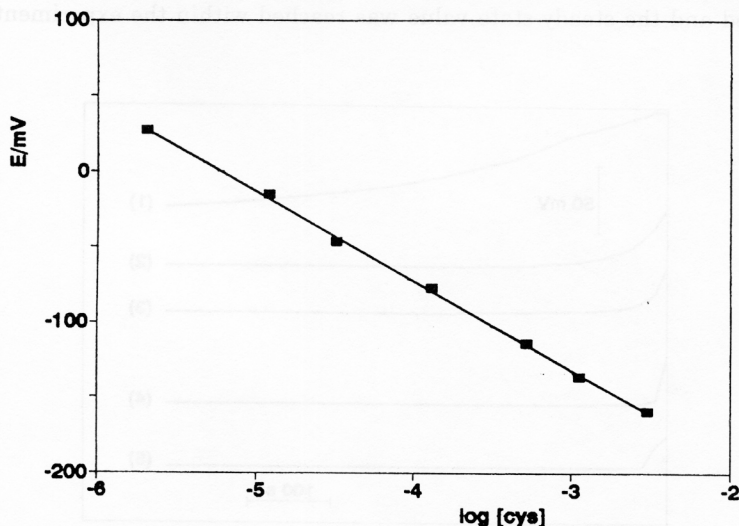


Figure 3. Response of the cell with iodide-ISE to the cysteine concentration in buffered solution ($\text{pH} = 5$) at constant ionic strength ($\mu = 0.20 \text{ M}$).

Solubility Product of RSAg

The potential response of the utilized electrode to silver ions in buffered nitrate solution is shown in Figure 4. The response measurements for Ag^+ were made by serial dilution. Buffered 0.2 M KNO_3 was used as a diluent solution.

In the same experimental solution, when the concentration of silver ions was 1.7×10^{-5} M, the known quantities of cysteine were added, and the sparingly soluble silver salt (RSAg) was formed in the reaction vessel. If an excess of RSAg material sits at the bottom of a saturated solution, the silver ion activity in solution is determined by the solubility product of the precipitate and the concentration of cysteine. Also, any irreversible adsorption of silver ions on the vessel surface will be compensated with this system, and a stable electrode potential can be expected.

Starting from theoretical consideration, during the experiment with silver ion or cysteine in solution, the electrode potential is determined by an activity of Ag^+ ion on the electrode surface. The experimental potential values are plotted in Figure 4. against the experimental activity of silver ion (filled squares) and calculated values (empty squares). For the experimental concentration range of silver ion, the silver activities have been calculated from molar concentrations and the activity coefficient at the experimental ionic strength. The calculation of pAg for the experimental concentration range of cysteine is based on the equation

$$E = E' - S \cdot \text{pAg} \quad (7)$$

where E , E' and S denote the cell potential after addition of cysteine, a conditional standard cell potential at the experimental slope, respectively.

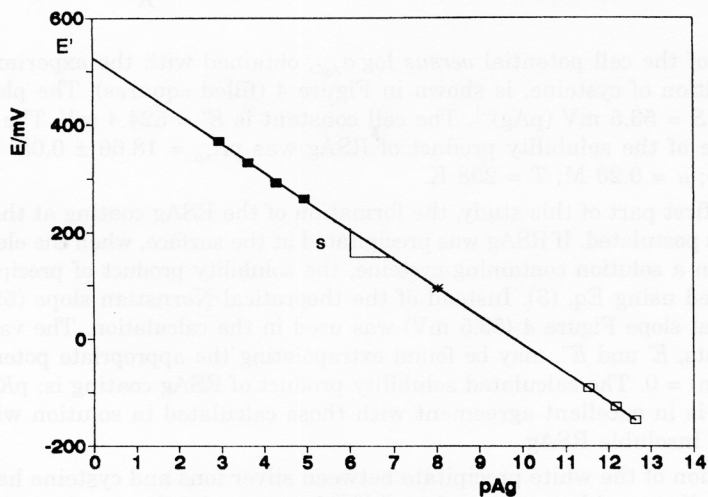


Figure 4. Relationship between the experimental potential values of the electrode and the experimental activity of silver ion (filled squares) and the calculated values (empty squares). The calculation of pAg in cysteine solution is based on Equation (7). See text. pAg calculated from the solubility product of silver iodide is marked with an asterisk.

When solution contains insoluble RSAg and cysteine in excess, the next equilibria exist in solution



and the cell potential can be given by

$$E = E' + S \log \left(\left\{ K_{\text{sp}}(\text{RSAg}) \frac{K + [\text{H}^+]}{K} \right\} / c \right) \quad (10)$$

where K_{sp} , K and c denote the solubility product, the dissociation constant of cysteine, $K = [\text{RS}^-][\text{H}^+] / [\text{RSH}]$, and the total or analytical concentration of cysteine in solution, respectively. The value of the dissociation constant of cysteine, $K = 3.16 \times 10^{-9}$, was taken from literature.²⁰

We have no information concerning the structure of precipitate composition, but for the calculation of the solubility product of RSAg we accept that the precipitate formed in acetic buffered solution contains only silver and cysteine with 1 : 1 stoichiometry. Solubility of the precipitate containing an anion with basic properties (RS^-) is dependent upon pH. The solubility product of RSAg, when pH is fixed and known, can be calculated using the next equation.

$$\text{p}K_{\text{sp}}(\text{RSAg}) = (E' - E) / S + \text{p}c + \log \frac{K + [\text{H}^+]}{K} \quad (11)$$

A plot of the cell potential *versus* $\log a_{\text{Ag}^+}$, obtained with the experimental data before addition of cysteine, is shown in Figure 4 (filled squares). The plot is linear with slope $S = 53.5 \text{ mV (pAg)}^{-1}$. The cell constant is $E' = 524.4 \text{ mV}$. The calculated mean value of the solubility product of RSAg was $\text{p}K_{\text{sp}} = 18.66 \pm 0.08$. Conditions were: pH 5; $\mu = 0.20 \text{ M}$; $T = 298 \text{ K}$.

In the first part of this study, the formation of the RSAg coating at the electrode surface was postulated. If RSAg was precipitated at the surface, when the electrode was immersed in a solution containing cysteine, the solubility product of precipitate could be calculated using Eq. (3). Instead of the theoretical Nernstian slope (59 mV), the experimental slope Figure 4 (53.5 mV) was used in the calculation. The values of the cell constants, E' and E'' , may be found extrapolating the appropriate potential $p(\text{ion})$ line to $p(\text{ion}) = 0$. The calculated solubility product of RSAg coating is: $\text{p}K_{\text{sp}} = 19.08$. This value is in excellent agreement with those calculated in solution with a large quantity of insoluble RSAg.

Formation of the white precipitate between silver ions and cysteine has been observed and discussed by many authors^{10,12,19} but the solubility product of RSAg has not been determined.

All these observations indicate that the utilized electrode potential satisfactorily follows cysteine concentration through the equilibria of RSAg precipitate forming at the membrane surface.

In conclusion, the proposed potentiometric method is a simple and rapid way of determining cysteine in the concentration range from 3×10^{-3} to under 10^{-5} M. The »native« behaviour of the applied commercial iodide-selective electrode, including its life time, is not being changed by repeating runs in the described experiment. Flow-injection determination of cysteine using a flow-through electrode with AgI-based membrane hydrophobized by PTFE is under further investigations.

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SAŽETAK

Izravno potenciometrijsko određivanje cisteina elektrodom selektivnom za jodidne ione

Marija Bralić i Njedomir Radić

Opisano je potenciometrijsko određivanje cisteina (Cys) u vodenoj otopini jodid-selektivnom elektrodom s membranom ($\text{Ag}_2\text{S} + \text{AgI}$). Ravnotežni potencijali zabilježeni u postupku razrijeđenja reakcijske otopine ili nakon dodatka standardne otopine pri stalnoj ionskoj jakosti i vrijednosti pH prikazani su u ovisnosti o $\log c(\text{Cys})$. Utvrđen je linearan odziv elektrode u području koncentracija od 3×10^{-3} do ispod 10^{-5} M s nagibom 58 mV $\{p(\text{Cys})\}^{-1}$. Promjena potencijala u ispitivanom koncentracijskom području cisteina RSH sugerira formiranje sloja RSAg na izloženoj površini membrane. Na temelju potenciometrijskih mjerenja određen je produkt topljivosti RSAg čija srednja vrijednost jest: $p\bar{K}_{\text{sp}}(\text{RSAg}) = 18.66 \pm 0.08$.