Flow Injection Analysis with Two Parallel Detectors:
Potentiometric and Spectrophotometric Determination of Thiols and Ascorbic Acid in Mixture

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Abstract: One flow injection analysis (FIA) procedure for the successive potentiometric and spectrophotometric determination of ascorbic acid (AA) and thiols (cysteine, cys; N-acetyl-L-cysteine, NAC; penicillamine, pen; glutathione, glu) in mixtures has been described. The potentiometric FIA signal is based on the reaction of formation the sparingly soluble salts, RSAg, between thiols (designated also as RSH) and Ag⁺ ions. Ascorbic acid has had no influence on potentiometric signal at any experimental concentration. The spectrophotometric FIA signal is based on redox-reaction of chosen compounds with 1,10-phenanthroline-iron(III) complex. For potentiometric determination of thiols and spectrophotometric determination of selected compounds a rectilinear calibration graphs were obtained.

Keywords: Flow-injection analysis, Thiols, Ascorbic acid, Potentiometry, Spectrophotometry

1. Introduction

Thiols, RSH, (N-acetyl-L-cysteine, NAC; cysteine, cys; penicillamine, pen; glutathione, glu), and ascorbic acid (AA) are very important biomolecules. They are well known as a cells premier antioxidant and detoxicant agent against “free radicals”. Thiols are also used as aid to the elimination
Determination of Thiols and Ascorbic Acid

of excess heavy metal ions. AA and RSH are essential compounds for metabolism of cells and indispensable for prevent or healing some diseases. Many pharmaceutical and cosmetic preparations contain these compounds and because that exist a need for fast, selective, precise, sensitive and low cost methods for their determination.

Several analytical techniques, for determination RSH or AA in pharmaceuticals, food or biological tissue, have been proposed: capillary electrophoresis with UV\(^1,2\) or with amperometric\(^3\) detector, liquid chromatography,\(^4,6\) fluorimetry,\(^7\) spectrofluorimetry,\(^8\) voltammetry,\(^9,10\) amperometry,\(^11,12\) potentiometry,\(^13,14\) spectrophotometry.\(^15,17\) Also, many potentiometric\(^18,20\) or spectrophotometric flow systems have been developed for determination of ascorbic acid or thiols in pharmaceuticals or in biological tissue. The cited potentiometric FIA methods\(^18-20\) were carried out in acidic medium, and many spectrophotometric determination of thiols or AA were carried out at pH in range from 4 to 6.\(^21,25\) In available literature, a few flow injection methods for the individual and simultaneous determination of AA or RSH have been found.\(^21,26\) Teshima et al.\(^21\) were proposed simultaneous flow injection spectrophotometric determination of AA and cys in mixture but small quantity of copper(II) as a catalyst had to be used.

Detectors based on electrochemical and optical detection coupled with flow injection system have been very popular for many applications.\(^27\) Potentiometry is the most widely used electroanalytical technique because of its simplicity, versatility, and low cost. UV-Vis spectrophotometry is also widely used in analytical laboratory as the detection technique for the flow injection analysis.

The goal of this work was to develop simple method for the determination of ascorbic acid and thiols in mixture, in acidic media, in the absence of catalytic effect and without previously separation. Based on the experimental results the equations for calibration lines for RSH compounds and (RSH + AA) mixtures have been calculated. The FIA system with two parallel placed detectors provides the successive potentiometric and spectrophotometric determination of these compounds in mixture.

2. Materials and Methods

2.1. Reagents and chemicals

All reagents were of analytical-reagent grade, and all the solutions were made up by doubly distilled water. For adjusting pH of reaction solutions (pH 2.8) acetate buffer was used. The stock solutions of thiols, \(1.0\times10^{-2} \text{ M: cis (Merck), NAC (Merck), pen (Fluka), glu (Sigma-Aldrich)}\), and AA (Merck), were prepared by dissolving an appropriate amount of these compounds in acetate buffer (pH 2.8). The stock solutions of iron (III), \(1.0\times10^{-3} \text{ M was prepared by dissolving appropriate amount of iron (III) ammonium sulfate dodecahydrate (Merck) in 250.0 mL 0.5 M sulfuric acid (Kemika) and standardized with 0.01 M solution of NaCl (Merck)}\). A \(2.0\times10^{-4} \text{ M 1,10-phenanthroline, phen (Kemika)}\) was prepared by dissolving an appropriate amount of reagent in 1.0×10^{-3} M sulfuric acid (Kemika). The stock solution of silver (I), \(1.0\times10^{-3} \text{ M, was prepared by dissolving an appropriate amount of silver nitrate (Kemika) in acetate buffer and standardized with 1.0\times10^{-1} \text{ M solution of NaCl (Merck)}\). Working solutions of RSH compounds, AA and mixture Fe(III)/phen \(\{c(\text{Fe}^{3+}) = 1.0\times10^{-2} \text{ M, c(phen) = 4.0}\times10^{-2} \text{ M}\}\) were prepared daily by diluting the stock solutions with acetate buffer, pH 2.8.

2.2 Apparatus

The manifold for flow injection analysis shown in Figure 1 was used. An ultraviolet-visible, UV-Vis, spectrophotometer (UV-1601 SHIMADZU, Kyoto, Japan) with flow through cell (80 µL) and potentiometer, MA 5740 ISKRA, with iodide ion selective electrode (IISE) and double junction
reference electrode (DJRE) combined with laboratory made flow cell “fall” type, were used as
detectors. An injection valve, 5020 RHEODINE, was used to inject an aliquot of sample solutions into
the flowing stream. One 8-channel peristaltic pump, IPC ISMATEC, was used to pump carrier and
reagents solutions. All components of FIA system were interconnected by silicone tubing of 1.5 mm
diameter. Two personal computers, which ensured continuous recording, storing and processing of
data, were used.

Figure 1. Schematic diagram of FIA system with potentiometric and spectrophotometric detector.
P - the flow thought potentiometric cell, “fall” type, with iodide ion selective electrode (IISE) and
double junction reference electrode (DJRE); S - spectrophotometer with flow cell (80 µL); V - two channels valve for
direction of flow stream to detectors; W - waste.

2.3 Procedure

In the flow system as shown in Figure 1, carrier solution (acetate buffer, pH 2.8), sample
solution and reagents were pumped at a flow rate of 6.0 mL/min. Reagents were: Ag⁺, 1.0×10⁻⁶ M, and
mixture Fe(III)-phen. An aliquot of sample solution (1.0 mL) which contains single compound or
mixture of two compounds (RSH + AA) was injected in the carrier stream and by using two channels
valve, V, (Figure 1) was directed to potentiometric or spectrophotometric detector. For potentiometric
measurements, the reaction solution was injected into the flow through cell, and then passed over the
sensor surface area like a waterfall. The reference electrode was immersed in reaction solution. For
spectrophotometric measurements the absorbance of iron(II)-phen complex, produced in reaction coil
(300 cm in length), was continuously monitored at 510 nm. The procedure for preparation the
potentiometric calibration graphs was as follows. Three sample aliquots (1.0 mL) were injected for
two times in the carrier stream sequentially. The first three aliquots contained one of the tested RSH
compounds. The next aliquots contained (RSH + AA)-mixture. The same procedure was repeated for
five different concentrations in the selected concentration range. On the same way the calibration
graphs for spectrophotometric measurements have been prepared. Since both the sample and reagent
solutions were prepared in acetate buffer, pH 2.8, the pH value of reaction solution in flow system remained constant. All measurements were carried out at room temperature.

2.4 Optimization of the flow injection analysis system

Flow injection system, which was used in these measurements, according to special demands of potentiometric and spectrophotometric detectors was optimized. The chosen experimental parameters of FIA system were enabled successive measurement with two detectors. The flow rate, 6.0 mL/min, and sampling volume, 1.0 mL, were a compromise between dispersion, diluting, the sensor response speed and measurement rate. The picked out flow rate allows reproducible rate of delivering the sample zone to the sensing surface of electrode or to the flow spectrophotometric cell. According to the kinetic of the occurring indicator reaction for spectrophotometric determination (forming of Fe(II)-phen complex), the reaction coil 300 cm in length was used. The flow spectrophotometric cell, 80.0 µL volume, and the special features of flow potentiometric cell (design, active volume, the place in FIA system) combined with chosen flow rate and sampling volume, allowed reproducible the peak height, the narrow peak and stable base line. In order to reduce the dissociation of the –SH group and allows optimal condition for signal forming reaction pH 2.8 was chosen. Reagents concentrations of 1.0×10⁻⁶ M for silver nitrate and 1.0 x 10⁻² M for Fe(III)-phen complex were selected as the compromise between the sampling rate, linear dynamic range and lower quantification limit.

3. Results and Discussion

The formal reduction potential for Fe(III)-phen complex, RSH compounds and AA have been calculated for the large pH range. In acidic solution (pH: 0-5) the calculated reduction potential for Fe(III)-phen/Fe(II)-phen couple was much more positive then for Fe(III)/Fe(II) couple. When pH of reaction solution decreases the formal reduction potentials of RSH and AA become more positive and reduction activity of these compounds decrease. With thermodynamic approach one can concluded that the formal reduction potential for Fe(III)-phen/Fe(II)-phen ($E^0_0 \approx +1.2$ V; pH 2.8) is enough large to provide the oxidation all tested compounds in spectrophotometric experiment. Also the standard reduction potential for Ag⁺/Ag(s) couple ($E^0 = 0.799$ V) is enough large to provide the oxidation the tested compounds in the potentiometric stream.

When a sample solution is directed to potentiometric detector at a concentration sufficiently high to cause precipitation of RSAg or redox process, the following reactions at and/or near the membrane surface may be expected to occur:

$$\text{AgI}\,\rightarrow\,\text{Ag}^+ + \text{I}^- \quad (1)$$
$$\text{RSH}\,\rightarrow\,\text{RS}^- + \text{H}^+ \quad (2)$$
$$\text{RS}^- + \text{Ag}^+ \rightarrow \text{RSAg}\,(s) \quad (3)$$

or

$$2\text{RSH} + 2\text{Ag}^+ + \text{H}_2\text{O} \rightarrow \text{RSSR} + 2\text{Ag}(s) + 2\text{H}^+ \quad (4)$$
$$\text{H}_2\text{A} + 2\text{Ag}^+ \rightarrow \text{DA} + 2\text{Ag}(s) + 2\text{H}^+ \quad (5)$$

where H₂A is reduced form of AA and DA is dehydrogenised AA.

Figure 2 shows the typical response of Ag⁺ sensing sensor to cys obtained by FIA. The similar response was obtained for other RSH compounds. Comparing the peak height obtained for cys and the peak height obtained for mixture (cys+AA) solution, with the same concentration of cys, it is evident that AA don’t influence on potentiometric detector response, at chosen experiment condition. According the experimental results we concluded that the both proposed reactions (equations 4 and 5) dont occur at the surface of sensor or in the streaming solution. The change in potential of the flow
potentiometric cell, under optimized conditions, follows the change of RSH concentration in the injected sample. The obtained peaks are sharp and reproducible. The signal returns to the base line within short time.

Figure 2. The change of the potential for cys (a-e) and cys in mixture with AA (a1-e1). c(cys)/M: a. 1.0×10⁻⁵, b. 3.0×10⁻⁵, c. 1.0×10⁻⁴, d. 3.0×10⁻⁴, e. 1.0×10⁻³. The same concentrations of cys were in mixtures. c(AA)/M: a1. 8.0×10⁻⁶ M, b1. 2.0×10⁻⁵, c1. 4.0×10⁻⁵, d1. 6.0×10⁻⁵, e1. 8.0×10⁻⁵. For experimental conditions and procedure see text.

A linear dependence between the peak height and the logarithm of concentration of compounds containing sulfur has been analyzed previously. Based on the experimental results collected in this work the equation for a calibration curve with good linearity ($R^2 = 0.9926$) has been calculated:

$$\Delta E = 361.4 - 67.1 \cdot (-\log c_{\text{cys}}/\text{M})$$  \hspace{1cm} (6)

For spectrophotometric determination of RSH compound and ascorbic acid in mixture the redox reaction of RSH compound and AA with Fe(III)-phen complex can be written as follows:

$$2 \text{RSH} + 2 \text{Fe(phen)}_{3}^{3+} \rightarrow \text{RSSR} + 2 \text{Fe(phen)}_{3}^{2+} + 2 \text{H}^+$$  \hspace{1cm} (7)

$$\text{H}_{2}\text{A} + \text{Fe(phen)}_{3}^{3+} \rightarrow \text{DA} + \text{Fe(phen)}_{3}^{2+} + 2 \text{H}^+$$  \hspace{1cm} (8)

Figure 3 shows typical spectrophotometric FIA signals that were obtained when cys, AA and mixture (cys + AA) have been injected. The similar signals were obtained when determination of NAC, AA and their mixture were investigated.
Figure 3. The change of absorbance for cys (a1-a5), AA (b1-b5) and their mixture (c1-c5). c(cys)/M: a1. 1.0×10⁻⁴, a2. 3.0×10⁻⁴, a3. 6.0×10⁻⁴, a4. 8.0×10⁻⁴, a5. 1.0×10⁻³; c(AA)/M: b1. 8.0×10⁻⁶, b2. 2.0×10⁻⁵, b3. 4.0×10⁻⁵, b4. 6.0×10⁻⁵, b5. 8.0×10⁻⁵. The concentrations of cys and AA in mixtures (c) were the same as above was written (a,b) and in the same direction.

The calculated calibration curves showed good linearity for a series of standards.

For cys ($R^2 = 0.9712$): $A = 0.6433 \, c(\text{cys})/\text{mM} - 0.0973$ \hspace{1cm} (9)

For AA ($R^2 = 0.9950$): $A = 0.0139 \, c(\text{AA})/\mu\text{M} - 0.0611$ \hspace{1cm} (10)

When signals recorded after injection of mixtures (cys + AA) had been reduced for cys signals the next equation, practically the same as equation 10, was calculated.

$$A = 0.0139 \, c(\text{AA})/\mu\text{M} - 0.0622$$ \hspace{1cm} (11)

All tested thiols injected alone or in the mixture with AA can be determined using this FIA system and potentiometric detector. When mixture of AA and cys or AA and NAC is injected and directed to the spectrophotometric detector both species take part in forming the recorded signal. So, AA can be determined by using spectrophotometric detector if concentration of thiol has been determined in the previous potentiometric experiment. In addition, when samples with AA and glu or AA and pen were injected in FIA system and directed to spectrophotometric cell only AA was contributed to recorded absorbance value. Glu and pen can be determined only by using potentiometric detector.

3.1 Optimization of the method

Concentrations of Ag (I), Fe(III) and phen in mixture and pH are variables that have influence on the response of IIE and spectrophotometric detector, as well as on the range and linearity of determination. Because of that they have been optimized.
The influence of Ag\textsuperscript{+} in concentration range from $1.0 \times 10^{-4}$ to $1.0 \times 10^{-6}$ M was studied for the determination of different concentration of cys: $1.0 \times 10^{-3}$ M, $1.0 \times 10^{-4}$ M, $1.0 \times 10^{-5}$ M. The sensitivity of method is better at $c(\text{Ag}^{+}) = 1.0 \times 10^{-6}$ M, thus that concentration was selected as the optimum value.

Effect of pH on spectrophotometric and potentiometric determination of thiols was studied performing determination trials of $1.0 \times 10^{-4}$ M cys in pH range between 1 and 5. The same pH range is tested for spectrophotometric determination of AA. The obtained absorbance and potentials increases with increasing pH of reaction solution. The solutions of RSH and AA are more stable at lowest pH. Also, the reproducibility of measurement is better at lowest pH. Taking this in consideration, as a compromise, the determination of chosen compound were carried out at pH = 2.8.

The influence of Fe(III) concentration was studied in the range from $2.0 \times 10^{-3}$ to $3.0 \times 10^{-2}$ at determination of cys ($5.0 \times 10^{-4}$ M) and AA ($4.0 \times 10^{-5}$ M). The mole ratio Fe(III)/phen was 0.25 and therefore the concentration range of phen in mixture solutions was from $8.0 \times 10^{-3}$ to $1.2 \times 10^{-1}$ M. The results show that the largest absorbance were obtained at $1.0 \times 10^{-2}$ M Fe(III), (Figure 4). That concentration was selected as the optimum value.

![Figure 4](image_url)

**Figure 4.** Effect of Fe\textsuperscript{3+} ions concentration on the absorbance. $c(\text{cys}) = 5.0 \times 10^{-4}$ M and $c(\text{AA}) = 4.0 \times 10^{-5}$ M; $c(\text{Fe}^{3+})/c(\text{phen}) = 0.25$

### 3.2 Interference Studies

The influence of concomitant species on the determination of RSH and AA was examined by applying the proposed method to a determination of cys and AA. The tested concentrations for cys and AA were $5.0 \times 10^{-4}$ M and $4.0 \times 10^{-5}$ M, respectively. The tolerance limit was taken as the amount of added species that caused an error less than ±3%. The results are given in Table 1 and Table 2.
Table 1. Tolerance limit for interferences for the spectrophotometric determination of 5.0×10^{-4} M cys and 4.0×10^{-5} M AA.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Tolerance limit ratio (mole_{substance}/mole_{cys})</th>
<th>Tolerance limit ratio (mole_{substance}/mole_{AA})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose, glucose, fructose, lactose</td>
<td>&gt;300^a</td>
<td>&gt;300^a</td>
</tr>
<tr>
<td>Ca^{2+}, Mg^{2+}, Na^{+}, SO_{4}^{2-}, NO_{3}^{-}</td>
<td>&gt;300^a</td>
<td>&gt;300^a</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Citric acid, tartaric acid, oxalic acid</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>HPO_{4}^{2-}, PO_{4}^{3-}</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>SO_{3}^{2-}</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

^aMaximum ratio tested

Table 2. Tolerance limit for interferences for the potentiometric determination of 5.0×10^{-4} M cys.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Tolerance limit ratio (mole_{substance}/mole_{cys})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose, glucose, fructose, lactose</td>
<td>&gt;300^a</td>
</tr>
<tr>
<td>Ca^{2+}, Mg^{2+}, Na^{+}, SO_{4}^{2-}, NO_{3}^{-}</td>
<td>&gt;300^a</td>
</tr>
<tr>
<td>Citric acid, tartaric acid, oxalic acid</td>
<td>55</td>
</tr>
<tr>
<td>HPO_{4}^{2-}, PO_{4}^{3-}</td>
<td>30</td>
</tr>
<tr>
<td>SO_{3}^{2-}</td>
<td>15</td>
</tr>
</tbody>
</table>

^aMaximum ratio tested

3.3 Analytical Applications

When the signal heights recorded with potentiometric detector are plotted versus negative logarithm of the RSH concentrations, p(RSH), the straight line is obtained by using the method of linear regression. Calibration graphs with good linearity for cys and NAC in the concentration range from 1.0×10^{-5} to 1.0×10^{-3} M and for pen in the range from 3.0×10^{-5} to 3.0×10^{-3} M were obtained. The calculated equations for calibration lines with linear regression coefficients (R^2) for RSH compounds and (RSH + AA) mixtures are presented in Tables 3 and 4. For spectrophotometric determination of selected compounds a rectilinear calibration graphs are obtained: i) for AA in the range from 8.0×10^{-6} to 8.0×10^{-5} M, ii) for NAC in two ranges (1.0×10^{-4} - 1.0×10^{-3} M; 1.6×10^{-5} - 1.6×10^{-4} M) and iii) for cys from 8.0×10^{-4} to 8.0×10^{-3} M (Table 5).

Table 3. Potentiometric detector and RSH standards injected. The calculated equations for calibration lines and linear regression coefficients, R^2.

<table>
<thead>
<tr>
<th>Thiol</th>
<th>Equation</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>cys</td>
<td>ΔE = 360.3 + 67.1 log c</td>
<td>0.993</td>
</tr>
<tr>
<td>NAC</td>
<td>ΔE = 347.0 + 65.1 log c</td>
<td>0.998</td>
</tr>
<tr>
<td>pen</td>
<td>ΔE = 385.3 + 81.1 log c</td>
<td>0.999</td>
</tr>
<tr>
<td>glu</td>
<td>ΔE = 236.0 + 42.3 log c</td>
<td>0.948</td>
</tr>
<tr>
<td></td>
<td>ΔE = 304.9 + 57.5 log c</td>
<td>0.994*</td>
</tr>
</tbody>
</table>

^*Values calculated based on three smaller concentrations.
Table 4. Potentiometric detector and (RSH + AA) standards injected. The calculated equations for calibration lines and linear regression coefficients, $R^2$.

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>cys+AA</td>
<td>$\Delta E = 360.6 + 66.9 \log c$</td>
<td>0.993</td>
</tr>
<tr>
<td>NAC+AA</td>
<td>$\Delta E = 348.1+65.4 \log c$</td>
<td>0.998</td>
</tr>
<tr>
<td>pen+AA</td>
<td>$\Delta E = 385.7 + 81.3 \log c$</td>
<td>0.999</td>
</tr>
<tr>
<td>glu+AA</td>
<td>$\Delta E = 307.9 + 57.9 \log c$</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>$*\Delta E = 307.9 + 57.9 \log c$</td>
<td>0.990*</td>
</tr>
</tbody>
</table>

*Values calculated based on three smaller concentrations.

Table 5. Spectrophotometric detector. AA and (RSH + AA) standards injected. The calculated equations for calibration lines and linear regression coefficients, $R^2$. Equations for RSH compounds injected in mixture have been calculated when signals recorded after injection of mixtures (RSH + AA) had been reduced for AA signals.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Single compound</th>
<th>Mixture (RSH + AA)</th>
<th>Single compound</th>
<th>Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>$A = 0.014c-0.061$</td>
<td>0.995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>$A = 0.643c-0.097$</td>
<td>0.971</td>
<td>0.972</td>
<td></td>
</tr>
<tr>
<td>NAC</td>
<td>$A = 1.484c-0.164$</td>
<td>0.977</td>
<td>0.975</td>
<td></td>
</tr>
<tr>
<td>NAD</td>
<td>$A = 0.001c-0.018$</td>
<td>0.993</td>
<td>0.987</td>
<td></td>
</tr>
</tbody>
</table>

*aConcentration range from 8.0 to 80 µM
*bConcentration range from 0.8 to 8.0 mM
*cConcentration range from 0.1 to 1.0 mM
*dConcentration range from 16 to 160 µM.

3.4 The real sample analysis

The method was applied for analysis of commercially available pharmaceutical samples, Tables 6 and 7. Mixture of cys and AA is contained in pharmaceutical preparation HAIRFACTOR. Samples of NAC are spiked with 100 mg AA, and sample of AA is spiked with 100.0 mg cys. Solution of real samples were prepared by dissolving one tablet of pharmaceutical preparation in an adequate volume of acetic buffer, pH = 2.8. Then, it was diluted with the same buffer in a 100.0 mL calibrated flask. Before diluting it was filtered, if necessary.
**Table 6** Results for the determination of NAC, cys and AA in pharmaceutical preparation by using potentiometric and spectrophotometric detector

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount Labelled</th>
<th>Found±SD (n = 5)</th>
<th>Recovery (%)</th>
<th>Spiked with</th>
<th>Found±SD (n=5)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWINLAB® NAC (N-acetyl-L-cysteine)</td>
<td>600.00 mg</td>
<td>592.50±1.73</td>
<td>98.75</td>
<td>AA</td>
<td>100.00 mg</td>
<td>99.21±1.2</td>
</tr>
<tr>
<td>Fluimukan® (N-acetyl-L-cysteine)</td>
<td>500.00 mg</td>
<td>494.75±0.97</td>
<td>98.95</td>
<td>AA</td>
<td>100.00 mg</td>
<td>98.92±1.0</td>
</tr>
<tr>
<td>HAIRFACTORS TWINLAB (L-cysteine; L-ascorbic acid)</td>
<td>167.00 mg cys 500.00 mg AA</td>
<td>165.93±1.48 497.65±1.23</td>
<td>99.36 99.53</td>
<td>---- -----</td>
<td>---- ----</td>
<td>---- ----</td>
</tr>
<tr>
<td>CalciumvitaC® (L-ascorbic acid)</td>
<td>500.00 mg</td>
<td>496.60±1.12</td>
<td>99.32</td>
<td>cys</td>
<td>100.00 mg</td>
<td>99.17±1.7</td>
</tr>
</tbody>
</table>

**Table 7** Results for the determination of NAC, cys in real samples by using potentiometric detector

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount Labelled</th>
<th>Found±SD (n = 5)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWINLAB® NAC (N-acetyl-L-cysteine)</td>
<td>600.00 mg</td>
<td>605.88±1.5</td>
<td>100.98</td>
</tr>
<tr>
<td>Fluimukan® (N-acetyl-L-cysteine)</td>
<td>500.00 mg</td>
<td>505.30±2.9</td>
<td>101.06</td>
</tr>
<tr>
<td>HAIR FACTORS TWINLAB (L-cysteine; L-ascorbic acid)</td>
<td>167.00 mg</td>
<td>165.28±2.3</td>
<td>98.97</td>
</tr>
<tr>
<td>CalciumvitaC® (L-ascorbic acid) spiked with 100 mg cys</td>
<td>100.00 mg</td>
<td>98.53±1.18</td>
<td>98.53</td>
</tr>
</tbody>
</table>
4. Conclusion

The proposed flow injection analysis (FIA) procedure for the successive potentiometric and spectrophotometric determination of AA and thiols (cys, NAC, pen, glu) in mixtures can be applied in analytical laboratory as a simple, fast and economic procedure. Also, good linearity between measured signal (absorbance) and concentration was achieved in the range of AA concentration from $8.0 \times 10^{-6}$ to $8.0 \times 10^{-5}$ M, for NAC in two ranges $1.0 \times 10^{-4}$ to $1.0 \times 10^{-3}$ M and from $1.6 \times 10^{-5}$ to $1.6 \times 10^{-4}$ M and for cys from $8.0 \times 10^{-4}$ to $8.0 \times 10^{-3}$ M. Calibration graphs with good linearity for potentiometric determination of cys and NAC in the concentration range from $1.0 \times 10^{-5}$ to $1.0 \times 10^{-3}$ M and for pen in the range from $3.0 \times 10^{-5}$ to $3.0 \times 10^{-3}$ M were obtained. The applicability of the proposed method was successfully demonstrated by analysis of pharmaceutical preparation.

References


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