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# Chemically prepared silver electrode for determination of N-acetyl-L-cysteine by flow-injection potentiometry

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This paper describes the use of the silver electrode by means of chemical pretreatment of the electrode surface with mercuric(II) chloride solution and potassium iodide solution in flow injection analysis (FIA). The electrode is used as a potentiometric sensor for the indirect determination of NAC in a carrier stream containing iodine. A one-channel flow system that consists of a peristaltic pump, injection valve, a silver wire electrode and a saturated calomel reference electrode (SCE) was used. Some typical FIA parameters such as flow rate, tube length and composition of the carrier stream were varied. The electrode is further characterised by a constant linear response within the concentration range for NAC between $4.0 \times 10^{-6}$ and $1.0 \times 10^{-3}$ M at the slope of $60.6 \pm 1.0$ mV/p(NAC). Some pharmaceutical products containing NAC were also tested. These results can be compared to the results obtained by the direct potentiometric titrations with silver nitrate and are also in good agreement with values declared by pharmaceutical manufacturers.

## 1. Introduction

NAC is an altered form of the amino acid cysteine. It is biologically active and used as a mucolitic agent and as an antidote in paracetamol poisoning [1]. Beside the mycolytic function NAC is a potent antioxidant. Medical studies suggest that NAC may help to prevent some types of cancer [2] and slows the progression of Alzheimer's disease [3]. It can also be used by people with HIV infection to enhance the immune function [4]. Therefore NAC is widely applied in different pharmaceutical products and is determined by several analytical methods. Spectrophotometric [5–9] or chromatographic [10, 11] determination of NAC is commonly used for quantitative analysis. In potentiometry the transformation of the active sulfhydryl group in NAC is used for its titrimetric determination. Commercially available silver electrode with silver nitrate as a titrant [12] and silver or platinum electrode with copper (II) acetate as a titrant [13, 14] was described for the determination of NAC in pharmaceutical preparations. A fluoride ion-selective electrode also enables the detection of NAC due to the reduction of NAC on $Fe^{3+}$ ions in acid fluoride solution [15]. In flow-injection analysis only the tubular AgI based membrane as a potentiometric sensor for NAC is described in the literature [16]. This electrode primary responds to the activity of silver ions. When solutions of NAC where injected in acidic stream of silver ions, NAC is forming sparingly soluble silver salts and NAC can be detected in a concentration range between $1.0 \times 10^{-4}$ and $1.0 \times 10^{-1}$ M at the slope of 62 mV/p(NAC) [17].

In previous investigations the reduction of iodine and detection of iodide formed with a home made iodide ion-selective electrode in FIA was described for the determination of vitamin C, glutathione [18] and cysteine [19]. In the present work the chemical treated Ag wire was used for the first time for the indirect determination of NAC in flow injection analysis. This method was also tested for determination of NAC in different pharmaceutical products.

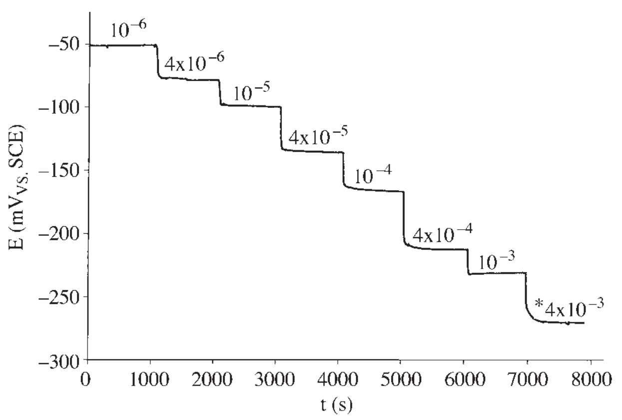
## 2. Investigations, results and discussion

After the silver wire has been immersed into the mercuric(II) chloride solution a homogeneous grey coating is formed on the surface, which turns yellow in the iodide solution. This chemical pretreatment of the silver wire is used to prepare a thin layer surface of AgI, which makes the electrode responsible for iodide ions. The compounds on the surface of the electrode are very important and directly connected with the potentiometric behaviour of the ion-selective electrode [18, 20].

In order to determine NAC, a solution containing iodine was used in a carrier stream. By mixing NAC with iodine, a stoichiometric amount of the iodide is formed due to reaction

$$2\,C_5H_9NO_3S + I_2 \rightarrow C_{10}H_{16}N_2O_6S_2 + 2H^+ + 2I^- \quad (1)$$

where $C_5H_9NO_3S$ denotes NAC and $C_{10}H_{16}N_2O_6S_2$ denotes NAC-disulfide. To confirm this reaction some tests were initially carried out where iodine was mixed with a known concentration of NAC. The solution was then pumped through the system to the electrode at a flow rate of 1.0 ml/min. As shown in Fig. 1, the electrode gives a rapid and reproducible response within the linear range between $1.0 \times 10^{-6}$ and $4.0 \times 10^{-3}$ M at the slope of $64.8 \pm 1.0$ mV/p(NAC), R = 0.998.



* Concentration of $I_2$ is $5 \times 10^{-3}$ mol/l.

Fig. 1: Response of the Ag/AgI wire electrode to the mixture of iodine ($10^{-3}$ M) and NAC (M)

In the next step, some typical FIA parameters, such as composition of the carrier stream, number of streams, flow rate and sample volume were varied in order to enlarge the linear concentration range, to increase the sampling rate and to maximise the accuracy and precision of the flow method.

The active sulfhydryl group in NAC can easily be oxidized in basic, neutral or weak acidic media and therefore the solution of perchloric acid was used as carrier stream. To constant concentration of the perchloric acid (0.1 M) iodine as a reagent solution was added. The concentration of iodine is upwardly limited by the solubility in water (0.29 g $I_2$/l ($H_2O$) [21]. Therefore a fixed concentration of iodine ($10^{-3}$ M) was used in the carrier stream. Potassium iodide at the concentration of $10^{-6}$ M was also added to the carrier stream to prevent the base line drift. Beside the effects to the position and stability of the base line its addition reduces response times and broadens the linear concentration range, while it also provides good reproducibility and stable measurements. The comparison between one and two-channel FIA using different compositions of carrier stream, reagent stream and tube length shows that the one-channel FIA described gives the most reproducible and precise results with a high sampling rate. In flow systems tubular shaped detectors are preferred because they allow undisturbed and continuous flow of the carrier stream through the sensing zone [22]. In our work the iodide ion-selective wire electrode in the FIA gave better results and shorter injections times compared to the tubular electrode due to the smaller active electrode surface of the coated wire.

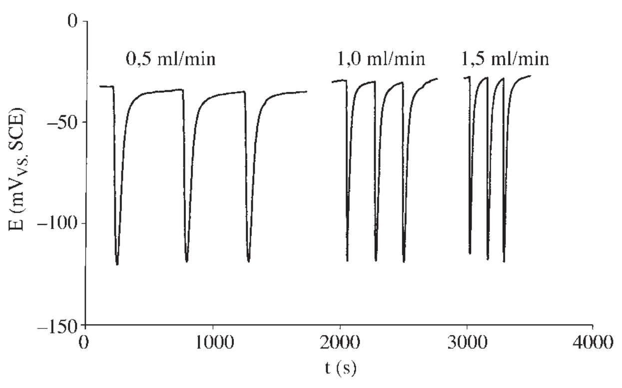


Fig. 2: Response of the electrode to 100 μl of injected NAC ($1.0 \times 10^{-4}$ M) at different flow rates

The flow rate of the carrier stream at the constant sample volume of NAC (100 μl) was varied as shown in Fig. 2. At the flow rate of 0.5 ml/min the electrode gave reproducible response to NAC but long response times are observed and the frequency of analysed samples is low. At higher flow rates (1.5–2.0 ml/min) shorter injection times are achieved but poor reproducibility is observed. The maximum ratio of peak height/reproducibility was found at 1.0 ml/min. Studying the volume of the NAC injected into the FIA the optimum was found at 100 μl. For low concentrations of NAC the injected volume can increase up to 200 μl.

In our experiments the electrode responds primarily to the concentration of free iodide ions at the sample solution electrode interface. The potential of the cell measured with the sensing ion-selective electrode is given by the equation

$$E = E' - S \log c(I^-) \tag{2}$$

where S is the response slope of the electrode mV/p(NAC), $c(I^-)$ the analytical concentration of iodide ions and E′ the electrode constant in mV. In the absence of the NAC the concentration of iodide ions at the surface of the electrode is described mainly as the sum of the iodide concentration in the sample solution and the iodide concentration coming as the result of the dissolution of the silver iodide at the electrode surface. If portions of the NAC were injected into the carrier stream reaction (1) must be considered and the potential is then presented by

$$E = E'' - S \log \{c(I^-) + dc(NAC)\} \tag{3}$$

where c(NAC) is the molar concentration of injected NAC, d the dispersion coefficient of the system and E″ represents the electrode constant as well as the reference-electrode and liquid-junction potentials and the activity coefficient of iodide ions ($f(I^-)$). Hence, $c(I^-)$ and d are kept constant while a linear dependence between the electrode potential and logarithm of concentration of NAC can be obtained according to the following equation

$$E = K - S \log c(NAC) \tag{4}$$

where K denotes the constant of the electrode system in mV.

The response of the electrode at optimum FIA parameters is given in Fig. 3. When the recorded signal heights in

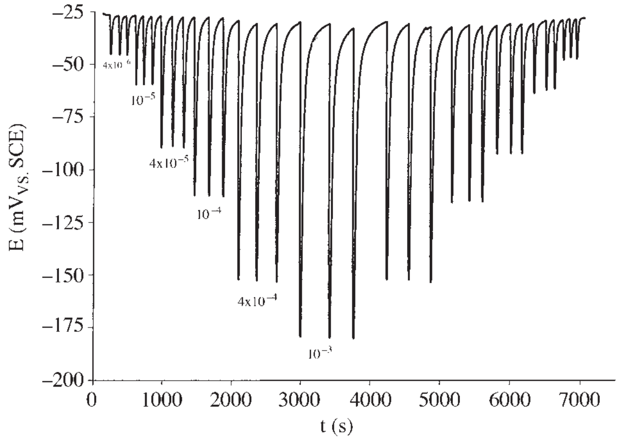


Fig. 3: Response peaks obtained for the injection of standard solutions of NAC (M) at optimum FIA parameters

**Table: Determination of NAC in pharmaceutical products with the volumetric method and the FIA**

| Sample | Number of replications | Declared amount in a sample (g) | Determined FIA (g) | Standard deviation (%) | Determined volumetric (g) | Standard deviation (%) |
|---|---|---|---|---|---|---|
| Mucobene® Ratiopharm | 9 | 0.200 | 0.199 | 1.8 | 0.192 | 0.6 |
| Mucobene® Ratiopharm | 9 | 0.600 | 0.596 | 0.3 | 0.609 | 0.2 |
| Cimexyl® Cimex | 9 | 0.200 | 0.196 | 2.2 | 0.193 | 0.6 |
| Flumikan® Lek | 9 | 0.200 | 0.200 | 0.3 | 0.193 | 0.2 |

mV are plotted to the negative logarithms of increasing NAC concentrations in the injected sample we obtain the value $60.6 \pm 1.0$ mV $\{p(NAC)\}^{-1}$ in the concentration range between $4.0 \times 10^{-6}$ and $1.0 \times 10^{-3}$ M with $R = 0.995$. The extrapolated intercept at log $c(NAC) = 0$ gives the value $K = -358 \pm 1.0$ mV vs. SCE. In the same concentration range for the decreasing series of injected NAC the slightly lower value $58.8 \pm 1.0$ mV $\{p(NAC)\}^{-1}$ at $R = 0.996$ was obtained. For the decreasing series the extrapolated intercept at log $c(NAC) = 0$ gives the value $K = -354 \pm 1.0$ mV vs. SCE.

In Fig. 4, the calibration plot received from Fig. 1 was compared to that from Fig. 3. In FIA experiments transient potential values were recorded whereas in continuous flow measurements steady-state potential values were reached. Considering the fact that a constant dispersion coefficient is present in FIA both plotted curves are very close to that obtained from the theoretical model [23].

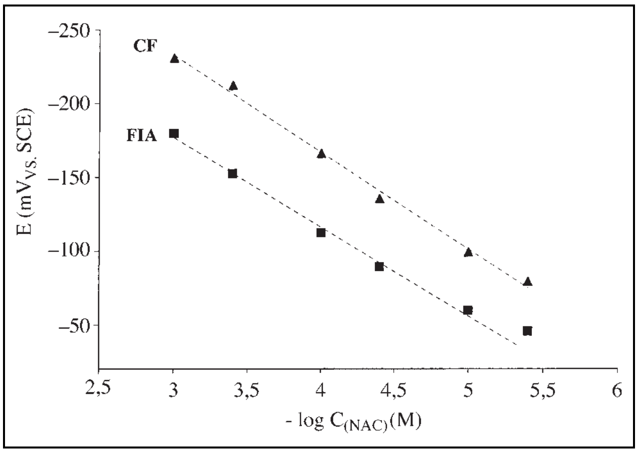


Fig. 4: Calibration plots for mixtures of iodine and NAC from the continous flow (Fig. 1) and from flow injection analysis (Fig. 3).

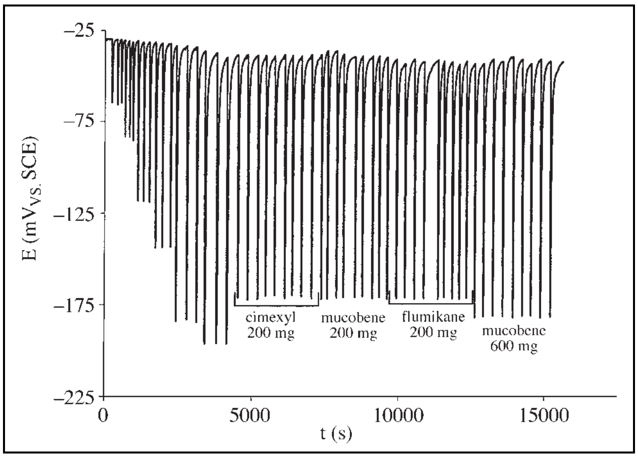


Fig. 5: Response peaks obtained for the injection of standard and sample solutions of NAC at optimum FIA parameters

Finally four pharmaceutical products (Mucobene® 200 mg and Mucobene® 600; Ratiopharm, Cimexyl® 200 mg; Cimex and Flumikan® 200 mg; Lek) containing NAC were analysed in FIA. After injecting a series of standard solutions of NAC three samples of each product were analysed in three replications Fig. 5. On the same samples direct potentiometric titrations with silver nitrate were carried out for comparison. The results for both methods are shown in the Table. The relative standard deviation (RSD) for potentiometric titrations was between 0.2% and 0.6%. Using FIA the RSD was slightly higher (between 0.3% and 2.2%) but still quite acceptable for potentiometric flow injection analysis. For both methods the average values of detected NAC in all tested samples are very close to the prescribed values given by the pharmaceutical producers. In FIA a frequency of 25 samples per hour can easily be achieved which is far more than with volumetric methods. Beside that in the flow injection analysis only non-toxical chemicals are applied and no extra preparation of the samples is necessary.

The chemically treated silver wire electrode demonstrates its applicability for the indirect determination of NAC in flow injection analysis. The developed method shows that simple chemical transformation of NAC can allow efficient and inexpensive determination that can be used even for routine work.

## 3. Experimental

### *3.1. Reagents and solutions*

All the chemicals used were of analytical grade of purity and all the solutions were prepared in doubly distilled water, except for the basic iodine solution (0.05 M) that was prepared in methanol.

A stock solution of NAC was prepared daily by dissolving 1.6320 g of NAC (Sigma) and diluted up to 100 ml with 0.1 M $HClO_4$. Standard working solutions were prepared by dilution of the appropriate aliquots of the stock solution of NAC with 0.1 M $HClO_4$ to cover concentration range from $1.0 \times 10^{-2}$ to $1.0 \times 10^{-6}$ M.

### *3.2. Preparation of the electrode*

Electrolytic pure silver wire (99.99% Ag) 1.0 mm in diameter and 12–15 mm long was connected to a coaxial cable and adhered into a glass tube with epoxy resin. First it was for 5–10 s treated with diluted $HNO_3$ (50% v/v). After being washed in doubly distilled water the electrode was dipped for 30 min into a 0.1 M solution of $HgCl_2$ and it was then thoroughly rinsed in doubly distilled water. Finally the electrode was dipped into 0.1 M KI for 24 h. The electrode is kept in 0.1 M KI and can be used for at least 10 days.

### *3.3. Apparatus*

The FIA system consists of a peristaltic pump (MCP CA-4, Ismatec) connected to an low pressure injection valve (model 5020, Rheodyne) through PTFE tubing of 0.8 mm i.d. The silver electrode was positioned 120 cm behind the injection valve. The wire electrode was placed direct by in the PTFE and the SCE was positioned next to the wire electrode in a small funnel ($V = 5$ ml).

All potentiometric data were recorded using mV/pH meter (type 301, Hanna Instruments) and downloaded to a personal computer through software HI 92000 (Hanna Instruments). All measurements were carried out at $298 \pm 0.1$ K.

### 3.4. Procedure

#### 3.4.1. FIA method

Tablets or powder samples containing NAC for flow analysis were dissolved in 50 ml of 0.1 M $HClO_4$ and diluted to 250 ml in a measuring flask with 0.1 M $HClO_4$. Sample solutions were prepared by dilution of appropriate aliquots of dissolved NAC with 0.1 M $HClO_4$. Standard and sample solutions were then injected into the carrier stream (0.1 M $HClO_4 + 10^{-3}$ M $I_2 + 10^{-6}$ M KI) at the flow rate (1.0 ml/min) in portions of 100 µl.

#### 3.4.2. Volumetric method

Samples containing NAC were dissolved in 250 ml of 0.1 M $HClO_4$. To a measured volume (5 or 20 ml) of this solution 25 ml of glacial acetic acid was added. The solution was then titrated with a standard solution of 0.01 M $AgNO_3$. Potentiometric titrations were carried out with silver sulfide electrode (model 94-16, Orion) and the end-point of titrations was found from a derivative plot [12].

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