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Potentiometric flow-injection determination of vitamin C and glutathione with a chemically prepared tubular silver electrode

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This paper describes the preparation and use of a tubular electrode by means of chemical pretreatment of a silver tube with mercuric (II) chloride solution and iodide solution in flow injection analysis (FIA). The electrode was used as a potentiometric sensor for the indirect determination of vitamin C and glutathione in a carrier stream containing iodine. A simple FIA system that consists of a peristaltic pump, an injection T valve, a tubular silver electrode and a saturated calomel reference electrode (SCE) was used. Some typical FIA parameters such as flow rate, tube length, sample volume and composition of the carrier stream were varied. After optimisation of these parameters, the electrode was further characterised by a constant linear response within the concentration range for the vitamin C between 5×10^{-5} and 5×10^{-3} M at the slope of 60.5 ± 1.0 mV/p (vitamin C). Glutathione has a linear concentration range between 5×10^{-5} and 10^{-2} M at a slope of 55.2 ± 1.0 mV/p (glutathione). The experimental slope was in good agreement with the theoretical values. Some pharmaceutical products containing vitamin C were also tested. These results can be compared to the results obtained by the standard volumetric method for the determination of vitamin C and are also in good agreement with values declared by pharmaceutical manufacturers.

1. Introduction

Vitamin C (l-ascorbic acid) can be determined by several analytical methods in various matrices [1]. In batch potentiometric measurements the indirect determination of vitamin C by the reduction of iodine and detection of formed iodide with the iodide ion selective electrode (I-ISE) is described [2, 3]. Measurements of some inorganic and organic compounds (also vitamin C) with I-ISE using iodate as an oxidant agent are suggested [4, 5]. Microtitrations of vitamin C with copper (II) solution, using a commercial copper (II) ISE as a potentiometric sensor in pharmaceutical preparations are described in the literature [6].

Spectrophotometric determination is commonly used in normal flow injection analysis (FIA) or FIA titrimetry analysing different samples of vitamin C. Titrimetry by FIA using Ce(IV) as titrant was used for determining vitamin C in pharmaceutical products [7]. FIA methods using chloramine-T and a starch-KI solution were compared and a chloramine-T reagent was used to determine vitamin C in urine [8, 9].

The electro analytical detection of the vitamin C in FIA is performed by coulometry or amperometry with a glassy carbon electrode [10].

Glutathione is a major constituent of cells and has several independent functions, in most cases it is a reductant [11]. For the determination of glutathione in low concentration regions cathodic stripping voltammetry can be used [12]. For the determination of the glutathione usage of the co-enzyme properties of glutathione and the detection of secondary products of glutathione is reported [13]. In the present work, the chemical treatment of an Ag tube is presented in order to prepare the potentiometric FIA electrode for vitamin C and glutathione.

2. Investigations, results and discussion

2.1. Surface analysis

After the silver tube has been immersed into the HgCl_2 solution a homogeneous grey coating is formed on the surface which turns yellow in iodide solution. On visual inspection the coating is solid and does not scale. The

depth profile diagrams obtained by Auger electron Spectroscopy (AES; PHI, model SAM 545A) show a quantitative elemental composition of atomic layers on the electrode surface (Figs. 1, 2). As can be seen in Fig. 2 the stoichiometric composition of the AgI is found only in the first atomic layers on the surface of the silver tube. The broadening of the iodide concentration profile is typical for the rough surface of the chemically etched metal surface [14, 15]. Mercury was not detected in this layer. The surface analysis by means of an electro microscope (SEM Microsan 9, Cambridge Ins., Ltd.) confirms the results of AES-analysis. During the first step (HgCl_2 pre-treatment) the direct displacement or "cementation" of Hg on Ag is known to occur. The driving force of this reaction comes from the difference of electrochemical potentials of the Hg/Hg^{2+} and Ag/Ag^+ systems (overvoltage and complexation effects being taken into account, too). Because of the very close normal potentials of the above systems and the presence of the Cl ions, the displacement of Hg on Ag is inhibited by a parallel formation of AgCl, which covers the Ag surface (Fig. 1). During the second step (KI treatment) the AgCl deposited on the Ag surface is trans-

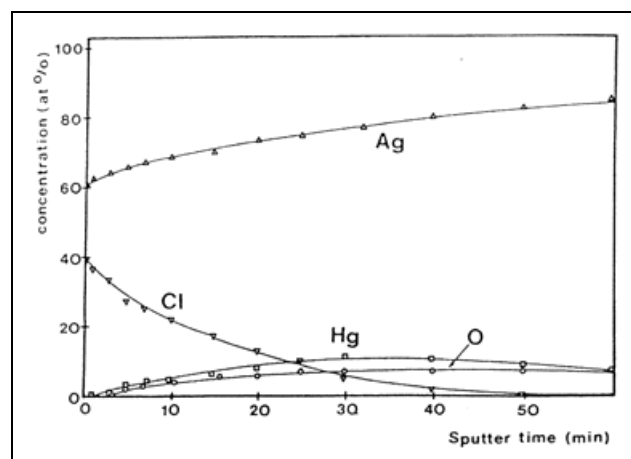


Fig. 1: AES composition-depth profile of the silver tube after treatment with a HgCl_2 solution obtained by sputtering with 1 keV Ar^+ ions at a 60° incidence angle and raster size of $10 \text{ mm} \times 10 \text{ mm}$

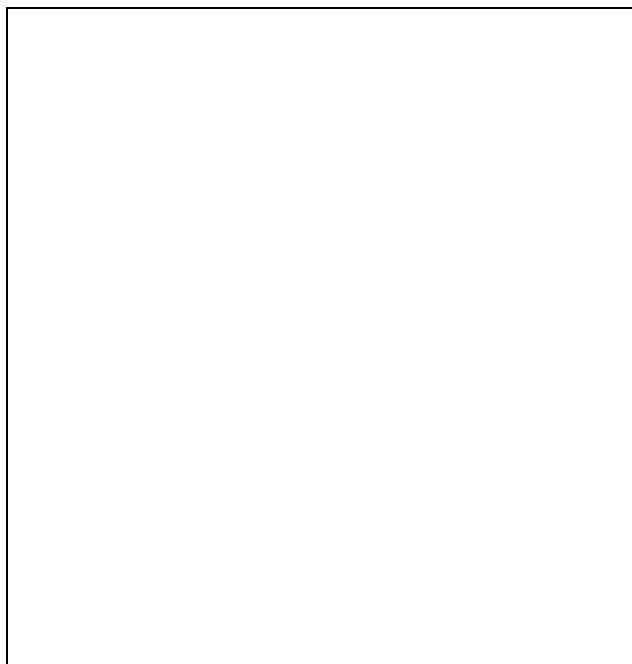
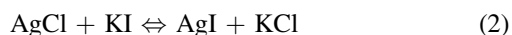


Fig. 2: AES composition-depth profile of the silver tube after treatment in HgCl_2 and the KI solution (for experimental conditions see Fig. 1)

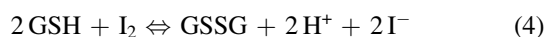
formed into AgI (Fig. 2). The overall reaction describing the whole procedure in the simplest case could be given by summing two consecutively occurring reactions:



The cementation processes are known to depend on many factors: temperature, stirring and the pH of the solution, metal surface conditioning and even position of the metal in the solution. Therefore it is difficult to produce an integral uniform single-phase AgI membrane of definite thickness.

2.2. Potentiometric behaviour

The tubular shape design of the potentiometric detectors is prevalent for FIA [16]. The described preparation procedure of the silver tube in solutions of HgCl_2 and KI was used to prepare a thin layer surface of AgI, which makes the electrode responsible for iodide ions. In order to determine vitamin C and glutathione, a solution containing iodine was used as a carrier stream. By mixing one of the reductants with iodine, a stoichiometric amount of iodide is formed:



where AA denotes ascorbic acid, DAA dehydroascorbic acid, GSH glutathione and GSSG glutathione disulfide, an oxidised form of glutathione.

To confirm these reactions some tests were initially carried out in which iodine was mixed with a known concentration of vitamin C or glutathione. After 5 min, the solution was pumped through the electrode at a flow rate of 2.5 ml/min. As shown in Fig. 3, the electrode gives a rapid and reproducible response within a linear range between 2×10^{-5} and 2×10^{-3} M at a slope of 60.8 ± 1.0 mV/p (vitamin C). For glutathione, a linear range between 2×10^{-6} and 2×10^{-3} M at a slope of 54.7 ± 1.0 mV/p (glutathione) was determined.

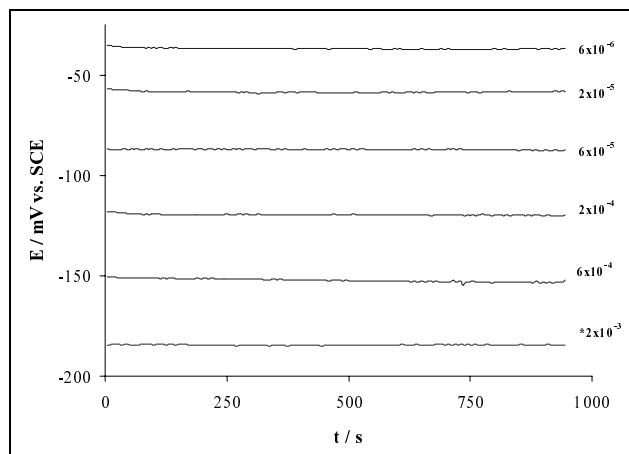


Fig. 3: Response of the Ag/AgI tubular electrode to the mixture of 10^{-3} M iodine and vitamin C (M); I_2 concentration: 3×10^{-3} mol/l

The electrode was conditioned and stored in 0.1 M KI and no loss of the Nernstian response as described for membrane Ag/AgI electrodes was observed [17].

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 maximise precision and accuracy, to enlarge the linear concentration range, and to increase the sampling rate.

The composition of the carrier stream was optimised to prevent the base line drift (addition of 10^{-6} M KI) and to obtain a maximal response to iodide ions. The concentration of iodine (10^{-3} M) is upwardly limited by its solubility in water [18]. The electrode can be used for approximately 10 days. After that time, a negative drift of the baseline occurs and new chemical pretreatment of the silver tube is necessary. Standard solutions and samples of vitamin C must be prepared daily because of the oxidation in the presence of atmospheric air [19].

The comparison between one and two-channel FIA using different compositions of carrier stream, reagent stream and tube length shows that the described (one-channel FIA) gives the most reproducible and precise results with a high sampling rate.

In our experiments the electrode responds primarily to the activity of iodide ions at the sample solution electrode interface. The potential of the cell measured with the sen-

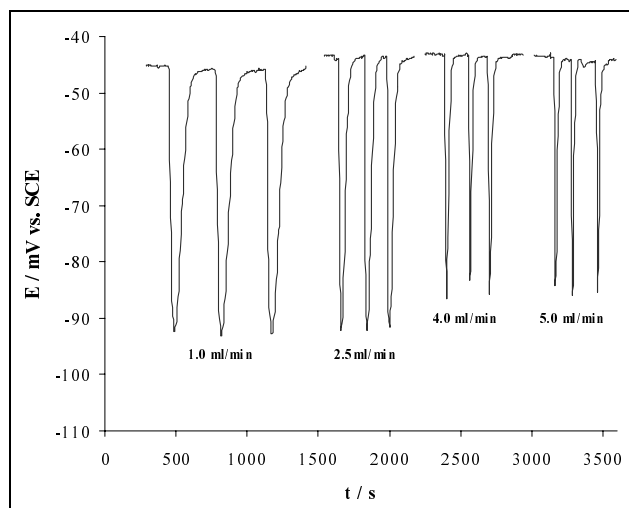


Fig. 4: Response of the electrode to 100 μl of injected vitamin C (5×10^{-4} M) at different flow rates

sing electrode is given by the equation:

$$E = E' - S \log a(I^-) \\ = E' - S \log \{ (f(I^-) c(I^-)) \} = E'' - S \log c(I^-) \quad (5)$$

where S is the response slope of the electrode and $c(I^-)$ the analytical concentration of iodide ions. The constant E'' represents the electrode constant as well as the reference-electrode and liquid-junction potentials and the activity coefficient of iodide ions ($f(I^-)$).

In the absence of vitamin C 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.

Hence, if the carrier stream does not contain vitamin C, the potential of the sensor can be expressed by the following equation:

$$E = E'' - S \log c(I^-) \quad (6)$$

where $c(I^-)$ is the concentration of iodide in the carrier stream.

If portions of the vitamin C were injected into the stream reaction (1) must be considered and the potential is then presented by:

$$E = E'' - S \log \{ c(I^-) + d_2c(\text{vitamin C}) \} \quad (7)$$

where the dispersion of the sample is represented by the constant d . Hence, $c(I^-)$ and d are kept constant while a linear dependence between the peak height and logarithm of concentration of vitamin C with the slope of 60.5 mV/p(vitamin C) can be obtained according to the following equation:

$$E = K - S \log c(\text{vitamin C}) \quad (8)$$

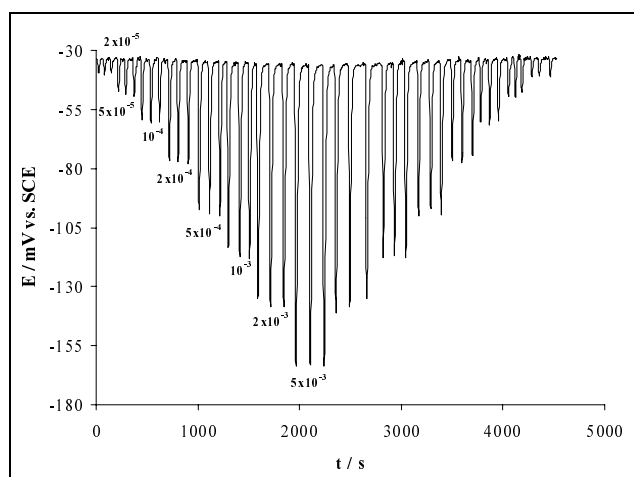


Fig. 5: Response peaks obtained for the injection of vitamin C (M) at optimum FIA parameters

For glutathione an equation similar to (7) can be written, adjusting it accordingly to eq. (4).

The flow rate Fig. 4 and the sample volume of vitamin C were varied. The maximum ratio of peak height/reproducibility was found at 2.5 ml/min. The optimum volume of the vitamin C injected into the FIA was 100 μ l. For low concentrations of vitamin C the injected volume can increase up to 250 μ l.

The response of the electrode at optimum FIA parameters is given in Fig. 5. For vitamin C, a constant linear response was found within the concentration range between 5×10^{-5} and 5×10^{-3} M ($R = 0.994$) at the slope of 60.5 ± 1.0 mV/p (vitamin C) (100 μ l). The extrapolated intercept at $\log c(\text{vitamin C}) = 0$ gives the value $K = -300 \pm 1.0$ mV vs. SCE.

In Fig. 6, the calibration plot from (Fig. 3) was compared to that in Fig. 5. Considering the fact that the same dispersion-diffusion coefficient [20] is present in both cases the plotted curves are close to the theoretical examples [21].

Optimum FIA parameters for glutathione were found at 250 μ l of the injected sample at the flow rate of 2.5 ml/min. The composition of the carrier stream used here was the same as described for vitamin C. The linear concentration range of the glutathione was between 5×10^{-5} and 10^{-2} M ($R = 0.996$) at slope of 55.2 ± 1.0 mV/p (glutathione). The extrapolated intercept at $\log c(\text{glutathione}) = 0$ gives the value $K = -281.5 \pm 1.0$ mV vs. SCE.

Finally some commercial tablets containing vitamin C were tested and the results obtained by the standard volumetric method and FIA are shown in the Table. The results are in good agreement with the values declared by the pharmaceutical manufacturers. The relative standard deviation (RSD) was between 0.8% and 1.8% which is

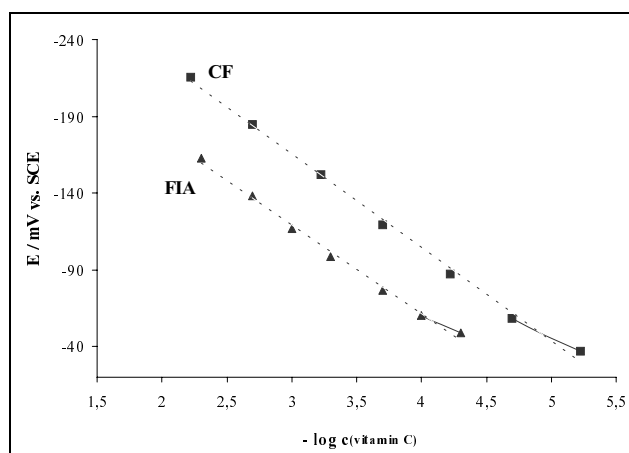


Fig. 6: Calibration plots for mixtures of iodine and vitamin C in the continuous flow (CF) (Fig. 3) and for FIA (Fig. 5)

Table: Determination of vitamin C in pharmaceutical products with the standard volumetric method and FIA

Sample	Number of samples	Declared amount in a tablet (g)	Determined FIA (g)	Standard deviation (%)	Determined volumetric (g)	Standard deviation (%)
Aspirin [®] plus C	6	0.240	0.244	1.3	0.243	0.8
Bayer						
Fortalgin [®] C						
Lek	6	0.240	0.234	1.7	0.238	0.8
Lekovit [®] C-Ca						
Lek	3	0.500	0.486	1.8	0.489	0.6
Ca-C 500 Sandoz [®]						
Krka	3	0.500	0.478	0.8	0.494	0.5

quite acceptable for potentiometric determinations. Using FIA, a frequency of 40 samples per hour can easily be achieved which is almost ten times more than using the standard volumetric method. Besides that no further preparation of the tablet sample (except dissolution and dilution) is necessary. Another advantage of the flow method is very low consumption of non-toxic chemicals. Thus, the described FIA method demonstrates its applicability for the determination of vitamin C and glutathione with simple and inexpensive detection for routine work or as a comparative analytical method.

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 vitamin C was prepared daily by dissolving 1.7666 g of vitamin C and diluted up to 100 ml with 0.1 M KNO₃. Standard working solutions were prepared by dilution of the appropriate aliquots of the stock solution of vitamin C with 0.1 M KNO₃ to cover concentration range from 2×10^{-3} to 6×10^{-6} M.

A glutathione stock solution was prepared by dissolving 3.1683 g of glutathione in 0.1 M KNO₃ and diluted up to 100 ml. Standard working solutions were prepared by dilution of the appropriate aliquots of stock solution of glutathione with 0.1 M KNO₃ to cover a concentration range from 2×10^{-3} to 2×10^{-6} M.

3.2. Preparation of the electrode

A pure silver (99.99%) electrolytic tube (internal diameter, 1.5 mm; length, 10 mm) was connected to a coaxial cable. This was first briefly treated (5–15 s) with HNO₃ (1 + 1). The electrode was then thoroughly rinsed with twice distilled water and immersed in a 0.1 M solution of HgCl₂ for 30 min. After another rinsing of the electrode with twice distilled water, the final step for electrode preparation was conditioning in 0.1 M KI for 24 h. Before and after use the electrode was stored in 0.1 M KI.

3.3. Apparatus

The FIA system consists of a peristaltic pump (MCP CA-4, Ismatec, Zürich) connected to an injection T valve (model SVP-3T/T, SGE, Australia) through teflon tubing of 1.5 mm i.d. The tubular silver electrode was positioned 60 cm behind the injection T valve. The saturated calomel electrode (SCE) with a 0.5 M KNO₃ salt bridge was placed at the outlet – 20 cm from the indicator electrode (connected also with a teflon tubing of 1.5 mm i.d.) in a glass container.

All potentiometric data were recorded using mV/pH meter (type ORION 920A) and downloaded to a personal computer. All measurements were carried out at 298 ± 0.1 K.

3.4. Procedure

3.4.1. FIA method

Vitamin C tablets for flow analysis were dissolved in 50 ml of 0.1 M KNO₃ and diluted to 250 ml in a measuring flask with 0.1 M KNO₃. Sample solutions were prepared by dilution of appropriate aliquots of dissolved

vitamin C tablets with 0.1 M KNO₃. Standard and sample solutions were then injected into the carrier stream (0.1 M KNO₃ + 10^{-3} M I₂ + 10^{-6} M KI) at the flow rate 2.5 ml/min in portions of 100 µl.

3.4.2. Volumetric method

Tablets containing vitamin C were dissolved in 60 ml of 0.3 M H₂SO₄. After that 2 g of solid KI and 50 ml of standard 0.01 M KIO₃ were added. The solution was immediately titrated with a standard 0.07 M Na₂S₂O₃ until the solution lost almost all of its pale yellow colour. Then 2 ml of starch indicator was added. The titration was completed when colourlessness of the titrated solution was observed [22].

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