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Direct Potentiometric Determination of N-acetyl-L-cysteine (NAC) in Real Samples by Using "home made" Iodide ISE

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Direct potentiometric method for determination of NAC in pharmaceuticals by using "home-made" iodide ISE is described. Iodide ISE membrane was made of AgI:Ag_2S:PTFE = 1:1:2. Proposed method is very inexpensive, simple and reasonably fast method for determination of NAC in acetic buffer, pH = 5 without pretreatment of pharmaceuticals and determination is based on the reaction between NAC and Ag^+ from electrode membrane. Described method has linear response range for NAC from 2×10^{-5} to 1×10^{-2} mol L^{-1} with limit of detection of 7.8×10^{-6} mol L^{-1}. Found concentrations of NAC are in very good agreement with declared ones with standard deviation values in range 0.13-3.41%.

Keywords: *N*-acetyl-L-cysteine, potentiometric, determination, "home-made" ion-selective electrode

1. INTRODUCTION

N-acetyl-L-cysteine (NAC) is modified amino acid cysteine, which is synthesized in the body either in taken with food. NAC, can significantly increase the level of glutathione, an antioxidant that is involved in many biological processes in the body. There are plenty of strong mucolitic properties and therefore is the treatment for the degradation of mucus by breaking disulfide bonds in proteins (e.g. *Fluimukan, TWINLAB®NAC*) and is useful in the treatment of emphysema, bronchitis, tuberculosis, bronchiectasis, amyloidosis and pneumonia. Also, it is used as an antidote in paracetamol poisoning. Thiol group of NAC can reduce free radicals formed as byproducts of metabolism.

Recently in literature there are few papers that describe direct potentiometric determinations of thiols/NAC[1,2,3]. Other proposed methods are based on different techniques, such as volatammetry [4,5] and spectrophotometry[6,7].

We are proposing a method suitable for determination of NAC in wide concentration range without pretreatment of pharmaceuticals. Proposed method is very inexpensive, simple and reasonably fast method. Using described potentiometric sensor for determination of NAC in pharmacueticals makes proposed method a very robust one. Also, preparation of membrane for used potentiometric sensor is very simple and can be more inexpensive if commercially available silver nitrate, potassium iodide and sodium sulphide would used.

2. EXPERIMENTAL

2.1. Reagents and chemicals

All needed solutions were prepared by solving certain amount of solid chemicals in suprapure water. Suprapure water (declared conductivity 0.04 μS cm^{-1}) was prepared by Millipore Simplicity.

Following chemicals were used: Potassium nitrate, KNO_3, p.a., Potassium iodide, KI, p.a., Silver nitrate, $AgNO_3$, p.a., Sodium sulphide, Na_2S, p.a., Sodium acetate, CH_3COONa, p.a., Acetic acid, CH_3COOH, p.a., Kemika (Croatia) and *N*-acetyl-L-cysteine, $C_5H_9NO_3S$, p.a., Merck (Germany)

2.2. Apparatus

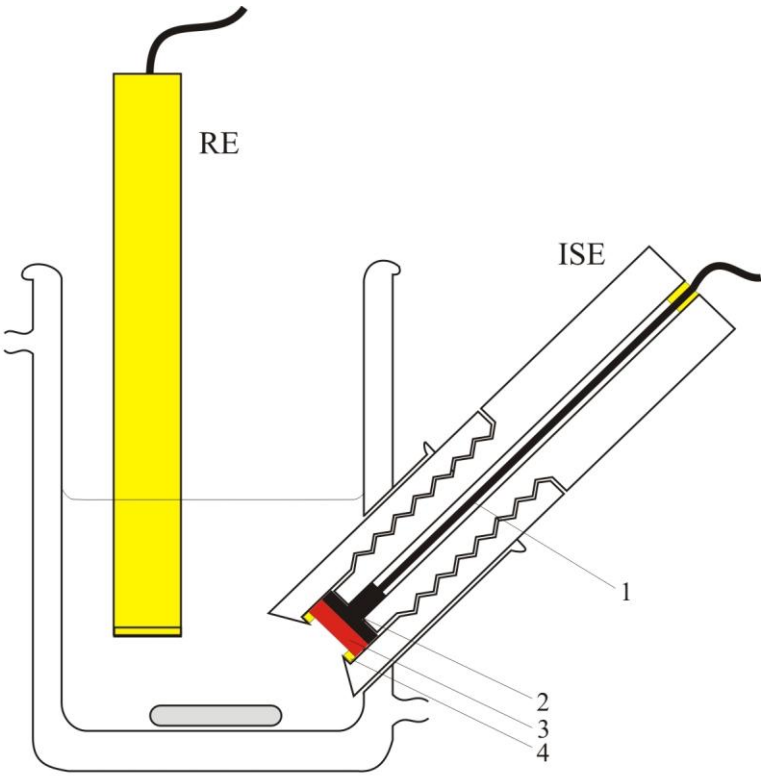

Figure 1. Potenciometric system with direct metal contact at membrane. 1) coaxial cable 2) solid-state disk made of stainless steel 3) AgI based sensor 4) silicon rubber

All parts of the multi-purpose solid-state electrode body were machined from PTFE or stainless-steel. A pressed pellet (2.0 mm i.d. and 1.2 mm long) was incorporated in the electrode body. The preparation and performance of the silver-iodide-based pellet hydrophobized by PTFE have been described previously[8]. Electrical connection, between the pellet and millivoltmeter, was provided through stainless-steel disk and coaxial cable.

The indicator electrode was an Orion 90-02 double junction reference electrode. Potentiometric data were recorded at room temperature with a millivoltmeter (Model MA 5740, Iskra, Ljubljana, Slovenia) coupled to a personal computer and recorder.

3. RESULTS AND DISCUSSION

"Classic" or "batch" potentiometric determination of NAC has been done by using previously described iodide ion-selective electrode. "Home-made" iodide ISE has been tested for response to iodide concentration. Change of concentration of I^- was performed by standard dilution method. During measurement, solution was stirred and kept at constant temperature of 25 °C. Results are shown at Figure 2.

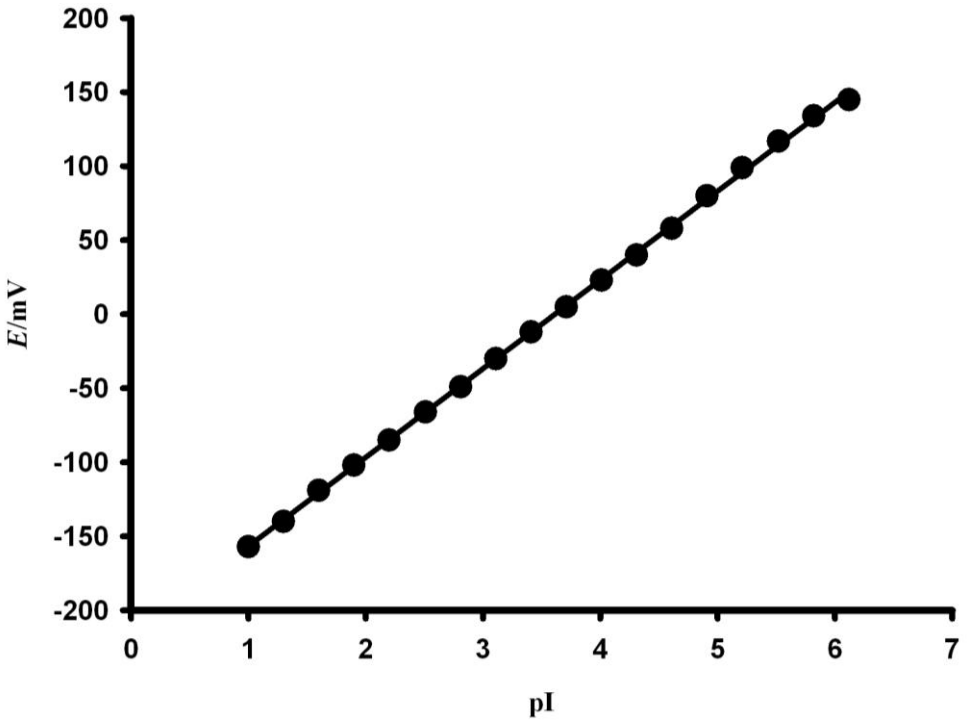

Figure 2. Response of "home-made" iodide ISE to iodide ions

Points on the graph represent experimental data and straight line was calculated by using method of linear regression. As it can be seen, iodide electrode, made of pressed pellet, linearly follows changing of I^- concentration in wide concentration range. Stable potential was reached in 1 minute. Potential change of 59.96 mV per decade of iodide concentration change was recorded, with

correlation coefficient of 0.9996, which is in good agreement with theoretical Nerstian slope for monovalent cations. "Home-made" iodide ion-selective solid-state electrode linearly follows I^- concentration changing with linear response in the concentration range of $I^- = 7.6\times10^{-7}$ -10^{-1} mol L^{-1} in acetic buffer, pH = 5. Response of "home-made" iodide ISE to NAC was based in reaction of sulphur in thiol's group of NAC with Ag^+ from silver iodide from electrode membrane by forming complex at membrane surface described by Radic & Dobcnik[9]. Same method for testing response of "home-made" electrode to NAC as to iodide ions was performed. Results are shown at Figure 3.

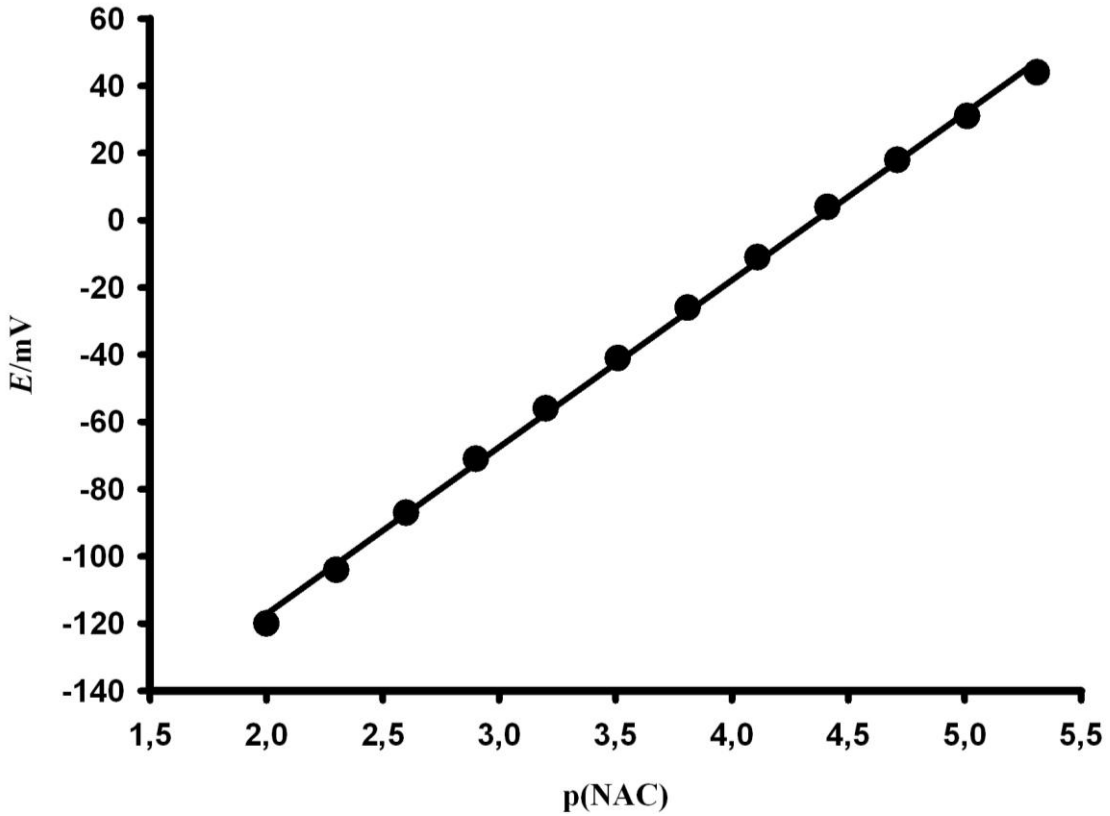

Figure 3. Response of "home-made" iodide ISE to NAC

Points on the graph represent experimental data and straight line was calculated by using method of linear regression. It can be seen that iodide electrode, made of pressed pellet, show linear dependence with changing of NAC concentration in the concentration range between 2×10^{-5} and 1×10^{-2} mol L^{-1}, with a detection limit of 7.8×10^{-6} mol L^{-1}. Stable potential was reached in about 1 minute. Potential change of 49.67 mV, for decade concentration change of NAC, with correlation coefficient of 0.9989 was recorded in acetic buffer, pH = 5. In previous works[1,2,10] was recorded linear response range of two concentration decade. In comparison of results obtained by Bralic & Radic[10] with commercial available iodide ISE and proposed method with "home-made" iodide ISE it can been seen that linear response range for NAC was extended and limit of detection was decreased for one concentration decade.

After doing potentiometric measurements with constant stirring speed, we preformed potentiometric measurements with different stirring speeds to investigate a speed influences at "home-made" iodide electrode response time. These experiments were performed at different stirring speeds of 200, 250 and 300 rotation per minute (rpm) and changing of concentration of NAC concentration by order of magnitude with adding standard solution of NAC, c(NAC) = 0.1 mol L^{-1}. Results are shown at Figure 4.

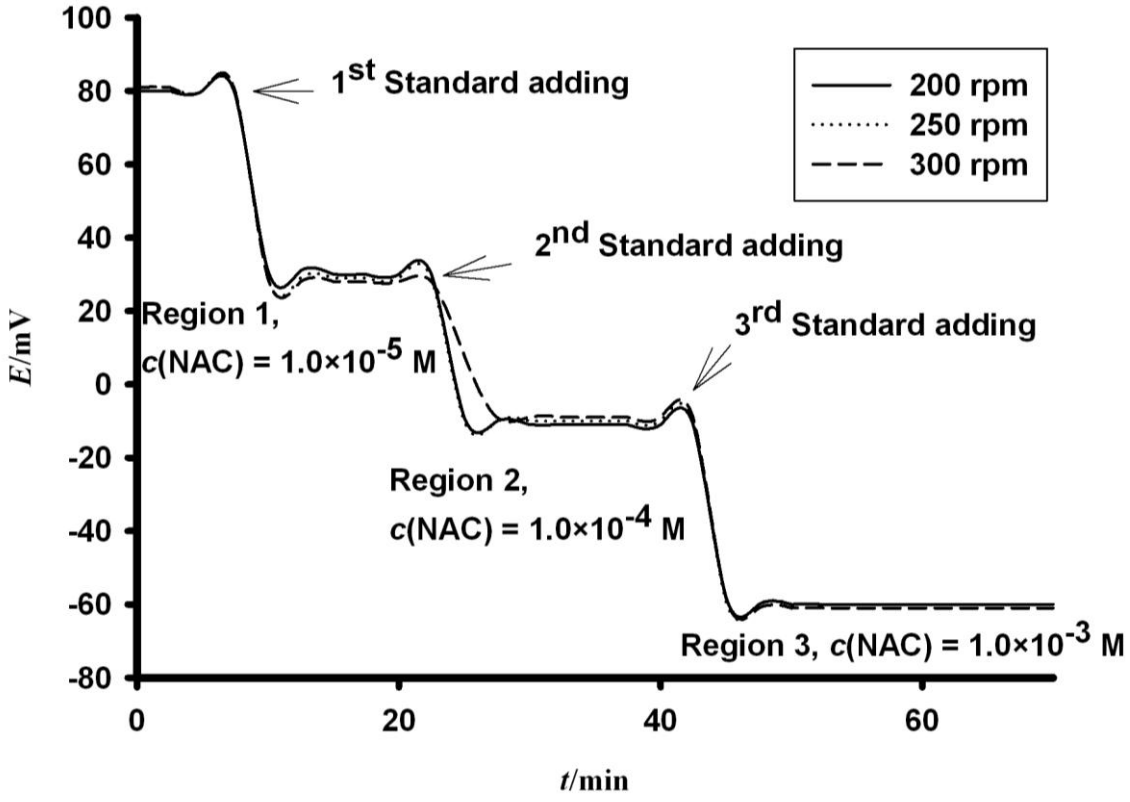

Figure 4. Influence of stirring speeds at "home-made" iodide ISE response to NAC

By increasing stirring speed according the Fick's Laws, more mass would be transported on membrane surface. It is important notice that hills and downhills at beginning and end of each region became from stop and start of stirring when NAC standard solutions were added. It can be seen at Figure 4 that is small difference in potential values (±7 mV) for different stirring speeds in region 1, at low NAC concentration (1.0×10^{-5} mol L^{-1}), where diffusion is limiting process. Tested NAC concentration of 1.0×10^{-5} mol L^{-1} is below limit of quantization for proposed method and according that are slightly differences in measured potential at different stirring speeds (region 1 at Figure 4). For NAC concentration of 1.0×10^{-4} mol L^{-1} (region 2 at Figure 4) only benefit of increasing stirring speed is shorter response time, especially for 300 rpm (compare dashed line with full and doted ones). For NAC concentration of 1.0×10^{-3} mol L^{-1} (region 3 at Figure 4) practically there is no benefits, because diffusion was not limiting factor anymore and response time at all tested stirring speeds were reasonably same. In this region stable potential was reached in about 55 seconds. Increasing stirring

speed on 350 rpm and above was counter effected because an air bubble was formed at membrane surface.

The influence of concomitant species on the determination of NAC was examined by applying the proposed method to a determination of NAC. The tested concentration for NAC was 1.0×10^{-4} mol L^{-1} which is 10 times lower concentration than NAC concentration in real samples that we obtained. The tolerance limit was taken as the amount of added species that caused an error less than ±5% what is similar with results obtained with another analytical techniques and methods[3,6]. The results are given in Table 1.

Table 1. Tolerance limits for interferences for the determination of 1.0×10^{-4} mol L^{-1} NAC

Substance	Tolerance limit ratio ($mole_{substance}/mole_{NAC}$)
Fructose, Glucose, Lactose, HCO_3^-	>300
EDTA, $S_2O_3^{2-}$	50
Cl^-, SCN^-	20
I^-, Br^-	10

Fructose, Glucose, Lactose, HCO_3^- were tested because they are some of components in pharmaceuticals. Other ions in Table 1 were tested because they build insoluble compounds with Ag^+ and can significantly reduce active places at membrane surface.

Described potentiometric determination of NAC was used for measuring concentration of NAC in tested pharmaceuticals (Table 2). Pharmacueticals' solutions were prepared by dissolving one tablet in appropriate volume of acetic buffer, pH = 5, and diluted with the same buffer in a 100.0 mL calibrated flask. We performed the recovery studies by adding the standard of NAC to the sample solution. Equation used for calculating NAC concentrations in tested samples was: E = K– S log c(NAC), R^2 = 0.9989.

Table 2. Determination of NAC in real samples

Fluimukan®	Labeled, mg	Found ±SD (%) (n=5)	Recovery (%)
	100	100.17±3.41	100.16
	200	199.67±0.91	99.84
	600	599.83±0.25	99.97

In table 2 can be seen that proposed method has positive error for small NAC concentrations and negative error for bigger NAC concentrations. SD values become smaller by NAC concentration increase.

Table 3. Recovery studies by adding the standard

Labelled/Taken, mg	Added, mg	Found±SD% (n = 5)	Recovery, %
100/5	10	15.3±1.03	102.0
	50	55.38±0.87	100.69
	100	105.92±0.23	100.88
200/5	10	14.78±0.88	98.53
	50	54.82±0.35	99.67
	100	104.02±0.08	99.07
600/5	10	14.78±0.57	98.53
	50	54.78±0.15	99.60
	100	104.22±0.13	99.26

In table 3 can be seen that proposed method is very suitable for direct potentiometric determination of NAC. Positive errors occur only in experiments with pharmaceutical containing 100 mg of NAC what can be explained that some constituent or more of them increases measured potential. Found values of tested pharmaceuticals are in very good agreement with declared ones with standard deviation values in range 0.13-3.41%.

When we compare results shown in table 3 with previously ones [1,2], it can be seen very similar recovery as we have got. It is important to say that we performed direct measurements without any pretreatment or adding any chemical reagents in light acidic solution (pH = 5) by getting stable potential in 1 minute and in other papers kinetic measurements were performed what takes at least 5 minutes per an analysis in acetic acidic solution (pH = 3) or sulfuric acid solution (pH < 1). Also, it is significant to stress that we performed all investigation using "home made" iodide ISE while others [1,2] used a commercially available iodide ISE.

4. CONCLUSIONS

"Home-made" iodide ion-selective electrode (ISE) is acceptable sensor in potentiometric determination of NAC in acetic buffer, pH = 5, without needed used pharmaceuticals pretreatment in concentration range c(NAC) = 2×10^{-5}-1×10^{-2} mol L^{-1}, Figure 3, with potential change of 49.67 mV per decade of NAC concentration. At experimental conditions NAC forms very stable complex in reaction with Ag^{+} from the surface of membrane and generate potential change. Found values of tested pharmaceuticals are in very good agreement with declared values with standard deviation values in range 0.13-3.41%. Stirring speed effect was observed in «classic» potentiometric experiment with measurements at speeds of 200, 250 and 300 rpm and it was found that increasing of stirring speed shorts response time of "home-made" iodide selective electrode. According the experiment data, we suggest stirring speed up to 300 rpm for applied "home-made" ISE. Stirring speeds above 300 rpm

cause air bubble forming at membrane surface because the used iodide ISE had been sink in solution under the angle, Figure 1.

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