Technical paper

Kinetic Potentiometric Determination of Penicillamine and *N*-Acetyl-L-Cysteine Based on Reaction with Iodate

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Received: 18-06-2008

Abstract

Optimization and application of simple kinetic potentiometric method for the determination of D-penicillamine (PEN) and *N*-acetyl-L-cysteine (NAC) in pharmaceutical is described. The method was based on the reaction between thiol and iodate, in the presence of small quantity of iodide. The changes of potentials, ΔE, in fixed time interval were measured with an iodide ion selective electrode *versus* a double-junction reference electrode. Good linearity between ΔE and negative logarithm of analyte concentration is achieved in the range of the studied drugs concentration from 1.0×10^{-6} to 1.0×10^{-4} M. The limit of detection for PEN is 9.0×10^{-8} M and for NAC is 1.0×10^{-7} M. The applicability of the proposed method to the determination these thiols in pharmaceutical preparation was demonstrated by investigating the effect of potential interferences and by analysis of commercial preparations.

Keywords: D-penicillamine, *N*-acetyl-L-cysteine, kinetic potentiometry, iodide ion selective electrode

1. Introduction

The thiols (RSH) are essential for metabolism of cells and indispensable for prevent or healing some diseases. Many pharmaceutical and cosmetic preparations contain these compounds. *N*-Acetyl-L-cysteine (NAC) and D-penicillamine (PEN) are often used thiols as therapeutic substances.

Several analytical techniques, for determination these thiols in pharmaceuticals or biological tissue, have been proposed: liquid chromatography,[1,2] fluorimetry,[3] spectrophotometry,[4–7] voltametry,[8,9] amperometry,[10,11] potentiometry.[12–14]

The reaction system with iodate and thiol compounds or other reductant compounds, in acidic media, is most often used for spectrophotometric determination due to formation of iodine extracted in CCl_4.[15,16]

The papers concerned with kinetic potentiometric determination of thiols in pharmaceutical preparation are very rare in the literature. No studies have been reported on the determination of *N*-acetyl-L-cysteine and D-penicillamine in pharmaceutical preparation using kinetic potentiometric determination based on the reaction between the mentioned analytes and iodate in the presence of very small quantity of iodide in an acidic medium.

2. Experimental

2. 1. Apparatus

A millivoltmeter (SevenMulti, Mettler Toledo), connected to a personal computer, was used for potentiometric measurement. Cell potentials were measured with an iodide ion selective electrode (DX327- I, Mettler Toledo) *versus* a double-junction reference electrode (90-02-00, Orion). Data are stored and processed with SevenMulti™ Transfer V1.1 software (Mettler Toledo).

2. 2. Reagents and Samples

Analytical grade reagents and deionized water were always used in this work. The standard solutions of thiols,

1.0×10^{-2} M, were prepared by dissolving appropriate amounts of these compounds (NAC from Merck, and PEN from Fluka) in deionized water. The working solutions of KI (1.25×10^{-4} M) and KIO_3 (2.50×10^{-3} M) were prepared by diluting of 1.0×10^{-2} M stock solutions. The optimum pH in reaction solution was achieved by dilution 0.50 M H_2SO_4.

Solutions of real samples were prepared by dissolving one tablet of a pharmaceutical preparation in 10.0 mL of 0.50 M H_2SO_4. Then, it was transferred to a 100.0 mL volumetric flask and diluted to volume with deionized water. Before diluting it was filtered, if necessary.

The stock solutions were stored in the refrigerator for five days. Working solutions were obtained by daily dilution of stock solutions.

2. 2. Procedures

In a reaction vessel were accurately pipetted 18.0 mL of deionized water, 5.0 mL of 0.50 M H_2SO_4 and 1.0 mL of 1.25×10^{-4} M KI . After immersing of the electrodes into reaction solution, the stirring and the beginning of measurements were started. The stable initial potential of the cell has been reached one minute after the beginning of measurement. When the stable potential had been reached (1 min after beginning of measurements), 1.0 mL of 2.50×10^{-3} M KIO_3 was added in the reaction vessel. Five minutes after that 1.0 mL of standard or sample solution was added in this reagent solution. The change of cell potential was continuously recorded at 3.0-second interval. The total time for one measurement was seven minutes. The final volume of reaction solution in the vessel was 25.0 mL. All measurements were performed under stirring conditions at room temperature.

A calibration graph for the experiment was constructed by plotting the change of potential, ΔE, recorded in the fixed-time interval (1 min after thiol was added), *versus* negative logarithm of the thiol concentration.

For spectrophotometric experiment the reaction solution was delivered from the reaction vessel to the flow cell with a peristaltic pump (IPC ISMATEC, Glattbrugg, Switzerland). All data were continuously recorded, stored, and processed by a personal computer.

3. Results and Discussion

The proposed method is based on redox reaction of iodate with thiols in acidic medium, in the presence of small quantity of iodide. Iodide is added in reaction solution because of stabilization of initial potential of iodide ion selective electrode (IISE).

Fig. 1A shows dynamic response curve of the cell, when NAC (1.0×10^{-4} M) was added in the reaction vessel.

3. 1. Optimization Method

The effect of the concentration of potassium iodide is studied in the range from 1.0×10^{-6} to 1.0×10^{-4} M, Fig. 2. The initial potential is unstable at concentration lower than 1.0×10^{-6} M. The analytical signal (ΔE/mV) has the highest value at concentration 1.0×10^{-6} M. On the other hand the initial potential is more stable and measurements are reproducible at higher concentrations of KI. Also, for the experiments for higher concentration of KI we need much more time to reach steady-state potential in the first step of redox reaction. Taking this in consideration, as a compromise, 5.0×10^{-6} M solution of KI was selected as optimum.

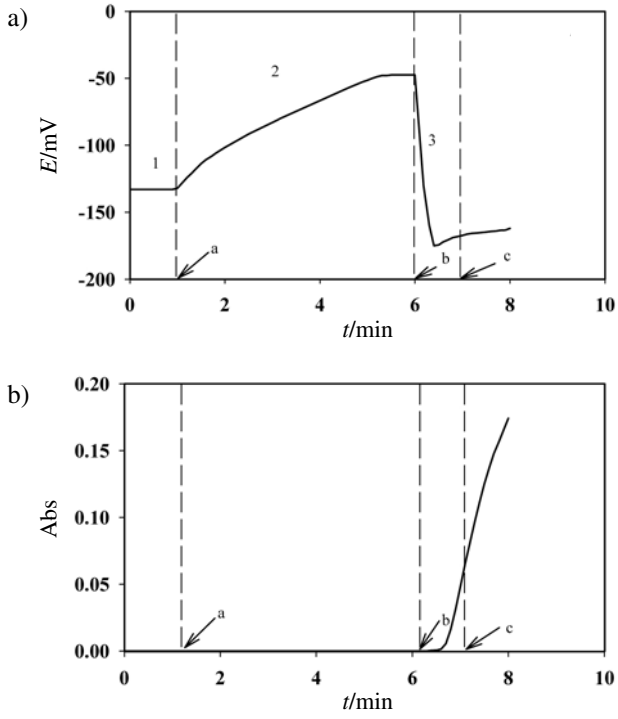

Figure 1. A) Dynamic response curve of the cell at determination of NAC, c(NAC) = 1.0×10^{-4} M. 1- area of initial stable potential; 2- area of reaction between iodide and iodate; 3- area of reaction between iodate and NAC. a. – Iodate was added, $c(KIO_3) = 1.0 \times 10^{-4}$ M, in acidic solution of iodide, c(KI) = 5.0×10^{-6} M. b. – NAC was added and corresponding potential E_1 was recorded c.- The fixed time in which potential E_2 was recorded and ΔE ($E_2 - E_1$) was calculated. B) Absorbance as a function of time for spectrophotometric experiment with starch: a.- Iodate was added, $c(KIO_3) = 1.0 \times 10^{-4}$ M, in acid solution of KI and starch, c(KI) = 5.0×10^{-6} M. b.- NAC was added, c(NAC) = 1.0×10^{-4} M. c.- The fixed time in which the change of potential was calculated.

The effect of potassium iodate was studied in concentration range from 1.0×10^{-5} to 5.0×10^{-4} M, Fig. 3. A KIO_3 concentration of 1.0×10^{-4} M was selected for further experiments.

Fig. 4 shows effect of concentration of H_2SO_4 on the analytical signal (ΔE/mV). The highest change of the po-

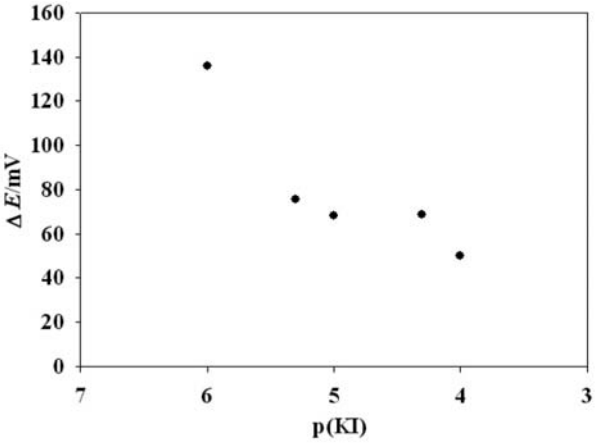

Figure 2. Effect of KI concentration on the change of potentials. Experimental condition: c(NAC) = 5.0 × 10^{-6}, c(KIO_3) = 1.0 × 10^{-4} M, c(H_2SO_4) = 0.1 M. Final volume: 25.0 mL

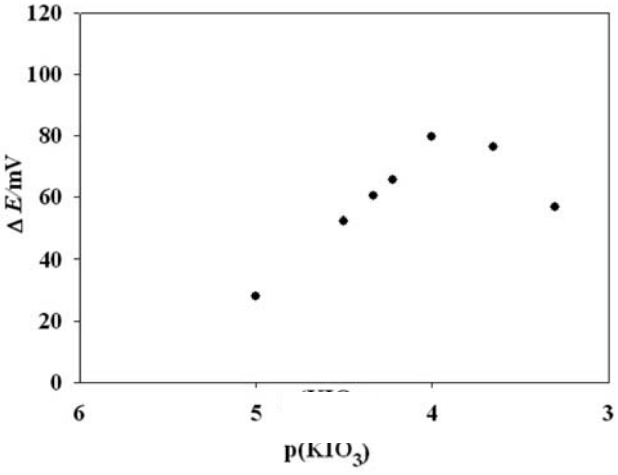

Figure 3. Effect of KIO_3 concentration on the change of potentials. Experimental condition: c(NAC) = 5.0 × 10^{-5} M, c(KI) = 5.0 × 10^{-5} M, c(H_2SO_4) = 0.1 M. Final volume: 25.0 mL

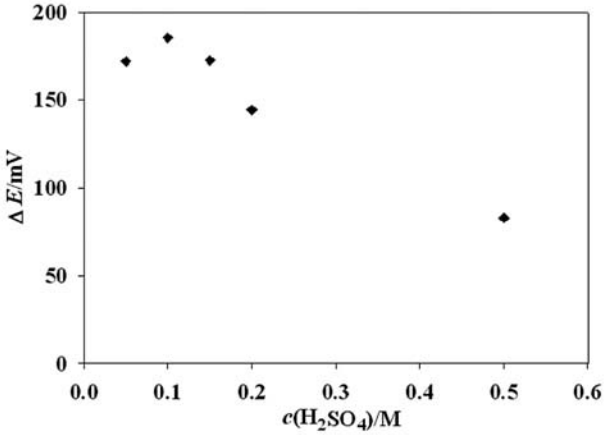

Figure 4. Effect of H_2SO_4 concentration on the change of potentials. Experimental condition: c(NAC) = 5.0 × 10^{-5} M, c(KI) = 5.0 × 10^{-6} M, c(KIO_3) = 1.0 × 10^{-4} M. Final volume: 25.0 mL

tential of used cell was obtained at 0.1 M H_2SO_4 which was applied in all experiments.

The optimum interval of time in which reaction between iodide and iodate occurred (first step of redox reaction) was tested. Three sets of determination underwent on a trial basis: thiol was added at 3 min, 5 min and 10 min, after iodate have been added. The five minutes period was selected as an optimum period of time for the first step of redox reaction.

Performed trials for this selected time have the optimal repeatability of measurements and the obtained coefficient of regression indicated good linearity.

3. 2. Interference Studies

The study of interferences for PEN and NAC determination was performed by applying the proposed method to a determination of NAC in presence of possible concomitant species. The tested concentration for NAC was 1.0 × 10^{-5} M. The tolerance limit was taken as the amount of added species that caused an error less than ± 5%. The results are given in Table 1.

Table 1. Tolerance limits for interferences for the determination of 1.0 × 10^{-5} M NAC.

Substance	Tolerance limit ratio (molesubstance/moleNAC)
Sucrose, glucose, fructose, lactose	1000*
Ca^{2+}, Mg^{2+}, Na^{+}, SO^{42-}, NO^{3-}	
Citric acid, oxalic acid	400
HPO_4^{2-}, PO_4^{3-}	>200*
Vitamin B	10
Methionine	1
SO_3^{2-}	0.2

* Maximum ratio tested

3. 3. Application Method

Under the optimized conditions previously given, rectilinear calibration graphs were obtained in the concentration ranges from 1.0 × 10^{-6} to 1.0 × 10^{-4} M, for both, NAC and PEN.

In order to evaluate the potential of the proposed method for analysis of a real sample, the method was applied for analysis of commercially available pharmaceutical samples. The validity of the proposed procedure was also assured by the recovery of standard additions. The results are summarized in Table 2.

4. Conclusion

In this work iodate and small quantity of iodide as a reagents system was used in kinetic potentiometric determination of D-penicillamine and *N*-acetyl-L-cysteine in pharmaceutical samples. The proposed method as a sim-

Table 2. Results for the determination of *N*-acetyl-L-cysteine and D-penicillamine in real samples

Sample	Amount Labelled	Amount Found ± SD (n = 5)	Recovery (%)	Amount Taken (mg)	Amount Added (mg)	Found ± SD (n = 3)	Recovery (%)
TWINLAB® NAC					10.00	13.16 ± 0.55	101.20
(N-acetyl-L-cysteine)	600.00 mg	598.44 ± 3.30	99.74	3.00	50.00	53.40 ± 0.75	100.75
					100.00	104.15 ± 0.45	101.12
Fluimukan®					10.00	11.87 ± 0.35	98.90
(N-acetyl-L-cysteine)	500.00 mg	495.90 ± 2.60	99.18	2.00	50.00	51.50 ± 0.55	99.04
					100.00	101.23 ± 0.45	98.30
Metalcaptase® 300					10.00	11.87 ± 0.55	98.90
(D-penicillamine)	300.00 mg	293.50 ± 3.00	97.83	2.00	50.00	51.32 ± 0.63	98.70
					100.00	100.7 ± 0.74	98.73

ple and inexpensive procedure was successfully applied for the determination of the studied drugs in the concentration ranges from 1.0×10^{-6} to 1.0×10^{-4} M.

5. References

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Povzetek

Opisujemo optimizacijo in uporabo enostavne kinetične metode za določitev D-penicilamina (PEN) in *N*-acetil-L-cisteina (NAC) v farmacevtskih pripravkih. Metoda je osnovana na reakciji med tiolom in jodatom v prisotnosti majhne vsebnosti jodida. Razlike v potencialu, ΔE, v enakih časovnih intervalih smo merili z uporabo jodidne ionoselektivne electrode. Metoda je linearna v območju koncentracij $1{,}0 \times 10^{-6}$ do $1{,}0 \times 10^{-4}$ M analita. Meji določitve sta PEN: $9{,}0 \times 10^{-8}$ M in NAC: $1{,}0 \times 10^{-7}$ M. Metodo smo uporabili v preiskavah farmacevtskih pripravkov.