


## APPLICATION OF THE KINETIC ENZYMATIC METHOD FOR BENZALKONIUM CHLORIDE DETERMINATION IN AEROSOL PREPARATION

O. Koval'ska, Assistant  
M. Blazheevskiy, Doctor of Chemistry, Full Professor  
National University of Pharmacy, Ukraine

A new kinetic photometric method for BAC determination based on an inhibition of the enzymatic (cholinesterase) reaction was proposed. The reaction rate was detected at unhydrolysed acetylcholine residue, which is determined by the amount of peracetic acid, produced during the impact of  $H_2O_2$  on it. Indicator reaction is a reaction of peracetic acid with 4-ethoxyaniline interaction that leads to the formation of azoxyphenetole with  $\lambda_{max} = 358 \text{ nm}$  ( $\lg \epsilon = 4.18$ ). The measurement velocity of changing of light absorption vs. time ( $\Delta A/\Delta t, \text{min}^{-1}$ ) give a chance to quantitatively determination of BAC. The results confirmed that the method is linear at concentrations ranging from  $1.0 \times 10^{-6} \text{ mol/L}$  to  $5.0 \times 10^{-6} \text{ mol/L}$ . Depending calibration equation  $\Delta A/\Delta t, \text{min}^{-1}$  from the village BAC has the form:  $\text{tg} \alpha = 5093 c + 0.0075$  ( $r = 0.999$ ). LOD was  $0.4 \times 10^{-6} \text{ mol/L}$ . The method was satisfactorily applied to the determination of BAC in nasal pharmaceutical preparation «Aqua-rinosol». The recovery was 99.9%, RSD = 2.7 % ( $n=5$ ,  $P=0.95$ ). This method is beneficial because high sensitivity and selectivity can be achieved using relatively simple technical equipment.

**Keywords:** Benzalkonium chloride, kinetic photometric method, determination, aerosol preparation.

Conference participants,  
National championship in scientific analytics,  
Open European and Asian research analytics championship

 <http://dx.doi.org/10.18007/gisap:msp.v0i13.1658>

### Introduction

«Aqua-rinosol» is a medicine for treatment of dryness inside the nose (nasal passages). It helps add moisture inside the nose in order to dissolve and soften thick or crusty mucus. In babies and young children with blocked nostrils who cannot blow their noses, this product helps to make the mucus easier to remove with a nasal bulb syringe. This helps relieve stuffiness and makes breathing easier. This product contains the purified gentle salt solution (also called saline or sodium chloride solution) and Benzalkonium chloride (BAC) as the preservative ( $w=0.015 \%$ ). It is a mixture of alkylbenzyltrimethylammonium chlorides of various even numbered alkyl chain lengths ( $R=C_8, C_{10}, C_{12}, C_{14}, C_{18}$ ). This product is a nitrogenous cationic surface-acting agent belonging to the quaternary ammonium group, especially inhibitors of acetylcholinesterase.

Considering the widespread use in pharmaceutical formulation the determination of low concentration preservative constitutes a challenging problem in current pharmaceutical analysis and is a topic of global interest. The standard method for determination of BAC in pharmaceutical compositions is reversed-phase high performance liquid chromatography [1-6].

### Experimental section

#### Instruments and apparatus

All the spectral absorbance measurements were made using the “photoelectric concentration colorimeter (CPC-2) (Zagorsky Optical & Mechanical plant, Russia).

pH value was measured with the laboratory ionomer I-160M (Belarus) by using EGL 43-07 pH glass electrode together with the auxiliary chloride silver electrode of EAL-1m3.1 type, standard with potassium chloride.

#### Materials and reagents

All chemicals and reagents used were of analytical grade: Pharmacopoeial acetylcholine chloride medication – 0.2 g per amp/5 ml (manufactured by “VECTOR” – State Science Center of virology and biotechnology of the Russian Federation” (Russia); dry protein drug of cholinesterase from horse serum – 8 mg / L (IV class) 22AE / mg (SMU “Biomed”, Russia). *N* - phenetidine (4 - ethoxyaniline - 98%) (SIGMA - ALDRICH); Series: A0281408s, New Jersey, USA; “Stabilized Hydrogen Peroxide 30-40%” (LLC “Inter - Synthes”, Boryslav, Ukraine). The content of hydrogen peroxide was determined by SPU according to the monograph “High-test hydrogen peroxide solution 27.5-31.0%.

High purity double distilled water was used throughout.

#### Preparation of the standard stock solution

Standard stock solution was prepared in the double distilled water: 0.09910 g of standard solution of Benzalkonium chloride (50%) were added into the 500 ml volumetric flask, and it was diluted up to the mark  $x$  (ppm). 0.1 ml of this solution were added into a 10 ml volumetric flask and was diluted up to 10 ml. From the standard stock solution, a standard solution was prepared containing  $2.12 \cdot 10^{-5} \text{ mol/L}$ . Alternatively, derived from the corresponding regression equation.

#### Preparation of the solution of Cholinesterase (ChE)

It was prepared in the double distilled water by adding 80mg of dry cholinesterase drug in volumetric flask, and it was shaken and diluted up to the mark. After that it was kept in the thermostate for 10 minutes at  $38^\circ\text{C}$  above Zero [7].

#### Phosphate buffer solution (pH 8.35) preparation

It was prepared by dissolving: 35.75 g of sodium hydrogen phosphate were added to 500 ml flask, then 300 ml of double-distilled water were added; the mixture was dissolved; Then 19 ml of 0.1 mol/L solution of hydrochloric acid were added, stirred and diluted with the double distilled water to 500.0 ml. The prepared pH solution is potentiometrically controlled.

## Hydrogen peroxide solution (10%).

It was prepared by dilution of the appropriate high-test hydrogen peroxide with double-distilled water. The exact hydrogen peroxide content in prepared 10% solution is determined permanganatometrically.

## P-phenetidine hydrochloride solution preparation (1%).

*N* - phenetidine hydrochloride (Ph) was extracted from the base by hydrogen chloride precipitation in the chloroform solution. Dissolve 1.00g *p*-phenetidine hydrochloride in 80 ml of double-distilled water in 100 ml capacity measuring bottle and dilute it to volume.

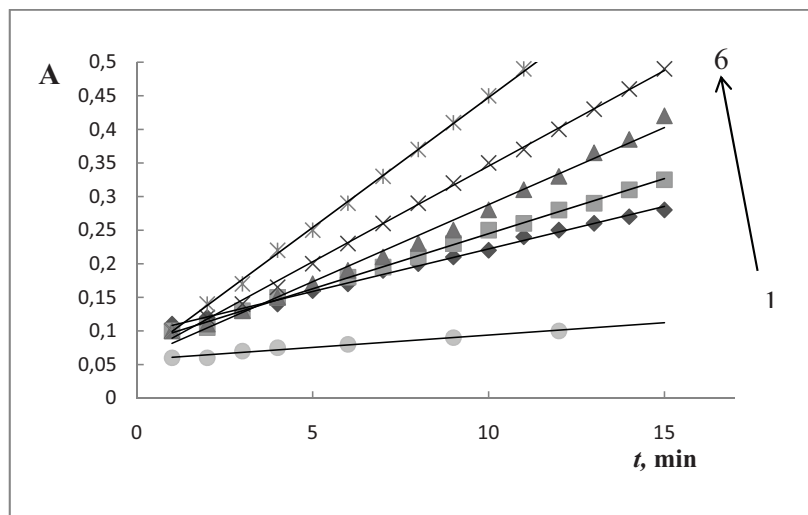
## Preparation of the enzyme substrate of the acetylcholine chloride solution (ACh).

The content of an ampoule of pharmacopoeia drug acetylcholine chloride (0.2 g) is dissolved in 200 ml of double-distilled water. For that, open an ampoule, add 4.0 ml of water with pipette, and shake until acetylcholine is completely dissolved. Then pour the acetylcholine solution into 200 ml capacity measuring bottle and dilute with the double-distilled water to the volume.

## General recommended procedure.

10.0 ml of 0.2 mol/L phosphate buffer solution (pH=8.3) were transferred in 20 ml graduated test tube with ground plug consistently. The accurate volumes of test solution (BAC or "Aqua-rinosol") over the concentration range of  $x$  mol/L were added into a standard flask.

After that, 2.0 ml of cholinesterase were added while stirring; a timer was switched on; each solution was thoroughly shaken up and thermostated for 15 min. Then 1.0 mL of 1% acetylcholine solution was added quickly; a timer was switched on; it was shaken thoroughly and thermostated for 10 min again. Then 2.0 ml of 10% hydrogen peroxide solution were added; kept for 10 min in the thermostat, and then 1.0 ml of 1% *p*-phenetidine solution (Ph) was added. The mix was diluted with the distilled water to volume of 20 ml. 5 ml drop of the solution was transferred to a 100 ml volumetric flask and made up to the mark with water. Suitable dilution was made to fit the applicable concentration range and the above described procedures were followed.



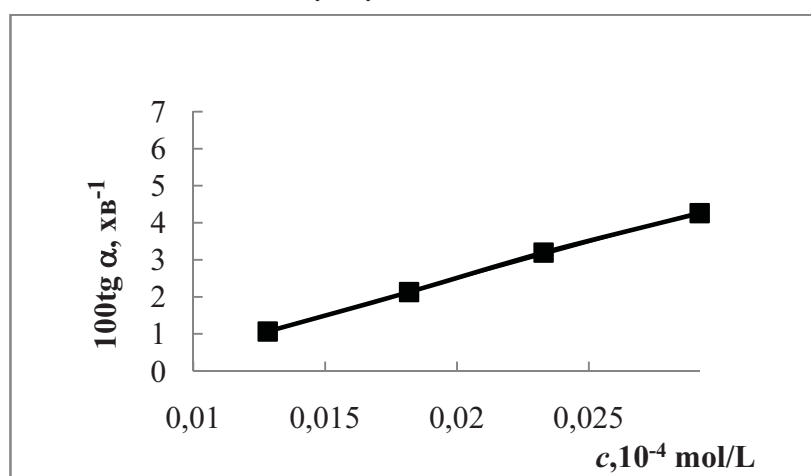
**Fig. 1: Kinetic curves of couple oxidation of *p*-phenetidine by hydrogen peroxide in presence of the system: 1 – ACh+ChE, 2- 5 – ACh+(ChE+BAC), 6 – ACh. Initial concentrations: pH 8.3 (phosphate buffer),  $w(\text{Ph}) = 0.05\%$  (w/v),  $c(\text{H}_2\text{O}_2) = 1.5 \cdot 10^{-2}$  mol/L,  $w(\text{ACh}) = 0.05\%$  (w/v),  $c(\text{ChE}) = 2.2$  AE/mL  $t = 37 \pm 0.1$  °C,  $\lambda = 358$  nm.**

The nominal concentration of the bottles was calculated either from calibration graph or using the regression equation.

## Results and discussion

The proposed method for BAC detection is based on an enzymatic (cholinesterase) reaction. Mechanism of analytical reaction and sensitivity of this method are the same as (or similar to) those in the human body. The conventional enzymatic method is based on the ability of cholinesterase to accelerate hydrolytic

decomposition of the neurotransmitter (substrate) acetylcholine to choline and acetic acid. The reaction rate is detected at unhydrolysed acetylcholine residue, which is determined by the amount of peracetic acid, produced during the impact of  $\text{H}_2\text{O}_2$  on it. Indicator reaction is a reaction of peracetic acid with 4-ethoxyaniline interaction, which leads to the formation of azoxyphenetole with  $\lambda_{\text{max}} = 358$  nm ( $\lg \epsilon = 4.18$ ). The measurement velocity of changing of light absorption vs time ( $\Delta A / \Delta t$ ,  $\text{min}^{-1}$ ) gives a chance to perform quantitative determination of BAC.



**Fig. 2: Dependence of the relative reaction rate on BAC concentration. Initial concentrations: pH 8.3 (phosphate buffer),  $w(\text{Ph}) = 0.05\%$  (w/v),  $c(\text{H}_2\text{O}_2) = 1.5 \cdot 10^{-2}$  mol/L,  $w(\text{ACh}) = 0.05\%$  (w/v),  $c(\text{ChE}) = 2.2$  AE/mL  $t = 37 \pm 0.1$  °C,  $\lambda = 358$  nm.**

Tab. 1.

Accuracy and precision of the proposed method

| No. | BAC taken*,<br>mol /L | BAC found,<br>$\bar{X} \pm \Delta X$ , mol /L | N | RSD<br>(P=0.95) | $(\bar{X} - \mu) \cdot 100\% / \mu$ |
|-----|-----------------------|---|---|-----------------|-------------------------------------|
| 1.  | $1.065 \cdot 10^{-6}$ | $(1.059 \pm 0.015) \cdot 10^{-6}$             | 5 | 2.52%           | 0.56%                               |
| 2.  | $3.195 \cdot 10^{-6}$ | $(3.179 \pm 0.05) \cdot 10^{-6}$              | 5 | 1.49%           | 0.50%                               |
| 3.  | $4.26 \cdot 10^{-6}$  | $(4.244 \pm 0.03) \cdot 10^{-6}$              | 5 | 0.63%           | 0.42%                               |

\*calculated according to the certificate.

According to the optical-time relations, the kinetic curves are plotted and the slope of the first 15 min is found (Fig. 1). According to the data received a slope - finite analyte concentration calibrated relation is obtained,  $c, \mu_{\text{mol}} / \text{L}$ .

A calibration curve equation is solved by the least squares method (Linear regression):  $\text{tg}\alpha = bc + a$ , where  $a$ ,  $b$  are Y-axis intercept and slope, ( $\text{tg}\alpha$ ,  $\text{min}^{-1}$ ) respectively. Switch on timer and every other minute scan photometrically each solution for 15 min on photoelectric colorimeter CPC-2, use colour filter №2 and 1.0 cm cuvette (Fig. 2).

The results confirmed that the method is linear at concentrations ranging from  $1.0 \times 10^{-6}$  mol/L to  $5.0 \times 10^{-6}$  mol/L. Depending on the calibration equation  $\Delta A / \Delta t$  ( $\text{tg}\alpha$ ),  $\text{min}^{-1}$  the BAC has the form:  $\text{tg}\alpha = 5093c + 0.0075$  ( $r = 0.999$ ). LOD was  $0.4 \times 10^{-6}$  mol /L. The precision and accuracy of the proposed method was studied by performing the experiment five times at three different concentration levels (low, medium and high) of BAC (table 1).

The method was satisfactorily applied to the determination of BAC in

nasal pharmaceutical preparation «Aqua-rinosol». The recovery was 99.9%, RSD = 2.7 % ( $n=5$ ,  $P=0.95$ ).

### Conclusion

The proposed kinetic-spectrophotometric method for the determination of BAC in pharmaceutical samples of «Aqua-rinosol» preparation described in this paper is simple, quick, inexpensive, and thus appropriate for routine quality control analysis of active drugs in the laboratories of hospitals, pharmaceutical companies and research institutions. It should also be suitable for the developing countries. The validation of the method shows that the results obtained are in good agreement with the standard Pharmacopoeial method.

### References:

1. Hashem A.I. Aani., J. Appl. Pharm. Sci. – 2016., Vol. 6, No. 5., pp. 80-89.
2. Kostić D.E., J. Chem. – 2012., Vol. 9, No. 3., pp. 1599-1604.
3. Gubin M., Pivovarova S.,

Pharmacy. – 2012., Vol. 4., pp. 9-12.

4. Kabeer AS, Ashish TP., J. Trace Anal. Food & Drugs. – 2013., Vol. 21, No. 1., pp. 14-21. <https://doi.org/10.7726/jtafd.2013.1002>

5. Hanni M.H. at all., Euras. J. Anal Chem. – 2015., Vol. 10, No. 1., pp. 46-67.

6. Metha J., Patidar K., Vyas N., E-Jornal Chem. – 2010., Vol. 7, No. 1., pp. 11-20. <https://doi.org/10.1155/2010/312379>

7. Blazheyevskiy M.Ye., Dyadchenko V.V., Pharm. J. – 2004., Vol. 2., pp. 52-58 (Ukrainian).

### Information about authors:

1. Mykola Blazheyevskiy – Doctor of Chemistry, Full Professor, National University of Pharmacy; address: Ukraine, Kharkiv city; e-mail: [blazejowski@ukr.net](mailto:blazejowski@ukr.net)

2. Olena Koval'ska – Ph.D. of Pharmaceutics, Assistant, National University of Pharmacy; address: Ukraine, Kharkiv city; e-mail: [olena\\_kovalska@mail.ru](mailto:olena_kovalska@mail.ru)

