Determination of *N*-Acetylcysteine in Tablets by Means of Chemiluminescence Inhibition Method

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The technique for quantitative determination of N-acetylcysteine in pharmaceutical preparations using a new chemiluminescence inhibitor – N-acetylcysteine in the system H_2L (luminol)–H_2O_2–Hemoglobin was developed. The objects of research were tablets containing N-acetylcysteine. The method of N-acetylcysteine quantitative determination in pharmaceuticals based on the inhibition of chemiluminescence in the system H_2L–H_2O_2–Hemoglobin was developed. The calibration curve was linear over the concentration range 0.06 – 0.82 μg/mL, LOD (3S) = 0.02 μg/mL, LOQ (10S) = 0.82 μg/mL. No interferences were observed in the presence of common components of the tablets such as microcrystalline cellulose, lactose monohydrate, corn starch, magnesium stearate, Opadry II 85F28751 White, polyethylene glycol 6000, polyvinyl alcohol, talc, titanium dioxide (E 171), citric acid, sodium bicarbonate, lemon flavor, adipic acid, povidone and aspartame (E 951). RSD = ± 1.45% (δ = –1.05%), RSD = ±0.64% (δ = –0.50%) and RSD = ±1.13% (δ = –0.16 %) for the "Acetylcystein 200 Heumann Brausetabletten" (Germany), "AC-FS" ("Farmastart", Ukraine) and "Acestad" tablets (Germany), respectively. The proposed method is promising for further research on the subject of its application for the determination of N-acetylcysteine in drugs, in the absence of ascorbic acid.

Keywords: N-acetylcysteine, luminol, inhibitor, chemilumeniscence method

Визначення *N*-ацетилцистеїну у пігулках методом інгібування хемілюмінесценції

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В роботі розроблено методики кількісного визначення N-Ацетилцистеїну в лікарських препаратах за його здатністю інгібувати хемілюмінесценцію в системі H_2L (Люмінол)–H_2O_2–Гемоглобін. Об'єктами дослідження були лікарські препарати, що містять N-ацетилцистеїн. Показано, що такі загально відомі допоміжні складники пігулок як мікрокристалічна целюлоза, лактоза, кукурудзяний крохмаль, магній стеарат, опудрювач Opadry II 85F28751 White, ПЕГ 6000, полівініловий спирт, тальк, титану діоксид (Е 171), цитратна кислота, натрій гідрогенкарбонат, цитратний ароматизатор, адипінова кислота, повідон та аспартам (Е-951) не впливають на результати визначення. Градуювальний графік зберігав лінійність в інтервалі концентрацій 0.06–0.82 мкг/мл, LOD (3S) = 0.02 мкг/мл, LOQ (10S) = 0.82 мкг/мл. Відносне стандартне відхилення (RSD) та правильності (δ) визначення N-Ацетилцистеїну у шипучих пігулках "Acetylcystein 200 Heumann Brausetabletten" (Німеччина), "AC-FS"(Україна) та "Acestad" (Німеччина) становили ±1.45% (δ = –1.05%), ±0.64% (δ = –0.50%) та ±1.13% (δ = –0.16%), відповідно. Запропонований метод є вельми перспективним для подальших досліджень на предмет застосування для здійснення кількісного визначення N-Ацетилцистеїну в інших лікарських препаратах за відсутності аскорбінової кислоти.

Ключові слова: *N*-Ацетилцистеїн, люмінол, інгібітор, метод хемілюмінесценції

N-acetylcysteine (*N*-acetyl-*L*-cysteine, NAC) is a substance that has an antioxidant effect due to the presence of a sulfohydryl group in its structure. NAC is able to increase the synthesis of glutathione, which is an important antioxidant factor in intracellular protection and provides support of functional activity and cellular morphological integrity. In medicine, NAC is used not only as an expectorant, pneumoprotective and cardioprotective agent, but also as a detoxifying agent for acute paracetamol poisoning. The literature provides medical research on the feasibility of using NAC in the complex treatment of certain types of cancer and Alzheimer's disease. NAC can also be used in the treatment of patients with HIV-infection as

a means to enhance the immune function of the body.

NAC is released in the form of effervescent tablets of 0.2 and 0.6 g, granules for the preparation of 2 % solution for internal use of 0.2 and 0.6 g, usually in combination with ascorbic acid, 20 % solution for inhalation by 5 and 10 mL in ampoules, as well as 5 % solution for injection in 10 mL and 10 % in ampoules of 2 and 3 mL.

At present, for quantitative determination of NAC various methods of analysis are usually used. British and European Pharmacopoeia recommend the quantitative determination of the content of the NAC in the substance by iodimetric titration in an acidic chloride-containing medium while cooling, in solution for injection – HPLC method [1, 2], and the US Pharmacopeia proposes for the quantitative determination of the NAC content in pure substance to use a HPLC method with a UV-detector [3]. As a variant of the iodimetric method for the determination of NAC in the substance and drugs in the absence of ascorbic acid, the method of peroxyacidimetric titration in an acidic medium in the presence of potassium iodide at room temperature was proposed [4]. Also, procedures are proposed for the sequential determination of ascorbic acid and NAC (after adding iodide) in drugs using as a titrant of diperoxyazelaic acid [5, 6] and potassium hydrogenperoxomonosulphate [7] with the fixation of the endpoint of the titration by the potentiometric method. For the quantitative determination of NAC voltamperometric [8, 9] and amperometric [10, 11] methods have been used.

The procedures for NAC determination by spectrophotometry [12, 3], as well as the method of chemiluminescent determination of NAC, based on the effect of chemiluminescence inhibition, which appears in the system of luminol – hydrogen peroxide – Cu(II) [14] and chemiluminescent determination of NAC by reaction with nitrate of 9-cyano-10-methylacridine [15] have been range of application.

Previously, in series of papers the advantages of using the chemiluminescence (CL) method for the quantitative determination of various medicinal and biologically active substances by the effect of inhibition of chemiluminescence in the system Luminol (H_2L) – H_2O_2 – hemoglobin (*Hb*) were shown [16, 17].

We have discovered the possibility of quantitative determination of NAC in the pharmaceutical preparations by CL method by the inhibition effect of the resulting chemiluminescence in the H_2L – H_2O_2 – *Hb* analytical system.

Experimental part and instrumentations

The objects of the study were: the pure substance of the *N*-acetylcysteine (*N*-acetyl-*L*-cysteine) produced by Moehs Catalana S.A. (the content of the main substance was 99.5 %, determined by the iodimetry according to the SPhU [18]) and pharmaceutical dosage forms containing *N*-acetylcysteine: "Acetylcystein 200 Heumann Brausetabletten", produced by Heumann Pharma GMBH Nürnberg Ein Unternehmen der Searle-Gruppe (Germany), 200 mg active substance; tablets coated with "AC-FS" produced by "Farmastart" (Ukraine), 200 mg of active substance and "Acestad" effervescent tablets produced by STADA Arzneimittel AG (Germany), 200 mg active substance.

Solutions were prepared by volume-weight method at 293 K. Double distilled water (DDW) was used to prepare the solutions in all cases.

Standard $1 \cdot 10^{-3}$ mol/L solution of Luminol (5-amino-2.3-dihydro-1.4-ftalazindion, H_2L, RPF «Synbias», Ukraine). 0.217 g of 3-Aminophthalhydrazide with qualification «R» was dissolved in 100 mL volumetric flask in 10 mL of 0.01 mol/L sodium hydroxide solution and brought to the mark by DDW. The solution was kept in a dark place.

0.1 mol/L solution of sodium hydroxide was used for medium pH stabilization, solutions pH was controlled by the laboratory potentiometer «Ionomer I-130» with glass electrode ESL-43-07 and silver-chloride electrode and laboratory ionomer I-130 (ZIP, Gomel, Belarus). All solutions were prepared with DDW.

Hydrogen peroxide (H_2O_2) 5.8 % (wt.) solution was prepared from 58 % high pure preparation (produced by LTD "Inter-Syntes", Boryslav, Ukraine) by its 10 times dilution with DDW: 10 mL was transferred into volumetric flask of 100 mL and volume was brought to the mark at 293 K. This solution was stored at reduced temperature of + 8 – 10 °C. The content of hydrogen peroxide in solution was controlled by perganatometric titration [18]. Working solution of H_2O_2 0.058 % (wt.) ($1.7 \cdot 10^{-2}$ mol/L) was obtained by the appropriate dilution of the original solution exactly 100 times. The working solution can be used throughout the day.

As a catalyst human blood hemoglobin (*Hb*) produsedby "Simko Ltd", Lviv, Ukraine was used. Hemoglobin solution 100 μg/mL was prepared by dissolving in a 100 mL volumetric flask of 10 mg hemoglobin in 50 mL DDW by heating and adding 1 mL of 1.0 mol/L sodium hydroxide solution. Volume was brought to the mark with DDW at 293 K and stirred. Working solution of hemoglobin was prepared by dilution with DDW of the initial one exactly 100 times. The working solution can be used throughout the day.

Standard solution sample (SSS) of NAC 2 mg/mL was prepared as follows: in a 100 mL volumetric flask 0.2000 g of NAC was dissolved and brought to the mark by DDW. Working solution of NAC was obtained by the appropriate dilution of the original solution in the required number of times for analysis.

The intensity of chemiluminescence was measured in relative units on the device with photoelectric multiplier FEU-84-A, using measurement of low currents IMT-0.5 and quick-acting (time constant 0.1 s) automatic potentiometer. Reaction that accompanies CL was performed in a cylindrical 30 mm diameter quartz cell with work volume of 10 mL. The following order of reagents mixing was

performed: to the mixture of luminol indicator in alkali solution and H_2O_2, with the presence or absence of NAC solution in control experiment, 0.50 mL of *Hb* was added with the help of dosage pipette P-1 and kinetic curve of chemiluminescence intensity (*I*) in relative units (*I*) – time (s) was registered. Dosage pipette is built in to the mobile keeper, that isolates photocatode of photoelectric multiplier from outside light, and further allows to work at the common lighting. All experiments were performed at 293 K. To characterize the inhibitory action of NAC on the maximum intensity of CL value I_o/I was calculated, where I_o – the maximum intensity of CL in the system H_2L – H_2O_2 – *Hb* (without NAC, control experiment), *I* – maximum intensity of CL in the same system with the addition of inhibitor: H_2L– H_2O_2 – NAC – *Hb* (working experiment).

The influence of the order of mixing of solutions and of concentrations of Luminol, sodium hydroxide, hydrogen peroxide, NAC and Hemoglobin solutions on the intensity of the appearing CL was studied and it was found that the mixing order is optimal, when the Hemoglobin solution is added in the end.

Construction of calibration graph. To chemiluminescent quartz cell solutions were added consistently as follows: 1.00 mL of $1\cdot10^{-3}$ mol/L H_2L, 0.50 mL of 1 mol/L sodium hydroxide solution, (10 – x) mL of DDW, where x is the total volume of all reagents and samples, (mL), 0.50 mL of $1.7\cdot10^{-2}$ mol/L H_2O_2 and 0.50 mL of dilute working-standard solution of NAC. Cell with the mixture was placed in chemiluminometer and 0.5 mL of working solution of hemoglobin with a concentration of 1 mg/mL was added.

Similarly, a control experiment with DDW instead of the use of dilute working-standard solution of NAC in the same amount was performed.

Results and discussion

As a result of the studies, it was found that under optimal conditions (c(NaOH) = 0.05 mol/L, $c(H_2O_2)$ = $8.53\cdot10^{-4}$ mol/L, $c(H_2L)$ = 10^{-4} mol/L, C(*Hb*) = $5\cdot10^{-2}$ μg/mL) NAC shows an observable inhibitory effect on the appearance of CL in the H_2L – H_2O_2 – *Hb* system. This phenomenon was used to elaborate a new procedure for quantitative determination of NAC in solutions of substance and drugs.

The presence of NAC in the system H_2L – H_2O_2 – *Hb* leads to a decrease in the maximum intensity of CL, indicating inhibition of CL reaction. This effect increases with increasing concentration of the inhibitor of the process. The dependence I_0/I on the concentration of NAC (μg/mL) was linear in the concentration range 0.06 – 0.82 μg/mL. CL calibration curve of NAC determination is presented in Fig. 1.

The linear equation is $I_o/I = 4.4 \pm 0.2\cdot C + 1.4 \pm 0.1$ (r = 0.999), where C is the concentration of NAC solution, μg/mL; I_0 – maximum intensity of CL in the absence of NAC (rel. un.), I – maximum intensity of CL in the presence of NAC (rel. un.), LOD (3S) = 0.02 μg/mL, LOQ

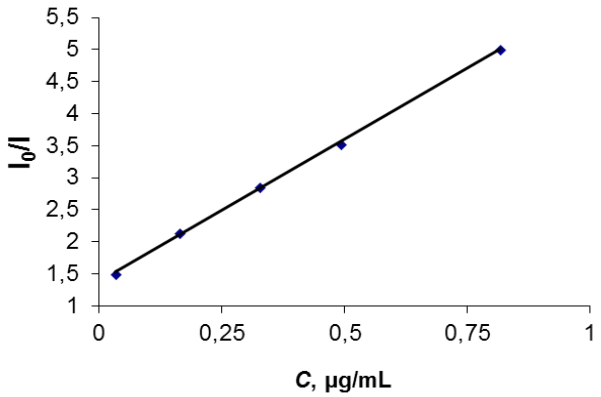

Fig. 1. Dependence of I_0/I v*s* the concentration of *N*-acetylcysteine in the chemiluminescence system H_2L – H_2O_2 – *Hb*.

The infuence of commonly used tablets excipients (microcrystalline cellulose – 95.0 mg, lactose monohydrate – 46,7 mg, corn starch – 8.0 mg, magnesium stearate – 0.3 mg, Opadry II 85F28751 White – 10.0 mg, polyethylene glycol 6000 – 75 mg, polyvinyl alcohol – 5 mg, talc – 50 mg, titanium dioxide (E 171) – 36 mg and citric acid - 350 mg, sodium bicarbonate – 450 mg, lemon flavor – 100 mg, adipic acid – 150 mg, povidone – 21mg, aspartame (E 951) – 20 mg) was investigated before the determination of the drugs in dosage forms. No interference could be observed with the proposed methods. This is evidenced in particular the results of the analysis we have obtained are presented in Table (δ < RSD). It has also been found that ascorbic acid and that contained in the combined medications with the NAC has a significant effect (inhibits chemiluminescence by more than 30% in the analytical system) on the analytical results, even in amounts well below the regulated (12.5 mg per tablet).

Quantitative determination of NAC in medical preparations was carried out by comparison with the standard, using linear range of the above-mentioned dependence of I_0/I *vs* NAC concentration.

Procedure of quantitative determination of NAC in "Acetylcystein 200 Heumann Brausetabletten" tablets 200 mg. Approximately 1.45 g of pounded tablets (accurately weighed) was dissolved in a 100 mL volumetric flask and brought to the mark with DDW. Similarly the NAC SSS was prepared by the volume-weighted method with a concentration of 2 mg/mL.

NAC working solutions were prepared by appropriate dilution immediately before the analysis (1: 200). When performing the experiment, a certain order of adding solutions was observed in accordance with the above (definition) method.

NAC content in *X* (g to one tablet) was calculated by the equation:

$$X = \frac{m_{st}\cdot(I_0/I)\cdot\overline{m}\cdot w\cdot 200}{\left(I_0/I_{st}\right)\cdot m_n\cdot 200\cdot 100\%},$$

where m_{st} – weight of NAC in SSS, g; (I_o/I) – maximum

value of I_o/I in working experiment, relative units; (I_o/I_{st}) – maximum value of I_o/I_{st} in SSS, relative units; $\overline{m}$ – average tablet weight (n = 20), g; m_n – mass of pounded tablets in a series used for analysis, g; w – content of the basic substance in the standard sample, %.

Procedure of quantitative determination of NAC in "AC-FS" coated tablets 200 mg. Approximately 360 mg of pounded tablets (accurately weighed) were dissolved in a 100 mL volumetric flask and brought to the mark with DDW. Similarly the NAC SSS was prepared by the volume-weighted method with a concentration of 2 mg/mL. NAC working solutions were prepared by appropriate dilution immediately before the analysis (1: 200). The following analysis was performed, as for determining the content of NAC in "Acetylcystein 200 Heumann Brausetabletten" tablets 200 mg; the content of NAC in grams in one pill (X) was calculated using the same formula.

Procedure of quantitative determination of NAC in "Acestsad" effervescent tablets 200 mg. Approximately 2 g of pounded tablets (accurately weighed) were dissolved in a 100 mL volumetric flask and brought to the mark with DDW. Similarly the NAC SSS was prepared by the volume-weighted method with a concentration of 2 mg/mL. NAC working solutions were prepared by appropriate dilution immediately before the analysis (1 : 200) . The following analysis was performed, as for determining the content of NAC in "Acetylcystein 200 Heumann Brausetabletten" tablets 200 mg; the content of NAC in grams in one pill (X) was calculated using the same formula.

The results of NAC quantitative determination are given in the Table 1.

Table 1. The results of NAC quantitative determination (n = 5, P = 0.95).

Dosage form of:	Found NAC, g	Metrological characteristics
"Acetylcystein 200 Heumann Brausetabletten" *N*-acetylcystein 0.200 g *(a = 0.201 g)	0.1995 0.1942 0.2021 0.1988 0.1997	$\overline{X}$ = 0.1989 S = ± 2.89·10^{-3} $S_{\overline{X}}$ = ± 1.29·10^{-3} $\Delta\overline{X}$ = ± 3.59·10^{-3} *RSD* = ± 1.45 % **δ = – 1.05 %
"AC-FS" *N*-acetylcystein 0.200 g *(a = 0.198 g)	0.1978 0.1954 0.1970 0.1986 0.1962	$\overline{X}$ = 0.1970 S = ± 1.265·10^{-3} $S_{\overline{X}}$ = ± 5.66·10^{-3} $\Delta\overline{X}$ = ± 1.57·10^{-3} *RSD* = ± 0.64 % **δ = – 0.05 %
«Acestad» *N*-acetylcystein 0.200 g *(a = 0.194 g)	0.1911 0.1937 0.1963 0.1920 0.1954	$\overline{X}$ = 0.1937 S = ± 2.20·10^{-3} $S_{\overline{X}}$ = ± 9.82·10^{-3} $\Delta\overline{X}$ = ± 2.73·10^{-3} *RSD* = ± 1.13 % **δ = – 0.16 %

Note: *a – NAC content by the certificate, g; **$\delta = (\overline{X} - a)100/a$.

Conclusions

The procedure was processed and the possibility of quantitative determination of *N*-acetylcystein in tablets using the method of chemiluminescence inhibition in the system $H_2L - H_2O_2 - Hb$ was shown.

No interferences were observed in the presence of common components of the tablets such as microcrystalline cellulose, lactose monohydrate corn starch, magnesium stearate, Opadry II 85F28751 White, polyethylene glycol 6000, polyvinyl alcohol, talc, titanium dioxide (E 171, citric acid, sodium bicarbonate, lemon flavor, adipic acid, povidone and aspartame (E 951).

RSD = ±1.45% (δ = –1.05%), *RSD* = ±0.64% (δ = –0.50%) and *RSD* = ±1.13% (δ = –0.16%) for the "Acetylcystein 200 Heumann Brausetabletten", "AC-FS" and "Acestad" tablets respectively; LOD (3S) of 0.02 μg/mL was achieved, LOQ (10S) = 0.82 μg/mL. The proposed method is promising for further research on the subject of its application for the determination of *N*-acetylcystein in drugs, in the absence of ascorbic acid which interferes with the determination.

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