DETERMINATION OF *N*-ACETYLCYSTEINE BY CHEMILUMINESCENCE METHOD

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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 as a mucolytic, expectorant and 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. 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.

Nowadays, for quantitative determination of NAC a variety of physico-chemical methods of analysis are usually used. SPhU and British Pharmacopoeia recommend the quantitative determination of NAC by iodometric titration in an acidic chloride-containing medium while cooling, and the US Pharmacopeia by chromatographic method. The method of supersaturation titration of NAC in an acidic medium in the presence of potassium iodide as a process catalyst is also known.

Now, for the quantitative determination of NAC a number of various instrumental methods are proposed, namely: the method of amperometric titration using perbenzoic acid; method of potentiometric titration using potassium hydrogenperoxomonosulphate and potentiometric determination of NAC using ISE; conductometric determination in aqueous medium using a solution of cuprum (II) sulphate as a titrant; voltamperometric determination on the electrode, modified by a film of osmium hexacyanocobaltate; various kinetic and spectrophotometric techniques, etc. The method of chemiluminescent determination of NAC, based on the effect of chemiluminescence inhibition, which appears in the system of luminol – hydrogen peroxide in the presence of a catalyst of cuprofluoric salts is of a great interest.

It is known that the method of chemiluminescence compared with other methods is faster, easier to perform, and also more sensitive and does not require the implementation of concentration or additional sample preparation.

We have discovered the possibility of quantitative determination of NAC in the substance by chemiluminescence method by the inhibition effect of the resulting chemiluminescence in the system Luminol – H_2O_2 – Hemoglobin (*Hb*). For the study, a NAC substance that meets the requirements of the SPhU was used.

The influence of the order of mixing of solutions and concentrations of Luminol, sodium hydroxide, hydrogen peroxide, NAC and Hemoglobin solutions on the intensity of the appearing chemiluminescence was studied.

As a result of the studies, it was found that the mixing order is optimal, when the Hemoglobin solution is added in the end. The presence of NAC in the system Luminol – H_2O_2 – *Hb* leads to a decrease in the maximum chemiluminescence intensity, indicating the inhibition in chemiluminescent reaction of luminol oxidation. This effect increases linearly with increasing concentration of the inhibitor of the process. Optimal concentrations of reagents in this chemiluminescent system are: c(NaOH) = 0.05 mol/L, $c(H_2O_2) = 8.53 \cdot 10^{-4}$ mol/L, $c(H_2L) = 10^{-4}$ mol/L, C(Hb) = $5 \cdot 10^{-2}$ μg·mL^{-1}.

The relatively clear and reproductive concentration dependence of the ratio of the maximum intensity of chemiluminescence in the absence and in the presence of NAC allowed the development of a proceeding for its chemiluminescent determination. (I0/ICL = 0.76 ± 0.07)·106c + 1.3 ± 0.2 (r = 0.995); where c is the concentration of NAC solution, M; I0 – maximum intensity of chemiluminescence in the absence of NAC (rel. un.), ICL – maximum intensity of chemiluminescence in the presence of NAC (rel. un.).

The calibration graph is linear in the range from 1.0× 10-7 to 5.0×10-6mol/L with detection limit of 4.27×10^{-7} mol/L, LOQ = 1.3×10^{-6} mol/L.

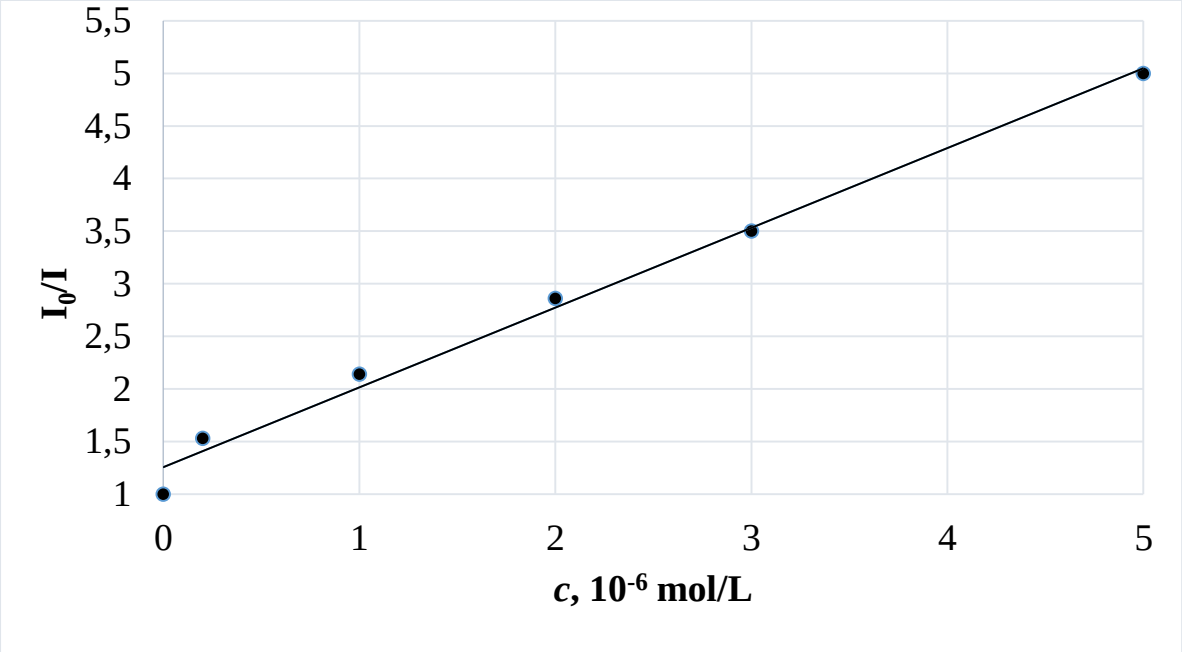

Fig. Dependence I_0/I on the concentration of *N*-acetylcysteine in the chemiluminescence system Luminol – H_2O_2 – *Hb*