# A CHEMILUMINESCENT METHOD FOR THE QUANTITATIVE DETERMINATION OF L-CYSTINE

Blazheyevskiy M.Ye., Bondarenko N.Yu.
***National University of Pharmacy*, *Kharkiv, Ukraine***
*tropikana2003@ukr.net*

*L*-Cystine is oxidized form of the well-known substitutable alpha-aminoacid of cysteine. It is known *L*-Cystine provides elasticity of keratin. This substance is included in the vitamin complexes to improve the appearance (skin and hair), biologically active additives and shampoos. The spectrum of drugs is wide and covers not only illnesses associated with the deterioration of the skin, but also cases of intoxication with heavy metals. As a food additive (E921) for the improvement of flour products, sodium and potassium salts of *L*-cystine are used. A drug with the same name *L*-Cystine exhibits antioxidant; Hepatoprotective; Detoxification Immunomodulatory Wound healing; Mucolytic and expectorant actions. Often, *L*-Cystine is used to treat diabetes, Alzheimer's disease, bronchitis, and protein deficiency. This substance is involved in the metabolism and helps with violations of connective tissue, it is also prescribed during rehabilitation after surgery and in diseases of the joints. The drug has two forms of release: capsules and ampoules. *L*-Cystine is also part of other combined medications. So, one sublingual tablet of the drug "ELTACIN" contains as active substances *L*-cystine 70 mg, glycine 70 ml, *L*-glutamic acid; Excipients: methyl cellulose 7.8 mg, magnesium stearate 2.2 mg.

The European Pharmacopoeia (EPh 8.0) for the quantitative determination of cystine recommends the method of inverse bromatometry. The sensitivity of the recommended method is limited by the relatively high concentration of titrant. *L*-Cystine in amino acids mixture have been determined, in biological media, in food or in pure form by a number of methods including their separation by thin-layer chromatography. The analytical methods used for its determination included coulometric and potentiometric titration, colorimetry, voltammetry, capillary electrophoresis, flow injection analysis after reduce to Cysteine with spectrofluorimetric detection and also using Raman microscopy combined chemometrics of Principal Component Analysis and Hierarchical Cluster Analysis.

A sensitive and simple chemiluminescent method for the determination of *L*-Cystine based on its inhibitory effect on the hemoglobin-catalyzed reaction of chemiluminescence oxidation of luminol with hydrogen peroxide was developed.

A key part of the sequence of reactions that lead to the emergence of CL through the formation of transannular luminol peroxide, and during decomposition light emitter is formed, is the radical anion $\cdot O\text{-}O^{-}$. In the available literature indications of CL inhibition during $H_2L$ oxidation by acceptors $\cdot O\text{-}O^{-}$ radical are presented. On the other hand, during the catalytic decomposition of $H_2O_2$ HO· radicals are typically formed. Very likely that the phenomenon of inhibition is caused by coordination of HO·radicals, and therefore the newly formed HOInh· radical recombination with superoxide $\cdot O\text{-}O^{-}$ radical, respectively:

$$HO^{\bullet} + Inh \longrightarrow HOInh^{\bullet}$$

$$^{\bullet}O\text{-}O^{-} + HOInh^{\bullet} \longrightarrow Inh\begin{matrix}OOH\\O^{-}\end{matrix}$$

The optimal concentrations of reagents (Luminol, NaOH, $H_2O_2$, Hemoglobin) have been determined, as well as the optimal reaction conditions (mixing order, pH, temperature, sample volume). All of the measurements were performed at the emission maximum of the oxidized form of luminol (425 nm). The optimal concentrations of the reagents were determined as follows: 0.1 mmol/L Luminol, 50 mmol/L NaOH, 0.85 mmol/L $H_2O_2$ and 0.05 μg/mL Hb in the reaction mixture. Analyses were carried out in buffer, 50 mmol/L NaOH, at room temperature. Chemiluminescence properties such as initial light intensity (*I*, rel. un.), area of emission (*S*, rel. un.) are studied, varying the concentration of all reactants using a multivariate factorial approach. The concentration of *L*-Cystine is in linear relationship with the percentage inhibition of system under the optimal experimental conditions. The calibration graph is linear in the range from $2.0\times 10^{-7}$ to $2.0\times10^{-6}$mol/L with detection limit of $4.3\times10^{-7}$ mol/L.

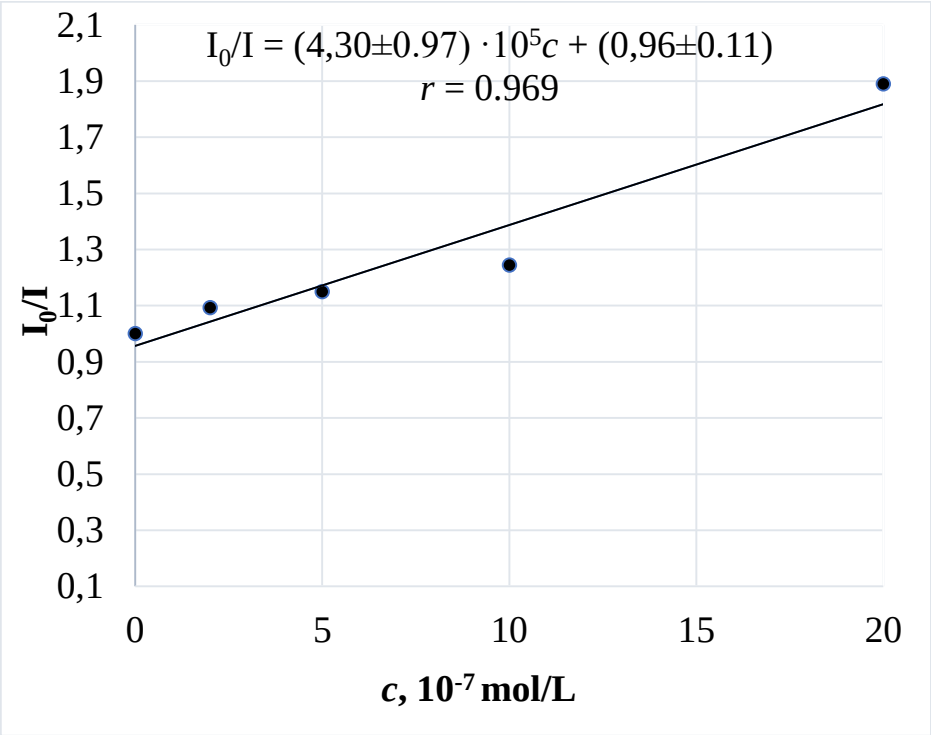


Fig. Dependence $I_0/I$ on the concentration of cystine in the chemiluminescence system Luminol – $H_2O_2$ – Hemoglobin

This method can be used for the determination of *L*-Cystine in tablet formulation with satisfactory results.