ABSTRACT

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Title of Diploma Thesis: Testing antioxidant activity using luminol chemiluminescence systém

The aim of the thesis was to compare various conditions for the testing of the antioxidant activity by means of the luminol chemiluminescent system in the flow system, and evaluation of dependence of the antioxidant efficacy on concentration. The antioxidant efficacy was described as dependency of the decrease of a chemiluminescence signal on the used concentration of an antioxidant. Efficacy of three substances – resveratrol, p-coumaric acid and trolox was observed. Trolox was used as a standard substance in evaluation of the antioxidant activity.

The chemiluminescence system was based on a chemiluminescent reagent. In this particular case we used luminol in a sodium hydroxide solution or in a sodium carbonate solution adjusted to pH 10 or without pH adjustment. Luminol demonstrated chemiluminescence emission after oxidation with hydrogen peroxide in the presence of catalysts. As the catalyst, potassium hexacyanoferrate(III) or potassium hexacyanoferrate(II) were used. The working voltage was set to 435 mV or to 750 mV during the measurement. The whole analysis was carried out in the sequential injection analysis (SIA) system that enables automation, high repeatability and the speeding up of the whole measuring process. The signal scanning was set to 60 seconds with the use of a stop-flow when the decrease of chemiluminescence occurs. Values of heights and peak areas were observed.

The antioxidant activity of resveratrol, p-coumaric acid and trolox with concentrations ranging from 2.44×10^{-7} up to 1×10^{-3} mol/l were observed under five different conditions of the measurement. The use of a hydrogen peroxide solution with the concentration of 10^{-4} mol/l, a potassium hexacyanoferrate(III) solution with the concentration of 10^{-3} mol/l, and a luminol solution with the concentration of 10^{-3} mol/l in a 0.1 M a sodium hydroxide solution appeared to be the most suitable.

Out of the three observed substances, resveratrol was the strongest antioxidant, trolox demonstrated a slightly weaker antioxidant activity and p-coumaric acid appeared to be the weakest antioxidant.