EVALUATION OF FOOD ANTIOXIDANT ACTIVITY BY PHOTOSTORAGE CHEMILUMINESCENCE

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Abstract

A sensitive and simple method for measuring antioxidant activity was developed, based on photostoragechemiluminescence generated from photolyzed acridine and strong bases. This method was used to evaluate the antioxidant activity of food additives by measuring the inhibition of chemiluminescence at a gradient of antioxidant concentrations. Adipic, tartaric, citric, ascorbic, malic and fumaric acid were employed as antioxidants. The method is more suitable for lipophilic compounds.

Keywords acridine, photolysis, chemiluminescence, antioxidants.

1. Introduction

Currently, there is a strong demand from the food industry to replace synthetic antioxidants by natural ones [1]. The introduction of new antioxidants requires reliable methods for the evaluation of their antioxidative activity, and a number of studies have been published on this subject [2-4]. Chemiluminescence (CL) measurements are often used to determine initial radical products by employing luminol or isoluminol based assays [5-12]. We have recently published the chemiluminescence of photo- or radiolyzed azaaromatics on reaction with reactive oxygen species [13-15]. We expected that addition of an antioxidant in the photolyzed mixture would scavenge the reactive oxygen species thus quenching the light reaction and leading to a simple and rapid method for the evaluation of antioxidant activity. In this paper we wish to report a method that employs photostoragechemiluminescence (PSCL) - a term that we have coined together to describe a photolysis process leading to stable chemiluminescent products and subsequent CL [16] - of acridine for the evaluation of the antioxidant activity of food additives, such as, \textit{adipic acid} (E 300), \textit{tartaric acid} (E 334), \textit{citric acid} (E 330), \textit{ascorbic acid} (vitamin C, E 300), \textit{malic acid} (E 296), \textit{fumaric acid} (E 297) and for comparison purposes, the vitamin E analogue, \textit{Trolox} (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid).

2. Experimental

Photolysis was performed in an ORIEL photochemical arrangement with the full spectrum of a 1000 watt xenon lamp. Photolysis was considered complete on disappearance of the characteristic absorption band of acridine at 357 nm (photolysis time 40 sec, concentration of acridine solution, 2 x 10^{-4} M). Further photolysis resulted in destruction of the photochemical products and lower CL. Also, the photolysis step is sensitive to moisture leading to less reproducible CL signals. Chemiluminescence measurements were performed on a 1250 Bio-Orbit luminometer with the timer circuitry disconnected. The light reaction of the blank (without antioxidant) was started by adding 250µl methanolic potassium hydroxide solution (1.5 M) into a mixture of 250µl photolyzed acridine (2x10^{-4} M in DMF) and 250µl pure DMF. The light intensity thus obtained
was compared with the light intensities from samples containing 250µl photolyzed acridine (2x10^{-4} M in DMF), 250µl antioxidant of various concentrations (10^{-2} - 10^{-6} M in DMF) and 250 µl of methanolic potassium hydroxide (1.5 M).

3. Results and discussion

As already mentioned we have employed the terms Photostorage chemiluminescence (PSCL) and Radiostoragechemiluminescence (RSCL) to describe a process in which in contrast to photo- and radiochemiluminescence where the photo- or radioproducts spontaneously proceed to emission of chemiluminescence, here, photolysis or radiolysis of azaaromatics leads to stable products, this being the first step of our process. Then addition of strong bases of higher concentrations triggers the second step (CL) of the azaaromatics PSCL.

Light emission on addition of methanolic potassium hydroxide into photolyzed DMF solutions of acridine was in the form of strong short bursts. Hydroxide anion produced concentration-dependent acridine CL intensities as shown in Fig.1 from which an optimum concentration of 1.5 M methanolic potassium hydroxide was established and employed for the evaluation of the antioxidant activities of food additives.

![Figure 1](image.png)

**Figure 1.** CL intensities vs. concentration of base; 250 µl methanolic potassium hydroxide added to 250 µl of photolyzed acridine (C=2 x 10^{-3}M) and 250 µl DMF.

The hydroxide anion-induced photostoragechemiluminescence of acridine was depressed by all antioxidants. Maximum antioxidant activity was observed at higher concentrations of all antioxidants (C=10^{-3}-10^{-2} M). Medium depressions were observed with fumaric, malic and tartaric acids at all concentrations used, compared to adipic acid, whereas citric acid and trolox showed the lowest antioxidant activity; the most marked inhibition was observed with adipic acid (Fig. 2). This signal is uniformly and effectively quenched by all antioxidants used in this work. As shown in Fig 2, adipic acid shows the highest antioxidant activity followed by malic acid, fumaric acid, tartaric acid, ascorbic acid and citric acid. For comparison purposes we also measured trolox, a Vitamin E analogue, usually employed as standard for the evaluation of antioxidants [17,18] and found that all food antioxidants investigated in this work were 2-3 times
more effective than trolox at higher concentrations between $10^{-5}$ and $10^{-3}$ M. At very low concentrations ($10^{-6}$ M) trolox and citric acid showed pro-oxidant activity, difficult to explain.

![Antioxidant activity graph](image)

**Figure 2.** Food antioxidant activity (%) at different antioxidant concentrations by the acridine PSCL method. The antioxidant activity was evaluated from equation $AA(\%) = 100 - \frac{I}{I_0} \times 100$, where $I$ is the CL intensity measured at various antioxidant concentrations and $I_0$ the CL intensity without antioxidant.

There is evidence that superoxide radical anion and hydrogen peroxide were involved in this reaction since the chemiluminescence signal is also produced by authentic hydrogen peroxide in alkaline solution. It is known that molecular oxygen is transformed to superoxide radical anion in the presence of electron donors, rapidly disproportionating to hydrogen peroxide and oxygen [19]. Hydrogen peroxide in alkaline solution exists as peroxide anion and readily reacts with the acridine photoproducts producing electronically excited acridone, whose return to the ground state gives the transient CL signal. It should be noted at this point that photooxygenated amines or amides also produce the superoxide radical anion and hydrogen peroxide via nitrones or nitroxyamines in protic solvents [20] and that it is very difficult to decide in this complex reaction which reactive oxygen species is responsible for the CL signal. It is possible that this signal is the result of the reaction of all ROS species with photolyzed acridine. It should be noted here that this method is advantageous in that isolation of the photoproducts is unnecessary prior to the CL measurement. The antioxidants of various concentrations were added to the mixture of acridine photoproducts and compared with each other directly.
4. Conclusions

Besides antioxidant evaluation, PSCL may be employed in the determination of such species in the region from $10^{-5}$ to $10^{-2}$ M where the drop in CL signals for all the antioxidants of this work is well described by a polynomial equation: $y = A + B_1X + B_2X^2 + B_3X^3$ where $y$ the logarithm of CL intensity and $x$ the logarithm of the antioxidant concentration. As many carboxylic acid are employed by the food industry in their water soluble salt form, the drawback of this method is that the PSCL assay is only usable with DMF soluble compounds, a drawback which, however, could turn into advantage for other industrial antioxidants such as tocopherols, di-tert-butylhydroquinone in the food industry or hindered phenolics and diphenylamines as lubricant and jet fuel additives etc.

References


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