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Preliminary Study for Detection of Hydrogen Peroxide Using a Hydroxyethyl Cellulose Membrane †

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Abstract: High concentration of biogenic amines (BA) is an indicator of deterioration of food and the determination of their concentration is an important method of food control. The hydrogen peroxide (H_2O_2) is a side product of the degradation of BAs by certain enzymes. It is presented an experimental technique grounded on chemiluminescence to measure small quantities of H_2O_2 with concentrations as low as 0.01%w/w up to 0.08%w/w. Luminol and cobalt hydroxide are added to hydroxyethyl cellulose to obtain an active membrane which will react with the sampling solution and the amount of total light emission is directly related to the H_2O_2 concentration.

Keywords: Biogenic amines; Chemiluminescence; Food storage

1. Introduction

Providing safe food to consumers has never been more important and more challenging for the food industry. In addition of being nutritious and appealing, food must be safe [1]. Deterioration is a complex process characterized by changes in food product that makes it unacceptable to be consumed [2]. However, microbial activity, the most common cause of deterioration, manifest as visible growth of slime, colonies and texture changes, due to polymer degradation, and development of off-odors and off-flavors [3]. High concentrations of biogenic amines (BAs) can be found as a consequence of microbial activity in foods such as wine, fermented meat and fish products, cheeses and fermented vegetables.

BAs are nitrogenous organic polar or semi-polar bases of low molecular weight. On the basis of their chemical structure these amines can be aliphatic (cadaverine, putrescine, spermine and spermidine), aromatic (phenylethylamine, tyramine), or heterocyclic (pyrrolidine, histamine) [4], and according to their number of amine groups they can be divided into monoamines (tyramine and phenylethylamine) and diamines (histamine, putrescine and cadaverine) [5]. Usually, these nitrogen compounds are formed mainly by microbial decarboxylation of amino acids and by amination and transamination of aldehydes and ketones.

Since the formation of BAs is used as an indication of food spoilage [6], the determination of their concentration in foods is an important method of food control [7]. As hydrogen peroxide (H_2O_2) is a side product of the degradation of BA by the enzyme diamine oxidase and produces

luminescence upon reaction with 5-amino-2,3-dihydrophthalazine-1,4-dione ($C_8H_7N_3O_2$), known as luminol [8], it was used chemiluminescence as an indirect method for detection of H_2O_2.

In this study we present the preliminary development of a small membrane based on hydroxyethyl cellulose combined with luminol and a catalyst which glows in the presence of H_2O_2 with concentrations as low as 0.01%w/w.

2. Materials and Methods

The luminol-based membrane was developed with luminol, sodium phosphate, cobalt (II) chloride hexahydrate, sodium lauryl sulphate, hydrogen peroxide (30%) and hydroxyethyl cellulose (Sigma Aldrich, Darmstadt, Germany). The procedure established by Miklicanin and Valzacchi [8] was refined to establish the experiment protocol.

In a first stage luminol (0.2 mg), sodium phosphate (8.6 mg), sodium lauryl sulphate (60 μL, 34.36 mmol/L) and hydroxyethyl cellulose (150 mg) was added to 10 mL of Milli-Q® water. The mixture was placed on a magnetic stirrer for 30 min and 1 mL was dispensed into home-made carrier, made of Teflon to decrease adhesion and facilitate removal of the membrane. The membranes were dried in an oven at 70 °C for 4 h then cooled and stored in a desiccator with vacuum.

A set of sample solutions with a concentration of 0.01 to 0.07%w/w of H_2O_2 were prepared by diluting a standard 30%w/w solution of H_2O_2. To all sample solutions was added cobalt hydroxide (200 μL, 5.0 mmol/L).

After drying, the membrane was carefully split into small pieces of the same size to increase the area of contact with the sample solution and placed inside a cuvette. The cuvette was placed on a small and portable acquisition system specially developed for this work containing a spectrometer (C12880MA from Hamamatsu) that collects part of the light emitted. The sample solution (250 μL) was added and the emission spectra were acquired being recording at every 100 ms and the total light detected was integrated up to 50 min. The same procedure was repeated to all H_2O_2 concentrations.

3. Results and Discussion

The light intensity variation emitted with a sample solution concentration of 0.01%w/w is represented in Figure 1a with a maximum peak of intensity at approximately 426 nm as expected [9]. A normalization of the data was carried out to eliminate fluctuations due to noise. The light emission spectra that characterize the reaction of peroxide with luminol over time, illustrated in Figure 1b, showed a clear difference between a concentration of 0.01% and 0.08% of H_2O_2.

It is evident that for a higher concentration, the peak of intensity is higher, and the reaction stays longer time. When the H_2O_2 concentration increases, it is observed a quasi-linear behavior of the total integral peak intensity, as shown in Figure 1c.

Although the data acquisition conditions are kept the same, some variability has been obtained in particular for higher concentrations. This can be justified because the light emission is not uniform, due to the unevenly rehydration of the membrane.

To verify the stability of the detection system the detection process was repeated four times for the same concentration. Figure 1d shows the relative data for three steps of different concentrations of H_2O_2. To check the stability and repeatability of the sensing scheme, similar samples were used to obtain several acquisitions. The system resolution, R, can be estimated considering the values obtained from two measurement linked with two different values of the concentration [10]

$$R = \frac{\sigma}{S} = \frac{\delta c}{\delta I}\sigma, \quad (1)$$

where the δc is the H_2O_2 concentration, S is the sensitivity to concentrations of H_2O_2 variations, δI is the difference in the total intensity of light detected and σ is the highest standard deviation associated with the light detection associated to a step variation of H_2O_2 concentration.

The resolutions obtained between levels 0.01 and 0.02%w/w; 0.02 and 0.03%w/w and 0.03 and 0.04%w/w were 0.0025, 0.00022 and 0.0008%w/w respectively.

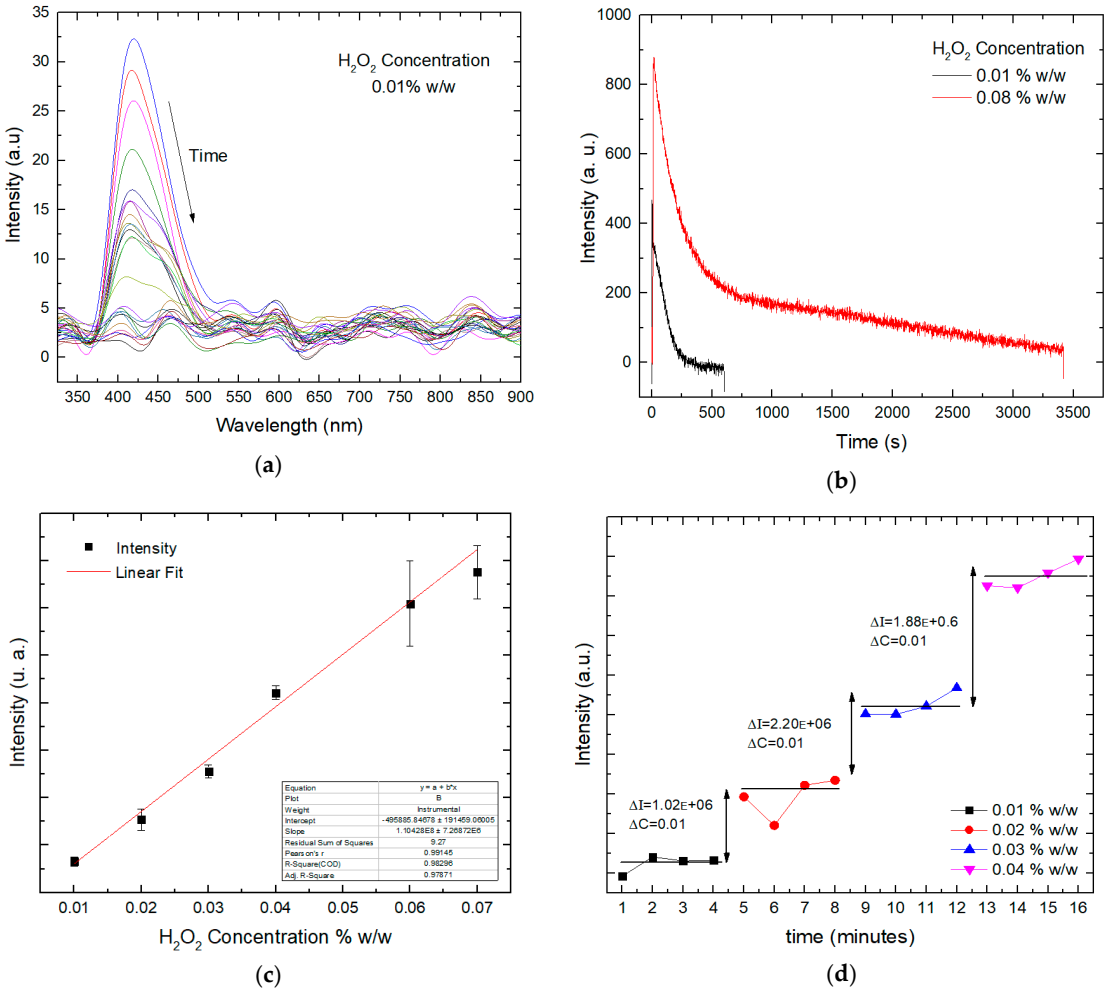

Figure 1. (**a**) Spectra showing the variation of the intensity of the light emission to the concentration 0.01%w/w as a function of the reaction time; (**b**) decay time of the spectra integral as a function of the chemical reaction; (**c**) integral of the decay time for each H_2O_2 concentration; (**d**) measured intensity when the H_2O_2 undertakes step increases.

4. Conclusions

A preliminary study for the detection of hydrogen peroxide using a hydroxyethyl cellulose membrane was presented. The results have shown that with this method it is possible to detect very low concentrations of H_2O_2 down to 0.01%w/w with resolutions better than 0.0025%w/w in the range up to 0.04%w/w. As the H_2O_2 concentration increased, an increase in light-emitting intensity and reaction time was observed following a quasi-linear behavior.

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Conflicts of Interest: The authors declare no conflict of interest.

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