DETERMINATION OF BISPHENOL F IN MUSTARD USING FLUORESCENCE

Alexandrina MOSCALU-LUNGU[1], Raluca-Ioana STEFAN-VAN STADEN[2],
Jacobus Frederick VAN STADEN[3]

Fluorescence was proposed for the determination of bisphenol F in mustard samples. Fluorescein was used as fluorophore in the assay of bisfenol F, to enhance the native fluorescence of bisphenol F. The limits of determination for bisphenol F were 10nmol/L for the native fluorescence, and 1nmol/L when fluorescein was used. The proposed method was validated using different mustard samples.

Keywords: bisphenol F, fluorescence, mustard

1. Introduction

Mustard is a thick paste with a sharp, stimulating taste, made from the ground seeds of a mustard plant(white or yellow mustard, *Sinapishirta;* brown or Indian mustard, *Brassica juncea;* or black mustard, *Brassica nigra*). When the seeds are crushed, elements are released to create an essence that has been described as "fiery" to the taste [1].The crushed seeds may be left in powdered form with the consumer adding water or they may be mixed with water, vinegar, or other liquids, as well as other flavorings and spices [1].

The important components of the seeds are sulfur compounds called glucosinolates (sinigrin in *B. juncea* and *B. nigra* and sinalbin in *S. alba*), which impart the flavor and pungency [2]. Stored whole and dry the seeds retain this flavor, but in the presence of moisture and the breaking of the seed’s cell, the enzyme myrosinase breaks down the glucosinolates to release sulfur, sugar, and hot principles called isotiocynates [2].

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[1] PhD student, Faculty of Applied Chemistry and Materials Science, Department of Analytical Chemistry and Environmental Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: alex_ly15@yahoo.com

[2] CS I, Prof. Habil, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, and Faculty of Applied Chemistry and Materials Science, Department of Analytical Chemistry and Environmental Engineering, POLITEHNICA University of Bucharest, E-mail: ralucavanstaden@gmail.com.

[3] CS I, Prof. Habil, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania, E-mail: koosvanstaden2012@yahoo.com

The yellow mustard releases a milder nonvolatile para-hydroxybenzyl isothiocynate, while seeds of the other two species release the strong, pungent, volatile allyl isothiocynate [3].

Bisphenol F [bis(hydroxyphenyl)methane] (Fig.1) is a diphenylalkane with a similar structure to bisphenol A. These molecules are involved in the production of epoxy resins and/or polycarbonate. Epoxy resins have a wide range of industrial applications, including the manufacture of lacquers, liners, adhesive plastics, and water pipes. They are also used in medical materials (e.g., dental sealants, oral prosthetic devices, and tissue substitutes) and coatings for packaging in contact with food [4,5].

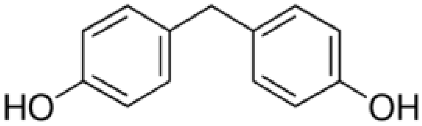

Fig. 1. The chemical structure of bisphenol F

Bisphenol F was formed during production of mustard under controlled laboratory conditions, but only when seeds of white/yellow mustard (*Sinapis alba*, synonym *Brassica alba*) were used. With seeds of brown (*Brassica juncea*) or black mustard (*Brassica nigra*) no formation of bisphenol F was observed. White/yellow mustard is used for the production of mild mustard types, while brown and black mustard are used for spicy/hot mustards. Based on the chemical structure of bisphenol F and in view of the mustard species different composition it may be concluded that bisphenol F is formed from the glucosinolate glucosinalbin. Glucosinalbin only occurs in white/yellow mustard, but not in brownor black mustard, which would explain the different levels of bisphenol F in mild and spicy/hot mustard types.

This paper proposed a fluorescence method based on native fluorescence of bisphenol F, but also on enhanced fluorescence using fluorescein as fluorophore for the determination of bisphenol F in mustard.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical grade and solutions were prepared using deionized water obtained from a Direct-Q3 Water Purification system (Millipore Corporation, France).

Bisphenol F, fluorescein, dimethyl sulfoxide, 2,2-diphenyl-1-picrylhydrazyl, dimethylsulfoxide, sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot H_2O$), sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$), Rhodamine B were purchased from Sigma-Aldrich (Milwaukee, USA). Phosphate buffer solution (PBS, 0.2 mol L^{-1}) with pH=7.45 was prepared using $NaH_2PO_4 \cdot H_2O$ and $Na_2HPO_4 \cdot 7H_2O$.

A stock solution of bisphenol F was prepared in dimethyl sulfoxide, to obtain a concentration of $1.00x10^{-3}$ mol L^{-1}. Serial dilutions of the stock solution of bisphenol F were done in PBS (pH =7.45) The solutions were kept in the refrigerator at 2-8 ºC. Working solutions were prepared daily by serial dilution method with PBS solution at pH =7.45.

2.2. Apparatus and methods

The fluorescence measurements were done with a spectrometer QE65000 from Ocean Optics (Dunedin, Florida) equipped with a xenon lamp (HPX 2000). The HPX-2000 Xenon Light Source is a high-power, high-intensity source that is especially useful for fluorescence applications and for other applications where a high-intensity lamp is necessary. Results are acquired and processed using a Spectra Suite software program. A Mettler Toledo pH-meter (model Seven Compact) was used in order to adjust the pH at desirable values.

All electrochemical measurements were performed with AUTOLAB/PGSTAT 302N (Metrohm) connected to a computer with a GPES software used to record the measurements.

A chronoamperometric method was used for the measurements of t_{off} and t_{on}, at a constant potential (125 mV vs. Ag/AgCl).

2.3. Sample preparation

Five samples of mustards, sample 1 were from classical mustard, sample 2 from pungent mustard, sample 3 from sweet mustard, sample 4 from pungent mustard and sample 5 from natural mustard produced by different companies purchased from retail market in the region of Bucharest, Romania were used for the validation of the fluorescence method used for the assay of bisphenol F. The samples were buffered with PBS (pH=7.45).

3. Results and discussions

Native fluorescence of bisphenol F showed a low intensity of the fluorescent signal. In order to enhance the sensitivity of the fluorescence detection of bisphenol F were checked two fluorophores: rhodamine B and fluorescein. Rhodamine B did not give a positive response compared to fluorescein that lead to a significantly enhancement of fluorescent signal.

3.1. Optimization of the working conditions

The concentration of fluorescein had to be optimized for the detection of bisphenol F. Therefore, solutions of different concentrations of fluorescein were prepared, and the intensity of the fluorescence signal was measured. The fluoresceine concentration range was between $1.00x10^{-9}$-$1.00x10^{-3}$ mol L^{-1}. Fig. 2 shows that the highest intensity of the fluorescence signal was obtained for a concentration of fluoresceine of $1.00x10^{-4}$ mol L^{-1} (log $1.00x10^{-4}$=-4), used for graphic representation).

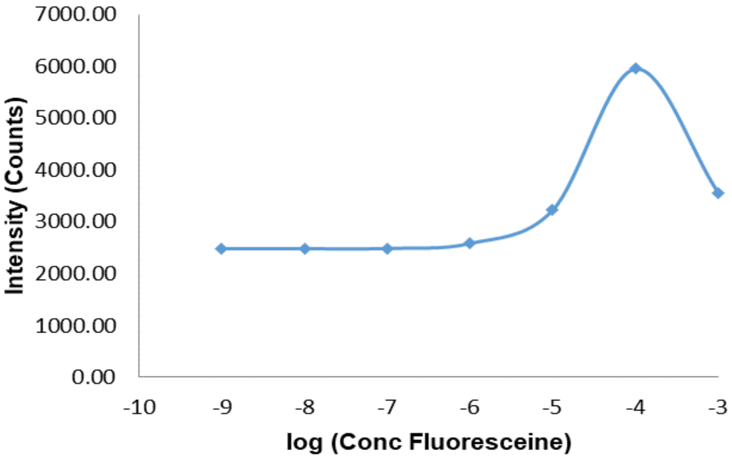

Fig. 2. The influence of the concentration of fluorescein on the intensity of fluorescence signal

3.2. Linearity and sensitivity obtained for the fluorescent assay of bisphenol F

The results obtained for the native fluorescence of bisphenol F were compared with the results obtained for bisphenol F samples with fluorescein as fluorescent probe.

For the native fluorescence of bisphenol F, the following parameters were obtained: the linear concentration range was between $1.00x10^{-8}$-$1.00x10^{-6}$ mol L^{-1} bisphenol F (Fig. 3). The linear regression equation was I=2588.7+ 9.00 $x10^{7}$xC (r=0.9987) where I is the fluorescence intensity and C is the bisphenol F concentration (mol L^{-1}). The limit of quantification was found to be $1.00x10^{-8}$mol L^{-1}. When fluorescein was used as fluorophore, the parameters obtained changed to: the linear concentration range was between $1.00x10^{-9}$and $5.00x10^{-7}$mol L^{-1}(Fig. 3). The linear regression equation was I=4894.8- $2x10^{-9}$xC(r=0.9996), where I is the fluorescence intensity and C is the bisphenol F concentration (mol L^{-1}). The limit of quantification was found to be $1.00x10^{-9}$ mol L^{-1}.

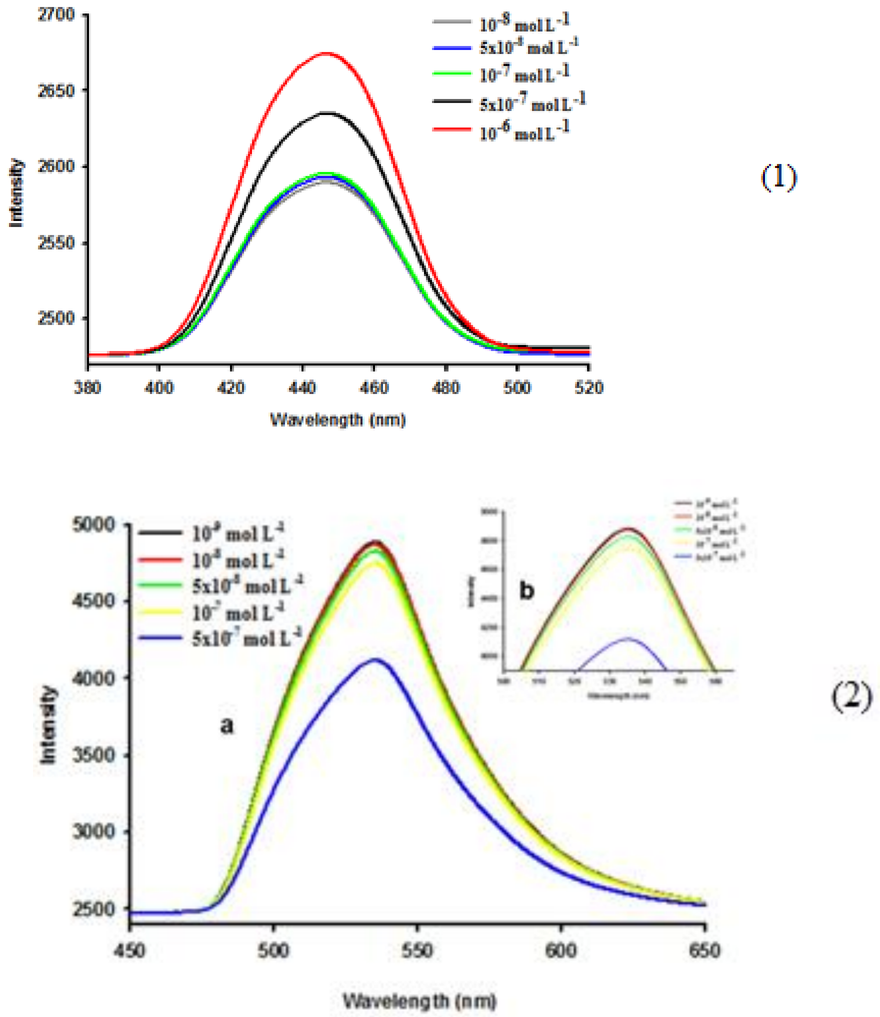

Fig. 3. Fluorescence spectra of different concentration of bisphenol F recorded for (1) native fluorescence of bisphenol F; (2) for bisphenol F with fluorescein as fluorophore showing (a) - overlaps with baseline; and (b) - enlargements.

A larger linear concentration range for bisphenol F was obtained by using the fluorescein as fluorophore for the detection of bisphenol F, the method having the ability to cover easily smaller and bigger concentrations of bisphenol F. Due to the decreasing of the limit of quantification, bisphenol F could be detected in mustard samples.

3.3. Determination of bisphenol F in mustard samples

The mustard samples were analyzed using fluorescence in two ways: using the native fluorescence of bisphenol F, and using the fluorescence generated by the addition of fluorescein to the mustard samples. The results obtained using the fluorescence methods were compared with those obtained using a different method – an electrochemical method based on a stochastic sensor [6] (based on the immobilization of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in carbon nanopowder (nC)) (Table 1). Fig. 4, shows some examples for the fluorescence spectra obtained using the two fluorescence methods.

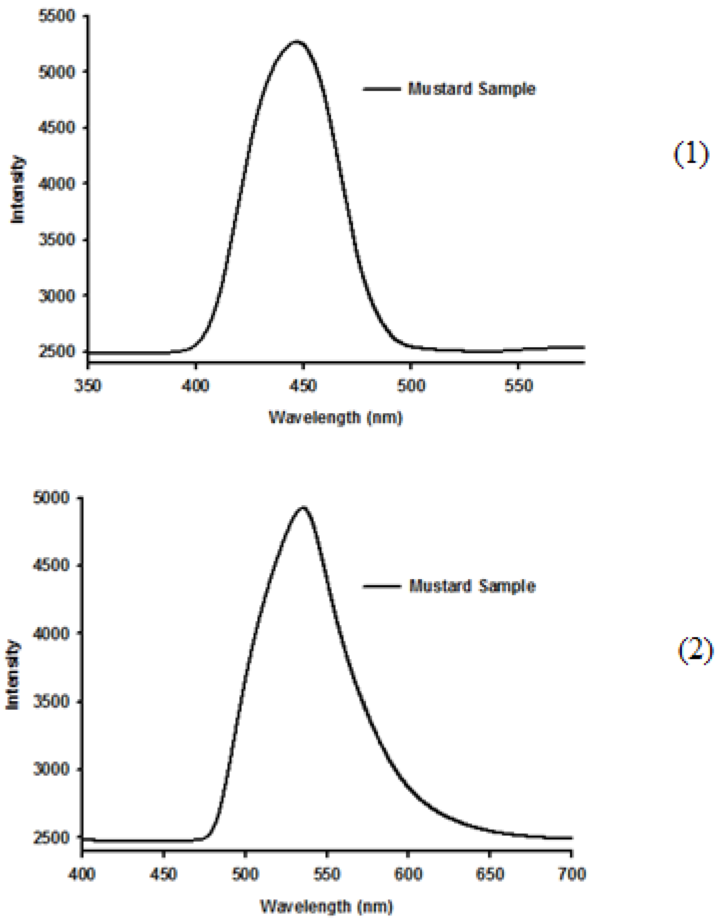

Fig. 4. Example of fluorescence spectrum for sweet mustard samples using: (1) native fluorescence, and (2) fluorescein as fluorophore.

Table 1 shows that there is a good correlation between the results obtained using the two fluorescence methods and the electrochemical method based on the stochastic sensor.

Tables 1

Determination of bisphenol F in mustard samples (N=10)

Mustard sample no.	ng BPF/1g mustard		
	Native fluorescence	Utilization of Fluorescein as fluorescent probe	Stochastic sensor based on DPPH/nC
1	6.48± 0.08	6.45±0.06	6.48±0.02
2	9.19±0.04	9.90±0.08	9.27±0.03
3	6.25±0.05	6.89±0.05	6.50±0.02
4	9.20±0.07	9.72±0.08	9.05±0.05
5	10.84±0.08	10.20±0.05	10.38±0.03

The advantage of using such method versus method proposed to date and also versus the stochastic method is that many food laboratories are equipped with fluorimeters and can detect bisphenol F from mustard with high reliability.

4. Conclusions

Utilization of fluoresceine as fluorophore for the determination of bisphenol F lead to the enhancement of the fluorescence signal, enlarging the linear concentration range, and decreasing the limit of determination of bisphenol F. The proposed method was used reliable for the determination of bisphenol F in mustard. The method is fast, cost effective, and efficient when used for the assay of bisphenol F from various food samples, as mustard.

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