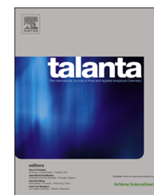
Talanta 132 (2015) 503–512

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta



Determination of traces of several pesticides in sunflower oil using organic phase immuno electrodes (OPIEs)

Elisabetta Martini, Mauro Tomassetti*, Luigi Campanella

Department of Chemistry, "Sapienza" University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy

ARTICLE INFO

Article history:
Received 9 June 2014
Received in revised form
22 August 2014
Accepted 2 September 2014
Available online 22 October 2014

Keywords:
Pesticides analysis
Sunflower oil
Immunosensors
Competitive step
Enzyme reaction
Organic phase

ABSTRACT

Testing for traces of different pesticides (triazinic, organophosphates and chlorurates), present in hydrophobic matrices such as sunflower oil was checked using new immunosensors working in organic solvent mixtures (OPIEs). The competitive process took place in an n-hexane–chloroform 75% (V/V) mixture, while the subsequent final enzymatic measurement was performed in decane using tert-butylhydroperoxide as substrate of the enzymatic reaction. A Clark electrode was used as transducer and peroxidase enzyme as marker. A linear response of between about 10 nM and 4 μM was usually obtained in the presence of sunflower oil. Immunosensors show satisfactory selectivity and precision and recovery tests carried out on commercial sunflower oil samples gave excellent results. Lastly, theoretical confirmation of the possibility that immunosensors can act positively in organic solvent mixtures was discussed on the basis of Hill's coefficient values.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

In recent years several authors [1–4] discovered that some antibodies were capable of retaining activity and selectivity in the non-aqueous phase, while, until recently, it was believed possible only in aqueous environment. Consequently a start was made also on the development of immunosensors working in the organic phase [5–7]. Even though the field of organic phase immunosensing is still not highly developed, the potential benefits of this approach for the measurement of poorly water-soluble analytes in organic solvent are manifold [6]; indeed many analytical methods involving non-polar analytes require solvent extraction prior to measurement. The analytical assay is therefore greatly simplified if the method is able to function effectively in non-polar solvent extract. However, several phytopharmaceutical compounds are more soluble in organic solvent than in aqueous solutions (see Table 1), and can therefore be rightly included in the category of compounds described above; also testing for them thus encounters difficulties linked to the low solubility of several pesticides in aqueous medium. These difficulties increased further when the low solubility in water solution of the analyte to be determined was coupled with the very low solubility of the real matrix in which the analyte was contained, as in the case of edible oily matrices. To overcome these difficulties, in the last two years, immunodevices were fabricated that are capable of operating in organic solvent, i.e. organic phase immuno electrodes (OPIEs) [8]. To this end, in a first investigation [8], in accordance with what is reported in literature [9–11], several different aspects of the new type of immunosensor were optimized. In particular different competitive formats [8,12] were tested and the results confirmed as the ELISA type format was found to be quite suitable [12]. Furthermore, the practicality of immobilizing the antibody used on the polymeric membrane support was confirmed [8,12]. However, the choice of the organic phase solvent was the most important and difficult point, although the factors governing antibody–antigen binding in organic solvent were clearly more complex and required further elucidation. Solvent properties such as hydrophobicity, molecular mass and fraction can all affect the binding process [6], but also the antigen and the real matrix solubility in the selected solvent play an important role. Furthermore, of equal importance is the fact of whether also the last enzymatic reaction involved in the enzyme immunoassay method was performed in the organic solvent or not. It is actually surprising that greater efforts appear not to have been made in this area [6], although basic research is not lacking [9–11,13–18]. Studies reported in literature concerning choice of solvent refer above all to alcoholic and hydroalcoholic solutions [19–22] and often do not agree among themselves [6,19–22] also because of the different antibody systems used in these studies. For this reason the organic phase immunoassay development has continued to rely on an empirical rather than mechanistic approach [6]. Also our approach to this problem was therefore of this type in our first research [8] involving the analysis of traces of triazinic pesticides in olive oil. A series of tests were thus carried out by

* Corresponding author. Tel.: +39 0649913722; fax: +39 0649913601.
E-mail address: mauro.tomassetti@uniroma1.it (M. Tomassetti).

http://dx.doi.org/10.1016/j.talanta.2014.09.004
0039-9140/© 2014 Elsevier B.V. All rights reserved.

Table 1
Comparison of solubility of several pesticides in chloroform and in water.

Pesticide	Solubility in chloroform ($g L^{-1}$)	Solubility in water ($g L^{-1}$)
Atrazine	52.0	0.028
Simazine	0.9	0.006
Carbaryl	200	0.040
Parathion	0.50	0.060
2,4-D	32	0.9
2,4,5-T	32	0.2

the present authors in previous work [8] using different solvents, different electrochemical transducers and different immunosensor constructions, or operating measurement format. This series of trials led to the development of suitable amperometric immunosensors for the analysis of triazinic, organophosphate and chlorurate pesticides in olive oil and olive oil by-products [8,23], with the competitive format of the immunological test (ELISA type) performed in 50% (V/V) chloroform n-hexane mixture, which was found [8] to be the best compromise among the different solubility requirements mentioned above, using as transducer a classical Clark electrode for oxygen with the external cup and the gas permeable membrane both made of PTFE and a pesticide–BSA conjugated hapten marked using horseradish peroxidase enzyme. These devices were certainly innovative compared to what had previously been reported in literature, but produced good results also for the applications in real olive oil samples and derivatives [8,23]. The success obtained in these researches involving pesticide measurement in olive oil matrices using the new OPIE aroused our interest in this field and so in the present research also the possibility of testing for the above pesticides in other kinds of edible oils was investigated. Since the literature contains several reports concerning the presence of traces of several pesticides such as atrazine, simazine, 2,4-D, 2,4,5-T and parathion in sunflower oil [24–29], it was decided to apply the OPIEs developed also to determine these pesticides in sunflower oil. Of course our approach was very similar to that successfully used for pesticide analysis in olive oil. However, also in this case the choice of solvent was a central point, considering that sunflower oil does not have exactly the same solubility as olive oil in the different organic solvents. For this reason measurements in the present work were performed in n-hexane–chloroform 75% (V/V) mixture instead of in the same 50% (V/V) mixture as used in the previous research aimed at measuring pesticide traces in olive oil [8].

2. Materials

2.1. Apparatus

The amperometric measurements were performed in a 5 mL thermostated glass cell at 23 °C under constant stirring. The Clark electrode, supplied by Universal Sensor Inc., New Orleans (U.S.A.), was connected to an amperometric biosensor detector provided by the same firm and to an analogue recorder mod. 868 Amel (Milan, Italy). In all experiments performed in the organic phase, the plastic cap of the electrodes was replaced by a PTFE cap.

2.2. Reagents and materials

Anti-atrazine monoclonal antibody, anti-dichloro-phenoxyacetic acid and anti-trichloro-phenoxyacetic acid antibodies, as well as atrazine and simazine carboxyderivative, dichloro-phenoxyacetic acid (i.e. 2,4-D) and trichloro-phenoxyacetic acid (i.e. 2,4,5-T), were provided by Dr. S. Eremin (Department of Chemical Enzymology, Faculty of Chemistry, Moscow State University, Russia). Anti-parathion was a commercial antibody and was obtained from Acris (Acris Antibodies, Herford, Germany). 1-Chloro-3-ethylamino-5-isopropylamino-2,4, 6-triazine (i.e. Atrazine), 6-chloro-N,N′-diethyl-1,3,5-triazine-2,4-diamine (i.e. simazine), N-tert-butyl-6-chloro-N′-ethyl-1,3,5-triazine-2, 4-diamine (i.e. tert-buthylazine), 2-amino-4-chloro-6-(isopropylamino)-s-triazin (i.e. atrazine desethyl), 1-naphthyl methylcarbamate (i.e. carbaryl), 2-methyl-2-(methylthio)propanal O-(N-methylcarbamoyl)oxime (i.e. aldicarb), diethyl 4-nitrophenyl phosphate (i.e. Parathion) were supplied by Pestanal Sigma-Aldrich (Sigma-Aldrich, Milan, Italy). Potassium chloride, dibasic and monobasic anhydrous potassium phosphate RPE, chloroform RPE, dichloromethane RPE and diethyl ether RPE were supplied by Carlo Erba Reagents (Carlo Erba, Milan, Italy). Ny+ Immobilon Affinity membrane (porosity 0.65 μm) was provided by Millipore (Millipore Corporation, Vimodrone, Milan, Italy). The biotinylation kit, supplied by Sigma Immunochemicals (Sigma, Milan, Italy), was composed of biotinylation reagent (BAC-SulfoNHS, namely biotinamido hexanoic acid 3-sulfo-N-hydroxysuccinimide ester), 5 M sodium chloride solution, micro-spin column (2 mL) (in practice, a small empty cylindrical vessel prepackaged with Sephadex G-50), 0.1 M sodium phosphate buffer pH 7.2, 0.01 M phosphate buffer saline (PBS) pH 7.4 (reconstituted with 1 L of deionised water to give 0.01 M phosphate buffer, 0.138 M NaCl, 2.7 mM KCl, pH 7.4); lastly extravidin® peroxidase (containing 0.2 mL of extravidin Peroxidase conjugate at 2.0 mg mL^{-1}, with 0.01% thimerosal). Phenol, dialysis membrane (art. D-9777), 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide, albumin (from bovine serum) (BSA) and TRIS (hydroxymethyl-aminomethane), tert-butylhydroperoxide solutions in decane solvent and TWEEN® 20, provided by Sigma-Aldrich (Sigma-Aldrich, Milan, Italy).

2.3. Samples

Edible sunflower oil samples, produced by the most important industrial Italian sunflower oil producer firms, were purchased from a local shop and stored in sealed glass bottles.

3. Methods

3.1. Immunosensor assembly and electrochemical format

The electrochemical transducer used was an amperometric gaseous diffusion amperometric electrode for O_2 determination (see Fig. 1). The transducer consisted of a Clark type electrode, equipped with PTFE cup and a gas permeable membrane of the same material. For the immunosensor assembly, in practice, three membranes were mounted on the PTFE cap of the Clark electrode, in the following order: the gas-permeable membrane, the dialysis membrane and the Immobilon membrane with the antibody immobilized on it. The membranes were kept in place by a nylon net and a PTFE O-ring. A constant potential of −650 mV with respect to an Ag/AgCl/Cl^- anode was applied to the Pt cathode of the oxygen electrode. Horseradish peroxidase enzyme was used as marker for immunocomplex detection. The cathode and anode of the Clark type amperometric electrode were immersed in an internal solution of phosphate buffer saline (PBS), pH 7.4 (reconstituted with 1 L of deionized water to give 0.01 M phosphate buffer, 0.138 M NaCl and 2.7 mM KCl). The current, which was measured and flowed in the external circuit, was obviously a function of the concentration of dissolved oxygen present in the internal solution. This concentration was regulated (through the diffusion of the oxygen across the gas permeable membrane) by the enzymatic reaction, which took place in decane (see Fig. 1); in this enzymatic reaction the reduction in the oxygen consumed was a function of the reduction in the concentration of the peroxidase enzyme present in the antibody complex formed on the Immobilon membrane as a result of the competitive process

Download English Version:

https://daneshyari.com/en/article/1242046

Download Persian Version:

https://daneshyari.com/article/1242046

Daneshyari.com