Full Paper

Quantitative Analysis of Prometrine Herbicide by Liquid–Liquid Extraction Procedures Coupled to Electrochemical Measurements

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Abstract
A sensitive method is proposed for the preconcentration and quantification of the herbicide Prometrine (PROM) at a liquid-liquid interface employing square-wave voltammetry. The preconcentration stage was based on liquid-liquid extraction methodology and the PROM quantification was carried out from the peak current of square-wave voltammograms. Under the experimental conditions employed, linear calibration curves in the concentration range 1.0×10^{-6} M – 5.0×10^{-5} M, with detection limit equal to 1.5×10^{-6} M were obtained without pretreatment of the samples. This linear range, as well as detection limit could be extended towards lower concentrations when a pretreatment procedure was employed. In this way, linearity of calibration curves between 8.0×10^{-8} M and 2.4×10^{-7} M and detection limit of 1.0×10^{-7} M, were observed. On the other hand, the standard addition method was also used as an alternative and an appropriated quantification technique for this system. A linear concentration range between 1.0×10^{-6} M and 2.7×10^{-5} M, with a correlation coefficient of 0.997, was obtained. This procedure has also a promising application in the separation of herbicides from other interferents, present in real samples, previous to their quantification.

Keywords: Prometrine, Triazine, Water/1,2-dichloroethane interface, Preconcentration, Square-wave voltammetry

1. Introduction

In the last years, the environmental pollution by pesticides has become in a serious problem especially in marine ecosystem. Due to their heavy use in agriculture and to their persistence, many of the compounds are present in surface and ground waters, and have to be considered a potential risk for marine life as well as for drinking water quality [1]. Low level detection of pesticides is important in many control process and environmental and food quality analyses [2]. For this reason, several techniques has been developed for this quantification, like HPLC [3], CG-MS, capillary electrophoresis [4], solid-phase microextraction coupling with GC [5–8] and with HPLC [9], immunosensor [10] and multibiosensor based on immobilized Photosystem II [11].

Herbicides such triazines are applied as pre- and post-emergent weed control agents to improve crop yields. The half-lives of these herbicides vary from weeks to several months, and under environmental conditions are usually degraded to compounds with better water solubility. Indeed, the most important physicochemical properties of these pesticides and their degradations products are the solubility in water and the capacity to be retained by the organic matter of the soil [12–13]. Environmental Protection Agency (EPA) has included triazines and their degradation products as a group of the Contaminant Candidate List (CCL) due to all potential risks stemming from this class of compounds [14]. For this reason, in many countries of Europe and North America the permitted level of pesticides in water is very low; in the case of triazines it is in order of $0.1~\mu g~L^{-1}$.

The traces quantification of pesticides in different kind of samples requires preconcentration techniques. In the past few years, new techniques were developed like liquid-liquid extraction, solid phase extraction, molecular imprinted polymers and carbon nanotubes, among others. These preconcentration procedures were employed before the quantification of the pesticide, coupled to different techniques like GC-MS, capillary electrophoresis, non-aqueous capillary electrophoresis and micellar electrokinetic capillary chromatography [1, 4, 9, 15–17].

Square-wave voltammetry (SWV) and differential pulse voltammetry are powerful electrochemical techniques with high sensibility employed for the quantification of many kinds of drugs on different electrodes [18–23]. Also they were successfully employed at liquid-liquid interfaces [24, 25]. In this way, they have been applied to the detection and extraction of aspartame and acesulfame K in real food samples [26]; dopamine [27]; insulin [28] and oligopeptides [29], employing conventional liquid–liquid interfaces or liquid–liquid microinterfaces within micromachined silicon membranes. SWV at a water/1,2-dichloroethane microinterface was also used to determinate the liposome–water partition coefficient of different β-blocker drugs [30].

In a previous paper [31], the transfer of three s-triazine herbicides, atrazine, propazine and prometrine, across the water/1,2-dichloroethane interface was investigated using cyclic voltammetry. A facilitated proton transfer mechanism from the aqueous to organic phase was demonstrated by the analysis of positive peak potential and peak current as a function of pH. It was shown that the determination of 2.5×10^{-5} M $- 5.0 \times 10^{-4}$ M concentration of herbicides in aqueous phase is possible under the experimental conditions employed.

The aim of the present paper is to improve the detection limit for the PROM quantification by applying a combined procedure consisting in a previous preconcentration stage, followed by SWV at a water/1, 2-dichloroethane interface. The preconcentration of the analyte in the organic phase is possible due to its high solubility and partition coefficient ($\log P = 3.34$ [31]) in this solvent.

The development of new procedures for analyte extraction and preconcentration has had a growing interest in analytical researches. In this sense, D. W. M. Arrigan et. al. have successfully employed electrochemistry at the interface between two immiscible electrolyte solutions as a modulated liquid – liquid extraction procedure, where ions as 4-octylbenzene-sulfonate [32, 33], tetraethylammonium [32, 33], propanolol [34], timolol [34] and food additives [26], could be selectively partitioned from a flowing aqueous phase to a stationary organogel phase, as a function of the applied interfacial potential difference. On another hand, S. Amemiya and co-workers [35] have developed a novel approach to preconcentrate and detect perchlorate at nanomolar level. This approach is based on the submicrometer thick plasticized poly(vinyl chloride) membrane spin-coated on the poly(3-octylthiophene)-modified gold electrode, where the liquid membrane serves as the first thin layer cell for ion transfer stripping voltammetry giving very low detection limits.

2. Experimental

The voltammetric experiments were performed in a four – electrode system using a conventional glass cell with 0.12 cm^2 interfacial area. Two platinum wires were used as counter – electrodes and the reference electrodes were Ag/AgCl. The reference electrode in contact with the organic solution was immersed in an aqueous solution of 1.0×10^{-2} M tetraphenyl arsonium chloride (TPhAsCl) (Sigma).

Cyclic and SW voltammograms were recorded using an Autolab (Eco-Chemie, Utrecht, Netherlands) equipped with a PSTAT 20 potentiostat and the GPES 4.3 software package. Typical SW instrumental parameters, unless otherwise stated, were: square-wave frequency $f = 8 – 40$ Hz, square-wave amplitude $E_{SW} = 35$ mV and scan increment $dE = 3$ mV.

The base electrolyte solutions were 1.0×10^{-2} M LiCl (Merck p.a.) in ultrapure water and 1.0×10^{-2} M tetraphenyl arsonium dicarbollyl cobaltate (TPhAsDCC) in 1,2-dichloroethane (DCE, Dorwill p.a.). TPhAsDCC was

prepared by metathesis of tetraphenylarsonium chloride (TPhAsCl, Sigma) and cesium dicarbollyl cobaltate (CsDCC, Lachema p.a.). The precipitate was recrystallized from a water : acetone mixture and then dried in an oven at 30 °C for two days.

The pH of the aqueous phase was adjusted within the range of 1.50 – 8.00 by addition of HCl (Merck p.a.) and LiOH (Merck p.a.) respectively.

The s-triazine employed, PROM, was of the highest purity available (Riedel de-Haën). PROM was added to the aqueous phase, in a concentration range between 1.0×10^{-8} M and 5.0×10^{-4} M. Also, PROM solution 2.0×10^{-3} M in organic phase was used for standard addition experiments. All measurements were carried out after equilibrating the two phases by agitation employing equal volumes. In this way, partition equilibrium was achieved before starting the electrochemical experiments. During the agitation, the PROM added to the aqueous phase completely partitions to the organic phase as it was demonstrated previously [31].

2.1. Preconcentration Procedure

Solutions of PROM in aqueous phase were prepared at pH 8.00, to ensure that the herbicide was in neutral form ($pK_a^{PROM} = 4.05$ [31]). The concentration was varied within the range from 1.0×10^{-8} M to 2.4×10^{-7} M. Volumes of 500.0 mL were stirred with three successive aliquots of 10.00 mL of DCE, up to a final volume of 30.0 mL. The resulting organic solution was evaporated at room temperature in a rotary evaporator to reduce the volume to 2.0 or 3.0 mL. Then, 5.00 mL of solution was prepared with this extract and TPhAsDCC adding DCE up to the final volume.

3. Results and Discussion

In a previous paper [31], we have demonstrated that PROM facilitates proton transfer from the aqueous to the organic phase, based on the analysis of positive peak potential and peak current as a function of pH employing cyclic voltammetry. Facilitated proton transfer is favored when the neutral specie, X, predominates over the protonated form HX$^+$ (i.e., at pH $\gg$ pK_a^w conditions) and when this X specie is highly hydrophobic. PROM has a high partition coefficient, $\log P = 3.34$ [31], so that this behavior was observed in the whole range of pH studied. Figure 1 shows the CV (a) and SWV (b) profiles corresponding to proton transfer from aqueous phase facilitated by the herbicide present in the organic phase, at different pH values. The response is compatible with a reversible facilitated transfer process, controlled by drug diffusion.

In electrochemistry at ITIES the effect of iR-drop is a very critical point. Depending on the experimental conditions significant R_Ω values arise as a consequence of the low electrical conductivity of the organic solvent, and efficient iR compensation is needed. We studied the influence of the
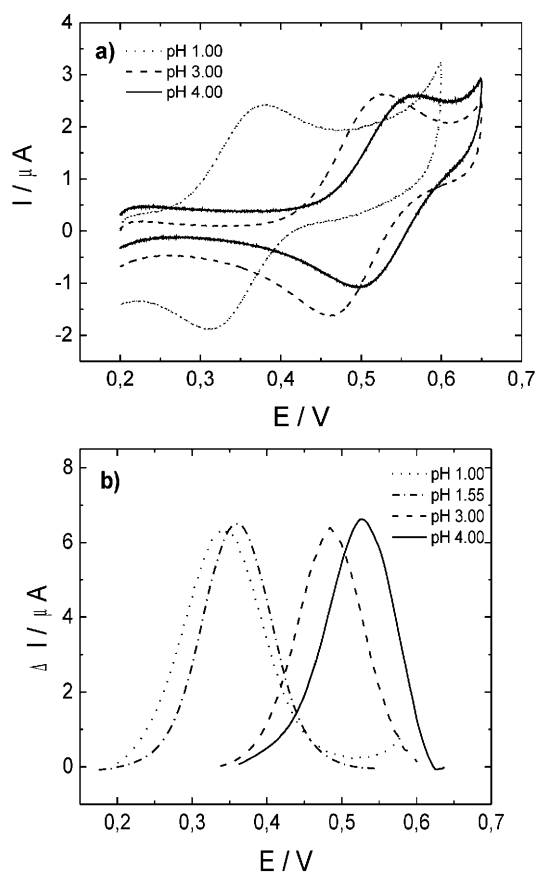
Fig. 1. a) Voltammetric profiles obtained for facilitated proton transfer in presence of PROM at different pH values, sweep rate: 50 mV s^{-1}. b) SWV voltammograms obtained for facilitated proton transfer in presence of PROM at different pH values. $E_{sw} = 35$ mV and d$E = 3$mV, $f = 8$ Hz. In both cases, pH (······) 1.00, (− · − · − ·) 1.55, (− − −) 3.00; (—) 4.00. Aqueous phase composition (APC): 1.0×10^{-2} M LiCl + 1.0×10^{-4} M PROM. Organic phase composition (OPC): 1.0×10^{-2} M TPhAsDCC.

uncompensated resistance at liquid – liquid interfaces employing SWV technique [25]. The study was based on the distortion of SW voltammetric profiles of tetraethyl ammonium transfer process across the water/1,2-dichloro-ethane interface when an uncompensated resistance, R_Ω, was present. The iR-drop effect was recognized analysing the variation of the SWV response with signal frequency. The nonlinear dependence of peak current on the square root of the frequency, the increase of half-peak width and the shift of peak potential towards more positive values were the indication of an uncompensated resistance, in agreement with the behavior predicted by V. Mirčeski et al. [36] for a reversible process.

To ensure that no resistance effect was present, the variations of SW voltammetric parameters at different pH values were analyzed and showed in Figure 2. As can be seen, peak current (ΔI_p) depends linearly on $f^{1/2}$, peak potential (E_p) and half – peak width ($\Delta E_{p/2}$) values were constant, independent on f (in the range $8 \leq f \leq 40$ Hz). This behavior indicates the absence of iR-drop effect on the voltammetric response.
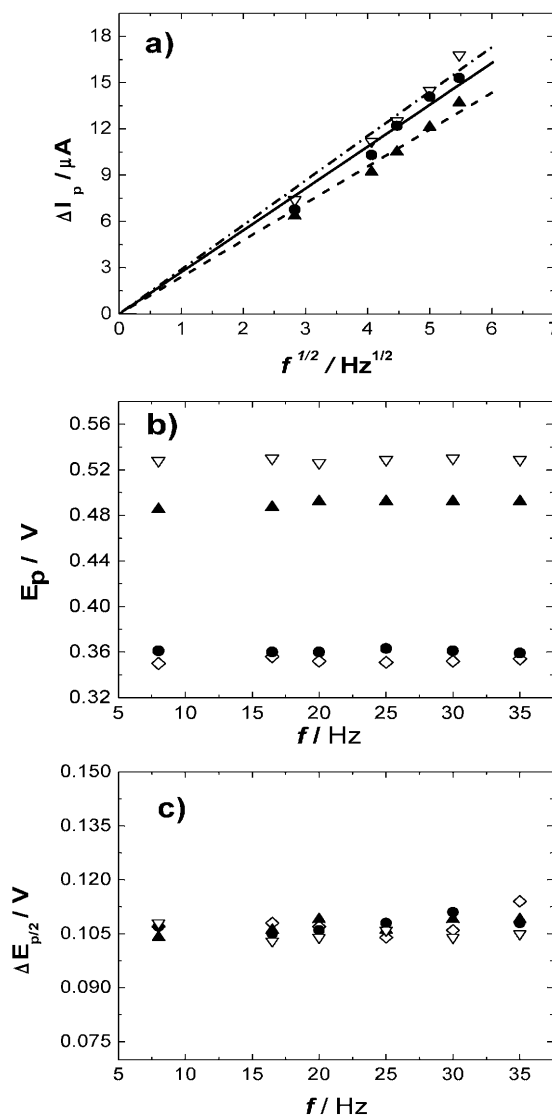

Fig. 2. Plots of a) ΔI_p vs. $f^{1/2}$; b) E_p vs. f; c) $\Delta E_{p/2}$ vs. f, at different pH values: (◆) 1.00; (●) 1,55; (▲) 3.00; (▽) 4.00. APC: 1.0×10^{-2} M LiCl $+ 1.0 \times 10^{-4}$ M PROM. OPC: 1.0×10^{-2} M TPhAsDCC. $E_{sw} = 35$ mV, d$E = 3$ mV.

3.1. Calibration Curves Before and After Preconcentration Procedure

Once found the optimal experimental conditions, the calibration curve was plotted employing ΔI_p values from SWV experiments. The calibration curve of PROM before preconcentration procedure was carried out at f equal to 8 Hz and pH 2.50. A linear range from 1.0×10^{-6} M to 5.0×10^{-4} M with a correlation coefficient of 0.9999 was obtained (not shown). The detection limit (DL) reached under these experimental conditions was 1.5×10^{-6} M, determined from standard deviation of a set of three replicates.

This DL value is lower than that obtained when calibrations curves are constructed employing I_p values from cyclic voltammetry experiments, but it is not lower enough to determine traces of PROM. For these reason, a preconcen-
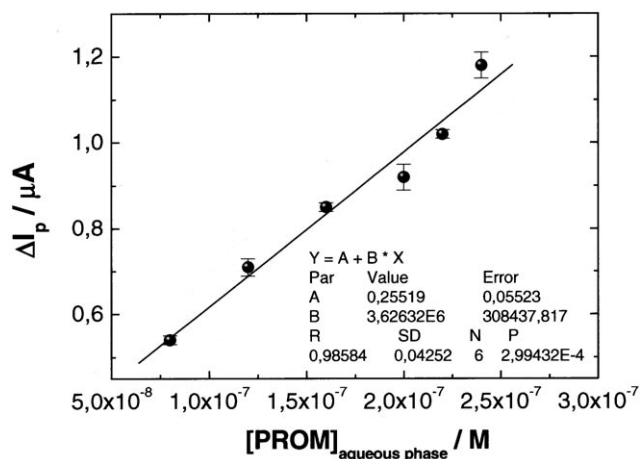
Fig. 3. Calibration curve of PROM employing preconcentration treatment. APC: LiCl 1.0×10^{-2} M, pH 2.00; OPC: TPhAsDCC 1.0×10^{-2} M + x PROM. This organic phase was obtained from preconcentration of PROM aqueous solutions in the concentration range: 1.0×10^{-8} M − 2.4×10^{-7} M (showed in x-axis). SWV parameters: $f = 8$ Hz, $E_{sw} = 35$ mV, $dE = 3$ mV.
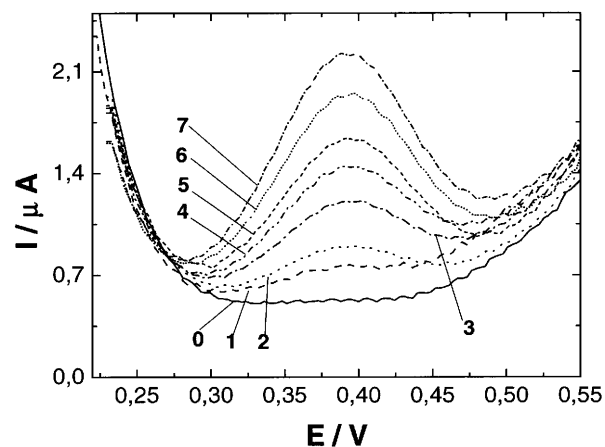

Fig. 4. SWV voltammetric profiles obtained after standard addition of a PROM 2.0×10^{-3} M solution to the organic preconcentrated phase. APC: LiCl 1.0×10^{-2} M, pH 2.00, f 8 Hz. OPC: TPhAsDCC 1.0×10^{-2} M + PROM (obtained by preconcentrating a 1.0×10^{-8} M PROM aqueous solution), with the following aggregates: 0) 0 µL; 1) 10 µL; 2) 20 µL; 3) 30 µL; 4) 40 µL; 5) 50 µL; 6) 60 µL; 7) 70 µL. SWV parameters: $f = 8$ Hz, $E_{sw} = 35$ mV, $dE = 3$ mV.

tration method was developed. This method consists in the extraction of PROM to the organic solvent, as it was described before. The herbicide was dissolved in aqueous phase in the concentration range 1.0×10^{-8} M to 2.4×10^{-7} M (corresponding to 1.0×10^{-6} M to 2.4×10^{-5} M in organic phase after the extraction). Figure 3 shows the variation of ΔI_p with the herbicide concentration in aqueous phase. A linear range of 8.0×10^{-8} M to 2.4×10^{-7} M was obtained. The correlation coefficient was 0.9858 and $DL = 1.0 \times 10^{-7}$ M was determined from standard deviation of a set of three replicates. It is important to notice that preconcentration procedure allows an increase of analytical sensibility of the technique.

It is worthwhile to remark that the extraction of the herbicide to the organic phase not only decreases the detection limit, but also, it offers the possibility of purification of the samples. This advantage can be very useful in the analysis of real samples, because they could have interferents or other contaminants more hydrophilic which would not transfer to the organic phase. Thus, it is possible to isolate the herbicide from the real aqueous matrix to the organic phase for its quantification.

3.2. Standard Addition Method

The addition of standards was carried out on a preconcentrated sample obtained from an aqueous solution containing 1.0×10^{-8} M PROM. In Figure 4 it can be seen the square-wave voltammograms obtained after the additions of different aliquots of PROM 2.0×10^{-3} M to the organic preconcentrated solution. The ΔI_p values obtained after each addition are shown in Figure 5 as a function of PROM added concentration. The correlation coefficient obtained from linear regression was 0.997. A 1.0×10^{-6} M initial concentration in the organic preconcentrated phase was deter-
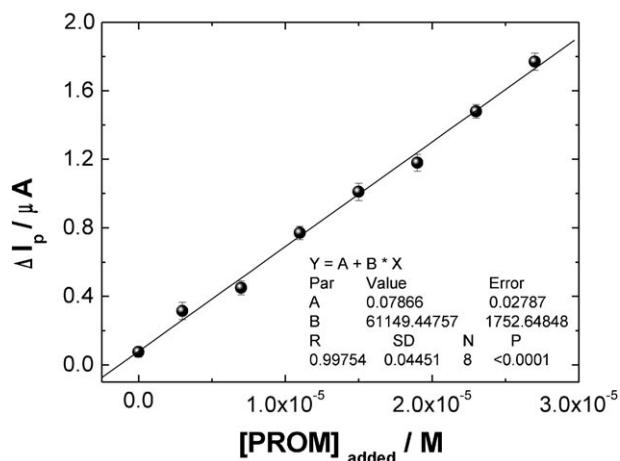

Fig. 5. Variation of ΔI_p with PROM concentration for standard addition experiments. The experimental conditions are the same as those in Figure 4.

mined from x-axis intercept. Indeed, this concentration value corresponds to PROM 1.0×10^{-8} M in the original aqueous phase. So that, these results allow concluding that standard addition method is appropriated for the quantification of PROM in preconcentrated samples.

4. Conclusions

Herbicide quantification in organic phase was possible employing SWV technique at a liquid-liquid interface; the detection limit found was 1.5×10^{-6} M. This DL value is very high for traces determinations of PROM required in environmental studies; however one of the advantages of these systems is the possibility of preconcentrating the

herbicide in the organic phase. The extraction of PROM to organic phase is favored for the high partition coefficient value and high solubility of this drug in 1,2-DCE. The aqueous:organic volume ratio equal to 500:30 and the later reduction of the volume of the organic phase enriched with PROM, by a factor of 6, yield an overall preconcentration factor of 100. The detection limit could be lowered to 1.0×10^{-7} M under these experimental conditions. Linear calibration curves in the concentration range between 1.0×10^{-6} and 5.0×10^{-5} M, without pretreatment, and between 8.0×10^{-8} and 2.4×10^{-7} M, carrying out the preconcentration procedure previous to electrochemical determination, were obtained. On the other hand, the standard addition method is highly efficient in this kind of systems and presents several advantages like less use of reactive and ease sample manipulation. A linear response in the concentration range between 1.0×10^{-6} and 2.7×10^{-5} M with correlation coefficient of 0.997 was obtained. The lower concentration value, in this case, corresponds to an aqueous PROM solution 1.0×10^{-8} M.

It is worthwhile to discuss the practical aspect of the procedure here proposed. In this sense, the extraction and preconcentration methods developed in references [26, 32 – 35] have several practical advantages over the present procedure, as the use of low organic phase volume, among others. Nevertheless, the results obtained in the this study justify evaluating the possibility of carrying out PROM preconcentration following the thin film approach [35] or the electrochemistry modulated liquid – liquid extraction procedure [32 – 35].

5. Acknowledgements

6. References

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