International Scholars Journals

African Journal of Chemistry ISSN: 4391-3199 Vol. 3 (1), pp. 102-113, January, 2016. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Potentiometric sensors for phenylureas based on their molecularly imprinted particles

Boutros M. El Batout[1,2]* and Ibrahim V. I. Youssef[1]

[1]Chemistry Department, College of Science, Qassim University, Buraidah 6644, KSA. [2]Chemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

Accepted 1 December, 2015

A molecularly imprinted polymer (MIP), with special molecule recognition properties of different phenylurea herbicides namely liuron (LU), isoproturon (IPU), diuron (DU), fenuron (FU) and methiuron (MU), was prepared by thermal polymerization in which phenylureas acted as the template molecules, methacrylic acid (MAA) acted as the functional monomer and ethylene glycol dimethacrylate (EGDMA) acted as the crosslinker. A biomimetic potentiometric field monitoring device was developed for the assessment of these phenylurea herbicides based on these newly synthesized imprinted polymers. The sensing elements were fabricated by the inclusion of phenylurea imprinted polymers in polyvinyl chloride (PVC) matrix. The sensors showed a high selectivity and a sensitive response to the template in an aqueous system. Electrochemical evaluation of these sensors revealed near-Nernstian response with slopes of 66.1±0.5 (r^2 = 0.998), 59.6±1.3 (r^2 = 0.997), 62.3±0.6 (r^2 = 0.998), 67.1±0.3 (r^2 = 0.998) and 71.5.0±0.4 (r^2 = 0.998) mV decade^{-1} with a detection limit of 1.0 × 10^{-5}, 7.1 × 10^{-6}, 1.3 × 10^{-5}, 1.8 × 10^{-5} and 1.6 × 10^{-5} mol L^{-1} with MIP/LU, MIP/IPU, MIP/DU, MIP/FU and MIP/MU membrane based sensors plasticized with DOP, respectively. The sensors were easily used in a double channel flow injection system and compared with a tubular detector. The method had the requisite accuracy, sensitivity and precision to assay phenylureas in water samples.

Key words: Phenylurea herbicides, potentiometric sensors, flow injection analysis (FIA), molecularly imprinted polymers.

INTRODUCTION

Herbicides represent about 50% of the demand for agricultural chemicals; their prolonged use involves the risk of their retention in crops and soils, from which in turn, due to washing and leaching processes, these substances pass to surface and ground waters (Barbash and Resek, 1996). Phenylureas are selective systemic herbicides commonly used in agriculture, alone or in combination, for the pre-emergence treatment of soil. Due to their polar nature, the increased possibility of them leaching from the surface to the water supply and water reserves, together with the emergence of potentially toxic degradation and metabolic products, may constitute a risk to human health (Ragsdale and Menzer, 1989; Lewis, 1992). Several techniques have been reported for phenylurea determinations (Suusse et al., 1996; Fenoll et al., 2012; Goger et al., 2001; Farran et al., 2004; Chicharro, 2005).

Over the past decade, molecular imprinting is a tool for the preparation of polymeric materials with high selectivity and affinity. The synthesis of MIPs involves the assembly of monomers around a template molecule, followed by polymerization in the presence of a cross-linker. Removal of the template molecule by extraction leaves sites specific for the template molecule as regards both shape and chemical functionality, thus enabling subsequent

*Corresponding author. E-mail:boutros2000@yahoo.com

recognition of the template. MIPs possess the advantages of physical robustness, high strength, resistance to elevated temperatures and pressures, and inertness towards acids, bases, metal ions and organic solvents compared to enzymes. MIPs have been extensively used in chromatographic separations (Kempe, 1995; Ansell et al., 1996; Takeuchi et al., 1999; Lai et al., 2001), antibody-mimics (Yilmaz et al., 1999; Beach et al., 1994), sensors (Sergeyeva et al., 1999; Kriz et al., 1995) and mimic enzyme catalysis (Ohkubo et al., 2001). In recent years, the applications of MIPs in potentiometric sensors have been extensively studied (Kamel et al., 2011; Kamel and Sayour, 2009; Kamel et al., 2012; Abd-Rabboh and Kamel, 2012; Kamel et al., 2010). The advantage of the association of MIP to potentiometric sensors is the avoidance of the need for template extraction from the host-tailored particle. This extraction may leave vacant recognition sites, ready for binding, which is a typical source of uncertainty in the determination or a sensitivity-limiting factor. In addition, there is no size restriction on the template compound because it does not have to diffuse through the membrane. Up to now, there are only few papers dealing with the phenylurea-based molecular imprinting studies (Tamayo et al., 2003; Martin-Esteban et al., 2001; Wang et al., 2005), but no potentiometric sensors based on MIP for monitoring this type of herbicides are reported in the literature.

In the present work, we describe sensitive potentiometric sensors for phenylureas (that is, linuron, isoproturon, diuron, fenuron and methiuron) based on their molecularly imprinted particles dispersed in 2-nitrophenyloctyl ether (NPOE) and embedded in polyvinyl chloride (PVC) matrix, for the monitoring of linuron (LU), isoproturon (IPU), diuron (DU), fenuron (FU) and methiuron (MU). Advantages of these sensors include the simplicity in designing, short measurement time, low cost, adequate precision, high accuracy, high analytical throughput, good response stability, low limit of detection and reasonable selectivity in the presence of many interferents.

EXPERIMENTAL

Equipments

Potentiometric measurements were performed at 25±1°C with Jenway digital pH/mV meter (model 3510) using the PVC membrane sensors based on MIP/ LU, MIP/IPU, MIP/DU, MIP/FU and MIP/MU particles in conjunction with an Orion Ag/AgCl double junction reference electrode (model 90-02) filled with 10% (m/v) KNO_3 solution in the outer compartment, and Ross glass pH combination electrode (Orion 81-02) was used for pH measurements. The potentials were measured for stirred solutions using the following electrochemical cell: Ag/AgCl/10^{-3}mol L^{-1} phenylurea herbicide/membrane /sample test solution/ Ag/AgCl double junction reference electrode.

The flow injection analysis (FIA) system manifold consisted of a two-channel Ismatec-MS REGLO model peristaltic pump. The manifold was connected with polyethylene tubing (Tygon, 0.7 mm i.d.) and an Omnifit injection valve (Rheodyne, Model 7125) with sample loop of 100 µL volume. The potential signals were recorded using an Jenway pH/mV meter (model 3510) connected to a PC through the interface ADC 16 (Pico Technology, UK) and Pico Log for windows (version 5.07) software.

Reagents and materials

All reagents were of analytical grade and used as received without further purification. Doubly distilled water was used throughout. High molecular weight poly (vinyl chloride) PVC, tetrahydrofuran (THF), and 2-nitrophenyl phenyl ether (2-NPPE) were obtained from Sigma-Aldrich (St. Louis, Mo). Methacrylic acid (MAA), ethyleneglycoldimethacrylate (EGDMA) and benzoyl peroxide (BPO) were obtained from Fluka (Ronkonoma, NY). Linuron (LU), isoproturon (IPU), diuron (DU), fenuron (FU) and methiuron (MU) (chemical structures are shown in Figure 1) were obtained from Riedel-deHaën (Seelze, Germany).

The herbicide stock solutions (10^{-3} mol L^{-1}) were prepared by dissolving an appropriate amount of the compound in methanol: water and kept in the dark at -5°C. Working solutions were prepared daily by diluting the stock solution with Britton-Robinson (BR) buffer (0.04 mol L^{-1} boric acid, 0.04 mol L^{-1} phosphoric acid and 0.04 mol L^{-1} acetic acid, pH was adjusted with 0.2 mol L^{-1} sodium hydroxide), covering the pH range from 2.0 to 7.0.

Synthesis of host-tailored polymers

For preparing MIPs, the template (0.5 mmol of herbicide) was placed in a glass tube (14.0 mm i.d) with the functional monomer (5.0 mmol MAA), the cross-linker (25.0 mmol EGDMA) and the radical initiator (0.30 mmol BPO), all dissolved in 5 mL acetonitril. The mixture was sonicated, degassed with nitrogen for 5 min, and placed in a water bath at 70°C for 30 min. Non-imprinted polymers (NIP) was also prepared in a similar way, excluding the template from the procedure. The resulting polymers were ground and sieved to particle sizes ranging from 50 to 150 µm. Extraction of the template molecule and washout of non-reacted species was carried out with methanol/acetic acid (4:1, v/v). All polymers both imprinted and non-imprinted were let dry at ambient temperature before use.

Sensor and detector preparation

The sensing membranes were prepared by mixing 15 mg

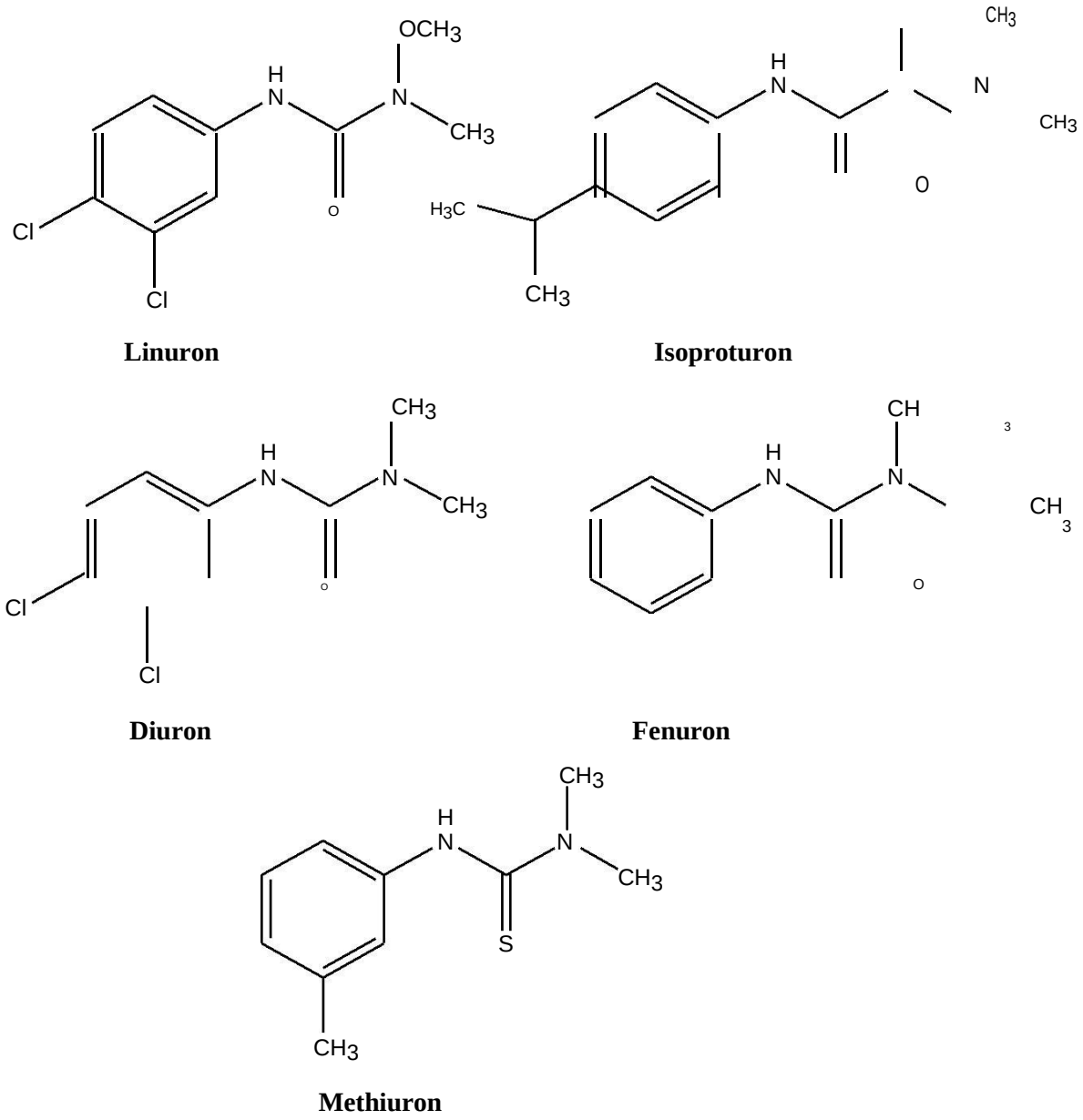

Figure 1. Chemical structures of phenylurea herbicides.

of the sensing polymer, 350 mg of the plasticizer (*o*-NPOE) and 200 mg of PVC and dissolved in ~ 3 mL THF in a glass ring (2.2 cm diameter) placed on a glass plate. The resulting mixture was left to stand overnight at room temperature to evaporate the solvent slowly. The resulting membrane was peeled off from the glass ring and discs of 9 mm i.d were cut out and glued onto a 7-mm i.d PVC body using THF. The tube was filled with 1.0 × 10^{-3} mol L^{-1} of the corresponding phenylurea herbicide as internal solution. A 3 mm diameter Ag/AgCl coated wire was used as an internal reference electrode. The sensors were conditioned by soaking in a 1.0 × 10^{-3} mol L^{-1} of their correspondence aqueous phenylurea herbicide solution for 24 h before use and were stored in distilled water between measurements. The sensors were stored in the same solution when not in use.

Detectors for flow injection analysis were prepared by mixing 15 mg of the sensing polymer, 350 mg of the plasticizer (*o*-NPOE), 200 mg PVC and 1.0 mg (K-TPB) and dissolved in ~ 3 mL THF. The clear solution was deposited drop wise on Tygon tube window of ≈ 0.5 cm length and 2 mm id. After each addition, the mixture was allowed to evaporate slowly at room temperature to yield a thin film. This operation was repeated until a membrane with a thickness of approximately 0.1 mm was formed. The sensor was conditioned by soaking in 1.0 × 10^{-3} mol

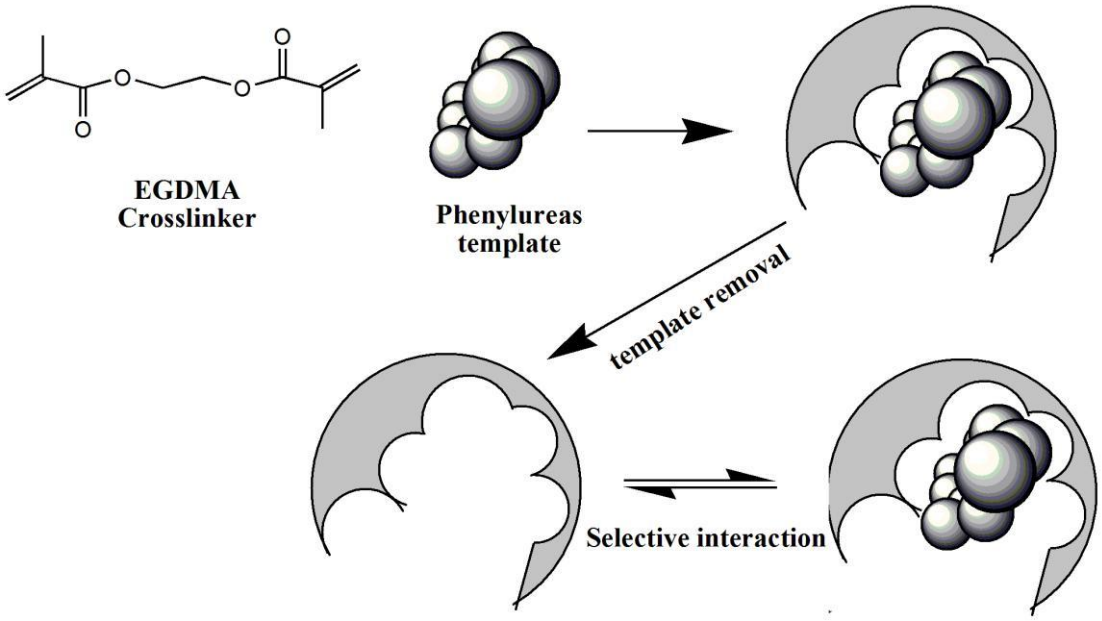

Figure 2. Protocols for preparation of the molecularly imprinted polymer and its recognition towards phenylureas herbicides.

L^{-1} of their corresponding phenylurea herbicide aqueous solution for 24 h and was stored in the same solution when not in use. The sensor was closely fitted in the tube at 10 cm distance from the valve. The end of the tube was placed in a beaker where a double-junction Ag/AgCl reference electrode was placed downstream from the detector just before the solution went to the waste. A carrier stream containing 1.0×10^{-2} mol L^{-1} acetate solution of pH 3.0 was pumped at a constant flow rate of 3.0 mL min^{-1}. To avoid slight pulsation originating from the peristaltic pump, grounding connection was made for flow system.

Binding experiments

The absence of phenylureas in the MIP particles was previously confirmed by measuring the absorbance of the washout solution at wavelength = 245 nm for MU, FU and IPU and at wavelength = 250 nm for DU and LU. The particles were repeatedly washed until the herbicide was no longer detected. The polymer was dried after at 60°C under vacuum until constant weight. Binding experiments were carried out by placing 20.0 mg of MIP washed particles in contact with 10.0 mL LU, IPU, DU, FU and MU solutions ranging from 0.2 to 5.0 mmol L^{-1}. The mixtures were stirred for 12 h at room temperature and the solid phase separated by centrifugation (3,000 rpm, 10 min). The concentration of free herbicide in the supernatant was detected by UV spectrophotometry at its corresponding wavelength. The amount of herbicide bound to the polymer was calculated by subtracting the concentration of free herbicide from its initial concentration. The data obtained were used for Scatchard analysis.

Analytical applications

Tap water samples were taken from the laboratory. It was filtered and then degassed with an ultrasonic bath. The samples were spiked with LU, IPU, DU, FU, or MU at a concentration of 5-10 µg mL^{-1} each. The working sensor and reference electrodes were immersed in the solution. The potential reading was recorded after reaching the equilibrium response (10 to 20 s).

RESULTS AND DISCUSSION

This study aimed to establish a simple and sensitive analytical system based on MIPs for the determination of some phenylurea herbicides. For this purpose, an electrochemical sensor was proposed utilizing the potentiometric determination method of bound analyte to the MIPs by electrochemical reaction. A schematic illustration for the molecular imprinting process is shown in Figure 2.

Equilibrium adsorption experiment

To understand how small molecules interact with adsorbent surface, the mode of binding and site distributions in the interaction, adsorption isotherms are an important tool. They plot the equilibrium

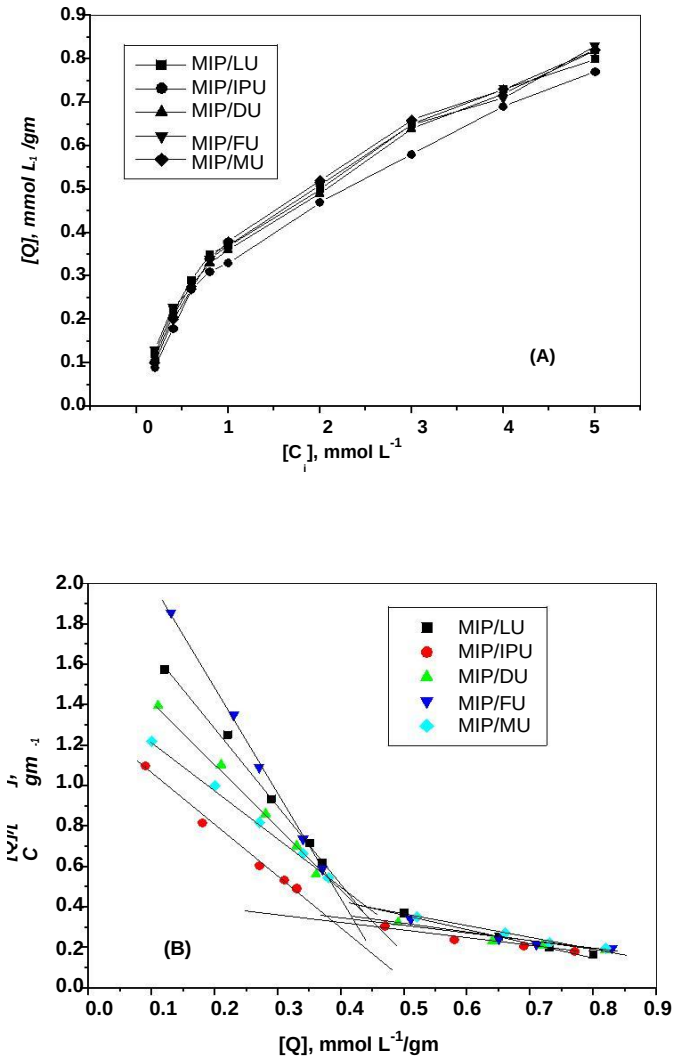

Figure 3. Binding isotherm (a) and Scatchard plot (b) for the phenylureas-imprinted (MI). *Q* = herbicide bound to 20.0 mg of the corresponding polymer; temperature = 25°C; volume = 10.0 mL; binding time: 12 h.

concentrations of bound ligand (adsorbate) versus free ligand. In liquid-phase applications of MIPs, a molecule in solution interacts with binding sites in a solid adsorbent. In the liquid-phase and after equilibrium the free ligand concentration becomes constant and is easily quantified to plot the corresponding adsorption isotherm (Guerreiro et al., 2011).

The static equilibrium adsorption experiments for the imprinted polymers were carried out by varying the initial concentration of the phenylurea herbicides in the range of 0.2 to 5 mmol L^{-1}. The adsorption isotherms are shown in Figure 3a.

It can be seen from the curve that the adsorbance of MIP increased with increasing of the initial concentration, but the adsorbance of NIP reached saturation for all tested herbicides when the initial concentration of the herbicide was beyond 0.8 mmol L^{-1}. Obviously, the adsorbance of MIP was bigger than that of NIP, which indicated that the cavities formed on MIP by selective bonding and the active binding sites in cavities determined that high affinity and specific recognition of MIP on the template were much larger than the non-selective bonding interaction. In studies on molecule imprinting, the Scatchard Model was often used to evaluate the binding characteristics of MIP, and the Scatchard equation can be described as shown by Yamamura et al. (1985):

$$[Q] / [C_{free}] = ([Q_{max}] - [Q]) / K_d \quad (1)$$

Where K_d is the dissociation constant of the binding site, $[Q_{max}]$ is the maximum binding capacity of the binding

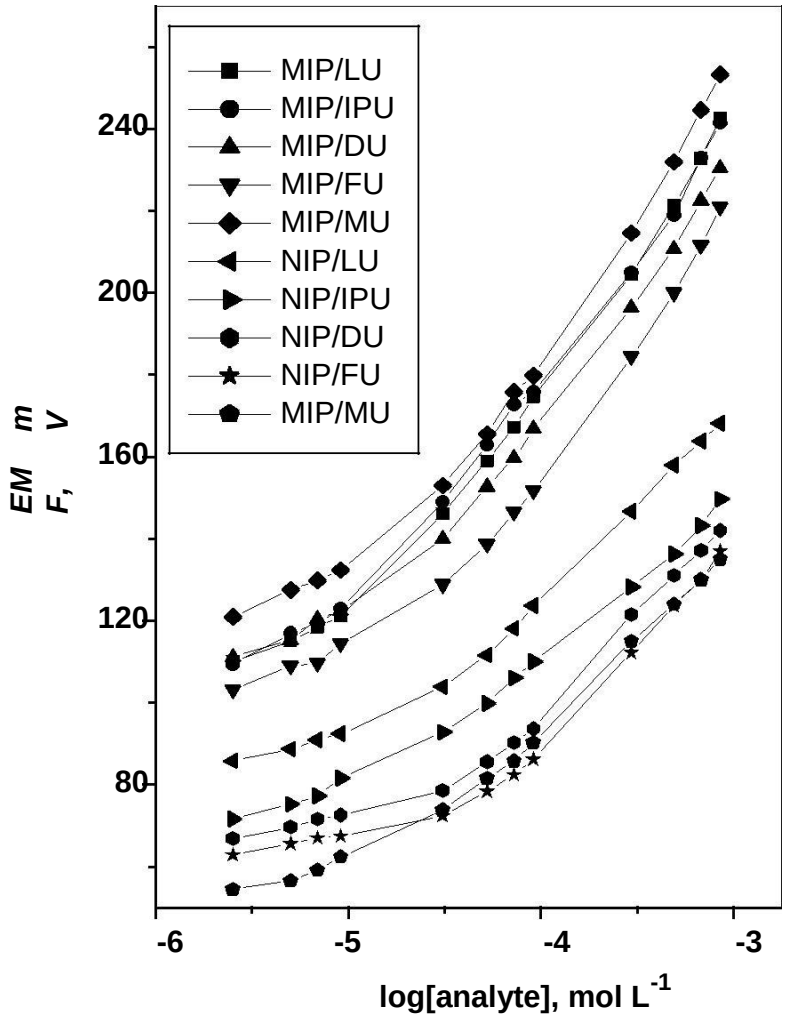

Figure 4. Calibration curve in acetate solution at pH 3.0 for different sensors.

site, and $[C_{free}]$ is the equilibrium concentration of the substrate in the supernatant. $[Q] / [C_{free}]$ was plotted versus $[Q]$ in Figure 3b. It illustrates that the binding sites of MIP for LU, IPU, DU, FU and MU were heterogeneous, but there were good linear relationships at both ends of the graph. According to this, it can be concluded that there existed two classes of binding sites with different affinities in the range of the different concentrations. It is probably because there were various interactions between the functional monomer (MAA) and imprinted molecules, and the interactions formed many kinds of complexes with different components. Various complexes have binding sites with different properties after polymerization. The data can be fitted according to the two sections of the linear relationship. The equilibrium dissociation constant K_{d1} and the apparent maximum amount Q_{max1} for the higher affinity binding sites can be calculated to be 0.259, 0.399, 0.303, 0.187 and 0.417 mmol L^{-1} and 0.534, 0.519, 0.538, 0.479 and 0.612 mmol g^{-1} for MIP/LU, MIP/IPU, MIP/DU, MIP/FU and MIP/MU, respectively. By the same treatment, K_{d2} and Q_{max2} for the lower affinity binding sites were calculated to be 1.450, 2.460, 2.411, 2.142 and 1.872 mmol L^{-1} and 1.030, 1.205, 1.244, 1.225 and 1.170 mmol g^{-1} for MIP/LU, MIP/IPU, MIP/DU, MIP/FU and MIP/MU, respectively.

ISEs analytical features

The synthesized MIPs were incorporated into the PVC membrane and were tested as sensing materials in the proposed potentiometric sensors. The potential response obtained with the sensors prepared with MIP/LU (sensor I), MIP/IPU (sensor II), MIP/DU (sensor III), MIP/FU (sensor IV) and MIP/MU (sensor V) membrane and their blank membranes is given in Figure 4. As seen from the figure, the sensors exhibit linear potentiometric response

with lower limit of linear range of 2.5×10^{-5}, 1.0×10^{-5}, 3.1×10^{-5}, 5.3×10^{-5} and 5.3×10^{-5} mol L^{-1}, and detection limits of 1.0×10^{-5}, 7.1×10^{-6}, 1.3×10^{-5}, 1.8×10^{-5} and 1.6×10^{-5} mol L^{-1}, for ISE's [I], [II], [III], [IV] and [V], respectively. All sensors exhibit near-Nernstian slopes of 66.1±0.5 (r^2 = 0.998), 59.6±1.3 (r^2 = 0.997), 62.3±0.6 (r^2 = 0.998), 67.1±0.3 (r^2 = 0.998) and 71.5.0±0.4 (r^2 = 0.998) mV decade^{-1}, respectively. The potential response obtained with the sensors prepared with NIP/LU (sensor VI), NIP/IPU (sensor VII), NIP/DU (sensor VIII), NIP/FU (sensor IX) and NIP/MU (sensor X) membrane exhibit also linear potentiometric response with lower limit of linear range of 3.1×10^{-5}, 3.1×10^{-5}, 5.3×10^{-5}, 1.0×10^{-4} and 3.1×10^{-5} mol L^{-1}, and detection limits of 1.3×10^{-5}, 1.0×10^{-5}, 3.1×10^{-5}, 3.1×10^{-5} and 1.4×10^{-5} mol L^{-1}, for ISE's [VI], [VII], [VIII], [IX] and [X], respectively. All sensors exhibit sub-Nernstian slopes of 45.8±0.7 (r^2 = 0.999), 38.5±0.9 (r^2 = 0.998), 48.1±0.6 (r^2 = 0.998), 51.5±1.1 (r^2 = 0.999) and 43.7±0.8 (r^2 = 0.998) mV decade^{-1}, respectively. The potentiometric response characteristics of the membrane sensors incorporating MIP and NIP as selective ion recognitions are shown in Table 1.

The repeatability of the potential reading for the sensors were examined by subsequent measurements in 5.0×10^{-4} mol L^{-1} immediately after measuring the first set of solution at 1.0×10^{-4} mol L^{-1} for each herbicide solution. The standard deviations of measuring emf for 5 replicate measurements obtained are 0.9 mV for the solution of 1.0×10^{-4} mol L^{-1} and 1.4 mV for the solution of 5.0×10^{-4} mol L^{-1}. This means that the repeatability of potential response of each sensor is good. The response properties of the sensors did not change obviously after the use of the sensor for three months. The validity of the proposed potentiometric method for determining each herbicide was assessed by measuring the range, lower limit of detection (LOD), accuracy (recovery), precision or repeatability (Cv_w), between-day variability (Cv_b), linearity (correlation coefficient) and sensitivity (slope) (Taylor, 1987). Data obtained with six batches (six determinations each) of each herbicide solution are shown in Table 1.

Effect of pH and response time

The influence of the pH on the potential response of sensors was tested using 10^{-5} and 10^{-4} mol L^{-1} of the corresponding herbicide over the pH range of 2 to 8. Adjustment of pH was carried out using NaOH and/or HCl. The pH-potential profiles showed that the membrane sensors display good stability and constant potential reading over the pH range of 2.5 to 4 for sensors I, II, IV, VI, VII and X, and 2.5 to 4.5 for sensors III, V, VIII and XI.

The time required to achieve a steady-state potential of the sensors within ±0.8 mV of the final equilibrium value was examined after successive immersion of the sensors in a series of their corresponding herbicide solutions, each has 10 fold differences, from low to high concentrations. The response time was <15 s for all herbicide solutions of concentrations in the linear range of calibration curves indicating a fast response of the sensors. The potentials remained constant for ~10 min (drift < 0.5 mV). The standard deviations of the potential reading of both sensors, for 6 identical measurements over a period of 2 months were calculated.

Sensors selectivity

The selectivity behavior of ISEs is defined by the ion exchange constants which depend on the selectivity of complexation as well as on the standard free energies of the respective ions in the aqueous and organic phases (Bakker and Pretsch, 2001). The former requisite suggests the use of ligands that strongly bind the preferred ion and only weaken all the others (Bakker et al., 1997), as the mechanism of selectivity is mainly governed by stereospecific and electrostatic aspects, being the lipophilic environment dictated by the plasticizer. The selectivity coefficients obtained for the proposed electrodes using the match potential method (MPM) (Umezawa et al., 2000) are summarized in Table 2.

The typical selectivity order of MIP/LU and MIP/IPU based sensors with membrane plasticized with *o*-NPOE is: LU > DU > MU> IPU > FU> phenylurea > phenylalanine >urea > NH_4^+ > K^+ > Na^+ > Ca^{2+} and IPU > LU > DU > FU> phenylalanine > phenylurea > urea > NH_4^+ > K^+ > Ca^{2+} > Na^+, respectively. For MIP/ DU, MIP/FU and MIP/MU based sensors with membrane plasticized with *o*-NPOE, the selectivity order is: DU > LU > FU> MU > IPU > phenylalanine > phenylurea >urea > NH_4^+ > K^+ > Na^+ > Ca^{2+}, FU > MU > DU> LU > IPU > phenylurea ~ phenylalanine >urea > NH_4^+ > K^+ > Na^+ > Ca^{2+} and MU > FU > IPU> LU > DU>phenylalanine>phenylurea >urea > NH_4^+> K^+> Na^+> Ca^{2+}, respectively.

Flow injection potentiometry

For the routine control of an analyte, FIA setup is of regular selection, in view of their versatility, simplicity and suitability for large-scale analyses. The flow assembly was double-channel, and the potentiometric sensor was accommodated in a flow cell of tubular configuration, allowing full membrane/sample contact (Figure 5).

MIP/LU, MIP/IPU, MIP/DU, MIP/FU and MIP/MU membrane based sensors were used in this study for showing the best analytical features for the simplest membrane composition. A sample loop (100 µL) for phenylureas solutions ranging from 1.0×10^{-5} to 1.0×10^{-3} mol L^{-1} at pH 3.0 with a 0.01 mol L^{-1} acetate carrier solution and flow rate of 3.0 mL min^{-1} was chosen to study the potentiometric response (slope in mV $decade^{-1}$) of the proposed sensors. Main analytical features recorded under optimum flow conditions are presented in

Table 1. Potentiometric response characteristics of phenylureas sensors.

Parameter	MIP/LU	MIP/IPU	MIP/DU	MIP/FU	MIP/MU	NIP/LU	NIP/IPU	NIP/DU	NIP/FU	NIP/MU
Slope (mV decade^{-1})	66.1±0.5	59.6±1.3	62.3±0.6	67.1± 0.3	71.5± 0.4	45.8±0.7	38.5±0.9	48.1±0.6	51.5±1.1	43.7±0.8
Correlation coefficient (r^2)	0.998	0.997	0.998	0.998	0.998	0.999	0.998	0.998	0.999	0.998
Linear range (mol L^{-1})	2.5×10^{-5}	1.0×10^{-5}	3.1×10^{-5}	5.3×10^{-5}	5.3×10^{-5}	3.1×10^{-5}	3.1×10^{-5}	5.3×10^{-5}	1.0×10^{-4}	3.1×10^{-5}
Detection limit (mol L^{-1})	1.0×10^{-5}	7.1×10^{-6}	1.3×10^{-5}	1.8×10^{-5}	1.6×10^{-5}	1.3×10^{-5}	1.0×10^{-5}	3.1×10^{-5}	3.1×10^{-5}	1.4×10^{-5}
Working range (pH)	2.5-4.0	2.5-4.0	2.5-4.5	2.5-4.0	2.5-4.5	2.5-4.0	2.5-4.0	2.5-4.5	2.5-4.0	2.5-4.5
Response time (s)	<15	<15	<15	<15	<15	<15	<15	<15	<15	<15
Standard deviation, σ_v(mV)	1.8	1.5	1.1	0.9	1.1	0.7	0.6	1.2	1.7	1.2
Accuracy (%)	95	96	98.6	99.5	99.3	96.7	98.1	96.5	94.3	95.6
Precision, CV_w (%)	0.8	0.7	0.6	0.4	0.4	0.7	0.9	0.7	0.5	0.6
Between-day variability, CV_b (%)	1.6	1.0	0.9	0.8	0.9	1.1	0.7	1.2	0.9	0.8

Table 2. Potentiometric selectivity coefficients ($Log\ K^{pot}_{i,B}$) of phenylureas PVC membrane sensors.

Interferent, B	MIP/LU	MIP/IPU	MIP/DU	MIP/FU	MIP/MU
Linuron (LU)	0	-1.9	-0.7	-1.7	-1.8
Isoproturon (IPU)	-2.0	0	-2.1	-2.2	-1.3
Diuron (DU)	-0.5	-2.0	0	-1.6	-2.1
Fenuron (FU)	-2.1	-2.1	-1.2	0	-0.9
Methiuron (MU)	-1.6	-1.8	-1.8	-1.1	0
Phenylurea	-2.9	-3.2	-2.8	-2.7	-2.6
Phenylalanine	-3.0	-3.1	-2.6	-2.7	-2.4
Urea	-3.2	-3.3	-3.4	-3.6	-3.1
NH_4^+	-3.9	-4.2	-4.1	-3.8	-4.0
K^+	-4.1	-4.3	-4.2	-4.4	-4.1
Na^+	-4.5	-4.5	-4.7	-4.6	-4.4
Ca^{2+}	-4.6	-4.4	-4.8	-4.9	-4.6

Table 3. The sensors gave slopes of 42.5 ± 1.2, 41.5 ± 0.9, 38.9 ± 1.4, 78.9 ± 0.4 and 77.7 ± 0.8 mV decade^{-1} with detection limits of 2.24 ± 0.3, 2.06 ± 0.8, 2.33 ± 0.1, 4.11 ± 0.2 and 6.02 ± 0.3 µg mL^{-1} and sample output of 18-20, 20-22, 22-25, 30-35 and 32-36, respectively, as shown in Figure 6a to e.

Analytical application: Monitoring of phenylureas in water samples

The method was used to determine phenylureas in water samples from agricultural sources. The water was mixed and spiked with 6 to 10 µg mL^{-1} phenylureas. A good agreement was found between added and found amounts of herbicide. Mean values of four independent determinations were 6.0, 7.5, 8.5, and 10 µg mL^{-1}. Results of the potentiometric analysis conducted in steady state showed recoveries ranging from 95 to 102.6%, 91.6 to 98.8%, 85 to 98.6%, 91.6 to 98.6% and 91.0 to 98.8% for MIP/LU, MIP/IPU, MIP/DU,

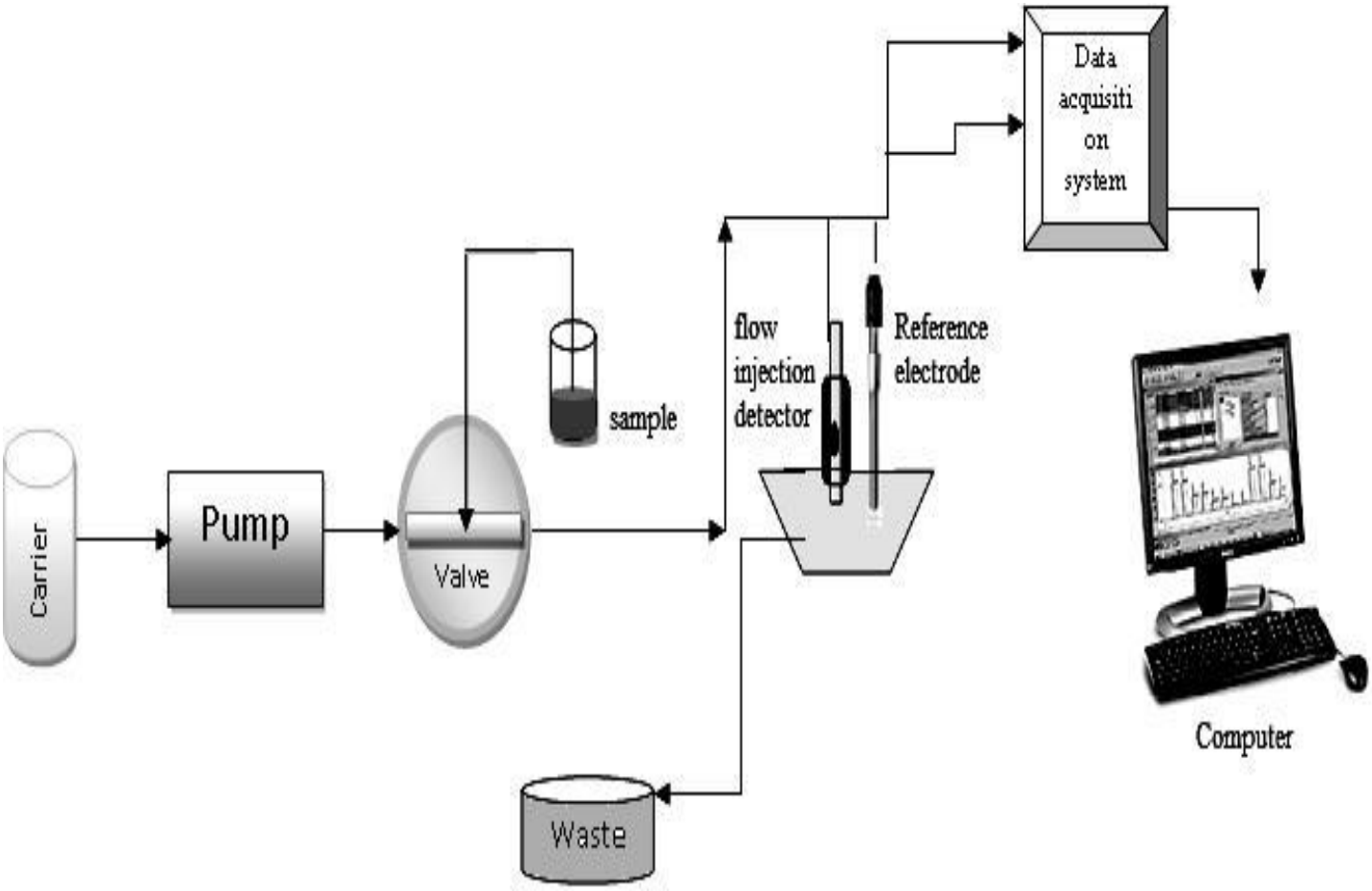

Figure 5. FIA manifold for the evaluation of the tested herbicides. A 0.01 mol L^{-1} carrier acetate buffer solution pH 3.0; loop sample 100 µL; and flow rate 3 mL min^{-1}.

Table 3. Response characteristics of phenylureas sensors under FI operation.

Parameter	MIP/LU	MIP/IPU	MIP/DU	MIP/FU	MIP/MU
Slope, mV $decade^{-1}$	42.5±1.2	41.5±0.9	38.9±1.4	78.9±0.4	77.7±0.8
Correlation coefficient, r	0.998	0.997	0.997	0.998	0.991
Lower detection limit, µg mL^{-1}	2.24±1.2	2.06±0.8	2.33±0.1	4.11±0.2	6.02±0.3
Optimum flow rate, mL min^{-1}	3	3	3	3	3
Life span, week	8	8	8	8	8
Output, sample h^{-1}	18-20	20-22	22-25	30-35	32-36

MIP/FU and MIP/MU membrane based sensors, respectively. As presented in Table 4, the results obtained for the analysis of water samples presented a good accuracy and demonstrates the applicability of the sensors for routine analysis without a prior separation. To confirm whether there was a statistically significant difference between the means of static potentiometric sets of results, the *t*-student value was calculated. The *p* two-tail was 0.06, below the theoretical value (3.18), confirming that there are no significant differences between the means.

Conclusions

Molecular imprinting technique was employed to produce phenylureas host-tailored sensors for potentiometric transduction. The sensors displayed good potentiometric analytical features capable of discriminating the different

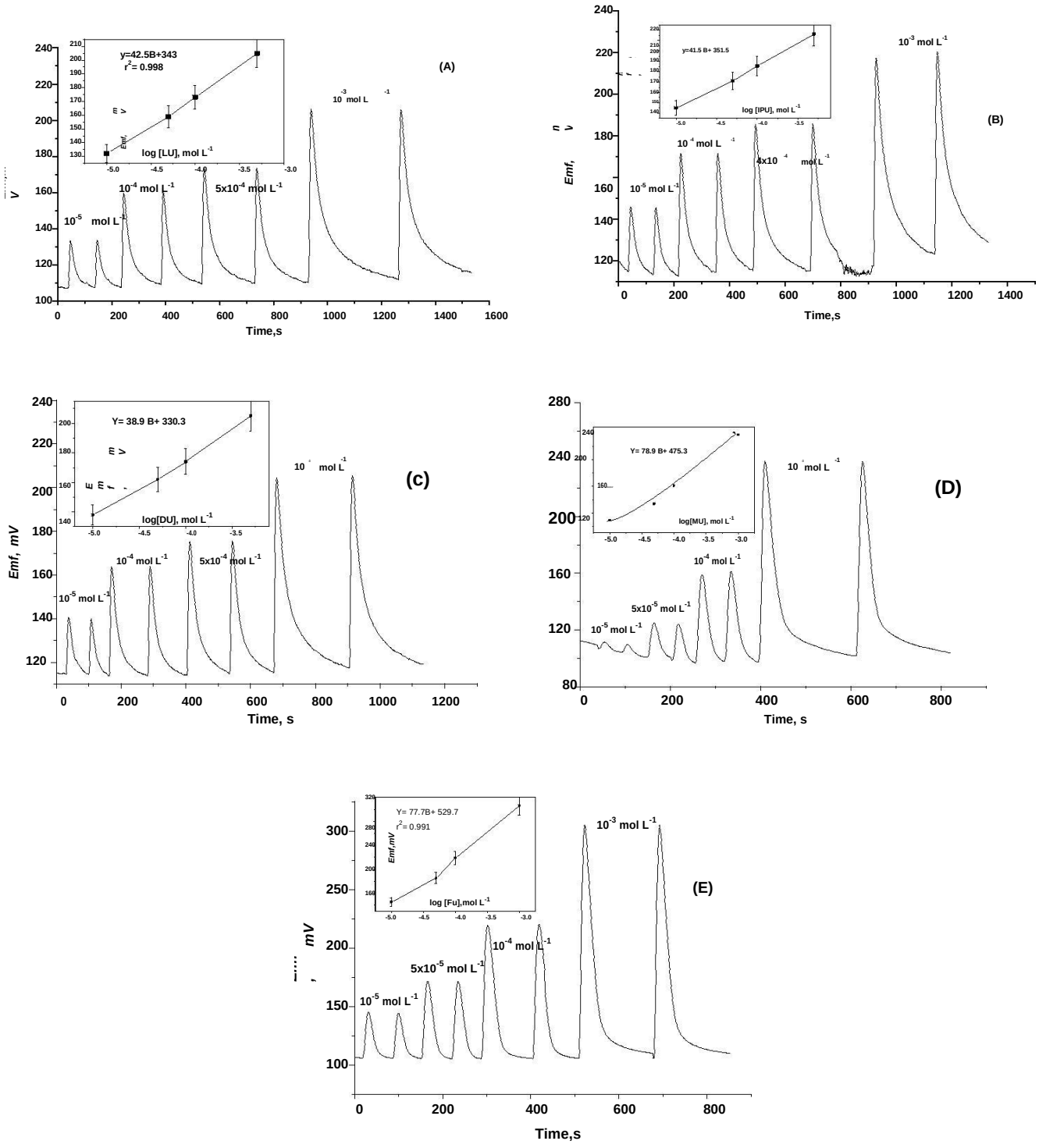

Figure 6. Transient potentiometric signals obtained in duplicate for phenylureas membrane based sensors. (A) MIP/LU; (B) MIP/IPU; (C) MIP/DU; (D) MIP/FU; (E) MIP/MU.

phenylureas herbicides in aqueous media. Simplicity in designing, short measurement time, good precision, high accuracy, high analytical throughput, low detection limit and good selectivity are the advantages of these sensors. The sensors were successfully applied in analysis of phenylureas in water samples both in steady state and in

Table 4. Application of the proposed method to determination of phenylureas in water samples spiked with different amounts of phenylureas.

Sample	Amount added	Concentration of phenylureas, µg mL^{-1}																			
		MIP/LU				MIP/IPU				MIP/DU				MIP/FU				MIP/MU			
		Static		FIA		Static		FIA		Static		FIA		Static		FIA		Static		FIA	
		Found	Rec. %	Found	Rec. %	Found	Rec. %	Found	Rec. %	Found	Rec. %	Found	Rec. %	Found	Rec. %	Found	Rec. %	Found	Rec. %	Found	Rec. %
Sample 1	6.0	5.7±0.1	95.0	5.8±0.3	96.6	5.5±0.2	91.6	5.4±0.3	90.0	5.2±0.1	86.6	5.1±0.3	85.0	5.5±0.4	91.6	5.6±0.1	93.3	5.8±0.2	96.6	5.6±0.3	93.3
Sample 2	7.5	7.7±0.2	102.6	7.4±0.1	98.6	7.1±0.2	94.6	7.3±0.5	97.3	7.4±0.2	98.6	7.1±0.2	94.6	7.0±0.3	93.3	7.4±0.1	98.6	7.1±0.3	94.6	7.4±0.1	98.6
Sample 3	8.5	8.4±0.3	98.8	8.2±0.4	96.5	8.3±0.1	97.5	8.4±0.1	98.8	7.9±0.3	92.9	8.1±0.4	95.3	7.9±0.7	92.9	8.1±0.2	95.3	8.4±0.1	98.8	8.0±0.4	94.1
Sample 4	10	9.7±0.4	97.0	9.5±0.1	95.0	9.3±0.4	93.0	9.8±0.3	98.0	9.4±0.2	94.0	9.6±0.3	96.0	9.3±0.3	93.0	9.7±0.1	97.0	9.8±0.1	98.0	9.1±0.3	91.0

flowing media. The proposed method is simple, of low cost, precise, accurate and inexpensive regarding reagent consumption and equipment involved. The main goal of these new sensors is to be used as a screening method; however, its detection limit is still higher than the desired limit.

ACKNOWLEDGMENT

The authors acknowledge the financial support from Deanship of Scientific Research, Qassim University, by means of project 1071/2012.

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