

2. Experimental

2.1. Chemicals and materials

Ethyleneglycol dimethacrylate (EDMA), methacrylic acid (MAA), styrene and 4-vinylpyridine (VPD) were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). 2,2'-Azobis-(isobutyronitrile) (AIBN) was obtained from Janssen Chimica (Geel, Belgium). Polyvinyl alcohol (PVA), polyvinyl chloride (PVC), 2,4-dichloro-1-nitrobenzene, oxalic acid and citric acid were obtained from Fluka Chemie AG (Buchs, Switzerland). Dibutyl phthalate was purchased from Unilab (Auburn, Australia). EDMA was purified by extraction with 10% CaCO₃, washing with water, drying over anhydrous sodium sulfate and subsequent distillation under reduced pressure. MAA was purified by distillation under reduced pressure. Five regulated HAAs: trichloroacetic acid (TCAA), dichloroacetic acid (DCAA), monochloroacetic acid (MCAA), dibromoacetic acid (DBAA) and monobromoacetic acid (MBAA), which have been regulated by the US EPA, plus one non-regulated HAA, tribromoacetic acid (TBAA) were studied of this work. These standards were obtained from Fluka Chemie AG (Buchs, Switzerland), except TCAA that was purchase from Merck K.G. (Darmstadt, Germany). All solvents were analytical grade and were dried with a molecular sieve before use. Working standard solutions were prepared daily depending on their concentrations. All solutions were stored at 4°C.

2.2. Preparation of TCAA-imprinted polymer

Bulk polymerization (BP) method

The procedure of bulk polymerization for the preparation of TCAA-imprinted polymer was modified from the method of Vlatakis et al. [11] as follows. 0.4 g of TCAA (2 mmol), 1.1 g of VPD (12 mmol), 9.3 g of EDMA (0.30 mol) and 0.12 g of AIBN (0.36 mmol) were dissolved in 25 ml of acetonitrile. Then, the mixture was degassed under vacuum, sonicated for 5 min, and purged with nitrogen for 5 min. The polymerization of the monomeric mixture was performed at 60°C for 18 h. The bulk polymer was ground to a fine powder in a pestle and mortar and sieved through a 100 mesh-sieve giving particles in the size range 1-100 μm . The TCAA template molecules were extracted from the polymer particles with a Soxhlet apparatus using methanol as a solvent. The extraction of polymer was confirmed to be complete from the disappearance of acidic proton in $^1\text{H-NMR}$ (CCl_4) measurements together with the absence of TCAA in a methanol rinse of polymer, as verified by gas chromatographic method (see detail in the analysis part). Finally, the polymer particles were dried under vacuum and stored at ambient temperature until use. Non-printed polymer (nonMIP) included as the control polymer was prepared in an identical manner except that template molecule was omitted from the process.

Multi-step swelling polymerization (MSP) method

The procedure of the multi-step swelling polymerization used in synthesis of TCAA-imprinted polymer was as described previously [12]. In the present case, one milliliter of 0.1% polystyrene aqueous solution was agitated with a microemulsion consisting of 0.48 ml of dibutyl phthalate, 0.02 g of sodium lauryl sulfate and 5 ml of distilled water at room temperature for 20 h. Then, the microemulsion prepared from 0.375 g of AIBN, 5 ml of acetonitrile, 12.5 ml of distilled water and 10 ml of 4.8% PVA solution in water

was added. After 2 h, the monomeric mixture consisting of 5.0 g of EDMA (160 mmol), 0.63 g of VPD (7.0 mmol), 0.02 g sodium lauryl sulfate, 12.5 ml of distilled water and 10 ml of 4.8% PVA solution was added. The emulsion was kept stirred at room temperature for 2 h before the addition of the TCAA template (0.33 g, 1.65 mmol). The polymerization was then performed at 50°C under nitrogen stream for 24 h and the resulting polymer was isolated by pouring the mixture into 250 ml of acetonitrile. Finally, the template molecule was extracted from polymer using the same procedure as that used in the BP method. The control polymer of this case was prepared identically to its MIP, but in the absence of template.

2.3. Binding study of the MIPs

The selective recognition of BP-based MIP and MSP-based MIP was examined in the direct binding system, using nonMIPs for control experiments. For this purpose, two series of TCAA analogous compounds; (1) the six halogenated acetic acids (halo acids) such as TCAA, DCAA, MCAA, TBAA, DBAA and MBAA, and (2) the three non-halogenated acetic acids (non-halo acids) such as acetic acid, oxalic acid and citric acid, were employed as the substrate molecules. In a typical binding assay, 500 mg of the polymer were added in 5 ml of the acetonitrile solution containing 5 mM of each analyte of interest or in methanol (blank) and stirred overnight at room temperature. Afterwards, the polymer was filtered, rinsed, dried and sonicated with 3 ml of methanol for 20 min. The polymer particles were then removed by filtration and the resultant

liquid was analysed for the quantity of acid using the methods described in the following sections.

2.4. The analysis of haloacetic acids

The assays of the haloacetic acids in bulk solution obtained from the binding experiment were carried out by using a Hewlett-Packard 6890 Series gas chromatograph equipped with a ^{63}Ni electron capture detector maintained at 350°C . A $1.0\ \mu\text{l}$ of sample was injected into the GC column using a Hewlett-Packard 7683 Series Automatic Liquid Sampler and a split injection (split ratio of 1:50) at the temperature of 250°C . Separations were obtained on a HP-5 capillary column (10 m x 0.25 mm I.D., 0.25 μm coating thickness, Hewlett-Packard Co., CA, USA). A temperature program of GC oven was set from 45°C (5 min) to 100°C (5 min) at $2^\circ\text{C}/\text{min}$ then 250°C (10 min) at $20^\circ\text{C}/\text{min}$. Helium was used as the carrier gas at a flow rate of 0.3 ml/min, and nitrogen was used as the make-up gas (40 ml/min). The linearity of the calibration curves was validated down to $10\ \mu\text{g}\ \text{ml}^{-1}$ in methanol.

2.5. The analyses of non-halo acids

The analyses of non-halo acids were performed by potentiometric titration. A 2.0 ml of a sample was placed in a 150 ml vessel. Subsequently, 5 milliliters of 2 M potassium chloride were added to adjust the ionic strength of the sample to 0.1 M. The solution was diluted to 100.0 ml with distilled water. The mixture was then stirred and

titrated with a standard solution of 0.01 M sodium hydroxide. The pH values were recorded at each 0.1 ml of titrant volume added. The plot of the titrant volume versus pH was made to determine the end-point.

2.6. The assay of HAAs in the water samples by LLE-GC-ECD

The water samples (and the water sample spiked with TCAA or a series of HAA) were extracted and analysed by GC-ECD using a Hewlett Packard Model 6890 Gas Chromatograph. The sample preparation was carried out using the EPA method outlined in the elsewhere [13]. In the present case, a 1000 ml of the water sample was dosed with 50 mg of ammonium chloride and 2-3 drops of 6 M HCl. Then 100 ml of water containing 5 ml of concentrated sulfuric acid and 40 $\bar{0}$ g of sodium sulfate was added and the solution extracted with 5 ml of methyl *tert*-butyl ether (MTBE) spiked with the internal standard, 2,4-dichloro-1-nitrobenzene (10 μ g). A 3 μ l aliquot of the extract was introduced into the GC by splitless injection at an injector temperature of 200°C and the transfer line temperature of 280°C. The separations were achieved on a HP-5 capillary column (30 m x 0.25 mm, 0.25 μ m film thickness). The carrier gas flow rate was at 1.0 ml/min. The oven temperature was held constant at 35 °C for 10 min and then ramped to 75°C at a rate of 5 °C/min for 5 min, 135°C at a rate of 5°C/min for 2 min, and 185°C at a rate of 25°C/min.

Under the analysis conditions described above, good linearity for the analysis of HAAs was observed in the range of 1-500 μ g l⁻¹. The concentration of analyte in the

sample was determined and calculated from its standard curve. In addition, the percentage analyte recovery was calculated by dividing the amount of analyte found in the sample by that in the reference standard and multiplying this ratio by 100.

2.7. The immobilisation of the MIP in membrane

The sensing element was incorporated into a sensor by immobilising the MIP in a membrane base made by casting PVC, using dibutyl phthalate as a plasticizer. The membrane preparation protocol was optimised to produce the membrane having the appropriate physical properties (flexibility and mechanical stability). For this purpose, the ratio of MIP to PVC was varied while the amounts of solvent and plasticizer were kept constant. The optimum membrane property took place at a MIP / PVC ratio of 1:2, which was maintained for making the ground polymer immobilised membranes. The details of the immobilisation procedure are given as follows. 1 g of PVC and 1.5 g of dibutyl phthalate were dissolved in 6 ml of THF and then 500 mg of the MIP was dispersed uniformly in the solution. After thorough mixing, the suspension was cast on a glass plate (90 mm diameter). The THF was allowed to evaporate under atmospheric conditions for 3 h and under vacuum for at least 24 h. Membrane with a thickness of 430-460 μm and the surface area 4.90 cm^2 was obtained in this way. Four pieces of membrane were cut using a cork-borer (30 mm diameter). NonMIP-immobilised membranes were prepared similarly for use in the reference sensors. In addition, undoped membranes were made in the same way but without any MIP particles. The amount of polymer immobilised in membrane was determined by subtracting the weight of the undoped membrane from the weight of the doped membrane. The amount of

MIP immobilised in membrane was about 70 mg and the percent relative standard deviations, %RSD ($n = 6$) of the amount of BP-based polymer and MSP-based polymer, embedded in a membrane, were 3.75 and 2.07, respectively.

2.8. Sensor and operating procedures

In this study, a four-electrode conductometer was employed as the transducer of the proposed sensor to give a wide range of ion concentrations and the operating frequencies. The conductometric sensor designed for this work comprised a conventional electrochemical cell and the MIP-immobilised membrane. The electrochemical cell used here was of a simple design, with a circular window between two chambers placed face to face, made "in house". Two cylindrical cavities each capable of holding 12 ml of solution were drilled out of two Perspex blocks. These cavities in the two half-cells were 25 mm in diameter. A rubber O-ring was set into grooves in the Perspex on each side to form an airtight seal when they were clamped together with the disc of membrane (30 mm diameter) between half-cells. The assembly was then placed on a magnetic stirrer plate with magnetic follows in each half-cell. A calomel electrode and an Ag/AgCl electrode (in 3 M KCl) were placed in the each of the chambers. In a typical experiment, 0.1 M phosphate buffer solution containing 35 mM NaCl was used as the background solution (except when the effects of electrolyte and buffer pH were being studied). All measurements were carried out at 25°C. The rest potential of the sensors was measured in distilled water at the beginning of each assay, and prior to sample application. The impedance analysis of the sensor was performed by applying an alternating potential (220 V) to the electrodes

with a frequency between 0.2 kHz and 30 kHz, which was generated by a frequency wave-form generator made in house (New South Wales, Australia), connected across an 18 kΩ resistor. The output voltage was read with a two-channel oscilloscope at the values corresponding to the steady state response. These potential values were used for the impedance analysis. The impedance spectra were made as the absolute values of the complex impedance ($|Z|$) and conductivity (G_{eff}) of membrane fabricated in sensor were calculated using the following equation:

$$|Z| = (V_0/R) / V_z$$

$$G_{\text{eff}} = (1/|Z|) / 2\pi\omega$$

where R is the resistance across the A.C. voltmeter input, V_0 is the voltage measured of electrodes, V_z is the voltage measured of membrane, and ω is the A.C. frequency applied to sensor.

In order to define the optimum sensor technological parameters of sensor the impedance or conductivity of sensor (membrane) was measured and presented in this work. For the sample measurement of sensor, the signal response towards the analyte of sensor was reported as $\Delta|Z|/|Z|$ (%), where $\Delta|Z|$ is the impedance shift response to the addition of analyte of interest in the measured solution and $|Z|$ is the measured impedance. Moreover, the selectivity of MIP sensor was affirmed by comparing in the signal responses of MIP sensor and reference sensor having a nonMIP membrane, as different pair mode. The selectivity of sensor was examined by comparing IC_{50} of analogs to IC_{50} of TCAA. The IC_{50} is obtained from the inflexion point of the calibration curves and represents the polymer content at which 50% of the polymer binds with

the analyte. TCAA was considered to give 100% selectivity and all other HAAs were related to this value.