

Development of trichloroacetic acid sensor based on molecularly imprinted polymer membrane for the screening of complex mixture of haloacetic acids in drinking water

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Received 6 January 2003; received in revised form 1 July 2003; accepted 21 July 2003

Abstract

This work shows developing conductometric sensor based on molecularly imprinted polymer (MIP) for the screening of complex mixture of haloacetic acids (HAAs) in drinking water. The recognition of the HAAs was achieved by trichloroacetic acid (TCAA)-imprinted polymers synthesised from the copolymerization of 4-vinylpyridine (4-VPD) and ethylene glycol dimethacrylate (EDMA) in the presence of the TCAA template in acetonitrile, either by bulk polymerization (BP) method or by a multi-step swelling polymerization (MSP) method. TCAA-imprinted polymer of both methods was tested for re-binding with the template and its analogs. It was found that these polymers could bind selectively to the template molecule and HAA derivatives. HAA measurements were carried out by the application of the polyvinyl chloride membrane fabricated with TCAA-imprinted polymer on conductometric sensors. The technological parameters (operating frequency, membrane composition, ionic strength and medium pH) for the sensors were identified and optimised in respect to the response to TCAA, using sensor fabricating with BP-based MIP as a model. The selectivity of the sensors constructed with MIPs made by either that of the two imprinting methods was also investigated, which the influence of the method of imprinting on the binding strength and selectivity of the recognition element embedded in sensor was observed. The sensors showed high sensitivity and selectivity for the response toward TCAA, the sensor modified with MSP-based MIP being better. In addition, the sensors, particularly when was constructed with MSP-based MIP exhibited good cross-reactivities with a wide range of HAAs, which is useful for the screening of the group of HAA usually present in chlorinated water in complex mixtures. Thus, the sensor modified with MSP-based MIP was chosen for analytical application. The calibration of this sensor was determined, showing the good linear graphs ($R^2 > 0.970$) for HAAs over the concentration range of 25–1000 $\mu\text{g/l}$ and the detection limit of each HAA in the range 0.2–5.0 $\mu\text{g/l}$. Moreover, the results in real analysis of the sensor indicate the simplicity and reliability of the method. The present work demonstrated that the sensor based on TCAA-imprinted polymer is a fast and sensitive screening method of HAAs in drinking water.
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Keywords: Molecularly imprinted polymer; Disinfection by-products; Haloacetic acids; Membranes; Conductometric sensor

1. Introduction

Chlorination is the most widely practised disinfection technique since it has dramatically reduced the incidence of waterborne diseases, and improved the quality of life [1]. However, chlorination of drinking water has been identified as contributing to the formation of a wide variety of disin-

fection by-products (DBPs). With this has come public concern regarding adverse health effects, since numerous DBPs including some haloacetic acids are toxic and cancer risks [2,3], leading to the increased regulation of DBPs in drinking water.

Currently, five haloacetic acids (HAAs) are regulated by the US environmental protection agency (EPA) [4]. These are monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dibromoacetic acid (DBAA), dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA). A level of 60 $\mu\text{g/l}$ has been introduced for the sum of these five chemicals,

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under Stage 1 of D/DBP rule [5]. Water utilities in the US must comply with ruling or else face heavy penalties. While, there are non-mandatory guidelines in several countries, such as Canada and Australia. In spite of having safe drinking water laws, inadequate funding for enforcement may cause non-compliance, which this is a problem found in several other countries. In addition, the use of additional water purification systems for the post-treatment of chlorinated water is not always helpful for extracting any residual DBPs. Therefore, the use of chlorinated disinfectants in the purification of water calls for the measurement of HAAs as well as other DBPs in drinking water. The traditionally practised methods for the direct determination of carboxylic acids by electrochemical methods give poor specificity. More sophisticated methods for the specific determination of HAAs, such as LC-MS, GC-MS and CE, need lengthy procedures, expensive and bulky equipment with a high power demand and well-trained operators for the instrumentation. It is therefore necessary to develop a simple and fast screening method, capable of complementing the established methods; numerous samples can be collected and further verified with established methods for the “positive” samples, so that the number of samples required is reduced. Apart from that, analysts at drinking water utilities could screen any water having a possible risk of DBPs contamination without high skill levels. For such prospect, the use of sensors seems to be the most promising means to give fast, cheap, simple and continuous measurements.

A sensor is defined as an analytical device incorporating a sensing element that is intimately connected to a transducer, which may be electrochemical, optical, piezoelectric, magnetic or thermometric [6]. To date, many sensors have been developed for determining particular substances, including environmental pollutants and clinical substances. A wide variety of naturally occurring recognition systems (such as cells, antibodies, enzymes, and receptors) are commonly used as sensing elements in sensor technology. Besides these, artificial recognition materials, such as MIPs have also found for the use in sensors [7–10]. A MIP is a synthetic polymer possessing selective molecular recognition properties because of recognition site within the polymer matrix that are complementary to the analyte molecule in the shape and positioning of functional groups. It is well known that the stability of MIPs is superior to that of biological recognition materials, so that a sensor modified with a MIP is easily stored and operated and has a long lifetime. Recently, a conductometric sensor combining the specificity of MIP with the high sensitivity of an electrochemical transducer was developed for detection of atrazine and its derivatives [7–9]. The advantages of conductometric sensors that they are simple and relatively cheap because they do not need any reference electrode. Also, they can be simply assembled using membrane technology, which suits them for large-scale production.

As MIPs would be applied for the use in the aqueous solution, it is important to make MIPs capable of recog-

nition in this medium. However, the preparation of imprinted polymers in aqueous solution has proven to be a difficult task, because the water molecules interfere with the hydrogen-bonding interactions between the functional monomer and the template molecule. One way to overcome this problem is to make the polymer in an organic solvent. Here, we prepared the MIPs against TCAA using the basic monomer 4-vinylpyridine (4-VPD) as the functional monomer and acetonitrile as the porogen. Ethylene glycol dimethacrylate (EDMA) was chosen as cross-linking monomer since it is the most commonly used cross-linker, which can dissolve in this organic solvent.

The purpose of this work was to develop a fast and sensitive screening method based on conductometric sensors for detection of HAAs in drinking water. To explore a sensing element for HAAs, TCAA-imprinted (and non-imprinted polymers) were prepared by either bulk polymerization (BP) or by multi-step swelling polymerization (MSP); both types of imprinted polymer were examined for their recognition ability, using non-imprinted polymers for the control experiments. The selective response of the conductometric sensors having the TCAA-imprinted polymers as sensing elements was investigated. Moreover, the sensor giving high cross-reactivity with the widest range of HAAs was determined in calibration characteristics (sensitivity, operational and linear concentration range and detection determination limits). The constructed sensor was used for the analysis of drinking water. The recovery of the analyte in spiked water samples was scrutinized and the results were verified by liquid–liquid extraction combined with gas chromatography–electron capture detector (LLE–GC–ECD).

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: TCAA, DCAA, MCAA, DBAA and 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 (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

2.2.1. 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. And 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 template extraction of polymer was confirmed to be complete from the disappearance of acidic proton in ¹H NMR (CCl₄) measurements together with the absence of TCAA in a methanol rinse of polymer, as verified by gas chromatographic method (see Section 2.4). Finally, the polymer particles were dried under vacuum and stored at ambient temperature until use. Non-printed polymer (non-MIP) included as the control polymer was prepared in an identical manner except that template molecule was omitted from the process.

2.2.2. 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 methanol. 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 non-MIPs 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 ⁶³Ni electron capture detector maintained at 350 °C. A 1.0 µl 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 × 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 °C/min then 250 °C (10 min) at 20 °C/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 µg/ml 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 ml 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 two to three drops of 6 M HCl. Then 100 ml of water containing 5 ml of concentrated sulfuric acid and 40 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 \times 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 earlier, good linearity for the analysis of HAAs was observed in the range of 1–500 μ g/l. 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. Five hundred milligram 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 at 40 °C for at least 24 h. Membrane with a thickness of 430–460 μ m and the surface area 4.90 cm² was obtained in this way. Four pieces of membrane were cut using a cork-borer (30 mm diameter). Non-MIP-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, %R.S.D. ($n = 6$) of the amount of BP-based polymer and MSP-based polymer, embedded in a membrane, were 3.75 and 2.07, respectively.

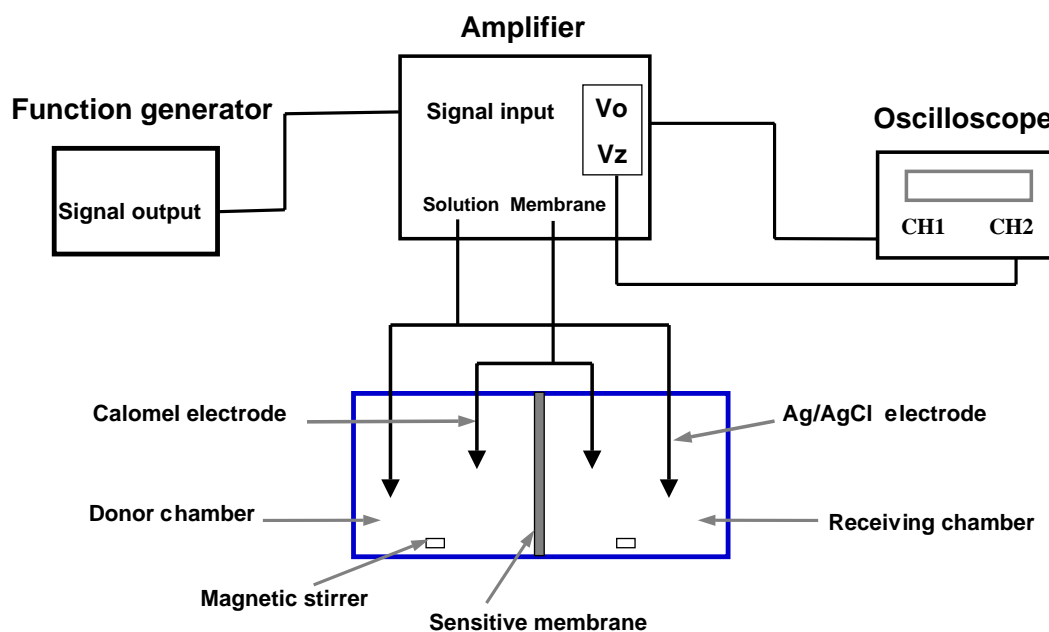
2.8. Sensor and operating procedures

In this study, a conductometer was employed as the transducer of the proposed sensor. To ascertain that the cell voltage was primarily due to the membrane potential (not the solution or electrodes) a four electrode system was used. In this system, the additional electrodes were placed close to the membrane surface. The two original electrodes were situated further away from the membrane surface on either side. A constant current was passed as usual between the two outer electrodes, while the inner electrodes were used to measure the voltage drop across the membrane. As shown in [Scheme 1](#), the conductometric sensor 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 equipped with a magnetic stir bar. 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 and 30 kHz, which was generated by a frequency wave-form generator made in house (New South Wales, Australia), connected across an 18 k Ω 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:

$$|Z| = \frac{V_0/R}{V_z}$$

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

where R is the resistance across the ac voltmeter input, V_0 the voltage measured of electrodes, V_z the voltage



Scheme 1. An illustration of an experimental set-up for conductometric sensor based on molecularly imprinted polymer membrane.

measured of membrane, and ω the ac 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 $|Z|$ 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 non-MIP 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.

3. Results and discussion

3.1. The recognition selectivity of TCAA-imprinted polymers

In this study, the TCAA-imprinted polymers produced by the copolymerization of 4-vinylpyridine monomer and ethylene glycol dimethacrylate cross-linker in the presence of the TCAA template either by bulk or multi-step swelling polymerization technique were examined for their recognition property. Table 1 lists the microgram adsorption of TCAA or its analogs per gram of the two types of TCAA imprinted-

non-imprinted polymers, in acetonitrile medium. The value of the cross-reactivity, CR (%), which is obtained from the adsorption value of the particular MIP for the analog relative to that for TCAA, is also listed. For the halo acids, the adsorption value for the MIP is considerably larger than that for the non-MIP in both techniques, which this result verifies the selectivity due to the imprint of the MIPs. In general, the MIPs of the two imprinting methods exhibit similar trends of their recognition selectivity to halo acids, in that the adsorption value is the largest for TCAA and is comparable for DCAA, MCAA and DBAA, whereas it is least for TBAA. Except in case of MBAA, only the MIP prepared by the MSP method gives a large value for this. From the result obtained, it is apparent that the MIPs have high cross-reactivity with the structurally closely related TCAA. In addition to above observations, non-specific adsorption of non-halo acids is noticed, from the comparable adsorption of MIP and non-MIP for both techniques. In two classes of TCAA analogous compounds, halo acid having halogen substituent group on the side chain shows high binding with the MIPs relative to non-halo acid, suggesting that the TCAA-imprinted polymers prepared have high specificity for the haloacetic acids.

The order of the recognition selectivity of MSP-based MIP for HAAs was TCAA > DCAA > MCAA > DBAA > MBAA > TBAA, while that of BP-based MIP was TCAA > DCAA > DBAA > MCAA > MBAA > TBAA. Typically, hydrogen-bonding and proton transfer interactions giving rise to ionic species are central to the non-covalent binding of the template molecule onto the MIP, although other interactions, such as hydrophobic, dipole-dipole, van der Waals may play an important role [14]. Due to the presence of the carboxylic group on the

TCAA molecule and the amino groups on the functional monomer, two different interactions, hydrogen-bonding and ion–ion events can be formed. The rank order in selectivity of both TCAA-imprinted polymers is clear, which suggests that electrostatic interaction via hydrogen-bonding is the driving force for the binding of HAAs onto the MIPs. Additionally, through careful analysis of the chemical structure of the lower members of the series, it becomes clear that optimal fit of ligand in the TCAA binding site is also necessary to the selective recognition.

From the result obtained, it appears that both imprinting methods can produce a polymer with binding sites having shape and size selection, and having the orientation of functional groups to recognise the TCAA molecules. The MIPs shows multiple selectivity towards HAAs, implying that their recognition behaviour is group specific rather than ligand specific, and that they are therefore suitable as recognition elements for the screening of the group of HAAs commonly present as mixtures in chlorinated water.

A comparison of cross-reactivity values (Table 1) obtained from the two types of TCAA-imprinted polymers reveals that the MSP-based MIP cross-reacts better with a wide range of HAAs than the BP-based MIP. It is also evident from the adsorption data that the MSP-based MIP shows higher affinity for the TCAA or TCAA analog than the BP-based MIP. Therefore, the TCAA-imprinted polymer prepared by the MSP method was chosen as the sensing element in the proposed sensor. However, in the early stage of developing the sensor, a relatively large amount of polymer is required for this purpose; whilst bulk polymerization method offers an easy and fast synthesis process, leading to a high yield of polymer and a reduction in the cost of the experiments. For these reasons, the MSP-based polymer was replaced by the BP-based polymer for the investigation of the technological parameters in the following sections. Moreover, we determined the capability as well as the recognition selectivity of both BP-based and MSP-based

MIPs fabricated into the sensors and use these data to justify selection of the sensor for analytical application.

3.2. The investigation of the technological parameters

3.2.1. Influence of the current frequency on the sensor response

In the present work, the effect of operating frequency on the sensor response was studied to identify the optimum operating frequency. For this purpose, ac impedance spectra of the sensor were measured over the frequency range 0.2–30 kHz using the TCAA concentration upto 1 $\mu\text{g/l}$. Fig. 1 shows the effect of the applied frequency on the impedance of the sensor. It can be seen that the impedance response of the membrane sensor initially increases with increasing TCAA concentration but flattens out at high concentrations. The result also demonstrates that the membrane resistance is sensitive to the presence of TCAA molecules in the solution. As TCAA is very strong acid ($\text{p}K_{\text{a}} = 0.9$) the deprotonated TCAA may be driven electrochemically from the bulk solution into the membrane and adsorbed in the polymer domain, preferentially at MIP binding sites. It is presumed that the TCAA ion cannot transport through PVC membranes, since other organic ions as salicylate have been shown not to be iontophoresed across a plasticized PVC membrane [15]. TCAA molecules adsorbed in the membrane feasibly cause the impedance change of the sensor as that the membrane resistance is increased. The mechanism of increase of the membrane resistance by TCAA is not yet known, but this may be due to the formation of ion-pairs between TCAA ions and the vinyl pyridine units of the imprinted polymer or a reduction in PVC chain mobility by the interaction of the TCAA molecule at the selective sites embedded in the PVC membrane [16]. Furthermore, for the same concentration of TCAA, there was no proportional increase in the impedance response of sensor for the high frequencies, particularly at high concentration of

Table 1
Adsorption data obtained from TCAA-imprinted and non-imprinted polymers obtained by two different polymerization methods

Substrate	Imprinting method					
	BP			MSP		
	MIP	Non-MIP	CR (%)	MIP	Non-MIP	CR (%)
Halo acids						
Trichloroacetic acid (TCAA)	10.44	0.83	–	13.07	1.02	–
Dichloroacetic acid (DCAA)	10.12	0.52	97	13.01	0.20	99
Monochloroacetic acid (MCAA)	10.02	2.21	96	11.48	<0.01	88
Tribromoacetic acid (TBAA)	0.70	0.33	7	1.13	0.60	3
Dibromoacetic acid (DBAA)	7.41	1.93	71	12.48	2.19	95
Monobromoacetic acid (MBAA)	1.24	0.41	12	8.58	1.45	66
Non-halo acids						
Acetic acid	2.48	2.24	24	2.89	2.75	22
Oxalic acid	1.35	1.18	13	1.50	1.30	11
Citric acid	1.06	0.96	10	1.15	1.16	9

BP: bulk polymerization method; MSP: multi-step swelling polymerization method. The adsorption value is expressed as microgram of substrate adsorbed per g polymer. CR (%), cross-reactivity is obtained from the adsorption value of MIP for the analog compared with that for the TCAA.

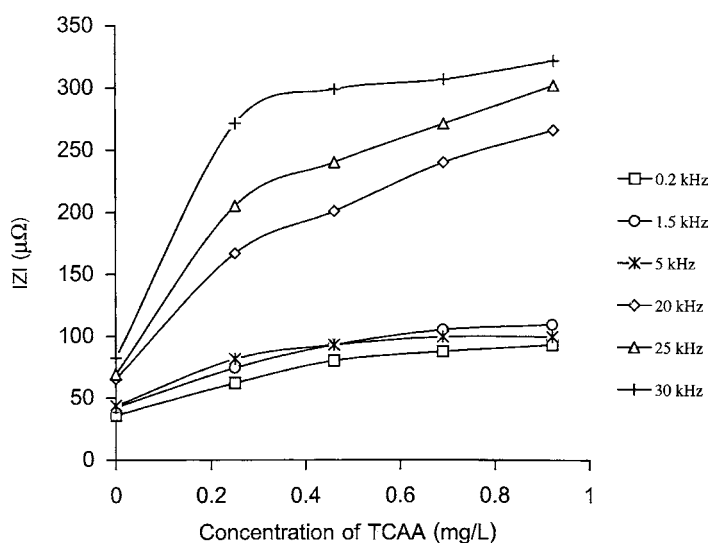


Fig. 1. Effect of the ac frequency on the impedance responses of the MIP sensor at various concentrations of TCAA. Measurements were carried out in 0.1 M potassium phosphate buffer, containing 35 mM sodium chloride.

TCAA. This may be caused by the high ac frequency making the membrane pores expand [15,17] hence the analyte can transport across the membrane. The diffusion of the analyte leads to variation of impedance response of sensor. As is evident in Fig. 1, an ac frequency of 1.5 kHz gives a reasonable impedance response of sensor over the widest concentration range of TCAA, so that this frequency has been employed in all subsequent experiments.

3.2.2. Influence of the membrane composition on the sensor response

The membrane prepared here gives a stable signal response of sensor within 10 s, being faster than that of several membranes prepared for other sensors [8,9]. Previous work on the potentiometric detection of organic acids using electrodes modified by PVC membranes incorporating monocyclic hexa-amines demonstrated that phthalate plasticizers could facilitate the penetration of the carboxylic acid into the PVC-based membrane [18].

In addition, it was found that the quantity of the casting solvent affects the characteristics of the membrane. With a low volume of the casting solvent (much <6 ml), the polymer resin was too viscous to spread and form a membrane. By contrast, the specified volume of 6 ml (or even a volume up to 8 ml) yielded not only the good spreading of the solution in forming as membrane but also a good mechanical strength and stability of membrane. With double this volume (12 ml), the electrical potential of sensor was not consistent, but gradually increased and eventually reached steady-state at the same value as the resting potential. This is because the increased amount of solvent leads to the formation of pores in the immobilised membrane, which allows the diffusion of the analyte to the another chamber, hence the variation of membrane potential with time. This explanation is supported by micrographs from a scanning electron microscope

(Jeol Series JSM 5200, CA, USA), showing the formation of big holes in the membrane prepared using 12 ml of casting solvent (see Fig. 2a) as contrasted with the smooth skin layer of the membrane prepared using 6 ml casting solvent (see Fig. 2b).

The amount of polymer also has a significant influence on the sensor response. As is evident from Fig. 3, increasing the amount of MIP promotes increased impedance response of sensor. This can be explained that the numeral binding sites increased with increase in the amount of MIP. However, a low amount of MIP (<40 mg) gives a very soft layer membrane. On the other hand, higher amounts of MIP (more than 70 mg) results in a slower response of the sensor and poorer mechanical strength of the membrane due to the reduction in content of the membrane base.

3.2.3. Influence of the NaCl concentration on the sensor response

Generally, the drawback of conductometric sensors is their poor specificity, particularly when the measurement is performed in buffers with a high capacity and high ionic strength. In the present study, the influence of the ionic strength on the sensor response was investigated. It can be seen from Fig. 4 that increasing the NaCl concentration up to 35 mM leads to an increased conductivity in both the MIP sensor and the reference sensor. However, a salt concentration more than 35 mM induced a decrease in conductivity of those sensors, although the conductivity should increase with salt concentration. Indeed, the conductivity behaviour of our sensors is similar to that of the cross-linked polyurethane acrylate electrolytes prepared in previous work [19,20]. It is noteworthy that the sensor fabricated with the MIP-immobilised PVC membrane had an ionic conductivity similar that of polymeric solid electrolytes. It has been established that synthetic polymers,

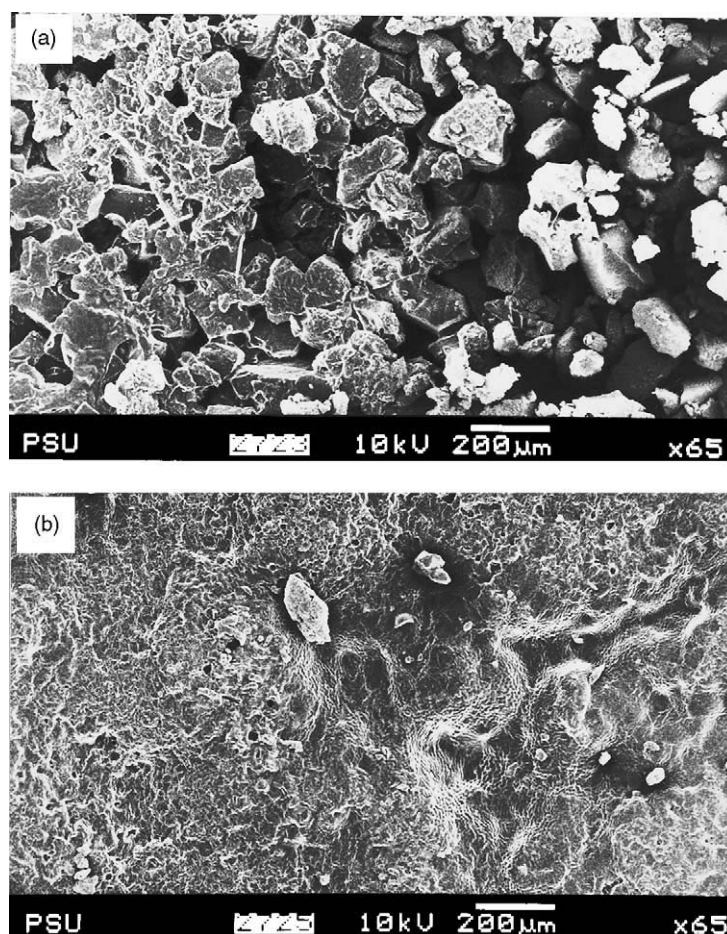


Fig. 2. Scanning electron micrograph of the immobilised membrane prepared using: (a) 12 ml; (b) 6 ml casting solvent.

such as polyethylene oxide, poly(methyl methacrylate), polyacrylonitrile and polyvinyl chloride exhibit ionic conductivity in the presence of excess electrolytes, such as LiCl and NaCl [21–23]. Such behaviour is attributed to the mobility of the ions coupled with the segmental motion of the polymer chains. On the basis of the explanation given in previous work [19,20] the decrease in conductivity of sensor with increasing in salt concentration may be a reflection of two opposing effects, namely an increase in the number of charge carriers and a decrease in the free volume. Although increase in salt concentration leads to increase in the number of charge carriers, increasing the salt concentration also increases the transport of the salt (Na^+) in membrane and the formation of transient cross-link with the PVC polymer, which restricts the segmental motion of the polymer chains. This result leads to a decrease in the mobility of the charge carriers, and hence the conductivity of sensor decreases. In addition, increasing in salt concentration may lead to the formation of charge-neutral contact ion pairs, which do not contribute to conductivity [24,25].

Despite this variation in sensor conductivity with salt concentration there was a significant difference in the conductivity obtained from MIP sensor and that from the reference sensor, which is apparent in the NaCl concentration

range between 10 and 125 mM (Fig. 4), and which indicates the selectivity of the MIP sensor to TCAA. In all cases of that difference, the conductivity response of reference sensor was always larger than that of MIP sensor. It is pertinent to note that the difference of conductivity of the MIP sensor and the reference sensor in the absence of TCAA were very small: $G_{\text{eff-reference sensor}}/G_{\text{eff-MIP sensor}} = 1.05$. Moreover, the role of ionic strength on the selective response of sensor was significant since the selectivity of the sensor ($G_{\text{eff-reference sensor}}/G_{\text{eff-MIP sensor}} = 17.5$) was the highest at a NaCl concentration of 75 mM.

The present study demonstrates that although there is an effect of electrolyte on the sensor response, the sensor comprising of MIP immobilised membrane and the conductometric transducer studied can give high specificity with TCAA and a stable and reasonable signal response throughout the salt concentration range studied.

3.2.4. Influence of the medium pH on the sensor response

The influence of pH of the sample medium on conductivity response of the sensor was examined over the pH range 3–9. It is evident from Fig. 5 that increasing in the sample pH leads to a decrease in sensor conductivity. On the other hand, changing the pH did not effect the conductivity of the

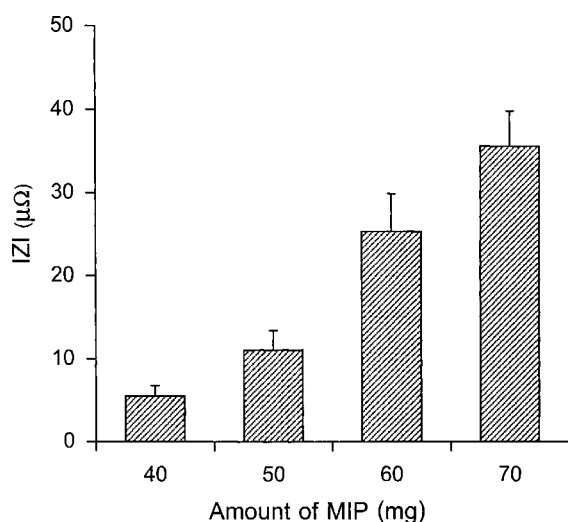


Fig. 3. Effect of the amount of BP-based MIP on the sensor response upon 1.20 $\mu\text{g/ml}$ TCAA. Each value represents the average of three independent measurements. Measurements were carried out in 0.1 M potassium phosphate buffer, containing 35 mM sodium chloride.

reference sensor. The difference in conductivity responses of MIP sensor and reference sensor over the pH range studied was apparent and this difference was more pronounced at high buffer pH. It is well known that in aqueous medium the hydrogen-bond interaction between MIP and ligand is suppressed. Also, ionization of TCAA is greater at high pH than at low pH due to its strong acidity. But from the results obtained, the selectivity of MIP sensor was larger at high pH values (pH 7–9). Hence in aqueous environment the main force of binding between the MIP and TCAA molecule is most likely derived from ionic interaction between the deprotonated carboxylic group and functional monomer. It is noted that the value of difference of the conductivity re-

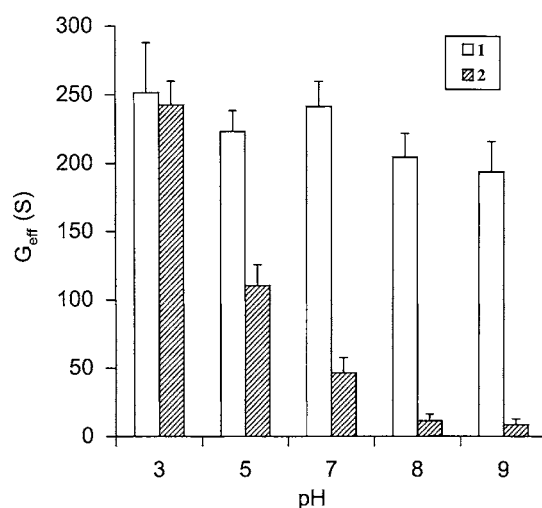


Fig. 5. Effect of pH on the sensor response upon 1.20 $\mu\text{g/ml}$ of TCAA for: (1) reference sensor; (2) MIP sensor. Each value represents the average of three independent measurements. Measurements were carried out in 0.1 M potassium phosphate buffer, containing 35 mM sodium chloride.

sponses obtained from MIP sensor and reference sensor was essentially constant in the interval of pH 7–9, though the maximum selectivity was evident at pH 9. However at very high pH, the mechanical properties of the membrane are poor. In order to have a compromise between the selectivity and mechanical property of the selective membrane, the sensor was measured at pH 7 of sample solution.

3.3. Concentration dependence of the sensors

The dependency of the sensor response upon the concentration of the TCAA was investigated using sensors constructed with MIPs made by either that of the two

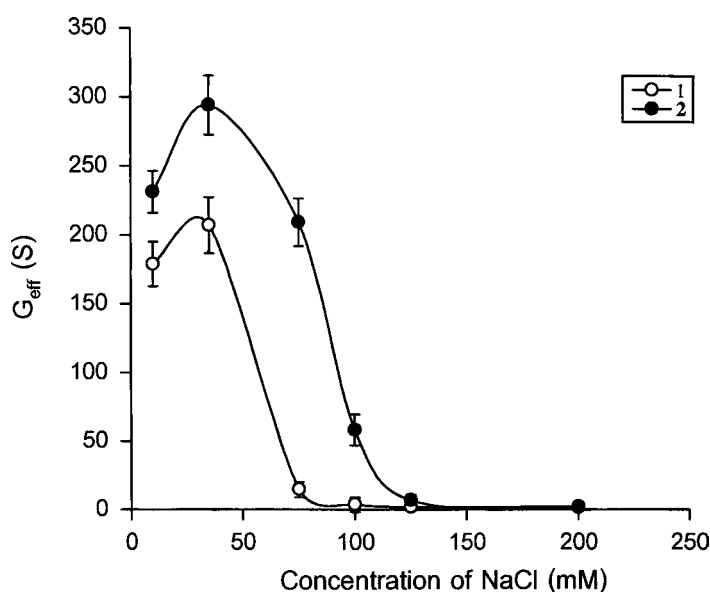


Fig. 4. Effect of NaCl concentration on the sensor response upon 1.20 $\mu\text{g/ml}$ of TCAA for: (1) MIP sensor; (2) reference sensor. Each value represents the average of three independent measurements. Measurements were carried out in 0.1 M potassium phosphate buffer.

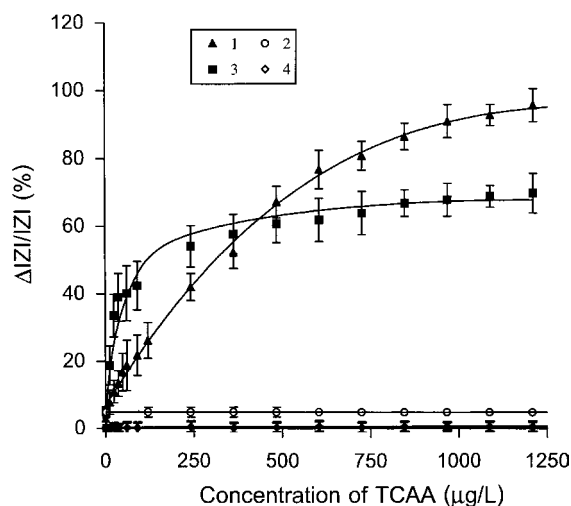


Fig. 6. Concentration dependence of the impedance response to TCAA for sensor incorporated with: (1) BP-based MIP; (2) BP-based non-MIP; (3) MSP-based MIP; (4) MSP-based non-MIP. Each point represents the average of six independent measurements. Measurements were carried out in 0.1 M potassium phosphate buffer pH 7, containing 35 mM sodium chloride.

imprinting methods. As can be seen from Fig. 6, MIP sensors give a signal response initially rises with an increase in the concentration of TCAA and then starts to level off. While both reference sensors show very low signal response with much less changes in the sensor responses upon the additional TCAA. The sensor response of reference sensors is apparently caused by weak non-specific adsorption of the polymer materials. The apparently hyperbolic curves shown in case of MIP sensors suggest that the substrate bind to only one active site on the MIPs. Moreover, the sensitivity of the sensor derived from MSP-based MIP is better than that of the sensor derived from BP-based MIP. In contrast, the level of the steady-state response of the sensor derived from BP-based MIP was appreciably larger than that of the sensor derived from MSP-based MIP. These results reveal that the imprinted sites of MSP-based MIP are more accessible, but less active than that of BP-based MIP, though the latter conclusion is in conflict with the findings in the adsorption studies. Possibly, MSP-based MIP shows a lower selectivity in the sensor. Ordinarily, the polymer prepared by MSP method has imprints that are close to the particle surface allowing easier diffusion of ligands to binding sites, the aforementioned role of the receptor sites in the case of MSP-based MIP is quite reasonable. For the alteration in activity of the receptor sites of MSP-based MIP when incorporated in sensor this may be explained by the presence of the dibutyl phthalate plasticizer in the membrane, which this is added primarily to improve the mechanical properties of the membrane. Owing to the polarity of the plasticizer, it may solvate the ionized TCAA, leading to a decrease in complexation between TCAA and the MIP, while this additive may promote the penetration of TCAA molecule into the membrane. This view is reason-

able in the sense that the plasticizer preferentially disrupts the interaction of TCAA with the easily accessible site and hence increased the extent of the effects for MSP-based MIP.

3.4. The selectivity and sensitivity of the sensors

Most MIP syntheses are performed in an organic medium, and studies on ligand binding of the MIP are then very often conducted using these organic solvents as the incubation medium. However, a number of previous studies have demonstrated that imprint binding selectivity is different in aqueous medium from that in organic solvents [26–28]. Consequently, the applicability of the MIP relies on the assay format. The selectivity of TCAA-imprinted polymers exposed to an aqueous solution of HAAs was examined under optimised sensor conditions. The results revealed the steady-state response of the MIP sensors more than 25% for HAAs and the negligible change in signal response of the reference sensors (data not shown). The cross-reactivity values of both MIP sensors responding to TCAA and analogs were shown as histograms, in Fig. 7.

In sensor experiments, the MIPs show a high selectivity with TCAA and its analogs similar to the binding experiments. However, the selectivity profiles observed from these two assay formats are different and this difference is much more pronounced in the case of MSP-based MIP. This may be interpreted as arising from a difference of the solvents used in the two experiments. For example, in the organic solvent (acetonitrile) electrostatic interaction via hydrogen-bonding is likely to be the dominating factor governing selective recognition; by contrast, in an

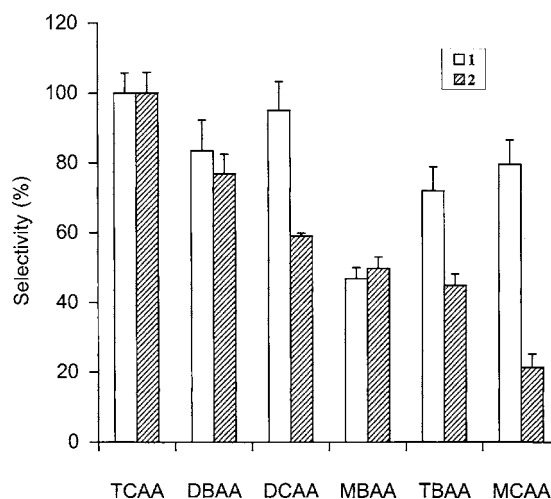


Fig. 7. Selectivity of (1) sensor modified with MSP-based MIP and (2) sensor modified with BP-based MIP for six HAAs at a concentration giving IC_{50} . Note that IC_{50} designates the polymer content at which 50% of the polymer is blocked by the analyte. Each value represents the average of three independent measurements for all HAAs except TCAA that the average of six independent measurements was shown.

aqueous medium, acid–base interactions are likely to play a more important role in the recognition mechanism, as is evident from the pH results. Furthermore, a difference between the selectivity profiles of two imprinted polymers in the sensor measurements was noticed, namely TCAA > DBAA > DCAA > MBAA > TBAA > MCAA in the case of MSP-based MIP and TCAA > DCAA > DBAA > TBAA > MCAA > MBAA in the case of BP-based MIP. This situation suggests that there has been a change of imprint binding selectivity of the MIPs because of their incorporation into the sensor. Also, the differences in the selectivity profiles of the two MIPs may be attributed to the plasticizing effect being different for these polymers. As discussed before, the plasticizing effect is far more pronounced in the case of MSP-based MIP. Although the selectivity profiles of the MIPs were different, these polymers generally cross-react with chlorinated haloacetic acid better than with brominated haloacetic acid for the same degree of halogen substitution. Also, the tri- or di-substituted HAA cross-reacted greater than the mono-substituted HAA. Thus, it seems that the halogen atoms make a significant contribution to recognition by the MIPs.

The sensor containing MSP-based MIP has a higher cross-reactivity with the HAAs tested (except MBAA) than that containing BP-based MIP. Likewise, the former sensor gives a large cross-reactivity value (more than 65%) for each HAA, which is beneficial for the simultaneous measurement of HAAs. In addition to that this sensor offers the linear dynamic range of HAA concentrations that covers the regulation limit of HAAs. Therefore, the sensor modified with MSP-based MIP was chosen for the real analysis of HAAs in a water sample.

3.5. Calibration and limits of detection

In this study, the calibration characteristics of sensor fabricating with MSP-based MIP were identified. The calibration graphs were obtained from the plot of logarithm of signal response of the sensor ($\log |\Delta Z|/|Z|$) versus the logarithm of the concentrations ($\log C$) of TCAA and its analogs. The calibration data are shown in Table 2. The linearity of those was observed over the HAA concentration range of 25–1000 $\mu\text{g/l}$,

depending on the compound. The concentration graphs are linear with a correlation coefficient greater than 0.970 for each HAA. The limits of detection as obtained from extrapolation of linear segments of the calibration graph (IUPAC recommendation) were in the range 0.2–5.0 $\mu\text{g/l}$.

3.6. The real analysis of the conductometric sensor

The sensor incorporated with MSP-based MIP was employed for the analysis of bottled water as obtained from a local supplier, either as spiked with or without either TCAA or a series of HAAs in representative quantities. Moreover, a method for analysis of HAAs in water, LLE–GC–ECD (modified US EPA method) was used for the verification the content of HAA in the water samples. Table 3 displays the recovery data obtained from the samples analysed with the sensor and with LLE–GC–ECD. The results show the concentration dependence of the recovery of analysis of either TCAA or mixed HAAs in LLE–GC–ECD method. At low spiking TCAA or mixed HAAs concentration, there were high differences in the recoveries obtained from the sensor and from LLE–GC–ECD. However, the precision of the sensor was better than LLE–GC–ECD, in that the relative standard deviation for the results from three separately prepared samples of the sensor was the range of 0.1–3% while the maximum relative standard deviation of LLE–GC–ECD was 12%. This is due to the fact that assay with the sensor are done without the need for any separations. In the determination of the bottled water spiked with the various levels of the sum of six HAAs, the sensor gave high recovery and repeatability for the group analysis of six HAAs.

The result for the sum of the six HAAs in the bottled water sample was 4.28 $\mu\text{g/l}$ when was analysed by LLE–GC–ECD, while this result was 7.22 $\mu\text{g/l}$ for the analysis with the sensor. Since the US EPA regulations require that the concentrations of the five HAAs added together (total HAAs) equal 60 $\mu\text{g/l}$ or less in drinking water the TCAA sensor seems to be suitable for determining HAAs in these samples. Moreover the large reading of the sensor indicates that the sensor has high inherent sensitivity and selectivity for the group analysis of HAAs. This is because the sensor has relatively high cross-reactivity with a wide range of HAAs. In general, the determination with LLE–GC–ECD is applicable only to DCAA, MCAA and TCAA but low sensitivity to TBAA and the other brominated acetic acids. The low sensitivity of LLE–GC–ECD for the brominated haloacetic acids can be explained by the poor methylation efficiency of these compounds with the acidic methanol used in this procedure [13]. Because the sensor is only semi-quantitative method the quantitative analyses of individual HAAs in the water sample by this means cannot be carried out. The relative content of each HAA in the bottled water sample was determined using the LLE–GC–EC method: the result was DCAA 0.5%, MBAA 6.5%, MCAA 72%, TCAA 3% and DBAA 18% (the TBAA content was below LOD).

Table 2
Calibration data obtained for the analysis of six HAAs by a sensor containing with MSP-based MIP

Compound	Slope	Intercept	Working range ($\mu\text{g/l}$)	R^2	LOD ($\mu\text{g/l}$)
TCAA	0.198	0.296	25–850	0.986	1.0
DCAA	0.254	0.254	40–1000	0.991	4.2
MCAA	0.241	0.241	10–660	0.980	4.2
TBAA	0.286	0.286	5–250	0.978	0.2
DBAA	0.336	0.336	15–150	0.983	0.5
MBAA	0.309	0.309	20–250	0.976	5.0

Table 3
Recovery data for the sensor method and the LLE–GC–ECD method

Analyte	Spike ($\mu\text{g/l}$)	Sensor method		LLE–GC–ECD method	
		%Recovery ^a	%R.S.D.	%Recovery ^a	%R.S.D.
TCAA	30	105.95	2.26	81.46	12.39
TCAA	60	101.52	0.1	99.58	12.26
TCAA	90	101.09	0.45	103.45	4.66
Sum 6 HAAs	5 each	107.85	4.92	60.53	3.04
Sum 6 HAAs	10 each	103.85	0.47	84.74	2.84
Sum 6 HAAs	15 each	102.88	1.61	95.69	2.56

Sum 6 HAAs refer to the mixture of TCAA, DCAA, MCAA, TBAA, DBAA and MBAA altogether.

^a Mean value ($n = 3$).

4. Conclusions

Conductometric sensors based on TCAA-imprinted polymer have been developed under the optimum sensor technology, showing a good response to TCAA and other HAAs with desirable sensitivity and selectivity. The influence of ionic strength and pH on sensor response towards TCAA was found, however, the selective response of the sensor for this compound was shown. The change in selectivity profile of the sensing elements when incorporated in sensor was also observed. The sensor derived from MSP-based MIP showed better cross-reactivities for selective response to HAAs than the sensor derived BP-based MIP. Moreover, the applicability of the sensor was proved by validation. The results indicate that the sensor based on TCAA-imprinted MIP is applicable for screening of complex mixtures of HAAs in drinking water. The fabrication of sensing material in sensor, as membrane, is easy, even if the mechanical property of the obtained membrane needs to be more improved to increase the strength in use of the sensor. The stability of the sensor is also good, in that its analytical performance is unchanged after being stored for more than 3 months at room temperature. The present study demonstrated the specific use of the proposed sensor for the screening of HAAs in drinking water.

Acknowledgements

The authors gratefully acknowledge the financial support from Prince of Songkla University, Thailand. Helpful comments from Professor Franz L. Dickert (Institute of Analytical Chemistry, University of Vienna, Austria) are acknowledged.

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