Molecularly imprinted polymer-modified electrode for on-line conductometric monitoring of haloacetic acids in chlorinated water

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Abstract

A conductometric sensor for on-line testing of haloacetic acids has been developed based on lab-on-chip device incorporated with an integrated miniaturised liquid-handling system. The sensor utilises a molecularly imprinted polymer (MIP) synthesized by the interaction between trichloroacetic acid (TCAA) template and a functional monomer, 4-vinylpyridine (VPD), together with cross-linking polymerisation of ethylene glycol dimethacrylate (EDMA). The ability of this MIP to change its conductivity in the presence of the target molecule into the imprint cavity has been used to develop the sensor, which responds well to TCAA in a continuous flow system with relatively good linearity, although this depends on the applied frequency. Thermal influences on the resistance of the sensor were in the order of 1.45% resistance signal variation per Kelvin at 3 kHz. The sensor showed high specific sensitivity to the target analyte and a stable and reasonable signal response in a solution containing inorganic anions. The sensitivity (range 0.5–5 μg l−1) and selectivity achieved with standard TCAA and five other haloacetic acids (HAAs) (dichloro-, monochloro-, tribromo-, dibromo-, and monobromoacetic acid) in water was good. Minimum sample volume required is 2.5 ml and the assay time is 2 min. The sensor has successfully been applied to haloacetic acid determination in domestic and commercial drinking water samples.

Keywords: Molecularly imprinted polymers; Disinfection by-products; Charge transfer complex; Haloacetic acids; Flow-through electrochemical system

1. Introduction

Haloacetic acids (HAAs) are disinfection by-products of the chlorination of drinking water that are known to cause serious health problems [1,2]. For example they are reported to cause an increased risk of certain cancers, in particular of bladder [3] and rectal [4] cancers. Instrumental methods such as ion chromatography, GC–ECD, GC–MS and LC–MS, for determining HAAs are time consuming, complicated and expensive for routine analysis. Fast and sensitive screening methods could be an essential complement to these sophisticated instrumental methods. Importantly, a special electrode technique offers the possibility of on-line testing for HAAs.

Electrochemical type of sensors (e.g. voltammetric, potentiometric, conductometric and capacitance) are becoming important tools in medical, biological and environmental sciences due to their simplicity, high sensitivity and relative cheapness [5,6]. The interest in conductometric sensors has been generated by these advantages. Conductometric transducer consisting of an interdigitated array electrode on a planar surface is suitable for miniaturization and mass production using photolithography or screen-printing technology [7]. Normally conductometric is the least selective of the electrochemical techniques, as it is difficult to distinguish between different ions. However, the sensitivity and specificity of this system can considerably be improved by incorporating it with highly selective recognition elements (either biological or synthetic substances).

Molecularly imprinted polymers (MIPs) are attractive as versatile and inexpensive materials capable of molecular recognition [8–10]. MIPs consist of highly cross-linked polymers that

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are synthesized in the presence of a template molecule [11,12]. Subsequent removal of the template leaves a cavity that retains affinity and selectivity for the template. The cavity is analogous to the 'active site' of enzymes. Interest in the use of MIPs in electrochemical sensors is increasing [13–16]. This is due to the advantage that the MIP gives to the electrochemical sensing device, both in terms of the stability obtained from the abiotic property of the MIP and the ease of operation and storage of the device when fabricated with the MIP. Nevertheless, the transformation of MIP into a measurable electrochemical signal is considered to be an important factor, as this has been found to be an obstacle in developing this type of material in electro-chemical sensor technology [16,17].

The aim of this work was to construct a conductometric sensor for on-line detection of haloacetic acids in chlorinated water by using molecularly imprinted cross-linked poly(ethylene glycol dimethacrylate-co-4-vinylpyridinium) salts specific for trichloroacetic acid (TCAA) and structurally similar compounds as the recognition element. The sensor was designed as part of a lab-on-chip and has been successfully incorporated into a miniaturised flow-through cell. An integrated sensor specific for the target analyte was fabricated using gold screen-printing to deposit the interdigitated conductometric electrodes at the glass plate, with measurement based on detection of MIP-containing artificial recognition sites for TCAA and its analogues. Additionally, the ability of molecularly imprinted cross-linked poly(ethylene glycol dimethacrylate-co-4-vinylpyridinium) salts to change its conductivity due to the placement of the target molecule into the imprint cavity has been used to develop a detector for HAAs. The vinylpyridinium entity fabricated into the MIP would form charge-transfer complexes with the target analyte HAAs, which would produce the continuous conducting phase of the insulating poly(ethylene glycol dimethacrylate) cross-link [18,19]. Electrically-conducting MIP thin-films from cross-linked poly(ethylene glycol dimethacrylate-co-4-vinylpyridine) have been produced at the interdigital transducer surface by free-radical cross-linking copolymerisation of a functional monomer, 4-vinylpyridine (VPD) and a cross-linker, ethylene glycol dimethacrylate (EDMA) in the presence of TCAA in acetonitrile.

In this paper, the sensor fabrication and the performance of the flow-through haloacetic acid sensitive conductometric sensor are presented. The new flow-through conductometric sensor was applied to haloacetic acid determination of real-life samples.

2. Experimental

2.1. Chemicals and materials

Ethylene glycol dimethacrylate (EDMA) 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). Polydimethylsiloxane and hardener (Sylgard 184) were obtained from Dow Corning Corporation (MI, USA). Trichloroacetic acid (TCAA) was purchased from Merck K-G (Darmstadt, Germany). Dichloroacetic acid (DCAA), monochloroacetic acid (MCAA), dibromoacetic acid (DBAA), monobromoacetic acid (MBAA), tribromoacetic acid (TBAA) and malonic acid were obtained from Fluka Chemie AG (Buchs, Switzerland). VPD and EDMA were purified by distillation under reduced pressure. All solvents were analytical grades and dried with molecular sieves prior to use. Working standard solutions were prepared daily.

2.2. Fabrication of the sensor device

The sensor devices fabricated in this study were of thick-film type. The gold paste was screen-printed on a pre-cleaned borosilicate glass (1 mm thickness and 15 mm × 20 mm) with a pair of comb-type Au electrodes having interdigital distances of 0.5 mm and overall area 11 mm × 8 mm (Fig. 1A). This electrode unit was sintered at 550 °C for 3 h. The thickness after sintering was about 1 μm as measured by an atomic force microscopy (AFM) method. The procedure for immobilization of MIP on electrodes was as follows. TCAA (14 mg, 0.08 mmol) was dissolved in 1 ml of acetonitrile. Appropriate amounts of VPD and EDMA were admixed, followed by the addition of AIBN (9 mg, 0.05 mmol). These monomeric mixtures were purged with a stream of nitrogen gas for 1 min, and pre-polymerised at 65 °C for 1 min in a water-bath. The viscous polymer (6 μl) was spread evenly over the electrode pattern of the interdigitated conductometric gold electrode placed in a chamber. Subsequently, the chamber was flushed with nitrogen gas for 1 min to remove the radical scavenger oxygen before being closed and oven-heated at 70 °C for 18 h. After the immobilization process, the electrode was washed with deionised water to remove the template molecules. A non-imprinted polymer (NIP) electrode, which was included as the control, was prepared in the same way as the MIP electrode, but in the absence of the TCAA template. Elemental analysis of the MIP thin-film coated on electrodes was performed on a JSM-5800 LV electron microscope (Jeol, MA, USA) equipped with an Oxford Instruments LINK-ISIS 300 X-ray detector and microanalysis system. The morphology of deposited film was inspected by an atomic force microscope (AFM) using a Nanoscope III Scanning tunnel microscope (Digital Instruments Inc., Santa Barbara, CA). The thickness of the films (see Fig. 1B) was determined by scratching with a needle and measuring depth of the scratches using an AFM (Digital Instruments, CA, USA) with a Nanotec Entrinica WSSM scanning probe microscopy software version 3.0 Beta 8.1 (Digital Instruments, CA, USA).

2.3. Optimization of polymer composition

The optimal amount of functional monomer and cross-linking monomer required for the manufacture of the MIP film on interdigital electrode was verified. A set of molecularly imprinted polymer films and the corresponding non-imprinted polymer films were immobilized on interdigitated electrodes using the procedure described in Section 2.2, and their electrical conductivity and adhesive properties were determined. These polymeric films were synthesized with different initial amounts of cross-linking monomer and different mole ratios of functional
monomer to template. The optimal amount of EDMA cross-linker was examined by varying the amount of EDMA as 55, 65, 70, 75 and 80 mol%, while the mole ratio of monomer to template was kept constant at 2:1. Further, the functional monomer (VPD) was applied at five different molar ratios with respect to the template with a fixed amount of cross-linking monomer (EDMA). These molar ratios were 1, 2, 4, 6 and 8 mol monomer to 1 mol of template. In the case of non-imprinted polymer film to which no template is added the amounts of VPD still relates to these stoichiometric ratios. The electrical resistance in air ($R_a$) of the prepared thin-films on electrode was obtained by means of an HP 4254 Precision LCR. In a typical resistance measurement, the electrode was connected to HP 4254A Precision LCR meter (Agilent Technologies, Palo Alto, CA) by soldering copper wires on coppered gold electrode contacts. The AC frequency of 1 kHz was used and the operating voltage was 100 mV.

2.4. Sensor fabrication

A diagram of the flow-through conductometric sensor system is shown in Fig. 1C. The glass plate that constituted the integrated sensor array and wire connections with electrode-contacts, was coupled with a 16 mm x 13 mm, 3 mm thickness home-made silicone pad (obtained from the commercial polydimethylsiloxane and hardener). This silicone pad has a 11 mm x 9 mm x 1 mm depth cavity created by molding. Through holes of 1 mm diameter for sample inlet and outlet were drilled through the cavity. Assembly of the thin-film sensor array with the silicone pad, followed by incorporation into a Perspex housing-box of 4.0 cm x 3.0 cm x 0.4 cm gave the analytical microsystem with a total internal volume of 90 ml. This integrated miniaturised device not only minimised the sample volume, but also considerably reduced the total assay time.

The sampling and conductometric analysis system of the sensor consists of a liquid port for delivery of water sample from a sample-reservoir, a peristaltic pump, a thermo set and the flow-through microcell/integrated MIP sensor incorporated with a conductometric apparatus controlled by a computer program developed in-house. The function of this system was on-line and the minimum sample volume required was 2.5 ml. The stand-alone sensor was operated at a continuous flow rate of 3 ml min$^{-1}$ driven by Ismatec peristaltic pump (MCP-Process Series, Ismatec SA, Wertheim-Mondfeld, Germany). The sensor array output signals were monitored using a network analyser (HP 4254A Precision LCR, Hewlett Packard, Germany), which read the resistance signals from the sensor array with subsequent display on the Laptop screen. The resistance measurement of the sensor was performed by applying an alternating potential (100 mV) to the electrodes with a frequency between 100 Hz and 1 MHz. At the initial measurement, the electrical resistance of the sensors was measured in deionised water as a reference. The measurements were carried out at room temperature (24 ± 1 °C). The signal was allowed to reach a constant value before subsequent addition of the analyte. The sensitivity of the MIP-based sensor was measured as a function of the changes in the resistance of the polymer upon exposure to TCAA or other HAA analogs with concentrations from 0.0005 to 500 mg l$^{-1}$. A control experiment was carried out with the corresponding NIP-based sensor. In addition, technological parameters (operating frequency, temperature and electrolyte) for the sensor were identified with regard to the response to TCAA, using the electrode giving high resistance change with good adhesion of the...
deposited film as a model. For the sample measurement using the sensor, the signal response towards the analyte of sensor was reported as $\Delta R_a$, where $\Delta R$ is the resistance shift response to the addition of known amounts of the analyte of interest. Every experiment was carried out in triplicate on any particular day of experimentation.

3. Results and discussion

3.1. The MIP-based electrode-fabrication of the sensor device

The electrically conducting MIP thin-film of poly-(ethylene glycol dimethacrylate-co-vinylpyridine) selective for haloacetic acids, was prepared at the gold electrode surface by thermal polymerisation using TCAA as the print molecule. Ethylene glycol dimethacrylate (EDMA) was chosen as the cross-linking monomer, due to its anticipated property of giving a high stability to the polymer, with corresponding rigidity of imprint structure. Vinylpyridine (VDP) was adopted as functional monomer for the imprinting of TCAA, because its amine group is capable of interacting with the acid group of the template. Besides, VDP forms a charge-transfer complex with strong acidic molecules [18,19]: these can produce the continuous conducting phase of an insulating polymer (ethylene glycol dimethacrylate) cross-link, enabling the signals of ligand binding to be detected.

The immobilization of MIPs and the corresponding NIPs on the surface of gold interdigitated electrodes was successful using the method described here. A sketch of sensor-device showing film arrangement and electrode disposition over glass plates is given in Fig. 1. Both MIP and NIP gave a detectable signal on the conductometer when examined as dry layers on gold electrode. The cleaning profiles of both MIP and the corresponding NIP were studied. For this, the deposited thin-film of both MIP and the corresponding NIP prepared with 2:1 monomer:template ratio and 65 mol% EDMA was soaked in 100 ml-water, and resistance change of resistance in air ($\Delta R_a$) was calculated from the resistance obtained at the start and at the end of washing. An increase in resistance of MIP after washing with water for 4 h, was maximal with EDMA content from $2.7 \times 10^8$ to $7.6 \times 10^8$ $\Omega$ for the MIP film and from $6.9 \times 10^8$ to $8.5 \times 10^8$ $\Omega$ for the corresponding NIP film 4 h after soaking in water. The $\Delta R_a$ of MIP ($4.9 \times 10^8$ $\Omega$) was almost three times higher than that of the corresponding NIP ($1.6 \times 10^8$ $\Omega$). The changes in resistance of the corresponding NIP probably arise due to the removal of un-reacted monomers and some parts of the non-adhered polymer. The electrodes fabricated with either MIP or NIP, either before or after washing in water, gave a resistance signal higher than the plain electrode ($R_a = 7.5 \times 10^5$ $\Omega$).

The increase in the resistance signal of the MIP film (after polymerisation) when exposed to water can be explained in terms of a change in the surface conductivity of the polymer as template is gradually eluted by the water. This means that the electrical conductivity of MIP before template extraction is produced by the interactions of template at the imprint. The change in conductivity of MIP films corresponds to the charge transfer interactions of TCAA and polymer. Additional evidence supporting the formation of charge transfer complexes between MIP and TCAA was an emerald-green coloring of only the MIP after polymerisation, which was eliminated by washing with water. This suggests that in the dry state, poly(ethylene glycol dimethacrylate-co-vinylpyridinium) exists in the form of acid-base complexes of TCAA and VPD monomer. The increase in resistance of MIP after washing with water suggests dissociation of the template from the imprint sites. In the case of the NIP-based sensor, no TCAA molecules were involved in the polymerisation process. Therefore the vinylpyridine on the polymer backbone is unable to be quaternized and the imprint cavities are not produced in the polymer network due to the fact that TCAA was not used in NIP polymerisation.

The interaction of template with MIP is apparently reversible. It is interesting that the incorporation of TCAA into the VPD-EDMA polymer not only enables the polymer to be conductive, but also improves the mechanical and adhesive properties of the film coating of the MIP. This may be because the charge-transfer complex between TCAA and VPD ions, which may swell and/or dissolve in water and can be crippled down by water flow from the surface of glass, as this is indicated by only HAA is released out from the MIP but not MIP during washing. After washing, the polymer morphology was examined using AFM. Fig. 2a and b show the AFM topographic images of MIP and non-imprinted polymers, respectively. AFM topographies of the MIP-film surface exhibit ridges and roughness, while smooth and uniform deposition is evident with the film of non-imprinted polymer. This result indicates the differences in the morphologies of polymer prepared in the presence and absence of the template molecule.

3.2. The MIP-based electrode-optimisation of polymer composition

The optimal composition of MIP film was examined in terms of resistance change as well as adhesive properties. The resistance change of the MIP due to the template extraction was examined in parallel experiments with non-imprinted polymers. Additionally, the imprint capacity, reflecting the efficiency of recognition of the MIPs was interpreted by comparing the resistance variation of MIP with the corresponding NIP. As seen in Fig. 3, there is a dependency in the degree of the $\Delta R_a$ as a function of the cross-linker amount for MIP, whereas this is not the case for NIP. Lower concentrations of EDMA (55, 65 and 70) give a higher resistance change than higher concentrations of EDMA (75 and 80). The imprinting factor, which represented the effect of the imprinting process, was the ratio of $R_a$ of MIP-based electrode to $R_a$ of the corresponding NIP-based electrode after washing with water for 4 h, was maximal with EDMA content between 55 and 65 mol%. However, the low amounts of EDMA
resulted in a poorer mechanical strength of the film. Therefore an EDMA content of 65 mol% was chosen to prepare the MIP for use in further investigations.

As shown in Fig. 4, further increase in the molar ratio of VPD to template shows a decrease in the resistance of MIP, but this is not evident with NIP. A maximum imprinting factor of 5.8 was obtained at a molar VPD to template ratio of 1:1, suggesting that the MIP forms 1:1 complexes with the TCAA molecules. Although a 1:1 monomer:template ratio is preferred, the 2:1 monomer:template ratio gave higher mechanical strength with good stability of film than the 1:1 monomer:template ratio. The results of this study suggest that the physical property of the film varies with the functional monomer:cross-linker ratio, and that the 2:1 monomer:template ratio may be suitable for combining with 65 mol% content of EDMA to produce a MIP film with good physical properties. Also, the 2:1 monomer:template ratio gave a relatively large value of imprinting factor (4.4). The low relative standard deviation (2.6%, n = 3) indicates good and reproducible coating. Since the MIP thin-film prepared with 2:1 monomer:template ratio and 65 mol% EDMA gives a high recognition selectivity to the template with good stability of film coated, this film type has been chosen as the recognition material for the development of a HAA sensitive conductometric sensor for on-line HAA analysis. Subsequent elemental analyses of MIP thin-film of this film type, which is layered on gold electrode, revealed an elemental composition of the film (and the gold paste) as C 45.9%, O 3.6%, Na 8.7%, Mg 3.0%, Al 1.3%, Au 0.10%, Ca 22.3% and Ag 15.1%. The thickness of MIP thin-film layered in place on the gold surface and on the glass surface was determined to be about 1 and 2 μm, respectively. The recorded resistance (R0) of the film on electrode was found to be about 80 kΩ in deionised water at 3 kHz at room temperature (24 ± 1°C). The percentage coefficient of variation for the R0 values of the MIP electrode obtained for three different batches of the interdigitated conductometric gold electrode was found to be 0.1%.
3.5. Conductometric sensor response

MIP-based films, particularly made of hydrophobic (ethylenglycoldimethacrylate) polymer, perform poorly in aqueous medium. For instance, the hydrophobic MIP based film is hardly wet and can be easily removed from the surface of metal and glass when soaked in water. While TCAA-imprinted poly(ethylenglycoldimethacrylate-co-4-vinylpyridinium) offered a good adhesion in aqueous medium due to polyelectrolytic nature of the polymer. The sensor fabricated with this MIP responded to analyte in water rapidly (30 s) with a steady response obtained within 2 min. The response time shown with the prepared MIP conductometric sensor is fairly rapid when compared to the other MIP conductometric sensors using the membrane electrode that gave response time in the order of 30 min [14]. The quick response of this MIP sensor may be explained by both the transducer system and the sensing material utilized in the present study. Basically, the transducer system using interdigitated array electrode has close proximity of the sensing element to the transducer, leading to rapid measurement due to low diffusion times.

As can be seen, repeated incremental additions of TCAA to polymer, and it may also enhance the penetration of water in the polymer matrix leading to an easier diffusion of TCAA into polymer. Hence the sensor responds quickly. It appears that the penetration of water into the MIP film is easier than into the NIP film, as is evident from the initial baseline of sensors, Fig. 5.

As can be seen, repeated incremental additions of TCAA, there is a decrease in the resistance of the MIP sensor (see Fig. 5). The effect of various concentrations of TCAA on the resistance signal of NIP-based sensor is negligible. This suggests that the interaction of TCAA with the MIP fabricated in water solution is derived from ionic interaction between the deprotonated carboxylic group and functional monomer. The formation of ion-pairs between TCAA ions and the vinylypyridinium ions of the imprinted polymer causes charge transfer interaction within the MIP. Consequently, the conductivity of the MIP film is likely to be generated by the overlapping electron π-orbitals which form extended conduction and valence bands. Ordinarily, charge transfer complexes have an anisotropic quasi-one-dimensional π-conducting structure with a delocalized charge carrier, while their chain structure leads to a strong coupling of electron states and to conformational excitations to quasi-one-dimensional system [18,19].

3.4. Effect of experimental parameters on the sensor signal

Since the sensing element of MIP-based sensor demonstrates a semiconducting polymer property and factors such as applied frequency, temperature and electrolytes are expected to have an effect on the conductivity of the semiconducting polymers [18,19]. Therefore variation in these factor may affect the signal response of the sensor.

3.4.1. Effect of applied frequency

The effect of the applied frequency on the resistance response of MIP-based sensors was studied over an ac frequency range 0.1–1000 kHz using TCAA concentrations up to 70 μg l\(^{-1}\) (Fig. 6a). The results show that increasing the applied frequency decreases the electrical resistance of the sensor at all concentrations of TCAA used (0–70 μg l\(^{-1}\)). The resistance at lower frequencies represents the electron diffusion process in the poly(VPD-co-EDMA) film. As shown in Fig. 6a, the resistance of the sensor is sensitive to the presence of TCAA molecules in the solution, and on the applied frequency. The resistance variation on the addition of TCAA at the high frequency region was shown to be lower than that at the low frequency region. A reasonable signal response of sensor was obtained in the ac frequencies ranging 1–10 kHz. A frequency of 3 kHz was therefore chosen for operating the sensor in all remaining experiments.

3.4.2. Effect of experimental temperature

The influence of variation of the experimental temperature on the resistance response of the sensor upon exposure to TCAA (200 μg l\(^{-1}\)) was investigated over the temperature range 299–333 K. As seen in Fig. 6b, the resistance of MIP sensor gradually decreases with increasing temperatures from 299 to 333 K. In contrast, the NIP-based sensor shows only a slight effect of temperature on the resistance at temperatures between 299 and 333 K, although the resistance of the sensor at the elevated temperature 333 K is much lower. As MIP and NIP films were prepared with the same monomer composition, differences in the conductivity behaviour are thus due to the imprinting effect.

The electrical conductivity of MIP sensor obeys the Arrhenius equation:

\[
\frac{1}{\Delta R} = A \exp \left(\frac{-E_a}{RT}\right)
\]
Fig. 6. (a) Effect of the ac frequency on the resistance responses of the MIP sensor at various concentrations of TCAA. Measurements were carried out at room temperature. (b) Effect of temperature on the resistance response upon 200 μg l<sup>−1</sup> of TCAA for: reference sensor (dashed lines) and MIP sensor (thick lines), at various frequencies. Top right is Arrhenius plot for the electrical conductivity of MIP electrode. (c) Effect of NaCl on the resistance shift response of (a) MIP sensor and (b) reference sensor at 3 kHz and at room temperature. Responses were initiated by the addition of 200 μg l<sup>−1</sup> TCAA.

where \( A \) is the pre-exponential factor, \( E_a \) the activation energy, \( R \) the gas constant and \( T \) is the temperature (see Fig. 6b, top right). A 35 K increase in temperature above room temperature, the resistance of the MIP-based sensor decreased from 10<sup>5</sup> to 10<sup>4</sup> Ω. The results obtained confirms that the MIP has semiconductive like behaviour of \( \pi \)-type conjugative polymers [19–21]. Accordingly, the main effect of temperature variations on the conductivity of the MIP sensor may be explained by a change of charge-transfer as temperature varies. The activation energy derived from the Arrhenius type of temperature dependence of conductivity in the case of the MIP sensor may be associated with the excitation energy for thermal generation of binding site in the polymer. The resistance change obtained at different temperatures also allows us to calculate the activation energy of the binding site for MIP; the activation energy of 10.2 kcal/mol was calculated from the data. The result obtained in this study demonstrated that temperature changes could have strong influences on the sensitivity of the sensor. Thus, measurement using this sensor should be carried out under controlled steady temperature.

3.4.3. Effect of electrolytes

The effect of the electrolyte on the resistance shift response of the developed sensors was investigated. The data show that increasing concentration of NaCl in sample solutions containing 200 μg l<sup>−1</sup> TCAA increases the magnitude of the sensor responses in both MIP- or NIP-based sensors (Fig. 6c). The increased conductivity observed with both MIP and NIP sensors when exposed to the analyte and electrolytes (NaCl) can be explained by the enrichment of the analyte and counter ions in the polymer particles, which gives a high local concentration of charged species close to the gold electrode surface in the sensing device. It can be seen (Fig. 6c) that there is a difference in the 200 μg l<sup>−1</sup> TCAA-signal response of MIP and NIP sensors at every concentration of electrolyte studied. This difference seems to be constant independent of further addition of the electrolyte. Where the difference occurred, which is apparent in the NaCl concentration range between 0.2 and 3.4 mg l<sup>−1</sup> (0.01–200 mM), the conductivity response of MIP sensor was always higher than that of NIP sensor, indicating the greater affinity with the target analyte of the former sensor.

3.5. Concentration dependence on signal response of sensor

As can be seen from Fig. 7, the MIP sensor gives a signal response primarily rising with an increase in the concentration of TCAA, while reference sensor shows a negligible shift of resistance response to the added TCAA. At higher concentrations of TCAA (>100 μg l<sup>−1</sup>) the response of MIP sensor seems to be constant which is probably explained by the saturation of the recognition sites in the imprinted polymer. This suggests
that the adsorbed analyte produces appropriate binding of analyte molecule at binding sites on the MIP film. The adsorption behaviour of the MIP film fabricated in sensor can be fitted to the Langmuir isotherm expressed as

$$\Omega = \frac{\Omega_{\text{max}} \cdot K_c \cdot c}{1 + K_c \cdot c} \quad (2)$$

where \(\Omega_{\text{max}}\) is the maximum value of the resistance response (\(\Omega_1\)), \(K_c\) the modified adsorption equilibrium constant and \(c\) is the concentration of the analyte. Plot of \(\frac{1}{c}\) versus \(\frac{1}{\Delta R_s}\) yielded a straight line with a single apparent binding constant of \(9.3 \pm 0.9262\) M\(^{-1}\). The affinity of the TCAA-imprinted polymer for its original template molecule is similar to those of the other MIPs reported in literatures [13,22].

### 3.6. Analytical characteristics

The calibration curves constructed from the resistance shift parameter dependency provided reasonable results. There was a linear relationship between the shift resistance of the MIP sensor and the logarithm of the concentrations (log \(c\)) \((R^2 > 0.97)\) of TCAA, in the range of 0–100 \(\mu g \cdot l^{-1}\), and the other five HAAs (DCAA, MCAA, TBAA, DBAA and MBAA) in the range of 1–500 \(\mu g \cdot l^{-1}\), depending on the compound, and the mixture of total six HAAs in the range of 10–400 \(\mu g \cdot l^{-1}\). The calibration data are shown in Table 1. The limits of detection as obtained according to \(3 \times SD/m\) criterion, where \(m\) is the linear calibration and \(SD\) were estimated as the standard deviation (\(n = 3\)) of the signal response for HAAs, were in the range 0.5–5 \(\mu g \cdot l^{-1}\) for the various HAAs. The US Environmental Protection Agency (USEPA) [23] has set a maximum contaminant level of 60 \(\mu g \cdot l^{-1}\) for the five commonly occurring acids namely TCAA, DCAA, MCAA, DBAA and MBAA in the stage 1 of the disinfection by-product regulation. Again, this is to be lowered in the coming years to 30 \(\mu g \cdot l^{-1}\). Within this regulation, DCAA should never be present, and TCAA concentrations should not be more than 30 \(\mu g \cdot l^{-1}\) [24]. The MIP electrode can thus detect HAAs at concentrations well below the maximum permitted concentrations.

### 3.7. Selectivity of the sensor

To assess the overall selectivity of the MIP electrode, the effects of structurally related haloacetic acid compounds such as DCAA, MCAA, TBAA, DBAA, MBAA and non-haloacetic acid compounds such as malonic acid and acetic acid, on sensor conductivity were examined. The results revealed that the NIP sensor exhibited relatively little response to both haloacetic acid and non-haloacetic acid compounds (data not shown). For the MIP sensor, non-haloacetic acid compounds, either acetic acid or malonic acid, in the 10–500 \(\mu g \cdot l^{-1}\) concentration range, generated negligible change in the electrical conductivity signal of the sensor (see Fig. 8). This indicates a definite imprint effect of the MIP coated on electrodes. On the other hand the concentrations of structurally related haloacetic acid compounds resulted in significant shift of the resistance of the sensor. The results indicate that the HAA analogues are capable of induc-

![Fig. 7. Concentration dependence of the sensor response to TCAA for: (a) MIP sensor and (b) reference sensor. Each point represents the average of three independent measurements. Measurements were carried out in distilled water at 3 kHz and at room temperature.](image)

![Fig. 8. Calibration curves for TCAA, DCAA, MCAA, TBAA, DBAA and MBAA obtained in the steady state on-line system using the developed MIP sensor.](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Slope</th>
<th>(R^2)</th>
<th>Working range ((\mu g \cdot l^{-1}))</th>
<th>LOD ((\mu g \cdot l^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>TCAA</td>
<td>38.89</td>
<td>0.992</td>
<td>0–100</td>
<td>0.5</td>
</tr>
<tr>
<td>DCAA</td>
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<td>0.993</td>
<td>1–500</td>
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<td>MCAA</td>
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<td>0.986</td>
<td>5–500</td>
<td>5.0</td>
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<tr>
<td>TBAA</td>
<td>5.81</td>
<td>0.972</td>
<td>10–500</td>
<td>5.3</td>
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<tr>
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<td>0.985</td>
<td>40–400</td>
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<tr>
<td>MBAA</td>
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<td>0.987</td>
<td>30–400</td>
<td>1.2</td>
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<tr>
<td>Six HAAs</td>
<td>117.23</td>
<td>0.980</td>
<td>10–400</td>
<td>5.6</td>
</tr>
</tbody>
</table>

* Refers to TCAA, DCAA, MCAA, TBAA, DBAA and MBAA altogether.
Water filtration system (home) 1.2
±
Bottled water C (25 l) 1.0
±
Bottled water B (1 l) 0.9
±
±
Bottled water A (1 l) 0.8

Sample HAA concentration measured (method 552.2) was in excellent agreement with that obtained as a detection method for this group of HAAs. Since the selectivity values of HAAs (particularly MCAA, MBAA, DBAA) are relatively low than those for DCAA, MCAA and TCAA, there may be a possibility of inter-anionic competition for receptor in MIP cavity, and TCAA could have preference over other five HAA analogs in terms of rebinding.

3.8. Sample analysis

The haloacetic acid conductometric sensor was used as an analytical tool for haloacetic acid determination in four water samples, including three brands of commercial bottled water (1 and 25 l containers) and a municipal tap water (with home filtration system). These water samples were assayed directly. A calibration curve of TCAA alone was prepared in the range of 0.5–10 μg l⁻¹ and compared to that measured for HAA in the samples. A method for analysis of HAAs in water, LLE–GC–ECD recommended by the USEPA [25] (method 552.2) was also used to verify the amounts of HAA in the samples obtained by the conductometric sensor. The results obtained for analyses of each sample by the sensor and the method 552.2 are displayed in Table 2. The measurement of HAAs with the USEPA method revealed that only TCAA was contained in the samples. The results for HAAs analysis by the sensor method was in excellent agreement with that obtained with the USEPA method. For validation purposes a recovery study was performed by spiking the above water samples with increasing amounts of TCAA and other HAAs at various concentrations by the sensor method (Table 3).

Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>HAA concentration measured (μg l⁻¹)</th>
<th>µgl⁻¹ added (TCAA)</th>
<th>µgl⁻¹ added HAAs (Rec)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottled water A (1 l)</td>
<td>0.0 ± 0.10</td>
<td>0.7 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>60 ± 2</td>
</tr>
<tr>
<td>Bottled water B (1 l)</td>
<td>0.0 ± 0.10</td>
<td>0.6 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>57 ± 2</td>
</tr>
<tr>
<td>Bottled water C (25 l)</td>
<td>0.8 ± 0.00</td>
<td>3.0 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>Water filtration system (home)</td>
<td>1.2 ± 0.07</td>
<td>1.2 ± 0.02</td>
<td>1.2 ± 0.02</td>
<td>104 ± 2</td>
</tr>
<tr>
<td>Total</td>
<td>0.56 ± 0.00</td>
<td>3.2 ± 0.00</td>
<td>1.2 ± 0.02</td>
<td>95 ± 2</td>
</tr>
</tbody>
</table>

Note that only TCAA was detected in all samples. The value in parenthesis is mean percent recovery of three independent measurements using only one electrode. Total six HAAs refers to TCAA, DCAA, MCAA, TBAA, DBAA and MBAA altogether. Expected concentrations are amounts added plus the amounts already present in the water sample.
Emmert et al. [26] proposed criteria for consideration of an 
1 or 2 min and sample volume requirement is low. Recently, 
HAA cannot be used on-line. The readout can be obtained within 
potential is important because other methods for measuring 
advantages. Most important is on-line detection. This on-line 
haloacetic acid assays this novel device offers considerably more 
water.

as well as for fast environmental monitoring of the drinking 
traditional analytical techniques used for measurement of HAAs 
related compounds. The sensor would be complementary with the 
an early warning system for measuring these toxic compounds 
conditions.

ing HAAs in water. Moreover, the sensor exhibited the same 
detection limit show the suitability of this sensor for measur-
ing and measuring of HAAs, which are often presents in water 
as complex mixtures. The achieved sensitivity ranges and the 
detection limit show the suitability of this sensor for measur-
ing HAAs in water. Moreover, the sensor exhibited the same 
recognition characteristics over 3 months when storage in dry 
conditions.

With its current sensitivity, the sensor can be operated as an 
ear warning system for measuring these toxic compounds in 
water sources. The sensor would be complementary with the 
traditional analytical techniques used for measurement of HAAs 
as well as for fast environmental monitoring of the drinking 
water.

Compared to the traditional conductometric techniques for 
haloacetic acid assays this novel device offers considerable more 
advantages. Most important is on-line detection. This on-line 
potential is important because other methods for measuring 
HAAs cannot be used on-line. The readout can be obtained within 
2 or 3 min and sample volume requirement is low. Recently, 
Emmert et al. [26] proposed criteria for consideration of an 
ideal analyzer that could be used to monitor five regulated 
HAAs concentrations directly in drinking water distribution sys-
tems; namely it should operate in real or near real-time; provide 
method detection limits (MDLs) in the range of 0.5–5 µg l⁻¹ 
with mean percent recoveries of 100 ± 5% and percent rela-
tive standard deviations (%R.S.D.) of less than 10%; not suffer 
from total organic carbon interferences (i.e. acetic acid and mal-
onic acid); be principally automated; require fairly low operator 
skill level; be compared directly with USEPA methods in real 
world situations; be inexpensive. Our proposed sensor prac-
tically meets all of these criteria. A patent for this developed 
sensor has been applied for in Thailand (ref No. 107046), and a 
worldwide patent application will follow.

4. Conclusions

A flow-through haloacetic acid sensitive conductometric sensor 
based on the electrically-conducting imprinted polymer of 
cross-linked poly(ethylene glycoldimethacrylate-co-4-
vinylypyridinium) on screen-printed interdigitated electrode has 
been developed for on-line testing of haloacetic acids and shows 
a good response to trichloroacetic acid template and structurally 
related compounds. The sensor has potential for use in detect-
ing and measuring of HAAs, which are often presents in water 
within the sensor.

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