

# Trichloroacetic Acid-Imprinted Polypyrrole Film and Its Property in Piezoelectric Quartz Crystal Microbalance and Electrochemical Sensors to Application for Determination of Haloacetic Acids Disinfection By-Product in Drinking Water

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**ABSTRACT:** A molecularly trichloroacetic acid (TCAA) imprinted non-crosslinked polypyrrole (TCAA-MIPpy) has been successfully prepared and evaluated in its properties for both piezoelectric quartz crystal microbalance (QCM) and electrochemical means of detection, and application of such sensors for determination of haloacetic acid concentrations in water. Haloacetic acid selectivity was demonstrated in gravimetric or in electrochemical sensors via either cyclic voltammetric or interdigitated conductometric (IDC) analyses studies upon comparison with nonimprinted polypyrrole and TCAA-imprinted polypyrrole. The measurement results with all these systems revealed that the prepared molecularly imprinted polymer can recognize TCAA well from struc-

turally similar compounds. The TCAA-MIPpy incorporated with only the IDC transduction system produced the specific signal for TCAA detection in aqueous environment, which is useful for the quantifying TCAA disinfection by-product in water. The analytical application for determination of TCAA in real-life samples was readily achieved with the incorporation of TCAA-MIPpy in the IDC analysis system, the results revealing the high sensitivity and selectivity of the method. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 106: 3861–3871, 2007

**Key words:** molecularly imprinted polymer; conducting polymer; voltammetry; inter-digitated conductometry; quartz crystal microbalance

## INTRODUCTION

Molecularly imprinted polymer (MIP), a synthetically derived artificial receptor, has been attracting considerable research interest in the field of chemical and biomimetic sensors.<sup>1,2</sup> Since such receptors are not affected by harsh conditions during the measurement processes, they can be used both in organic and aqueous solvents. In addition, the cost of obtaining MIPs is lower, and their preparation and storage easier than for biological recognition materials. MIPs can be synthesized by means of molecular imprinting, which involves copolymerization of functional monomers with appropriate cross-linkers in the pres-

ence of a template molecule, and subsequent removal of template molecules with a suitable solvent. MIPs prepared under suitable conditions have recognition cavities that are complementary to the template molecules, and their performance can be investigated for the recognition of several analyte species, including biological compounds, drugs, and agrochemicals.<sup>3,4</sup>

To date, MIPs are almost always prepared with cross-linking monomers, such as acrylates, styrenes, or silanes. This may be because these materials yield imprinted polymers with potentially high molecular recognition and with good physical and mechanical stability of the polymer layer. Over two decades, noncrosslinked conducting polymers such as polypyrroles, polyvinylchlorides, polyphenols, polyporphyrin IX, and polyphenylenediamines have been applied as molecular recognition systems for detection of anionic species in electrochemical type sensors.<sup>5,6</sup> Among these conducting polymer materials,

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polypyrroles have many attractive features as selective recognition elements for chemical sensing purposes: they can be used at neutral pH, and their stable films can conveniently be polymerized on several substrate materials. Non-crosslinked polymeric materials, including polypyrrole, have been found to have recognition ability for certain inorganic anions through recognition mechanisms of both size and shape selectivity.<sup>7,8</sup> However, sometimes the addition of a molecular recognition compound, e.g. polyoxyanion macrocyclics, into these non-crosslinked polymers is required to assure the specificity for the analyte.<sup>9,10</sup> In generating recognition towards target analytes in a noncrosslinked conducting polymer, tailor-made methods utilizing molecular imprinting polymerization is an alternative approach to yield specific detection for analytes of interest.<sup>11,12</sup> This approach has advantages over the methods involving the insertion of molecular recognition molecules into the non-crosslinked conducting polymer. For example, the molecular imprinting polymerization method can overcome the problems associated with the use of entrapment techniques, such as incompatibility of polymer layer with a selective molecule, problematic accommodation of the selective species, and several concerns about the mechanical properties, and processibility of the noncrosslinked polymer layer embedding the additional molecule.

Trichloroacetic acid (TCAA), a disinfection by-product, is produced from chlorination during the treatment of water.<sup>13</sup> TCAA and analogs such as dichloroacetic acid (DCAA), monochloroacetic acid (MCAA), tribromoacetic acid (TBAA), dibromoacetic acid (DBAA), and monobromoacetic acid (MBAA), belong to haloacetic acid (HAA) disinfection by-products group. These HAAs have been found to have potentially mutagenic and carcinogenic effects in animals and man.<sup>14,15</sup> Many countries, e.g. USA, Canada, and Australia, have set regulations with the maximum permissible levels of TCAA and related HAAs to control HAAs concentrations in both domestic and commercial bottled drinking waters.<sup>16,17</sup> The analysis methods for determination of the HAA concentrations, as recommended by the USEPA involves chromatography, i.e., IC, GC-MS, GC-ECD.<sup>18,19</sup> The chromatographic techniques, even though they are sensitive and reliable for measurement of HAA concentrations in water, are complicated, costly and time-consuming, which makes such chromatographic systems unsuitable for routine analysis of HAAs in water. Indeed, continuous online monitoring of the regulated HAA disinfection by-products would be of interest to governmental agencies involved in the supply of domestic water, to industries producing bottled water, and to environmental agencies for routine monitoring of contaminants in rivers, lakes, and water reservoirs.

Thus, to achieve this goal, a reliable analysis system must be developed which meets all the essential criteria for such an analyzer, e.g. real-time operation, low cost, short response time, specificity, sensitivity, and reliability.

The aim of the present study was to seek a specific and reliable method for direct detection of HAA disinfection by-products in drinking water by means of molecularly imprinted polypyrrole (MIPpy) sensing element incorporated with a suitable transducing analysis system. In generating a predefined HAA selectivity for noncross-linked polypyrrole by molecular imprinting technique, the electropolymerization of pyrrole at appropriate voltage was carried out in the presence of TCAA as a template. This TCAA-MIPpy was created as a thin-film onto surface of both a cyclic voltammetric (CV) and inter-digitated conductometric (IDC) transduction unit, and also on a piezoelectric quartz crystal microbalance (QCM) transduction unit. The recognition property and sensing performance of TCAA-MIPpy integrated in all these transducer systems has been assessed, for the application for these sensors for the determination of HAA concentrations in drinking water.

## EXPERIMENTAL

### Chemicals and materials

Reagent grade pyrrole was purchased from Fluka Chemie AG (Buchs, Switzerland). TCAA was obtained from Riedel-deHaen (Seelze, Germany). Dichloroacetic acid (DCAA), monochloroacetic acid (MCAA), dibromoacetic acid (DBAA), and monobromoacetic acid (MBAA) were purchased from Fluka Chemie AG (Buchs, Switzerland). Malonic acid was obtained from Aldrich Chemical Company (Milwaukee, WI). Working standard solutions were prepared daily depending at appropriate concentrations. All solvents used (methanol and acetic acid) were of analytical grade and used as received without further purification.  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ , NaCl, HCl, and KCl (Merck, Darmstadt, Germany) of analytical grade were used for the preparation of buffer solutions. Buffer solutions were prepared providing different pH acceptor solutions. A HCl-KCl buffer was prepared by mixing 0.2 mM HCl and 0.2 mM KCl in different proportions for pH 0.7 and 1. A phosphate buffer solution was prepared by adding appropriate proportions of 0.2 mM  $\text{K}_2\text{HPO}_4$  and 0.2 mM  $\text{KH}_2\text{PO}_4$  for pH values between 6 and 8. An acidic phosphate buffer solution was prepared by mixing 0.2 mM  $\text{K}_2\text{HPO}_4$ , 0.2 mM  $\text{KH}_2\text{PO}_4$ , and 0.2 mM HCl to give pH 4.

An AT-cut quartz disc 15 mm with gold electrodes and a resonance frequency of 10 MHz was employed for investigations. The electrode facing the aqueous

phase had a diameter of 4.5 mm, while that facing the gas-phase had a diameter of 2.5 mm; the electrode was prepared by screen-printing techniques. The IDC devices fabricated in this study were of thick-film type. The gold paste (Aldrich, WI) was screen-printed on a 1-mm thick borosilicate glass ( $12 \times 18 \text{ mm}^2$ ) with a pair of 10-finger Au electrodes having both an electrode width and electrode gap of 0.5 mm. The screen-printed electrode was finally sintered in an oven at  $550^\circ\text{C}$  for 3 h. The thickness of gold layer after sintering was about  $1 \mu\text{m}$  as measured by an atomic force microscopy (AFM) method. Cyclic voltammetry was performed using a  $\mu$ -Autolab three-electrode system (Eco Chemie, B.V. Utrecht, The Netherlands). Electropolymerization was accomplished on a platinum plate auxiliary electrode with 2 mm diameter as the working electrode, and Ag/AgCl as reference electrode.

#### **Immobilization of TCAA-imprinted polypyrrole on the surface of the transducer**

The immobilization of TCAA-imprinted polypyrrole on surface of either the QCM transducer, or the IDC transducer, was performed by means of a Coulostat E524 at room temperature ( $25 \pm 1^\circ\text{C}$ ). For this purpose, the electropolymerization of pyrrole was conducted on Coulostat E524 galvanometer (USA) at a current density of  $2 \times 10^{-1} \mu\text{eq/s}$  for 50 s in an aqueous solution containing 0.1M pyrrole and 0.1M TCAA, under a nitrogen atmosphere. The nonimprinted polymer (NIP) films, which were employed as controls, were prepared in the same manner as the MIP films but using 0.25M KCl instead of 0.1M TCAA for improving electrical sensitivity in NIP solution. The coated electrode was subsequently washed with five portions of 50 mL deionized water for at least 3 h to extract the template molecules.

For immobilization of the TCAA-imprinted polypyrrole onto the voltammetric transducer surface, the platinum electrode was immersed in a buffer solution containing of 0.25M pyrrole and 0.5M TCAA. Electropolymerization was carried out at current density of  $0.1 \text{ mA cm}^{-2}$  for 1.5 h. The film coat was then washed in deionized for 1 h. A control electrode was prepared using the same procedure in every case, but in the absence of template molecule. It was found that each MIP electrode yielded similar responses, but not exactly identical, so that a set of one MIP and one of corresponding NIP-coated electrodes was freshly prepared for each experiment.

#### **Piezoelectric QCM-analytical detection of analyte**

The coated quartz electrode was mounted in a self-made measuring cell with a 250  $\mu\text{L}$  volume. The quartz crystal was in contact with the liquid on one

side only. The 100 mL of sample solution was pumped sequentially through a thermostat set at  $25^\circ\text{C}$  and was passed into the measuring cells which contained the quartz sensor (2 mL/min). The measurements of sample solution and standard were made on a network analyser (HP 8572C) with home-built oscillator circuits and self-programmed processing software. For measurement of each test solution, the frequency of the imprinted polypyrrole polymer on QCM electrode was recorded in parallel experiments with the corresponding NIP polymer. The response of sensor exposed to a solution of the analyte was reported as frequency shift response ( $-\Delta F$ ) which is the difference between the values of frequency shift of the MIP electrode and frequency shift of the NIP electrode. Various concentrations of standard TCAA and analogs solution ( $0.1\text{--}100 \text{ mg L}^{-1}$ ) were measured. Calibration data for the TCAA-MIPpy-QCM sensor was obtained using concentrations of standard TCAA and analogs ranging from 0.1 to  $100 \text{ mg L}^{-1}$ . Every measurement was carried out in triplicate.

#### **Electroanalytical detection of analyte**

The electrochemical interaction of the prepared TCAA-MIPpy film with TCAA and its analogs was evaluated using both a differential pulse voltammetric analysis and inter-digitated conductometric analysis. Optimized conditions for preparation of the polymers and for rebinding measurements were identified before evaluation of the sensor recognition properties for each MIP film. In addition, calibration data for both the developed CV and IDC analysis system were determined, by incorporation of the TCAA-MIPpy film as means of detection.

##### **Differential pulse voltammetric analysis**

Differential pulse voltammetric analysis of reversible oxidation peak of TCAA and analogs was conducted in a 150 mL-glass cell with a three-electrode potentiostatic unit. The MIP-coated electrode was used as working electrode. CV signals were recorded at  $0.05 \text{ mV s}^{-1}$  and a potential window between  $-0.8$  and  $1.6 \text{ V}$ . Differential pulse voltammetric signals were recorded with a potential window between  $-0.8$  and  $0.6 \text{ V}$  at  $0.025 \text{ mV s}^{-1}$  and at pulse applied of  $25 \text{ mV}$ . Various concentrations of standard TCAA and its analogs, in the range of  $0.1\text{--}1000 \text{ mg L}^{-1}$  were analyzed, all measurements being performed in triplicate.

##### **Inter-digitated conductometric analysis**

The coated-IDC electrode was fabricated in a self-made measuring cell with a 100  $\mu\text{L}$  volume. Sample

solution (2 mL) was pumped sequentially at a flow rate of 2 mL/min through a thermostat set at 25°C and then into the measuring cell, which contained IDC sensor. The conductometric measurements were made on a 4284A precision LCR meter (Hewlett Packard, Germany) connected with a laptop computer. The resistance data were processed using software developed in-house. All measurements were carried out in deionized water at room temperature (26°C). The resistance measurement of the IDC sensor was performed by applying an alternating potential (100 mV) to the electrodes with an optimized frequency of 3 kHz. An initial measure of the electrical resistance of the sensors was recorded with deionized water as a reference. The resistance change of the TCAA-imprinted pyrrole was measured as a function of the changes in the resistance of the polymer upon exposure to TCAA or other HAA analogs at concentrations from 1 to 120  $\mu\text{g L}^{-1}$ . A series of standard solution of TCAA and analogs (1–120  $\mu\text{g L}^{-1}$ ) were added to the test solutions. Signal response of the sensor towards the analyte was reported as  $1/\Delta R$ , where  $1/\Delta R$  is the conductivity shift response of the MIP electrode exposed to each sample solution, where  $\Delta R$  is obtained from the change in resistance response as different, increasing amounts of analyte are added.

## RESULTS AND DISCUSSION

### Preparation of TCAA-imprinted polypyrrole films

In this study, the polypyrrole thin films, prepared in presence of TCAA as template were integrated into both a QCM and IDC transducer and CV transducer by using galvanostatic and CV deposition method. TCAA, a strong acid ( $\text{pK}_a = 0.95$ ), which was the template of this work, allowed the production of an imprinted polymer film of pyrrole with conducting properties. During the imprinting, the pyrrole monomer was expected to incorporate a trichloroacetate anion to compensate cationic charge carried by the non-crosslinked polypyrrole produced. After the removal of TCAA molecules by washing, a cavity with shape, and size complementary to TCAA was created within the network of the polypyrrole film, presumably at the surface.

### Piezoelectric QCM experiments

Piezoelectric QCM, that measures mass loading on the conducting electrode surface of the crystal, is one of useful transducers for analysis of analyte in a bulk solution. QCM is remarkably mass sensitivity, is relatively inexpensive and easy to fabricate on an electropolymerizable polymer material. Thus, incorporating TCAA-MIPpy with this transduction system

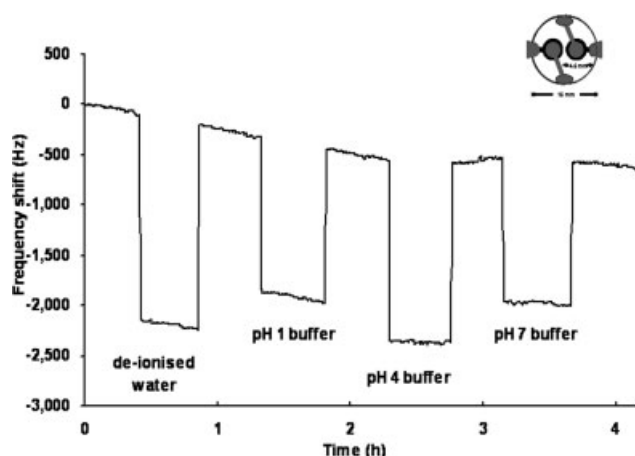
**TABLE I**  
The Effect of Layer Thickness, and Monomer : Template Mole Ratio, on Frequency Shift Responses of the TCAA-MIPpy Coated on QCM Electrode to TCAA at a Concentration Level of 100  $\mu\text{g L}^{-1}$

Parameter	Frequency shift <sup>a</sup> (Hz)	
	MIP	NIP
Polymer thickness (kHz)		
8	1220	153
15	2850	168
20	3200	182
35	3450	237
Monomer:template mole ratio		
1 : 1	1700	200
1 : 2	1200	400
1 : 3	1000	300
1 : 4	800	300

<sup>a</sup> Mean frequency shift of three independent measurements using three electrodes. The RSD values are less than 0.1%.

would possibly afford an analyser that can be used for the routine analysis of HAA concentrations in drinking water. Several parameters (polymer layer thickness, monomer:template mole ratio, medium pH, electrolyte concentration) can affect the rebinding property and sensing performance of the TCAA-MIPpy in QCM transduction system. Thus those factors were verified before determining in the interaction of the prepared TCAA-MIPpy film with the TCAA analyte.

The various layer thicknesses of the TCAA-MIPpy coated on the QCM transducer surface varied electrochemically in the range of 8–35 kHz were examined in the frequency shift response of the sensor (Table I). The frequency shifts increase with increase of the polymer thickness, with the greatest shift occurring between 8 and 15 kHz, and the shift-increase relatively stable between 20 and 35 kHz. In contrast, the frequency shifts with the NIP are only slight. Layer height of the TCAA-MIPpy coated on the QCM was optimum at about 22 kHz which equals to 870 nm thickness as calculated by the Sauerbrey equation.<sup>20</sup> This would suggest that the imprint site is not only generated at the polymer surface, but also deep inside the matrix of the non-crosslinked polypyrrole layer. At the optimum layer height the imprint cavities are expected to nearly fill-up with the molecular pendants of TCAA. Besides, the measurement results with the electrode imprinted with a various mole ratio of monomer and template revealed that imprinting effect, i.e. the difference of frequency shift of MIP electrode and NIP electrode, was the highest at 1 : 1 mole ratio of monomer:template (Table I). The mechanism for the generation of the binding cavities for the TCAA template in non-crosslinked polypyrrole is proposed as follows: an oligomer of polypyrrole formed during



**Figure 1** Frequency shifts of the TCAA-imprinted polypyrrole-coated QCM at various background  $10 \text{ mg L}^{-1}$  TCAA concentrations, there is a reversible signal when washing the electrode with pure deionized water. The small insert demonstrated the QCM electrode pattern.

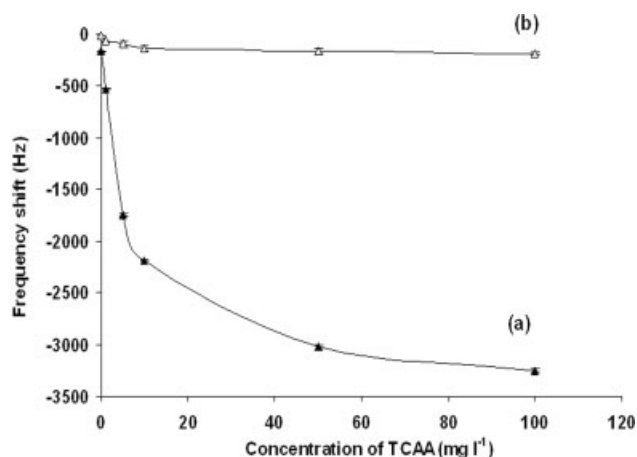
the early stage of electropolymerization attracts the TCAA carboxylate anions to compensate its cationic species located at the backbones, and then this repeatedly occurs until the reaction is complete in the predefined time interval; eventually the pores or channels that have a shape and size complementary with the template molecules are produced inside an engineered network of polypyrrole molecules.

The ionic interaction of the template and recognition site in the TCAA-MIPpy may be affected by some ionic species in the background solution resulting in QCM frequency shift response of the TCAA-MIPpy. Therefore, the effect of the pH of background solution was also investigated in pure deionized water and buffer solutions at pH 1, 4, and 7. As shown in Figure 1, the TCAA-MIPpy gives the highest frequency shifts for the  $10 \text{ mg L}^{-1}$  (ppm) TCAA solution in deionized water, when compared with the other media. In the buffer at pH 4, the interaction between TCAA and cationic species of the MIP was strongest, compared with the other buffers. In addition, the results, in Figure 1, show that the interaction of TCAA with MIP can be reversible in pure deionized water.

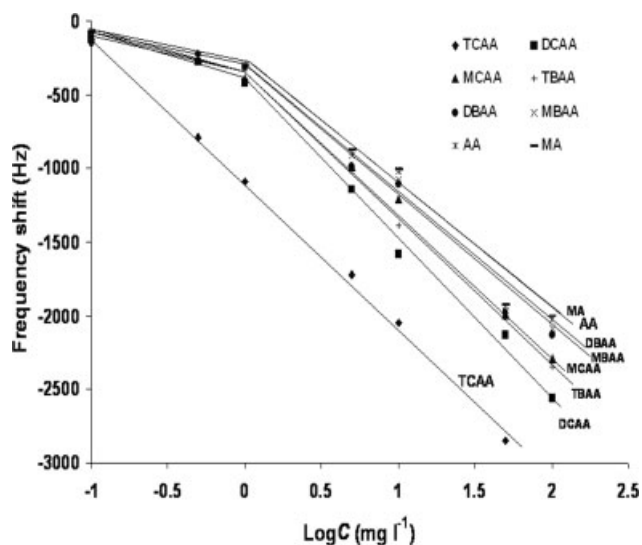
The dependency of the frequency shift response on TCAA and its analog concentration in the range of  $0.1\text{--}100 \text{ mg L}^{-1}$  was investigated for both the TCAA-MIPpy and the corresponding non-imprinted polymer-coated electrode. Figure 2 shows that the TCAA-MIPpy coated electrode has a good response with the added TCAA in water sample solutions in the predetermined concentration range. The frequency shifts responses of the MIP electrode are higher as the concentration of TCAA is increased. In contrast, the NIP-coated electrode showed a negligible

shift of frequency shift response to TCAA (Fig. 2). This suggests that the imprinting procedures have produced cavities with a high affinity for the TCAA in MIP. Saturation of recognition sites with the template molecules occurred at the higher concentration ( $100 \text{ mg L}^{-1}$ ) of added TCAA.

The putative binding sites of the TCAA-imprinted polypyrrole-coated QCM were identified, by using noncompetitive analyses method, using five structurally related HAA analogs (DCAA, MCAA, TBAA, DBAA, and MBAA) and non-HAA analogs such as acetic acid (AA) and malonic acid (MA) as the substrates. The dependency of the sensor response on concentration of TCAA and analogs was determined in the range of  $0.1\text{--}100 \text{ mg L}^{-1}$ . Both five HAA analogs and the non-HAA analogs, malonic acid, and acetic acid in the  $0.1\text{--}100 \text{ mg L}^{-1}$  concentration range generated change in the frequency shift signal of the MIP electrode, but the level of frequency shift responses depended on the compound and its concentration, as shown in Figure 3. The plot of  $-\Delta F$  values versus the logarithm of concentrations ( $\log C$ ) of TCAA showed a good linear relation over the TCAA concentration range of  $0.1\text{--}100 \text{ mg L}^{-1}$  with the correlation coefficient ( $R^2$ ) of 0.995. By contrast, the calibration curves generated with both five HAA analogs and two non-HAA analogs were linear in the two ranges of  $100\text{--}1000 \mu\text{g L}^{-1}$  with correlation coefficients in a range of  $0.980 \leq R^2 \leq 0.995$ , and  $1\text{--}100 \text{ mg L}^{-1}$  with correlation coefficients in a range of  $0.978 \leq R^2 \leq 0.998$ . This behavior could be explained as considering that, at low analyte concentration the analogs bound loosely with the imprint sites and stripped off while running the analyte solution, but at high analyte concentration the analogs bound greatly with the MIP binding sites due to decrease in conductivity of the polypyrrole MIP film with excess HAA anions in solution. The limit of



**Figure 2** The frequency shift of (a) the TCAA-imprinted polypyrrole-coated QCM and (b) the control upon interaction with TCAA at various concentrations.



**Figure 3** The frequency shift response of the TCAA-MIPpy coated QCM to TCAA and TCAA structurally related compounds at various concentrations.

detection obtained from extrapolation of linear segments of the calibration graph with 3  $S/m$  criterion, where  $m$  is the linear calibration and  $S$  were estimated as the standard deviation ( $n = 3$ ), was  $35 \mu\text{g L}^{-1}$  for TCAA and  $40 \mu\text{g L}^{-1}$  for five HAA analogs.

With the gravimetric-analytical detection method used, the amount of TCAA and analogs adsorbed on the polymer layer can be estimated and binding characteristic of the imprinted layer can be obtained by Scatchard analyses studies. Table II shows the binding data for the analysis of TCAA and analogs in the range of 1–100  $\text{mg L}^{-1}$  by the noncompetitive binding assay using Scatchard analysis.<sup>21</sup> As shown in Table II that the association constant ( $K_a$ ) and binding capacity ( $B_{\text{max}}$ ) values were highest in the case of TCAA template when compared with those for the analogs. This may be because that the recognition sites generated in the polypyrrole matrix actually have shape and size complementary with TCAA molecule.

The specificity of the TCAA-MIPpy-QCM in the assay ranges of between 100 and 1000  $\mu\text{g L}^{-1}$  and 1 and 100  $\text{mg L}^{-1}$  was evaluated by measuring the cross-reactivity or CR (i.e. the ratio of  $E_{\text{max}}$  measured for the analog to that of TCAA, where  $E_{\text{max}}$  is the analyte concentration that gave maximum  $-\Delta F$  signal in an assay range). The data are collected in Table II. The results revealed that TCAA-MIPpy-QCM exhibited low specificity to all the HAA and non-HAA analogs in range of 100–1000  $\mu\text{g L}^{-1}$  ( $\text{CR} \leq 27\%$ ). On the contrary, in range of 1–100  $\text{mg L}^{-1}$  TCAA-MIPpy-QCM displayed some degree of specificity to the HAAs structurally related to TCAA, i.e., the cross-reactivity of TCAA-MIPpy-QCM for DCAA, TBAA and MCAA is 1.5–3 times higher than that

achieved for DBAA, MBAA, and other two non-HAA analogs (acetic acid and malonic acid). This suggests that analogs with different halogen atoms and degree of substitution were recognized to varying degrees depending on their similarity to the template molecule. The non-halogen substituted compounds were poorly recognized by the MIP.

### Voltammetric experiments

Voltammetric transduction detection involves monitoring the current generated upon application of a potential sweep. This transduction system is considered to be the most selective electrochemical technique, since the oxidation or reduction potential of a particular substrate is its intrinsic property. Several parameters such as pH employed during polymer synthesis, measurement pH, current density, as well as deposition time can affect the signal response of the voltammetric measurements. Therefore, the effects of these parameters were examined with respect to the recognition ability of the polymer layer on the electrodes. The data are summarized in Table III.

The effect of synthesis pH, and measurement pH, in the range of pH 4–7 on the signal response of the MIP electrode was studied, using the TCAA-imprinted polypyrrole film prepared at a 1 : 1 mole ratio of template and pyrrole with a deposition time of  $5.4 \times 10^3$  s and current density of  $0.1 \text{ mA cm}^{-2}$ . The coated electrode with non-crosslinked polypyrrole produced in the presence or absence of TCAA template showed significantly different current responses to the template molecule in cyclic voltammetry (Fig. 4). The template molecule-dependent

**TABLE II**  
Binding Characteristics ( $K_a$  and  $B_{\text{max}}$ ) for Analyses of TCAA and Analogs on the TCAA-MIPpy Coated QCM Electrode and Cross-Reactivities Related to TCAA (CR) When the Assay is Conducted for an HAA Concentration Ranging from 100 to 1000  $\mu\text{g L}^{-1}$ , and from 1 to 100  $\text{mg L}^{-1}$

Compound	$K_a$ ( $\text{mM}^{-1}$ )	$B_{\text{max}}$ (nmol)	CR <sup>a</sup> (%)	
			100–1000 $\mu\text{g L}^{-1}$	1–100 $\text{mg L}^{-1}$
TCAA	84.7	61.5	100	100
DCAA	30.5	53.2	27	74
MCAA	29.7	47.2	23	60
TBAA	31.4	48.3	25	62
DBAA	25.4	46.5	20	41
MBAA	25.6	45.3	20	39
Acetic acid	24.3	44.5	19	26
Malonic acid	23.8	44.3	18	25

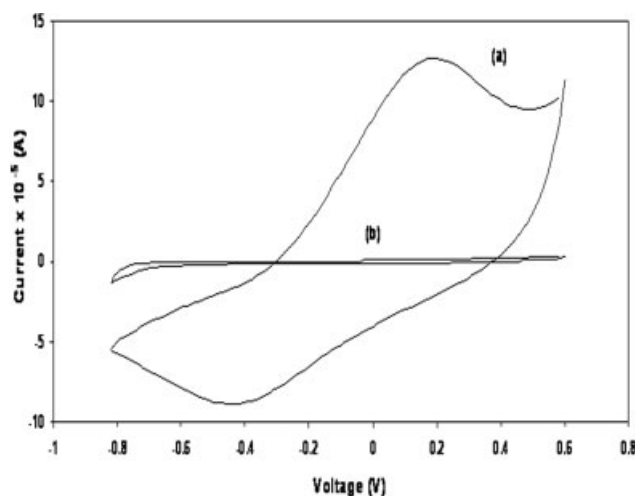
<sup>a</sup> The specificity of the electrode to TCAA analog at a concentration giving  $E_{\text{max}}$ .  $E_{\text{max}}$  is defined as the analyte concentration that gives maximum QCM frequency shift signal in an assay range.

**TABLE III**  
Effects of Certain Parameters on the Current Density Responses of the MIP-Coated Electrode and the NIP-Coated Electrode

Parameter	Current peak height <sup>a</sup> ( $10^{-5}$ A)	
	MIP	NIP
Synthesis pH		
0.7	1.8 (pH 0.7), 1.8 (pH 1), 5.3 (pH 4), 9.3 (pH 7)	4.3 (pH 0.7), 4.6 (pH 1), 3.9 (pH 4), 5.4 (pH 7)
1	2.3 (pH 0.7), 3.6 (pH 1), 5.0 (pH 4), 5.0 (pH 7)	4.3 (pH 0.7), 4.5 (pH 1), 3.9 (pH 4), 0.2 (pH 7)
4	1.3 (pH 0.7), 1.3 (pH 1), 3.8 (pH 4), 4.0 (pH 7)	0.0 (pH 0.7), 0.0 (pH 1), 0.0 (pH 4), 0.0 (pH 7)
7	2.3 (pH 0.7), 2.1 (pH 1), 4.7 (pH 4), 8.2 (pH 7)	4.7 (pH 0.7), 1.4 (pH 1), 0.2 (pH 4), 0.0 (pH 7)
Current density ( $\text{mA cm}^{-2}$ )		
0.1	61.0	0.0
0.5	8.6	0.0
1.0	0.0	0.0
Deposition time (h)		
0.5	5.5	0.0
1.0	6.1	0.0
1.5	7.0	0.0
2.0	7.0	0.0
Monomer : template molar ratio		
1 : 1	6.9	0.0
1 : 1.3	6.6	0.0
1 : 1.6	6.1	0.0
1 : 2	7.0	0.0

<sup>a</sup> Mean current peak height of three independent measurements using two electrodes, and the RSD values are less than 0.1%. The pH value in parenthesis is pH of measurement.

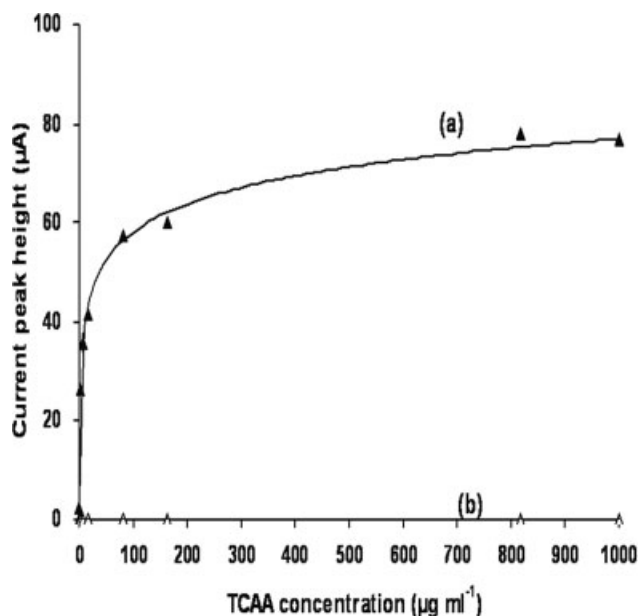
current change for the MIP-coated electrode compared to the control electrode indicated the specificity of the imprinted polymer coated on to the electrode (Fig. 5).



**Figure 4** The voltammogram for (a) TCAA-imprinted polypyrrole-coated electrode prepared at 1 : 1 mole ratio of template and pyrrole, at a deposition time of  $5.4 \times 10^3$ , and at a current density of  $0.1 \text{ mA cm}^{-2}$  in comparison with that for (b) nonimprinted polymer when exposed to a solution of TCAA template.

The results revealed that the differences in the signal response of imprinted polypyrrole and corresponding nonimprinted polymer depended on the synthesis and measurement pH used. As shown in Table III, the current peak height of the imprinted polypyrrole at the higher measurement pHs is higher than at the lower measurement pHs for every synthesis pH. However, the current response of the NIP is not significantly affected by the change of the synthesis pHs, except at pH 4, which gives none of current peak height for NIP at every measurement pH, indicating that the imprinted polypyrrole layer has the greatest selectivity at this pH of synthesis. The differences in signal responses of the imprinted polypyrrole and corresponding non-imprinted polymer were largest at pH 7 of measurement to TCAA template when compared to the other pH. The results showed that the synthesis pH of 4 and the measurement of pH 7 afforded high specificity and reasonable transducing signal to TCAA-MIPpy coated on electrode. The current change in the TCAA-MIPpy coated electrode may be due to ionic interactions of the anionic species in TCAA molecule and cationic species in polypyrrole backbones, allowing increased continued electropolymerization within the non-crosslinked polypyrrole network.

A preliminary evaluation of current density effect in voltammetry detection of TCAA-imprinted polypyrrole was performed by a pulse differential voltammetry analysis. The TCAA-imprinted polypyrrole



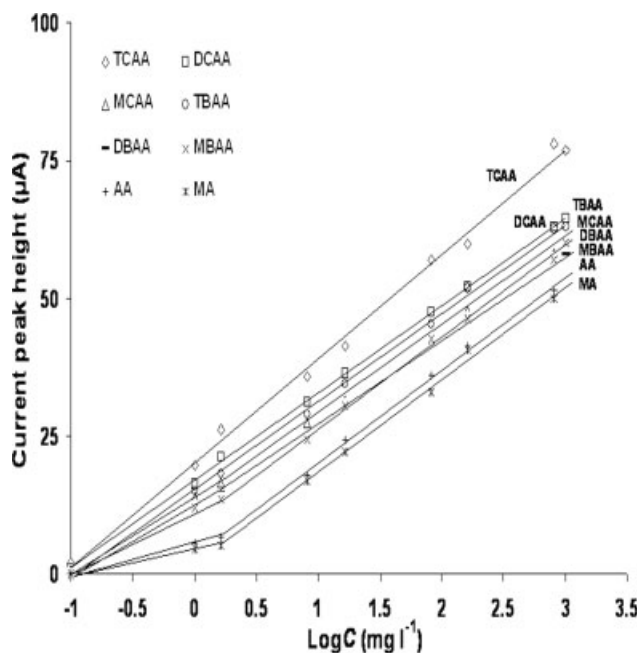
**Figure 5** Effect of increasing concentrations of TCAA on the electrochemical signal response of (a) the TCAA-imprinted polypyrrole and (b) nonimprinted polypyrrole-coated voltammetric electrode, at a deposition time of  $5.4 \times 10^3$ , and at a current density of  $0.1 \text{ mA cm}^{-2}$ .

film was prepared at template:pyrrole mole ratio of 1 : 1 with a current density of  $0.1 \text{ mA cm}^{-2}$  and deposition time of  $5.4 \times 10^3 \text{ s}$  and synthesis pH of 4, and measured in pH 7 phosphate buffer. As shown in Table III, increasing the current density results in the decreased current peak height of the TCAA-imprinted polypyrrole film. The lowest current density of  $0.1 \text{ mA cm}^{-2}$  was sufficient to keep the TCAA-imprinted polypyrrole film in the low oxidized state, without causing significant over-oxidized state moieties, so that the polypyrrole films were expected to carry the positive charges on their backbone, allowing the binding of TCAA anion.

The deposition time used during polymer synthesis is another factor determining the amount of polymer deposited on the electrode surface. The TCAA-imprinted polypyrrole films were prepared with various deposition times; these were investigated to ascertain their effect on current peak height. Increasing the deposition time from 0.5 h up to 1.5 h resulted in a significant increase in current peak height, as shown in Table III. The current peak height did not increase significantly at deposition times longer than 1.5 h. This may be due to the saturation of the number of template binding sites on the surface of polymer when the deposition time was increased. For electrodes imprinted with a various mole ratio of monomer and template, the current response appeared to be constant at about 1 : 1 mole ratio of monomer : template, as compared with the electrode imprinted at a higher molar ratio of monomer and template (see Table III). It was found that different sets of the MIP electrodes gave similar current ranges (mean peak height =  $60.6 \mu\text{A}$ ,  $n = 3$ ), with maximum variations of about 1.2%. The electroactivity of the polypyrrole MIP film deteriorated after the tenth cycle and continued to decrease with each following cycle.

As shown in Figure 5, as an increased amount of TCAA was added, there was an overall increase in the current generated with the MIP-coated electrode. The NIP electrode showed little or no change with addition of TCAA. This suggests that the binding of the template molecule to the MIP increases the current signal of the voltammetric transducer system, presumably because of increase in charge transferring within the non-crosslinked polypyrrole network.

Further, the selectivity range of the TCAA-imprinted polypyrrole-coated CV was studied by noncompetitive ligand binding assay, using TCAA and the same analogs as used in the QCM experiments. The results revealed that the NIP-based sensor exhibited relatively little response to both HAA and non-HAA compounds (data not shown). A calibration plot of current response versus the (logarithm) concentration of TCAA or analogs for the



**Figure 6** The current response of the TCAA-MIPpy coated CV to TCAA and analogs at various concentrations.

MIP sensor is shown in Figure 6. Similar to the QCM assay, in the CV assay the analogs showed shift of calibration curve. As can be seen, in Figure 6, the current peak height of the MIP sensor increased at a more rapid rate between 1 and  $100 \text{ mg L}^{-1}$  and at a lower rate between  $100$  and  $1000 \mu\text{g L}^{-1}$  in case of the two HAAs (i.e. DBAA and MBAA) and non-HAAs, acetic acid and malonic acid. In the  $100$ – $1000 \mu\text{g L}^{-1}$  concentration range, non-HAA analogs, either acetic acid or malonic acid generated small change in the current signal of the MIP sensor. By contrast, TCAA and other HAA analogs resulted in significant shift of the current peak height of the MIP sensor with different magnitude depending on the compound, namely  $\text{TCAA} > \text{DCAA} > \text{TBAA} > \text{MCAA} > \text{DBAA} = \text{MBAA}$ . These results confirm that the TCAA-MIPpy binds the template molecule strongly but that the HAA analogs were recognized to varying degrees dependent on their similarity to the template molecule. TCAA-MIPpy on the CV transducer system cross-reacted with chlorinated-HAA better than with brominated-HAA for the same degree of halogen substitution. In addition, the tri- or di-substituted HAA cross-reacted greater than the mono-substituted HAA. This implies that the size and number of the halogen atom substituted in HAA may be a key factor for an efficient recognition of HAAs by the polypyrrole MIP.

The calibration curves constructed from the current shift parameter dependency provided reasonable results (Fig. 6). There was a linear relationship



between the current response of the MIP sensor and the logarithm of the concentrations of TCAA and other two HAAs (DCAA and TBAA) in the concentration range of  $100 \mu\text{g L}^{-1}$  to  $1000 \text{mg L}^{-1}$  with correlation coefficients in a range of  $0.993 \leq R^2 \leq 0.999$ . Also, the peak currents of MCAA, DBAA, MBAA, acetic acid, malonic acid measured on the MIP sensor have good linear relations with its (logarithm) concentrations in the two ranges of  $100\text{--}1000 \mu\text{g L}^{-1}$  with correlation coefficients in a range of  $0.990 \leq R^2 \leq 0.998$ , and  $1\text{--}100 \text{mg L}^{-1}$  with correlation coefficients in a range of  $0.993 \leq R^2 \leq 0.999$ . The limits of detection for TCAA and HAA analyses examined, as described in the QCM study, were between  $35$  and  $50 \mu\text{g L}^{-1}$ , well below the permissible limits concentration of TCAA in drinking water, that are set by the WHO and the USA organization.

### Inter-digitated conductometric experiments

Miniaturized planar electrodes or inter-digital conductometric (IDC) transducers have become important electronic transducers in medical, biological and environmental diagnostics. This electronic transducer system is usually of compact design, easy to use, inexpensive and portable. In a sensor constructed with IDC, the sensitive layer is coated on the electrode of the transducer. The close proximity of the sensing element to the transducer enhances the response time, leading to rapid measurement due to low diffusion times. The incorporation of TCAA-imprinted polypyrrole into an IDC sensor would be expected to show high sample throughput, so that single measurements could be delivered in real time. Since the polypyrrole used for the construction of MIP has a conductive property, and factors such as applied frequency, temperature and electrolytes can have an effect on the conductivity of the conducting polymers,<sup>22</sup> variation in these factors may affect the signal response of the TCAA-MIPpy on the IDC. The thickness of the TCAA-MIP on IDC electrode, synthesized as described in the earlier section, was about  $817 \text{nm}$  when measured with AFM (Digital Instruments, Santa Barbara, CA).

The results of measurement of conductivity responses of the electrodes at various current frequencies ranging from  $20 \text{Hz}$  to  $1 \text{MHz}$ , when exposure to  $100 \mu\text{g L}^{-1}$  TCAA solution, revealed an increase in conductivity of both MIP and NIP-coated electrodes in the order of  $0.02\%$  per kilohertz at room temperature [ $25 \pm 1^\circ\text{C}$ ]. A reasonable signal response of the MIP sensor was obtained in the lower frequency region, rather than in higher frequency region; all subsequent measurements were therefore performed at an operating frequency of  $3 \text{kHz}$ . It was found that temperature influenced the sensor signal in the order of  $1.1\%$  conductivity

change per Kelvin at  $3 \text{kHz}$ . Therefore, for high accuracy of sensing measurements using the pyrrole-based IDC electrode were carried out under controlled fixed temperature.

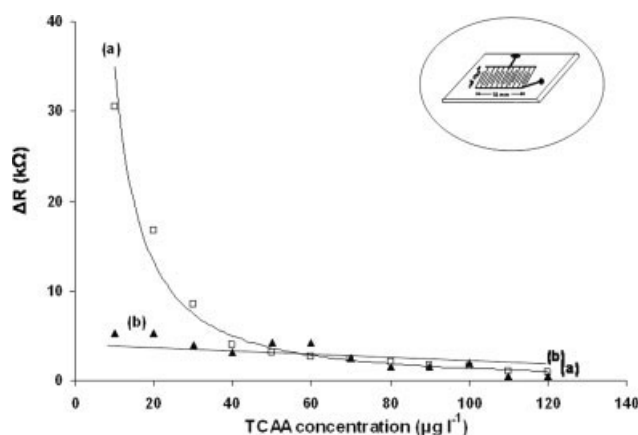
When the signal responses of the TCAA-MIPpy and corresponding nonimprinted polymer for water solutions containing  $0.2M \text{HCl}$ ,  $\text{K}_2\text{PO}_4$  or  $\text{KH}_2\text{PO}_4$ , and  $100 \mu\text{g L}^{-1}$  TCAA were measured under the optimized conditions, both MIP and nonimprinted electrodes gave negative conductivity shift between  $80$  and  $105 \Omega^{-1}$ . The anionic ion of these inorganic substances may lead to the formation of ion-pairs with cationic species in polypyrrole backbones, which do not contribute to conductivity. The effect of the common electrolyte, NaCl on the signal response of IDC sensor was also investigated. The signal responses of the MIP-coated IDC sensor for water solutions containing NaCl salt between  $1$  and  $30 \text{g L}^{-1}$  ( $0\text{--}500 \text{mM}$ ) were measured under the optimized conditions. Increasing concentrations of NaCl in sample solutions containing  $100 \mu\text{g L}^{-1}$  TCAA resulted in the increased conductivity response of both MIP- and NIP-coated electrode, for instance  $5 \text{mM}$  NaCl caused  $10$  times increase in the magnitude of  $100 \mu\text{g L}^{-1}$  TCAA-conductivity response for either MIP or NIP-coated electrode. Indeed, the conductivity behavior of TCAA-imprinted polypyrrole in the presence of NaCl salt is similar to that of the conducting poly (vinyl chloride) membrane containing the TCAA-imprinted poly(vinylpyridine-co-ethylene glycoldimethacrylate) particles in our previous work,<sup>23</sup> and which had an ionic conductivity similarly to solid polymer electrolytes.<sup>24</sup> Accordingly to this, the explanation in terms of the movement of the  $\text{Na}^+$  ions bound with the mobile non-crosslinked polypyrrole chains would be given to such increase in electrical conductivity of the polypyrrole film in the presence of excess NaCl salt. Independency of addition of the electrolyte upon signal responses of MIP sensors with NIP sensors was observed only at low concentrations of NaCl ( $<5 \text{mM}$ ). Thus, measurements using the pyrrole-based electrode should be carried out under low concentration of  $\text{Na}^+$  ion.

The template molecule-dependent conductivity changes of the TCAA-imprinted polypyrrole coated IDC electrodes compared to the NIP-coated electrodes is shown in Figure 7. Increasing the amount of TCAA results in an overall increase in the conductivity change at TCAA concentration up to  $120 \mu\text{g L}^{-1}$  with the MIP electrode only, whilst there were tiny changes in the conductivity responses for the NIP electrode. The increase in the conductivity signal of the polypyrrole MIP film when exposed to TCAA in water solution might be caused by the change in surface conductivity of the polypyrrole material. Recently, the previous work<sup>25</sup> has shown that polypyrrole films deposited on the gold electrode are

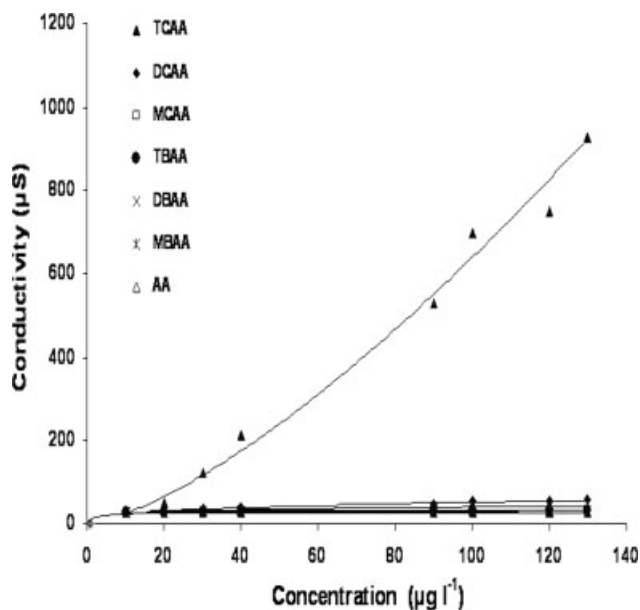
p-type conjugative polymer, and can become more conductive and more electrochemically active at more positive polarization in an anodic process. Therefore, possibly, the binding of the template molecule to the MIP may increase continued transduction phase of the TCAA-MIPpy film through charge transfer.

Specificity of the TCAA-imprinted polypyrrole-coated IDC was examined as with the QCM or CV studies. Figure 8 shows the conductivity shift responses of the MIP-coated electrode to the HAA and non-haloacetic analogs. Both 5 HAA analogs and two nonstructurally related analogs (acetic acid and malonic acid) in the 1–120  $\mu\text{g L}^{-1}$  concentration range showed little or no change in the electrical conductivity signal of the MIP-coated electrode. This result demonstrates that the MIP selectively recognizes TCAA on the IDC transducer integrated with the TCAA-MIPpy. Although the HAA analogs used for this study contained halogen-substituted carboxylic acid groups as the template, they contained also different halogen atoms and different degree of substitution which this may cause the pendent of HAAs molecule can not fit properly into the template sites, hence restraint in the conductivity signals. Or another word, the HAA analogs might have formed only charge neutral contact ion-pairs with the quaternary amine of the TCAA-MIPpy, which this can not trigger signal.

The extrapolation of the linear segment of the curve between the conductivity response ( $1/\Delta R$ ) and the logarithm of the concentration showed the 1–120  $\mu\text{g L}^{-1}$  (ppb) sensitivity range of TCAA analyses using IDC analysis. The detection limit for TCAA achieved with the IDC assay is at  $1.0 \mu\text{g L}^{-1}$ . The LOD value for TCAA analyses by the IDC sensor is lower than the LOD achieved by the QCM and CV



**Figure 7** Conductivity changes of (a) the TCAA-MIPpy coated IDC in comparison to (b) the nonimprinted polymer-coated electrode at various concentrations of added TCAA. Inset: sketch of the IDC electrode.



**Figure 8** Conductometric responses for TCAA-MIPpy coated IDC at various concentrations of TCAA and analogs.

sensors, when integrating these transduction systems with the TCAA-MIPpy thin-film. However, the working range for QCM and CV analyses is broader than that for IDC analysis. The assay time for the IDC was as fast as 3 min, and the MIP-coated electrode is reusable at least 50 times without any change in its sensing property. Stability of the coated electrode was good when stores at room temperature in dry-air condition, and the electrode can be used for at least 1 month and retains the same sensor characteristics during this period.

### Analysis of drinking water samples

For analytical application, the TCAA-MIPpy integrated with QCM or CV or IDC transduction system was examined for analysis of TCAA in real-life samples. Four drinking water samples obtained from domestic and commercial supplies (sample A, B, C, and D) were subject for this study. The gas chromatographic-electron capture detection method in conjunction with liquid-liquid extraction recommended by the USEPA<sup>26</sup> was used as the standard method to verify the amounts of HAA in the water samples. The measurement results revealed that the limits of detection of HAAs with either the QCM or the CV sensor were at present higher than concentrations found in these four drinking water samples. For validation purposes a recovery study of TCAA analyses by the QCM and CV sensor was performed by spiking those four water samples with three different amounts of TCAA standard solution (0.1, 10,

and  $100 \text{ mg L}^{-1}$ ). Recoveries range between 87 and 103% and relative standard deviations less than 3.5% were achieved, demonstrating the applicability of the QCM and CV assays to the analysis of samples that contain (ppm) TCAA.

The concentration levels of sample A, B, C, and D when assayed with the IDC sensor were 1.4, 1.8, 1.0, and  $1.0 \text{ } \mu\text{g L}^{-1}$ , respectively. Assay of samples by the USEPA method showed only TCAA in the samples at concentration levels of 0.8, 1.2, 0.9, and  $1.0 \text{ } \mu\text{g L}^{-1}$  in water sample A, B, C, and D, respectively. It was evident that the TCAA disinfection by-product in the real-life samples can effectively be measured with the IDC sensor embedded with TCAA-MIPpy as sensing element. From the results obtained, the IDC analysis method incorporated with TCAA-MIPpy as sensing element shows high specificity and sensitivity for TCAA analyses, and can therefore be suitable for routine monitoring of the concentration of individual TCAA disinfection by-product which usually contain in chlorinated drinking water.

## CONCLUSIONS

In this study, molecularly TCAA imprinted non-crosslinked polypyrrole thin-film has been prepared and studied in its property on the voltammetric, inter-digital conductometric, or piezoelectric QCM sensors. The TCAA-imprinted polypyrrole incorporated with the QCM transduction system shows a TCAA-dependent frequency shift response over the concentration range of  $0.1\text{--}100 \text{ mg L}^{-1}$ , whilst TCAA-imprinted polypyrrole incorporated with CV transduction system shows a TCAA-dependent current response over the concentration range of  $0.1\text{--}1000 \text{ mg L}^{-1}$ . The TCAA-imprinted polypyrrole integrated with IDC transduction system is specifically sensitive towards TCAA ( $\sim 1 \text{ } \mu\text{g L}^{-1}$ ), which is useful for the determining trace TCAA in chlorinated drinking water. The TCAA-imprinted polypyrrole incorporated into the QCM and CV transduction systems showed similar sensitivity and specificity characteristics. The mechanism of signal generation by the TCAA-imprinted polypyrrole incorporated in QCM transduction system was supposed to involve with the binding of the TCAA molecule at the MIP binding sites by acid-base interactions, resulting to mass shift of electrode. For the TCAA-imprinted polypyrrole incorporated with CV transduction system, the electron transferring within the oxidized polypyrrole MIP film doped with TCAA molecule was supposed to contribute to the specific current signal for detection. Besides, the mechanism of the generation of the conductivity signals for the TCAA-imprinted polypyrrole incorporated with IDC transduction

system referred to the continuous conducting phase of the p-type conjugative polypyrrole-based MIP, which increases when there was binding of the TCAA molecule to the MIP. The study demonstrates the feasibility of using TCAA-MIPpy sensing element incorporated with IDC analysis systems to quantify TCAA (the most common HAA species found in drinking water) in both domestic and commercial bottled drinking waters.

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