CHAPTER 4

TRICHLOROACETIC ACID IMPRINTED POLYPYRROLE AND VOLTAMMETRIC TRANSDUCER

4.1. Principle of voltammetric transducer (Dai *et al.*, 2002)

Voltemmetric sensors generate the sensing response from the redox peak current characteristic of the analyte under a sweep of the electrode voltage over a range of redox potentials associated with the target redox reaction. As is the case in amperometric sensors, the conjugated conducting polymer in the voltammetric sensors may act either as a redox-active material to reduce the redox potential for the analyte of interest, and hence reduce the influence of background and interfering currents, or merely as the substrate for immobilizing a redox mediator molecule. Unlike the amperometric sensors, however, the voltammetric sensors offer an additional advantage with which redox signals for reference molecules added to the sample can be simultaneously measured to improve accuracy. Comparing to optical, mass and thermal sensors, voltammetric sensors are especially attractive because of their remarkable detectability, experimental simplicity and low cost. They have a leading position among the presently available sensors that have reached the commercial stage and which have found a vast range of important applications in the fields of clinical, industrial, environmental and agricultural analyses.

There are several types of voltammetric techniques, depending on the shape of the applied potential function (Bergamini *et al.*, 2006). For linear sweep voltammetry (LSV) and cyclic voltammetry (CV), the potential applied changes linearly with time. When the potential sweep is not a linear function but comprises constant increments on a linear ramp (differential pulse voltammetry, DPV) or a square wave function (square wave voltammetry, SWV), these techniques could offer better sensitivity, because they offer better signal-to-noise ratios. However, SWV has hot yet been used with these types of sensors. CV has the advantages of allowing the density of template units at the surface to be estimated through an easy coulometric analysis of the resulting redox peaks, as it sometimes used to be done to determine the optimum incubation

time. In general, the measurement medium for all these techniques is an inert electrolyte buffer solution, probably to facilitate ionic interactions between template and polymer.

In recent years, a voltammetric transduction based on molecularly imprinted polypyrrole has been focused considerably. Structurally, polypyrrole can provide a free electron for transfer in a polymer chain during an electrochemical reaction. The polypyrrole film possesses a positive charge allowing the formation of imprints with anionic template molecules that subsequently exhibit good selectivities.

4.2. Objective

To evaluate the use of trichloroacetic acid imprinted polypyrrole as sensitive material on the voltammetric electrode for selective determination haloacetic acids in drinking water.

4.3. Method

4.3.1. Immobilization of imprinted polypyrrole layer on the working electrode surface

For immobilisation of the TCAA-imprinted polypyrrole onto the voltammetric transducer surface, the platinum electrode was immersed in a buffer solution containing of 0.25 M pyrrole monomer and 0.5 M TCAA as shown in Fig. 4.1.

Electropolymerisation was carried out at current density of 0.1 mA cm^{-2} for 1.5 h. The coated film was then washed in de-ionised water for 1 h. A control electrode was prepared using the same procedure in every case, but in the absence of template molecule. The MIP coated electrode and the corresponding non-imprinted coated electrode were freshly prepared for each experiment.

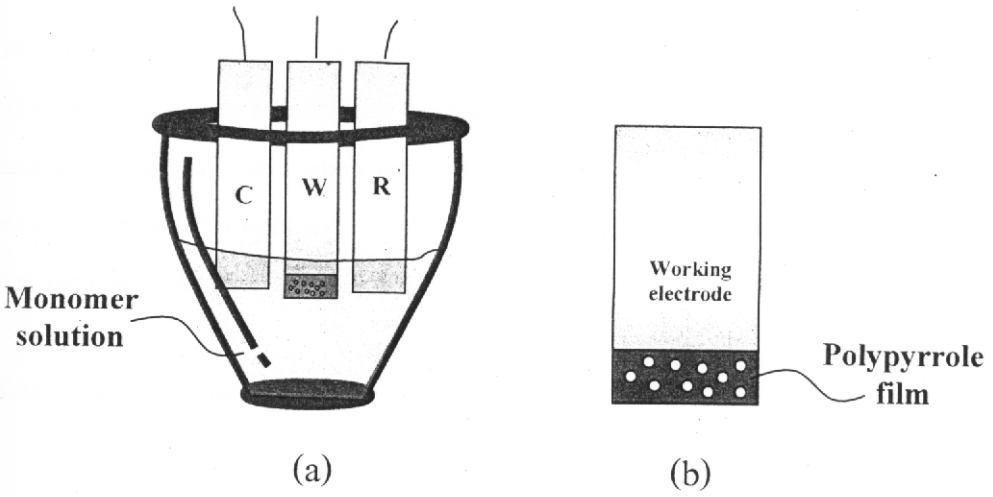


Fig. 4.1. (a) Electrochemical coating imprinted polypyrrole onto the surface of platinum working electrode in a glass cell. C, W and R represented counter electrode, working electrode and reference electrode, respectively. (b) Enlarged image of coated polypyrrole layer on platinum working electrode.

4.3.2. Electroanalytical detection of analyte

The electrochemical interaction of the prepared TCAA-MIPpy film with TCAA and its analogs was evaluated using both a cyclic and differential pulse voltammetric 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 the developed voltammetric analysis system were determined, by incorporation of the TCAA-MIPpy film as means of detection. 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. Cyclic voltammetric signals were recorded at 0.05 mVs^{-1} and a potential window between -0.8 and +1.6 mV. Differential pulse voltammetric signals were recorded with a potential window between -0.8 and +0.6 V at 0.025 mVs^{-1} and at pulse applied of 25 mV. All measurements were carried out in a phosphate buffer solution at room temperature with a stream of nitrogen gas, by changing the sample liquid in reservoir. Peak currents were measured by subtraction of the base line. The amount of adsorption sites was calculated by the coulumetric

method using the equation: $Q = \int_{t_1}^{t_2} I dt$, where Q is number of adsorption sites, I is current (A) and t is time (s). The area representing the number of adsorption was obtained by integration of the current between time at starting point of the curve (t_1) and time at the end of curve (t_2). Various concentrations of standard TCAA and its analogs, in the range of 0.1-1000 mg l^{-1} (ppm) were analysed, all measurements being performed in triplicate.

4.4. Material and equipment

4.4.1. Material

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).

Analytical grade pyrrole was purchased from Fluka Chemie AG (Buchs, Switzerland) and its chemical structure is displayed in the Fig. 4.2.

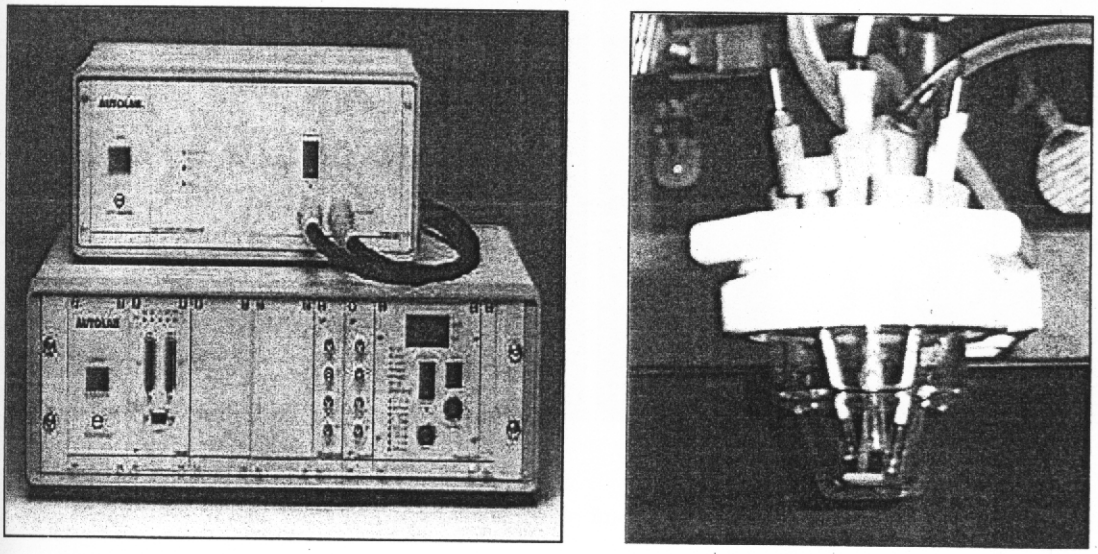
Fig. 4.2. The chemical structure of conducting monomer used to prepare polypyrrole film

All chemicals for preparing buffer solution (K_2HPO_4, NaH_2PO_4, NaCl, HCl and KCl) were analytical grade and were obtained from Merck (Darmstadt, Germany).

All solvents used in this work were analytical grade and were dried with 4 A pore sized molecular sieve before use. Working standard solutions were prepared daily.

4.4.2. Equipment

A μ-Autolab three-electrode system (Eco Chemie, B.V. Utrech, The Netherlands) (Fig. 4.3a) was used to prepare electropolymerized polymer and perform cyclic or differential pulse voltammetry. This instrument composes of a platinum rod electrode as the auxiliary electrode, platinum rod with 2 mm of diameter and Ag/AgCl as working and reference electrode, respectively (Fig. 4.3b).



(a) (b)

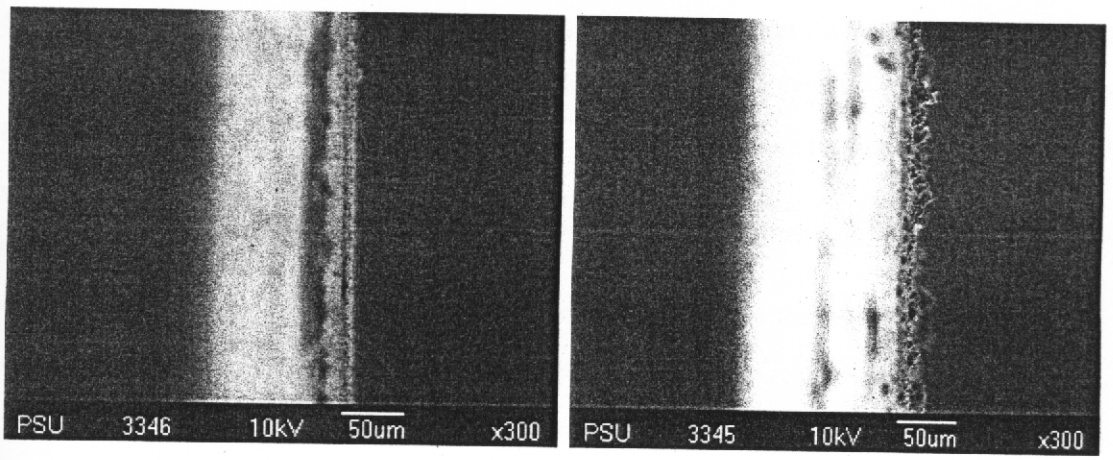
Fig. 4.3. (a) μ-Autolab three-electrode system. (b) The arrangement of auxiliary, working and reference electrode in glass cell.

4.5. Results and discussion

4.5.1. Preparation of polpyrrole film onto voltammetric electrode surface

Polypyrrole film can be easily prepared onto the metal surface of electrode by electrodeposition owing to redox reaction occurred at the electrode surface. The polymerization of this monomer commences from the electron withdrawing process of pyrrole monomer by cathode electrode that can be accepted electron in the system. The active form of positively charge oligomer backbone can propagate the reaction by further interacting with the other

monomer molecule to form the black solid at the electrode tip. Several conducting surfaces have been accomplished integrating with this polymer to be used as sensors selective for various analytes of interest. The successful preparation of polypyrrole film on the platinum electrode surface in this work using electrochemical polymerization is shown in the Fig. 4.4. The flat surface in case of bare electrode (Fig. 4.4a) became rougher and thicker with the process of polymer deposition (Fig. 4.4b).



(a) **(b)**

Fig. 4.4. SEM micrographs of (a) bare electrode and (b) imprinted polypyrrole coated platinum working electrode using 300 x resolution

This process can be obtained very easily by immersing the platinum electrode into the mixture solution of monomer and template in the pH controlled buffer. The coating experiment was started with electrical flowing into the cell through platinum rod counter electrode. Even through this method showed a very promising mean in case of preparation, however, the polymer film generated had low stability and low robustness after several usages. Therefore, in every experiment the polymer coated electrode was freshly prepared in order to assure their good signal transduction.

4.5.2. The influence of synthesis conditions and polymer composition on signal response of voltammetric sensor

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 (Albano and Sevilla, 2007; Ho *et al.*, 2006; Tamm *et al.*, 2007; Zanganeh and Amini, 2007). 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 4.1.

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 5.4×10^3 s and current density of 0.1 mA cm^{-2}. The coated electrode with non-cross-linked polypyrrole produced in the presence or absence of TCAA template showed significantly different current responses to the template molecule in cyclic and differential pulse voltammetry. The template molecule-dependent current change for the MIP-coated electrode compared to the control electrode indicated the specificity of the imprinted polymer coated on to the electrode (see Fig. 4.5). The results also revealed that the differences in the signal response of imprinted polypyrrole and corresponding non-imprinted polymer depended on the synthesis and measurement pH used.

Table 4.1

Parameter effects in the current peak height response of the MIP coated electrode and the non-MIP coated electrode. (Each experiment was performed in triplicate with freshly prepared electrode)

Parameter	Current peak height (10^{-5} A)	
	MIP	NIP
Synthesis pH	pH 0.7, pH 1, pH 4, pH 7*	pH 0.7, pH 1, pH 4, pH 7*
0.7	1.8, 1.8 , 5.3, 9.3	4.3, 4.6, 3.9, 5.4
1	2.3, 3.6, 5.0, 5.0	4.3, 4.5, 3.9, 0.2
4	1.3, 1.3, 3.8, 4.0	0.0, 0.0, 0.0, 0.0
7	2.3, 2.1, 4.7, 8.2	4.7, 1.4, 0.2, 0.0
Current density (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
1.3 : 1	7.1	0.0
1.6 : 1	6.1	0.0
2 : 1	7.0	0.0

* pH of measurement

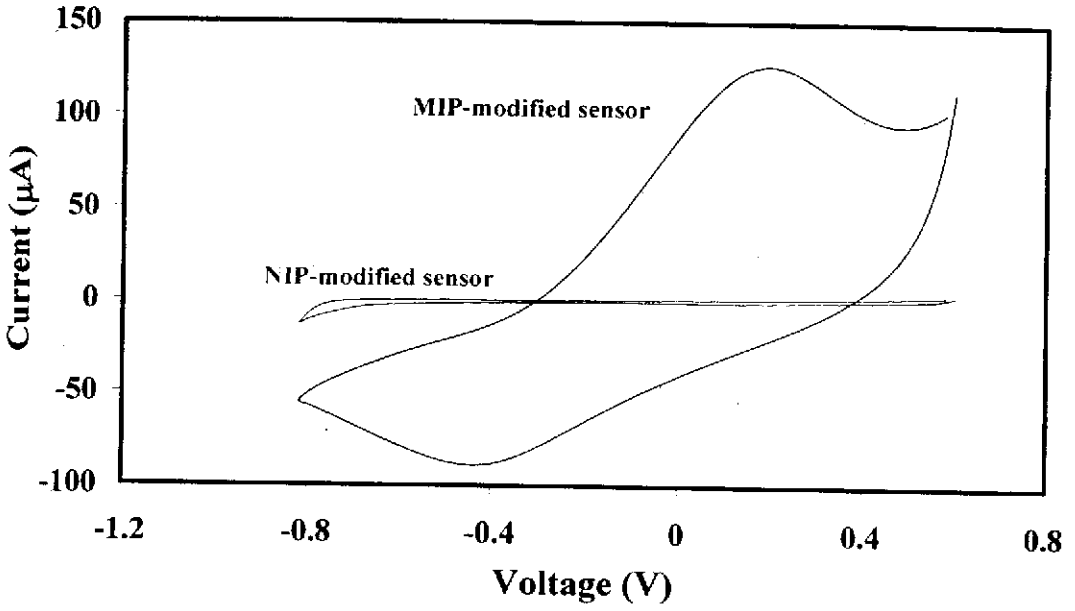


Fig. 4.5. The cyclic voltammogram for (a) TCAA-imprinted polypyrrole coated electrode prepared at 1:1 mole ratio of template and pyrrole, at a deposition time of 5.4 x 10^3 s, and at a current density of 0.1 mA cm^{-2} in comparison with that for (b) non-imprinted polymer when exposed to a solution of TCAA template.

As shown in Table 4.1, 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. In contrast, the current response of the non-imprinted polypyrrole is not significantly affected by the change of the synthesis pHs, except at pH 4, which gives none of current peak height for non-imprinted polypyrrole 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.

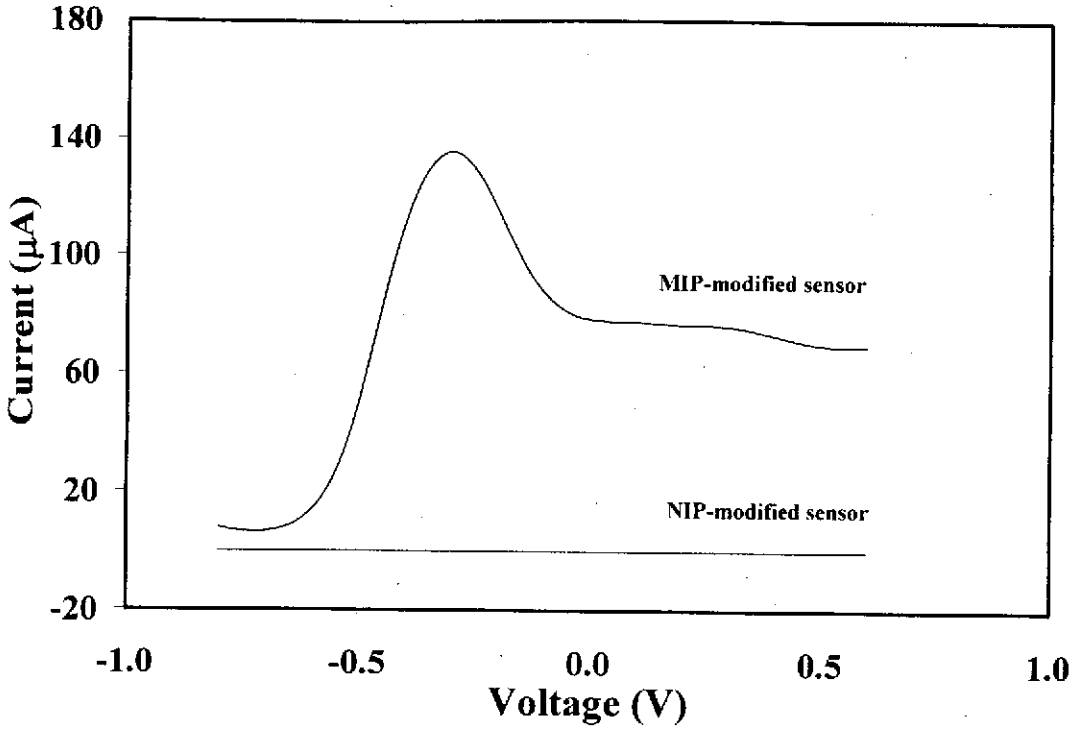


Fig. 4.6. The differential pulse voltammogram for (a) TCAA-imprinted polypyrrole coated electrode prepared at 1:1 mole ratio of template and pyrrole, at a deposition time of 5.4 x 10^3 s, and at a current density of 0.1 mA cm^{-2} in comparison with that for (b) non-imprinted polymer when exposed to a solution of TCAA template.

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 film was prepared at template:pyrrole mole ratio of 1:1 with a current density of 0.1 mA cm^2 and deposition time of 5.4 x 10^3 s and synthesis pH of 4, and measured in pH 7 phosphate buffer. As shown in Table 4.1, increasing the current density resulted in the decreased current peak height of the TCAA-imprinted polypyrrole film. It is probably due to the polypyrrole polymer undergoing further oxidation into a higher over-oxidation state when a higher current density is applied for a certain period of time. The lowest current density of 0.1 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 the TCAA template.

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 4.1. 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 4.1). Different sets of the MIP electrodes gave similar current ranges (mean peak height = 60.6 μA, n =3), with maximum variations of about 1.2%. 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 non-MIP coated electrodes was freshly prepared for each experiment.

4.5.3. Concentration dependence and analytical characteristics of voltammetric sensor

As shown in Fig. 4.7, as an increased amount of TCAA was added, there was an overall increase in the current generated with the MIP-coated electrode. The control 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 due to the increased charge transferring within the non-cross-linked polypyrrole network.

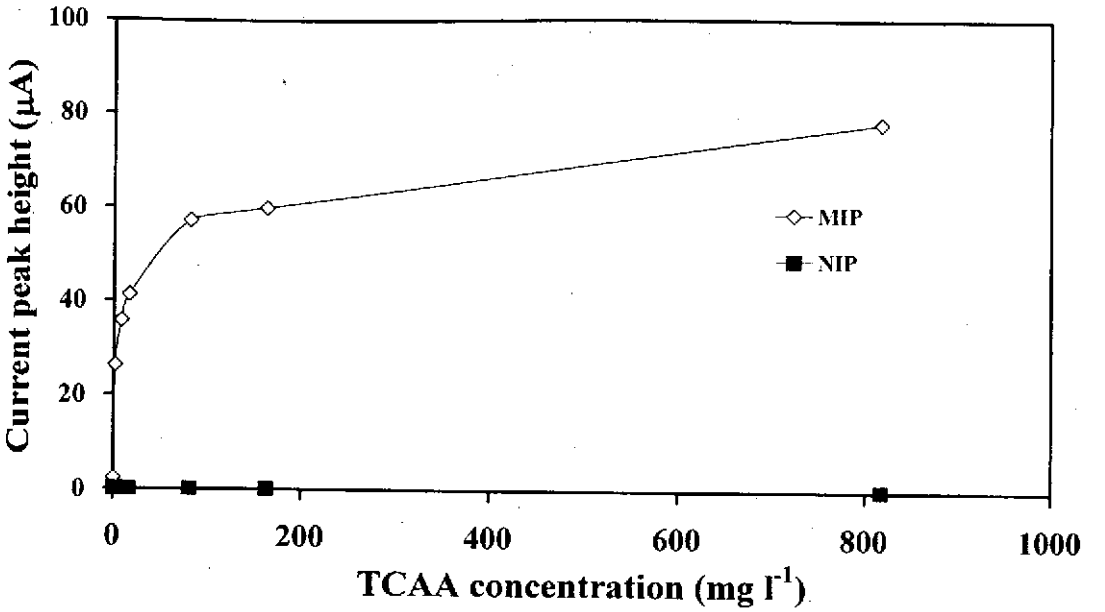


Fig. 4.7. Effect of increasing concentrations of TCAA on the electrochemical signal response of (a) the TCAA-imprinted polypyrrole and (b) non-imprinted polypyrrole coated voltammetric electrode, at a deposition time of 5.4×10^{3}, and at a current density of 0.1 mA cm^{2}.

Further, the selectivity range of the MIP electrode was examined, using five structurally related haloacetic acid compounds such as DCAA, MCAA, TBAA, DBAA, MBAA and two non-haloacetic acid compounds such as malonic acid and acetic acid as the analytes. The results showed that the NIP sensor exhibited relatively little response to both haloacetic acid and non-haloacetic acid compounds. In case of the MIP sensor, non-haloacetic acid compounds, either acetic acid or malonic acid, in the 0.1-1000 mg l^{-1} concentration range, generated negligible change in the current signal of the sensor, as shown in Fig. 4.8, Five HAA analogs resulted in significant shift of the current peak height of the sensor with different magnitude depending on the compound. This result indicates that the HAA analogs were recognized to varying degrees dependent on their similarity to the template molecule. Generally, TCAA-MIPpy on the voltammetric transducer system cross-react with chlorinated haloacetic acid better than with brominated haloacetic acid for the same degree of halogen substitution. While, the tri- or di-

substituted HAA cross-reacted greater than the mono-substituted HAA. This suggests that the size and number of the halogen atom substituted in haloacetic acid may be a key factor for an efficient recognition of HAAs by the polypyrrole MIP.

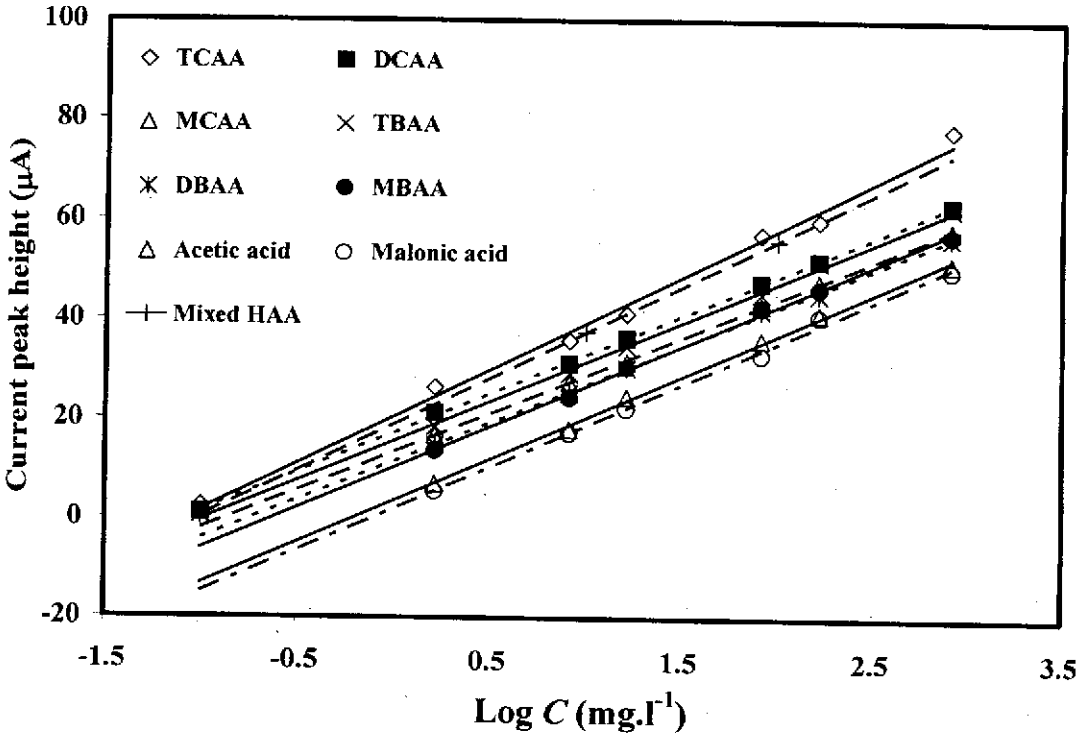


Fig. 4.8. Calibration curve of TCAA and analogs (individually) and the cross-reactivity of the TCAA-MIPpy coated voltammetric electrode to the mixture of total 6 HAAs.

The calibration data obtained from the plot of current shift response of the sensor and logarithm of analyte concentration provided reasonable results and is shown in Table 4.2. There was a linear relationship between the shift current peak height of the MIP sensor and the logarithm of the concentrations (R^2 > 0.99) of TCAA and five HAAs (individually) and the mixture of total 6 HAAs in the range of 0.1-1000 mg l^{-1}, depending on compound. The limits of detection for TCAA and HAA analyses were roughly between 35 and 50 μg l^{-1}, regarding to 3 *S/m* criterion, where *m* is the linear calibration and *S* were estimated as the standard deviation (n = 3), well below the maximum permissible limits concentration of TCAA in drinking water (60 μg l^{-1}), that are set by the WHO and the USA organization.

Table 4.2

Calibration data of sensor responded with TCAA and five other analogs in the concentration range between 0.1 and 817 ppm

Analyte	Working range (ppm)	R^2	LOD (ppm)
TCAA	0.1 - 817	0.9931	0.046
DCAA	0.1 - 817	0.9996	0.042
MCAA	1.63 - 817	0.9996	0.034
TBAA	0.1 - 817	0.9985	0.045
DBAA	1.63 - 817	0.9988	0.034
MBAA	1.63 - 817	0.9980	0.037

4.5.4. Analysis of drinking water samples

The developed CV analyse method, using the TCAA-MIPpy as a sensing element, was applied to measurement of HAA concentrations in real-life samples. Four drinking water samples obtained from domestic and commercial supplies (same as IDC and QCM sample analysis) were analysed. The LLE-GC-ECD method recommended by the USEPA was used as the standard method to verify the amounts of HAA in those water samples. The limits of detection of HAAs with CV-TCAA-MIPpy sensor are at present higher than concentrations found in these four drinking water samples. Assay of samples by the USEPA method revealed only TCAA in the samples at concentration levels of 0.8, 1.2, 0.9 and 1.0 $\mu g\ l^{-1}$ in water sample A, B, C and D, respectively. These results indicated that the HAA analyses with the IDC method were in good agreement with that obtained with the recommended method.

Recovery studies of TCAA analyses by the CV-TCAA-MIPpy were carried out with the samples after spiking with two different amounts of TCAA standard solution (0.1, 10 and 100 mg l^{-1}) and total 6 HAAs standard solution (each 0.0167, 1.67 and 16.7 mg l^{-1} or total 0.1, 10 and 100 mg l^{-1}). The analysis data for TCAA in spiked water samples by the CV method are summarized in Table 4.3. Recoveries range between 76 and 95% and % RSD values less than 3.5% were achieved with the CV analysis method. The result obtained in the present study

demonstrates the reproducibility and precision of the assay with the CV-TCAA-MIPpy sensor for the analysis of HAAs in drinking water samples.

Table 4.3

Analysis data for HAAs spiked in four brands of commercial bottled water and a municipal tap water with home filtration system by the votammetric-based assay

Compound/spiked concentration	Measured[a], mg l^{-1} after adding HAAs (% recovery)	
	Bottled water (supermarket , 1 l)	Bottled water (local supplier, 1 l)
TCAA 0.1 mg l^{-1}	93 ± 0.1	95 ± 0.1
TCAA 10 mg l^{-1}	92 ± 0.0	93 ± 0.0
TCAA 100 mg l^{-1}	84 ± 0.2	91 ± 0.2
Total six HAAs[c] 0.1 mg l^{-1} (0.0167 mg l^{-1} each)	92 ± 0.2	94 ± 1.0
Total six HAAs[c] 10 mg l^{-1} (1.67 mg l^{-1} each)	91 ± 1.3	93 ± 0.1
Total six HAAs[c] 100 mg l^{-1} (10.67 mg l^{-1} each)	80 ± 1.6	89 ± 0.0

Table 4.3 (Continued)

Compound/spiked concentration	Measured[a], mg l^{-1} after adding HAAs (% recovery)	
	Bottled water (local supplier, 25 l)	Water filtration system (home)
TCAA 0.1 mg l^{-1}	90 ± 3.5	89 ± 0.2
TCAA 10 mg l^{-1}	86 ± 1.0	85 ± 0.3
TCAA 100 mg l^{-1}	81 ± 0.2	82 ± 0.2
Total six HAAs[c] 0.1 mg l^{-1} (0.0167 mg l^{-1} each)	85 ± 1.6	89 ± 1.1
Total six HAAs[c] 10 mg l^{-1} (1.67 mg l^{-1} each)	81 ± 1.4	84 ± 1.9
Total six HAAs[c] 100 mg l^{-1} (10.67 mg l^{-1} each)	76 ± 0.4	81 ± 0.0

[a] Expected concentrations are amounts added plus the amounts already present in the water sample (mean ± R.S.D, $n = 3$)

[b] With home filtration system.

[c] Total six HAAs refers to TCAA, DCAA, MCAA, TBAA, DBAA and MBAA altogether.