Piezoelectric quartz crystal sensor for rapid analysis of pirimicarb residues using molecularly imprinted polymers as recognition elements

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Abstract

The newly developed molecularly imprinted polymer (MIP) as sensing material for the piezoelectric quartz crystal (PQC) sensor was developed for fast and onsite determination of pirimicarb in contaminated water. Out of the three MIP particles prepared by conventional bulk polymerization and precipitation polymerization in chloroform or acetonitrite, the best MIP were prepared by precipitation polymerization in chloroform which produced particles with uniform spherical shape and mean particle diameter at nanosized range of about 50 nm. The sensor fabricated can achieve a steady-state response within 5 min, a very short response time as compared with the traditional MIPs-coated PQC sensor reported in the literature. The sensor developed exhibited good selectivity (low response to atrzine, carbaryl, carbofuran and aldicarb, pesticides with similar structures as pirimicarb) and highly sensitive response to pirimicarb with a linear working range from 5.0 x 10^{-6}mol/L to 4.7 x 10^{-3}mol/L, following a regression equation of $-\Delta F = 0.552+1.79 \times 10^{6}C$ (r = 0.9988), a repeatability (R.S.D., n=5) of 4.3% and a detection limit of 120 μg/L (S/N= 3, n=5). The MIP-coated PQC sensor developed was shown to provide a sensitive and fast sensor for onsite determination of pirimicarb in water extracted from vegetables with satisfactory recoveries from 96-103% and repeatability (R.S.D., n=5) from 4.6-7.1% for real samples at practical pirimicarb concentration range (8.0 x 10^{-6} mol/L to 2.0 x 10^{-4}mol/L).

1. Introduction

The problem of environmental contamination by persistent pesticide causes a major concern due to the accumulation of their residues in the environment and human tissues. Owing to its broad spectrum of biological activity, pirimicarb [2-(dimethylamino)-5, 6-dimethyl-4-pyrimidinyl dimethylcarbamate], an extensively used carbamate insecticide for pest control, has progressively replaced the more toxic organochlorinated (OC) and organophosphorinated (OP) pesticides [1] in recent years. Pirimicarb is suspected carcinogen and mutagen [2]. It inhibits acetylcholinesterase and shows neurotoxic effect such as the generation of a series of uncontrolled nerves pulse [3]. Thus, its residues at the surface of sprayed vegetables and in run-off water discharged to the environment have to be controlled and monitored. An analytical methodology capable to produce a quick result onsite is needed, in particular to protect the public against vegetables contaminated with carbamate pesticides due to their premature harvest at a time when the vegetable price is running high in the market.

Analytical methods such as GC [4], HPLC [5] and Capillary electrophoresis [6] have been developed for the determination of pirimicarb. However, they are laboratory-based methods which are too slow to produce results in case of an outbreak of carbamate contamination of vegetables. The analytical method based on the inhibition of acetylcholinesterase is not very sensitive and gives too many undesirable false positive results [7]. A screening method using HPTLC has recently been developed for the determination of carbamate residues in vegetables [8]. However, the sensitivity at mg/kg level is too low. As carbamate contamination occurs

mostly at the surface of vegetables, the use of a sensitive and selective sensor to monitor its concentration quickly on-site in eluted water after washing the suspected vegetable samples may provide a better solution to the problem.

Various chemical sensors and biosensors have recently been developed based on the piezoelectric quartz crystal (PQC) [9, 10 and 11] due to its high portability, cost-effective, fast response and good sensitivity. However, the success of a good PQC sensor highly depends on the coating material which should be stable, highly selective and sensitive towards the analyte that it intends to monitor. To develop a suitable PQC sensor for pirimicarb, the coating must be highly sensitive and selective for pirimicarb at trace levels in the presence of a high organic matrix as expected from vegetables.

The newly developed molecularly imprinted polymers (MIPs) may provide an answer. Molecular imprinting technology is derived from the concept of creating designed recognition sites in macromolecular matrices by means of template polymerization. This technique is based on the in situ co-polymerization of cross-linkers and functional monomers that form complexes with template (imprinted) molecules prior to polymerization. After removing the template molecules from the polymerized material, binding sites are left behind, showing complementarity to the template in a subsequent rebinding experiment. The development of MIP for analytical application has been initially focused on using MIP as packing material for HPLC and capillary columns [12,13], or as selective material for cleanup and pre-concentration in SPE [14]. For example, MIP has been developed for pre-concentration of carbamates prior to subsequent

electrochemical detection [15]. For chromatographic application, the particle size is normally in micron size and the kinetic of interaction is less demanding as compared to sensor application.

Various papers have been published for the development of MIP as sensing material on the surface of PQC for analytical application [16, 17]. The MIPs used as coating for PQC sensor were mostly prepared by the traditional bulk polymerization method. The bulk polymer prepared is ground into small but irregular particles prior to coating them onto the surface of PQC electrode for sensor application. The bulk polymerization method produces polymer particles with irregular and random shape and a wide size distribution, both of which are undesirable for sensor coating. Moreover, the process of crushing and sieving of the polymer after polymerization could destroy the imprinted sites and a substantial number of the cavities may shrink after the removal of the template using polar organic solvents [18]. When a non-covalent approach is used, the pre-polymerization step in which template and monomer have to form a stable complex, is an ill-defined process. As a consequence, complexes with different template to monomer stoichiometry can be formed [19, 20] and thus, the MIPs obtained possess a heterogeneous binding site distribution that limit their applicability (in the form of broad peaks in chromatography and non-linear response in sensors) and selectivity. As a result, the response time of the PQC sensor coated with MIP particles prepared by bulk polymerization was often found to be as long as 40 minutes, and with highly fluctuating signals [21]. This is not suitable as a coating for PQC to deliver quick results for on-site determination in case of pesticide contamination of vegetables.

In order to overcome these drawbacks, several polymerization strategies for the preparation of spherical particles with a narrow particle size distribution and a more homogeneous binding site distribution have been proposed in the literature [22, 23, 24]. The use of precipitation polymerization [25] has been suggested to produce MIP micro-spheres with desired characteristics. This methodology is in essence conducting polymerization of the mixture (template, monomer and cross-linker) in the presence of porogen at a higher amount than previously used in the bulk polymerization method.

Various approaches for the synthesis of MIPs using pirimicarb as template will be investigated and the material prepared are characterized using SEM to identify desirable particle morphology with suitable surface interaction and strong binding with pirimicarb. The quality of the MIP coatings on the PQC sensor will be studied using the electrochemical impedance method and cyclic voltammetry. The working conditions of the PQC sensor will be optimized and factors affecting its analytical performance discussed. In light of the results obtained, the applicability of the PQC sensor for the determination of pirimicarb in water and vegetables is assessed and discussed.

2. Experimental

2.1. Reagents and standards

All standard solutions were prepared from analytical reagent (AR) or equivalent grade chemicals unless otherwise stated. 2, 2'-Azobissobutyronitrile (AIBN) was purchased from No.4 Chemical Reagent Company of Shanghai, whereas ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA), polyvinyl pyrrolidone (PVP) and polyvinyl chloride (PVC) were purchased from Aldrich (Miwaukee, WI, USA). The deionized (D.I.) water used in the experiment was prepared by demineralization of the doubly quartz-distilled water using the Milli-Q system.

The pirimicarb stock solution (0.010 mol L^{-1}) was prepared by dissolving the compound (0.012 g) in 5.0 ml of a mixture of acetonitrile/water (1:9, v/v). Other standard solutions were prepared by appropriate dilution of the stock solution using D.I. water. The Britton–Robinson buffer solution was prepared by mixing each component acid at 0.20 mol L^{-1} with pH adjusted using sodium hydroxide.

Stock solutions of atrazine [2-chloro-4-(ethylamino)-6-(isopropyamino)-1,3,5-triazine], terbutylazine [2,4-bis(isopropylamino)-6-chloro-s-triazine], carbaryl [1-naphthyl-*N*-methylcarbamate], carbofuran [2,2-dimethyl-2,2-dihydrobenzofuranyl-7-N-methylcarbamate; 2,2-Dimethyl-2,3-dihydro-7-benzofuranyl-N-methylcarbamate] and aldicarb [2-methyl-2-

(methylthio)propionaldehyde O-(methylcarbamoyl)oxime] (Riedel-de Haaen) were prepared as described above for the interference study.

2.2. Apparatus and equipment

The 9M Hz At-cut quartz crystals with gold or silver coating on both sides were purchased from International Crystal Manufacturing Co. Inc., Oklahoma City, OK. A self-constructed oscillator circuit powered by a 5 volts D.C. voltage regulator was used to resonate the piezoelectric crystal with frequency output monitored by a frequency counter (Heathkit model IM-4120). The quartz crystal was bonded to a glass tube using silicone rubber and only one side of the crystal was coated with MIP which was allowed to contact the solution. Cambridge Leica (Model Stereo Scan 440) Scanning Electron Microscope was used for the characterization of the MIPs prepared.

The electrochemical impedance spectra and cyclic voltammograms of the PQC electrodes were measured in a solution containing 3.0% NaCl, 1.0 mmol/L $K_4Fe(CN)_6$ and 1.0 mmol/L $K_3Fe(CN)_6$. The electrochemical impedance measurements were carried out in an electrochemical cell with a three-electrode configuration by an EG&G 263 potentiostat and a PAR model 5201 lock-in amplifier controlled by a microcomputer under the M398 software. The measured frequencies were ranged from 0.001 to 10 Hz under a 5mV AC modulation on the controlled potentials. Five points per decade were measured. The cyclic voltammetry experiment was

conducted by the Princeton Applied Research model 263 potentiostat under software-control (EG&G model 270).

2.3. Procedures for the preparation and rebind study of MIP

The template molecule (pirimicarb, 1.0 mmol) and the monomer (MAA, 4.0 mmol) were added to a 25 ml round-bottom flask for dissolution in 20 mL of acetonitrile or chloroform for precipitation polymerization, or in 5 ml of chloroform for bulk polymerization. After left in contact for 5 min, the cross-linker (EDMA, 20 mmol) and the initiator (AIBN, 2.0 mmol) were added. The mixture was purged with N_2 for 5 min and the flask was sealed under this atmosphere. After 24 h of polymerization in a thermostated water bath at 60 ℃, the MIP particles prepared by the precipitation method were collected by centrifuge, whereas the MIP monolith prepared by bulk polymerization was crushed, ground, and wet-sieved with water. Particles within 25-30 μm range were collected for subsequent use. The corresponding non-imprinted polymers (NMIPs) were obtained following the same procedures without the addition of the template molecule.

The particulates were extensively washed with methanol/ acetic acid solution (9:1, v/v) until the template could not be detected (λ =245 nm) in the extraction solvent. Then the particles were washed several times with methanol until the pH of the extracted solvent was neutral. Finally, the solvent was removed by centrifugation and the particles were dried under vacuum.

For rebind performance study, the polymer particles (10 mg) prepared were mixed with a 2.0 mL solution containing a known concentration of pirimicarb (0.001–10.0 mmol L^{-1}). The mixtures were incubated for 16 h under continuous shaking in a horizontal shaker (KS-260B, IKA-WERKE GMBH & CO.KG, Germany) at room temperature. After incubation, the mixture was centrifuged using a high-speed centrifuge. The supernatant was withdrawn and the concentration of unbound pirimicarb was determined by UV absorption measurement at 245 nm using a UV spectrometer (HP UV-visible 8453). Data from triplicate measurements were averaged for Scatchard analysis.

2.4. Procedures for PQC sensor operation

The coating of the PQC sensors by the polymer particles prepared was carried out using the following procedures: 30 mg of fine polymer powders were suspended into 5 ml THF containing 10 mg of PVC powders. About five microliters of the suspension were spread onto the Ag-electrode surface of the PQC while rotating at a definite speed. After THF was evaporated at room temperature in air, a polymer coating was formed on the Ag-electrode. The frequency shift was controlled to about 8.0×10^3 Hz.

Before the determination of pirimicarb, the MIP-coated PQC sensor was stabilized in 2.0 ml of Britton-Robinson (B-R) buffer (pH 7) for several minutes until the frequency (f_0) was stable. A series of pirimicarb standard solutions at different concentrations were then injected into the

detection cell using a micro-syringe. The measurements were carried out in a stirred buffer solution and the temperature of the cell was maintained at 25.0 ± 0.1 ℃. The frequency (f_i) of the MIP-coated PQC sensor at different times were recorded. The frequency shift was calculated as $\Delta f_i = f_i - f_0$. After measurement, the MIP-coated PQC sensor was washed three times with methanol–acetic acid (1:1, v/v), following by three washes with doubly distilled water. Each wash was lasted for 10 min. The regenerated PQC sensor was then ready for reusing.

3. Results and discussion

3.1. Preparation and characterization of MIPs

In the present work, three different types of MIP particles (MIP-P1, MIP-P2 and MIP-B) were prepared. MIP-P1 and MIP-P2 were prepared by the precipitation polymerization method using acetonitrile and chloroform as porogen respectively. MIP-B was prepared by bulk polymerization method (Table 1). The morphologies of the MIP polymers prepared by the precipitation polymerization and the traditional bulk polymerization were investigated using SEM with results shown in Fig. 1. It can be seen from the SEM micrographs that the use of precipitation polymerization produces uniformly distributed microspherical particles with size of 300 nm for MIP-P1 using acetonitrile as the porogen and size of 50 nm for MIP-P2 using chloroform as the porogen. As a dilute monomer solution was used as a starting solution with

careful control of the separation point during precipitation polymerization, uniformly distributed microspheres were obtained [26]. On the other hand, the MIP-B particles produced by bulk polymerization with subsequent grinding showed random and irregular shapes, and a wide size distribution.

The recognition of MIP as sensing material to template is due to the functional group and shape complementarity between the template molecules and the 3-D structure at the binding sites in the imprinted polymer. When MIP is in contact with the template solution, the template will be absorbed onto the polymer matrix. It has been reported that there is a linear relationship between the frequency shift ($-\Delta f$) and the absorbent mass (Δm) within a definite concentration range in the liquid phase for a PQC sensor. When the system is at equilibrium,

$$\Delta f = -KC^{1/n} \quad (1)$$

where K and n are numerical parameters and minus indicates a decrease in frequency with increasing mass, whereas C is the concentration of the analyte [27].

The response time of the sensor is defined as the time interval from the injection of the template solution until the attainment of a steady resonant frequency (frequency shift <2 Hz in 5 min). It should also be mentioned that for a MIP-sensor, with a thicker membrane, a longer response time was required. Thus, the frequency shift due to the MIP membrane for all the sensors was kept to about 8 KHz to assist comparison. According to the Sauerbrey equation, the thickness of the membrane of the sensors was 0.44 μm (assuming that the density of different membranes is the same as 1.0 g cm^{-3}). The same conditions were used to determinate pirimicarb

using the three MIPs prepared. It was found (Fig 2A) that the MIP-P2 modified electrode showed the fastest response time as compared to the other two MIPs for the same amount of pirimicarb in solution (9.0×10^{-4} mol/L), achieving a steady state within 5 min after injection, which is very fast as compared to that reported in the literature using traditional MIPs-coated PQC sensor. Moreover, the fast response time could be maintained over a large concentration range of pirimicarb (5.0×10^{-6} to 6.0×10^{-3} mol/L).

The fast response time is attributed to the effect of the particle shape and sizes of the MIP coatings. For MIP-P2 with uniformly distributed particle morphology, the response time was less than 5 minutes after injection. For MIP-P1 with larger spherical particle size of about 300 nm, the response time was over 15 minutes, and for MIP-B with irregular shape and wide particle size distribution, the response time was over 40 minutes. Thus, the use of smaller and more uniform particles in the sensor coating allowed faster mass transfer rate of the analytes to the recognition sites in the sensor coating. For the bulk MIP polymer, complexes with different template to monomer stoichiometry were formed during the pre-polymerization step as the result of using a high concentration of template and monomer. Thus, the MIPs obtained gave a heterogeneous binding site distribution, limiting the rate of mass transfer. On the other hand, the polymers prepared using the precipitation method with more homogeneous binding sites, narrower size distribution and well-defined binding sites [19] allowed a faster mass transfer rate than MIP-B. With the smallest particle size and a uniformly size distribution, MIP-P2 was found to produce the fastest steady-state response as compared to the other two polymers.

The affinity of different polymers was studied using the Scatchard analysis. The amounts of primicarb bound to the MIPs particles were plotted against their initial concentrations as shown in Fig. 3. The amount of pirimicarb bound to the polymers, Q, was calculated by subtracting the amount of unbound pirimicarb from that of initial pirimicarb added to the mixture. Binding data were obtained by the following Scatchard equation: Q/[Pirimicarb] = $(Q_{max} - Q)/K_D$, where Q_{max} is the apparent maximum number of binding sites and K_D the equilibrium dissociation constant. Q_{max} and K_D were determined from the intercept and slope, respectively, when Q/[Pirimicarb] was plotted *versus* Q.

The amount of pirimicarb bound to the MIPs at equilibrium Q was found to increase with increasing initial concentrations of pirimicarb. The saturation binding data were used in the Scatchard equation to estimate the binding properties of MIPs. From the Scatchard analysis for three different types of polymer, there are two distinct sections clearly within the plot which can be regarded as straight lines. This indicates that the binding sites could be classified into two distinct groups with specific binding properties (the higher affinity-binding site and the lower affinity binding site) for each polymer [28]. Fig. 4 showed the scatchard plot for polymer MIP-P2. According to the slope and intercept of each Scatchard plot line, the equilibrium dissociation constant (K_D) and the apparent maximum number (Q_{max}) of each affinity-binding site can be calculated. The results showing the dissociation constant and maximum number of binding sites of the three MIPs synthesized using different method are given in Table 2.

The result of the Scatchard analysis shows that the apparent maximum number of binding sites (Q_{max}) of the precipitation polymer is higher than that of the bulk polymer. This can be attributed to the loss of the binding sites in the polymeric matrix during the crushing and sieving step during the preparation of MIP-B. The microspherical MIP particles prepared using the precipitation method possessed higher surface areas and more complementary sites than those produced by the bulk polymerization [29]. Comparing MIP-P2 and MIP-P1, the apparent maximum number of binding sites (Q_{max}) at MIP-P2 is higher than that at MIP-P1. This is attributed to the smaller particle size of MIP-P2 and the different media used for polymerization. Thus, MIP-P2 was more suitable to be used as sensing materials for PQC sensor as the sensor's frequency response (Δf) to the concentration (C) of sample solution is related to the total amount of template bound to MIPs in the solution.

In summery, as MIP-P2 showed the fastest response time of about 5 minutes and the largest apparent maximum number (Q_{max}) of binding sites, it was selected to fabricate the PQC sensors for fast and onsite monitoring of pirimicarb with results shown in the next section.

3.2. Fabrication and characterization of MIP-coated PQC electrode

Experiments using electrochemical impedance (EIS) measurements and cyclic voltammogram (CV) were conducted to characterize the surface of the PQC electrode prepared. Fig. 5 shows the Niquist plot of the electrochemical impedance spectra obtained from the MIP-

coated PQC electrode in solution containing 3.0% NaCl, 1.0 mmol/L $K_4Fe(CN)_6$ and 1.0 mmol/L $K_3Fe(CN)_6$, where Zre and Zim represent the real impedance and imaginary impedance respectively. The Niquist plot can be divided into two parts: the high frequency range controlled by electrode kinetics, and the low frequency range controlled by diffusion. Plot (a) shows the impedance spectra of a bare PQC electrode carefully cleaned three times by washing with conc.H_2SO_4 following by distilled water. A straight line was obtained in the impedance spectra at all frequency range investigated. The results showed that the kinetic for electron transfer at the surface of electrode was not limiting and the reaction was controlled mainly by diffusion. The impedance spectra of the PQC electrode modified with MIP-P2 particles were shown in plot (b). It comprised of an arc in the high frequency range and a line in the low frequency range. The magnitude of the arc was found to increase with increasing thickness of the coating (not shown in the figure). The result observed was probably due to the increase in the contact resistance and the charge transfer resistance at the MIP-coating/electrode interface. At the low frequency range of plot (b), the line was observed to incline at an approximately 45 degree to the real axis, indicating that the mass transport of ions occurred purely by diffusion through the homogeneous MIP phase in this frequency range [30].

The cyclic voltammograms (Fig. 6) obtained before (A) and after (B) the modification of PQC electrode with the MIP-P2 particles showed a clear reduction in the peak current after modification of the PQC surface with the MIP-P2 particles. The results showed an increase in the charge transfer resistance after MIP-P2 modification, probably due to the blocking of the electron

transfer of the ferri-/ferrocyanide couple by the MIP-P2 particles coated at the surface of the PQC electrode. In another words, interferences due to electrochemically active impurities or dissolved oxygen are expected to be reduced. After soaking for 24 h, the CV and EIS spectra did not find to be changed, indicating a stable MIP-P2 coating structure at the PQC electrode surface even though it was porous after coating with PVC and MIP-P2 particles. The good integrity of the coating structure indicates a suitable PQC sensor for the determination of pirimicarb in water.

As polyvinyl pyrrolidone (PVP) and polyvinyl chloride (PVC) were commonly used as adhesive for binding MIP particles on the sensing surface of PQC electrode and thus they were expected to have strong effect on the structure of the coating layer. To study their effect on the performance of the PQC sensor, equal amounts of PVP and PVC were used to fabricate MIP-PQC sensor. The signal of the MIP-PQC sensor using PVP as the adhesive was found to be highly variable, while the MIP-modified PQC sensor using PVC as the adhesive produced a more stable signal. Thus, in the present work, PVC was used as the adhesive for the fabrication of MIP coating on the surface of the PQC electrode. To investigate the effect of PVC on the determination of pirimicarb, a PVC-coated PQC sensor was prepared by dissolving 40 mg PVC in 5 ml THF prior to dropping the solution onto the Ag-electrode. The shift of frequency observed was about 8 KHz.

As shown in Fig. 7, the PQC electrode coated with PVC only showed a very small and constant response to pirimicarb up to 6.0 mmol/L. Thus, very weak adsorption had occurred between PVC and the template molecule. In contrast, the MIP-P2/PVC-coated electrode showed

a very large response factor to the increase in the pirimcarb concentration. The response must be coming from a strong interaction between MIP-P2 and pirimicarb. In comparison to the bare PQC electrode and the PQC electrode coated with NMIP by non-imprinted polymer prepared using the same method as MIP-P2, they were all showing low response towards pirimicarb. Thus, the interaction between MIP-P2 and pirimicarb is probably due to the special recognition binding sites existed at the MIP particles which caused the shift of frequency of the PQC electrode upon binding with pirimicarb.

3.3. Analytical performance and applicability study

For a molecularly imprinted polymer, the binding sites are produced in situ by copolymerization of functional monomers and cross-linkers around the template molecules pirimicarb. In the present work, MAA (methacrylic acid) was used as the functional monomers with carboxyl functional groups. The binding of the template to the imprinted polymers is either through the hydrogen bond, electrostatic force and/or charge transfer between the functional groups and the imprinted molecules. Thus, the pH of the tested solution exhibited a great effect on the sensor response. Britton-Robinson (B-R) buffer was used to buffer solution pH at the range between 2.0–12.0. At a pirimicarb concentration of 1.0×10^{-3} mol/L and pH ranging between 4.0–9.0, the frequency shift was found to increase slowly at pH up to about 8 (Fig. 8). When the pH was increased above 9.0, the frequency shift was found to increase sharply.

However, there was a drop in the frequency shift when the pH was below 4. The observations are interpreted as follow. When the pH is above 9.0, pirimicarb is not stable because of hydrolysis [31]. The appearance of additional hydrolysis products probably elevates the change in the analytical signal. When pH is below 4.0, de-protonation of the organic acid molecules at the binding sites is low (as the binding sites are mainly made up by the carboxylic groups as the functional group). Thus, the response of the MIP-coated PQC sensor is decreased under low pH conditions.

In addition, at pH range between 4-7, a fast response was obtained using the MIP-P2 modified PQC sensor. With the consideration of both the frequency shift and the response time, the use of a buffer solution at pH 7 provides the best condition for pirimicarb determination as it gives not only high sensing sensitivity but also provides a fast response time within 5 minutes. Thus, the buffer solution is adjusted to pH 7 for the determination of pirimicarb.

To illustrate the high selectivity of MIP to the imprinted molecules due to a specific arrangement of the functional groups of the monomer units around the print molecules to form a specific 3-D structure, atrazine was chosen as the structure analogue of the template. Potential interferents such as carbaryl, carbofuran and aldicarb were selected for study as all possessed the N-methyl carbamate structures as shown in Fig. 9. All pesticides selected possessed nitrogen atoms like pirimicarb that could also form hydrogen bond with the polymer. Their interference to the MIP-coated PQC electrode were studied under the same working conditions at concentrations of between 0.9 to 6.0 mmol/L. The results were given in Fig. 10, showing no obvious

interference when the MIP-coated sensor was used to determine pirimicarb. However, when NMIP was used as coating for the PQC sensor, all the pesticides studied showed similar response. The high selectivity of the MIP-coated PQC sensor is thus attributed to the specific recognition sites at MIP which are complementary to the template in terms of size, shape and arrangement of the functional group whereas these sites are absence in coatings of NMIPs.

To estimate the lifetime of the MIP-P2 modified PQC sensor, pirimicarb solutions with a constant concentration (5.0×10^{-4} M) were tested over a prolonged period under the same testing and washing procedure with initial (Δf_1) and final frequency shifts (Δf_i) recorded for each test. The recovery was calculated as the ratio of the MIP-sensors' frequency shift before washing to that after washing ($\Delta f_i/\Delta f_1$). The results showed recoveries over 95 % after 30 consecutive tests. Thus, the procedure for washing out the analytes absorbed onto the PQC sensor is effective to remove them and the nano-sized MIP-P2 particles are strongly adhered onto the surface of PQC electrode during the lifetime. The results suggest a shelf-life of at least 3 months for the MIP-coated PQC sensors.

Under the optimized conditions using 50 nm microspheric molecularly imprinted polymer (MIP) as the coating for PQC sensor, the MIP-P2 modified PQC sensor displayed a linear working range for pirimicarb from 5.0×10^{-6} mol/L to 4.7×10^{-3} mol/L (Fig. 5) which follows a regression equation of $-\Delta F = 0.552 + 1.79 \times 10^{6}$ C ($r = 0.9988$). The response time is short and at about 5 minutes for the whole concentration range. The sensor developed exhibited high selectivity to pirimicarb against other pesticides with related molecular structures such as atrazine,

carbaryl, carbofuran and aldicarb in concentrations between 0.9 to 6.0 mmol/L. At a pirimicarb concentration of 5.0×10^{-4}mol/L, a repeatability of 4.3% (R.S.D., n= 5) was obtained. The limit of detection was 120 μg/L, calculated according to 3 times signal to noise ratio (n=5). SPE could be used for pre-concentration [32, 33] to lower the detection limit for trace pirimicarb determination.

To test the applicability of the technique developed, the concentrations of pirimicarb were determined in six water samples extracted from vegetables, which were spiked with the herbicide to concentrations ranging between 8.0×10^{-6} mol/L to 2.0×10^{-4}mol/L. The recoveries obtained (average of 5 replicates of the six different samples) were found to vary from 96% to 103% and repeatability (R.S.D, n=5) from 4.6 to 7.1% with a mean value of 6.1%. Details on the recovery test on real samples are tabulated in Table 3. In summary, the MIP-coated PQC sensor developed was shown to provide a sensitive and fast sensor for onsite determination of pirimicarb in water extracted from vegetables with satisfactory recoveries at practical concentration range.

4. Conclusions

To meet the need of a fast, accurate and sensitive onsite monitoring method for the determination of pirimicarb in water extract from vegetables, a MIP-coated PQC sensor is developed in the present work. Three different types of MIP particles were prepared by precipitation polymerization method using acetonitrile (MIP-P1) and chloroform (MIP-P2) as

porogen and by traditional bulk polymerization method (MIP-B). The MIP-P2 prepared by precipitation polymerization using $CHCl_3$ as porogen was found to produce the best coating material for PQC sensing application, in particular those with particles at nano-sized range below 50 nm, and with spherical shape and narrow size distribution. The MIP-P2 coated PQC sensor was found to give rise to a stable and repeatable signal (RSD = 4.3 % and n=5 for 5.0×10^{-4}mol/L pirimicarb) and a fast response achieving a steady signal in about 5 minutes, a very fast response time as compared to traditional MIPs-coated PQC sensor as reported in the literature. The MIP-coated PQC sensor has been applied for direct determination of pirimicarb in water, giving a linear working range from 5.0×10^{-6}mol/L to 4.7×10^{-3}mol/L, following a regression equation of $-\Delta F = 0.552+1.79 \times 10^{6}$ C (r = 0.9988) and a detection limit of 120 μg/L (S/N = 3, n=5). The MIP-coated PQC sensor developed was shown to provide a sensitive and fast sensor for onsite determination of pirimicarb in water extracted from vegetables giving satisfactory recoveries from 96-103% and repeatability (R.S.D., n=5) from 4.6-7.1% at practical pirimicarb concentration range (8.0×10^{-6} mol/L to 2.0×10^{-4}mol/L).

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Table 1

Conditions for the preparation of three different types of molecularly imprinted polymers

Polymer designation	Synthesis method	Template /mmol	Monomer /mmol	Cross-linker /mmol	Porogen /ml
MIP-P1	Precipitation	Pirimicarb/1	MAA/4	EDMA/20	Acetonitrile/20
MIP-P2	Precipitation	Pirimicarb/1	MAA/4	EDMA/20	Chloroform/20
MIP-B	Bulk	Pirimicarb/1	MAA/4	EDMA/20	Chloroform/5.0

Table 2

The dissociation constants and maximum number of binding sites for three different types of molecularly imprinted polymers investigated.

Polymer designation	High affinity binding site		Low affinity binding site	
	K_D/mol L^{-1}	Q_{max}/ μ mol g^{-1}	K_D/mol L^{-1}	Q_{max}/ μ mol g^{-1}
MIP-P1	2.15×10^{-4}	211	20.5×10^{-4}	550
MIP-P2	1.49×10^{-4}	223	40.1×10^{-4}	1266
MIP-B	1.25×10^{-4}	134	14.3×10^{-4}	427

K_D: dissociation constants; Q_{max}: maximum number of binding sites

Table 3

Recovery of pirimicarb from spiked samples extracted from vegetable by PQC sensor

Sample	Pirimicarb spiked (10^{-4}mol/L)	Pirimicarb Found (10^{-4}mol/L)	Repeatability* (% RSD, n=5)	Recovery (%)
1	0.08	0.078	6.9	98
2	0.16	0.164	7.1	103
3	0.32	0.307	4.8	96
4	0.64	0.666	6.6	99
5	1.28	1.242	4.6	97
6	2.00	1.920	5.7	96

* RSD = Relative Standard Deviation

Caption of figures:

Fig. 1. The SEM micrographs showing MIP particles prepared by different methods.

(A) MIP-P1; (B) MIP-P2; (C) MIP-B

Fig. 2. The response to pirimicarb by PQC sensors coated with MIPs prepared by different methods.

(A) The frequency shifts of different PQC sensor to pirimicarb in pH 7.0 buffer solution;

(B) The time profile for the PQC response during the adsorption of pirimicarb onto the MIP-P2 modified PQC electrode in pH 7.0 buffer solution.

Each arrow of the seven injections indicated (1-7) represents the addition of one portion of 200 μL of 0.01 M pirimicarb to the pH 7.0 buffer solution. The initial buffer solution is 2.0 mL. After the first injection, the concentration of pirimicarb in solution is 9.0×10^{-4} mol/L.

Fig.3. The binding isotherm of different polymers to pirimicarb. (◀) MIP-P2; (●)MIP-P1; (■) MIP-B.

Fig.4. Scatchard plot for binding study between MIP-P2 and pirimicarb.

Fig. 5. Electrochemical impedance spectra of the PQC sensor in contact with electrochemically active solution. Solution contains: 3.0 % NaCl, 1.0 mmol/L $K_4Fe(CN)_6$ and 1.0 mmol/L $K_3Fe(CN)_6$; Frequency range:10Hz-1mHz ; (a) bare PQC electrode; (b) PQC electrode modified with MIP-P2.

Fig. 6. Cyclic voltammograms of the PQC sensor in contact with electrochemically active solution. Solution contains : 3.0 % NaCl, 1.0 mmol/L $K_4Fe(CN)_6$ and 1.0 mmol/L $K_3Fe(CN)_6$; (A) bare PQC electrode; (B) PQC electrode modified with MIP-P2.

Fig.7. The response to pirimicarb by PQC electrodes prepared by different methods. [pirimicarb]= 0.9-6.0 mmol/L; Buffer solution pH =7.0; (◊) bare PQC electrode; (◄) PQC electrode coated with PVC only; (○) PQC electrode coated with NMIP; (■) PQC electrode coated with MIP-P2 using PVC as adhesive.

Fig. 8. The effect of pH on the frequency shift (A) and response time (B) of the MIP-P2 modified PQC sensor. [Primicarb] =1.0×10^{-3} M.

Fig.9. Potential interferents with molecular structure similar to pirimicarb.

Fig.10. The response of the MIP-coated PQC sensor to different pesticides with molecular structure similar to pirimicarb. (a) pirimicarb; (b) atrazine; (c) carbofuran; (d) carbaryl; (d) aldicarb.

FIG. 1

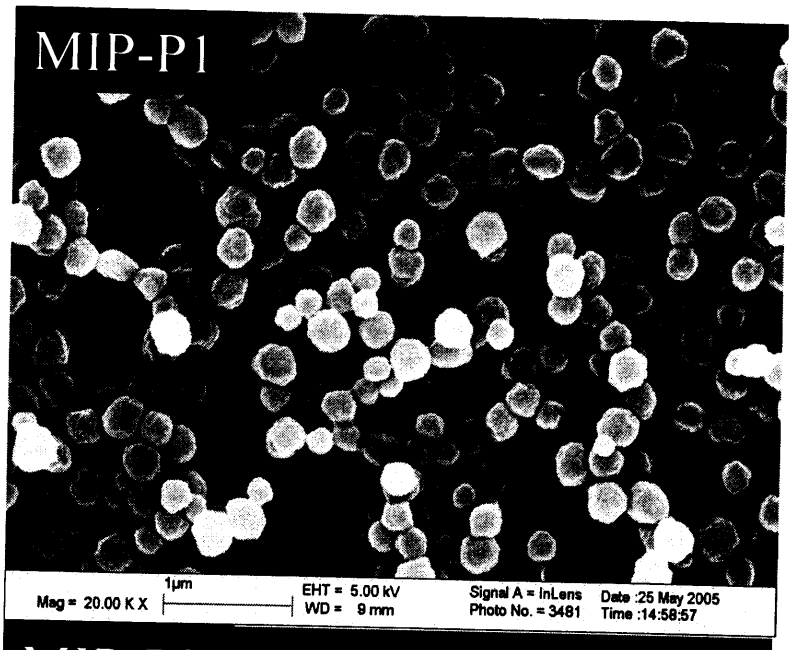


FIG. 1A

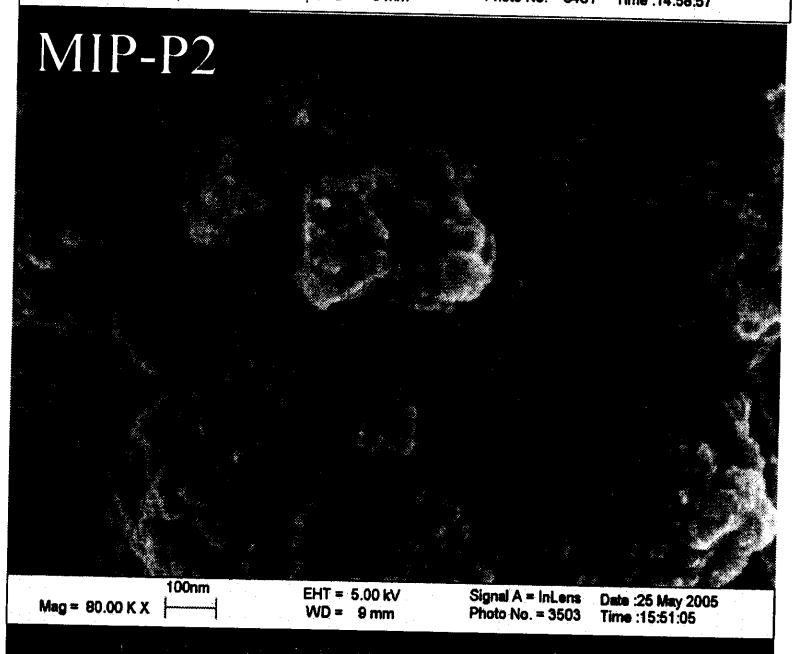


FIG. 1B

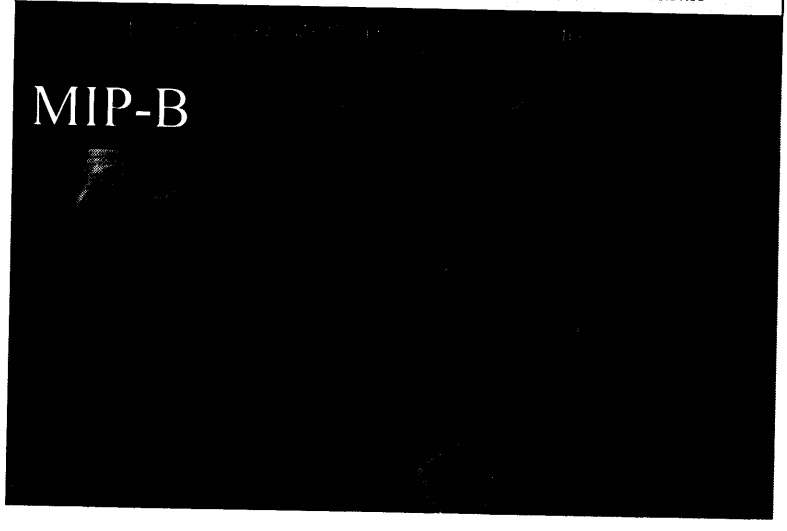


FIG. 1C

FIG. 2

FIG. 2A

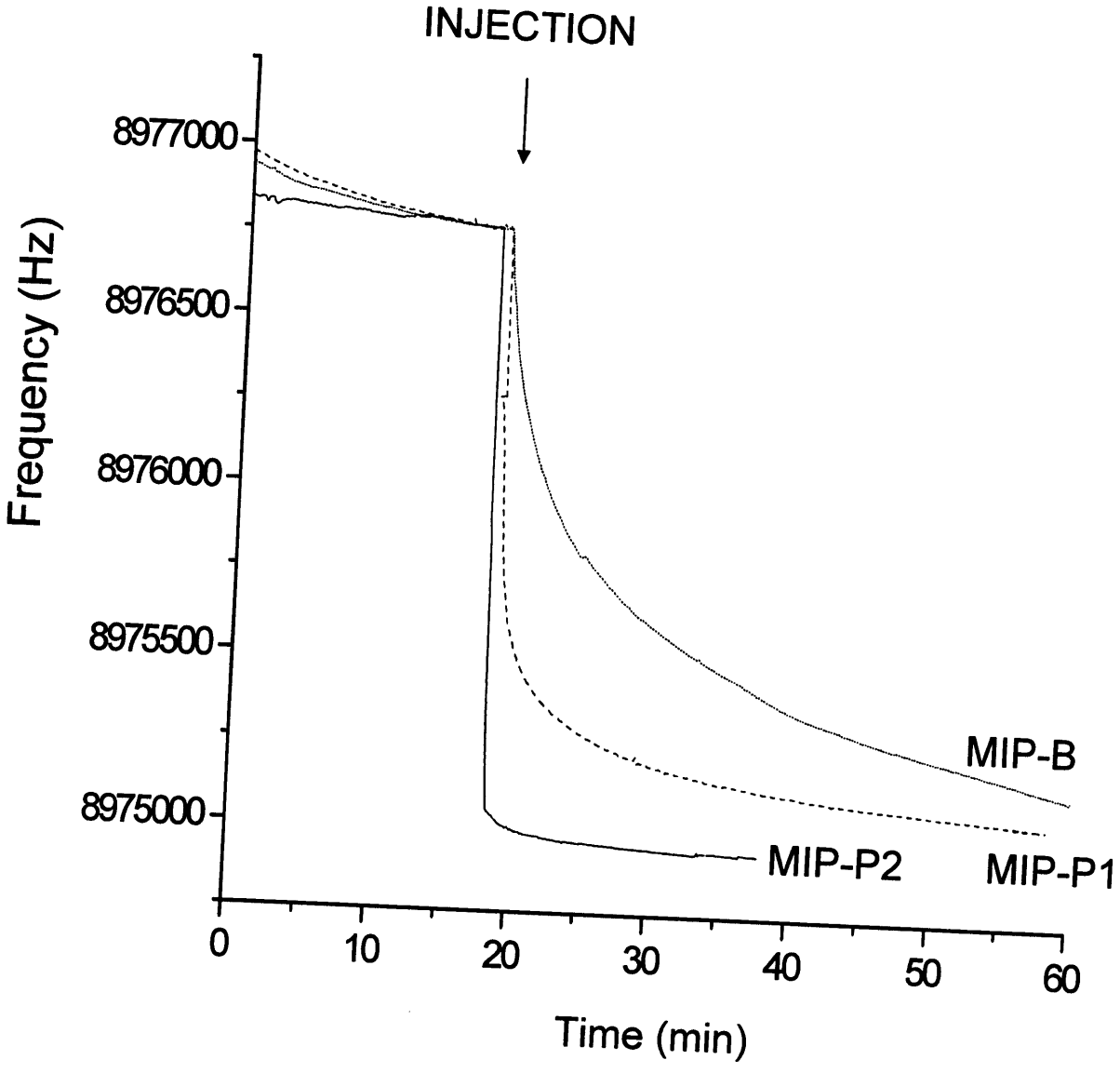

INJECTION
8977000
8976500
8976000
8975500
8975000
Frequency (Hz)
MIP-B
MIP-P2
MIP-P1
0
10
20
30
40
50
60
Time (min)

FIG. 2B

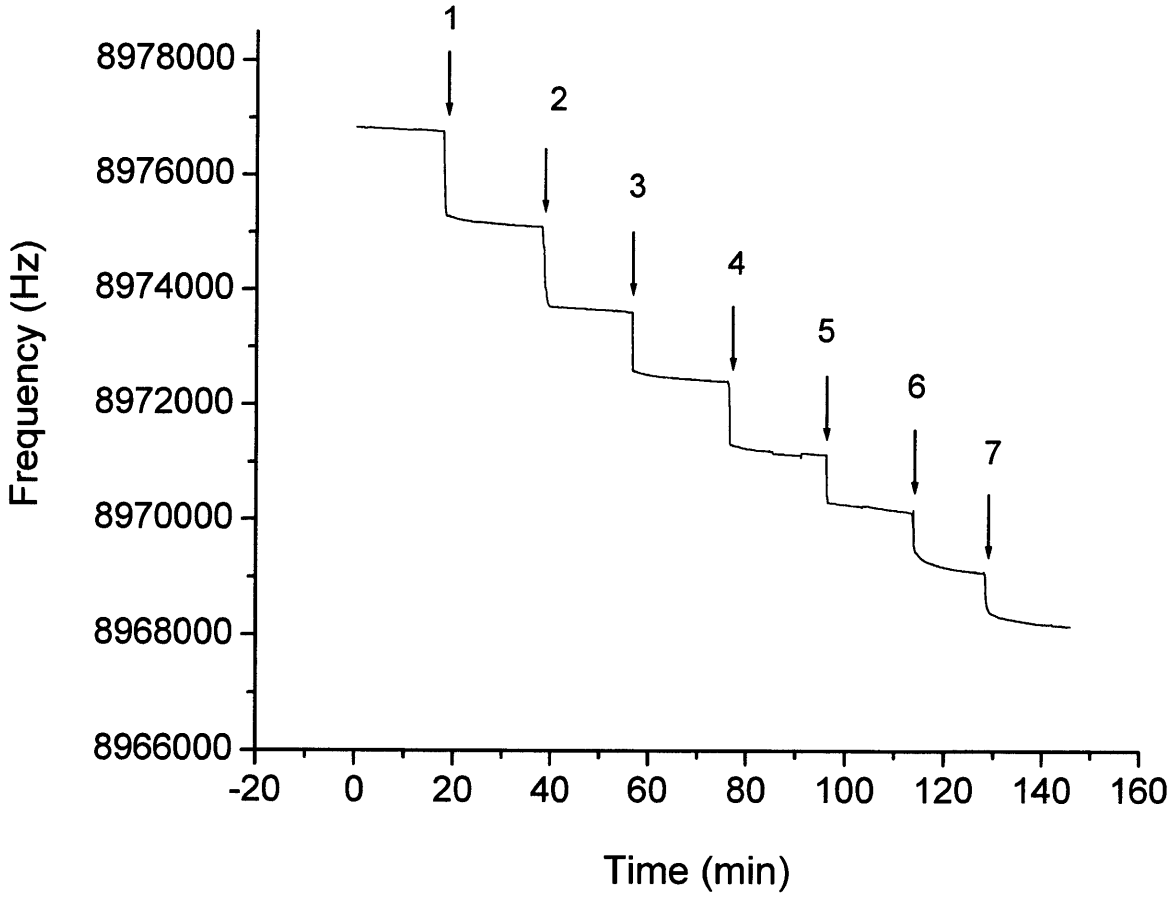

1
2
3
4
5
6
7
8978000
8976000
8974000
8972000
8970000
8968000
8966000
Frequency (Hz)
-20
0
20
40
60
80
100
120
140
160
Time (min)

FIG. 3

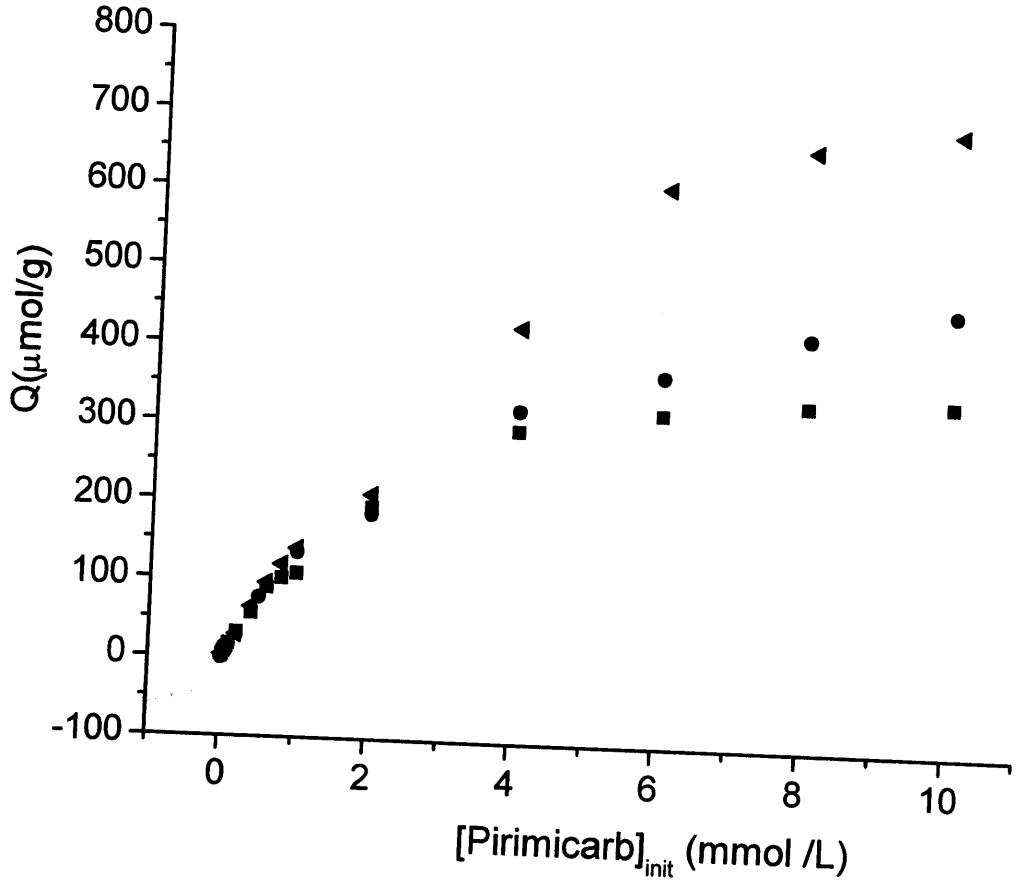

800
700
600
500
400
300
200
100
0
-100
Q(µmol/g)
0
2
4
6
8
10
[Pirimicarb]init (mmol /L)

FIG. 4

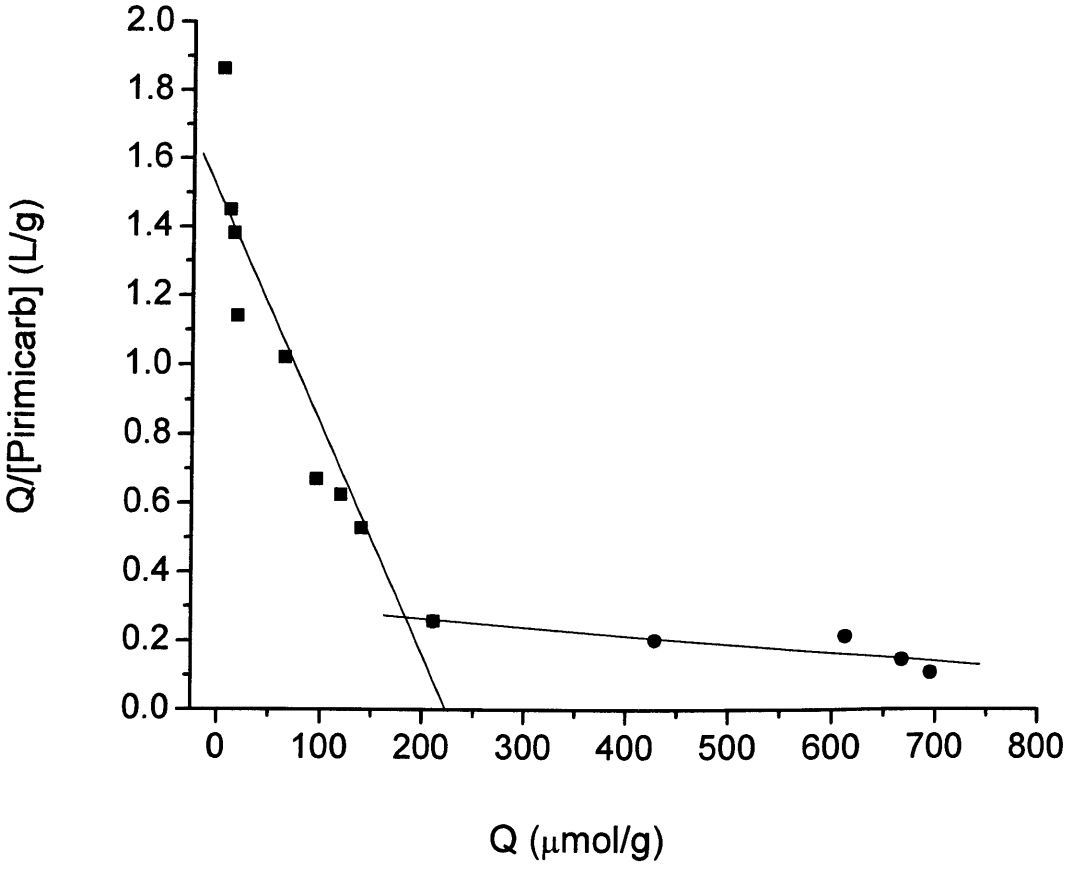

Q/[Pirimicarb] (L/g)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
600
700
800
Q (μmol/g)

FIG. 5

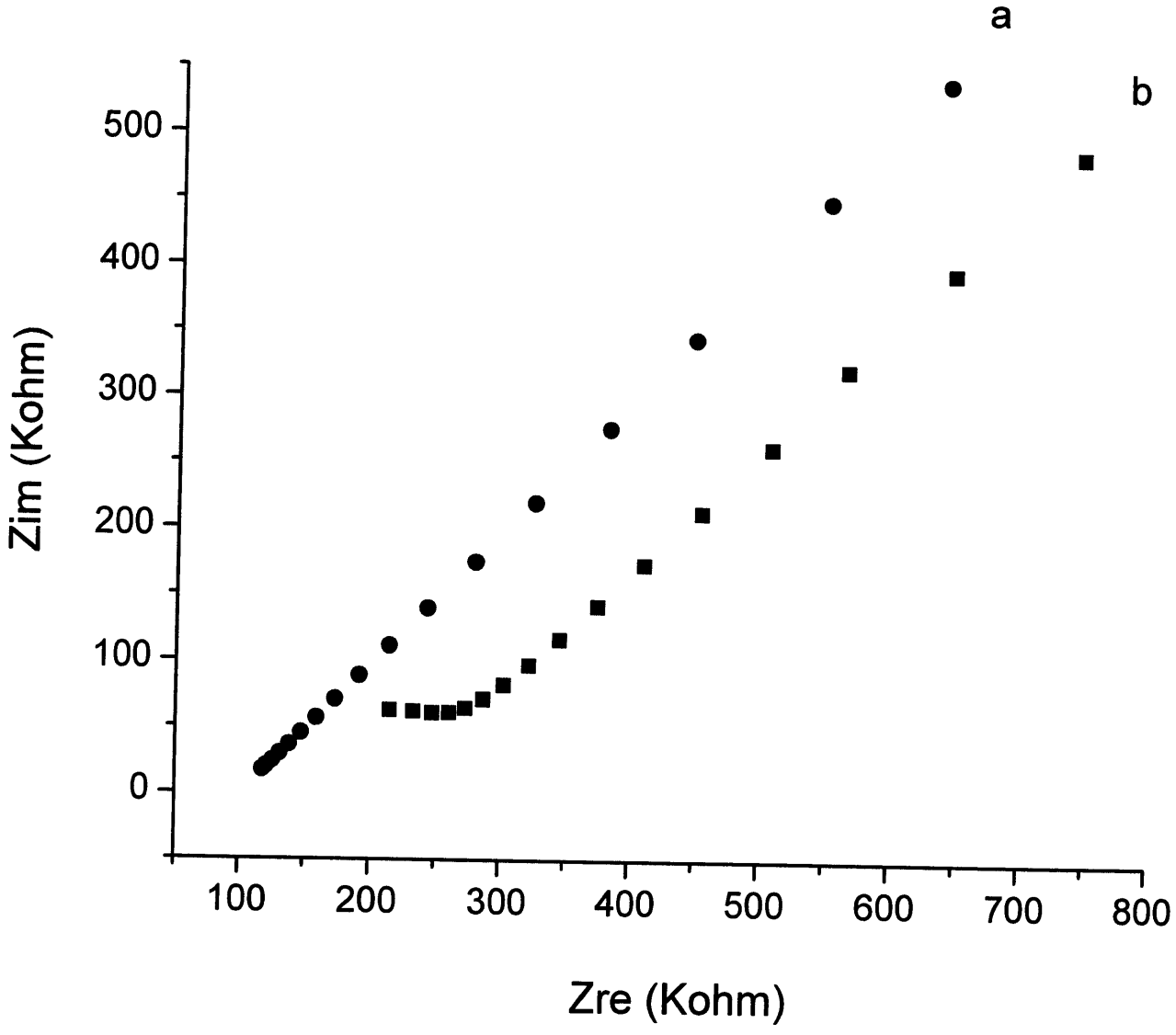

a
b
500
400
300
200
100
0
Zim (Kohm)
100
200
300
400
500
600
700
800
Zre (Kohm)

FIG. 6

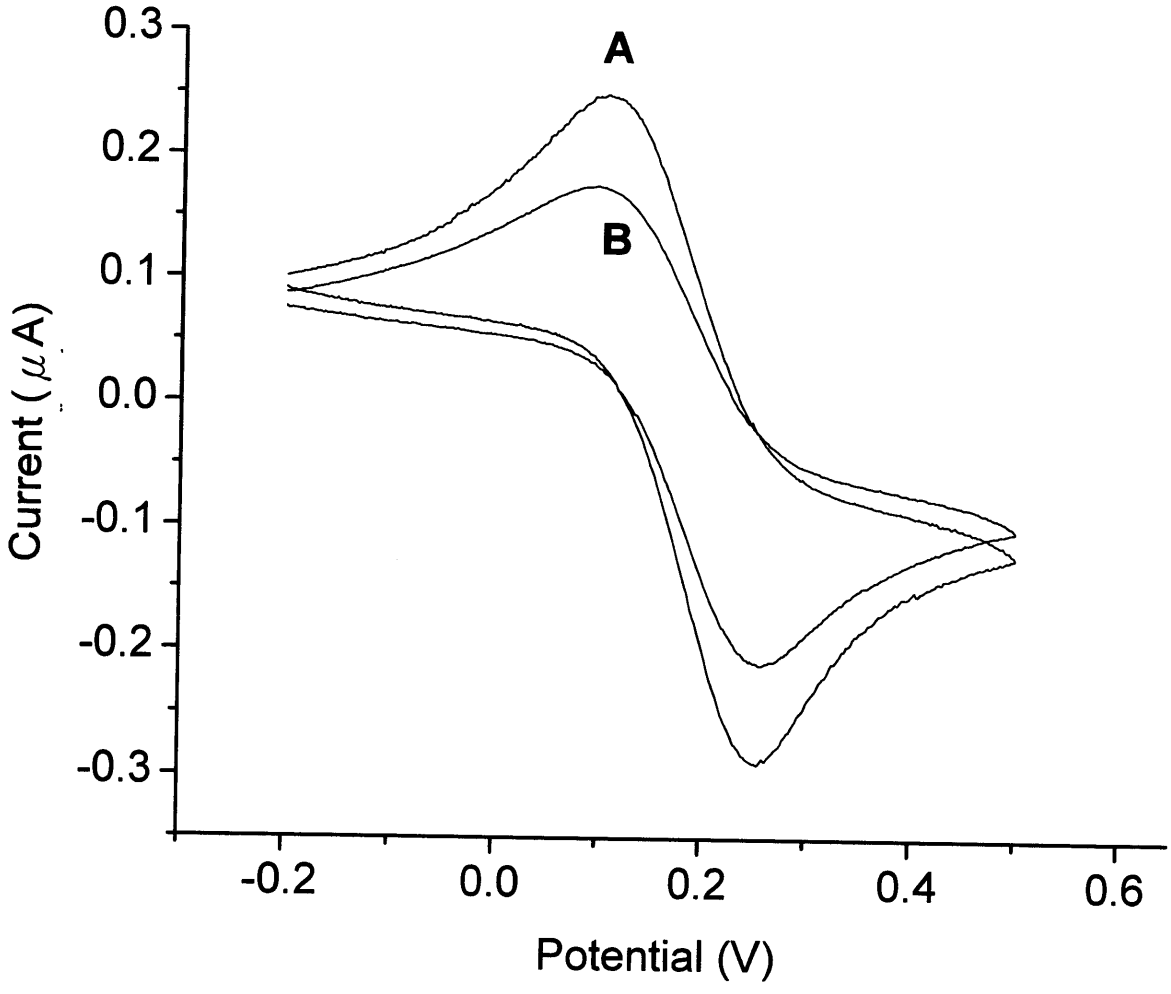


FIG. 7

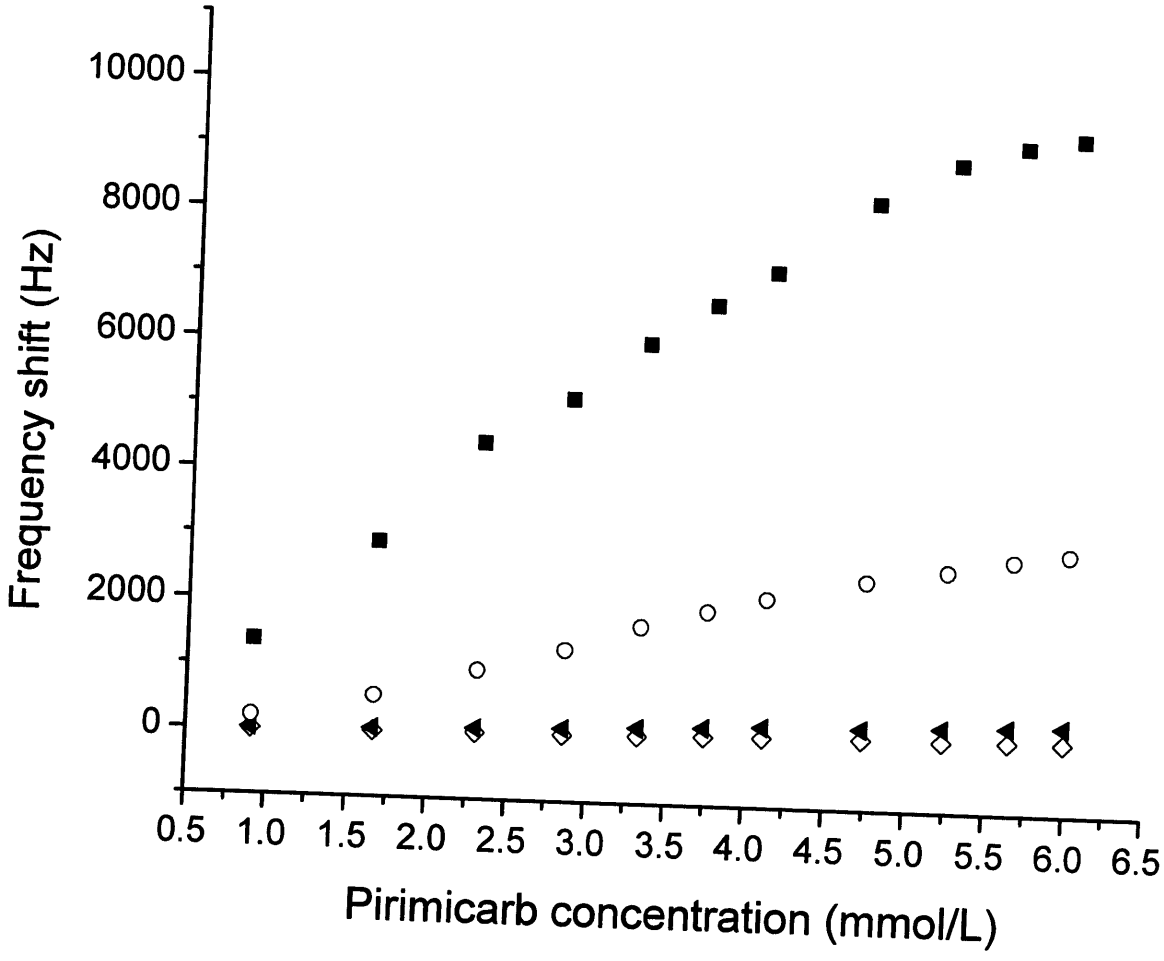

Frequency shift (Hz)
10000
8000
6000
4000
2000
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
Pirimicarb concentration (mmol/L)

FIG. 8

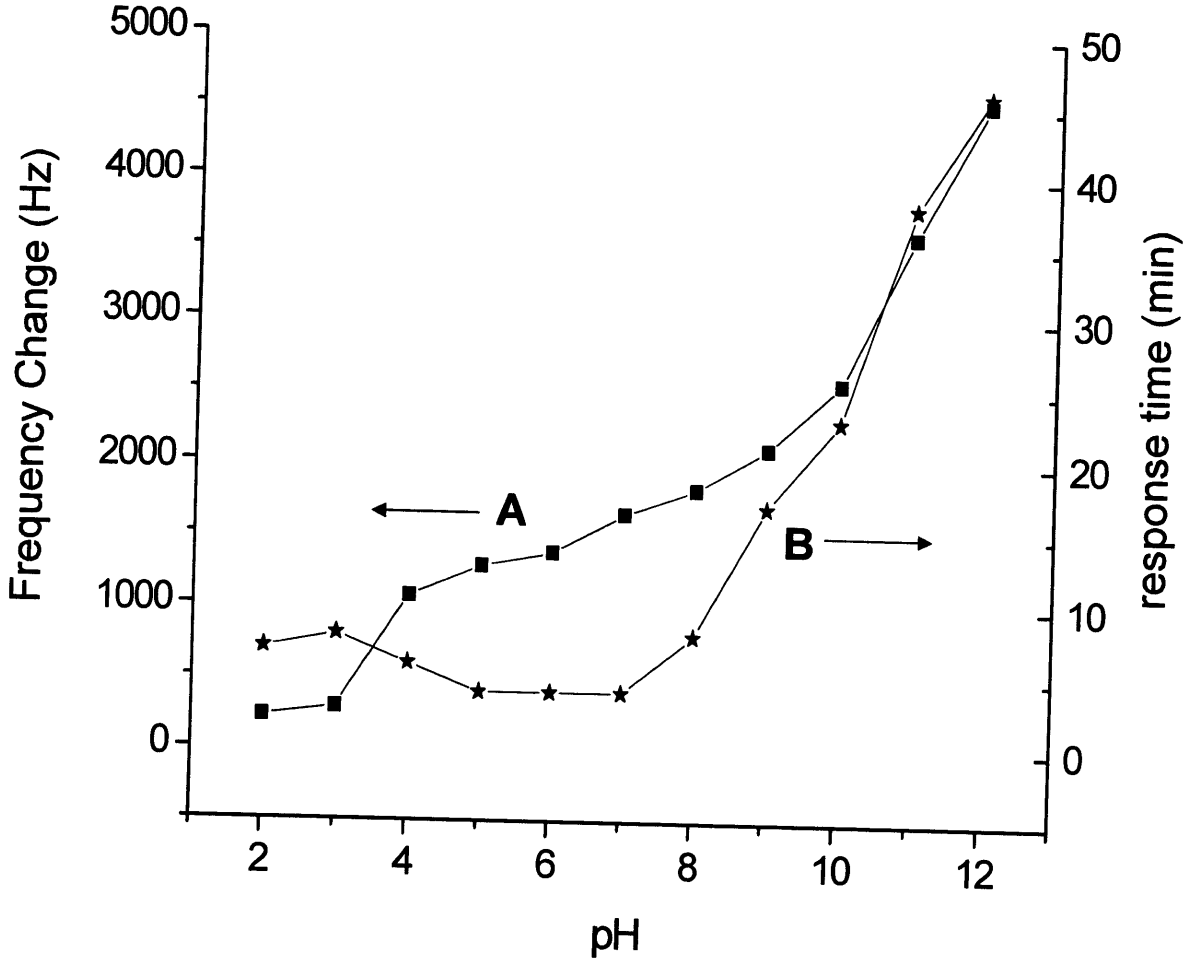
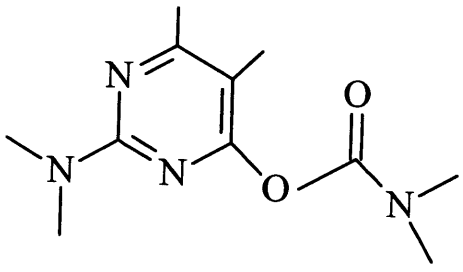
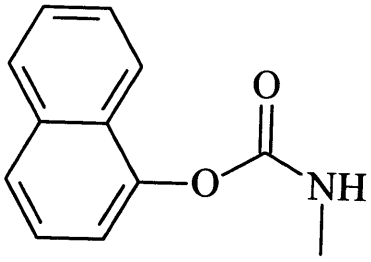

Frequency Change (Hz)
5000
4000
3000
2000
1000
0
response time (min)
50
40
30
20
10
0
A
B
2
4
6
8
10
12
pH

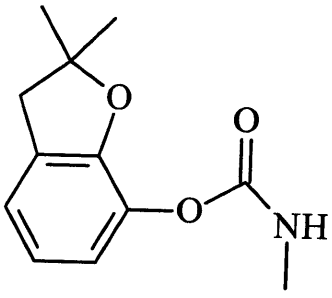
FIG. 9



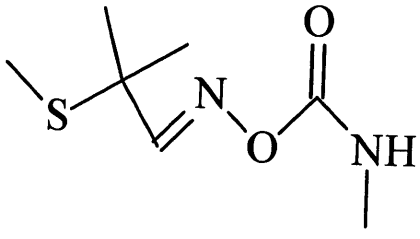
pirimicarb



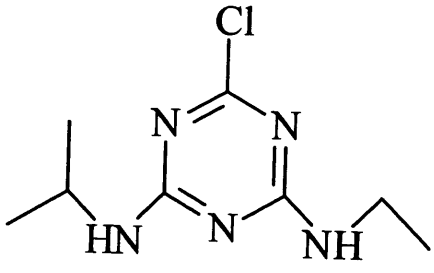
carbaryl



carbofuran

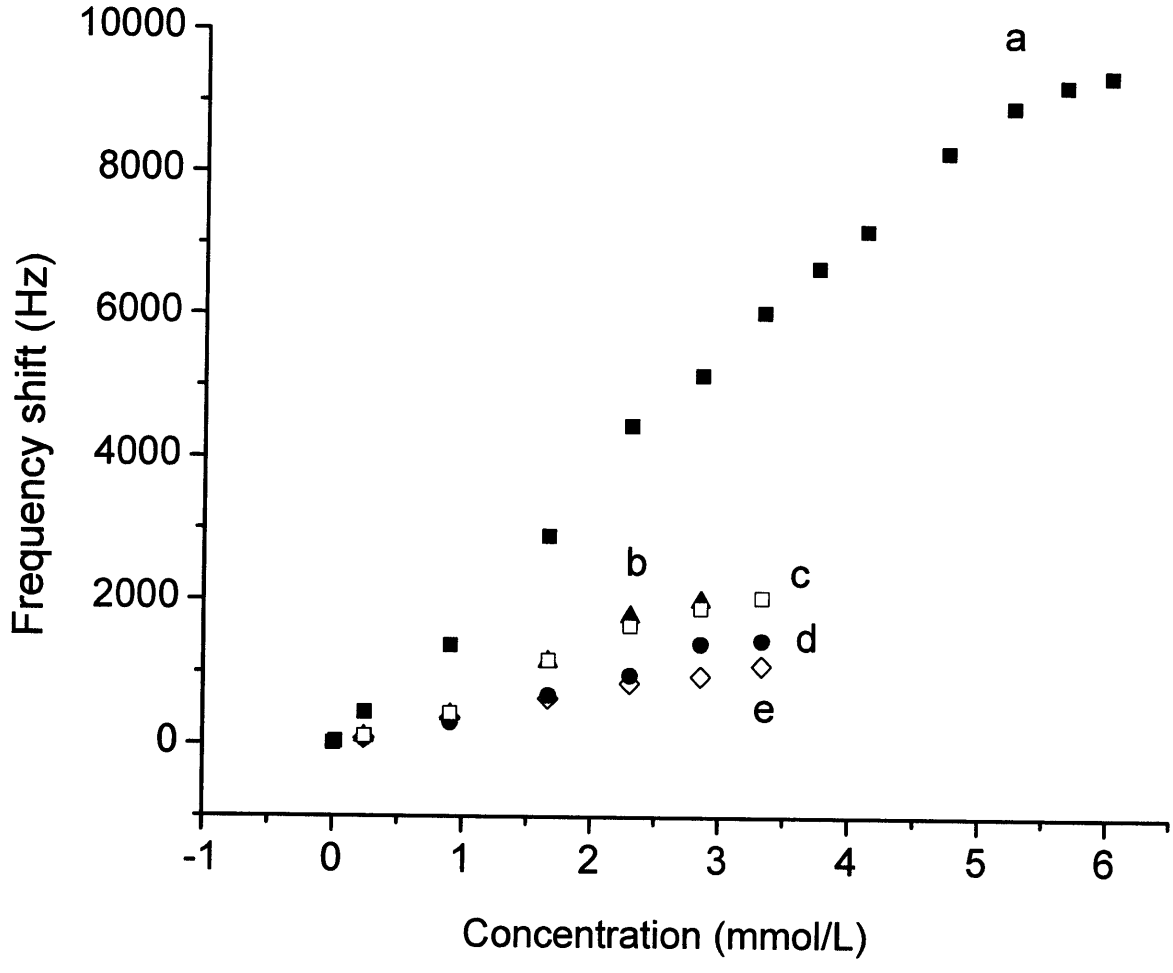


aldicarb



atrazine

FIG. 10


10000
8000
6000
4000
2000
0
Frequency shift (Hz)
-1
0
1
2
3
4
5
6
Concentration (mmol/L)
a
b
c
d
e