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Evaluation of Inhibition Efficiency for the Detection of Captan, 2,3,7,8-Tetrachlorodibenzodioxin, Pentachlorophenol and Carbosulfan in Water: An Electrochemical Approach

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Abstract A novel bio-analytical method has been devised based on the change in catalytic activity of acetylcholinesterase (AChE) enzyme induced by captan, carbosulfan, 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and pentachlorophenol (PCP) for the investigation of inhibition efficiency and sensitivity using Pt/ZnO/AChE/Chitosan bioelectrode. The inhibition curves of captan, carbosulfan, TCDD and PCP were similar to Michaelis–Menten curve. TCDD held the minimum inhibitor Michaelis–Menten constant (K_M^I) value (10.2 nM) in comparison with PCP (10.9 nM), carbosulfan (14.5 nM) and captan (7.9×10^3 nM). The maximum inhibition of AChE enzyme by captan was about 100 %, which was much higher than that of TCDD (72.7 %), PCP (68.1 %) and carbosulfan (47.7 %). The calculated theoretical sensitivity was in the order of TCDD > PCP > carbosulfan > captan. Comparing with TCDD (35.3 %), PCP (47.8 %) and carbosulfan (20.9 %), only the inhibition efficiency of captan (55.0 %) was the maximum. The developed bioelectrode exhibited high recovery and low relative standard deviation in local tap water samples.

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Pesticides are toxic compounds used in agriculture to control rodents, weeds, bacteria, insects and other pests (Juraske et al. 2009). These pesticide residues enter into ground water through soil. The concentration of these pesticide residues in the ground water is increasing day by day. These pesticide residues not only affect the ecosystem but also cause several health problems to humans. The toxic compounds also cause neurological disorder, abdominal pain, depression, myocardial malfunctioning, respiratory problems and even death (Sassolas et al. 2012; Dhull et al. 2013; Gahlaut et al. 2012). Even though threshold limits for pesticide residues have been framed on the national and international level, detection of pesticide residues at these levels still remains a challenge (Sassolas et al. 2012; Dhull et al. 2013; Gahlaut et al. 2012).

Conventional analytical methods used to detect pesticide residues are high performance liquid chromatography, gas chromatography, thin layer chromatography, mass spectrometry, colorimetry and capillary electrophoresis (Sassolas et al. 2012; Dhull et al. 2013; Gahlaut et al. 2012). However, these analytical methods are expensive, time consuming and require trained manpower (Sassolas et al. 2012; Dhull et al. 2013; Gahlaut et al. 2012). As these pesticides have the ability to irreversibly (or reversibly) inhibit the serine hydroxyl group present in the active site of acetylcholinesterase (AChE) enzyme (Dhull et al. 2013), considerable attention has been given to the development of AChE electrochemical biosensor for the detection of pesticides in ground water (Sassolas et al. 2012; Dhull et al. 2013; Gahlaut et al. 2012). Different pesticide residue has

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diverse mode of degree of inhibition to AChE enzyme activity, which can be utilized as an indicator to select the highly toxic pesticide residue.

In this work, four pesticides namely, captan, 2,3,7,8-tetrachlorodibenzodioxin (TCDD), pentachlorophenol (PCP) and carbosulfan were considered for the investigation of sensitivity and inhibition efficiency test. As per the report of World Health Organization allowed daily intake of carbosulfan, captan and TCDD in human is 0.01, 0.1 mg kg day^{-1} and 1–4 pg kg day^{-1} respectively. According to European Union, the maximum concentration level of PCP in drinking water is 1.87 nM. People exposed to high concentrations of captan experienced eye irritation, including burning, itching and tearing. Owing to this reason, Singh et al. (2009) developed sensor for the detection of captan within the range of 0.831–53.228 μM in contaminated water. On the other hand, low concentrations of carbosulfan, TCDD and PCP affect immune response and central nervous system. Hence micromolar concentration of captan and nanomolar concentration of carbosulfan, TCDD and PCP were considered in the design of AChE inhibition based electrochemical biosensor.

Although a number of works on the inhibition of AChE enzyme activity for the detection of pesticides in ground water have been reported, there were few reports on electrochemical sensor studies of comparison of multi-pesticide inhibition efficiency by estimating the maximum percentage inhibition (I_{max}%) and inhibitor Michaelis–Menten constant (K_M^I).

As the inhibition curves followed Michaelis–Menten relationship, Du et al. (2007) correlated inhibition efficiency of carbaryl, malathion, dimethoate and monocrotophos to AChE enzyme activity with the K_M^I value, which was calculated from the Lineweaver–Burk equation. In addition, Du et al., [Du et al. 2008] also correlated sensitivity to AChE with degree of inhibition. For sensitivity and inhibition efficiency calculation, Du et al., considered I_{max}% and K_M^I. Since practical pesticide biosensor has lower and upper detection limit, it is necessary to consider I_{max}% and I_{min}% for the accurate estimation of inhibition efficiency and sensitivity. Considering these three parameters, a mathematical model for comparison of inhibition efficiency and sensitivity was developed.

Materials and Methods

Carbosulfan, captan, TCDD, PCP, zinc acetate, acetylthiocholine chloride (ATChCl) (≥99 % purity) with molecular weight of 19,773 g M^{-1} and AChE from electricus eel with specific activity (E.C. 3.1.1.7, activity: 200–1000 U mg^{-1}) were obtained from Sigma-Aldrich, USA. Chemicals namely dibasic sodium phosphate dehydrate, monobasic sodium phosphate monohydrate, 0.5 wt% chitosan in 1 % acetic acid (degree of deacetylation of 82.5 %, molecular weight: 140,000 g mol^{-1}), potassium hydroxide and sodium hydroxide were purchased from Merck India Ltd., India. KCl saturated Ag/AgCl reference electrode (CHI111, 0.5 mm diameter), Platinum (Pt) wire counter electrode (CHI115, 0.5 mm diameter) and Pt working electrode (CHI102, 2 mm diameter) were purchased from CH Instruments, Inc., USA. All solutions and reagents were prepared using deionized water (Millipore, USA).

The fabrication of modified electrodes to detect captan, carbosulfan, TCDD and PCP in tap water is as follows: 50 μL of chitosan and 1 mg of ZnO powder were added to 100 μL of phosphate buffered saline (PBS, pH 7.4) and sonicated at room temperature for 15 min. This was followed by the addition of 10 μL of AChE enzyme to the above mixture and the mixture was sonicated for less than a minute. Finally, 3 μL of this solution was taken and coated onto the Pt electrode's surface. The Pt/ZnO/AChE/Chitosan modified bioelectrode was used as a working electrode, Pt wire and Ag/AgCl saturated in 0.1 M KCl were used as the auxiliary and reference electrodes respectively. All the experiments were performed at room temperature. Optimization of experimental variables for pesticide biosensor is given in Table 1. In this work, the prepared ZnO particles used in the fabrication of Pt/ZnO/AChE/Chitosan exhibited mean diameters of 40, 163 and 240 nm which were employed as nanointerfaces for the determination of captan, carbosulfan, TCDD and PCP respectively. Due to the differences in the size of ZnO nanoparticles, the immobilized AChE amount varied in between 0.25 and 0.35 μU mL^{-1} which resulted in the change in number of catalytic active sites. This in-turn altered the rate of reaction which resulted in variation in the optimized experimental variables namely scan rate, incubation time, operating pH and saturating ATChCl.

As the enzyme inhibition curves were similar to Michaelis–Menten curve, the Michaelis–Menten equation can be rewritten as

$$I\% = \frac{I_{max}\%[I]}{K_M^I + [I]} \quad (1)$$

where, I% is the percentage inhibition, I_{max}% is the maximum percentage inhibition achieved by the linear sweep voltammetric system, [I] is the concentration of the inhibitor and K_M^I is the inhibitor concentration at which the degree of inhibition is 50 % of the I_{max}%.Dividing numerator and denominator of Eq. (1) on right hand side by I_{max}% and K_M^I, we get,

$$I\% = \frac{[I]}{\theta_1 + \theta_2[I]} \quad (2)$$

Table 1 Optimization of experimental variables for pesticide biosensor construction

Optimized variables	Captan	Carbosulfan	TCDD	PCP
Scan rate (Vs^{-1})	0.08	0.11	0.055	0.055
AChE amount ($\mu U\ mL^{-1}$)	0.25	0.30	0.35	0.35
pH	8.0	7.4	8.0	8.0
Saturating ATChCl (mM)	1.0	0.08	1.4	1.4
Incubation time (min)	15	20	30	30
Synthesis of ZnO nanoparticle				
$Zn(CH_3COO)_2$:NaOH	0.1:0.4	0.2:0.5	0.3:0.5	0.3:0.5
Annealing temperature	393 K for 6 h	393 K for 12 h & 1023 K for 4 h	423 K for 1 h, 333 K for 24 h & 823 K for 1 h	423 K for 1 h, 333 K for 24 h & 823 K for 1 h

$$I\% = \frac{\theta_3[I]}{1+\theta_4[I]} \tag{3}$$

where, $\theta_1 = \frac{K_M^I}{I_{max}\%}$, $\theta_2 = \frac{[I]}{I_{max}\%}$,$\theta_3 = \frac{I_{max}\%}{K_M^I}$ and $\theta_4 = \frac{1}{K_M^I}$. For simulation purpose, the Eqs. (1, 2, 3) are represented as modified Michaelis–Menten model 1, 2 and 3 respectively. In Eqs. (1, 2, 3), $I_{max}\%$ at saturating inhibitor concentration is fixed. However, it fails to fix lower detection limit (i.e., lowest inhibitor concentration required to inhibit immobilized enzyme ($I_{min}\%$)). Hence modified Michaelis–Menten model 1, 2 and 3 with $I_{min}\%$ as an intercept is (Eqs. 1, 2, 3) included.

$$I\% = \frac{I_{max}\%[I]}{K_M^I+[I]} + I_{min}\% \tag{4}$$

$$I\% = \frac{[I]}{\theta_1+\theta_2[I]} + I_{min}\% \tag{5}$$

$$I\% = \frac{\theta_3[I]}{1+\theta_4[I]} + I_{min}\% \tag{6}$$

For simulation purpose, the Eqs. (4, 5 and 6) are represented as modified Michaelis–Menten model 4, 5 and 6 respectively. When $[I] = K_M^I$, Eq. (4) becomes

$$I\% = \frac{I_{max}\% + 2I_{min}\%}{2} \tag{7}$$

It is expected that K_M^I is equal to the concentration of inhibitor $[I]$ when $I\% = \frac{I_{max}\%-I_{min}\%}{2}$. However, the Eq. (4) gives $I\% = \frac{I_{max}\%+2I_{min}\%}{2}$ when $[I] = K_M^I$, which may lead to inaccurate estimation of K_M^I. Hence for the precise estimation of K_M^I and $I_{max}\%$, the Eq. (4) is rewritten as,

$$I\% = \frac{[I]}{K_M^I+[I]}(I_{max}\% - I_{min}\%) + I_{min}\% \tag{8}$$

where, K_M^I is the concentration of inhibitor $[I]$ at $I\% = \frac{I_{max}\%-I_{min}\%}{2}$. For simulation purpose, the Eq. (8) is represented as modified Michaelis–Menten model 7. Usually, an enzyme could access the pesticide in linear fashion only when the pesticide concentration is below the Michaelis–Menten constant. When K_M^I is much greater than $[I]$, $K_M^I + [I]$ is approximately equal to K_M^I.Substituting $K_M^I + [I] \approx K_M^I$and rearranging Eqs. (1, 4 and 8),

$$I\% = \frac{I_{max}\%}{K_M^I}[I] \tag{9}$$

$$I\% = \frac{I_{max}\%}{K_M^I}[I] + I_{min}\% \tag{10}$$

$$I\% = \frac{I_{max}\% - I_{min}\%}{K_M^I}[I] + I_{min}\% \tag{11}$$

where, $\frac{I_{max}\%}{K_M^I}$, $\frac{I_{max}\%}{K_M^I}$ and $\frac{I_{max}\%-I_{min}\%}{K_M^I}$ estimated from Eqs. (1, 4 and 8) represent theoretical sensitivity of the immobilized enzyme towards different pesticides in which theoretical sensitivity refers to the ratio of maximum change in degree of inhibition to Michaelis–Menten constant.

The inhibition efficiency of a pesticide (Eq. 12) can be defined as the ability of a pesticide to inhibit the active sites of enzyme to a greater extent so as to affect the binding of substrate with the active sites of enzyme. In other words, it is the efficiency of a pesticide in inhibiting the conversion of substrate to product.

$$Inhibition\ efficiency(\%) = \frac{Practical\ sensitivity}{Theoretical\ sensitivity} \times 100 \tag{12}$$

Substituting $theoretical\ sensitivity = \frac{I_{max}\%}{K_M^I}$ in Eq. (12), we get,

$$Inhibition\ efficiency = \frac{Practical\ sensitivity}{I_{max}\%/K_M^I} \times 100 \tag{13}$$

Similarly, substituting $theoretical\ sensitivity = \frac{I_{max}\%-I_{min}\%}{K_M^I}$ in Eq. (12), we get,

$$Inhibition\ efficiency = \frac{Practical\ sensitivity}{(I_{max}\% - I_{min}\%)/K_M^I} \times 100 \quad (14)$$

From the Eqs. (13 and 14), it is observed that the *Inhibition efficiency* can be calculated by knowing the values of $I_{max}\%$, $I_{min}\%$ and K_M^I. By knowing the values of $I_{max}\%$, $I_{min}\%$, $I\%$ and K_M^I, the unknown pesticide [*I*] concentration in tap water sample can be estimated from Eqs. (1, 4, 8).Rearranging the Eq. (1) to solve for [*I*],

$$[I] = \frac{I\%K_M^I}{I_{max}\% - I\%} \quad (15)$$

In order to determine [*I*] from Eq. (4), we need to rearrange Eq. (4):

$$[I] = \frac{(I\% - I_{min}\%)K_M^I}{I_{max}\% + I_{min}\% - I\%} \quad (16)$$

Similarly, making rearrangements in Eq. (8) to calculate [*I*] gives us,

$$[I] = \frac{(I\% - I_{min}\%)K_M^I}{I_{max}\% - I\%} \quad (17)$$

Results and Discussion

Under optimized conditions, the inhibition of captan (1 nM), carbosulfan (1 nM), TCDD (1 nM) and PCP (1 nM) on the electrochemical response of different sizes of ZnO nanoparticles modified Pt bioelectrodes (Pt/ZnO/AChE/Chitosan) was studied (Fig. 1). Captan, carbosulfan, TCDD and PCP pesticides showed high degree of inhibition in Pt/ZnO of 40 nm/AChE/Chitosan, Pt/ZnO of 164 nm/AChE/Chitosan and Pt/ZnO of 240 nm/AChE/Chitosan bioelectrodes respectively. Hence in this work, Pt/ZnO of 40 nm/AChE/Chitosan, Pt/ZnO of 164 nm/AChE/Chitosan and Pt/ZnO of 240 nm/AChE/Chitosan bioelectrodes were employed for the detection of captan, carbosulfan, TCDD and PCP respectively.

Schematic illustration of AChE catalysis and the principle of pesticide determination is shown in Scheme 1. The Pt/ZnO/AChE/Chitosan electrode converted acetylthiocholine to thiocholine and acetic acid. However, in the presence of pesticide, the proposed Pt/ZnO/AChE/Chitosan bioelectrode detected captan, carbosulfan, TCDD and PCP based on the inhibition of immobilized AChE enzyme by these pesticides using cyclic voltammetry. After inhibition, the residual signal was recorded and the degree of inhibition was calculated to estimate inhibition efficiency and sensitivity.

Figure 2a–d shows the effects of captan, carbosulfan, TCDD and PCP on the response of Pt/ZnO/AChE/Chitosan bioelectrode. With an increasing concentration of captan, carbosulfan, TCDD and PCP, the linear sweep voltammetric response decreased linearly in the range of 0.05–6 μM, 10–30, 3–13 and 1–10 nM and then tended to a stable value. The decrease in current density was due to the inhibition of pesticide on AChE enzyme activity, which reduced the interaction between AChE enzyme and acetylthiocholine chloride. Thus the hydrolysis rate was affected. The calculated percentage inhibition can be related to the interaction between pesticide and AChE enzyme. The inhibition curves were similar to Michaelis–Menten behaviour. Hence Lineweaver–Burk, Hanes-Woolf, Eadie-Hofstee, Eadie-Scatchard and modified Michaelis–Menten models (1–7) were chosen in the following experiments for sensitivity and inhibition efficiency analysis. The fitting of Michaelis–Menten models with experimental data was carried out using Levenberg–Marquardt algorithm. Based on statistical and physical criteria, the most appropriate model was selected.

Akaike information criterion (AIC) test for modified Michaelis–Menten model comparison was performed to find out which modified Michaelis–Menten model was the best fit for the same data set. In the case of captan (AIC = 6.4), TCDD (AIC = 7.7) and PCP (AIC = 7.2), the best fit was observed in modified Michaelis–Menten model 7, as they showed low AIC value. Similarly, in the case of carbosulfan, the best fit was noticed in modified Michaelis–Menten model 1, 2 and 3 with AIC value of 2.6. Moreover, 83.7 % and 50–100 %, 99.7–100 %, 89.7–100 % of Akaike weight observed in modified Michaelis-Model 1, 2, 3 (for carbosulfan) and 7 (for captan, TCDD and PCP) also indicated

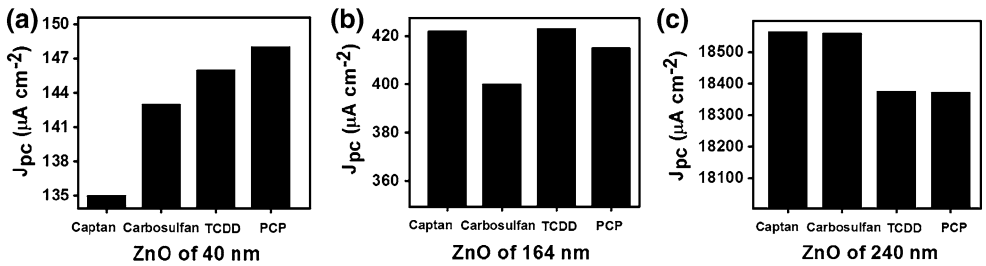

Fig. 1 The inhibition of captan (1 nM), carbosulfan (1 nM), TCDD (1 nM) and PCP (1 nM) on the electrochemical response of different sizes of ZnO nanoparticles modified Pt bioelectrodes (Pt/ZnO/AChE/Chitosan) under optimized conditions

Scheme 1 Schematic illustration of AChE catalysis and the principle of pesticide determination

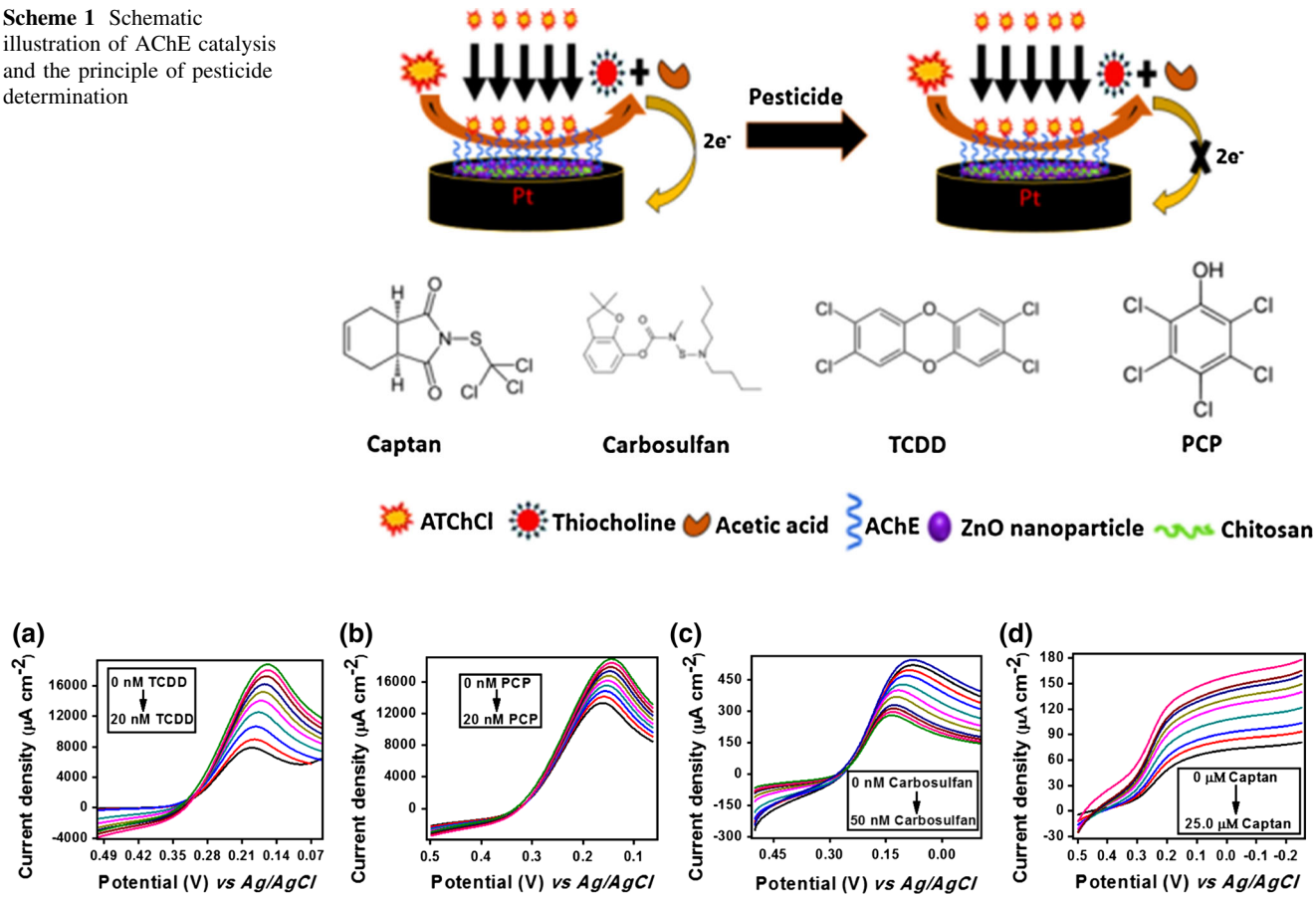

Fig. 2 Effect of **a** TCDD, **b** PCP, **c** carbosulfan and **d** captan on the response of Pt/ZnO/AChE/Chitosan bioelectrode

that the fitted models were correct. Even though Akaike information criterion test results showed best fit of modified Michaelis–Menten model 1, 2 and 3 in carbosulfan dataset, the precise and accurate estimation of K_M^I have remained limited. The limitation in the accurate estimation of K_M^I was because of the absence of I_{min}% in the Michaelis–Menten models 1, 2 and 3. Hence for accurate K_M^I estimation, Michaelis–Menten model 7 was considered.

The K_M^I values calculated using Michaelis–Menten model 7 for captan, carbosulfan, TCDD and PCP were found to be 7.9×10^3, 14.5, 10.2 and 10.9 nM respectively. Their corresponding inhibition efficiency %, I_{max}%, theoretical and practical pesticide sensitivities determined from the inhibition curves were 55 %, 20.9 %, 35.3 % & 47.8 %, 100 %, 47.7 %, 72.7 % and 68.1 %, 11.3×10^{-3}, 3.5, 6.4 and 5.8 % nM^{-1} and 6.2×10^{-3}, 0.7, 2.2 and 2.8 % nM^{-1} respectively.

It can be observed that TCDD exhibited the lowest K_M^Ivalue, which indicated that there was a good affinity between AChE enzyme and TCDD pesticide. Owing to the good affinity between AChE enzyme and TCDD, the immobilized AChE enzyme was highly sensitive towards TCDD. Even though the immobilized AChE enzyme was highly sensitive towards TCDD, the TCDD pesticide couldn't inhibit the serine hydroxyl group of AChE completely. Whereas, captan inhibited the active sites of immobilized AChE enzyme completely. These results also showed that captan had the higher ability to inhibit the active sites of AChE enzyme when compared with other pesticides. Due to the high AChE enzyme inhibition by captan, the catalytic electrochemical reaction of thiocholine at the surface of ZnO/AChE modified Pt electrode got affected to a greater extent, which in-turn resulted in lower electrochemical response. All these results clearly demonstrated that captan is the best inhibiting pesticide.

The predictive ability of modified Michaelis–Menten models is shown by 100 % recovery and low root mean square error of cross validation (RMSECV) value, which resulted in low relative prediction error (RPE) value. In addition, to assess the accuracy of modified Michaelis–Menten models, correlation coefficient (r) between the added and predicted concentrations of captan, carbosulfan, TCDD and PCP was also compared. Only the modified Michaelis–Menten models 4, 5, 6 and 7 (for captan detection: recovery = 103.5 %, RMSECV = 0.3, RPE = 2.3×10^{-2} and r = 0.9 and for carbosulfan detection: recovery = 100.2 %,

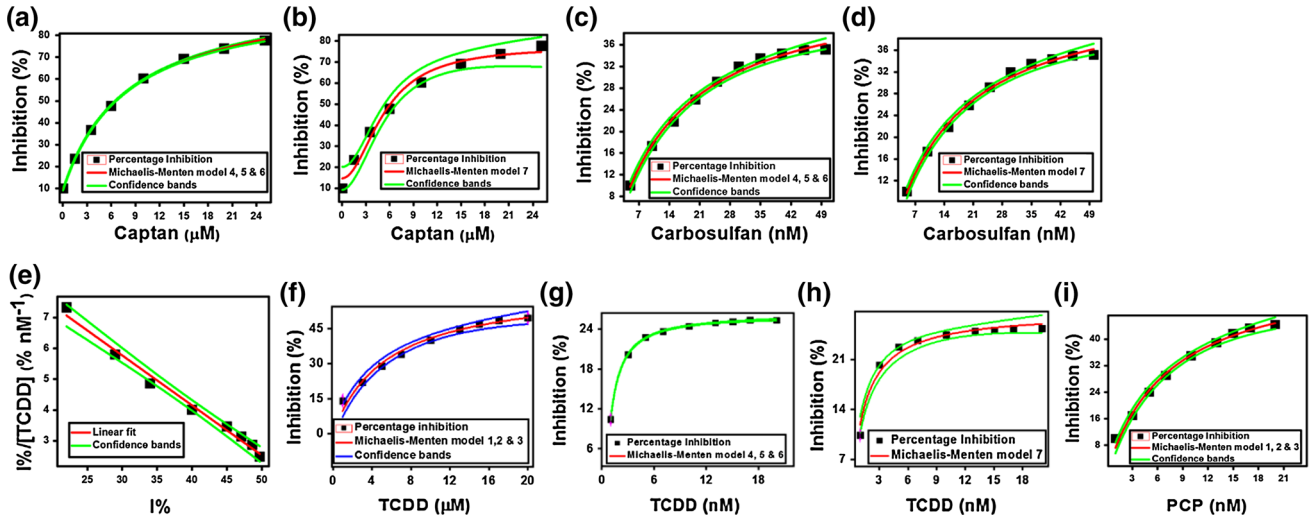

Fig. 3 Calibrated inhibition curves for captan, carbosulfan, TCDD and PCP: **a** modified Michaelis–Menten models 4, 5, 6 and **b** 7 for captan detection, **c** modified Michaelis–Menten models 4, 5, 6 and **d** 7 for carbosulfan detection, **e** Eadie-Scatchard model, **f** modified Michaelis–Menten models 1, 2, 3, **g** 4, 5, 6 and **h** 7 for TCDD detection and **i** modified Michaelis–Menten models 1, 2 and 3 for PCP detection

RMSECV = 2.2, RPE = 7.1×10^{-2} and r = 0.9), Eadie-Scatchard (for TCDD detection; recovery = 107.6 %, RMSECV = 0.5, RPE = 4.3×10^{-2} and r = 0.9) and modified Michaelis–Menten models 1, 2 and 3 (for PCP detection; recovery = 104.2 %, RMSECV = 0.4, RPE = 3.8×10^{-2} and r = 0.9) gave best results in validation (Fig. 3a–i). The calibrated equations are,*For captan detection:*

$$I\% = \frac{90.8[captan]}{8.0 + [captan]} + 9.3 \quad \text{or} \quad I\% = \frac{[captan]}{8.0 + [captan]}(90.7) + 9.3 \tag{18}$$

For carbosulfan detection:

$$I\% = \frac{51.0[carbosulfan]}{14.6 + [carbosulfan]} - 3.2 \quad \text{or} \quad I\% = \frac{[carbosulfan]}{14.6 + [carbosulfan]}(51.0) - 3.2 \tag{19}$$

For TCDD detection:

$$I\% = \frac{65.6[TCDD]}{6.2 + [TCDD]} \tag{20}$$

For PCP detection:

$$I\% = \frac{62.0[PCP]}{7.6 + [PCP]} \tag{21}$$

The Pt/ZnO/AChE/Chitosan bioelectrode detected TCDD, PCP, carbosulfan and captan over a range of 1–20 nM, 1–20 nM, 5–50 nM and 0.05–25 μM with a detection limit of 1, 1, 5 and 50 nM respectively. All these results clearly showed that the proposed calibrated models (Fig. 2a–f) were reliable for the quantification of captan, carbosulfan, TCDD and PCP in local tap water samples. In order to estimate intra-assay precision and accuracy, three replicates of local tap water samples at five different carbosulfan, captan, TCDD and PCP concentration were measured on the same day (Table 2). The captan, carbosulfan, TCDD and PCP concentrations in local tap water sample were estimated using the calibrated equations (Eqs. 22–25).*For captan detection:*

$$[Captan] = \frac{(I\% - 9.3)8.0}{100 - I\%} \tag{22}$$

For carbosulfan detection:

$$[Carbosulfan] = \frac{(I\% - 3.2)14.6}{47.7 - I\%} \tag{23}$$

For TCDD detection:

$$[TCDD] = \frac{6.2I\%}{65.6 - I\%} \tag{24}$$

For PCP detection:

$$[PCP] = \frac{7.6I\%}{62.0 - I\%} \tag{25}$$

As shown in Table 2, the recoveries obtained for captan, carbosulfan, TCDD and PCP varied in the range of 99.0 %–103.2 %, 99.7 %–100.2 %, 99.8 %–100.2 % and 99.7 %–100.1 % respectively, showing that the calibrated models are reliable for the practical detection captan, carbosulfan, TCDD and PCP. In order to study the accuracy of the calibrated models, correlation between added and predicted concentrations of captan, carbosulfan, TCDD and PCP was compared. A correlation coefficient of 0.9 suggested that

Table 2 Determination results of AChE biosensors after spiking 2, 4, 6, 8 and 10 μM concentrations of captan, carbosulfan, TCDD and PCP in tap water samples (n = 3)

Pesticide	Recovery (%)	RSD (%)	RMSECV	RPE (%)
Captan	99.0–103.2	0.1–2.2	0–0.1	0.2–3.1
Carbosulfan	99.7–100.2	0–0.1	$1.0 \times 10^{-2} - 3.0 \times 10^{-2}$	0.1–0.2
TCDD	99.8–100.2	4.9×10^{-2}–0.2	$1.0 \times 10^{-2} - 3.0 \times 10^{-2}$	$6.9 \times 10^{-2} - 0.2$
PCP	99.7–100.1	4.6×10^{-2}–0.2	$1.0 \times 10^{-2} - 3.0 \times 10^{-2}$	$6.4 \times 10^{-2} - 0.2$

there existed a linear correlation between added and predicted concentrations of captan (for captan: $Captan_{predicted}$ [μM] = 1.0 $Captan_{added}$ [μM] + 3.5×10^{-2}), carbosulfan (for carbosulfan: $Captan_{predicted}$ [μM] = 1.0 $Captan_{added}$ [μM] + 3.5×10^{-2}), TCDD (for TCDD: $TCDD_{predicted}$ [μM] = 1.0 $TCDD_{added}$ [μM] + 13.9×10^{-2}) and PCP (for PCP: $PCP_{predicted}$ [μM] = 1.0 PCP_{added} [μM] − 4.0×10^{-2}). In all the four cases, the slope was approximately equal to one indicating that the predicted and added pesticide concentrations were equal.

In addition, student's paired *t* test at 95 % confidence interval between the added and predicted concentrations of captan, carbosulfan, TCDD and PCP was conducted. The critical tabulated t-value was higher than the calculated experimental t values. All these results showed that there were no significant differences between the added and predicted concentrations of captan, carbosulfan, TCDD and PCP. Moreover, RPE and RMSECV of added captan, carbosulfan, TCDD and PCP in local tap water samples were 0.2 %–3.1 % & 0 %–0.1 %, 0.1 %–0.2 % & $1.0 \times 10^{-2} - 3.0 \times 10^{-2}$, $6.9 \times 10^{-2} - 0.2$ % & $1.0 \times 10^{-2} - 3.0 \times 10^{-2}$ and $6.4 \times 10^{-2} - 0.2$ % & $1.0 \times 10^{-2} - 3.0 \times 10^{-2}$ respectively, suggesting acceptable accuracy of the proposed calibrated models.

A relative standard deviation of 0.1–2.2, 0–0.1, $4.9 \times 10^{-2} - 0.2$ and $4.6 \times 10^{-2} - 0.2$ % was observed for captan, carbosulfan, TCDD and PCP, indicating satisfactory degree of reproducibility of the proposed calibrated models. In order to evaluate the precision of the calibrated models, F-test was used at 95 % confidence interval. F-test results exhibited that there were no significant differences between the standard deviation of mean predicted and added concentrations of captan, carbosulfan, TCDD and PCP. Thus it was concluded that the calibrated models can detect captan, carbosulfan, TCDD and PCP in local tap water samples with high accuracy and precision.

To the best of our knowledge, there is no report on sensitivity (unit: % M^{-1}) for TCDD, PCP and carbosulfan biosensors based on enzyme inhibition. On the other hand, Singh et al. (2009) reported sensitivity for captan biosensor. It can be seen that the proposed captan biosensor exhibited low sensitivity (sensitivity = 6.2×10^{-3} % nM^{-1}) than the previously reported captan biosensor based on glutathione-s-transferase inhibition (sensitivity = 8.6×10^{-3} % nM^{-1}). Even though the sensitivity of the developed captan was lower than the previously reported captan biosensor based on glutathione-s-transferase inhibition, the captan pesticide inhibited active sites of AChE enzyme to a greater extent ($I_{max}\% = 100$) than that of in the active sites of glutathione-s-transferase enzyme ($I_{max}\% = 72$).

A mathematical model for inhibition efficiency and sensitivity was successfully developed. Based on this method, pesticide quantification has been made simple while inhibition efficiency and sensitivity analysis have been improved. This novel method was also applied for the quantification of pesticide residues in tap water samples. The recovery results suggested a good quantification of pesticide in tap water. The proposed method could be extended to other inhibitors for their applications in developing sensors based on enzyme inhibition. The developed method could be applied to screening of the naturally clean samples, like tap, spring water.

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