Asian Journal of Chemistry; Vol. 27, No. 7 (2015), 2445-2451

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.17912

Interaction of Chlorpyrifos with Purine Bases: A Study of Electrochemical Measurement and Density Functional Theory Calculation

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Received: 29 April 2014; *Accepted*: 30 June 2014; *Published online*: 30 March 2015; AJC-17053

Interactions of chlorpyrifos with the purine bases involving guanine, adenine and xanthine were investigated, using cyclic voltammetry, anodic differential pulse voltammetry, electrochemical impedance spectroscopy and density functional theory calculations. Bonding constants of the chlorpyrifos-purine base complexes were calculated. The global density functional theory descriptors including chemical potential, electronegativity and electrophilicity index were employed to evaluate the chemical reactivity of chlorpyrifos and purine bases. The condensed Fukui function was calculated to reveal the reactive sites of chlorpyrifos and purine bases. The interaction energies between chlorpyrifos and the purine bases were also calculated. Chlorpyrifos interacts as an electrophile with guanine, adenine and xanthine, showing a certifiable toxicity to the purine bases. The interactions of chlorpyrifos with the purine bases follow the order of guanine > adenine > xanthine. Compared with other two chlorpyrifos-purine base complexes, the chlorpyrifos-guanine complex exhibits larger bonding constant, higher charge transfer and more negative interaction energy.

Keywords: Chlorpyrifos, Purine base, Interaction, Electrochemical measurement, Density functional theory.

INTRODUCTION

Organophosphate (OP) pesticides have played an important role in agriculture to control the major insect pests, chlorpyrifos (CPF), *i.e.* O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate being one of the effective common. chlorpyrifos has been widely used throughout the world[1-3], to control the insect pests of crops, fruit, vegetable and ornamental plants, as well as to control flies, mosquitoes and household pests. The excessive use of chlorpyrifos results in the widespread existence in environments[4,5]. A major pathway for unintentional introduction of chlorpyrifos into aquatic ecosystems is surface runoff from fields treated by this kind of organophosphate[6]. Human exposure to low levels of chlorpyrifos *via* various pathways such as ingestion of contaminated food and water, inhalation of particles and contact with agricultural residues[7-9], has also been quantified.

Chlorpyrifos can impact a non-target toxicity to aquatic organisms and mammals[10,11]. Chlorpyrifos was reported to be safe for humans with low doses because of no obvious symptoms[12]. However, it should be noted that chlorpyrifos poses a much more danger to children than adults. Children in the womb exposed to chlorpyrifos have an increased risk of mental delays and an increased occurrence of pervasive developmental disorders[13]. Chlorpyrifos, like other organophosphates, is well known that the primary toxicity is due to the ability to inhibit the acetylcholinesterase activity, adverse effect on synthesis of biomacromolecules and the interaction with neurotransmitter receptors[14]. Chlorpyrifos can induce the formation of reactive oxygen species[15], thereby increasing genomic instability and contributing to the initiation as well as the progression of cancer[16]. Furthermore, chlorpyrifos has exhibited hepatotoxicity, nephrotoxicity and immunological abnormalities[17,18].

Up to now, numerous investigations of chlorpyrifos toxicity have extensively focused on dietary exposure studies of aquatic organisms and small animals. Besides the disadvantage of time-consuming, another major drawback of such investigation is that they reveal little about mechanism toxicity of chlorpyrifos. Compared with the dietary exposure studies, the electrochemical techniques such as cyclic voltammetry (CV) and anodic differential pulse voltammetry (ADPV), have become applicable for understanding the interactions of pollutants with biomacromolecules[19,20]. The electrochemical impedance spectroscopy (EIS) technique can be applied to detect biochemical molecules[21,22]. Based on the changes of electrochemical impedance spectra, EIS may be suitable for elucidating the poison of hazardous substances[23]; whereas, few studies of EIS technique for the interactions between hazardous materials and biomolecules were reported. In addition, density

functional theory (DFT) calculations have been confirmed to be useful in determining the structural and energetic properties of isolated molecules[24]. Particularly, DFT chemical reactivity parameters involving chemical potential (μ), electronegativity (χ), electrophilicity index (ω) and condensed Fukui function (FF) are competent for elucidating interactions between molecules and biomolecules[24,25]. Among the conventional DNA nitrogen base biomolecules, guanine and adenine have been confirmed to be more sensitive to pollutants than thymine and cytosine[26,27]. Therefore, the purine bases as the chosen target, are suitable for evaluating the toxicities of environmental pollutants. To our best of knowledge, little attention has been paid to the interaction of chlorpyrifos with the purine bases considering the mentioned-above three electrochemical techniques combined with DFT simulations.

The aim of this study is to extend the application of electrochemical techniques for evaluating the toxicities of pollutants and to shed light on some aspects of the molecular mechanism of potential toxicity of chlorpyrifos. In this research, cyclic voltammetry (CV), anodic differential pulse voltammetry (ADPV) and electrochemical impedance spectroscopy (EIS) were used to illustrate the interactions of chlorpyrifos with guanine, adenine and xanthine. The DFT reactivity descriptors μ, χ and ω were considered to explore the chemical reactivity of chlorpyrifos, guanine, adenine and xanthine. The local reactivity index of condensed Fukui function (f_k^+ for electrophilic attack and f_k^- for nucleophilic attack) was also calculated to reveal the reactive sites of the four molecules. Furthermore, the contributions of charge transfer (ΔN) and interaction energies (ΔE) to the reactivity of chlorpyrifos with the purine bases at 298 K were calculated. To some extent, we believe that this work will be of benefit for the conception of an interaction model of biomolecule with this organophosphate pesticide which would be valuable for further studies on the genotoxicity of organophosphates.

EXPERIMENTAL

Reagents of chlorpyrifos (CPF), guanine, adenine and xanthine were of analytical grade and were used as received. Chlorpyrifos and the purine bases were dissolved using anhydrous ethanol and 1 mol/L NaOH, respectively. The concentrations of stock solutions containing chlorpyrifos and purine bases were 1×10^{-3} and 5×10^{-3} mol/L. The working solutions were prepared by diluting the stock solution to appropriate volumes with a 100 mmol/L phosphate buffer solution. All chemicals and reagents were supplied by Jingchun Scientific Co., Ltd. (Shanghai, China).

Electrochemical measurements: Electrochemical measurements were carried out at 298 K under atmospheric pressure, using an electrochemical workstation (CHI 650 C, Chenhua Co. Ltd., Shanghai, China). A three stand electrode cell was employed in the electrochemical investigations. A glassy carbon with a diameter of 2 mm was used as working electrode; a Ag/AgCl electrode and a platinum foil served as reference electrode and auxiliary electrode, respectively. The working electrode was polished with silicon carbide paper (grit #1000, Kailai Abrasive Technology Co. Ltd., Qingdao, China) for 1-2 min and rinsed with deionized water.

All aqueous samples were prepared using a buffer solution of 100 mmol/L phosphate and 10 mmol/L NaCl adjusted to pH 8 with 1 mol/L NaOH. The solutions were deaerated by purging with pure nitrogen for 0.5 h. Cyclic voltammetry was done from -1.5 to 1.5 V at a scan rate of 100 mV/s, anodic differential pulse voltammetry (ADPV) between -1.5 and 1.5 V at 50 mV/s. The electrochemical impedance spectroscopy (EIS) measurements were performed at a 1 V anodic polarization potential. The employed amplitude of the sinusoidal signal was 5 mV and the tested frequencies ranged from 10^5 to 10^{-2} Hz. EIS measurement was carried out from high frequency to low frequency. All measurements were performed in triplicate.

Computational details: All calculations for density functional theory (DFT) were performed with Materials Studio Dmol^3 package (version 4.1, Accelrys Inc., San Diego, USA)[28]. Structural optimizations were performed at the generalized gradient approximation level with the spin unrestricted approach. Double numerical plus polarization functions and the becke exchange functional in conjunction with the Lee-Yang-Parr correlation functional were employed. All electron as the core treatment pattern was used. Pulay's direct inversion of iterative subspace technique and a small electron thermal smearing value of 0.005 Hartree were employed to accelerate self consistent field convergence. The convergence tolerance of energy and the self consistent field tolerance were set as 1×10^{-5} Hartree and 1×10^{-6}, respectively. The optimized structures of chlorpyrifos and the purine bases are shown in Fig. 1. The values of condensed Fukui function (FF) for chlorpyrifos and the purine bases were calculated using Mulliken population analysis. The Mulliken atomic charges were assessed by the technique of electrostatic potential derived charges. For the study of chlorpyrifos-purine base complexes, a conductor-like screening model (COSMO)[28] was used for the simulation of aqueous interaction.

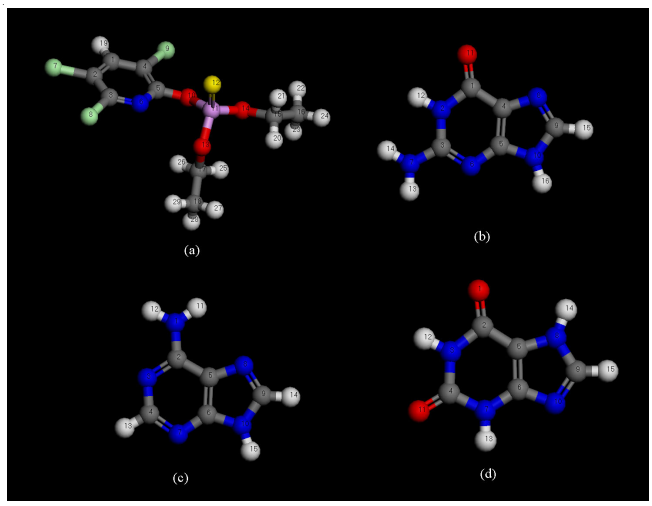


Fig. 1. Optimized structures of chlorpyrifos (CPF) and the purine bases with the atoms numbering scheme adopted in this study: Atom types are denoted by sequence number as follows: (a) chlorpyrifos (19-29 -hydrogen; 1-5,15-18-carbon; 10,13,14-oxygen; 6-nitrogen; 7-9-chlorine; 11-phosphorus; 12-sulfur); (b) guanine (12-16-hydrogen; 1,3,4,5,9-carbon; 11-oxygen; 2,6-8,10-nitrogen); (c) adenine (11-15-hydrogen; 2,4,5,6,9-carbon; 1,3,7,8,10-nitrogen); (d) xanthine (12-15-hydrogen; 2,4-6,9-carbon; 1,11-oxygen; 3,7,8,10-nitrogen)

RESULTS AND DISCUSSION

Cyclic voltammetry (CV): The cyclic voltammetry curves of guanine solutions with and without the existence of chlorpyrifos are shown in Fig. 2a. As indicated in Fig. 2a, an oxidation peak at 680 mV with a peak current of 0.72 mA/cm^2 for the pure guanine solution can be observed, corresponding to the oxidation of -NH_2 group[29]. Compared with that of the pure guanine solution, however, the cyclic voltammetry curve of chlorpyrifos-guanine solution shows a decrease in current density of 0.24 mA/cm^2 and a negative shift in potential of 61 mV. The changes of current densities and potentials for chlorpyrifos-adenine and chlorpyrifos-xanthine mixed solutions (Fig. 2b and c) are similar to that of chlorpyrifos-guanine solution. The coexistence of chlorpyrifos reduces the current densities of adenine and xanthine solutions by 23.81 and 17.59 %, respectively.

The potential change, an indicator of the percentage of interaction calculated by eqn. 1[26], was used to evaluate the influences of chlorpyrifos on the purine bases. Herein, E_{base} and $E_{CPF\text{-}base}$ are the potentials of the free purine base and the chlorpyrifos-purine base complex.

$$\% \text{ Interaction} = \frac{E_{base} - E_{CPF\text{-}base}}{E_{base}} \times 100 \quad (1)$$

In comparison with free purine base solutions, the presence of chlorpyrifos induces potential shifts of guanine, adenine and xanthine solutions by 8.97, 8.04 and 4.44 %. Interactions of chlorpyrifos with the purine bases follow the descending order: guanine > adenine > xanthine. Chlorpyrifos shows a more remarkable affinity for guanine than other two purine bases, indicating the prominent toxicity to guanine.

Anodic differential pulse voltammetry (ADPV): The interactions of chlorpyrifos with purine bases were investigated by the anodic differential pulse voltammetry (ADPV) technique. All voltammetric titrations were performed by keeping the concentrations of purine bases as 100 µmol/L, while the concentrations of chlorpyrifos varied in the range of 0-1.6 µmol/L (Fig. 3a to c). The anodic peak current is related to the oxidation of amido group. In the chlorpyrifos-purine base mixed solution, the current titration equation was described by eqn. 2[30]:

$$\frac{1}{c_{CPF}} = K_f \frac{1 - A}{1 - (i / i_0)} - K_f \quad (2)$$

where, c_{CPF} is the concentration of chlorpyrifos, K_f is the bonding constant of the chlorpyrifos-purine base complex. i_0 and i are the peak currents of the purine base without and with the presence of chlorpyrifos. A is a proportional constant. By plotting of $1/c_{CPF}$ *versus* $1-(i/i_0)$, K_f of chlorpyrifos interacting with the purine bases can be obtained.

K_f of chlorpyrifos bonding with guanine, adenine and xanthine is 3.23×10^6, 7.95×10^5 and 5.17×10^5, respectively. Thus, chlorpyrifos exhibiting a stronger toxicity to guanine than adenine and xanthine. In addition, it should be noted that

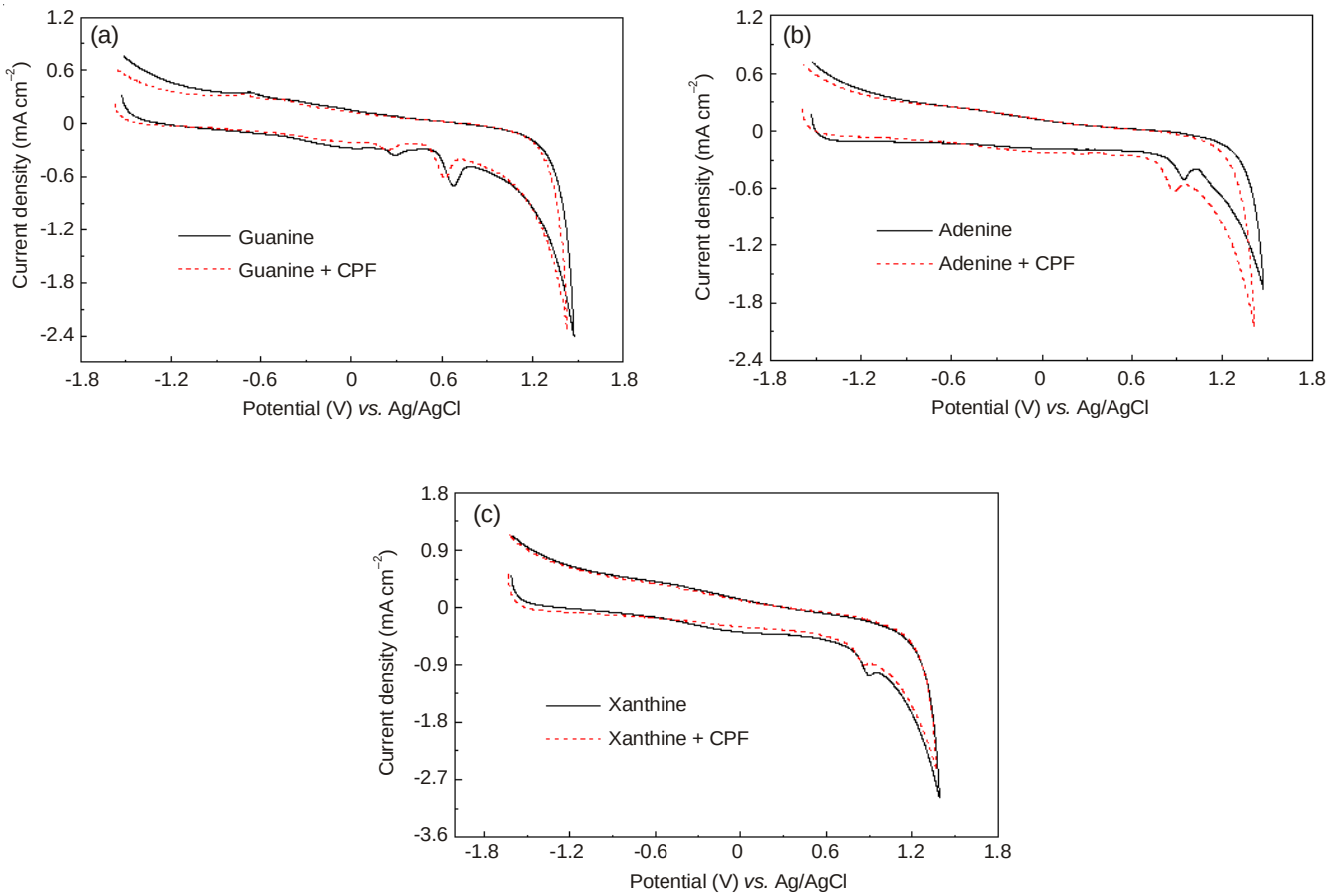


Fig. 2. Cyclic voltammograms of the chlorpyrifos-purine base solutions: (a) Chlorpyrifos-guanine; (b) Chlorpyrifos-adenine; (c) Chlorpyrifos-xanthine. pH: 8; c(guanine) = c(adenine) = c(xanthine) = 100 µmol/L; c(CPF) = 1.2 µmol/L; Temperature: 298 K; scan rate: 100 mV/s

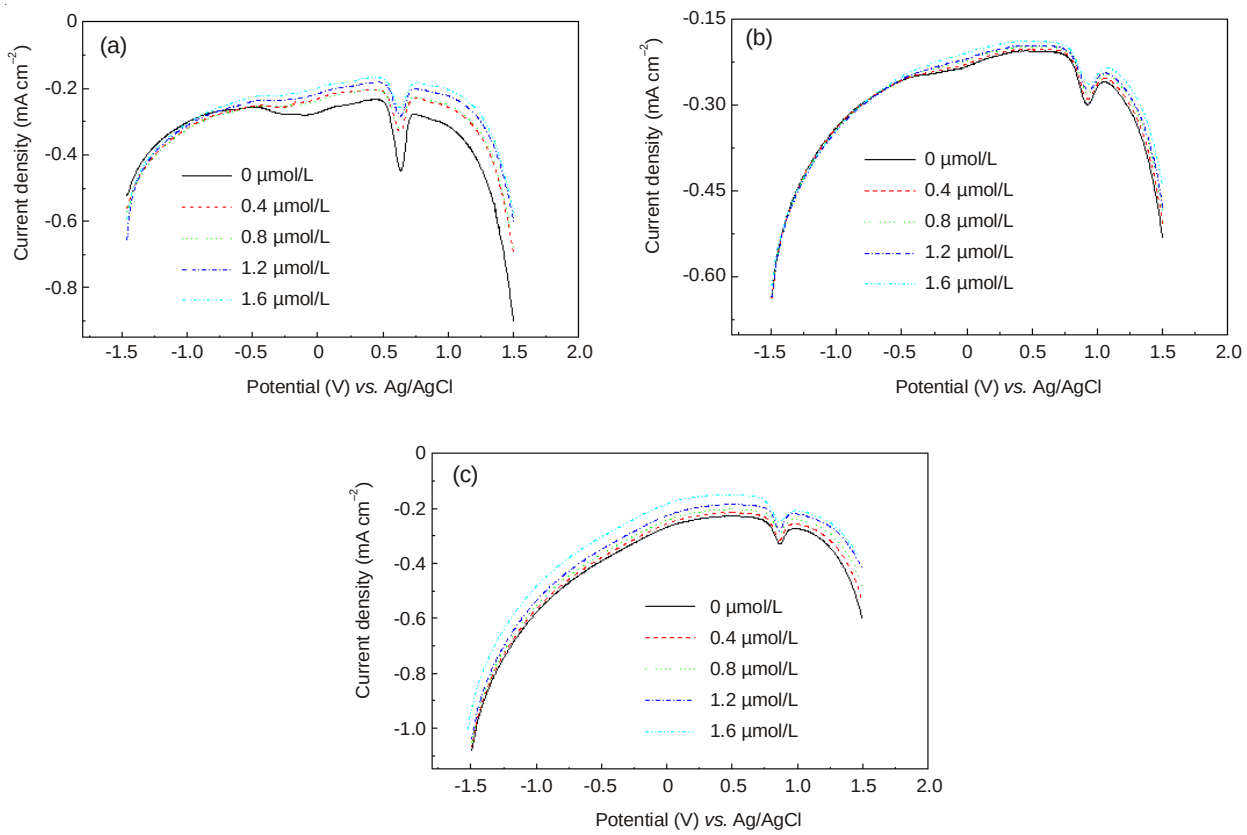


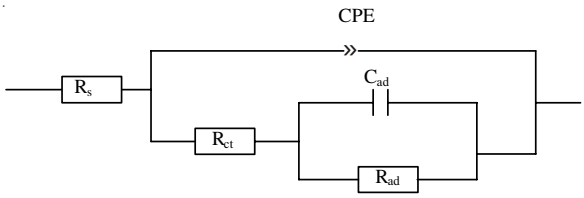
Fig. 3. Anodic differential pulse voltammograms of the chlorpyrifos-purine base solutions: (a) chlorpyrifos-guanine; (b) chlorpyrifos-adenine; (c) chlorpyrifos-xanthine. pH: 8; c(guanine) = c(adenine) = c(xanthine) = 100 μmol/L; c(CPF) = 0-1.6 μmol/L; Temperature: 298 K; scan rate: 50 mV/s

ADPV plots have a linear correlation coefficient (R^2) higher than 0.98, suggesting that chlorpyrifos interacts with the three purine bases to form the 1:1 complexes[30].

Electrochemical impedance spectroscopy (EIS): The EIS data of purine base solutions without and with the presence of chlorpyrifos (Fig. 4) were fitted using a modified randles equivalent circuit shown in **Scheme-I**. In this model, a constant phase element (CPE) was introduced to model the electrical double layer of the electrode/electrolyte interface. The necessity of CPE in place of pure capacitance is due to the dispersed distribution of Nyquist diagrams in high frequency domain[31]. The double layer capacitance (C_{dl}) of the electrode was estimated using eqn. 3[32]:

$$C_{dl} = Y_0 \times (R_s^{-1} + R_{ct}^{-1})^{n-1} \quad (3)$$

where, Y_0 regarded as a capacity parameter, is the CPE coefficient; the dimensionless CPE exponent (n) is related to the constant phase angle. R_s and R_{ct} are the solution resistance and the charge transfer resistance. C_{ad} and R_{ad} are the adsorption capacity and resistance, respectively.



Scheme-I: Modified Randles electrical equivalent circuit compatible with the Nyquist diagrams shown in Fig. 4

The values of the circuit elements obtained by fitting the experimental results are shown in Table-1. Compared with the values of R_{ct} and R_{ad}, the solution resistance (R_s) can be ignored. The values of n are in the ranges of 0.87-0.94, suggesting the capacity characteristic of CPE. In the chlorpyrifos-contained guanine solution, in comparison with the free guanine solution, R_{ct} and R_{ad} increase considerably by 43.42 and 19.29 %. On the contrary, the double layer capacitance (C_{dl}) and adsorption capacity (C_{ad}) reduce from 2.14 to 1.42 mF/cm^2 and from 1.65 to 1.24 mF/cm^2, corresponding to a 33.64 and 24.85 % decrease, respectively. The existent chlorpyrifos shows a more

TABLE-1
ANALYZED PARAMETERS OF ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY (EIS)

System	R_s (Ω cm^2)	Y_0 (mS s cm^{-2})	n	C_{dl} (mF cm^{-2})	R_{ct} (kΩ cm^2)	C_{ad} (mF cm^{-2})	R_{ad} (kΩ cm^2)
Guanine	7.62	1.08	0.94	2.14	2.81	1.65	2.54
Adenine	8.86	0.47	0.91	1.26	6.44	1.28	5.93
Xanthine	8.69	0.41	0.90	1.15	8.84	1.11	8.16
CPF-guanine	7.41	0.67	0.92	1.42	4.03	1.24	3.03
CPF-adenine	8.46	0.32	0.88	1.21	7.07	1.25	6.31
CPF-xanthine	8.77	0.24	0.87	1.07	9.86	1.04	9.28

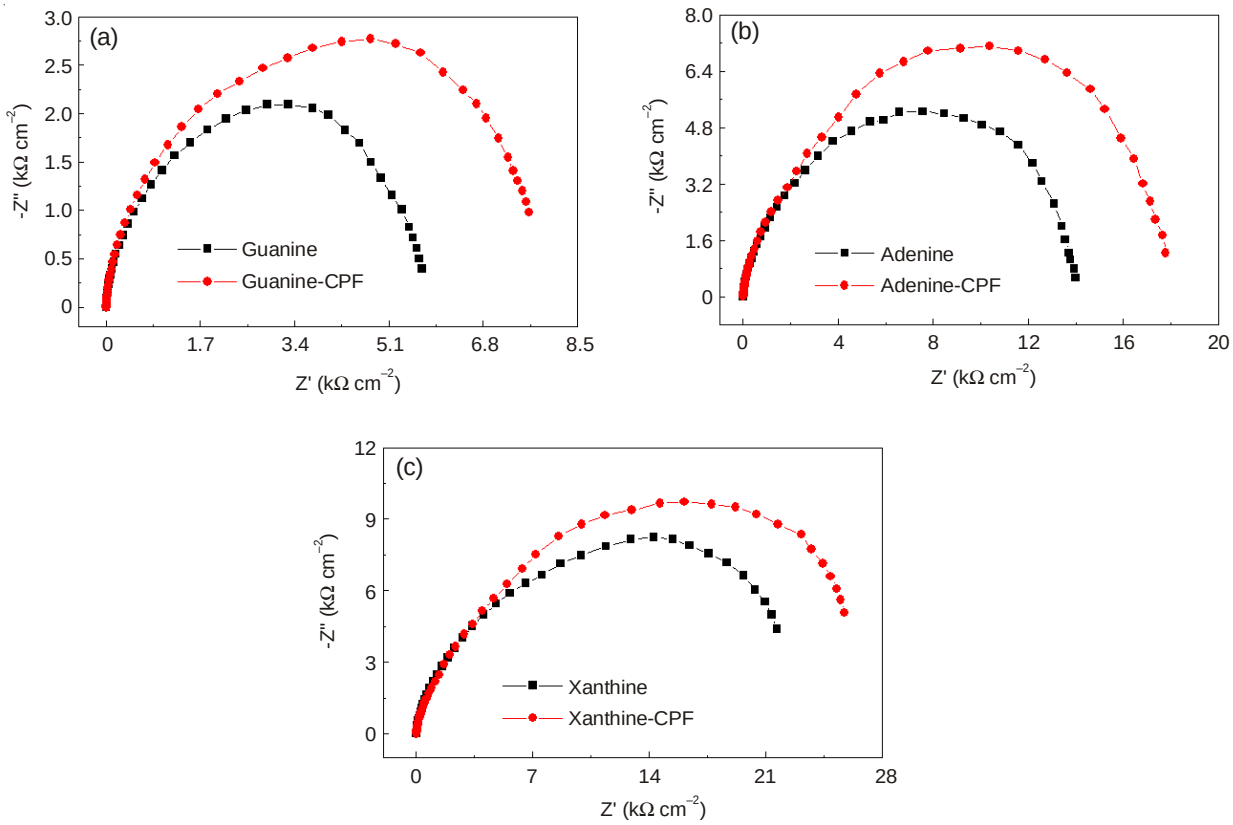


Fig. 4. Electrochemical impedance spectrum of the chlorpyrifos-purine base solutions: (a) chlorpyrifos-guanine; (b) chlorpyrifos-adenine; (c) chlorpyrifos-xanthine. pH: 8; anodic polarization potential: 1 V; c(guanine) = c(adenine) = c(xanthine) = 100 μmol/L; c(CPF) = 1.2 μmol/L; Temperature: 298 K

notable effect on the process of charge transfer than the adsorption process. EIS spectra of adenine and xanthine solutions with the presence of chlorpyrifos (Fig. 4b and c) show the similar trends to that of chlorpyrifos-guanine mixed solution. As chlorpyrifos was added to adenine and xanthine solutions, the increases of R_{ct} and R_{ad} and the decreases of C_{dl} and C_{ad} for both two purine base solutions are observed. Therefore, the interactions of chlorpyrifos with adenine and xanthine are also confirmed. In comparison with EIS spectra of adenine and xanthine solutions, it should be noted that guanine shows smaller resistances of charge transfer and adsorption.

In the chlorpyrifos-containing mixed solutions, rates of heterogeneous charge transfer and adsorption for the purine bases at the electrode/electrolyte interface are hindered, because of the presence of chlorpyrifos. With the presence of chlorpyrifos, the decreases of charge transfer of the three purine base solutions are consistent with the shift of peak potential and decreasing the peak current in CV and ADPV, indicating that an intermolecular action between chlorpyrifos and the purine base takes place. The existent chlorpyrifos exerts a more negative effect on guanine than adenine and xanthine for the charge transfer at the electrode/solution interface.

DFT calculations

Global DFT indices: Calculated energies of HOMO, LUMO and the band gaps ($\Delta E_{LUMO-HOMO}$) of chlorpyrifos, guanine, adenine and xanthine are shown in Table-2. Chlorpyrifos shows a strong ability of accepting electrons because of the lowest LUMO energy; whereas, guanine shows a strong capacity of donating electrons due to the highest HOMO energy. Within the conceptual framework of DFT, the chemical activity descriptors of chemical potential (μ), electronegativity (χ) and the electrophilicity index (ω) of chlorpyrifos and the three purine bases, were calculated using eqns. 4-6[33,34]:

$$\mu = \frac{(E_{HOMO} + E_{LUMO})}{2} \quad (4)$$

$$\chi = -\mu = -\frac{(E_{HOMO} + E_{LUMO})}{2} \quad (5)$$

$$\omega = \frac{(E_{HOMO} + E_{LUMO})^2}{4(E_{LUMO} - E_{HOMO})} \quad (6)$$

where, E_{HOMO} and E_{LUMO} are the energies of the highest occupied and the lowest unoccupied molecular orbitals

TABLE-2
CALCULATED HOMO, LUMO, BAND GAP ($\Delta E_{LUMO-HOMO}$), CHEMICAL POTENTIAL (μ), ELECTRONEGATIVITY (χ), AND ELECTROPHILICITY (ω) IN HARTREE FOR CHLORPYRIFOS, GUANINE, ADENINE, AND XANTHINE

Molecule	E_{HOMO}	E_{LUMO}	$\Delta E_{LUMO-HOMO}$	μ	χ	ω
Chlorpyrifos	-0.2233	-0.0931	0.1302	-0.1582	0.1582	0.1922
Guanine	-0.1884	-0.0511	0.1373	-0.1198	0.1198	0.1044
Adenine	-0.1995	-0.0585	0.1410	-0.1290	0.1290	0.1180
Xanthine	-0.2097	-0.0756	0.1341	-0.1426	0.1426	0.1517

(HOMO and LUMO). The electronegativity (χ) as shown in Table-2, decreases in the order of chlorpyrifos > xanthine > adenine > guanine. Hence, during the interactions, chlorpyrifos acts as an electrophile (electron acceptor); while guanine, adenine and xanthine serve as the nucleophiles (electron donors).

It well known that a molecule with the high value of electrophilicity index (ω) shows the great propensity of attracting electrons[34], so ω can be used to quantify the tendency of a molecule to soak up electrons. The calculated values of ω (Table-2) follow the order of chlorpyrifos > xanthine > adenine > guanine, indicating that chlorpyrifos owns an obvious capacity of accepting electrons. On the contrary, among the purine bases, guanine shows the strongest ability of contributing electrons. The differences in χ and ω between chlorpyrifos and guanine are prominent. Thus, it can be inferred that chlorpyrifos shows a more notable intermolecular action with guanine than adenine and xanthine.

DFT index of charge transfer (Δ*N*): The DFT indices of μ, χ and ω are related to the properties of an isolated molecule. However, the DFT index of charge transfer (Δ*N*) defined by eqn. 7, will be helpful to get a complete picture of interaction between two molecules[35].

$$\Delta N = \frac{(E^{B}_{HOMO} + E^{B}_{LOMO} - E^{A}_{HOMO} - E^{A}_{LUMO})}{2(E^{A}_{LUMO} + E^{B}_{LUMO} - E^{A}_{HOMO} - E^{B}_{HOMO})} \quad (7)$$

where, the superscripts of A and B were used to describe the reactants A and B, respectively. If $\Delta N < 0$, electrons flow from A to B, *i.e.*, A acts as the electron donor and B as electron acceptor. Furthermore, a high absolute value of Δ*N* between two molecules indicates the strong interaction of them[35].

Δ*N* Values of chlorpyrifos interacting with guanine, adenine and xanthine are -0.1437, -0.1077 and -0.0588. The negative values of Δ*N* indicate that guanine, adenine and xanthine act as the nucleophiles; chlorpyrifos serves as an electro-phile. The absolute value of Δ*N* between chlorpyrifos and guanine ($\Delta N_{guanine \rightarrow chlorpyrifos}$) is 1.33- and 2.44-fold levels of $\Delta N_{adenine \rightarrow chlorpyrifos}$ and $\Delta N_{xanthine \rightarrow chlorpyrifos}$. Therefore, the ability of chlorpyrifos interacting with the purine bases is in the following order: guanine > adenine > xanthine.

Condensed Fukui function: The condensed Fukui function (FF) can describe the reactivity of an atom in a molecule: an atom with a higher Fukui function value indicates more reactivity than others[24]. In general, two kinds of condensed Fukui function for an atom k in a molecule, namely f_k^+ and f_k^-, can be obtained. A site with maximum values of f_k^+ and f_k^- can be considered as the active attack site for nucleophile or electrophile. f_k^+, f_k^- and atomic charges of chlorpyrifos, guanine, adenine and xanthine were calculated. It should be mentioned that all hydrogen atoms are not considered because of the small absolute Fukui function values of them. The negative Fukui function value of an atom can be assigned that the electron density is depleted or accumulated from this particular site[34]. Compared with that of other atoms in chlorpyrifos molecule, the electrophilic characteristic of C_1 atom is most prominent (Table-3). Therefore, in the interactions of chlorpyrifos with guanine, adenine and xanthine, C_1 atom will be attacked by the purine bases. As indicated in Table-4, N_6 atom of guanine, N_1 atom of adenine and O_{11} atom of xanthine are suitable for the sites of chlorpyrifos attack, because of the high values of f_k^- and the negative atomic charges of them.

TABLE-3
CALCULATED VALUES OF f_k^+, f_k^- AND ATOMIC CHARGE FOR CHLORPYRIFOS

Atom	f_k^+	f_k^-	Atomic charge
C_1	0.178	0.021	0.044
C_2	0.023	0.025	-0.018
C_3	0.091	0.028	-0.133
C_4	0.056	0.026	-0.063
C_5	0.068	0.024	0.467
N_6	0.107	0.010	-0.298
Cl_7	0.093	0.066	-0.061
Cl_8	0.115	0.064	-0.066
Cl_9	0.111	0.066	-0.057
O_{10}	0.019	0.036	-0.617
P_{11}	-0.006	0.056	1.560
S_{12}	0.043	0.429	-0.689
O_{13}	0.006	0.018	-0.622
O_{14}	0.003	0.027	-0.631
C_{15}	-0.001	-0.016	0.110
C_{16}	0.000	0.000	-0.194
C_{17}	-0.005	-0.007	0.103
C_{18}	0.001	0.000	-0.196

TABLE-4
CALCULATED VALUES OF f_k^+, f_k^- AND ATOMIC CHARGE FOR GUANINE, ADENINE, AND XANTHINE

Atoms of the purine bases		f_k^+	f_k^-	Atomic charge
Guanine	C_1	0.170	0.053	0.434
	N_2	0.035	0.016	-0.403
	C_3	0.043	0.050	0.531
	C_4	0.026	0.104	0.053
	C_5	0.095	0.062	0.354
	N_6	0.067	0.113	-0.483
	N_7	0.033	0.101	-0.360
	N_8	0.089	0.072	-0.433
	C_9	0.102	0.100	0.216
	N_{10}	0.021	0.030	-0.307
	O_{11}	0.149	0.111	-0.569
Adenine	N_1	0.073	0.157	-0.356
	C_2	0.115	0.048	0.347
	N_3	0.045	0.073	-0.456
	C_4	0.116	0.060	0.196
	C_5	0.043	0.084	0.107
	C_6	0.045	0.061	0.355
	N_7	0.102	0.106	-0.454
	N_8	0.068	0.069	-0.441
	C_9	0.136	0.085	0.225
	N_{10}	0.031	0.029	-0.301
Xanthine	O_1	0.144	0.107	-0.540
	C_2	0.151	0.049	0.454
	N_3	0.028	0.015	-0.404
	C_4	0.033	0.050	0.602
	C_5	0.038	0.131	0.127
	C_6	0.098	0.079	0.368
	N_7	0.032	0.082	-0.372
	N_8	0.057	0.048	-0.280
	C_9	0.139	0.088	0.240
	N_{10}	0.053	0.062	-0.433
	O_{11}	0.053	0.119	-0.546

Interaction energies (Δ*E*): The interaction energies (Δ*E*) between chlorpyrifos and the purine bases at 298 K were

calculated using eqn. 8, to illustrate the comparative stabilities of the chlorpyrifos-purine base complexes.

$$\Delta E = E(\text{CPF-purine base}) - E(\text{CPF}) - E(\text{purine base}) \quad (8)$$

where, E(X) is the COSMO-corrected total energy of species (X). The calculated interaction energies (ΔE) of chlorpyrifos bonding with guanine, adenine and xanthine are -4.98, -4.37 and 1.04 kJ/mol. Stabilities of the chlorpyrifos-purine base complexes are in the order of chlorpyrifos-guanine > chlorpyrifos-adenine > chlorpyrifos-xanthine. chlorpyrifos shows a stronger interaction with guanine than adenine and xanthine, indicating that chlorpyrifos exhibits a remarkable toxicity to guanine.

Compared with those toxic studies of aquatic organisms and small mammals exposed to organophosphate pesticides, the electrochemical measurements and DFT calculations with the advantages of convenience and time-saving, are competent for evaluating toxicities of organophosphates. Although this work was limited to investigate the interactions of chlorpyrifos with some simple biomolecules, we confirm that chlorpyrifos has shown a certifiable toxicity to the functional biomolecules. Based on the results of this research, it should be mentioned that the further study of chlorpyrifos toxicity to protein biomolecules such as bovine serum albumin and human serum albumin deserves to be considered.

Conclusions

The interactions of chlorpyrifos with guanine, adenine and xanthine were investigated using cyclic voltammetry, anodic differential pulse voltammetry, electrochemical impedance spectroscopy and DFT calculations. The conclusions are listed as follows:

• The data of electrochemical measurements are in accordance with the results of DFT simulations, showing that the combined technique of them is suitable for evaluating the interactions between organophosphate pesticides and biomolecules.

• Chlorpyrifos acts as an electrophile in interactions with guanine, adenine and xanthine; conversely, the three purine bases serve as the nucleophiles. C_1 atom of chlorpyrifos is assigned to the reactive site for the electrophilic interaction with the purine bases.

• Chlorpyrifos shows a certifiable toxicity to the functional biomolecules, which is confirmed by the electrochemical measurements and DFT calculations. The toxicity of chlorpyrifos to the purine bases follows the order: guanine > adenine > xanthine.

ACKNOWLEDGEMENTS

This work was supported by Hebei Provincial Natural Science Foundation of China (Grant No. B2012203037).

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