Int. J. Electrochem. Sci., 5 **(2010)** 1587 - 1596

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Electrochemical Investigation of DNA Interaction with Melamine and its Related Compounds (MARCs)

Hao-Yu Shen[*], *Hui-Min Zheng, Ning Zhu, Yang-Qing Liu, Jing Gao, Jia Li*

Biological and Chemical Engineering School, Ningbo Institute of Technology, Zhejiang University; Ningbo, 315100, China
[*]E-mail: hyshen@nit.zju.edu.cn

Received: 28 August 2010 / *Accepted:* 15 September 2010 / *Published:* 1 November 2010

The interaction of melamine (MM) and its related compounds (MARCs), *i.e.* Ammeline (AMN), Ammelide (AMD), Cyanuric acid (CA) with native Herring Sperm DNA (HS-DNA) has been investigated by cyclic voltammetric studies at glassy carbon electrode (GCE) in phosphate buffer (PBS at pH 7.0). It was observed that the electrochemical processes of the 4 MARCs at a bare GCE were quasireversible. By adding HS-DNA into the MARCs solutions, a decrease in reduction peak current of MARCs with a slight negative shift in peak potential values was observed. The reduction peak current (I_{pc}) of any of the MARCs, either in absence or presence of HS-DNA, varied linearly with scan rate (v) rather than $v^{1/2}$, which indicated that the electrode process was controlled by adsorption step. The number of electrons transferred per molecule (n) of MM or the other three MARCs was calculated to be *ca.* 2. Electrochemical parameters (charge transfer coefficient α and the standard rate constant k_s) of free MARCs and binding compounds were obtained according to Laviron theory. The interactions between MARCs and HS-DNA were presumed in groove mode with the interaction constants to be 10^4~10^5 L·mol^{-1}.

Keywords: Melamine (MM) and its related compounds (MARCs), herring sperm DNA (HS-DNA), cyclic voltammetry, groove mode

1. INTRODUCTION

Melamine (MM) is a triazine-based widely used industrial chemical. The transform of melamine and its related compounds, ammeline (AMN), ammelide (AMD), and cyanuric acid (CA) normally can be realized by hydrolysis [1, 2]. The dissociation constants pK_a are 5.0 for MM, 4.5 and 9.4 for AMN, 1.8, 6.9, and 13.5 for AMD, and 6.9 for CA, respectively [1, 2]. Melamine (MM) and its related compounds (MARCs) were assumed to be of equal potency and would be referred to

collectively in the assessment as melamine compounds by the US Food and Drug Administration (FDA) [1].

Since it contains 66.6% of nitrogen by mass, MM had been added to raw material, such as wheat gluten, rice protein, even infant formula and milk products, to fake high protein contents by some illegitimate merchants. The 2007's pet food incident and the following year's milk powder scandal were notorious typical examples. Although, the clinic symptoms of the sufferers with melamine poisoning are related to kidney stones or bladder urolithiasis, other potential harmful effects are still uncertain [1-3]. A study performed by FDA described the risk to human health associated with eating products from animals that have been fed with MARCs [1]. High dosage of MM has shown to be carcinogenic for male rats [4]. Although great attention has recently been paid to establish analytic methods for the determination of MARCs, whether or not there are some interactions bewteen MARCs and DNA have not been reported.

The interaction between DNA and other molecules is important in life sciences and has attracted considerable interest, because it is related to the replication and transcription of DNA *in vivo*, mutation of genes and related variations of species in character, action mechanism of DNA-targeted drugs, *etc* [5-12]. Recently, researches on the interaction between DNA and some harmful chemicals, such as enviromental pollutants, pesticides, *etc,* gradually become hot topics as a main way for the investigation of DNA damage, as well as the understanding of toxic mechanism [6, 9, 12]. A variety of methods, such as gel electrophoresis [13], footprinting technique, X-ray crystallography [14], fluorescence [15], UV/visible spectroscopy [16], NMR [17], *etc*., have been used to investigate this interaction. Recently, electrochemical method has become more and more widely used for the investigation of the DNA interaction as it has been testified to be of high sensitivity, relatively low cost, direct monitoring and simplicity [18-26]. Equilibrium constants (K) for the interaction of the small moleculars with DNA can be obtained from shifts in peak potentials or currents, and the number of base pair sites involved in binding (s) *via* intercalative, electrostatic, or hydrophobic interactions can be obtained from the dependence of the current passed during oxidation or reduction of the bound species on the amount of added DNA. In some cases it should also be possible to obtain kinetic data from current and potential mesurements [27, 28]. Moreover, investigation of the electrochemical behavior of small molecule compounds by means of electrochemical techniques has the potential for providing valuable insights into the redox reaction of these molecules in living body [29].

In this work, the interactions of native Herring Sperm DNA (HS-DNA) with melamine (MM) and its related compounds (MARCs), *i.e.* Ammeline (AMN), Ammelide (AMD), Cyanuric acid (CA) have been investigated by cyclic voltammetric studies at glassy carbon electrode (GCE) in phosphate buffer (PBS at pH 7.0). The results showed that the electrochemical processes of the 4 MARCs, either in absence or presence of HS-DNA, were adsorption controlled quasireversible. The charge transfer coefficient α and the standard rate constant k_s of free MARCs and binding compounds were obtained by kinetic studies. The interaction constants between MARCs and HS-DNA were found to be 10^4~10^5 $L \cdot mol^{-1}$. It was presumed that the MARCs could interact with DNA by groove mode, which were consisted with our previous findings obtained *via* spectrophotometric, spectrofluorometric, melting temperature (T_m), and viscosimetric techniques [30, 31]. We hope this work will provide some useful

information for the evaluation of the safety performance of MARCs through understanding their interaction with DNA.

2. EXPERIMENTAL

2.1. Chemicals and reagents

Melamine (MM) and cyanuric acid (CA) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), while ammeline (AMN) and ammelide (AMD) were purchased from Yan Shing Industrial Co., Ltd. (Tokyo, Japan). The range of purity was 96-99%.

Herring Sperm DNA was purchased from Shanghai Dechemical Co., Ltd (Shanghai, China). The stock solution of DNA was prepared by dissolving DNA in 0.05 mol/L of phosphate buffer (PBS) at pH 7.0 (the mixture solution of 0.05 mol/L of Na_2HPO_4 and NaH_2PO_4) and dialyzing exhaustively against the same buffer for 24 h, and used within 5 days.

A solution of DNA gave a ratio of UV absorbance at 260 and 280 nm more than 1.8, indicating that DNA was sufficiently free from protein. The DNA concentration of the stock solution was determined by UV spectrophotometry, in properly diluted samples, using the molar absorption coefficient 6600 $L \cdot mol^{-1} \cdot cm^{-1}$ at 260 nm; the stock solution was stored at 4 ^oC. An individual stock solution for each compound containing 1×10^{-4} $mol \cdot L^{-1}$ MM or AMD, 4×10^{-4} $mol \cdot L^{-1}$ CA was prepared by dissolving an appropriate amount of the individual MARCs in PBS buffer, while AMN solution at concentration of 4×10^{-4} $mol \cdot L^{-1}$ was prepared by dissolving an appropriate amount of AMN in a 50:50 (v/v) mixture of ethanol and 0.05 $mol \cdot L^{-1}$ aqueous sodium hydroxide solution.

A fresh working solution was prepared daily by diluting the stock solution with PBS buffer and used for different studies.

2.2. Apparatus

The cyclic voltammetry studies were carried out by using LK-2006 electrochemical system (Lanlike Co. Ltd, Tianjing, China). Electrochemical cell consisted of a glass container with a cap having holes for introducing electrodes and nitrogen.

The cell was then maintained oxygen free by passing nitrogen over the solution. The reference electrode used was saturated calomel electrode (SCE), while the auxillary and working electrodes were platinum foil and glassy carbon electrode (GCE), respectively.

In a typical cyclic voltammetric experiment of the reaction mixture consisted of melamine (MM) or its related compounds (MARCs) and DNA in PBS buffer, a stream of nitrogen was passed over them and the reaction mixture was thermostated.

The three electrodes were connected to a computer controlled potentiostat and required potential scan rate, current sensitivity, initial potential and final potential were given and the resulting current was measured as a function of applied potential.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of MARCs and their interaction with HS-DNA in solution

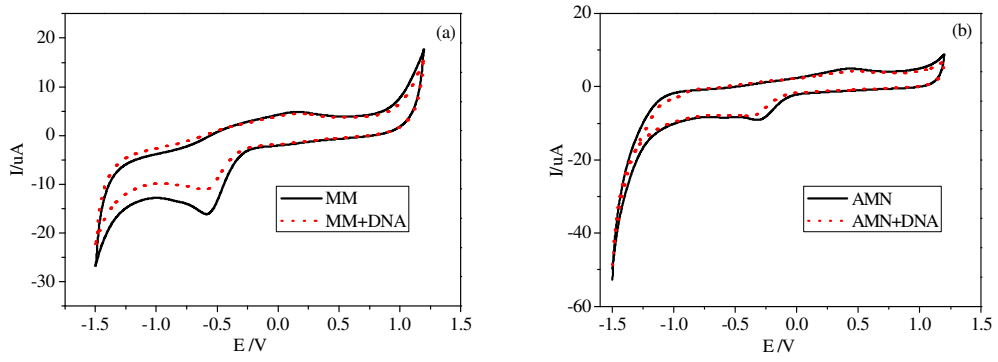
Typical cyclic voltammetric curves of the MARCs with concentration at 1.0×10^{-5} mol·L^{-1} for MM, 5.0×10^{-5} mol·L^{-1} for AMN, 1.0×10^{-5} mol·L^{-1} for AMD, and 5.0×10^{-5} mol·L^{-1} for CA, in absence or presence of HS-DNA (5.0×10^{-5} mol·L^{-1}) in 0.05 mol·L^{-1} PBS (pH 7.0) with a scan rate of 100 mV ·s^{-1} were shown in Figure 1.

A pair of redox peaks for any of the 4 MARCs in absence of HS-DNA appeared using a bare GCE in the range of -1.5 to +1.5 V (vs. SCE). The cathodic peak potential (E_{pc}) was at -0.59 V for MM, -0.32 V for AMN, -0.56 V for AMD, and -0.51 V for CA, with a scan rate of 100 mV ·s^{-1}, respectively, while the anodic peaks for all the 4 MARCs were not obvious. The reduction peak current of any of the 4 MARCs was obviously much higher than the oxidation peak current. The current ratio of the cathodic peak current to the anodic one (I_{pa}/I_{pc}) was 0.43, 0.69, 0.58, and 0.50, for MM, AMN, AMD, and CA, respectively. The separation between the anodic and cathodic peak potential ($\Delta E_p = |E_{pc} - E_{pa}|$) was 820 mV, 850 mV, 900 mV, and 800 mV, accordingly. These results indicated that the electrochemical processes of the 4 MARCs at a bare GCE were quasireversible.

As shown in Figure 1, when HS-DNA was added into the MARCs solutions, decreases in reduction peak currents of MARCs (from -16.44 μA to -11.17 μA for MM, -9.10 μA to -7.88 μA for AMN, -9.29 μA to -9.25 μA for AMD, and -11.85 μA to -10.63 μA for CA, respectively) with a slight negative shift in peak potential values (from -0.59 V to -0.60 V for MM, -0.32 V to -0.45 V for AMN, -0.56 V to -0.57 V for AMD, and -0.51 V to -0.57 V for CA, respectively) were observed.

3.2. Kinetic Investigation

Cyclic voltammetric experiments of the MARCs in absence and presence of HS-DNA in 0.05 mol·L^{-1} PBS (pH 7.0) with varied scan rate were recorded. The curves in presence of HS-DNA were taken as expamples shown in Figure 2. It was observed that the reduction peak current (I_{pc}) of any of the MARCs, either in absence or presence of HS-DNA, varied linearly with scan rate (v) rather than $v^{1/2}$. The results were summarized in Table 1, which indicated that the electrode processes were controlled by adsorption step [32].



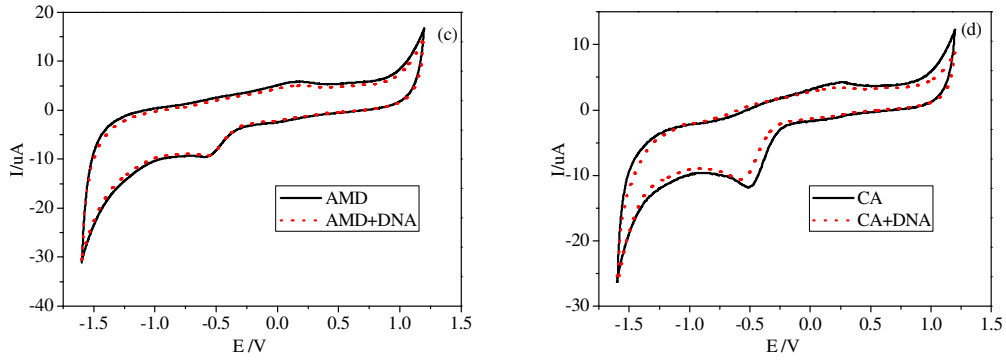


Figure 1. Cyclic voltametry (CV) of MARCs, MARCs-DNA system ((a) MM (b) AMN, (c) AMD, and (d) CA, c(MM) = 1.0×10^{-5} mol·L^{-1}, c(AMN) = 5.0×10^{-5} mol·L^{-1}, c(AMD) = 1.0×10^{-5} mol·L^{-1}, c(CA) = 5.0×10^{-5} mol·L^{-1}, c(DNA)=5.0×10^{-5} mol·L^{-1}, 0.05 mol·L^{-1} PBS (pH 7.0), with a scan rate of 100 mV ·s^{-1})

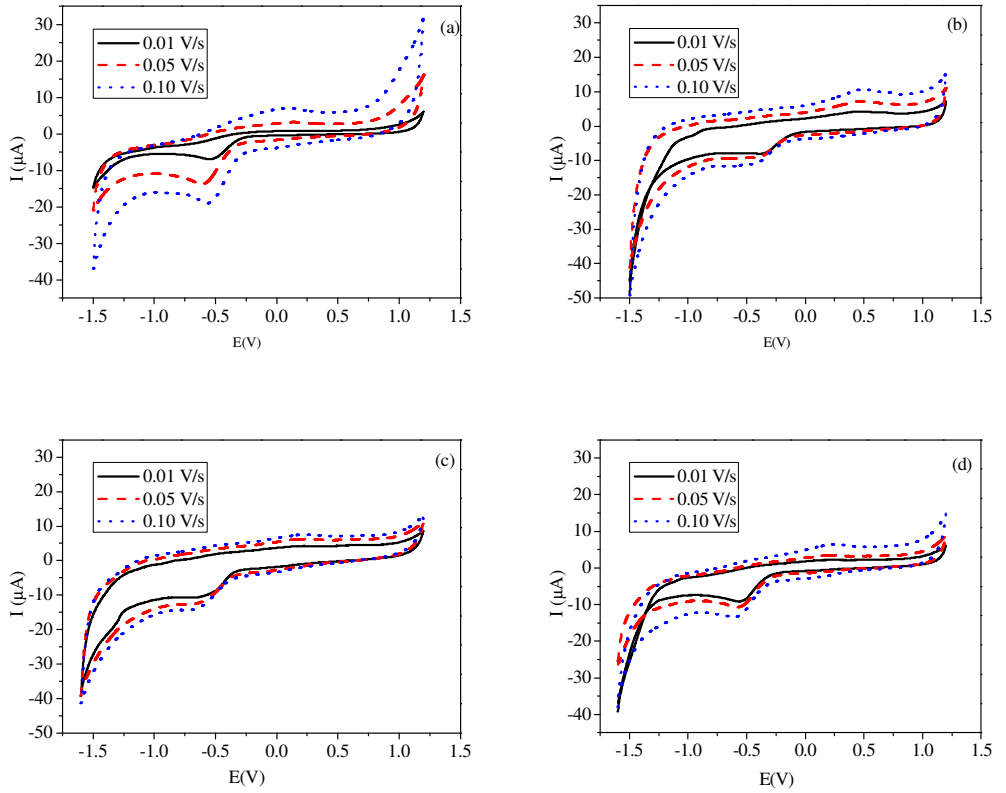


Figure 2 Cyclic voltametry (CV) of MARCs in presence of HS-DNA (c(DNA)=5.0×10^{-5} mol·L^{-1}) at different scan rate ((a) MM-DNA (b) AMN-DNA, (c) AMD-DNA, and (d) CA-DNA, , c(MM) = 5.0×10^{-5} mol·L^{-1}, c(AMN) = 5.0×10^{-5} mol·L^{-1}, c(AMD) = 1.0×10^{-5} mol·L^{-1}, c(CA) = 5.0×10^{-5} mol·L^{-1}, 0.05 mol·L^{-1} PBS (pH 7.0))

For an adsorption controlled quasireversible electrochemical process, the relationship between the the peak current (I_p) and the coulomb of adsorption (Q_a) obeys the following formula [33]:

$$I_p = n^2F^2A\Gamma v/4RT \quad (1)$$

$$\text{Since, } Q_a = nFA\Gamma \quad (2)$$

$$\text{Then, } I_p = nQ_aFv/4RT \quad (3)$$

where R is the universal gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$), T is the Kelvin temperature (T), F is the Faraday constant (96487 $C \cdot mol^{-1}$), n is the number of electrons transferred in reaction, A is the surface area of the working electrode (cm^2), v is the scan rate ($V \cdot s^{-1}$), Γ is the surface concentration of adsorption ($mol \cdot cm^{-2}$), Q_a is the coulomb in the process of adsorption (C), and I_p is the peak current (A).

By integrating the peak areas of the reduction peaks of the MARCs, Q can be obtained, therefore, the number of electrons transferred per molecule (n) of the MARCs were calculated as listed in Table 1. The number of electrons transferred per molecule (n) of any of the MARCs was found to be ~2.

Table 1. The relationship between I_p and v, the number of electrons transferred per molecule (n) of MARCs

MARC	v($V \cdot s^{-1}$)	I_p(μA)	$I_p \sim v$	Q (μC)	n	n_{av}
MM	0.01	-9.63	I_p = -129.17 v - 8.8893 R^2 = 0.9626	-49.72	1.99	2.05
	0.03	-12.35		-20.63	2.05	
	0.05	-16.44		-15.94	2.12	
	0.07	-18.73		-14.32	1.92	
	0.10	-20.88		-9.84	2.18	
AMN	0.01	-7.78	I_p = -26.696 v - 7.6245 R^2 = 0.9806	-42.51	1.88	2.04
	0.03	-8.42		-13.60	2.12	
	0.05	-9.10		-9.54	1.96	
	0.07	-9.62		-6.39	2.21	
	0.10	-10.15		-5.16	2.02	
AMD	0.01	-7.26	I_p = -51.873 v - 6.9959 R^2 = 0.9691	-36.75	2.03	2.06
	0.03	-9.25		-16.77	1.89	
	0.05	-9.29		-8.88	2.15	
	0.07	-10.73		-7.95	1.98	
	0.10	-12.14		-5.59	2.23	
CA	0.01	-6.66	I_p = -79.593 v - 7.488 R^2 = 0.9676	-33.72	2.03	2.02
	0.03	-10.25		-16.56	2.12	
	0.05	-11.85		-13.10	1.86	
	0.07	-13.40		-9.84	2.00	
	0.10	-14.96		-7.36	2.09	

As discussed above, the reduction peak current (I_{pc}) of any of the MARCs varied linearly with scan rate (v) rather than $v^{1/2}$, indicating that the electrode process was controlled by an adsorption step. According to Laviron theory [33], for an adsorption controlled process, the relationship between E_p and lg$v$ should obey the following formula.

$$E_p = E_p^{0'} + 2.3RT/\alpha nF[\lg(RT/nF)\, k_s\,] - 2.3RT/\alpha nF \lg v \qquad (4)$$

where E_p is the peak potential (V), $E_p^{0'}$ is the formal potential (V), which can be obtained from the intercept of the resulted lines by plotting of $E_p \sim v$ [32, 33], R is the universal gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$), T is the Kelvin temperature (T), F is the Faraday constant (96487 $C \cdot mol^{-1}$), n is the number of electrons transferred in reaction, k_s is the standard rate constant (s^{-1}), α is the charge transfer coefficient, v is the scan rate ($V \cdot s^{-1}$).

By plotting of $E_p \sim \lg v$, the charge transfer coefficient α and the standard rate constant k_s before and after adding HS-DNA can be obtained. The results were listed in Table 2.

Table 2. The main electrochemical kinetic parameters of the MARCs and MARCs-DNA systems

MARC /MARC-DNA	$E_p \sim v$	$E_p^{0'}$ (V)	$E_p \sim \lg v$	α	$k_s(s^{-1})$
MM	E_p = -1.0662 v - 0.5237 R^2 = 0.9504	-0.52	E_p = 0.1032 lg$v$ - 0.7222 R^2 = 0.986	0.28	0.26
MM-DNA	E_p = -0.8309 v - 0.6627 R^2 = 0.9793	-0.66	E_p = -0.1244 lg$v$ - 0.8668 R^2 = 0.9641	0.24	0.28
AMN	E_p = -0.5564 v - 0.2884 R^2 = 0.9701	-0.29	E_p = -0.1303 lg$v$ - 0.4805 R^2 = 0.9928	0.23	0.59
AMN-DNA	E_p = -1.34 v - 0.3008 R^2 = 0.9782	-0.3	E_p = -0.1535 lg$v$ - 0.5777 R^2 = 0.9884	0.2	0.23
AMD	E_p = -1.0804 v - 0.6624 R^2 = 0.9542	-0.52	E_p = -0.1041 lg$v$ - 0.8229 R^2 = 0.9806	0.28	0.26
AMD-DNA	E_p = -0.8305 v - 0.6627 R^2 = 0.9792	-0.66	E_p = -0.1254 lg$v$ - 0.8684 R^2 = 0.9669	0.24	0.42
CA	E_p = -0.5039 v - 0.487 R2 = 0.9913	-0.49	E_p = -0.0694 lg$v$ - 0.6051 R^2 = 0.9866	0.43	0.66
CA-DNA	E_p = -0.6544 v - 0.5357 R^2 = 0.9887	-0.54	E_p = -0.0874 lg$v$ - 0.6854 R^2 = 0.9787	0.34	0.5

3.3. Thermodynamic studies

For further investigation the intensity of the interaction between the MARCs and DNA, cyclic voltametric experiments of MARCs with the varied concentrations in absence and presence of HS-DNA (c(DNA)＝5.0×10^{-5} $mol \cdot L^{-1}$) were carried out. The results showed that the difference of the

reduction peak current in absence and presence of DNA ($\triangle I_p$) gradually increased with the concentration of the MARCs and finally reached to a flatform (shown in Figure 3 (insert, (a) for MM (a), (b) for AMN, (c) for AMD, and (d) for CA), which was the typical phenomenon for an adsorption controlled quasireversible electrochemical process.

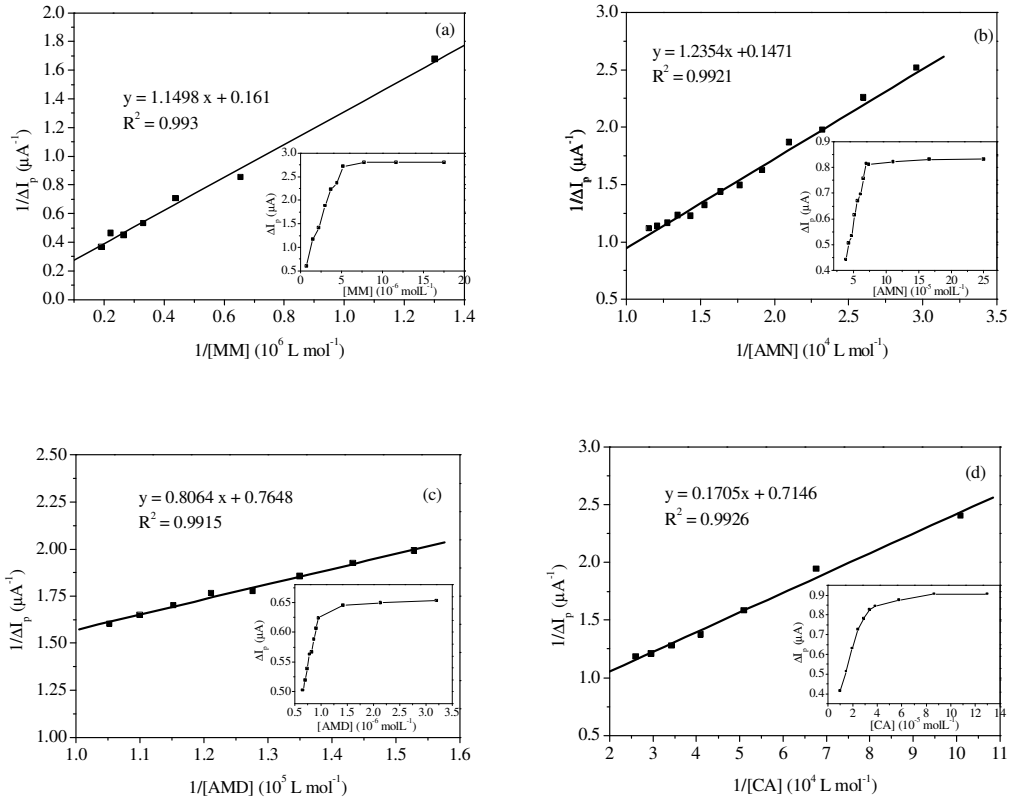


Figure 3. Relationship curves between ΔI_p^{-1} and $[MARCs]^{-1}$ (insert: Relationship curves between ΔI_p and [MARCs]; $\triangle I_p$: the diference of peak currents before and after addition of DNA; [MARCs]: the equilibrium concentration of MM (a), or AMN (b), or AMD (c), or CA(d))

Table 3. The thermodynamic parameters of MARC-DNA interactions

MARC	K ($L \cdot mol^{-1}$)		
	Electrochemical (CV) method (this work)	Electronic spectra [30, 31]	Fluorescence studies [30, 31]
MM	1.4×10^5	1.02×10^5	9.5×10^4
AMD	1.1×10^4	2.9×10^4	2.2×10^4
AMN	9.5×10^4	1.2×10^5	3.8×10^5
CA	4.2×10^4	4.3×10^4	4.3×10^4

Thus, the interaction constants were evaluated by double reciprocal equation as listed in eq. 1 [33].

$$\frac{1}{\Delta I_{\mathrm{p}}} = \frac{1}{\Delta I_{\mathrm{pmax}}} + \frac{1}{K\Delta I_{\mathrm{pmax}}} \times \frac{1}{[\mathrm{MARC}]^{s}} \qquad (5)$$

Where $\triangle I_{\mathrm{p}}$ and $\triangle I_{\mathrm{pmax}}$ are the difference and the maxium difference of the reduction peak current in absence and presence of DNA, respectively, while c(MARC, mol·L^{-1}) is the concentration of any of MARCs added, and K (L ·mol^{-1}) is the interaction constant of MARCs-DNA, s is the binding number for MARCs to DNA. Taken different values of s (s = 1, 2 .... n), by plotting of $1/\triangle I_{\mathrm{p}}$ *vs* $[1/c(\mathrm{MARC})]^{s}$, K can be obtained from the slope and intercept of resulted curves. In the present work, when s = 1, a good linear relationship between $1/\triangle I_{\mathrm{p}}$ *vs* $[1/c(\mathrm{MARC})]$ can be obtained, as shown in Figure 3. The K values for the MARC-DNA complexes were summarized in Table 3, which were consisted with our previous findings obtained *via* spectrophotometric, spectrofluorometric methods [30, 31].

The value of K, the interaction constants of MARC-DNA, was ~10^5 for any of the MARCs, which was at least 100 times smaller than reported examples of traditional intercalating mode [5, 34-36], such as ethidium, proflavin, daunomycin, cryptolepine, and chlorobenzylidine. These results furtherly illuminated that the interactions between DNA and MARCs did not follow traditional intercalating mode, while the conformation changes of DNA may be realized *via* the groove binding interaction with MARCs. These results were consistant with our previous findings obtained by spectrophotometric, spectrofluorometric, melting temperature (T_m), and viscosimetric techniques [30, 31]. Our previous thermodynamic studies suggested that the interaction processes were exergonic favored ($\triangle H$<0) and entropy disfavored ($\triangle S$ <0), which indicated that hydrogen bonds might be the main acting force in the binding of the MARCs and DNA.

4. CONCLUSIONS

In summary, the electrochemical behavior of each MARC has been investigated by cyclic voltammetric studies at glassy carbon electrode (GCE) in phosphate buffer (PBS at pH 7.0). The electrochemical processes of the 4 MARCs at a bare GCE were shown to be quasireversible adsorption controlled. The number of electrons transferred per molecule (n) of any of the MARCs was calculated to be *ca*. 2. The electrochemical parameters (charge transfer coefficient α and the standard rate constant k_s) of free MARCs and binding compounds were obtained. The interaction constants between MARCs and HS-DNA were found to be 10^4~10^5 L·mol^{-1}.

References

1. FDA Interim Melamine and Analogues Safety/Risk Assessment. May 25, 2007.
2. R. Muñiz-Valencia, S. G. Ceballos-Magaña, D. Rosales-Martinez, R. Gonzalo- Lumbreras, A.

Santos-Montes, A, Cubedo-Fernandez-Trapiella, R. C. Izquierdo- Hornillos. *Anal. Bioanal. Chem.,* 392 (2008) 523
3. P. Suchy, E. Strakova, I. Herzig, J. Sta, R. Kalusoca, M. *Interdisc. Toxicol.,* 2 (2009) 55
4. NTP (National Toxicology Programme), Carcinogenesis bioassay of melamine (CAS No. 108-78-1) in F344/N rats and B6C3F1 mice (feed study). *National Toxicology Program technical Report Series*., 245 (1983) 1
5. E. C. Long, J. K. Barton. *Acc. Chem. Res.,* 23 (1990) 271
6. A. Hartwig. *Chemico-Biological Interact.,* 184 (2010) 269
7. P. G. Mantle, V. Faucet-Marquis, R. A. Manderville, B. Squillaci, A. Pfohl- Leszkowicz. *Chem. Res. Toxicol.,* 23 (2010) 89
8. K. J. Jang, G. Y. Yeo, T. S. Cho, G. H. Eom, C. Kim, S. K. Kim. *Biophys. Chem.,* 148 (2010) 138
9. S. Kashanian, S. Askari, F. Ahmadi, K. Omidfar, S. Ghobadi, F. A. Tarighat. *DNA Cell Biol.* 27(10) (2008) 581
10. C. Y. Qiao, S. Y. Bi, Y. Sun, D. Q. Song, H. Q Zhang, W. H. Zhou. *Spectrochim. Acta A.,* 70 (2008) 136
11. F. A. Tanious, D. Y. Ding, D. A. Patrick, R. R. Tidwell, W. D. Wilson. *Biochem.,* 36 (1997) 15315
12. F. Ahmadi, F. Bakhshandeh. *DNA Cell Biol.,* 28(10) (2009) 527-533.
13. A. Z. Li, J. L. Qi, H. H. Shih, K. A. Marx, *Biopolymers,* 38 (1996) 367
14. J. K. Barton, *Comments Inorg. Chem.,* 3 (1985) 321
15. W. Zhong, J. H. Yu, Y. Liang, *Spectrochim. Acta* A., 59 (2003) 1281
16. J. Pastor, J. G. Garcia-Navio, J. J. Vaquero, J. Alvarez-Builla, F. Gago, B. Pascual-Teresa, M. Pastor, M. M. Rodrigo. *J. Org. Chem.,* 62 (1997) 5476
17. K. Sandstrom, S. Warmlander, M. Leijon, A. Graslunda, *Biochem. Biophys. Res. Commun.,* 304 (2003) 55
18. M. T. Carter, M. Rodriguez, A. J. Bard. *J. Am. Chem. Soc.,* 111 (1989) 8901
19. S. Rauf, J. J. Gooding, K. Akhtar, M. A. Ghauri, M. Rahmana, M. A. Anwar, A. M. Khalid. *J. Pharmac. Biomed. Anal.,* 37 (2005) 205
20. J. Wang, M. Ozsoz, X. Cai, G. Rivas, H. Shiraishi, D. H. Grant, M. Chicharro, J. Fernandes, E. Palecek, *Bioelectrochem. Bioenerg.,* 45 (1998) 33
21. S. F. Wang, T. Z. Peng, C. F. Yang, *J. Electroanal. Chem.,* 544 (2003) 87
22. M. Forjta, *Electroanalaysis,* 14 (2002) 1449
23. X. Q. Lu, Y. N. Chen, J. Chen, Y. Zhang, L. M. Zhang, M. R. Li. *Int. J. Electrochem. Sci.,* 1 (2006) 130
24. W. Sun, M. X. Yang, K. Jiao. *Int. J. Electrochem. Sci.,* 2 (2007) 93.
25. J. B. Raoof, M. S. Hejazi, R. Ojani, E. H. Asl. *Int. J. Electrochem. Sci.,* 4 (2009) 1436.
26. H. Ilkhani, M. R. Ganjali, M. Arvand, P. Norouzi. *Int. J. Electrochem. Sci.,* 5 (2010) 168
27. S. Riahi, A. Mashhadi, S. Eynollahi, M. R. Ganjali, P. Norouzi. *Int. J. Electrochem. Sci.,* 5 (2010) 955
28. T. R. R. Naik, H. S. B. Naik. *Int. J. Electrochem. Sci*., 3 (2008) 409
29. Z. S. Yang, D. P. Zhang, H. Y. Long, Y. C. Liu. *J. Electroanal. Chem.,* 624 (2008) 91
30. H. Y. Shen, F. Zhu, W. Shi, Z. Chen, Q. Q. Pan. *Acta Chim. Sinica.,* (2010) in press
31. H. Y. Shen, Y. Q. Liu, J. Gao, H. M. Zhen, N. Zhu, J. Li. *DNA Cell Biol.,* Submitted.
32. A. J. Bard, L. R. Faulkner. *Electrochemical Methods: Fundamentals and Applications,* Second edition, John Wiley & Sons, Inc. NewYork, (2001)
33. E. Laviron. *J. Electroanal. Chem.,* 101 (1979) 19
34. J. B. Chaires, N. Dattagupta, D. M. Crothers. *Biochemistry,* 21(1982) 3933
35. K. Bonjean, M.C. de Pauw-Gillet, M. P. Defresne. *Biochemistry,* 37 (1998) 5136
36. W. Zhong, J. Yu, Y. Liang, K. Fan, L. Lai. *Spectrochim. Acta A.,* 60 (2004) 2985