ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 217–225

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Voltammetric studies of the interaction of lumazine with cyclodextrins and DNA

M.S. Ibrahim *, I.S. Shehatta [1], A.A. Al-Nayeli

Chemistry Department, Faculty of Science, UAE University, PO Box 17551, Al-Ain, United Arab Emirates

Received 25 July 2001; received in revised form 6 September 2001; accepted 25 September 2001

Abstract

The interaction of lumazine, an antibacterial drug, with α-, β-cyclodextrins and DNA in aqueous solution was studied by differential pulse stripping voltammetry and cyclic voltammetry as well as UV–vis spectroscopy. The electrochemical and absorption spectral data indicated a 1:1 complex formation of lumazine with α-, β-cyclodextrins and DNA. The nature of the process, taking place at the hanging mercury drop electrode, was clarified. It was found that the complexation of lumazine molecules enhances the stacking interactions which might facilitate the formation of a perpendicularly stacked layer of lumazine-α-cyclodextrin complex on the electrode surface. Based on the variations in the current or absorbance, the formation constants and consequently, the Gibbs energy of these complexes were determined. The small size cavity of α-cyclodextrin was found to have a greater affinity for lumazine than the β-cyclodextrin. Moreover, the interactions of lumazine-α-cyclodextrin or lumazine-β-cyclodextrin inclusion complex with DNA have been investigated by means of voltammetry. The results suggest that lumazine displayed high affinity for DNA and the inclusion complex decomposed when it binds to DNA.

Keywords: Voltammetry; Lumazine; Antibacterial drug; Interactions; Cyclodextrins; DNA

1. Introduction

Much attention was paid in the last decades to the binding of small molecules with cyclodextrin (CD) and deoxyribonucleic acid (DNA). DNA binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA [1]. A variety of small molecules interacts reversibly with double-stranded DNA, primarily through three modes: (i) electrostatic interactions with the negative-charged nucleic sugar-phosphate structure, which are along the external DNA double helix and do not possess selectivity; (ii) binding interactions with two grooves of DNA double helix; and (iii) intercalation between the stacked base pairs of native DNA. Heterocyclic dyes, such as ethidium, anthracyclines, phenothiazines and acridine

* Corresponding author. Permanent address: Chemistry Department, Faculty of Science, Assiut University, Assiut 71516, Egypt. Tel.: +971-3-7064-229; fax: +971-3-7671-291.
E-mail address: mohamedI@uaeu.ac.ae (M.S. Ibrahim).
[1] Permanent address: Chemistry Department, Faculty of Science, Mansoura University, Mansoura, Egypt.

0731-7085/02/$ - see front matter
PII: S0731-7085(01)00624-0

derivatives interact through intercalation with the planar, aromatic group stacked between base pairs [2–4]. The interactions of DNA with nucleic-acid-binding molecules have been extensively studied over the past years using various techniques, including spectroscopic and X-ray crystallographic methods [5]. In the recent years, electrochemical investigations of nucleic-acid-binding molecules and DNA interactions [6–9] can provide a useful complement to the previously used methods of investigation, such as spectroscopic methods, e.g. for nonabsorbing species, and yield information about the mechanism of intercalation and the conformation of adduct.

As well known, cyclodextrins (CD) are a kind of polysaccharides made up of six to eight D-glucose monomers connected at 1 and 4 carbon atoms, and they can provide a hydrophobic cavity in aqueous solution for the hydrophobic molecules or groups to form inclusion complexes [10,11]. This important characteristic make it possible for cyclodextrins to be used as biomimetic enzyme models [12,13], drug delivery systems [14] and electrode reaction modifiers [15].

Both DNA and cyclodextrin are common in having hydrophilic coat/hydrophobic core structure. Thus, an aromatic ring either staching between nucleobase pairs or incorporated in CD cavity is the main driving force for the binding of an intercalator into double-stranded DNA and a guest molecule to CD, respectively.

These stimulate us to investigate the complexing properties of CD and DNA as hosts for an antibacterial drug substance namely lumazine as guest model. Lumazine is one of the pterin compounds which are known as important antibacterial substances [16]. It has been observed that lumazine inhibits the growth of *Methanobacterium thermoautotrophicum* and *Methanococcus voltae* [17]. The application of the phase-selective a.c adsorptive stripping voltammetry for determination of lumazine has been recently reported by us [18].

In this paper, the interaction of lumazine (LMZ) with CD (α- or β-) or DNA has been studied by differential pulse stripping voltammetry and cyclic voltammetry as well as UV–vis spectroscopy. Furthermore, the interactions of the CD inclusion complexes of LMZ with DNA were investigated by voltammetry. From the decrease in the peak current, or from the variation in the absorption spectra, the formation constant (K_f) of the LMZ–CD or LMZ–DNA interaction was obtained.

2. Experimental

2.1. Chemicals and solutions

Calf thymus deoxyribonucleic acid (ct-DNA), α-CD, β-CD and lumazine (2,4-pteridinediol) were obtained from Sigma and were used without further purification. Stock solutions of LMZ or DNA were prepared by dissolving an appropriate amount of the drug or DNA in H_2O and stored at 4 °C. The concentration of the stock solution of ct-DNA (5.43×10^{-5} M in nucleotide phosphate, NP) was determined by UV absorbance at 260 nm using the molar extinction coefficient = 6600 M^{-1} cm^{-1}. The concentration of the analyte determined voltammetrically or spectroscopically in the buffer solution used remain constant over at least 12 h. Stock solutions of α-CD and β-CD were prepared by dissolving the desired weights in water. The supporting electrolyte was 0.25 mol l^{-1} sodium phosphate buffer. All chemicals were reagent grade (Merck, Darmstadt). Double distilled water was used to prepare the solutions. The pH was measured using a pH-meter Model M64 (ORION) with accurate to ± 0.05.

2.2. Apparatus and methods

Differential pulse stripping voltammetry (DPSV) and cyclic voltammetry (CV) were carried out using a PAR Model 264A polarographic analyzer/stripping voltammetry in conjunction with a PAR model 303A HMDE and advanced X–Y recorder model RE0150. An Ag/AgCl saturated KCl reference electrode and a Pt wire auxiliary electrode were used. A PAR model 305 stirrer was also used for DPSV measurements. The ultraviolet and visible absorption spectra were obtained using a Shimadzu UV-2101PC, UV–vis scanning spectrophotometer. All measurements were carried out at 25.0 ± 0.5 °C.

The test solution was placed in a polarographic cell and de-oxygenated by passing nitrogen for 15 min. In the case of DPSV the accumulation was performed on the HMDE and the solution was stirred at the accumulation potential of $E_{acc} = -0.2$ V for 30 s. The voltammetric response was obtained using a pulse repetition time of 0.2 s, within an amplitude of 100 mV and a scan rate of 20 mV s^{-1} (or 100 mV s^{-1} for CV, unless stated otherwise). When preconcentration was done in stirred solutions, a quiescent period of 15 s was allowed before the potential scan was started.

Current and absorption titrations were performed by keeping constant the concentration of LMZ while varying the concentrations of CD or DNA. The current titration equation was described as follows [19]:

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

where, C_H is the concentration of CD or DNA, K_f is the apparent formation constant, i_0 and i are the peak currents without and with CD or DNA. A is the proportional constant. The condition of using Eq. (1) is that a 1:1 association complex is formed and C_H is much larger than the total concentration of LMZ in solution. Whereas, the formation constant can be evaluated spectrophotometrically according to the following equation [20,21];

$$\frac{A_0}{A-A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}\frac{1}{K_f[CD]} \quad (2)$$

where A_0 and A are the absorbances of the free guest and the apparent one, ε_G and ε_{H-G} are the absorption coefficients of the guest and complex, respectively. Thus, if Eqs. (1) and (2) fit the experimental data, this may suggest that the complex of LMZ with CD or DNA is a 1:1 association complex.

3. Results and discussion

3.1. Formation of LMZ–CD inclusion complex

The inclusion phenomena were investigated by cyclic voltammetry and differential pulse cathodic stripping voltammetry which are sensitive electrochemical methods and permit the collection of excellent data at low concentration of electroactive guest [20,21]. The cathodic stripping voltammogram of free LMZ gave cathodic peak at -0.2, -0.75 and -0.875 V for pH 2.0, 7.1 and 9.0, respectively. This cathodic peak reflects the $2e^-/2H^+$ reduction of the pteridine ring to yield the 5,8-dihydro derivative [22]. A detailed examination of the electrochemical behaviour of LMZ has been carried out by us [18] using both cyclic voltammetry and ac voltammetry.

Addition of cyclodextrins (α- or β-CD) to aqueous solutions of LMZ causes changes in the voltammogram of the latter (Fig. 1 is a representative example). With the increase in the amounts of cyclodextrins, the cathodic peak potentials shifted in a negative direction, and at the same time, the cathodic peak current decreased. These results are attributed to the formation of the inclusion complexes according to the equilibrium of the following reaction,

$$LMZ + CD \rightleftharpoons LMZ\text{–}CD \quad (3)$$

where the LMZ–CD is the association complex between LMZ and a given cyclodextrin. The decrease of the peak current can also be explained by the smaller diffusion coefficients of the inclusion complexes. The formation of inclusion complexes between LMZ and cyclodextrin are markedly affected by pH value of the solution. Strong interactions are observed at pH 9.2 phosphate buffer compared to media with other pH values, in which the cathodic peak current decreased to about 20, 10 and 50% for pHs 2.0, 7.2 and 9.2, respectively, of that in the absence of β-CD. The latter behaviour reflects that the anionic species of LMZ are strongly interacted with cyclodextrins. It was also found that the peak potential is shifted to more negative values with increasing pH and the peak height decreases markedly at pH ≥ 2.0. Furthermore, the effect of buffer constituents on the interaction of LMZ with cyclodextrins was also studied in the presence of several supporting electrolytes at pH 9.2, e.g. borate, Britton–Robinson and phosphate buffers. Best results (with respect to strong interaction and reproducibility) were obtained in the

phosphate buffer solution. Therefore, phosphate buffer (pH 9.2) was employed throughout this study.

Several instrumental parameters, such as drop size, scan rate and pulse amplitude, which directly affect the voltammetric response, were optimized. The chosen working conditions were 1.2×10^{-2} cm^2, 20 mV s^{-1} (or 100 mV s^{-1} for CV) and 100 mV, respectively. The stripping currents were not modified when varying the rest period. The chosen value, 15 s, is sufficient to allow the formation of a uniform concentration of the analyte in the mercury drop and to ensure that the subsequent stripping step is performed in a quiescent solution.

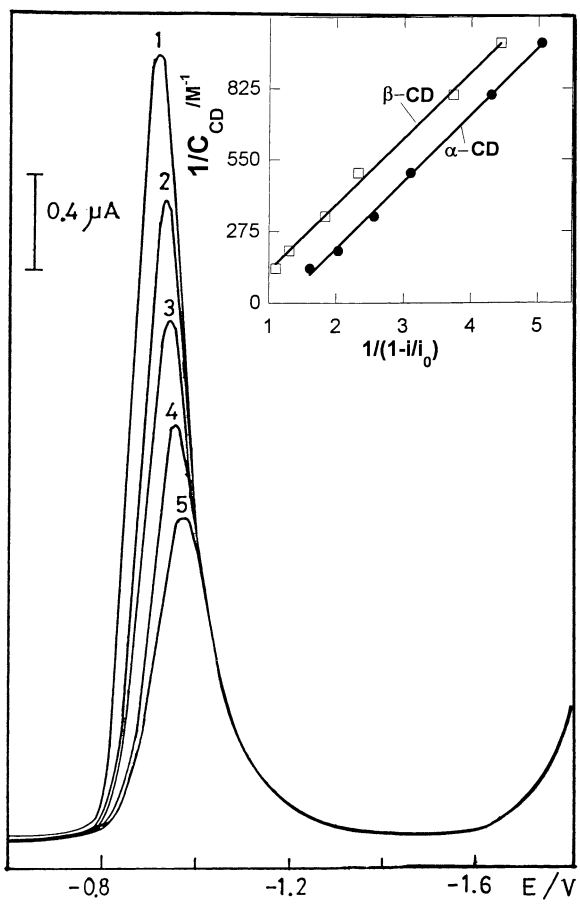


Fig. 1. DPS voltammograms of 5×10^{-5} mol l^{-1} LMZ at HMDE in phosphate buffer (pH 9.2) in the absence (1) and presence of (2) 1.0, (3) 2.0, (4) 5.0, and (5) 7.5 mM α-CD. Scan rate 20 mV s^{-1}, accumulation time 30 s and pulse amplitude 100 mV. Inset: the plot of $1/C_{CD}$ to $1/(1 - i/i_0)$.

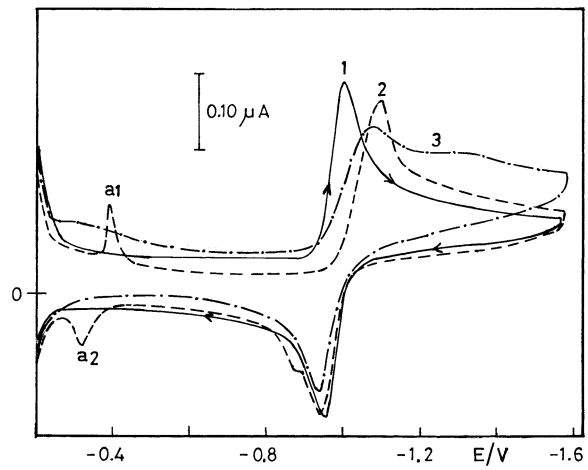


Fig. 2. Cyclic voltammograms for 5×10^{-5} mol l^{-1} LMZ solution obtained in phosphate buffer (pH 9.2) using a scan rate of 100 mV s^{-1} and accumulation potential of -0.2 V. (1) LMZ only, (2) $1 + 5 \times 10^{-3}$ M α-CD, (3) $1 + 5 \times 10^{-3}$ M β-CD.

According to the decrease of peak currents with the concentrations of α-CD and Eq. (1), the following equation was obtained: $1/C_{\alpha\text{-CD}} = 2.57 \times 10^2/(1 - i/i_0) - 3.06 \times 10^2$ with a linear correlation coefficient (r) of 0.993. Similarly, the experimental data of the decrease of peak current with β-CD corresponded well to Eq. (1) and the following equation was obtained: $1/C_{\beta\text{-CD}} = 2.54 \times 10^2/(1 - i/i_0) - 1.28 \times 10^2$ with $r = 0.993$. This revealed that the inclusion complex of LMZ with α-CD or β-CD was a 1:1 association complex and the formation constants (K_f), which are usually used to characterize the inclusion phenomena of CD system [23], were 3.06×10^2 and 1.28×10^2 M^{-1} for LMZ-α-CD and LMZ-β-CD, respectively, as calculated from the y-intercepts.

The inclusion phenomena of the electroactive LMZ were also studied using cyclic voltammetry. Typical cyclic voltammetric behaviour of LMZ in the absence, and presence, of α-CD or β-CD at pH 9.2 are shown in Fig. 2. Summaries of cyclic voltammetric results are given in Table 1. The cyclic voltammetric behaviour of pure LMZ shows one cathodic peak and the corresponding oxidation peak in the potential range of -0.2 to

−1.5 V at the HMDE (curve 1 in Fig. 2). The separation of the anodic and the cathodic peak potentials, ΔE_p, was 44 mV at 100 mV s^{-1}, indicating a quasireversible, two-electron redox process. The formal potential, $E^{\circ\prime}$, taken as the average of E_{pc} and E_{pa}, was −0.978 V in the absence of CD.

The addition of an excess of α-CD or β-CD in the LMZ solution caused the couple of redox peaks currents to diminish considerably (curves 2 and 3 in Fig. 2). The solutions of the complexed guests in the presence of 5×10^{-3} mol l^{-1} of α-CD or β-CD yield the cathodic peak potentials shifted in a negative direction (Table 1). $E^{\circ\prime}$ shifted to more negative potentials by 47 and 28 mV in the presence of α-CD and β-CD, respectively, revealed that the reduction of LMZ molecules become more difficult when they were included into the cavity of α-CD or β-CD to form an inclusion complex [23]. This result may be attributed to a better fit between the LMZ and the small α-CD cavity. Such cavity permits a much closer contact with the LMZ than that of β-CD. In presence of α-CD or β-CD, the difference ΔE_p between anodic and cathodic peak potentials is 150 or 137 mV, respectively. These values are larger than that expected for a reversible two-electron transfer reaction which is given by $57/z$ mV, where z is the number of electrons transferred in the process [24], indicating that the irreversibility of the electron-transfer process was maintained under this condition. The decrease of the peak current observed upon addition of α-CD or β-CD is due to the lower diffusion coefficient of cyclodextrin complexes compared to that of the free guests. CV peak potentials were independent of scan rates (v) in the range of 20–100 mV s^{-1} and the peak currents were proportional to the square root of scan rates both without and with α-CD or β-CD. The slope of the linear plot of i_p versus $v^{1/2}$ without CD (23.7 nA $mV^{-1/2}$ $s^{1/2}$) was more than that with CD (e.g. with β-CD 16.9 nA $mV^{-1/2}$ $s^{1/2}$), suggesting the diffusion coefficient of the free form of LMZ ($D = 6.88 \times 10^{-6}$ cm^2 s^{-1}) was larger than that of the complexed form of LMZ with CD ($D = 3.13 \times 10^{-6}$ cm^2 s^{-1}). At higher v ($v \geq 200$ mV s^{-1}), broadening of ΔE_p was observed ($\Delta E_p > 150$ mV), possibly due to the onset of kinetic complications, as well as a dependence of ΔE_p on v was obtained (e.g. $\Delta E_p = 150$ mV at 100 mV s^{-1} and [α-CD] = 5.0 mM, while $\Delta E_p =$ 250 mV at 500 mV s^{-1} and [α-CD] = 5.0 mM).

The less efficient complexation of LMZ and β-CD is probably due to the bigger cavity of β-CD when compared to α-CD, making it possible to accommodate a greater amount of water around the included LMZ. The small-size cavity of α-CD offers the best protection of LMZ from bulk water; on the other hand, LMZ is not sufficiently big to completely fill the β-CD cavity and as a result even the included molecule is exposed to water molecules with which it probably shares the cavity.

Further evidence for the formation of a better fit between the LMZ and the small α-CD cavity is given by the appearance of two additional small peaks in the CV, denoted a_1 and a_2 located at ca. −0.400 and −0.325 V, respectively (Fig. 2). The intensity of the a_2 peak is always less than the a_1 peak. The peaks are seen only in presence of

Table 1
Cyclic voltammetric parameters of LMZ in absence and presence of cyclodextrins and DNA

Substances	E_{pc}[a] (V)	E_{pa}[a] (V)	$E^{\circ\prime}$ (V)	ΔE_p (mV)	i_{pc}[a] (nA)	i_{pa}[a] (nA)	i_{pc}/i_{pa}
LMZ	−1.000	−0.956	−0.978	44	275	162	1.69
LMZ–α-CD	−1.100	−0.950	−1.025	150	250	150	1.66
LMZ–β-CD	−1.075	−0.937	−1.006	137	225	125	1.80
LMZ–DNA	−1.000	−0.937	−0.969	62	237	156	1.52
LMZ–α-CD–DNA	−1.000	−0.937	−0.969	62	212	150	1.41
LMZ–β-CD–DNA	−1.002	−0.933	−0.968	69	212	125	1.69

Analytical conditions as in Fig. 2.

[a] All values are average of five measurements ($E \pm 50$) mV and ($I \pm 25$) nA.

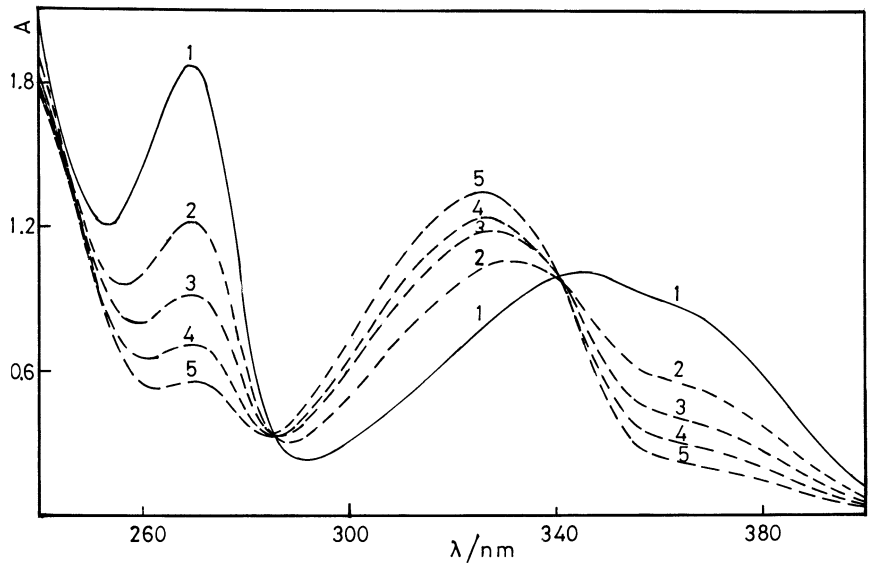


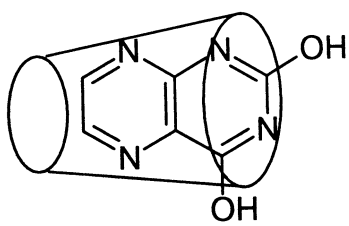
Fig. 3. UV–vis spectra of 2.0×10^{-4} mol l^{-1} LMZ in phosphate buffer (pH 9.2) in the absence (1) and presence of (2) 1.0, (3) 2.0, (4) 3.0 and (5) 4.0 mM β-CD.

α-CD, and appear to reflect capacitive changes as re-orientation process occurs. The two peaks are clearly related to one another, the a_1 peak represents the re-orientation of the adsorbed LMZ molecules from the flat to the perpendicular orientation, and the a_2 peak is likely due to the re-orientation of the molecules back to the original flat orientation. This indicating that complexation of the LMZ molecules enhances the stacking interactions and hence would be expected to facilitate the formation of a perpendicularly stacked layer of LMZ–α-CD complex on the electrode surface. Similar behaviour was obtained previously by us [25,26].

The formation of inclusion complex between LMZ and cyclodextrin could be further confirmed by a spectroscopic experiment. The ultraviolet and visible absorption spectra of LMZ in the absence, and presence, of β-CD are shown in the Fig. 3. It is noticed that upon addition of β-CD, the absorption maximum of LMZ at 270 nm shifted towards longer wavelength, whereas, the absorption maximum at 345 nm has a hypsochromic shift. However, the UV–vis absorbance changed differently with increasing concentration of β-CD and two isosbestic points at 285 and 340 nm were observed indicating the formation of 1:1 complex. At wavelength 270 and 360 nm, the absorbance decreased upon addition of β-CD, while at 327 nm, the absorbance increased. This phenomena may be explained by the presence of two different electron transitions, $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$. According to the symmetry forbidden rule, the $\pi \rightarrow \pi^*$ transition in LMZ may be less intense in the case of being included inside the β-CD cavity than of being exposed in the aqueous solution. While, the hydrophilic part of the LMZ molecule (–OH) remains, as far as possible, at the outer face of the complex, this gives a higher probability for the $n \rightarrow \pi^*$ transition. The spectral data further proved the formation of the inclusion complex of LMZ with β-CD or α-CD, and the formation constants, K_f, of these complexes can be determined according to Benesi–Hildebrand equation [27], under the condition of the 1:1 inclusion complex formation equilibrium with an excess concentration of β-CD or α-CD compared to the total concentration of LMZ. According to Eq. (2), from an $A_0/(A - A_0)$ versus 1/[CD] plot, the ratio of the intercept to the slope gives the value of the formation constant. Our experimental data are shown in Table 2. We can see that the

formation constant of the LMZ–α-CD complex is larger than that of LMZ–β-CD. This means that LMZ fits better into the cavity of α-CD, as the following supposed model, in which the planar part of LMZ molecule may enter the hydrophobic cavity of α-CD and the hydrophilic group (–OH) may stick out of the cavity.

Moreover, the fact that the interaction of LMZ with CD is weakened by the addition of methanol (K_f is decreased by approximately ten times in presence of 20% methanol–water mixture) indicates that the binding process is a hydrophobic driven one. The decrease in the K_f and consequently, the Gibbs energy ($-\Delta G$) values with the addition of methanol confirm that water molecules play an important role in the association process. The decrease in the polarity of the medium with the addition of methanol is unfavourable for hydrophobic interaction between LMZ and CD.



3.2. Interaction of LMZ with DNA

The typical cyclic voltammograms without and with DNA are shown in Fig. 4a. In the presence of DNA, the peak currents decreased and the anodic peak potential slightly shifted (19 mV) in a positive direction but the cathodic peak potential did not change (Table 1). The reason of the decrease of peak current was that the apparent diffusion coefficient decreased and also the apparent concentrations of the electroactive species decreased [28]. Small change of anodic peak potentials was observed, revealing that the interaction of the reduced form of the LMZ molecule with DNA was the same as that of its oxidized form [28]. The peak potentials (E_{pc}, E_{pa}) were independent of the scan rates (v) in the range of 20–100 mV s^{-1} and the peak currents (i_{pc}, i_{pa}) were proportional to the square root of scan rates in the presence of DNA. ΔE_p was 62 mV in the presence of DNA, indicating that DNA did not affect the electron transfer process of LMZ molecules. Moreover, the experimental data of the decrease of DPSV peak current with DNA corresponded well to Eq. (1). According to experimental data (at pH 9.2), the following equation was obtained $1/C_{DNA} = 8.749 \times 10^3/(1 - i/i_0) - 1.54 \times 10^4$ with $r = 0.997$, revealing that the binding complex of LMZ molecule with DNA (per nucleotide phosphate) was a 1:1 association complex. The binding constant of LMZ to DNA was calculated from the y-intercept to be 1.54×10^4 M^{-1}, as shown in Fig. 5.

The formation of a new adduct of LMZ with DNA could be further confirmed by a spectroscopic experiment (data not shown). The LMZ solution exhibited peculiar hypsochromic and bathochromic shifts in the absorption spectra on binding to DNA, a typical characteristic of DNA intercalation [29,30]. It is considered that such peculiar variation in spectroscopic behaviour was also due to the overlap between the electron clouds of LMZ and the stacked base planes of DNA [31].

Table 2
Formation constants and Gibbs free energy of LMZ–CD and LMZ–DNA complexes calculated from the results of voltammetry and spectroscopy (average ± S.D., $n = 5$)

The complex	pH	Voltammetry		Spectroscopy		
		K_f (M^{-1})	$-\Delta G$ (kJ mol^{-1})	K_f (M^{-1})	$-\Delta G$ (kJ mol^{-1})	λ_{max} (nm)
LMZ–α-CD	9.2	$(3.06 \pm 0.034) \times 10^2$	14.18 ± 0.012	$(2.70 \pm 0.10) \times 10^2$	13.87 ± 0.090	235
LMZ–β-CD	9.2	$(1.28 \pm 0.012) \times 10^2$	12.03 ± 0.051	$(1.31 \pm .0.08) \times 10^2$	12.08 ± 0.077	270
LMZ–DNA	7.2	$(1.65 \pm 0.030) \times 10^4$	24.07 ± 0.150	$(1.74 \pm .0.06) \times 10^4$	24.21 ± 0.065	229
	9.2	$(1.54 \pm 0.025) \times 10^4$	23.90 ± 0.210	$(1.60 \pm 0.07) \times 10^4$	23.99 ± 0.054	235

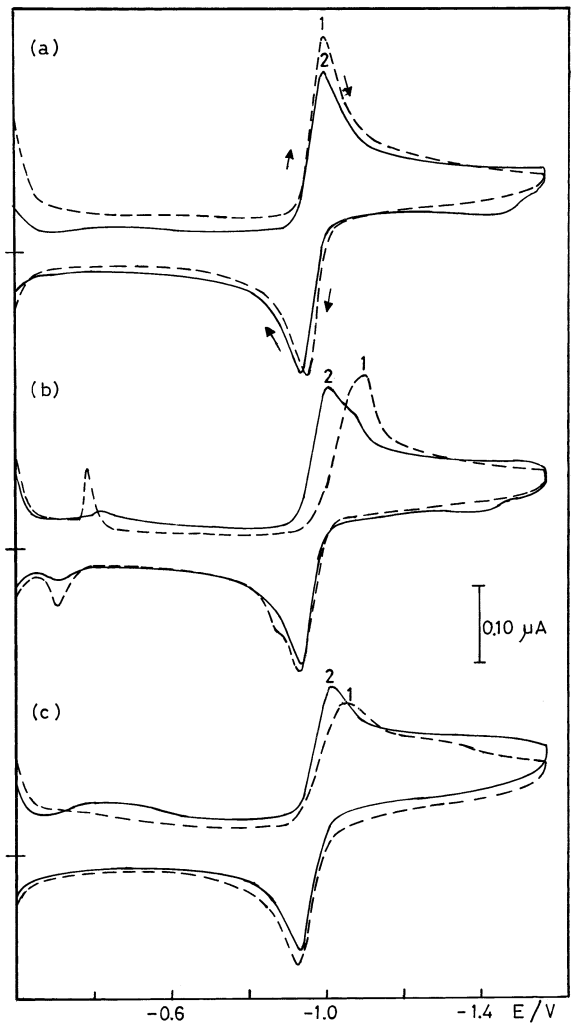


Fig. 4. (a) Cyclic voltammograms for 5×10^{-5} mol l^{-1} LMZ solution in the absence (1) and presence of (2) 1.02×10^{-4} M DNA; (b) cyclic voltammograms for (1) 5×10^{-5} mol l^{-1} LMZ + 5×10^{-3} M α-CD and (2) $1 + 1.36 \times 10^{-5}$ M DNA; (c) cyclic voltammograms for (1) 5×10^{-5} mol l^{-1} LMZ + 5×10^{-3} M β-CD and (2) $1 + 1.36 \times 10^{-5}$ M DNA. Other conditions as in Fig. 2.

Based on the variations in the absorbance, the formation constant (K_f) of LMZ–DNA complex was determined according to Eq. (2), as mentioned above. Our experimental data at pHs 7.2 and 9.2, which were in agreement, within the experimental error, with those obtained electrochemically, are listed in Table 2.

3.3. Interaction of LMZ–α-CD and LMZ–β-CD complexes with DNA

The effect of the addition of DNA to the complexed LMZ with either α-CD or β-CD was investigated using cyclic voltammetry (Fig. 4b,c). It was observed that, in presence of DNA, the redox potential of LMZ–α-CD or LMZ–β-CD complex shifted to a more positive value and the peak currents of CVs were decreasing with increasing concentrations of DNA. If the inclusion complex does not decompose, the redox potential would have not change by adding DNA. But, in fact, the redox potential shifted to the potential at which LMZ was oxidized or reduced in absence and presence of DNA as shown in Table 1. This suggested that the inclusion complexes of LMZ–CDs decomposed when interact with DNA. Accordingly, the interaction of LMZ with DNA is more favoured and thus the CD is replaced by DNA to form intercalate with LMZ. This is quite reasonable due to the higher stability of LMZ–DNA complex than LMZ–CDs complexes (Table 2). The results also indicate that the ΔE_p for LMZ–DNA, LMZ–α-CD–DNA and LMZ–β-CD–DNA are the same 62 mV, this suggested that, the existence of α-CD or β-CD did not affect the interaction of LMZ with DNA, and the inclusion complex decompose when it binds to DNA.

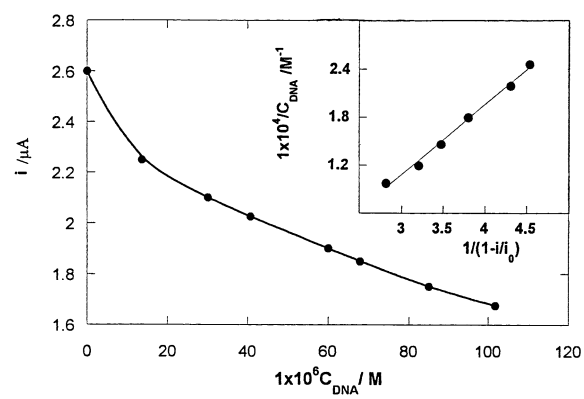


Fig. 5. The dependence of peak currents on the concentration of DNA. Inset: the plot of $1/C_{DNA}$ to $1/(1 - i/i_0)$.

4. Conclusion

In this paper, the electrochemical and UV–vis spectroscopic results demonstrated that the LMZ molecules can be included within CDs in aqueous solutions. It was also found that, the small-size cavity of α-CD offers the best protection of LMZ from bulk water; on the other hand, LMZ is not sufficiently big to completely fill the β-CD cavity. The results presented here show also that, LMZ displayed high affinity for DNA. Furthermore, the existance of α-CD or β-CD did not affect the interaction of LMZ with DNA and the inclusion complex decomposed when it binds to DNA. In general, small molecules which are not amenable to spectroscopic methods, either because of weak absorption bands or because of overlap of electronic transitions with those of the DNA molecule, can potentially be studied via voltammetric techniques to characterize the interaction of any electroactive species with cyclodextrins or DNA. Moreover, the voltammetric method for calculating the formation constant give more accurate, more sensitive, simpler, quicker and easier to operate automatically. These investigation are of potential importance in understanding the mechanism of interaction and recognition of the drugs in the living body.

Acknowledgements

The authors would like to thank the UAE University Research Council for financial support.

References

[1] M.J. Waring, in: G.C.K. Roberts (Ed.), Drug Action at the Molecular Level, Maemillar, London, 1977, p. 167.
[2] M.J. Waring, J. Mol. Biol. 13 (1965) 269.
[3] J.B. Chaires, N. Dattagupta, D.M. Crothers, Biochemistry 21 (1982) 3933.
[4] L.P.G. Wakelin, G.J. Atwell, G.W. Rewcastle, W.A. Denny, J. Med. Chem. 30 (1987) 855.
[5] J.K. Barton, Comm. Inorg. Chem. 3 (1985) 321.
[6] O. Vrana, V. Brabec, Bioelectrochem. Bioenerg. 19 (1988) 145.
[7] E. Palecek, V. Kolar, F. Jelen, Bioelectrochem. Bioenerg. 23 (1990) 285.
[8] X. Chu, G.-L. Shen, J.-H. Jiang, T.-F. Kang, B. Xiong, R.-Q. Yu, Anal. Chim. Acta 373 (1998) 29.
[9] M.S. Ibrahim, Anal. Chim. Acta 443 (2001) 63.
[10] D. Hallen, A. Schon, I. Shehatta, I. Wadso, J. Chem. Soc. Faraday Trans. 88 (1992) 2859.
[11] I. Shehatta, React. Funct. Polym. 28 (1996) 183.
[12] I. Tabushi, Acc. Chem. Res. 15 (1982) 66.
[13] F. Cramer, W. Kampe, J. Am. Chem. Soc. 87 (1965) 1115.
[14] W. Saenger, Angew. Chem. Int. Ed. Engl. 19 (1980) 344.
[15] T. Matsue, M. Fujhira, T. Osa, J. Electrochem. Soc. 129 (1982) 1681.
[16] R.L. Blakely, The Biochemistry of Folic Acid and Related Pteridine, Frontiers of Biology Series, vol. 13, Wiley, New York, 1969, pp. 1–569.
[17] R.K. Nagur-Anthal, E.V. Worrell, D.P. Nagle, Arch. Microbiol. 166 (1996) 136.
[18] M.S. Ibrahim, Fresenius J. Anal. Chem. 367 (2000) 189.
[19] G.C. Zhao, J.J. Zhu, J.J. Zhang, H.Y. Chen, Anal. Chim. Acta 394 (1999) 337.
[20] X.J. Dang, J. Tong, H.L. Li, J. Inclusion Phenom. 24 (1996) 275.
[21] M.Y. Ni, Y. Wang, H.L. Li, Pol. J. Chem. 71 (1997) 816.
[22] G. Dryhurst, Electrochemistry of Biological Molecules, Academic Press, New York, 1977, pp. 324–357.
[23] J. Taraszewska, A.K. Piasecki, J. Electroanal. Chem. 226 (1987) 137.
[24] P. Hemmerich, C. Veeger, H.C.S. Wood, Angew. Chem. Int. Ed. Engl. 4 (1984) 671.
[25] M.E. Ahmed, M.S. Ibrahim, Y.M. Temerk, A.M. Kawde, Electrochim. Acta 41 (1996) 2883.
[26] M.S. Ibrahim, Electrochim. Acta 40 (1995) 1913.
[27] H.A. Benesi, J.H. Hildebrand, J. Am. Chem. Soc. 71 (1949) 2703.
[28] M.T. Carter, M. Rodriguez, A.J. Bard, J. Am. Chem. Soc. 111 (1989) 8901.
[29] S. Takenaka, T. Ihara, M. Takagi, J. Chem. Soc. Chem. Commun. (1990) 1485.
[30] R. Fukuda, S. Takenaka, M. Takagi, J. Chem. Soc. Chem. Commun. (1990) 1029.
[31] Z.Y. Wang, R. Han, Pharmacentical Treatment of Tumor, People's Health Press, Beijing, 1987, pp. 72–74 (in Chinese).