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Fabrication of layer-by-layer deposited multilayer films containing DNA and its interaction with methyl green

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Abstract

Multilayer films were fabricated by layer-by-layer electrostatic deposition techniques between poly(diallyldimethylammonium chloride) (PDDA) and calf thymus DNA (CT DNA) on glassy carbon and quartz substrates. Electrochemical impedance spectroscopy (EIS), Fourier transform infrared (FTIR) spectroscopy and UV-vis spectroscopy demonstrated the uniform assembly of PDDA/DNA multilayer films, and X-ray photoelectron spectroscopy confirmed the elemental composition of the films. Moreover, the interaction of DNA in PDDA/DNA films with methyl green was investigated by UV-vis spectroscopy and circular dichroism (CD).

Keywords: Calf thymus DNA; Layer-by-layer; Spectroscopy; Methyl green

1. Introduction

Fabrication of organic thin films based on spontaneous molecular assembly has been considered as one of the powerful approaches to create novel supramolecular systems [1,2]. Since Iler [3] first described the formation of multilayer assemblies by spontaneous adsorption of the alternating layers of positively and negatively charged colloids on a charged surface, a layer-by-layer deposition technique has been widely used to construct organized ultrathin films of a variety of materials for numerous applications, including inorganic nanoparticles [4–6], dyes [7–13], and biological macromolecules [14,15]. Also, many

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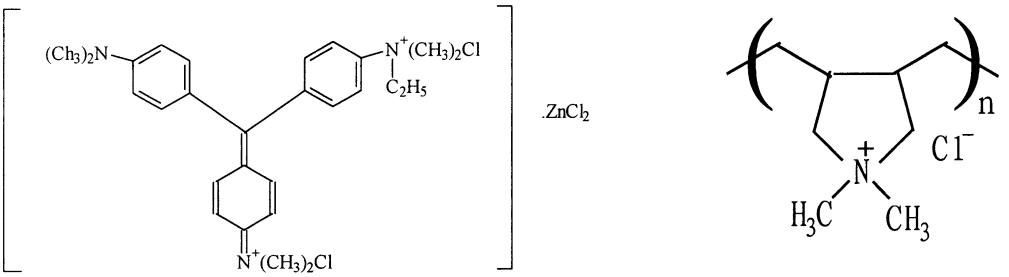
techniques have been applied to characterize these multilayer films, such as UV-vis spectroscopy [16,17], small-angle X-ray reflectivity [13,18], Fourier transform infrared (FTIR) [17], single particle light scattering [19], ellipsometry [20,21], scanning electron microscopy [17] and atomic force microscopy [22]. Recently, the layer-by-layer deposition method has been paid more and more attention as a promising technique for the fabrication of multilayer films. There are at least two main factors that make this approach interesting. One great advantage over other molecular deposition methods is that it is very easy to obtain multilayer films through electrostatic interaction of oppositely charged polyelectrolytes without any dedicated or sensitive equipment. Another benefit is that the adsorption is carried out from aqueous solutions, which also makes this technique environmentally attractive.

In recent years, the development of DNA-related sensors has attracted much attention directed to gene analysis, detection of genetic disorders, tissue matching and forensic applications [23–25]. In previous work, DNA was immobilized by covalently binding with the surface of substrates [23,26,27]. This method, however, is greatly limited, due to the lack of desirable substrate materials. Moreover, the chemical reactions between the substrate surfaces and DNA always lead to damage of the structure of DNA. To overcome this problem, the layer-by-layer deposition method is promising and has been successfully used to immobilize DNA on quartz substrate [28]. However, due to its unique double helix structure, DNA can interact with some small molecules [29]. In this aspect, much research has been carried out on the interactions between DNA and small molecules in aqueous solution, while few reports concerned their interactions in films [30].

In this paper, we report the fabrication of layer-by-layer deposited multilayer films containing DNA and its interaction with methyl green on both conducting and non-conducting substrates. For conducting substrate, poly(diallyldimethylammonium chloride) (PDDA) and calf thymus DNA were alternately deposited on 4-aminobenzoic acid-modified glassy carbon, and the resulting multilayer films were characterized by electrochemical impedance spectroscopy (EIS) and FTIR spectroscopy. For the non-conducting substrate, PDDA/DNA multilayer films were assembled on quartz and characterized by UV-vis spectroscopy and X-ray photoelectron spectroscopy. We also investigated the interaction of DNA in multilayer films with a cationic dye, methyl green. IR and CD experiments also prove that DNA in the films remains biologically active and is not



MG **PDDA**

Fig. 1. Molecular structures of methyl green (MG) and poly(diallyldimethylammonium chloride) (PDDA).

denatured. It is very significant to study some DNA-related biological phenomena in a biomimic membrane.

2. Materials and methods

2.1. Materials

The chemical structures of methyl green (MG) and poly(diallyldimethylammonium chloride) (PDDA) used are shown in Fig. 1. Calf thymus DNA (sodium salts, DNA, Sigma Chemical Co.) and PDDA (MW 400 000 to 500 000, Aldrich Chemical Co.), 4-amino-benzoic acid (4-ABA, Aldrich Chemical Co.) were used without further purification. MG was bought from Shanghai No.1 Chemical Co. (Shanghai, China), and used after being recrystalized from ethanol. All of the other chemicals were of analytical grade and used as received. All solutions were prepared using super-pure water from a Millipore system (Milli-QII, Millipore Inc.).

2.1.1. Substrate cleaning and preconditioning

Two kinds of substrates were used in the experiments: quartz slides and glassy carbon electrodes. For quartz slides, prior to the deposition of the multilayers, the quartz slides were cleaned in Piranha solution (H_2O_2/H_2SO_4 — 3:7 v/v) maintained at 80°C in a hot water bath and then washed thoroughly with super-pure water. Extreme caution must be exercised while using Piranha solution, as H_2O_2 forms an explosive mixture with H_2SO_4 and is extremely corrosive. The Piranha solution treatment removed all traces of organic materials sticking to the quartz surface, in addition to making the surface hydrophilic. Then the quartz slides were used to alternately deposit the PDDA/DNA multilayers.

For glassy carbon substrates, prior to use, the glassy carbon electrodes were polished with 1.0 and 0.3 μm α-Al_2O_3 powders successively and sonicated in water for approximately 2 min after each polishing step. Then the glassy carbon electrodes were sonicated in ethanol, washed with anhydrous ethanol and dried with high-purity nitrogen stream. Following the above procedure, the glassy carbon was covalently modified with 4-ABA using the method previously described [31]. After the modification, the electrodes were rinsed with ethanol and water successively, and sonicated for approximately 5 min in water to remove any physically adsorbed material. The modified electrodes were then used to deposit alternately the PDDA/DNA multilayers.

2.1.2. Self-assembly of multilayer films

Multilayer films of PDDA/DNA on quartz slides or 4-ABA modified glassy carbon electrodes were obtained by the layer-by-layer deposition technique through electrostatic interaction between PDDA and DNA. Briefly, the previously cleaned and preconditioned substrates were dipped in the aqueous solution of PDDA (approx. 0.5 mg/ml) for 30 min, thereby allowing sufficient time for the adsorption of PDDA onto the substrates. The substrates were then rinsed with water and dried in N_2 stream. After that, the substrates were dipped into DNA solution (0.1 mg/ml, pH 6.8, buffered with NaAc-HAc) for approximately 7 min, with water rinsing and N_2 drying. Again, the substrates were dipped into PDDA solution for approximately 7 min, with water rinsing and N_2 drying. Multilayer films of PDDA/DNA can be obtained by repeating the last two steps in a cyclic fashion.

2.2. Methods

2.2.1. Electrochemical measurements

The electrochemical measurements were performed in a conventional single compartment cell at room temperature. A three-electrode setup was employed with an Ag/AgCl (saturated KCl) electrode as the reference electrode, a Pt wire as a counter electrode, and a 4-ABA modified glassy carbon as the working electrode.

The cyclic voltammetric measurements were performed with a CHI 610 voltammetric analyzer (CH Instruments, Inc. USA). The electrochemical impedance measurements were carried out using a Solartron potentiostat (Model 1286) connected to a Solartron frequency response analyzer (Model 1250). Impedance measurements were taken in the presence of a 5-mM $K_3[Fe(CN)_6]$/

$K_4[Fe(CN)_6]$ (1:1) mixture in 10 mM phosphate buffer solution (consisting of 0.1 M KCl, pH 6.8) at frequencies ranging from 0.1 to 65 500 Hz using a 10-mV sinusoidal voltage centered around $E^{0\prime}$ of $Fe(CN)_6^{3-/4-}$. Before electrochemical measurements, the analytical electrolytes were purged with nitrogen for at least 15 min.

2.2.2. Spectra characterizations

UV-vis adsorption spectra of the DNA films and dye-incorporated DNA films on quartz slides were recorded in a UV CONTTRO 922 (Switzerland) Spectrophotometer.

Reflection-absorbance FTIR spectroscopy was carried out with a Nicolet 520 FTIR spectrometer (USA) equipped with a variable angle specular reflectance accessory (Specta Tech., USA), a DTGs detector, Omnic E.S.P. software and using 300 scans. Spectral resolution was 2 cm^{-1}. For recording the FTIR spectra, the PDDA/DNA films were prepared on modified glassy carbon substrates.

X-Ray photoelectron spectroscopy (XPS) was recorded with an ESCALab-MKII surface microanalysis system equipped with a standard Mg $K_{\alpha 1,2}$ radiation source (VG Co., UK). The data were obtained at room temperature.

Circular dichroism (CD), the difference in absorbance of left and right circularity polarized light was recorded from 200 to 800 nm. The experiments were performed at 25°C on a 62A DS CD spectrometer (AVIV, USA).

3. Results and discussion

3.1. Modification of the glassy carbon electrode by 4-ABA and multilayer assembly of PDDA / DNA on the modified glassy carbon electrode

In our experiments, the multilayer assembly of DNA and PDDA was made on two substrates. One is the quartz slide, and the other is the glassy carbon electrode. To realize the assembly of DNA and PDDA on the glassy carbon electrode, the glassy carbon surface was modified by 4-ABA through amine cation radical formation in anhydrous ethanol solution, as described in our previous work [31]. The 4-ABA modified glassy carbon is stable and difficult to be removed, which is very important in practical application of chemical and biological sensors. More importantly, this functionalized glassy carbon surface is covered with a terminal carboxyl group and therefore can be used as a charge-rich precursor for adsorbing oppositely charged substances by electrostatic interaction under proper pH conditions.

In our previous work, fabrication of monolayer and multilayer films of polyoxometalates and their catalysis application have been reported on this modified glassy carbon electrode [31]. Herein, we try further to extend its application to assemble multilayer films of PDDA/DNA on this modified glassy carbon surface. Firstly, PDDA was adsorbed through electrostatic interaction in a pH 6.8 solution. Then, a DNA monolayer was formed on it. Multilayer films of PDDA/DNA can be obtained by repeating the above procedures. EIS and FTIR spectra were used to characterize the growth of PDDA/DNA multilayer films on the modified glassy carbon electrode.

3.1.1. EIS of the self-assembly multilayer films in the presence of $Fe(CN)_6^{3-}$ / $Fe(CN)_6^{4-}$

EIS has been proved to be a powerful tool for the investigation of the electrode process [32]. It is based on the measurement of the response of the electrochemical cell to a small amplitude alternating potential. The response is often analyzed using the complex-impedance presentation and the results are interpreted in terms of an equivalent electrical circuit. The possibility of varying the perturbing frequency within a very wide interval enables it to provide a wealth of information on the kinetic process as well as on ohmic resistance, double-layer capacitance and charge-transfer resistance. Bruening has investigated the permeability and stability of layered polyelectrolyte films by EIS [33]. This technique has also been successfully used to characterize the formation of multilayer films in our lab [34].

Fig. 2 illustrates the result of complex plane

impedance spectroscopy on the modified electrode with various PDDA/DNA layer numbers, which are measured at the formal potential of $[Fe(CN)_6]^{3-/4-}$. From Fig. 2A, impedance plots show a quarter circle with no visible Warburg line, indicating kinetic control of the electron-transfer process [35]. Also, the diameters of the quarter circle in the impedance spectra increase with the stepwise formation of the multilayers. For simplicity, a modified Randles circuit is used to fit the experimental data, which includes R_s (solution resistance), in series with parallel R_{ct} (charge-transfer resistance) and C_{dl} (double-layer capacitance). Fig. 2B shows the relationship between the charge-transfer resistance R_{ct} and layer number. Because both DNA and PDDA are non-conductive, the PDDA/DNA multilayer films block the electron-transfer of $[Fe(CN)_6]^{4-/3-}$, leading to the increase of R_{ct} with the layer number. A good linear relationship between R_{ct} and the layer number indicates the uniform deposition of PDDA/DNA multilayer films.

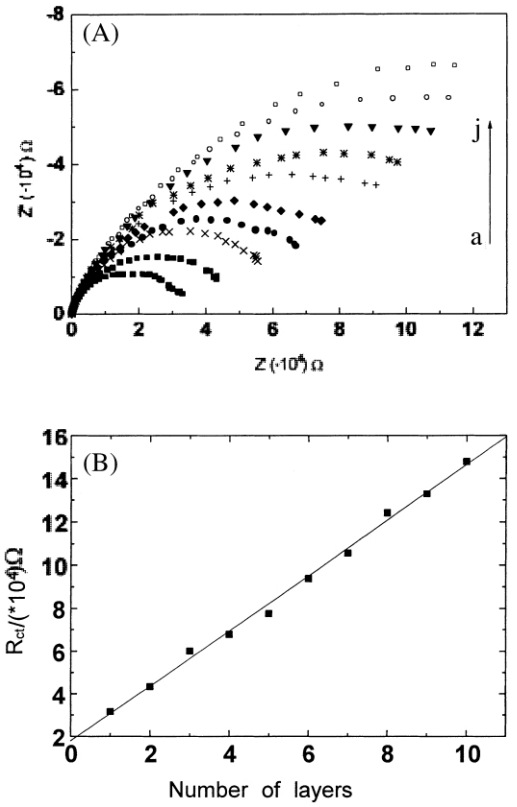


Fig. 2. (a) Electrochemical impedance spectra of PDDA/DNA multilayer films in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$ + 10 mM PBS + 0.1 M KCl. Bias voltage: 10 mV; frequency range: 0.1–65 500 Hz; number of layers:1; 2; 3; 4; 5; 6; 7; 8; 9; and 10. (b) Plot of the charge-transfer resistance R_{ct} vs. number of layers.

3.1.2. FTIR spectra characterization of the PDDA / DNA multilayer films

Further evidence for uniform multilayer growth of PDDA/DNA assemblies is obtained from FTIR spectra. Schlenoff has determined the water and ion pairing in multilayers made from poly(styrenesulfonate) and poly(diallyldimethylammonium chloride) using infrared spectroscopy [36]. Here, the growth of the PDDA/DNA multilayer films was monitored by FTIR spectra.

Fig. 3a shows the FTIR spectra of DNA in PDDA/DNA multilayer films (from down to up: 0, 2, 4, 6, 8, 10 layers) on a modified glassy carbon electrode. In order to avoid the absorption of water, the wavenumber range of 1250–950 cm^{-1} was used. PDDA has no marked infrared adsorption in the wavenumber range, while DNA has characteristic infrared absorption. Five typical infrared absorption bands at 1229, 1149, 1105, 1050 and 968 cm^{-1}, respectively, were observed for DNA [37,38]. The band at 1229 cm^{-1} was assigned to the antisymmetric stretching vibration mode of PO_2^-, which is typical for B-form DNA. The bands at 1149 and 1105 cm^{-1} were attributed to the vibration of the furan nucleus. The band at 1050 cm^{-1} was assigned to the symmetric stretching vibration mode of PO_2^- coupled with the C5′–O5′ ribose stretch. The band at 968 cm^{-1} was assigned to the in-plane stretching vibration mode of the nucleic bases due to C=C and C=O. Fig. 3b shows the plot of intensities of signature absorption bands for DNA at 1229 and 1105 cm^{-1} as a function of the number of layers deposited. The intensity of both bands increased proportionately with each additional layer. This is additional evidence that the assembly of PDDA/DNA multilayers was formed uniformly.

The FTIR data also show that the deposited

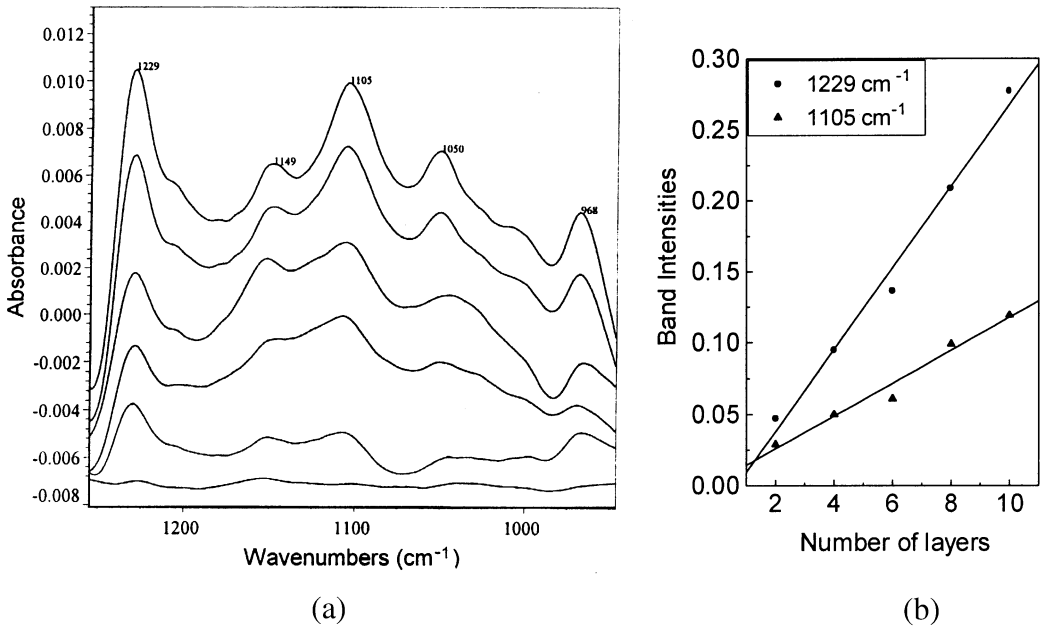


Fig. 3. (a) Fourier transform infrared (FTIR) spectra of DNA in PDDA/DNA multilayer films on a modified glassy carbon electrode. Number of layers: 2; 4; 6; 8; 10. (b) Plot of adsorption bands for DNA at 1229 and 1105 cm^{-1} as a function of the number of layers.

DNA in the PDDA/DNA multilayers is not denatured and keeps its B-form (further evidence from CD spectra is shown in the following part). From a biological point of view, this is very important in DNA-related biological application. For example, the design of DNA-based biosensors is one of the tasks in applied biophysics and analytical biotechnology and has attracted substantial recent research efforts [39,40]. Usually, DNA is immobilized by covalently binding with the surface of the transducer [39]. The chemical reactions between the transducer surface and DNA may lead to damaged DNA structure in the binding position, which is not desirable. Therefore, it is necessary to develop methods of immobilization of DNA on different transducer surfaces without destruction of the native DNA structure. The method presented here is promising in this aspect, and hence used to investigate DNA-related biological phenomena and develop DNA biosensors.

3.2. The multilayer assembly of PDDA / DNA on quartz slides

3.2.1. X-Ray photoelectron spectroscopy (XPS) on PDDA / DNA multilayer films

XPS was used to obtain information about the elemental composition of the films and confirm the adsorption of PDDA/DNA multilayers. Three characteristic elements including N, O and P were detected in our experiments. Typical XPS results for PDDA/DNA multilayers are shown in Fig. 4. A characteristic phosphor peak (P2p) is observed at 132.1 eV (Fig. 4A). An N1s signal appears at approximately 400 eV with a distinct shoulder peak at higher binding energy positions. Curve fitting of the N1s signal gives three different peaks with binding energies of 401.4, 399.1 and 397.7 eV, which on the basis of their peak positions were assigned to protonated nitrogen in PDDA, base ring and $-NH_2$ group in DNA, respectively (Fig. 4B). Curve fitting of the O2p

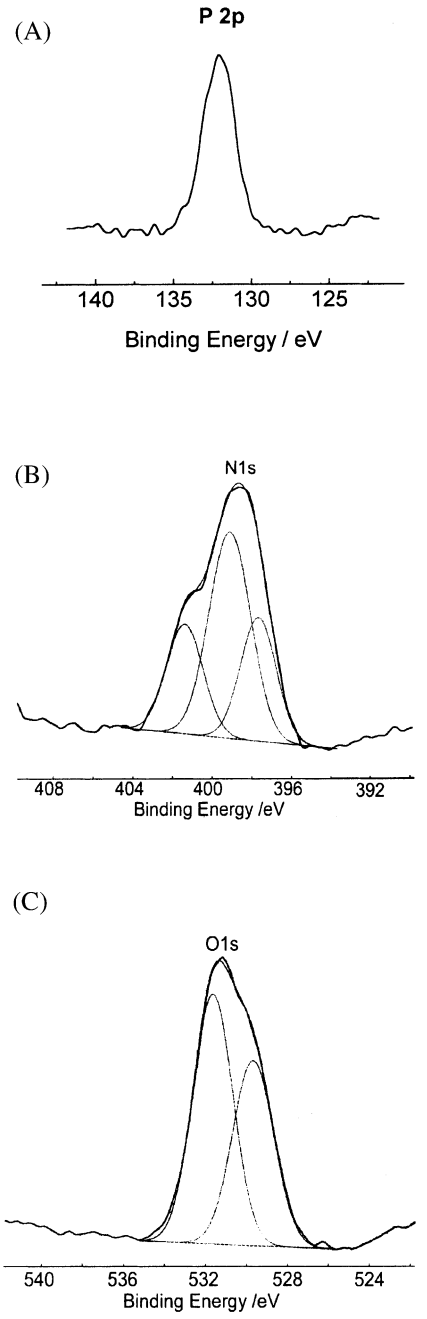


Fig. 4. X-Ray photoelectron spectra of PDDA/DNA multilayer films for the elements, nitrogen (A), phosphor (B) and oxygen (C).

signal gives two different peaks with binding energies of 531.9 and 529.7 eV, which on the basis of their peak positions were assigned to PO_2^- and deoxyribose in DNA, respectively (Fig. 4C) [41]. Although XPS data presented here do not indicate any direct interaction between PDDA and DNA in the films, they clearly demonstrate that PDDA and DNA are assembled on quartz substrates.

3.2.2. UV-vis adsorption spectroscopy characterization of multilayer growth of PDDA / DNA assemblies

Multilayer film growth on quartz slides can be easily monitored by UV-vis absorption spectroscopy. Fig. 5 shows the UV-vis absorption spectra changes of PDDA/DNA multilayers (2, 4, 6, 8, 10 layers). The UV-vis absorption spectrum has a band at 260 nm in the range of 200–350 nm, which is the characteristic absorption band of DNA. The linear increase of the absorbance as a

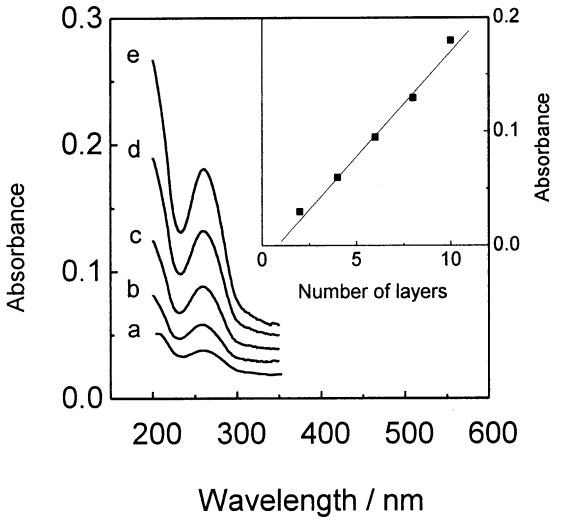


Fig. 5. UV-vis adsorption spectra of PDDA/DNA multilayer films as a function of the number of depositions (from bottom to top, 2, 4, 6, 8, 10 layers were deposited), inset: absorbance of the multilayers films at 260 nm as a function of the number of layers.

function of the number of layers suggests that the amount of DNA deposited each time is constant, as can be seen in the inset of Fig. 5.

3.3. Interaction of DNA in PDDA / DNA multilayer films with methyl green (MG)

Over the past 15 years, the binding interaction between DNA and small organic molecules and their transition metal complexes has received intense interest for different applications [42–44]. For example, dye complexes have been used as probes of DNA duplexes and models of designing new and promising anticancer agents for clinical use. Generally, dyes can bind DNA through three binding modes: intercalation; outside binding without self-stacking; and outside binding with self-stacking along the nucleic acids surface [45–48]. In this context, most research has been carried out in aqueous solution and few reports concerned investigation on their interaction in film on solid substrates.

Here we investigated the interaction of DNA with methyl green in DNA/PDDA multilayer films. It should be noted that many organic molecules and their transitional metal complex can interact with DNA. Therefore, the approach presented here provides a unique way to investigate their interaction in biomimic environment.

In our previous work, the interaction of divalent organic cationic dye, MG, with calf thymus DNA has been investigated by electrochemical, UV-vis and circular dichroism (CD) spectroscopic techniques [49]. In this part, the interaction of DNA with MG in PDDA/DNA multilayer films on quartz substrate was studied by UV-vis and CD spectroscopies.

For UV-vis and CD spectroscopic measurements, a maximum of 24 layers PDDA/DNA were assembled on quartz substrate in the experiment. The assembled DNA films were then immersed into 5 mM aqueous solution of the MG for a certain time. After this, the films were pulled out from the aqueous solution and washed with copious super-pure water for measurements.

Fig. 6a shows a characteristic absorption for DNA in PDDA/DNA multilayers at 260 nm, while Fig. 6b is the UV-vis spectra of DNA after

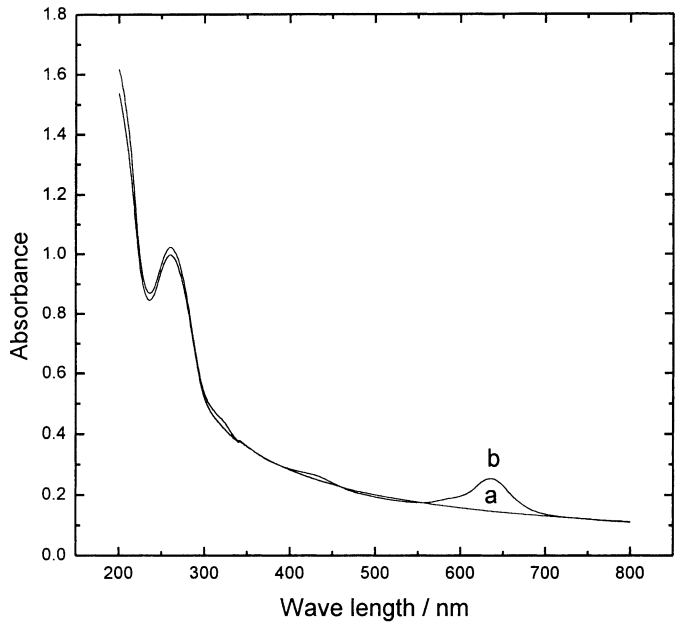


Fig. 6. UV-vis absorption of DNA in PDDA/DNA films before (curve a) and after (curve b) 1 min exposure in a 5-mM MG solution.

being immersed into a 5-mM MG aqueous solution for 1 min. Besides the similar absorbance for DNA at 260 nm, a new band appeared at 640 nm, which is the absorption of CT DNA-MG [50]. No change of the peak intensity at 640 nm was found even by extending the immersion time of DNA films in MG, indicating that the interaction between DNA and MG is very fast. More detailed information on the interaction of DNA and MG in films was obtained by CD spectroscopy. It is well-known that CT DNA is a B-form DNA in aqueous solution [51,52].

Fig. 7 shows the CD spectra of DNA in PDDA/DNA films before (curve a) and after (curve b) exposure in a 5-mM MG solution. As can be seen from curve a, a negative peak at 223 nm and a positive peak at 252 nm were obtained. This is different from the CD spectrum of DNA in aqueous solution. In aqueous solution, the B-form DNA is characterized by a negative peak at 245 nm and a positive peak at 274 nm. The shape of the CD spectrum for DNA in PDDA/DNA films was similar with that in aqueous solution, though both peaks shifted approximately 22 nm in films. Therefore, we consider that DNA takes a B-form in the multilayer, which was supported by the induced CD spectrum of MG while it interacted with DNA in multilayer films.

MG displays no CD spectrum in the absence of nucleic acids. As shown in curve b of Fig. 7, however, an induced CD (ICD) spectrum of MG is observed after it interacted with DNA films. Compared to curve a, besides the CD signal of DNA in the range of 200–300 nm, induced CD signals of MG appeared: a negative band at 420 nm and a positive band at 650 nm, that just like an exciton CD. The result of ICD indicates that there must be other forces involved than purely an electrostatic one with a phosphate backbone. It shows that MG is bound in a groove of the helix. The exciton ICD is explained as follows: MG is outside binding to the surface of DNA helix, which allows interaction between close lying molecules along the DNA helix and independent of the DNA sequence. A positive CD band corresponding to the interacting MG chromophore bears a right-hand helical relationship to one another, as expected for binding to the right-handed helix of the DNA [45–48,53,54]. This is

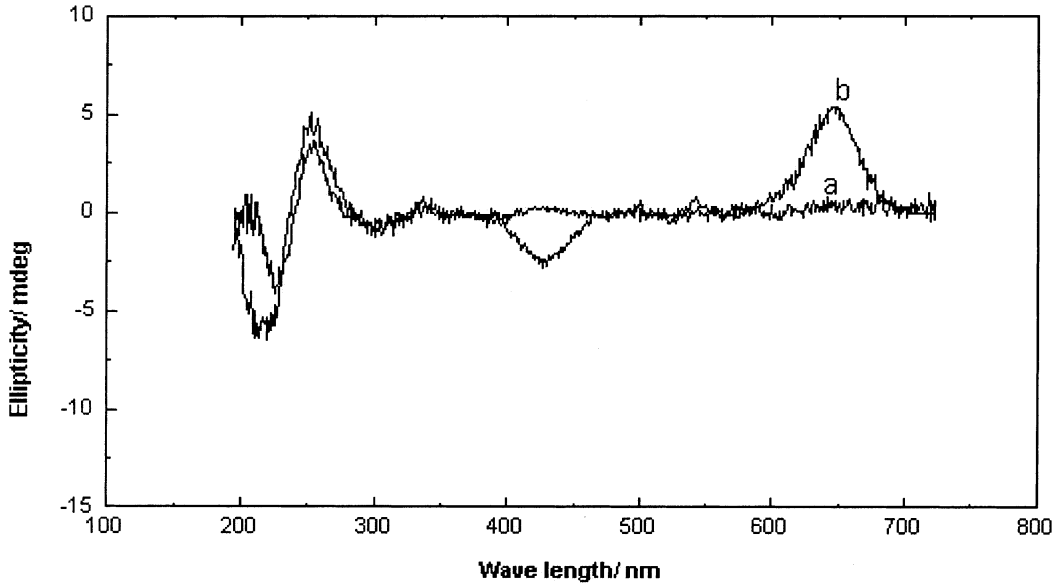


Fig. 7. CD spectra of DNA in PDDA/DNA films before (a) and after (b) exposure in a 5-mM MG solution.

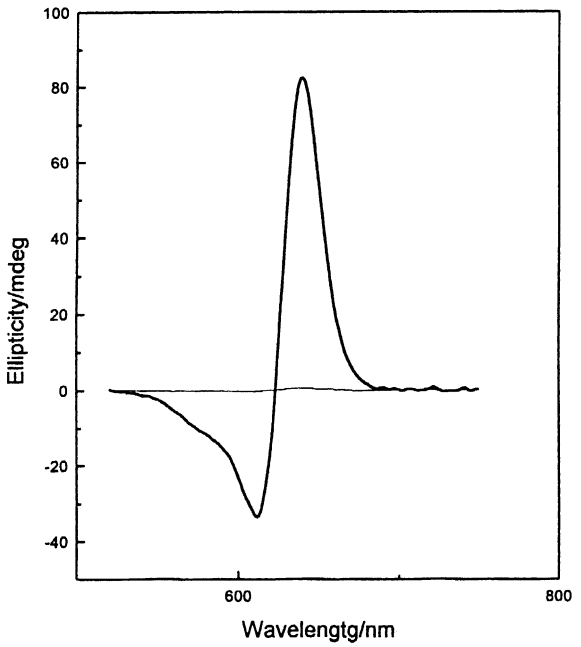


Fig. 8. Induced CD spectrum of MG in the presence of DNA in aqueous solution.

different from that in aqueous solution. In aqueous solution, a positive band at 639 nm and a negative band at 612 nm were observed for the ICD spectrum of MG in the presence of DNA, as shown in Fig. 8. This also supports the results from FTIR: DNA remains in its native structure in multilayer films.

4. Conclusion

In this paper, the multilayer films of PDDA/DNA have been fabricated on glassy carbon and quartz substrates by a layer-by-layer deposition method. Electrochemical impedance spectroscopy, Fourier transform infrared spectroscopy and UV-vis spectroscopy were used to characterize the uniform assembly of PDDA/DNA. X-Ray photoelectron spectroscopy confirms the elemental composition. The results of UV-vis spectroscopy and circular dichroism show that DNA in the multilayer films keeps its native structure and can interact with methyl green. The approach presented in this work provides a unique way to investigate the interaction of DNA and other molecules in a biomimic environment.

Acknowledgements

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