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Interaction of anticancer drug methotrexate with DNA analyzed by electrochemical and spectroscopic methods

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ABSTRACT

Electrochemical DNA biosensor was used to study the interaction of methotrexate (MTX) with DNA immobilized on the bare surface of glassy carbon electrode (GCE). The binding mechanism of MTX with DNA was elucidated by using constant current potentiometric technique further supported by UV–Visible and FT-IR studies. The decrease in guanine peak area was used as an analytical signal for the interaction of drug with DNA in acetate buffer solution at pH 4.2 (20% ethanol). The binding constant (K) value calculated for MTX was $3.821 \times 10^5\ M^{-1}$. UV–Visible studies indicated hyperchromic and hypsochromic shifts in the maximum absorption bands of MTX after interaction with DNA. FT-IR investigations of MTX–DNA interaction revealed significant changes in the characteristic IR absorption bands of all the bases and phosphate groups of DNA. Furthermore, the shift of characteristics bands of C=O, N–H, C–H and O–H groups of MTX endow evidence for the interaction of MTX with DNA supporting the intercalative binding between them.

1. Introduction

There are several types of interactions associated with drugs that bind DNA. These include: intercalation, non-covalent groove binding, covalent binding/cross linking, DNA cleaving and nucleoside-analog incorporation. These binding interactions involve changes to both the DNA and drug molecules due to complex formation. Side groups of drug molecules can make additional contacts with edges of base pairs or sugar-phosphate backbone in major or minor groove or at the surface-phosphate backbone, or at the surface of the DNA double-helix either by hydrogen bonding or electrostatic interactions.

Nuclear magnetic resonance (NMR), mass spectroscopy (MS), FT-IR and Raman spectroscopy, molecular modeling techniques, equilibrium dialysis, electric linear dichroism, capillary electrophoresis, surface plasmon resonance, DNA foot printing etc. are different techniques that have been used to study the drug–DNA interactions. Electrochemical methods have witnessed wide applications that can be applied not only for fundamental studies but also in the practical applications due to their high selectivity, rapidness and low cost instrumentation (Rauf et al., 2005). Different electrochemical techniques being used to study drug–DNA interaction are cyclic voltammetry (CV), anodic stripping voltammetry (ASV), chronopotentiometric stripping analysis (CSA) and potentiometric stripping analysis (PSA; Ozkan, 2009).

The interaction of sildenafil citrate (Viagra) and ciprofloxacin with DNA was studied using an electrochemical DNA biosensor by Nawaz et al. (2006) and Rauf et al. (2007). PSA and differential pulse voltammetry (DPV) at DNA-modified glassy carbon electrode was used to elucidate the binding mechanism of these drugs. The decrease in the guanine oxidation peak area or peak current was used as an indicator for the interaction in 0.2 M acetate buffer (pH 5).

Methotrexate (MTX), 2, 4-diamine-N10-methylpteroyl-glutamic acid)), is a folic acid analog with an amino group substituted for the hydroxyl group at the C4 position on the pyridine ring, which converts the molecule to a tight-binding inhibitor of the enzyme dehydrofolate reductase (DHFR). MTX prevents cancer cells to sustain purine and pyrimidine synthesis (Chow and Rubin, 1998). The affinity of MTX for DHFR is about one thousand-fold to that of folate, thereby, MTX inhibits the synthesis of DNA, RNA, thymidylates and proteins. MTX is used in the clinical treatment of various human neoplastic disorders i.e., childhood acute leukemia (Farber et al., 1948; Saxena et al., 2009), head and neck cancer (Levitt et al., 1973), and micro metastases of osteosarcoma (Frei et al., 1975), whereas its polyethylene esters have been used as drug delivery system (Yousefi et al., 2010).

Many mutagens including methotrexate fit into the space between two adjacent base pairs, this is called intercalating. Most intercalators are aromatic and planar molecules included ethidium,

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daunomycin, doxorubicin, thalidomide, etc. In order for an intercalator to fit between base pairs, the bases must separate, distorting the DNA strands by unwinding of the double helix. This inhibits both transcription and DNA replication, causing toxicity and mutations. DNA intercalators are often carcinogens, and other molecules like benzopyrene diol epoxide, acridnes, and aflatoxin and ethidium bromide being well known examples.

Interaction of methotrexate with DNA has been studied using different electrochemical techniques. The anodic voltammetric behavior of methotrexate at GCE in acetate buffer (pH=3.6) solution using cyclic, square-wave voltammetric and chronocoulometric techniques was investigated by Gao et al. (2007), who reported oxidation of methotrexate was an irreversible diffusion-controlled process and the method was applied for the determination of methotrexate in diluted human urine successfully. Interaction of dsDNA with the combination of methotrexate and emodin showed these drugs forming intermolecular hydrogen bonding that enhanced the anticancer activity of methotrexate (Zhou et al., 2009). Moreover, methotrexate can be used to discriminate between dsDNA and ssDNA due to its different interaction with these molecules. Wang et al. (2009) explored the adsorptive voltammetric behaviors of methotrexate on DNA-modified electrode using cyclic voltammetry (CV) and square wave voltammetry (SWV) and observed that currents measured by SWV presented a good linear function of the concentrations of methotrexate with LOD of 5.0×10^{-9} mol L^{-1}. This method was successfully applied for the determination of methotrexate in pharmaceutical dosage and diluted human urine. Resonance light scattering (RLS), UV–Vis, fluorescence and NMR spectra have been used to study the interaction of methotrexate (MTX) with nucleic acids in aqueous solution in the presence of cetyl trimethyl ammonium bromide (CTMAB) in Briton–Robinson (BR) buffer at pH 9.30 by Cai et al. (2009).

This is the first report for methotrexate interaction with dsDNA using potentiometric stripping analysis (constant current) supported by UV–Visible and FT-IR spectra.

2. Materials and methods

2.1. Apparatus

The electrochemical DNA biosensor consisted of a glassy carbon electrode (Metrohm 6.1204.110, diameter=2 mm), an Ag/AgCl reference electrode (Metrohm 6.0733.100) and a glassy carbon rod (as counter electrode). The analytical signals of the oxidation of guanine were obtained by using CCPS technique with an Auto lab PGSTAT 12 (Eco Chemie, The Netherlands) and the GPES 4.9 software. The moving average baseline correction (peak width=0.001) was applied during data treatment which was smoothed by the Switzky and Golay method (level 4). The UV–Visible spectra were recorded by using a T80 UV–Vis spectrometer (PG Instruments Limited). FT-IR spectroscopy was performed using BX-FT-IR system (Perkin Elmer).

2.2. Reagents and preparation of stock solutions

Salmon sperm DNA (D1626), acetic acid (A6283) and sodium acetate (S653) were obtained from Sigma. Methotrexate tablets (2.5 mg) were purchased from Werrick Pharmaceuticals, Islamabad-Pakistan. Stock solution of different DNA concentrations were prepared by dissolving 2, 4, 6, 8, 10 and 12 mg of Salmon sperm DNA (D1626) in 1 ml of double distilled deionized water. The solution was stirred for 2 h till complete solubility of DNA.

Stock solution of methotrexate (2.5×10^{-5} M) was prepared in 0.2 M NaOH by stirring for 30 min. freshly prepared stock solutions were used during all experiments. Sodium acetate buffer solution 0.16 M HAc–NaAc (20% ethanol, pH 4.2) was used as supporting electrolyte

2.3. Electrode Pretreatment and immobilization of DNA

Before DNA immobilization, glassy carbon electrode (GCE) was polished with Alumina cleaning kit (Metrohm 62.80.2000) to obtain a mirror-like surface. The alumina was removed from the surface of the electrode by sonication, thoroughly washed by deionized water, and dried. For immobilization of DNA on the tip of GCE, it was inverted upside and Salmon sperm DNA solution was dropped on the electrode surface tip with the help of a micropipette. It was allowed to dry for 40 min at room temperature. Immobilization time was kept constant in all experiments. Potentiometric stripping conditions used were equilibration time, 60 s; constant current, +8 μA; and potential range, from 0 to +1.2 V. The amount of DNA immobilized on electrode tip was estimated by plotting the guanine peak area versus DNA concentration.

2.4. Interaction of MTX with DNA

DNA electrode was immersed into methotrexate solution in 0.16 M acetate buffer ($c_{HAc-NaAc}$) at pH 4.2 at open circuit for selected times. The electrode was rinsed after the accumulation of MTX and placed in MTX-free acetate buffer solution and constant current potentiometric voltammogram was recorded.

3. Results and discussion

3.1. Optimization of immobilization of DNA

Optimization of immobilization of dsDNA on the glassy carbon electrode (GCE) was carried out by immobilizing 2 μL of DNA from solutions of different concentrations (2, 4, 6, 8, 10 and 12 mg/ml). The dt/dE signal was plotted against voltage (V). The guanine base in the DNA was oxidized at +0.89 V that gradually shifted to +0.86 V with increase in initial concentration of immobilized DNA. The guanine oxidation peak area was determined and attributed to the concentration of DNA immobilized on the electrode surface. The guanine oxidation peaks showed a gradual increase in peak area with increasing DNA concentrations onto the glassy carbon electrode from (a–e in Fig. 1) and then leveled off (Fig. 1f) ensuring maximum surface coverage of GCE.

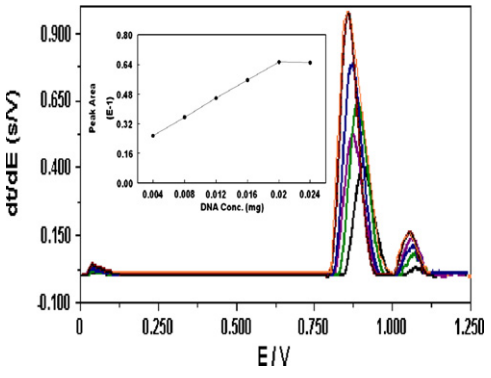

Fig. 1. Optimization of DNA immobilization on the glassy carbon electrode by using constant current potentiometric stripping analysis. Inset: plot of the different DNA concentrations against the guanine peak area obtained by immobilizing the corresponding DNA concentrations on glassy carbon electrode.

Different DNA concentrations were plotted against the guanine peak area (Fig. 1, inset). Peak area increased concomitantly with DNA concentration, showing maxima at 0.02 mg. However, by varying the initial concentration of stock solutions the amount of DNA immobilized to surface of GCE increased from 0.014 to 0.02 mg. (Nawaz et al., 2006). In this study, a thick film DNA biosensor was used impeding the undesired binding of drug molecules to the electrode surface.

3.2. Electrochemical studies of MTX–DNA interaction

3.2.1. Optimization of interaction time

The accumulation time for the methotrexate on the DNA-modified electrode was optimized (Fig. 1S supplementary material). The guanine peak current decreased with an increase in accumulation time. After 4 min of interaction, the decrease in guanine peak current leveled off and remained constant up to 5 min. Therefore, in all the experiments, 5 min interaction time was used which was reported earlier for interaction of ciprofloxacin with DNA (Nawaz et al., 2006). However, Zhou et al. (2009) reported 10 min stirring time for interaction of MTX with DNA using cyclic and differential pulse voltammetric techniques.

3.2.2. Optimization of pH

The potentiometric stripping analysis of methotrexate–DNA interaction was investigated within pH range of 4.0–8.0 in acetate buffer solution (Fig. 2S supplementary material). Results indicated that only the measurements made in acidic media produced stable responses and the best defined peak was obtained at pH 4.2. It was also observed that both the current and peak potential were affected by the concentration of acetate buffer. The potentiometric stripping analysis was carried out at 0.1, 0.16 and 0.2 M concentrations of acetate buffer. Sharp peak was obtained at $c_{HAc–NaAc}$ 0.16 M and above. Therefore, 0.16 M acetate buffer was chosen as the supporting electrolyte, producing only one anodic peak was observed indicating the irreversibility the reaction.

At pH < 4.2, peak current was very sensitive to the pH of solution and the stability of voltammogram was very poor. On the other hand, at pH > 7.2, the anodic peak almost disappeared which indicated that methotrexate was amenable to oxidation. Therefore, pH 4.2 was selected for interaction. At pH 4.2, the peak potentials for DNA oxidation was shifted from 0.89 to 0.94 V, predicting that oxidation of DNA on the electrode surface becomes recalcitrant, indicating a partly intercalation of MTX into DNA double-helix structure. Shift in peak potentials for DNA oxidation occurs after interaction with MTX at pH 7.2.However, by increasing MTX concentration from 2 to 3.6 μM, the peak potential for DNA oxidation shifted from 0.89 to 0.87 V. Zhou et al. (2009) also used same pH value (4.2) for studying interaction of methotrexate with DNA in combination with emodin. Our results are in agreement to that reported in literature using differential pulse voltammetry interaction of MTX with DNA showing partially intercalation.

3.2.3. Optimization of drug concentration

Potentiometric stripping analysis (PSA) of DNA–MTX interaction in HAc–NaAc (20% ethanol, pH 4.2) buffer solution is shown in Fig. 2. By increasing drug concentration from 0 to 3.6 μM the peak current of DNA decreased and t shifting the peak potential from 0.89 to 0.94 V, indicating that MTX partly intercalated into the DNA double-helix structure, rendering oxidation process on the electrode surface difficult.

Probably this peak current decrease can ascribed to the methotrexate–DNA complexation in solution. Positive peak potential shifts of intercalators were observed in the binding form via

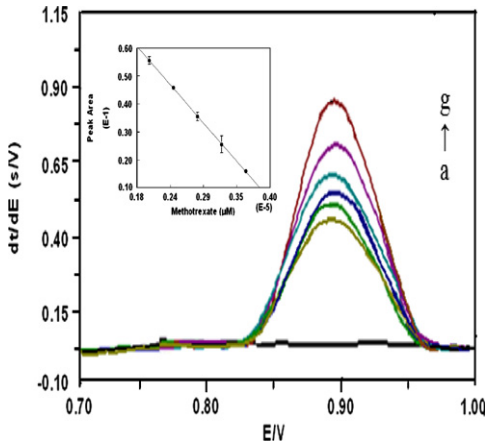

Fig. 2. Constant current potentiometric voltammograms for the interaction of methotrexate with dsDNA modified electrode. Oxidation signals of guanine at the bare GCE (a), DNA-modified electrode (g) and (b–f) after interaction with different concentrations (i.e., 2, 2.4, 2.8, 3.2 and 3.6 μM) of methotrexate for 5 min in 0.16 M acetate buffer (20% ethanol, pH 4.2). Inset): Calibration curve for the determination of methotrexate (2.0–3.6 μM) at dsDNA-modified electrode by using decrease in the guanine oxidation peak area.

hydrophobic interactions (Bard et al., 1989). The shift of peak potential indicated that the planar aromatic structure of methotrexate was expected to help out its intercalation into the DNA helix.

The calibration curve was obtained by plotting methotrexate concentration against the guanine oxidation peak area. By increasing the concentration of drug, guanine oxidation peak area was decreased. The PSA voltammograms of various concentrations of methotrexate demonstrated good linearity (Fig. 2, inset). The linear segment increased from 2.0×10^{-6} to 3.6×10^{-6} M (r=0.999). It is found that this method can detect 5.63×10^{-9} M methotrexate after 5 min accumulation time. The currents measured by SWV presented a good linear property as a function of the concentrations of methotrexate in the range of 2.0×10^{-8} to 4.0×10^{-6} mol L^{-1}, with detection limit (LOD) of 5.0×10^{-9} mol L^{-1} (Wang et al., 2009). The decrease in the signals of guanine oxidation peak from DNA-modified electrode was attributed to the binding of methotrexate to the guanine bases which could be explained due to shielding of the oxidizable groups of guanine.

DNA–MTX interaction was determined by using equation given below, slightly modified according to the nature of the immobilized molecules (Ibrahim, 2001).

$$\mathrm{DNA}+\mathrm{MTX}\rightarrow\mathrm{DNA}-\mathrm{MTX} \quad \text{(A)}$$

$$\log(1/\mathrm{MTX}) = \log K + \log\left(S_{DNA-MTX}/S_{DNA}-S_{DNA-MTX}\right) \quad \text{(B)}$$

where "K" is the binding constant, S_{DNA} represents the signal obtained just by the immobilized DNA alone and $S_{DNA–drug}$ represents the signal obtained by the formation of DNA–drug complex after the interaction of drug with DNA that was immobilized on the surface of electrode. "S" is the peak area in case of constant current potentiometery. By plotting log (1/drug) versus log ($S_{DNA–drug}/S_{DNA}-S_{DNA–drug}$), the value of binding constant was determined from the intercept of the straight line. Greater the binding constant values; greater will be the MTX–DNA interactions and vice versa. The plot of log (1/MTX) versus log ($S_{DNA–MTX}/S_{DNA}-S_{DNA–MTX}$) for MTX–DNA interaction gave a straight line having r value of 0.99 and the value of K determined from the intercept of the straight line was 3.436×10^{5} M^{-1}. (please see Fig. 3S in supplementary material)

Table 1 showed the decrease in guanine peak area with increase in concentration of methotrexate and corresponding values of binding constants (K). The experimental average value of "K" as calculated from Table 1 was 3.821×10^{5} M^{-1}.

Intercalation has been traditionally associated with molecules containing fused bi/tricyclic ring structures, atypical intercalators e.g., mitimycin, distamycin, etc., with nonfused rings systems may be more prevalent than previously recognized. Although DNA intercalators have been used extensively as antitumor, antineoplastic, antimalarial, antibiotic, and antifungal agents, not all intercalators are genotoxic. The presence of basic, cationic, or electrophilic functional groups is often necessary for genotoxicity (Snyder, 2007).

3.3. UV–Vis spectroscopy of MTX–DNA interactions

UV–Vis spectroscopy was used to confirm the formation of new products of drug with DNA. The absorption spectra of MTX interacting with DNA in range of 190–420 nm. The DNA has an absorption peak at about 258 nm. Different concentrations (0.02, 0.04, 0.06, 0.08, 0.1, 0.12 and 0.14 mg/ml of 0.16 M acetate buffer pH 4.2) from DNA stock solution (10 mg/ml de-ionized water) were used.

There was a concomitant rise in absorbance with increments in DNA concentrations from 0.02 to 0.1 mg/ml beyond which its increase was irregular (Fig. 3a). Therefore, 0.1 mg/ml concentration of DNA was selected for further experiments. Methotrexate has a sharp absorption peak at 208 nm, a peak at 256 nm and a broad absorption peak at about 302 nm. With an increase in the concentration of methotrexate (2, 2.4, 2.8, 3.2 and 3.6 μM), the value of all three absorption peaks increased (Fig. 3b). However, it is evident that the absorption peaks of MTX at 208 nm and 302 nm were not changed, while the new broad absorption peak at around 254 nm appeared to be the superposition of DNA (258 nm) and MTX (256 nm) spectra (Fig. 3c). The overall absorption decreased (hypochromic effect) and a slight shift of peak towards smaller wavelength region (hypsochromic effect), indicating the interaction (intercalation) between MTX and DNA. This was consistent with the result obtained by the electrochemical method.

When fixed amount of drug (2 μM) was allowed to interact with different concentrations of DNA (0.02–0.12 mg/ml of buffer; Fig. 3d), intercalational phenomena of the methotrexate with DNA could be explained by the presence of two different electronic transitions, π–π^* and n–π^* of methotrexate spectra. The increase in the concentration of added DNA enhanced the absorption peak height, i.e. hyperchromic effect of band II (π to π^* region) that could be related with enhanced intercalation of methotrexate into DNA and the complex formed accordingly may become more compact (Please see Fig. 4S and 5S in supplementary materials).

These findings are in accordance with that reported earlier (Janjua et al., 2009). This effect was also attributed to absorption of DNA and methotrexate in the same region of UV–Visible spectrum. Moreover, band I clearly showed that the λ_{max} shifted towards a longer wavelength (red shift) with the addition of Salmon sperm DNA. The increase in intensity of the absorption maxima (hyperchromic effect) of the drug or DNA binding molecules is a typical characteristic of DNA intercalation (Chen et al., 2005; Hajian and Tavokol, 2012, Ibrahim, 2001; Kashida et al., 2006). These spectral characteristics are attributed to the strong interaction of the drug chromophore with DNA bases. This hyperchromic effect provided the evidence for the MTX intercalation in DNA.

It has been reported that fagaronine, ethoxidine and methyl red intercalated into dsDNA and showed hypsochromicity upon addition of DNA (Kashida et al., 2006). Methotrexate on interaction with DNA showed both the hyperchromic and hypsochromic effects and this reflected the strong intercalative binding of MTX to DNA.

Table 1
Binding constant values for methotrexate MTX–DNA interaction at different MTX concentrations.

Ox. pot. (V)	DNA conc. (mg)	MTX conc. (μM)	Peak area	Binding constant ($K=M^{-1}$)
0.90	0.02	2.0	0.0555	8.851×10^4
0.91	0.02	2.4	0.0458	1.775×10^5
0.92	0.02	2.8	0.0355	3.0×10^5
0.93	0.02	3.2	0.0255	4.879×10^5
0.94	0.02	3.6	0.016	8.570×10^5

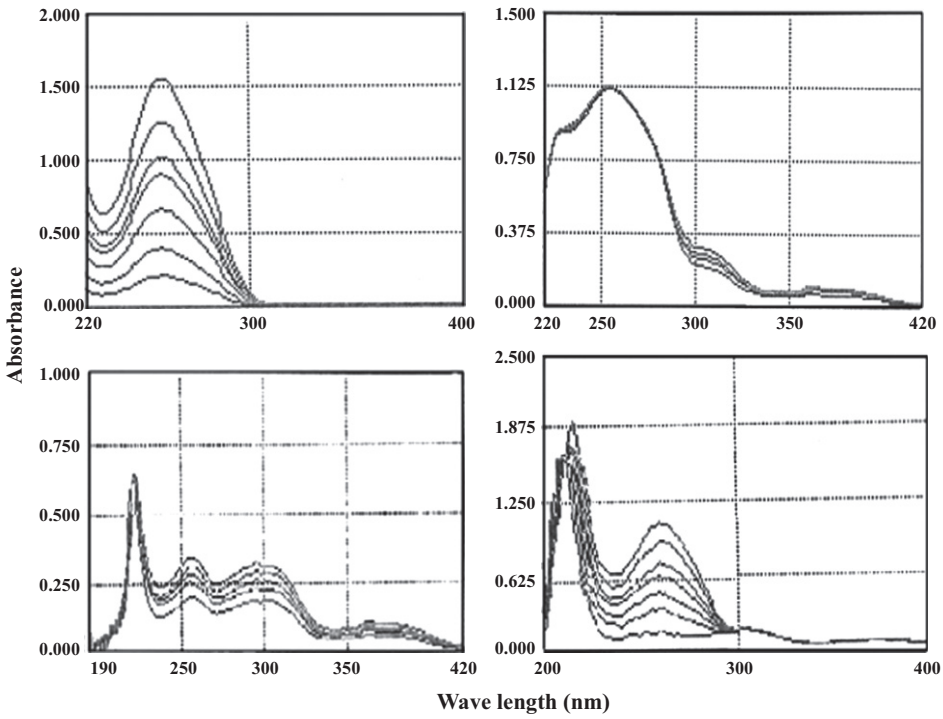

Fig. 3. UV–Visible spectra of DNA (a), MTX (b) and MTX–DNA interaction in 0.16 M HAc–NaAc (20% ethanol, pH 4.2) (c) DNA=10 μL and MTX=500–900 μL, (d) DNA =2–12 μL and MTX=500 μL.

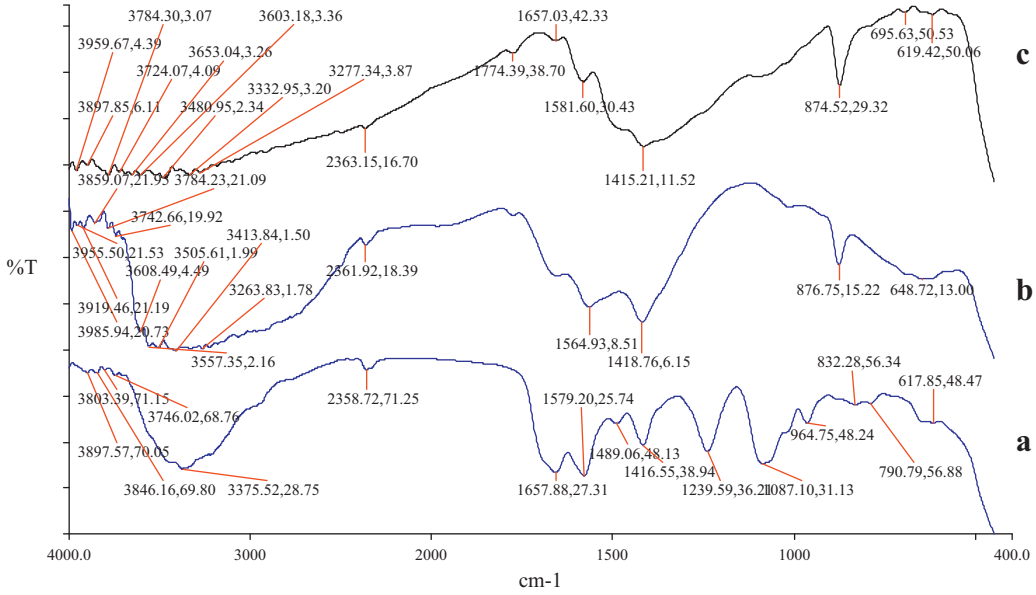

Fig. 4. FT-IR-spectra of DNA (a), MTX (b) and MTX–DNA complex (c).

3.4. *Fourier transform infrared (FT-IR) spectroscopy*

Vibrational spectroscopy (Raman and infrared) is often used to characterize the nature of drug–DNA interaction and to monitor the effects of various drugs on DNA structure (Morjani et al., 1993; Medina et al., 1998; Neault and Riahi, 1998). Fourier transform infrared (FT-IR) spectroscopy is used to determine drug binding sites, sequence preference and DNA secondary structure, as well as the structural variations of drug–DNA complexes in aqueous solution (Gal et al., 1993). The FT-IR spectral characterizations of DNA and the influence caused by adding methotrexate were studied in 0.16 M HAc–NaAc (pH 4.2, 20% ethanol) as shown in Fig. 4. For systematic evaluation, the FT-IR spectra were divided into three regions; functional group region (4000–1300 cm^{-1}), fingerprint region (1300–910 cm^{-1}) and aromatic region (910–650 cm^{-1}). The bands shown in the spectrum of DNA were identified according to the literature (Wang et al., 2001). The FT-IR spectral information about the occurrence of interaction could be easily gained by comparing the spectra of DNA (a) and MTX (b) with that of MTX–DNA complex (c). All the spectra were recorded in the region from 4000 to 400 cm^{-1} at 16 scans and 8 cm^{-1} resolution. Comparison of the IR spectrum of MTX–DNA complex (curve c) with that of DNA (curve a) revealed the appearance of some new absorption bands at 3653, 3481, 3333, 1775, and 696 cm^{-1}The absorptions at 1240, 1087 and 965 cm^{-1} disappeared with the appearance of new absorption at 1775 cm^{-1} providing evidence that MTX can interact with the phosphate groups of DNA by hydrogen bond or electrostatic interaction. Our results are in accordance with those reported for DNA–Indirubin interaction where also a new absorption band appeared at 1777 cm^{-1} after complex formation (Ye et al., 2005).

The appearance of strong absorption bands in the region of 4000–2500 cm^{-1} are usually due to stretching vibrations between hydrogen and some other atoms with a mass of 19 or less. In our studies, the DNA spectrum showed bands at 3898, 3846, 3803, 3746 and 3376 cm^{-1} which could be attributed to O–H, N–H and C–H stretching.

The other bands in this region were 1658, 1579, 1489, 1417 cm^{-1}. The bands in the 1800–1500 cm^{-1} region are due to C═O stretching, skeletal stretching and N–H bending vibrations of the base residues; absorption bands of DNA at 1658 cm^{-1}, corresponds to the vibrations of C_6═O of guanine and C_4═O of thymine (James et al., 1991) 1579 and 1489 cm^{-1} corresponds to adenine and cytosine respectively (Senthil and Sarojini, 2009) and 1417 cm^{-1} are due to ring vibrations of guanine.

The bands in the fingerprint region (1300–910 cm^{-1}) include the contributions from the complex interacting vibrations; in these studies, the DNA spectrum showed bands at 1240, 1087 and 965 cm^{-1} corresponding to the symmetric and antisymmetric stretching vibration of phosphate group of DNA (James et al., 1991). Strong absorption peaks appeared at 832 and 791 cm^{-1}, that may be due to the stretching of P–O, C–O and N–H out-of-plane bending vibrations or caused by the presence of aromatic rings. However certain non-aromatic molecules such as amines and amides can also contribute absorption in this region.

Increase in intensity of absorption band of DNA (1658 cm^{-1}) from 27% to 42% is associated with the shift of the band from 1658 to 1657 cm^{-1} which corresponds to the vibrations of C_6═O of guanine and C_4═O of thymine (James et al., 1991). The intensity changes of other DNA vibrations is also associated with the shift in the bands from 1579 (adenine, 26%) to 1581 cm^{-1} (30%) and from 1417 (ring vibrations of guanine, 39%) to 1415 cm^{-1} (12%); absorption band of DNA at 1489 cm^{-1} (cytosine) completely disappeared after interaction with drug. These changes are significant in the characteristic IR absorption bands of all the bases of DNA and reflected the intercalation mode of interaction of MTX with DNA.

Additional evidence for drug–DNA interaction was obtained from the spectral shifting of methotrexate vibrational frequencies upon DNA binding when major infrared bands of stretching vibrations of MTX shifted from 3743 to 3724 cm^{-1}, 3608 to 3603 cm^{-1} and 3264 to 3277 cm^{-1} (stretching vibration between hydrogen and some other atoms with mass of 19 or less i.e., C–H, N–H, O–H, etc.). The absorption bands due to stretching of carboxylate ions shifted from 1565 to 1581 cm^{-1} and 1419 to 1415 cm^{-1}. The absorption bands due to aromatic ring also shifted from 877 to 875 cm^{-1}. The shift of all these bands are evidence for a major MTX–DNA interaction through C═O, N–H, C–H or O–H groups of MTX. Moreover, the absorption bands at 3985, 3919, 3859, 3557, 3506, 3414 and 649 cm^{-1} disappeared from IR spectrum of MTX after addition of DNA.

4. Conclusions

The maximum amount of DNA that can be immobilized on the glassy carbon electrode is 0.02 mg. Guanine oxidation peak area decreased with increase in the concentrations of MTX. Optimum pH and interaction time of drug with DNA was found to be 4.2 and 5 min respectively. Value of binding constant was in the range of 10^{-5} for MTX showing intercalative and electrostatic mode of interaction with DNA. The UV–Vis studies revealed hyperchromic and hypsochromic nature of MTX–DNA interaction. However, FT-IR analysis of DNA, MTX before and after interaction showed the involvement of nitrogen bases, phosphate groups of DNA through hydrogen bonding, intercalative or electrostatic interactions. These studies show that electrochemical DNA biosensor can be helpful in understanding the drug–DNA interaction giving fast and reliable information about the affinity of chemical compounds and their mechanism of interaction with DNA.

Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.12.028.

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