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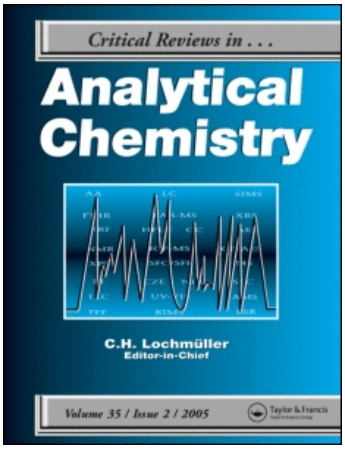

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Impedance Analysis of DNA and DNA–Drug Interactions on Thin Mercury Film Electrodes

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ABSTRACT: In this review we briefly summarize the results of impedance measurements (dependence of the differential capacitance C and/or impedance Z of the electrode double layer on potential E, C-E curves) or on frequency (Electrochemical Impedance Spectroscopy-EIS) of nucleic acids at mercury film electrode (MFE). Mercury film was plated either on a glassy carbon electrode (GCE) or pyrolytic graphite electrode (PGE). The dependence of the pseudocapacitance redox peak of echinomycin on concentration and on the ac voltage frequency was measured in bulk solution. Interaction of single- and double-stranded DNA with *bis*-intercalator echinomycin at nucleic acid-modified surface was studied using EIS with adsorptive transfer method.

KEY WORDS: electrochemical impedance spectroscopy, DNA, Intercalators, DNA at electrode surface.

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I. INTRODUCTION

The measurement of the impedance of the electrified interfaces started to be widely used for investigation of the interactions of nucleic acids and their components with electrode surface[1-20] since 1961, when I.R. Miller[1,2] has published his pioneering work on differential capacitance of the mercury electrode double layer in the solutions of nucleic acids. Differential capacitance of the electrode double layer is a sensitive indicator of the adsorption. When nucleic acids and/or nucleic acid bases, nucleosides, and nucleotides are adsorbed at the electrode surface, they remove from the surface the molecules and ions of the solvent and thus lower the value of the differential capacitance of the electrode double layer, because the solvent has usually much higher dielectric permittivity than nucleic acids.

In 1965 one of us (V.V.) found that nucleic acid bases posses an extraordinary high ability of self-association at the electrode surface and undergo a two-dimensional (2-D) condensation forming a monomolecular layer (self-assembled monolayer — SAM, a compact film).[3,21] The two-dimensional condensation was also observed with some of the halogen-, aza-, and methyl derivatives of common nucleic acid bases[18,22,23] and with most of the nucleosides[4,24] and nucleotides[25,26] commonly occurring in nucleic acids. The formation of a compact film at the electrode is characterized by the appearance of a well-defined capacitance "pit" on the capacitance-

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potential (*C*-E) curves. From the temperature dependence of the capacitance pit or from electrocapillary measurements the energy of the interaction between bases in the compact film[27-30] as well as the surface concentration of the adsorbed molecules and thus the area *A* required for one adsorbed molecule in the electrode surface can be calculated.[18,24,28,29,31] From the value of the area *A* the orientation of bases and nucleosides in the compact film thus can be determined.[27,28,31] The kinetic of the two-dimensional condensation of bases at the electrode surface[15,17,24,27,32-37] and the effect of ions of the solvent and substituents of bases on the film formation[18,22,23,29,32] were investigated.

With neutral bases the capacitance pit is usually observed near the potential of electrocapillary maximum (potential of zero charge, pzc). Exception in this respect are the bases with a large electric permanent dipole moment[38] like cytosine that forms the pit at negative potentials.[21,25,39-41] The halogen ions can induce a new potential region of condensation with cytosine[40] and with adenine.[35] The two-dimensional condensation of cytosine molecules at the mercury electrode surface is supported by protonation of a part of cytosine molecules.[41] *Ab initio* quantum chemical calculations have proven that the protonation significantly increases the stabilization energy of both stacked and hydrogen bonded cytosine dimer, which may support the two-dimensional condensation.[42]

The potential of maximum adsorption of polymeric nucleic acids or synthetic polynucleotides is usually around the pzc. From the positively or negatively charged electrode surface the nucleic acids are desorbed and adsorption-desorption (tensammetric) peaks appear on the C-E curves resulting from the sharp changes of the surface charges and/or surface coverage Θ within a narrow potential range. Thus, tensammetric peaks appear at potentials where $d\Theta/dE$ reaches maximum.

The use of the MFE and/or solid electrodes instead of mercury drop electrodes opened a new experimental field for the construction of DNA hybridization probes.[43-50] With solid electrodes[36,51-54] it was possible to use another experimental techniques studying the formation of self-assembled monolayers at the electrode surface such as scanning tunnelling microscopy (STM) and atomic force microscopy (AFM) imaging.[55-58] Single crystal gold electrodes have been used in widely electrochemical studies so that reliable results are obtained on well-defined surface structure. It was shown that uracil, uridine, cytosine, and cytidine may form condensed layers not only at the mercury electrode interface[18,59-63] but also at gold single crystal interface.[55,64-68] Both PNA and DNA oligomers displayed a strong adsorption onto the carbon electrode.[69]

The formation or dissolution of self-assembled monolayer (SAM) at the solid electrode interface was studied.[70-79] The potential windows for most of the solid electrodes is about 1 V more positive than for the MFE.[80-84] With MFE we can investigate the effect of the structure of the substrate of solid electrode on the adsorption or desorption of the nucleic acid in the same potential window as with a mercury drop electrode.

The MFE prepared by the deposition of mercury onto GCE has been used frequently for electrochemical studies during recent years.[47,83-85] On the other hand, there are only a few electrochemical studies working with the MFE prepared by the deposition of mercury onto PGE.[86] Therefore, we have compared the DNA adsorption and drug-DNA interactions at the mercury film deposited onto GCE and basal plane PGE.

II. MERCURY FILM ELECTRODES

Descriptions of different types of carbon materials and reactions observed at graphite electrodes were reviewed by Panzer.[87] Later

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more detailed studies about the effect of a carbon microstructure and morphology of different carbon substrates and PGE on the heterogeneous electron-transfer kinetics of some compounds were published using electrochemical methods,[88-92] Raman spectroscopy[89,90] and/or STM.[93,94] MFE prepared on GCE were used in electrochemical,[95-97] electrochemical quartz microbalance (EQCM),[96] and STM[98] studies.

We have used a PGE in a basal plane orientation or a GCE as the working electrodes. Mercury was plating on the GCE or basal plane PGE by electrochemical deposition. The working electrodes were immersed into 0.2 *M* $Hg(NO_3)_2$ water solution and treated at potential –1.0 V for 1 min 20 s (GCE) and 2 min 20 s (PGE), respectively. Figure 1 shows images from optical metallurgical microscope (zoom ratio 12.86) of the GCE and PGE covered or uncovered with a thin Hg film. Surface of the GCE (Figure 1A) is flat and glossy, surface of the basal plane oriented PGE (Figure 1B) is rough and corrugated. Figure1C shows surface of the GCE covered by Hg film (GCE|Hg), which is formed by droplets of mercury of different size. Consequently, flat and glossy surface of GCE covered by mercury is more rough and irregular. Figure 1D shows surface of the PGE covered by Hg film (PGE|Hg). It can be seen that the smaller corrugations were filled with the mercury; the mercury seems to form a continuous film at the surface of the PGE. The surface covered with a Hg film is smoother, but the general topology of the surface is not changed. Thus, the morphology and the surface properties of MFE depend on the underlying material of the electrode.

III. ELECTROCHEMICAL ANALYSIS OF NUCLEIC ACIDS

The electroactivity of nucleic acids at mercury electrode was discovered about 40 years ago.[8,99] It was found later that only nucleic acids bases are responsible for nucleic acids redox signals; adenine and cytosine were reduced at mercury electrode at neutral pH[100] and guanine produced a specific anodic signal explained by the oxidation of the guanine reduction product formed at highly negative potentials.[101] In addition, all nucleic acid bases produced anodic signals due to the formation of sparingly soluble compounds with the electrode mercury that were utilized for the cathodic stripping voltammetric determination of nucleic acid bases at submicromolar concentrations.[102]

On C–E curves and/or ac voltammograms, single-stranded (ss) DNA yields two peaks attributed to desorption and/or reorientation of DNA molecules. The desorption of DNA segments adsorbed via DNA sugar-phosphate backbone appears at about –1.2 V and is denoted as peak 1 in C-E curves.[103] At more negative potential (around –1.4 V) DNA segments adsorbed through hydrophobic base residues are desorbed, yielding peak 3. Double-stranded (ds) DNA produces tensammetric peak 1 as well.[1,2] At full electrode coverage the height of the peak 1 of ssDNA and dsDNA is almost the same. At hanging mercury drop electrode (HMDE) dsDNA yields also peak 3 (if potential is scanned from positive to negative values), which is smaller than peak 3 of ssDNA. This peak is connected with partial unwinding of dsDNA at negative potentials.[41,103-105] In unwounded (distorted) regions dsDNA is adsorbed at the electrode surface via bases like ssDNA. The unwinding probably takes place at the ends of the dsDNA molecules and/or around breaks of the single strand.[41,103-105]

In the 70 years the oxidizability of adenine and guanine residue in polynucleotides at the graphite surfaces was demonstrated.[106-109] Under conditions when monomeric adenine and guanine and their derivatives yield well-defined voltammetric oxidation current, nucleic acids containing these bases yield only poorly developed peaks on conventional linear sweep voltammograms at the graphite

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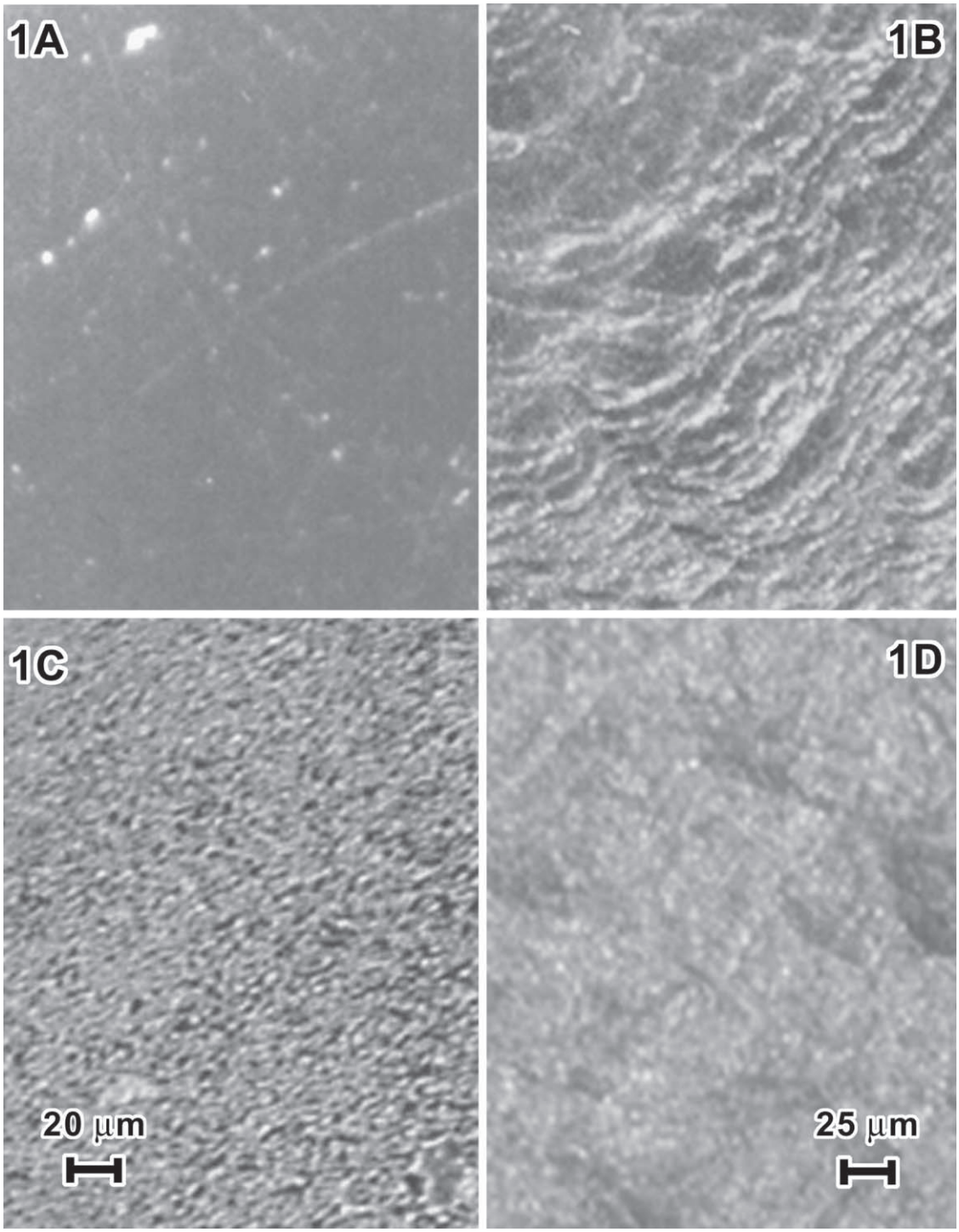

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FIGURE 1. The working electrodes were a laboratory-made pyrolytic graphite electrode (PGE) and glassy carbon electrode (GCE) covered by a thin Hg film. Before plating Hg film on the PGE and GCE, the electrode surfaces were carefully cleaned by mechanical polishing. Then the electrodes were washed by distilled water. Finally, the electrodes were cleaned in an ultrasonic bath for several minutes before measurement. Mercury was plating by electrochemical deposition. The PGE (Figure 1B) or GCE (Figure 1A) was immersed into 0.2 *M* $Hg(NO_3)_2$ water solution and treated at potential -1.0 V for 2 min 20 s or 1 min 20 s, respectively. The mercury was presented as a compact thin film on the PGE (Figure 1D) or as droplets of mercury of different size on the GCE (Figure 1C). The electrochemical active areas of the PGE|Hg or GCE|Hg are $A = 0.14$ cm^2 and $A = 0.04$ cm^2, respectively.

electrodes. However, differential pulse voltammetry at the graphite electrode gives rise to well-developed peaks of nucleic acids with peaks potentials at 0.9 and 1.2 V at around neutral pH.[108,110]

Recently, MFE have been employed for voltammetric analysis of nucleic acids. It was stated that surface of MFE is sufficiently stable and can be used for electrochemical studies of DNA in negative potential region.[43,47] Fojta and co-workers used GCE|Hg for measurements of redox and tensametric response of DNA, RNA, synthetic polynucleotides and peptide nucleic acids (PNA).[19,47,85]

The frequency dependence of the double layer impedance of DNA-modified GCE was studied by Brett[111] and of DNA-modified HMDE by Vetterl et al.[41] It was found that the desorption of ssDNA is accompanied by higher dielectric losses than the desorption of dsDNA. With ssDNA the desorption of more firmly bound bases is accompanied by higher dielectric losses then the desorption of the sugar-phosphate backbone.[41]

In the present article we compare the frequency response of the impedance of electrode double layer of three types of electrodes (HMDE, GCE|Hg, and PGE|Hg) modified with nucleic acids. We show that the frequency response depends on the underlying material of the electrode as it can be seen in Figure 2.

Figure 2A shows the frequency dependence of the impedance of electrode double layer modified by nucleic acids measured with the HMDE at potential of the tensammetric peak 3. Providing that the mercury-solution interface can be simulated by a parallel combination of C_1 (double layer capacitance) and R_2 (representing dielectric losses by tensammetric processes and/or charge transfer resistance by redox reactions) in series with the background electrolyte resistance R_1 the complex impedance plot should be a semicircle[112-117] the radius of which is smaller with lower R_2 values. From Figure 2A it can be seen that ssDNA has a smaller radius (lower R_2) than the dsDNA, which is in good accordance with earlier findings[41] and interpreted by a higher dielectric losses accompanying the desorption of denatured DNA. With the GCE|Hg the complex plane impedance plots of DNA exhibited arc shape as well (Figure 2B). With the PGE|Hg (Figure 2C) the complex plane impedance plots of DNA exhibited two arcs of different radius. The smaller arc (50 to 10 kHz) is observed not only with PGE|Hg modified by DNA but with background electrolyte as well and thus results obviously from the kinetic processes taking place at the PGE|Hg–electrolyte interface at higher frequencies. The semicircle with larger radius can be simulated by a parallel combination of C_1 and R_2 in series with the resistance R_1. The semicircle of ssDNA has a smaller radius than the semicircle of dsDNA both at HMDE and at the GCE|Hg or PGE|Hg, that is, the desorption of ssDNA is accompanied by a higher dielectric losses than the desorption of dsDNA at all three types of electrodes. The radius of the semicircles in the complex impedance plots in Figure 2 increases in the order PGE|Hg < GCE|Hg < HMDE both with ss and ds DNA. Thus, the desorption of DNA from MFE is accompanied by higher dielectric losses than the desorption from HMDE. The desorption of DNA from PGE|Hg is accompanied by higher dielectric losses than the desorption from GCE|Hg.

IV. IMPEDANCE ANALYSIS OF DNA–DRUG INTERACTIONS

One of the important type of interactions is the intercalation of a planar aromatic system inside the molecules of nucleic acids. This type of interaction enables the insertion of the intercalator between adjacent base pair of nucleic acid by changing torsion angles in the sugar-phosphate backbone. A number of these compounds belong to known carcinogens (polycyclic aromatic hydrocarbons, aromatic amines) or antitumor antibiotics (daunomycin, doxorubicin, bleomycin,

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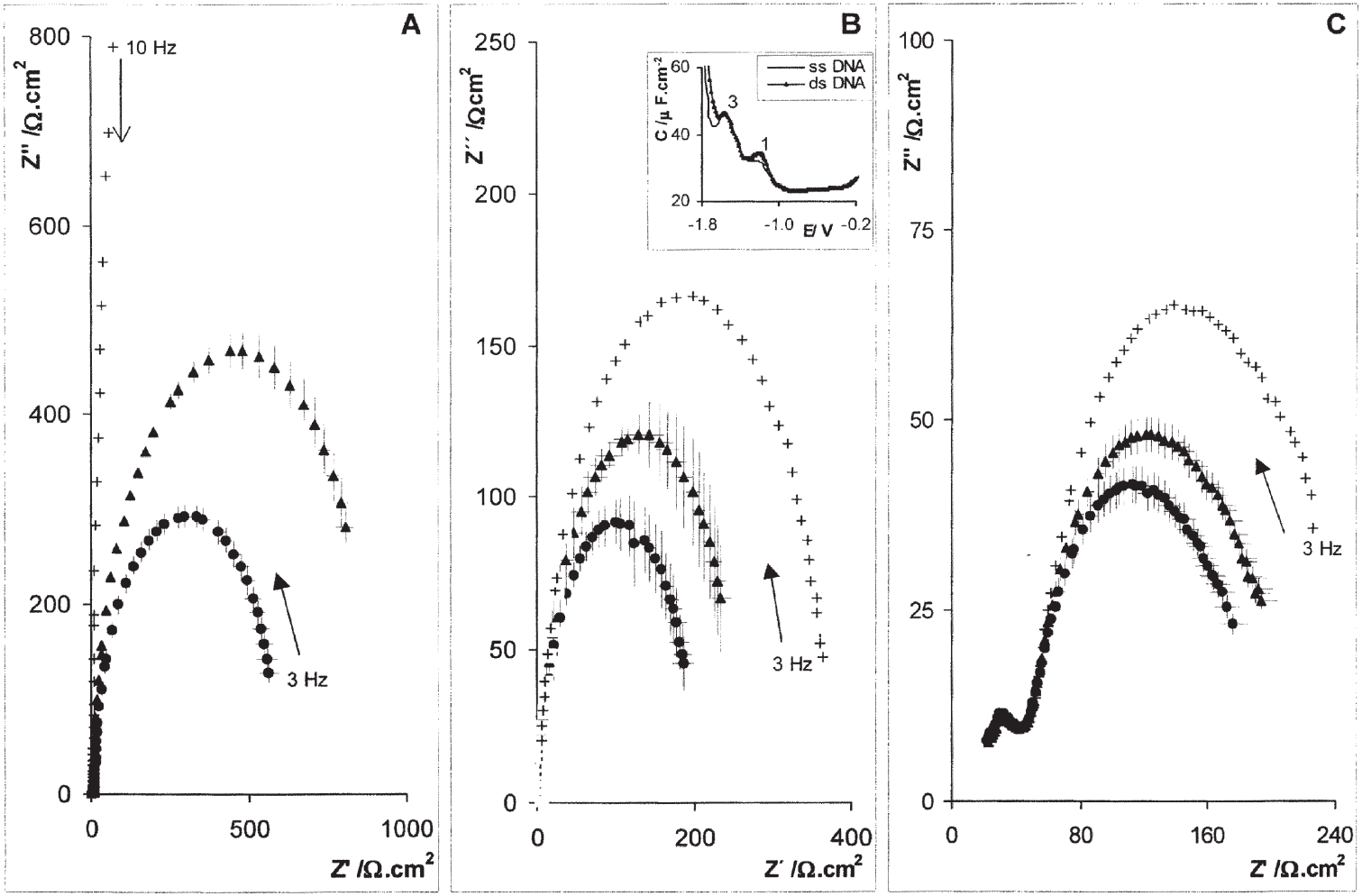

FIGURE 2. Complex impedance plot (Nyquist or Cole-Cole plots) of **(A)**, HMDE **(B)**, GCE|Hg and **(C)**, PGE|Hg. (+) bare electrodes, (_) electrodes modified by 100 μg/ml native (ds) DNA and (_) 100 μg/ml denatured (ss) DNA. The values are means ± standard deviations of five experiments. Frequency range was from 50 kHz to 3 Hz, ac voltage amplitude was 5 mV. Insert represents the dependence of the differential capacitance on potential at frequency 263 Hz of 100 μg/ml ds and ssDNA in 0.3 *M* AF electrolyte (at GCE|Hg). Potential scan was applied in direction from positive to negative potentials. Frequency scan was applied at potential –1.55 V (potential of desorption of bases of nucleic acids, peak 3).

echinomycin, etc.). Due to the interaction with daunomycin, conformational changes of dsDNA immobilized at carbon electrode surface were detected.[118,119] Using ac adsorptive transfer stripping voltammetry at HMDE conformational changes of DNA due to binding of DNA intercalators[120] were studied.

Echinomycin interacts with DNA[121] by inserting a planar aromatic chromophore (quinoxaline) between adjacent DNA base pairs, later the mechanism of binding was named as *bis*-intercalation.[122] Molecule of echinomycin binds to the minor groove of DNA duplex showing strong preference for the CpG sequence.[123-126] DNA-echinomycin complex inhibits DNA replication as well as transcription. Recently, it was found that quinoxaline antibiotics facilitate PNA-DNA complex formation.[127]

Quite recently we have studied interaction of nucleic acids with a quinoxaline antibiotics mainly with a bifunctional intercalator echinomycin by electrochemical techniques.

The capacitance measurements showed that echinomycin forms the pseudocapacitance redox peak around potential –0.53 V at the MFE (Figure 3A,B). The similar result was obtained using cyclic voltammetry and chronopotentiometry in connection with HMDE.[128] It was found that the height of this peak strongly depends on the frequency (Figure 3D). The detection limit of echinomycin at the MFE was about 10 n*M* in bulk of solution (Figure 3C).

When MFE modified with ss or dsDNA was tranferred into solution of AF electrolyte we could observed capacitance curve similar to curve obtained with ds or ssDNA presented in the bulk of solution (Figure 2). On the other hand, if MFE modified with ss or dsDNA was first dipped into solution of echinomycin and then into solution of AF, we could observe differences between behavior of ss and dsDNA. Contrary to ssDNA, dsDNA produced a new pseudocapacitance redox peak at about –0.53 V (Figure 4A,B). This results showed that dsDNA-echinomycin complex produced specific echinomycin signal in agreement with the strong binding of echinomycin to dsDNA by bis-intercalation.[125] Under the same condition interaction of echinomycin with ssDNA resulted in almost no echinomycin signal suggesting only very weak interaction of echinomycin with ssDNA at the electrode surface. Quite recently we have observed very similar results with DNA-echinomycin complex at the HMDE.[129] Capacitance measurements in connection with adsorptive transfer techniques and MFE are thus convenient method that can detect the presence of dsDNA at the electrode surface.

V. CONCLUSION

It was shown that PGE|Hg is appropriate for analysis of nucleic acids. The nonfaradaic capacitance (tensammetric) signals of DNA are highly sensitive to changes in DNA structure. The dielectric losses accompanying desorption of nucleic acid were determined using EIS at HMDE and MFE. The detection limit of echinomycin at MFE is at concentrations down to 10 n*M* using capacitance measurement. We found that with the aid of DNA-echinomycin complex it is possible to distinguish between a ss and dsDNA adsorbed at the MFE. Electrochemical impedance measurements (C-E curves and EIS) seem to be a powerful tool for the study of DNA-intercalators complexes.

VI. ACKNOWLEDGMENTS

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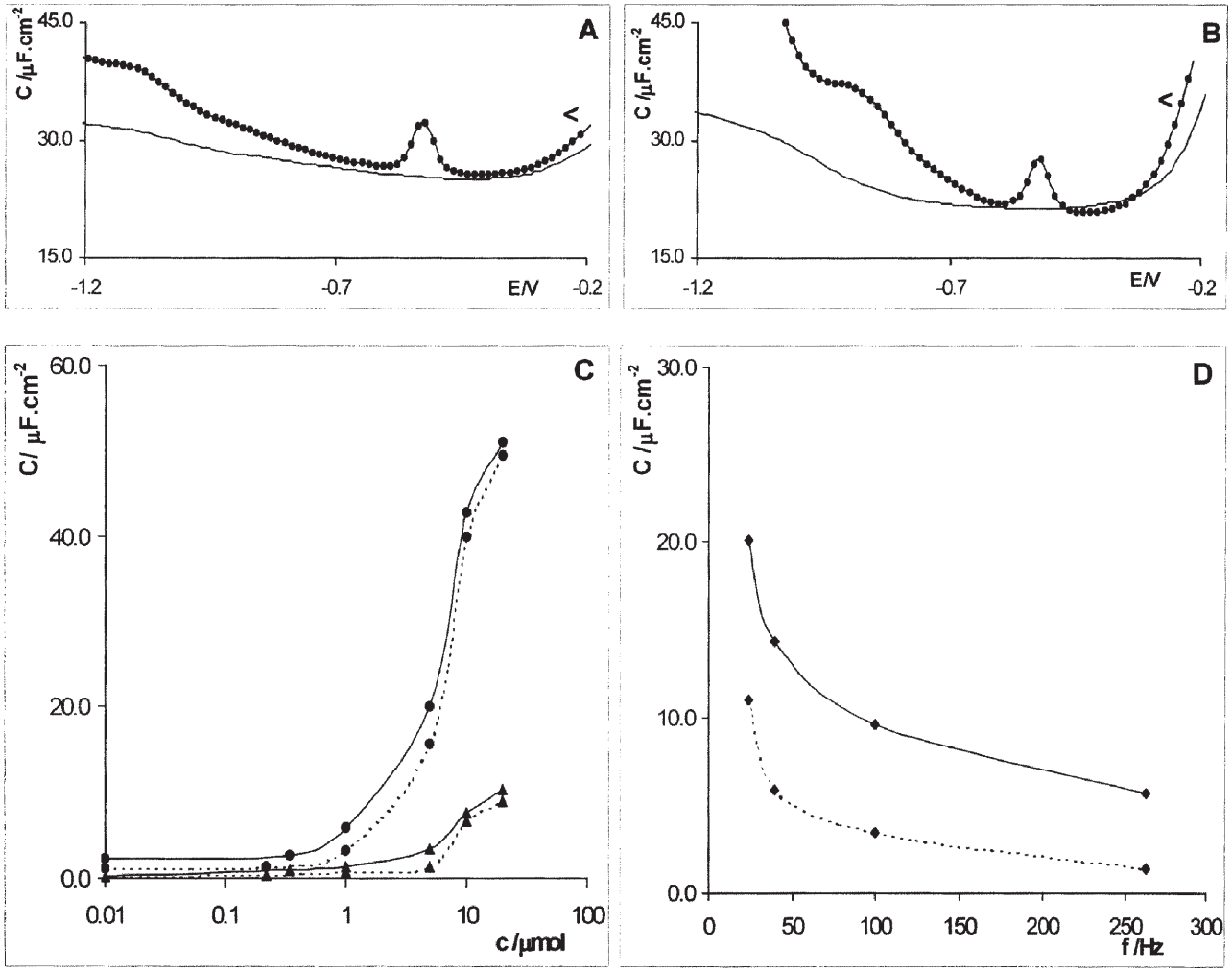

FIGURE 3. Dependence of differential capacitance on potential of 10 µ*M* echinomycin at the frequency 24 Hz **(A)** PGE|Hg and **(B)** GCE|Hg. **(C)** Concentration dependence of the pseudocapacitance redox peak of echinomycin at frequency (●) 24 Hz and (_) 263 Hz, (——) PGE|Hg and (- - -) GCE|Hg. **(D)** Dependence of pseudocapacitance redox peak of 5 µ*M* echinomycin on the frequency at (——) PGE|Hg and (- - -) GCE|Hg. Other conditions as in Figure 2.

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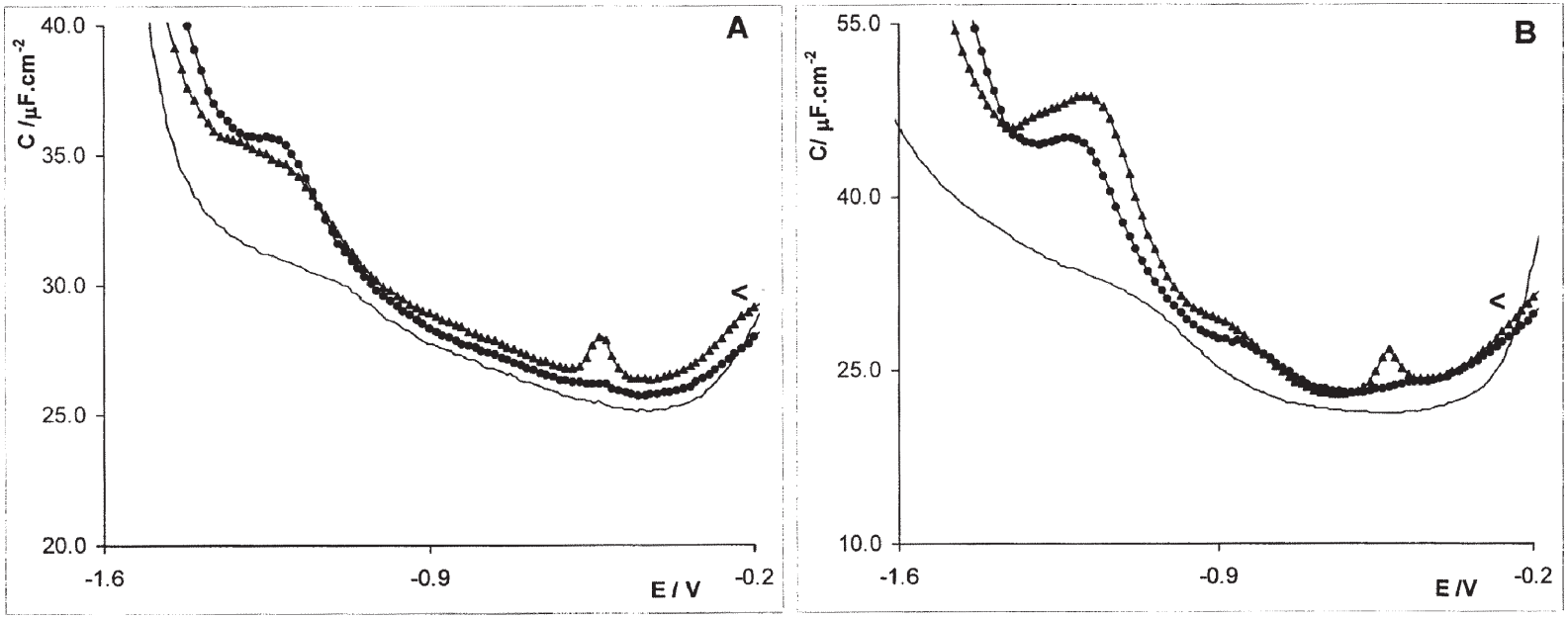

FIGURE 4. Dependence of differential capacitance on potential of complex 10 µ*M* echinomycin with (_) 100 µg/ml dsDNA and (●) 100 µg/ml ssDNA at **(A)** PGE|Hg and **(B)** GCE|Hg. MFE were immersed in a 35-µl solution containing 100 µg/ml of ss or dsDNA for the accumulation time: (a) t_A = 10 min (PGE|Hg) and (b) t_A = 7 min (GCE|Hg), respectively. After double washing in distilled water and background electrolyte solution (both at the time 20 s) the DNA-modified MFE was immersed into a 10 µ*M* echinomycin solution (drop volume was 35 µl) for accumulation time: (a) t_A = 90 s (PGE|Hg) and (b) t_A = 60 s (GCE|Hg), respectively. The double washing step was repeated as described above. After this procedure chemical modified MFE was immersed into background electrolyte and C-E or EIS measurements were performed. Other conditions as in Figure 2.

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