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Application of thin film mercury electrodes and solid amalgam electrodes in electrochemical analysis of the nucleic acids components: detection of the two-dimensional phase transients of adenosine

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Abstract

The optical diffractive (DOE)-based sensor was used to the study of the optical roughness of different carbon/graphite electrodes modified by mercury film (MFEs) and solid amalgam-alloy electrodes (S-MeAEs). The electrode surfaces were visualised by an optical metallurgical microscope. The adsorption of adenosine at the MFEs and S-MeAEs has been investigated by capacitance measurement. Some kinetics aspects, such as the influence of the surface morphology, nature of the substrate and thickness of the mercury film and amalgam-alloy on the formation of two-dimensional (2D) physisorbed adenosine adlayer on the MFEs and S-MeAEs, were studied.

Keywords: Mercury film electrodes; Solid amalgam electrodes; Roughness; Adenosine adlayers; Two-dimensional phase transition

1. Introduction

In 1965, Vetterl [1,2] found that nucleic acid bases posses an extraordinary high ability of self-association at the mercury electrode surface and undergo a two-dimensional (2D) condensation forming a monomolecular layer. It was shown later that the 2D condensation of organic molecules at the liquid indium amalgam surface could be studied as well [3]. Tadjeddine and Rahmani [4] showed, in the beginning of the 90 years, that surface plasmon excitation could be used to investigate the properties of a condensed film formed at the mercury–electrolyte interface in the presence of thymine.

The studies of the adsorption of the nucleic acid bases and nucleosides on various gold and silver single-crystal electrodes have provided clear evidence of the importance of the nature as well as of the crystallographic orientation of the electrodes on the process of the 2D condensation of organic molecules [5].

Our group recently showed that the carbon/graphite electrodes modified by mercury film electrodes (MFEs) can be successfully used to the investigation of the adsorption, 2D condensation and kinetics of the phase transients of the nucleic acid components in the same potential windows as with a mercury electrode [6–8]. However, the application of the MFEs based on the carbon/graphite substrates to the study of the 2D condensation of the nucleic acid components is restricted, because the mercury layer, which adsorb on the carbon/graphite substrate, is stable only short time (minutes). Due to this drawback of the MFEs, we have made an effort to apply the solid amalgam-alloy electrodes (S-MeAEs) to the study of the 2D condensation and kinetics of the phase transients of the nucleic acid components [9]. The longer lifetime of the amalgam-alloy layer (from hours to days) and its minimal toxicity is the main advantage of the S-MeAEs.

The aim of this work is to review the results of investigations of the adsorption and kinetic aspects of the phase

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transients of adenosine at the different carbon/graphite electrodes modified by MFEs and at the S-MeAEs.

2. Experiments

2.1. Electrode preparation and their electrochemical modification

Copper, platinum and silver wires (diameter 1.0 mm) were obtained from Advent Research Materials (purity 99.999%). Pyrolytic graphite electrodes (PGEs) were obtained from GE Advanced Ceramics as the basal plane-oriented (PGEb) and edge plane-oriented (PGEe) form. Glassy carbon electrodes (GCEs) were obtained from Metrohm. The working electrodes were mechanically polished and sonicated in triply distilled water [7].

The electrodeposition of the Hg on the carbon/graphite and polycrystalline metal substrates, that were immersed in 20 mM $Hg(NO_3)_2$ aqueous solution, was performed using potentiostat with a three-electrode system. The thickness h of the Hg layer deposited on the above-mentioned substrates was controlled by change of the deposition time t and current value I according to the Faraday law [7].

In the case of the amalgam electrodes, the second activation step of amalgamation process was followed. On the Hg-modified surface of the polycrystalline metal electrodes was applied the constant potential of -2.20 V for about 5 min into 0.2 M KCl solution. It can be assumed that the application of a high negative potential on the Hg-modified polycrystalline metal substrate causes disappearing of the liquid Hg, and the Hg layer transforms into solid amalgam-alloy. Really, earlier it was showed, that a high negative potential, where potassium amalgam is formed and where vigorous evolution of hydrogen takes place, can improve the properties of a Hg layer prepared on a metallic substrate [10].

2.2. Instrumentation

The diffractive optical element (DOE)-based sensor was examined to the detection of the surface morphology of the MFEs and S-MeAEs electrodes. The theory of the DOE sensor and technical arrangement of experiments for optical surfaces quality measurement was described in more detail in our previous publication [8]. To the visualisation of the surfaces of the working electrodes, the optical metallurgical microscope OLYMPUS SZX 9 (zoom ratio 9.05:1) was used.

The electrochemical measurements were performed with an AUTOLAB electrochemical system (Ecochemie, Utrecht, Netherlands) equipped with a potentiostat/galvanostat PGStat12 and a frequency-response analyser module (FRA 2). The counter electrode was a platinum wire and/or a graphite rod. The reference electrode was Ag|AgCl|3 M KCl.

3. Results and discussion

3.1. Roughness of the surfaces of the MFEs and S-MeAEs

From DOE sensor measurement, we found that the optical roughness R_a of the carbon/graphite electrodes used increases in the order: PGEe ($R_a = 0.0258$ μm) < GCE ($R_a = 0.0344$ μm) < PGEb ($R_a = 0.0404$ μm). The overall magnitude of the optical roughness R_a of the 2 μm Hg-modified carbon/graphite electrodes increases in the order: 2 μm Hg-modified PGEe ($R_a = 0.0215$ μm) < 2 μm Hg-modified PGEb ($R_a = 0.0322$ μm) < 2 μm Hg-modified GCE ($R_a = 0.0393$ μm) [8]. It means that the R_a value is higher in the case of Hg-modified GCE in comparison with unmodified GCE. This experimental result showed that the Hg is present on the 2 μm Hg-modified GCE surface in form of droplets (Fig. 1C). It was observed from the DOE measurements that the growth of the Hg-modification on the PGEb caused the smoother surface (decrease of the R_a value). It seems that Hg forms a really continuous film on the 2 μm Hg-modified PGEb substrate (Fig. 1B).

The surfaces of the polycrystalline metal electrodes after polishing with 1 μm diamond paste are rough with typical polishing lines running across the electrode surfaces (Fig. 1D–E). The optical roughness of the polycrystalline PtE was $R_a = 0.0361$ μm and polycrystalline CuE was characterised by $R_a = 0.0546$ μm. It was observed that the difference between the R_a of the modified and unmodified CuE has the lowest value. It seems that amalgam-alloy layer is more homogeneously distributed on the CuE than on the PtE and AgE. The overall magnitude of the R_a of the S-MeAEs increases in the order: 0.2 μm S-CuAE ($R_a = 0.0536$ μm) < 0.2 μm S-PtAE ($R_a = 0.0559$ μm).

3.2. Adsorption of adenosine at the MFEs and S-MeAEs

Earlier it was found that in acidic (pH 5) solution adenosine forms two different 2D physisorbed condensed layers at the Hg electrode (Fig. 1A) [2]. The first of these (region I) is located at more negative potential; the centre of this adlayer is around -1.3 V. The second 2D physisorbed film (region II) is formed at more positive potentials; the centre of region II is situated around -0.4 V (Fig. 1A). Regions I and II are manifested by capacitance pits on the C–E curves (Fig. 1).

Both of 2D condensed adenosine films were clearly detected on the 2 μm Hg-modified PGEb (Fig. 1B). On the 2 μm Hg-modified GCE, it was possible to detect clearly only the first (region I) 2D physisorbed adenosine adlayer (Fig. 1C). The second adenosine adlayer (region II) was poorly developed on 2 μm Hg-modified GCE (Fig. 1C). The region I of adenosine was detected on both the PGEb and GCE substrates modified with Hg layer down to 0.02 μm [8].

It was observed that adenosine forms the 2D physisorbed condensed adlayers I and II on the 0.2 μm S-PtAE (Fig. 1D)

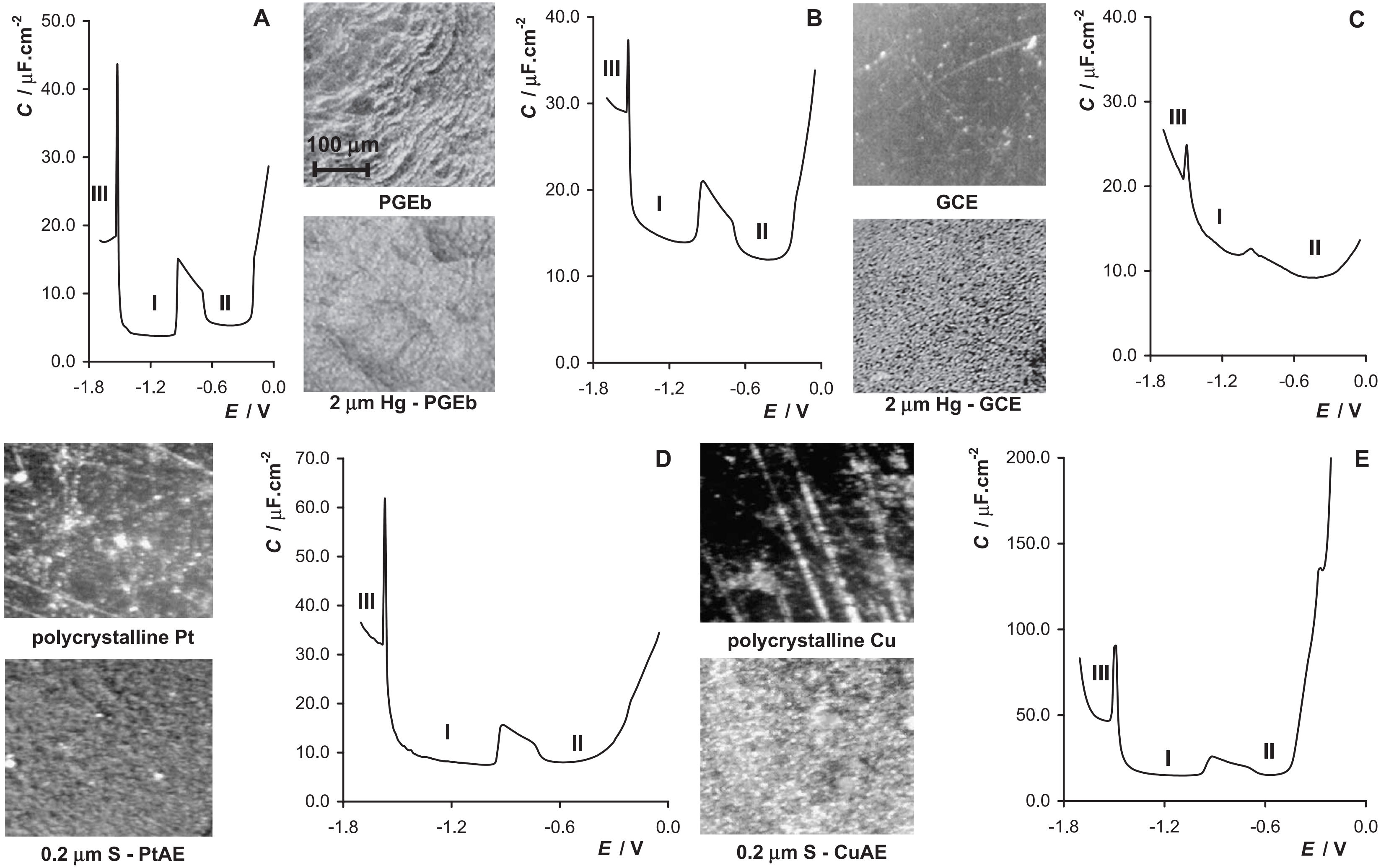

Fig. 1. Capacitance-potential curves of 14 mM adenosine in 0.1 M NaCl at pH 5 on the (A) mercury electrode, (B) 2 μm Hg-modified PGEb, (C) 2 μm Hg-modified GCE, (D) 0.2 μm S-PtAE and (E) 0.2 μm S-CuAE. The potential scan was performed from positive to negative potentials. The measuring frequency was 33 Hz; ac voltage amplitude was 5 mV. The different adsorption states are labelled as I, II and III. All measurements were performed at 5 °C. The inserts show microscope images of the unmodified and Hg modified (amalgam-alloy) electrode surfaces: (B) PGEb, (C) GCE, (D) Pt and (E) Cu. Images were recorded by an optical metallurgical microscope OLYMPUS SZX 9 (zoom ratio 9.05).

and 0.2 μm S-CuAE (Fig. 1E) as well. These 2D condensed adenosine adlayers could be clearly detected on the S-MeAEs, when the thickness of the amalgam-alloy layer is between 0.02 and 0.4 μm. On the other hand, the adsorption of adenosine is poorly developed on the 0.2 μm S-AgAE (not shown). The lifetime of the 2D condensed adenosine adlayers on the S-AgAE is very short (minutes) in contrary to the S-PtAE and S-CuAE substrates (hours) [9].

3.3. Kinetics of transformation of adenosine adlayers at the MFEs and S-MeAEs

In this contribution, we focused on the kinetics of phase transformations of the adenosine films starting from a dilute adsorption region (state III) to the 2D physisorbed adlayer (region I) at the above-mentioned surfaces. The kinetics of phase transformations was investigated by means of current transients using the double potential step techniques [11].

It was observed that the phase transients of III → I of adenosine are the fastest at the 0.02 μm Hg-modified PGEb (the lowest value of the t_{max}; t_{max} is the time, where the current reaches its maximum) and gradually drop to a slower value on both 0.2 μm Hg-modified PGEb and 2 μm Hg-modified PGEb (Fig. 2A). The similar dependence of the rate of the phase transients of III → I (characterised by the value of t_{max}) on the thickness of the Hg layer was observed on the Hg-modified GCE [7].

Fig. 2B shows the effect of the Hg layer thickness on the charge Q (i.e. area of the current peak on the j–t curves) for three phase transients of III → I of adenosine on the Hg-modified PGEb substrate. The potentials at which the j–t curves were measured are shown as values $|E_f - E_T|$ on the x-axis in Fig. 2B, where E_f is the final potential of the current transient and E_T is the true equilibrium transition potential. When the thickness of the Hg is 0.02 μm then the j–t curves of the phase transients of III → I of adenosine on Hg-modified PGEb are characterised by an initial exponential decay of the current followed by a poorly developed maximum ($Q \approx 0.3$ μC cm^{-2}; Fig. 2B). As long as the thickness of the Hg was about 2 μm, the pronounced current maxima on the j–t curves of the phase transients of III → I of adenosine on the Hg-modified PGEb were detected ($Q \approx 6.8$ μC cm^{-2}; Fig. 2B). The phase transients of III → I of adenosine on the 2 μm Hg-modified PGEb were

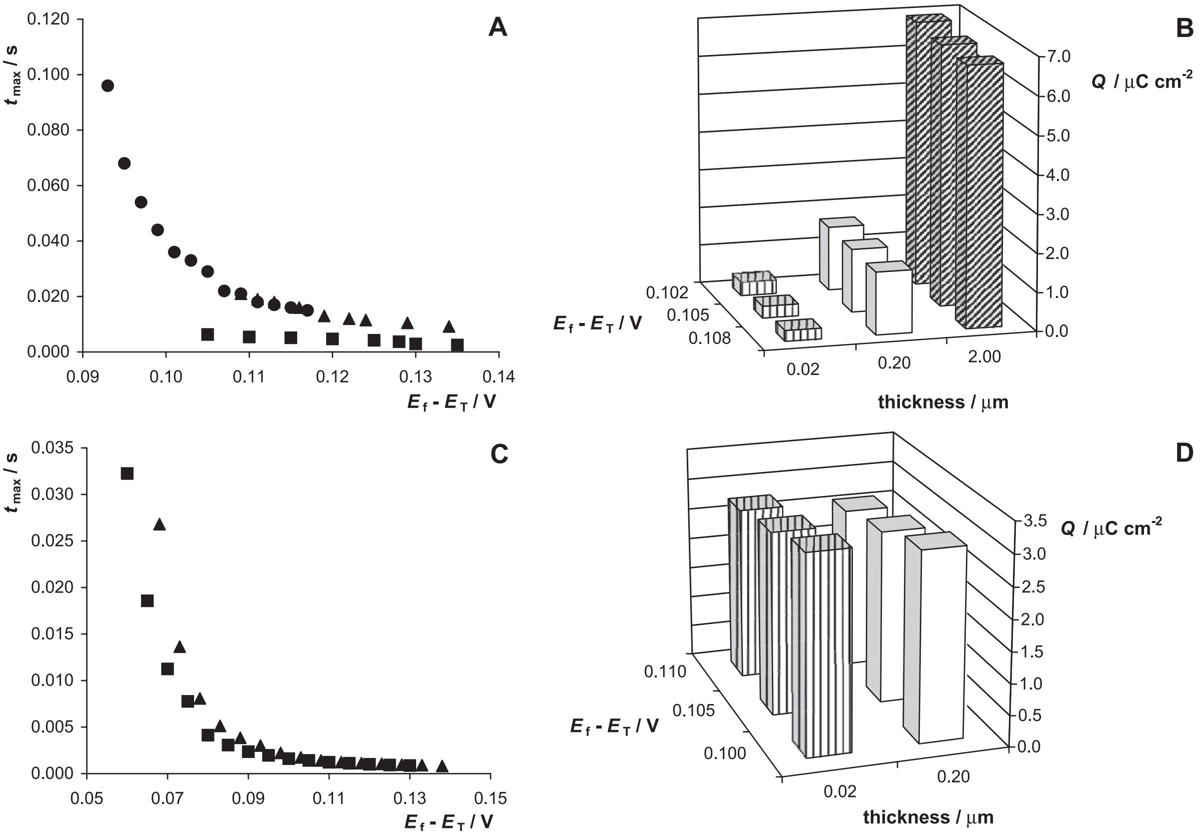

Fig. 2. (A) Dependence of the position of current maxima (t_{max}) on the potential $|E_f - E_T|$ and on the thickness of the Hg layer deposited on the PGEb substrate: (●) 2 μm, (▲) 0.2 μm and (■) 0.02 μm for the phase transients of III → I of 14 mM adenosine. (B) The effect of the Hg layer thickness on the charge Q (area of the current peak on the j–t curves) for three phase transients of III → I of 14 mM adenosine on the Hg-modified PGEb substrate. (C) Dependence of the position of current maxima (t_{max}) on the potential $|E_f - E_T|$ and on the thickness of the amalgam-alloy surface layer on the S-PtAE substrate: (▲) 0.2 μm and (■) 0.02 μm for the phase transients of III → I of 14 mM adenosine. (D) The effect of the amalgam-alloy surface layer thickness on the charge Q for three phase transients of III → I of 14 mM adenosine on the S-PtAE substrate. The measurement was performed in 0.1 M NaCl (pH 5) at 5 °C.

successful described by a model, which combines a Langmuir-type adsorption step with a nucleation according to a power law and linear growth mechanism [8]. Our measurements showed that the nucleation and growth process during phase transients of adenosine was not observed on the Hg-modified PGEe, which has higher resistivity [8].

From Fig. 2D, it can be seen that *j–t* curves of the phase transients of III → I on both 0.02 μm S-PtAE and 0.2 μm S-PtAE are characterised by pronounced current maxima. The areas of the current peaks on the *j–t* curves are slightly dependent on the amalgam-alloy thickness ($Q \approx 3.0$ μC cm^{-2}; Fig. 2D). The similar situation was detected for the phase transients of III → I on the S-CuAE substrates (not shown).

The phase transients of III → I on the 0.2 μm S-PtAE could be described by a model, which combines a Langmuir-type adsorption step with a nucleation according to a power law and linear growth mechanism. The phase transients of III → I on the 0.2 μm S-CuAE could be satisfactory described by a Langmuir-type adsorption followed an instantaneous nucleation and linear growth mechanism [9].

From Fig. 2C, it can be seen that the rate of the phase transients of III → I on the S-PtAE substrate is slightly dependent on the thickness of the amalgam-alloy (in this contribution, we applied the amalgam-alloy electrodes with thickness from 0.01 to 0.4 μm; for thickness of the amalgam-alloy higher than 0.5 μm, the fast formation of the liquid Hg droplet on the metallic substrate immersed into $Hg(NO_3)_2$ solution was started). After application of the S-CuAE was observed similar dependence [9].

4. Conclusion

The 2D condensation of adenosine was observed on the MFEs and S-MeAEs. For the study of the nucleation and growth process during phase transients of adenosine, it is appropriate to take the 2 μm Hg-modified PGEb substrate. The nucleation of adenosine can be studied on the 0.2 μm S-PtAE and 0.2 μm S-CuAE as well.

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