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Comparative Studies on the Adsorption and Association of 3-Methylxanthine and 7-Methylxanthine at a Charged Interface

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Summary. A systematic comparative study of the adsorption and association of 3-methylxanthine (*3MXan*) and 7-methylxanthine (*7MXan*) at mercury-solution interfaces in acidic, neutral, and alkaline buffer solutions was undertaken by ac voltammetry. At bulk concentrations of *3MXan* above a threshold value, the stacking interactions between vertically oriented molecules lead to a slow reorientation around E_{ecm}, and the molecules adopt a perpendicular orientation. The association of the adsorbed molecules of *7MXan* is hindered and is not effective in promoting base-base stacking interactions. The enhanced surface activity of *3MXan* compared to *7MXan* results from the different position of the methyl group in the purine moiety. The effect of some divalent metal ions on adsorption stages and association of the investigated compounds has been studied. The results indicate that the complexation of methylated xanthine enhances the stacking interactions and hence would be expected to facilitate the formation of perpendicularly stacked layers of *M*(II)-*MXan* complexes on the electrode surface. The adsorption parameters of the investigated compounds have been computed in absence and presence of Cu(II) at different *pH* values. The results are compared with the behaviour of xanthine.

Keywords. Adsorption; Association; AC voltammetry; Methylxanthines.

Vergleichende Untersuchungen zur Adsorption und Assoziation von 3-Methylxanthin und 7-Methylxanthin an einer geladenen Grenzfläche

Zusammenfassung. Eine systematische vergleichende Untersuchung zur Adsorption und Assoziation von 3-Methylxanthin (*3MXan*) und 7-Methylxanthin (*7MXan*) an Quecksilber-Lösungs-Grenzflächen wurde mittels AC-Voltammetrie in sauren, neutralen und basischen Pufferlösungen durchgeführt. Oberhalb einer bestimmten Grenzkonzentration kommt es durch Wechselwirkungen zwischen vertikal angeordneten *3MXan*-Molekülen zu einer langsamen Reorientierung um E_{ecm}, was zu einer senkrechten Anordnung führt. Die Assoziation von *7MXan* ist gehindert und erlaubt daher keine ausgeprägten Basen-Basen-Wechselwirkungen. Die erhöhte Oberflächenaktivität von *3MXan* gegenüber *7MXan* resultiert aus der unterschiedlichen Stellung der Methylgruppe am Purinrest. Der Effekt einiger zweiwertiger Metallionen auf die Adsorption und Assoziation von *3MXan* und *7MXan* wurde untersucht. Die Ergebnisse weisen auf eine Begünstigung der Wechselwirkungen hin; die Bildung von vertikal angeordneten Grenzschichten an der Elektrodenoberfläche sollte daher im Fall von *M*(II)-*MXan*-Komplexen erleichtert sein. Die Adsorptionsparameter der untersuchten Verbindungen wurden in Abwesenheit und in Gegenwart von Cu(II) bei verschiedenen *pH*-Werten berechnet. Die Ergebnisse werden mit dem Verhalten von Xanthin verglichen.

Introduction

Purine and pyrimidine derivatives play an important role in many biological processes. Some derivatives such as methylxanthines are compounds with a variety of pharmacological actions-increasing cardiac output, relaxing some smooth muscle, and causing stimulation of the central nervous system [1–3]. The surface activity of nucleic acid bases, nucleosides, and nucleotides at the mercury/solution interface has been studied in absence of metal ions by various authors [4–23]. It has been shown that the adsorption of monomeric purine and pyrimidine derivatives results in a lowering of the differential capacitance of the electric double layer next to the mercury electrode [4–6]. At relatively low bulk concentrations of these substances, a rather dilute adsorption layer is established up to a certain threshold value, whereas a condensed film is formed above this concentration within a certain potential range [24]. In the region of potentials at which the surface film is formed a kind of a pit appears on the capacitance curves or ac polarograms [4–6, 8, 14]. The mechanism of this film formation has been analyzed quantitatively by *Retter* [25].

Complexes containing the pyrimidine bases uracil and their thio derivatives [26], thymine [27] and cytosine [28], have been the subject of several studies in an attempt to determine their mode of binding to metal ions. In the purine bases, attention has been focused on adenine and guanine [29–31] as these are major constituents of DNA and RNA. This together with the recent finding that certain transitional metal complexes are potentially useful in cancer chemotherapy [32, 33] created a renewed interest in the study of the interaction of heavy metal ions with some purine derivatives.

The present paper is focussed on investigating the effect of *pH* on the interfacial behaviour of *3MXan* and *7MXan* in a wide range of bulk concentrations. The influence of complexation on the stacking interactions of these compounds has been elucidated. The results are compared with the similar type of adsorption behaviour of xanthine.

Results and Discussion

A survey of the adsorption behaviour of *3MXan* as a function of potential and *pH* is provided by recording phase-sensitive ac voltammograms (Fig. 1). In acidic and neutral solutions ($pH \leqslant 7.2$) and at relatively low bulk concentrations of *3MXan*, the out-of-phase ac current indicates a progressive decrease of the capacitive current around the electrocapillary point of zero charge of the pure supporting electrolyte ($P_{ZC} = -0.6$ V, $pH = 7.2$). This decrease corresponds to a progressive coverage of the electrode surface by a dilute adsorption layer [12, 14, 15]. At more elevated bulk concentrations, above the threshold value of *3MXan*, a sudden sharp decrease of the ac current is observed, giving a very sharply defined pit. As shown previously [24], the dilute adsorption layer reflects a flat orientation of the adsorbed species at the electrode surface; however, the pit points to a compact adsorption layer of stacked, vertically oriented adsorbed molecules. On the other hand, in alkaline solutions ($pH \geqslant 9.2$) the formation of a condensed film is hindered at all bulk concentrations. This is to be expected if the association of the adsorbed species depends predominantly on the stacking of the protonated or neutral molecules of *3MXan*. This

indicates that the adsorption of the anionic species of *3MXan* decreases the intermolecular association and the stacking interaction between the adsorbed molecules. This may be due to the repulsion of the partly negatively charged species of the adsorbed molecules by the negatively charged electrode. The depression of ac

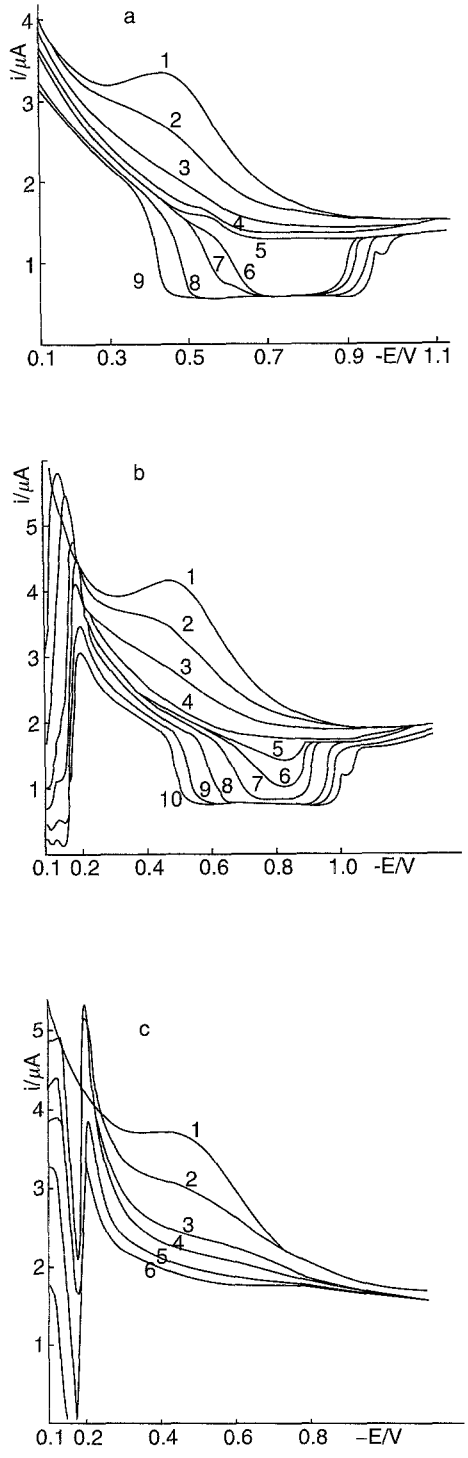

Fig. 1. AC capacitive current curves of *3MXan* at $pH = 3.2$ (a), 7.2 (b), and 9.2 (c); 0.5 *M B.R.* buffer (Cl^-), 5 °C, area of HMDE: $1.2 \times 10^{-2}\,cm^2$, scan rate: $5\,mV\,s^{-1}$, amplitude: $10\,mV_{pp}$, phase angle: 90°, frequency: 330 Hz, $t_s = 60$ s; (a); (1) 0.0, (2) 0.02, (3) 0.06, (4) 0.123, (5) 0.193, (6) 0.305, (7), 0.324, (8) 0.367, and (9) 0.415 *mM 3MXan*; (b): (1) 0.0, (2) 0.02, (3) 0.06, (4) 0.16, (5) 0.28, (6) 0.29, (7) 0.32, (8) 0.38, (9) 0.51, and (10) 0.588 m*M 3MXan*; (c): (1) 0.0, (2) 0.01, (3) 0.05 (4) 0.13, (5) 0.39, and (6) 0.51 m*M 3MXan*

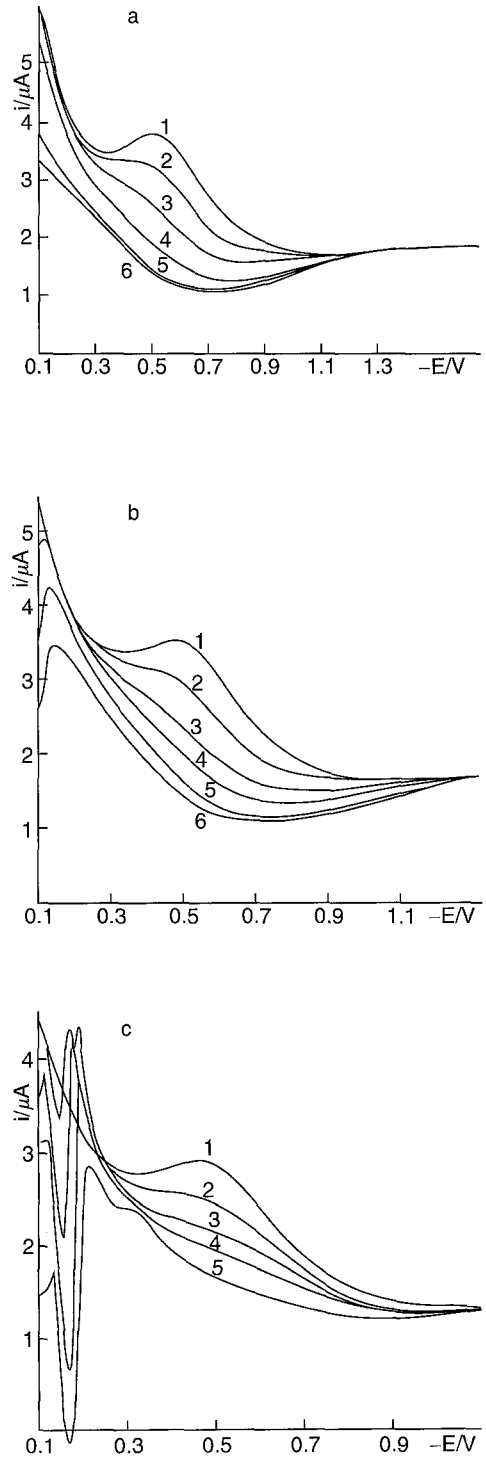

Fig. 2. AC capacitive current curves of *7MXan* at $pH = 3.2$ (a), 7.2 (b), and 9.2 (c); (a): (1) 0.0, (2) 0.01, (3) 0.03, (4) 0.16, (5) 0.44, and (6) 0.55 m*M* *7MXan*; (b): (1) 0.0, (2) 0.02, (3) 0.03, (4) 0.08, (5) 0.28, and (6) 0.44 m*M* *7MXan*; (c): (1) 0.0, (2) 0.02, (3) 0.04, (4) 0.15, and (5) 0.47 m*M* *7MXan*; other conditions as in Fig. 1

current in the potential range -0.1 V to -0.2 V (Figs. 1 and 2) corresponds to the adsorption of a Hg(II)–*3MXan* (or Hg(II)–*7MXan*) film. In this context, methylated xanthine like other purine compounds [34] interacts with mercury forming a Hg(II)–*MXan* compound. Moreover, previous work [35] has shown that the

adsorption behaviour of xanthine is rather similar to that of *3MXan*, except that the depression of the ac current is larger due to the increased number of adsorption sites. This indicates that the structure of the condensed film is similar in all cases and corresponds to a stacked orientation of the base residues due to base–base interactions.

The dependence of the ac capacitive current on *pH* and bulk concentration of *7MXan* is shown in Fig. 2. In acidic, neutral, and alkaline buffer solutions (*B.R.* buffer), the recorded capacitive ac component for *7MXan* indicates a dilute adsorption region with the molecules of *7MXan* adsorbed flat on the electrode surface. At more elevated bulk concentrations of *7MXan* ($> 1 \times 10^{-3}$ *M*) and in various buffer solutions, the association of the adsorbed molecules on the electrode surface is hindered and no condensed film is formed. The influence of the nature of the anions of the indifferent supporting electrolyte on the adsorption behaviour of *7MXan* was also studied. The effect of replacing the chloride anion in *B.R.* buffer ($pH = 3.2$) by various anions such as NO_3^-, SO_4^{2-}, and ClO_4^- results in a decrease of the capacitive ac signal, but no condensed film is obtained with the various anions. This behaviour indicates that *7MXan* is not effective in promoting base–base stacking interactions.

On comparison, the adsorption behaviour of *3MXan* and *7MXan* recorded under the same conditions shows that *7MXan* does not associate on the electrode at any bulk concentration, and no condensed film formation is obtained in the *pH* range from 3.2 to 9.2. Based on these observations, it is suggested that the enhanced surface activity of *3MXan* compared to *7MXan* results from the different position of the methyl group in the purine moiety. This indicates that the introduction of an electron releasing methyl group into the xanthine molecule at the pyrimidine moiety decreases the electrodeficiency and hence increases the binding between the π electron system and the electrode surface relative to *7MXan* where the methyl group is attached to the imidazol moiety. It has been shown previously [10, 36] that methylation of electron deficient purine [37] and pyrimidine [10] nuclei enhances the stacking interaction of the associated molecules. It is remarkable that small steric differences in the adsorbed molecules causing, for example, only a marginal change of pK_a values, yield a distinct change in the adsorption behaviour. This underlines the particular potentialities of ac voltammetry with phase-sensitive detection for the study of intermolecular forces which govern the formation of a compact film.

The effect of some divalent metal ions such as Cu(II), Cd(II), Co(II), and Ni(II) on the adsorption stages and association of methylated xanthine has been investigated. The ac capacitive current of *3MXan* and *7MXan* in presence of Cu(II) at *pH* 9.2, where these compounds do not associate at the electrode surface, was recorded as shown in Fig. 3. At bulk concentrations of *3MXan* or *7MXan* below 12 μM the voltammograms indicate a very sharply defined pit in the potential range from -0.2 V to -0.7 V. However, at bulk concentration above 33 μM *3MXan*, an additional pit in the ac voltammogram is observed as shown in Fig. 3a. The existence of two separated pits in which the association of Cu(II)–*3MXan* complexes takes place is explained by the surface rearrangement and reorientation of the adsorbed molecules at the electrode surface. The peaks at -0.2 V and -0.55 V (Fig. 3a) in the dip indicate reorientation to an arrangement where the Cu(II)–*3MXan* complex interacts strongly with the electrode surface. This results suggest that the complexation of the methylated xanthine enhances the stacking interactions and hence would

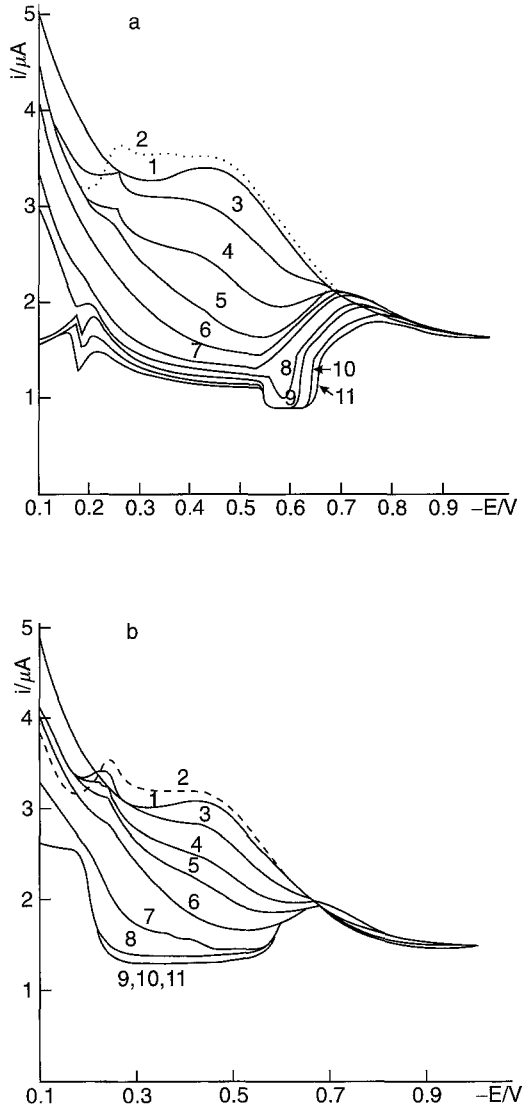

Fig. 3. AC capacitive current curves of *3MXan* (a) and *7MXan* (b) in presence of 90 μ*M* Cu(II) at $pH = 9.2$; (a): (1) background, (2) 0.0, (3) 1.78, (4) 3.51, (5) 5.17, (6) 6.78, (7) 12.7, (8) 33.6, (9) 61.3, (10) 158, and (11) 342 μ*M* *3MXan*; (b): (1) background, (2) 0.0, (3) 0.93, (4) 1.85, (5) 2.75, (6) 4.5, (7) 7.8, (8) 15.2, (9) 65.2, (10) 160, and (11) 350 μ*M* *7MXan*; other conditions as in Fig. 1

be expected to facilitate the formation of a perpendicularly stacked layer of the Cu(II)-*MXan* complex on the electrode surface (Table 1).

The adsorption behaviour of *3MXan* or *7MXan* in the presence of Cd(II), Co(II), and Ni(II) at $pH = 9.2$ was also investigated. By addition of 1 m*M* of these metal ions, the ac voltammograms of the investigated compounds indicated that a decrease of the capacitive signal but no condensed film formation is obtained. This reveals that Cd(II), Cu(II), and Ni(II) interact only weakly with the investigated compounds.

Additional information on the association of *3MXan* and *7MXan* in absence and presence of Cu(II) is supplied by the time dependence of the ac capacitive current (Fig. 4). The capacitive ac current measured at a constant mean electrode potential (E_{max}) slowly decrease at low bulk concentrations of *3MXan* to the first equilibrium value, corresponding to the dilute adsorption layer. For bulk concentration larger than the threshold value of *3MXan*, the current rapidly decreases to the second

Table 1. Adsorption parameters of dilute and compact layers of *Xan*, *3MXan*, and *7MXan* at various *pH* **values**

pH	$C \times 10^4$ (*M*)	*a*		$\beta \times 10^3$ ($l \cdot mol^{-1}$)		$-\Delta G^\circ \times 10^{-4}$ ($kJ \cdot mol^{-1}$)		$\Gamma_m \times 10^{-10}$ ($mol \cdot cm^{-2}$)	$\Gamma_m \times 10^{+14}$ ($molec \cdot cm^{-2}$)	S_m (nm^2)	$-E$ (V)
		dilute stage	compact stage	dilute stage	compact stage	dilute stage	compact stage				
Xanthine											
3.2	0.09	1.20	1.98	9.50	0.62	3.04	2.41	4.60	2.77	0.36	0.75
7.2	0.18	1.02	2.06	5.30	0.42	2.90	2.76	4.20	2.53	0.39	0.75
9.2[a]	–	0.98	–	3.50	–	2.81	–	–	–	–	0.45
9.2[b]	0.22	1.10	1.34	5.00	0.38	2.45	2.29	3.80	2.82	0.43	0.50
3-Methylxanthine											
3.2	2.00	1.21	2.00	8.40	0.50	3.01	2.40	2.80	1.68	0.59	0.65
7.2	3.20	1.09	2.11	4.50	0.36	2.88	2.82	2.20	1.32	0.75	0.80
9.2[a]	–	0.34	–	3.60	–	2.81	–	–	–	–	0.45
9.2[b]	2.20	1.33	1.96	4.60	0.30	2.87	2.24	2.50	1.51	0.66	0.50
7-Methylxanthine											
3.2	–	0.78	–	3.65	–	2.82	–	–	–	–	0.65
7.2	–	0.88	–	3.51	–	2.81	–	–	–	–	0.65
9.2[a]	–	0.75	–	3.36	–	2.80	–	–	–	–	0.50
9.2[b]	3.65	1.19	1.24	3.50	0.25	2.81	2.20	1.80	1.08	0.92	0.40

[a] In absence of Cu(II); [b] in presence of 90 μM Cu(II)

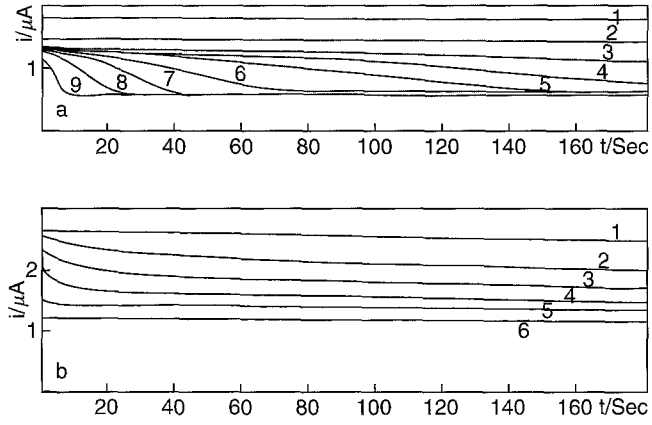

Fig. 4. Time dependence of the out-of-phase component of the ac current for *3MXan* (a) and *7MXan* (b) at $pH = 7.2$ (*B.R.* buffer $+Cl^-$); (a): (1) 0.0, (2) 0.08, (3) 0.10, (4) 0.32, (5) 0.33, (6) 0.41, (7) 0.47, (8) 0.52, and (9) 0.6 m*M* *3MXan*; (b): (1) 0.0, (2) 0.09, (3) 0.20, (4) 0.44, (5) 0.94, and (6) 2.6 m*M* *7MXan*; other conditions as in Fig. 1

equilibrium value characteristic of compact film formation. Once a compact film has been built up, there is no substantial influence from the solution; thus, the extent of the capacitive change accompanying the film formation depends only on the first stage of adsorption and may be governed by the steric arrangement of adjacent molecules. With *7MXan*, the capacitive current decreases slowly with increasing bulk concentration to the first equilibrium value, and no compact film formation is obtained. However, in presence of Cu(II) ions the second equilibrium value characteristics of compact film formation is obtained.

To follow the adsorption of *3MXan* and *7MXan* quantitatively at different *pH* values at the HMDE, the values of the capacitive ac current at a given bulk concentration were recorded. The dependence of the ac capacitive decrease, Δi_{ac} (the decrease of the capacitive ac current with respect to the i_{ac} value of the blank supporting electrolyte for a given bulk concentration) has the form of a two-step isotherm for *3MXan* (at $pH \leqslant 7.2$, Fig. 5) at the potential of maximal adsorption. On the other hand, at potentials more negative than the maximal adsorption potential the threshold value of the concentration for compact film formation increases appreciably. This shows that the threshold value for the compact adsorption stage depends on the *pH* value and the adsorption potential. The course of the concentration dependence of the ac capacity current decrease Δi_{ac} or the surface coverage θ for *7MXan* has the form of a one-step isotherm at different adsorption potentials and in various buffer solutions. This behaviour indicates that a rather dilute adsorption layer is formed, whereas a compact film, which would be reflected by a double-step isotherm, is not observed. The foregoing results indicates that the position of methyl group plays an important role in the formation of the compact film.

In order to calculate the adsorption parameters of *3MXan* and *7MXan* at various *pH* values, the experimental results were fitted to several adsorption isotherms. From a comparison of the experimental results with theory, it seems that the isotherm is of a *Frumkin* type given by Eq. (1) (θ: degree coverage; a: interaction

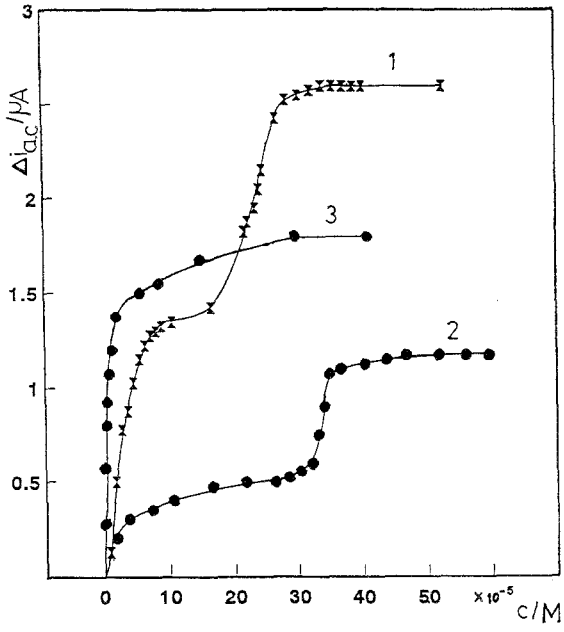

Fig. 5. Dependence of the capacity current decrease Δi_{ac} on the bulk concentration of *3MXan* at different *pH* values; (1) $pH = 3.2$ (−0.65 V), (2) $pH = 7.2$ (−0.8 V), and (3) $pH = 9.2$ (−0.45 V); other conditions as in Fig. 1

coefficient; β: adsorption coefficient; C: bulk concentration of *3MXan* or *7MXan*).

$$\theta(1-\theta)^{-1}\exp(-2a\theta) = \beta \cdot C \tag{1}$$

The interaction coefficient a was determined from the slope of the logarithmic plot of the *Frumkin* isotherm, and the adsorption coefficient β from the value at half coverage. The *Gibbs* energy of adsorption ($-\Delta G^\circ$) was then calculated from the adsorption coefficient β using Eq. (2).

$$\beta = (1/55.5)\exp(-\Delta G^\circ/RT) \tag{2}$$

Further information on the dilute and compact film formation of *3MXan* and *7MXan* in absence and presence of Cu(II) could be gained by computing the maximum excess concentration Γ_m using *Koryts* Eq. [38]

$$\Gamma_m = 0.736 \times 10^{-3} \cdot D^{1/2} \cdot C \cdot t^{1/2} \tag{3}$$

where C is the bulk concentration of *3MXan* or *7MXan* (mol·cm^{-3}) and D is the diffusion coefficient (cm^2·s^{-1}) which is calculated using the *Stoke-Einstein* equation. The value of the maximum surface concentration Γ_m was obtained from Eq. (3) by taking the time as the extrapolated time at which the linearized first portion of the time horizontal part, *i.e.* full coverage. The calculated values of the adsorption parameters of *3MXan* and *7MXan* at various *pH* values are given in Table 1. Moderatly high positive interaction coefficients a for dilute layer at various *pH* values for *3MXan* and *7MXan* indicate lateral attractive interactions of the adsorbed molecules. The low values of the adsorption energy indicate that the deviation from adsorption equilibrium is low and that equilibrium is established at relatively low bulk concentrations of *3MXan* or *7MXan*. The magnitude of the adsorption coefficient of *3MXan* for the compact adsorption stage at various *pH* values is significantly lower than in the dilute stage at the same *pH*. Nevertheless, the

interaction coefficient is generally increased in the compact stage due to enhanced possibilities for intermolecular attractive interactions resulting from the greater population of adsorbed molecules in the compact stage caused by the perpendicular orientation.

On comparison of the adsorption parameters of *3MXan* and *7MXan* and the similar type of adsorption behaviour of xanthine [35], we observed the following tendencies. This maximum surface concentration Γ_m for the compact stage in absence and presence of Cu(II) decreases in the order $Xan > 3MXan > 7MXan$; consequently, the threshold concentration value increases in the same order. At the same time, the adsorption coefficient β for the dilute stage decreases by a factor of 2 from *Xan* to *7MXan*. This lowering of the adsorptivity corresponds to a decrease in ΔG° by *ca.* 0.5 kJ·mol^{-1} for *7MXan*. This is connected with the steric effect of the methyl group attached to the imidazol moiety. The rather lower average surface areas for xanthine and *3MXan* in the compact film stage indicate a densely packed structure of the base residues of the compound oriented perpendicularly with respect to the surface of the electrode. Moreover, the average values of the surface coverage per molecule, S_m, for a compact adsorbed film are very close to those obtained for neutral and synthetic polynucleotides per adsorbed mononucleotide unit [39]. This indicates that in both cases the orientation in the adsorbed layer is characterized by a vertical position of the base units with respect to the surface of the electrode. This leads to the conclusion that the stacking of the base moieties in natural and biosynthetic polynucleotides is comparable to the compact film of the monomeric units adsorbed at the electrode interface.

Experimental

Chemicals and Solutions

3-Methylxanthine (*3MXan*) and 7-methylxanthine (*7MXan*) were obtained from sigma (USA) and used without further purification. Solutions containing different concentrations of the investigated compounds were prepared by dissolving a known amount of chemically pure product into a definite volume of tiply quartz-distilled water. The *Britton-Robinson* buffer was brought to a constant ionic strength of 0.5 *M* by addition of Na*X* ($X = Cl^-$, ClO_4^-, SO_4^{--}, or NO_3^-) and adjusted to the desired *pH*. All chemicals were reagent grade (Merck, Darmstadt). The *pH* was measured with a digital Radiometer *pH* Meter, Model pH M64.

Apparatus and Methods

A Princeton Applied Research (PAR) Model 174A polarographic analyzer coupled with a PAR Model 174/50 ac polarographic analyzer interface, a PAR Model 510 (lock-in-amplifier) phase detector, and a PAR Model 303A hanging mercury drop electrode (HMDE) were employed for ac voltammetric measurements. Phase-sensitive ac voltammograms were recorded with the phase angle adjusted to 90°, corresponding to the out-of-phase component of the ac current (capacitive current component). The amplitude of the ac voltage was 10 mV peak-to-peak, the scan rate of the ac ramp of the mean electrode potential was 5 mVs^{-1}, and the ac frequency has a value of 330 Hz unless stated otherwise. The time dependence of the ac component of the capacitive current at an adjusted constant dc electrode potential was obtained as described earlier [15].

The cell used was a thermostatted PAR cell equipped with a three-electrode system containing a hanging mercury drop electorde (HMDE) as the working electrode, a Ag/AgCl saturated KCl reference electrode, and a platinum wire as the auxiliary electrode. All measurements were carried out at

5 °C. Voltammograms were recorded on an advanced X-Y recorder Model RE0151 after deareation of the electrolyte solutions.

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