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Effect of azathioprine on the parameters of double Hg/chlorate(VII) interface layer in the presence of nonionic surfactants

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Abstract: The mixed adsorption of azathioprine and Triton X-100 or Tween 80 at the mercury/chlorates(VII) interface was described. The differential capacitance of the double layer C_d at the Hg/basic electrolyte interface was measured using the electrochemical impedance spectroscopy. The potential of zero charge E_z was determined applying streaming mercury electrode and the surface tension at the potential of zero charge γ_z was measured by the method of the highest pressure inside the mercury drop. The changes of determined parameters in the function of the supporting electrolyte concentration point with the participation of ClO_4^- ions in the adsorption process, as well as the electrostatic interactions between the dipoles of H_2O and azathioprine- Triton X-100 or azathioprine - Tween 80 in the double layer are shown.

Keywords: azathioprine, mixed adsorption layers, Triton X-100, Tween 80, adsorption parameters, water activity

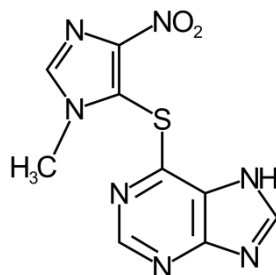
1. Introduction

In aqueous solutions, the adsorption on the mercury surface is mainly competitive between the basing electrolyte ions, water dipoles or organic molecules. Due to the quantitative composition of the basic electrolyte solution and the properties of the organic substance, competitiveness largely consists in displacing water molecules from the electrode surface and replacing them with the molecules of organic substances. In such systems the adsorption equilibrium is influenced by such parameters as: temperature, pH or ionic strength. The change in the structure of the interfacial area caused, for example, by the phenomenon of adsorption, plays an important role in metal electrodeposition processes, molecular or biomolecular electronics and research on biological membranes (Ościk, 1979).

Understanding the influence of the adsorption of organic compounds on the mechanism, speed of heterogeneous ions and electron transfer reactions is of potential practical importance. This can be used by experimentalists who deal only with practical aspects and focus on determining the parameters that optimize the conditions of biological processes. The fundamental importance are also the studies explaining the mechanisms of processes occurring on the electrodes and the conditions under which they take place. Therefore, the study of adsorptive phenomena at the electrode/solution interface seems to be justified in every respect.

The mixed adsorption of azathioprine (AZA) and neutral surfactants at the mercury/chlorates(VII) interface is described.

Azathioprine (AZA) is an example of antimetabolite of natural purine bases used in pharmacotherapy (Scheme 1). AZA is used as an immunomodulator in different fields: transplantology, dermatology, hematology and reumatologic or inflammatory bowel diseases (Kowalska, 2009; Zochowska et al., 2016). The drug biological activity mechanism is complex and may involve modulation of cellular metabolism in several ways. The first pathway is incorporation of active nucleotides into replicating nucleic acids and the other one is the action as a pseudofeedback inhibitor of *de novo* purine nucleotide synthesis.



Scheme 1. Azathioprine structure

During the research a mercury electrode, whose potential values are in the same order as of cell membrane potentials, was used. In addition, the surface of this electrode is characterized by hydrophobicity, similar to the surface of the biological membrane. Thus, it is possible to treat the surface of the electrode - adsorbate interactions as a model of interactions occurring on the surfaces of biological membranes.

2. Materials and methods

The double layer capacity was measured using the impedance method with the μ Autolab Fra 2/GPES (Version 4.9) frequency response analyzer (Eco Chemie, Utrecht, Netherlands). The reproducibility of the average capacity measurements was $\pm 0.5\%$. In the Electrochemical Impedance Spectroscopy, the optimal experiment operating conditions were as follows: first conditioning potentials -200 mV, start potential 200 mV, end potential -1600 mV, step potential 500 mV. For the whole polarization range, the capacity dispersion was tested at different frequencies changing between 200 and 1000 Hz. Adsorption equilibrium was not preserved in any of the systems. Therefore, to obtain proper equilibrium values of differential capacity, the linear dependence of capacity on the square element from frequency was extrapolated to the zero frequency. This procedure assumes that the impedance of the double layer is equivalent to a series of capacity-resistance combination and the rate of adsorption is diffusion-controlled (Nosal-Wiercińska, 2012; 2013).

The potential of zero charge E_z was measured for each solution using the method of streaming mercury electrode, with the accuracy of ± 0.1 mV. The interfacial tension γ_z between the mercury and the electrolyte solutions was measured with the earlier described conventional maximum bubble-pressure capillary electrometer. The surface tension values were determined with an accuracy of ± 0.2 mNm⁻¹.

The adsorption parameters were derived by the back integration of capacity-potential dependencies. No corrections for the effects of the medium on the activity of the supporting electrolyte and the activity coefficient of the adsorbate were made (Wandlowski and Kretschmer, 1986; Wiśniewska et al., 2012).

Analytical - grade reagents: azathioprine (Fluka), NaClO₄ (Fluka) and HClO₄ (Fluka), Triton X-100 (Sigma-Aldrich) and Tween 80 (Sigma-Aldrich) were used without further purification. The solutions were prepared from freshly, doubly distilled water. Before the measurements the solutions were deaerated using high - purity nitrogen which was passed over the solution during the measurements. 2 mol · dm⁻³ ($a_{\text{H}_2\text{O}}=0.927$), 4 mol · dm⁻³ ($a_{\text{H}_2\text{O}}=0.832$) and 6 mol · dm⁻³ ($a_{\text{H}_2\text{O}}=0.704$) chlorate(VII) solutions, mixed at proper ratios (x mol · dm⁻³ NaClO₄ + 1 mol · dm⁻³ HClO₄ (where $0 \leq x \leq 5$) were used as a basic electrolyte (Komorsky-Lovrič et al., 1993). The choice of chlorates(VII) determined their poor complexing and adsorption properties (Nosal-Wiercińska, 2013).

The solutions were prepared just before the measurements. The concentrations of AZA were in the range of $0.5 - 5.0 \cdot 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$ whereas these for surfactants were in the range $1 \cdot 10^{-6} - 1 \cdot 10^{-4} \text{ mol} \cdot \text{dm}^{-3}$.

The measurements were performed in a three - electrode cell containing: a dropping mercury-electrode (CGMDE) with a controlled increase rate and a constant drop surface (0.014740 cm^2), as a working electrode (MTM Poland); Ag/AgCl (3 M saturated solution of NaCl) as a reference electrode and a platinum spiral, as an auxiliary electrode at $25 \text{ }^\circ\text{C}$.

The critical micelle concentration (CMC) was determined using the viscosity method applying the rotational CVO 50 rheometer with the "double gap" measuring systems (Bohlin Instruments).

The measurements were made in chlorate(VII) - Triton X-100 or Tween 80 and in the presence of the azathioprine system. In the range of studied concentrations CMC was determined depending on viscosity of the studied solutions. A rapid change in the system viscosity was found at the concentration value corresponding to CMC.

3. Results and discussion

The comparison of the course of differential capacity curves (Fig. 1a, 1b) shows that the introduction of azathioprine into the chlorate(VII) solutions causes changes in the differential capacitance.

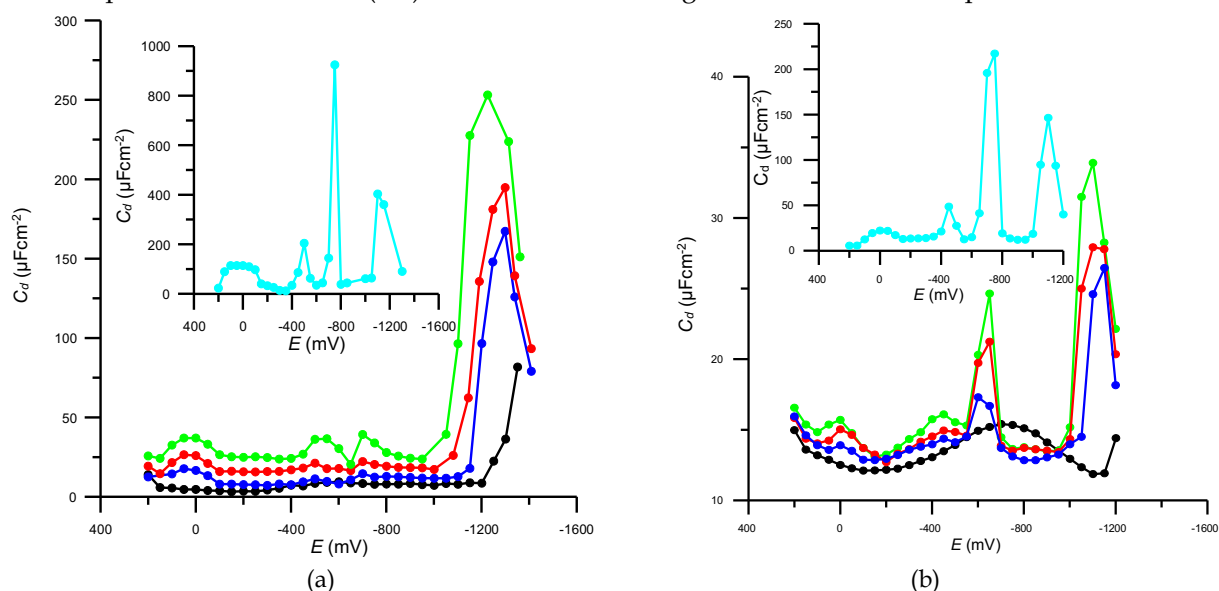


Fig. 1. Differential capacity - potential curves of double layer interface (a) Hg/ $2 \text{ mol} \cdot \text{dm}^{-3}$ and (b) Hg/ $6 \text{ mol} \cdot \text{dm}^{-3}$ chlorate(VII) with various concentrations of azathioprine: (●) 0, (●) $1 \cdot 10^{-5}$, (●) $5 \cdot 10^{-4}$, (●) $1 \cdot 10^{-3}$ (●) $5 \cdot 10^{-3}$ (in $\text{mol} \cdot \text{dm}^{-3}$)

In the area of the "hump" potentials (≈ -600 to -700 mV), occurring in chlorates(VII), after the introduction of azathioprine into the solution, the height of the hump grows. With the increasing azathioprine concentration in the basic electrolyte solution, a further increase in the capacitive hump can be observed, which takes rather the form of sharp peaks. This is most likely a consequence of the electrochemical reactivity of azathioprine which will adsorb specifically on mercury surface (Nosal-Wiercińska, 2012).

The area of more positive potentials (around 0 mV) will be used to illustrate appearing adsorption peaks which are not as well defined, whereas the area of very negative potentials (around -1000 to -200 mV) gives an image of desorption peaks. Both adsorption and desorption peaks increase with the increasing AZA concentration in the solution of the basic electrolyte. It should be also noted that the changes in the differential capacitance value in solutions with a high water activity ($2 \text{ mol} \cdot \text{dm}^{-3}$) (Fig. 1a) are definitely greater as compared to those with low water activity ($6 \text{ mol} \cdot \text{dm}^{-3}$) (Fig. 1b), which indicates participation of adsorptive and desorptive ions in ClO_4^- (Nosal-Wiercińska and Dalmata, 2010; Nosal-Wiercińska, 2013).

The differential capacity curves obtained for a $2 \text{ mol} \cdot \text{dm}^{-3}$ chlorate(VII) solution containing a constant amount of $5 \cdot 10^{-4} \text{ mol} \cdot \text{dm}^{-3}$ azathioprine and the increasing concentrations of Tween 80 (Fig. 2) indicate

changes in both the image of adsorption and desorption peaks and in the area of "hump" potentials (≈ -600 to -700 mV) as compared to the systems with azathioprine alone.

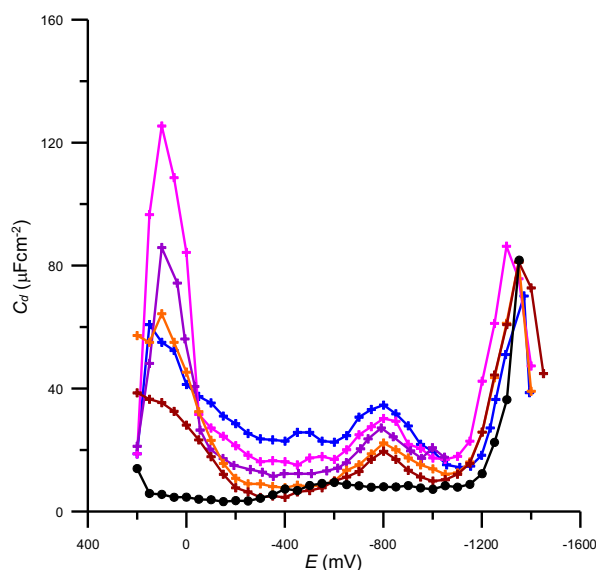


Fig. 2. Differential capacity – potential curves of double layer interface Hg / 2 mol·dm⁻³ chlorate(VII) (●) with 1·10⁻³ mol·dm⁻³ azathioprine and with various concentrations of Tween 80: (●) 5·10⁻⁶, (▲) 1·10⁻⁵, (◆) 3·10⁻⁴, (⊕) 4·10⁻⁴, (⊕) 5·10⁻⁴ (in mol·dm⁻³)

Adsorption peaks, much better defined, increase up to 3·10⁻⁴ mol dm⁻³ of Tween 80 concentration (this concentration corresponds to the critical micelle concentration in the presence of AZA, while CMC for Tween 80 is 5·10⁻⁵ mol·dm⁻³), and then decrease. This can be explained by the interactions of both AZA and Tween 80 molecules leading to the formation of a more or less compact structure of adsorption layers and also of synergistic effect connected with formation of mixed micelles in the basic electrolyte solution. However, one should not neglect the competitive adsorption (Szymczyk and Jańczuk, 2008; Nosal-Wiercińska et al., 2018). However, the shift of CMC in the presence of AZA, towards greater values of concentration suggest changes in the rate of mixed micelles formation.

The image of desorption peaks does not indicate any significant changes with the increase in the concentration of surfactant in the solution of the basic electrolyte. However, in the area (≈ -600 to -700 mV) evidently smaller peaks are observed. They are not sharp and do not dominate. This is probably associated with the effect of Tween 80 in shaping the adsorption equilibria.

A similar picture was found for the Triton X-100 system. It should be also noted that the CMC is shifted to a smaller value 5·10⁻⁵ (CMC for Triton X-100 = 1·10⁻⁴ mol·dm⁻³ and in the presence of AZA CMC = 2·10⁻⁵ mol·dm⁻³). The most probably the presence of AZA in the basic electrolyte accelerates the process of mixed micelles formation (Avranas et al., 2000; Munoz E. et al., 1992; Sotiropoulos et al., 1993).

However, for the chlorate(VII) solutions of much lower water activity, in the AZA – Tween 80 and AZA – Triton X-100 systems the critical micelle concentration was not observed. It is likely that high concentration of ClO₄⁻ ions and thus unfavourable thermodynamic conditions make aggregation and formation of micelles or mixed micelles impossible (Danov et al., 2019). Tables 1 and 2 present the values of the potentials of zero charge E_z and the values of the surface tension γ_z at the zero charge potential for the studied systems.

For all tested concentrations of chlorates(VII) with the increase of azathioprine concentration in the electrolyte solution, the values of E_z are shifted significantly towards the more positive potentials (Table 1). This suggests that the azathioprine molecule adsorb with a rather positive part of the molecule. The largest shift is observed in the solutions of chlorates(VII) with high water activity (Dalmata and Nosal-Wiercińska, 2008). The dependences of the potential of zero charge (Table 1) obtained for all the chlorates(VII) concentration examined point out specific adsorption of azathioprine on mercury. It can

Table 1. Potential of zero charge E_z vs. Ag/AgCl electrode and surface tension γ_z for E_z of chlorates(VII) solutions + different azathioprine systems

$10^3 C_{\text{azathioprine}} / \text{mol}\cdot\text{dm}^{-3}$	Chlorates(VII) solution concentration	$- E_z / \text{V}$	$\gamma_z / \text{m N m}^{-1}$
0.0	2 mol·dm ⁻³	0.483	477.5
0.5		0.291	465.5
1.0		0.193	456.0
5.0		0.032	440.0
0.0	4 mol·dm ⁻³	0.497	469.5
0.5		0.358	440.1
1.0		0.072	437.5
5.0		0.041	434.0
0.0	6 mol·dm ⁻³	0.528	471.2
0.5		0.382	441.8
1.0		0.224	437.0
5.0		0.072	432.3

Table 2. Potential of zero charge E_z vs. Ag/AgCl electrode and surface tension γ_z for E_z of chlorates (VII) solutions + $1\cdot 10^{-3}$ mol·dm⁻³ azathioprine + different surfactants concentration systems

$5\cdot 10^{-4}$ mol·dm ⁻³ azathioprine + $10^6 C_{\text{surfactants}} / \text{mol}\cdot\text{dm}^{-3}$	Chlorates(VII) solution concentration	$- E_z / \text{V}$		$\gamma_z / \text{m N m}^{-1}$	
		Triton X-100	Tween 80	Triton X-100	Tween 80
0	2 mol·dm ⁻³	0.291	0.291	465.5	465.5
5		0.291	0.290	465.0	464.0
20		0.291	0.285	464.7	463.7
50		0.280	0.282	464.0	462.0
100		0.290	0.273	463.5	460.0
300		0.289	0.240	462.5	450.0
400		0.288	0.222	460.1	446.0
500		0.287	0.204	458.5	441.2
0		4 mol·dm ⁻³	0.291	0.291	465.5
5	0.291		0.290	465.0	464.0
20	0.291		0.285	464.7	463.7
50	0.280		0.282	464.0	462.0
100	0.290		0.273	463.5	460.0
300	0.289		0.240	462.5	450.0
400	0.288		0.222	460.1	446.0
500	0.287		0.204	458.5	441.2
0	6 mol·dm ⁻³		0.382	0.382	441.8
5		0.380	0.379	439.8	438.0
20		0.376	0.375	438.9	436.0
50		0.362	0.366	438.0	431.5
100		0.352	0.360	437.7	428.0
300		0.346	0.348	537.5	424.0
400		0.340	0.330	436.0	419.0
500		0.338	0.291	433.8	410.0

be assumed that the specific interaction occurs through the sulphur atom of azathioprine donating their free electron pairs to mercury atom of the positively charged electrode surface (Nosál-Wiercińska, 2013).

The addition of Triton X-100 and Tween 80 causes further shift towards more positive potentials, in the case of Tween 80 these changes are much larger.

However, it should be noted that for the chlorates(VII) solution in concentration of $2 \text{ mol}\cdot\text{dm}^{-3}$, in the presence of the stable azathioprine concentration of $5\cdot 10^{-4} \text{ mol}\cdot\text{dm}^{-3}$ effect of E_z shift is distinctly shown in the presence of $5\cdot 10^{-5} \text{ mol}\cdot\text{dm}^{-3}$ of Triton X-100 and $1\cdot 10^{-4} \text{ mol}\cdot\text{dm}^{-3}$ of Tween 80 (Table 2). These values of surfactants correspond to the CMC values determined in the presence of AZA. Such dependencies are not observed in case of chlorates(VII) solution in concentration of $4 \text{ mol}\cdot\text{dm}^{-3}$ and $6 \text{ mol}\cdot\text{dm}^{-3}$, which evidences a significant role of ClO_4^- ions in the adsorption and desorption processes.

The values of surface tension γ_z at the zero charge potential (Tables 1-2) decrease for all studied systems as proved by the adsorption phenomenon (Gugała-Fekner et al., 2009). The values of surface tension (Table 2) at the zero charge potential γ_z decrease for all tested systems, which confirms the adsorption process. With the decreasing water activity adsorption properties in both systems develop. It should be noted that for the mixed adsorption layer AZA - Triton X-100 or AZA - Tween 80, these reductions are slightly smaller as compared to the system with only azathioprine. This may indicate a dominance of azathioprine in the development of adsorption equilibrium.

4. Conclusions

Azathioprine and forming mixed layers of AZA - Triton X-100 and AZA - Tween 80 adsorbed on the mercury surface change the structure of the interface area. Domination of specifically adsorbed azathioprine as well as formation of a more or less compact structure of adsorption layers and occurrence of a synergistic effect with creation of mixed micelles in $2 \text{ mol}\cdot\text{dm}^{-3}$ chlorates(VII) were pointed out to. However, in more concentrated chlorates(VII) solutions, no micelle or chemimicelle were found which can facilitate adsorption in the AZA - Triton X-100 and AZA - Tween 80 systems. However, it is known, that water molecules are the biggest obstacle to mercury adsorption. Therefore, it seems very beneficial to increase the concentration of chlorate ions which are largely capable of the change orientation of the water molecule on the electrode surface.

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