

Electrochemical oxidation of drug residues in water by the example of tetracycline, gentamicine and aspirin®

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Abstract Electro-chemical oxidation as a method to destroy drug residues like aspirin®, tetracycline or gentamicine in water was investigated with C-anodes (modified by manganese oxides) and Pt anodes. The mechanism of aspirin® and tetracycline oxidation and the influence of the biocide effect was observed using GC-MS and three different microbiological tests. In general, the biological availability increases with progressive oxidation of the antibiotics.

Keywords Analgesics; antibiotics; drug residues; electrochemical oxidation; wastewater treatment

Introduction

For many decades, people have been taking various biologically active organic compounds used as agents in medicines, like in analgesics, antibiotics, anabolics etc. Wrongly proportioned or non-specifically given medicines have a creeping impact on the environment. Through waste and wastewater, they find their way via groundwater or surface water and natural products back into the human organism, forcing them to “take the cure” once more. In this case, the biological activity of these substances has such a high power that if they get into municipal waste they cannot be rendered harmless, not even at the waste or wastewater treatment plants (Wizgall, 2002). To prevent this negative phenomenon, methods and means of the preliminary failure of many medicinal preparations which contaminate industrial, agricultural and other waste should be developed.

In this connection, the study of the possible application of electro-chemical oxidation of such medicaments as aspirin® (AS), salicylic acid (SA), tetracycline (TC) and gentamicine (GM) must be highly interesting. The available data show that the above mentioned medicines rush through municipal waste without any damage. However, it is known that the products of chemical transformation of complex organic compounds can have even higher biological activity than the reference substrates. This point requires a detailed biological testing of the solution being treated in the course of its chemical and electrochemical treatment. The general picture of the changing biological activity in the course of the chemical transformation of organic substrates becomes even more complicated, as the biological activity of the reference substrate and its transformation products can strongly depend on the type of biological test being used.

Therefore the main task of the present study is to define the impact of the electro-catalytic oxidation of the above mentioned medicines on their biological activity by means of various tests.

Methods

The electrochemical oxidation of organic substrates was run in a glass cell with a gas vent consisting of two tubes going out from an anode and a cathode area divided by a diaphragm.

The anode and 2 cathodes were located parallel to each other at a distance of 1–2 cm. Pt or steel plates served as a cathode. Pt plates with a surface of 1 or 10 cm², or the plates made of carbon fibre, modified by manganese oxides (C-anode) with a surface of 10 cm² were used as anodes. In the course of the electrolysis, the volume of gases liberated out of the anode area was measured, with samples being taken to determine the concentration of substances and the biological activity of the solution. A qualitative analysis of the liberated gases for CO₂ presence was made while passing them through the barite/water solution. Moreover 0.1 N H₂SO₄ or 0.1 N NaOH were used as basic electrolyte solutions wherein model solutions were made by different substrate contents.

The product analysis was run by means of mass-spectrometry and NMR. To determine the toxicity or the gradual deterioration of micro-organisms, different microbiological test methods (MBT) were executed with activated sludge from the municipal wastewater treatment plant of Hanover at different stages of the electrochemical process: MBT 1, methylene blue test to determine the oxygen demand according to the German Standard DEV H23; MBT 2, luminescent bacteria tests with *Vibrio fischeri* (acutely after 30 min and chronically after 24 hours) as an inhibition test on the microbiological activity according to EN-ISO 11348-1; and MBT 3, the so-called impedance test to determine the inhibition of the microbiological growth.

Results and discussion

MBT1 showed that in the process of the electrochemical oxidation of SA solutions having 0.02–0.2 g/l as initial concentration the degree of their biological deactivation (*p*) will grow as fast as the electrolysis duration increases (Figure 1). The solution with a low SA content can fully lose its biological activity as early as after 1 or 2 hours of electrochemical treatment. The use of the C-anode shows similar results (Figure 2). Such an increasing SA bioactivity, resulting from the electrolysis of its solution, is connected with the fact that (as NMR-analysis of reaction products has shown) the molecule of SA being oxidized at the first stage generates aromatic products. Also, at the next stage the break of a six-member ring of these connections occurs and aliphatic acids are formed, which, according to the received data, have a very low biological activity.

While studying SA electrochemical oxidation, similar results were obtained when using MBT 3, the impedance test. In Figure 3, the curve of micro-organism colony growth is given, registered according to the impedance change (full electric resistance to the alternative current) of the nutrient medium, containing micro-organisms. The colony growth in the absence of SA additives is shown by the reference line (RL). It becomes obvious that at such conditions the total number of the colony reaches 60% of the maximum within 45

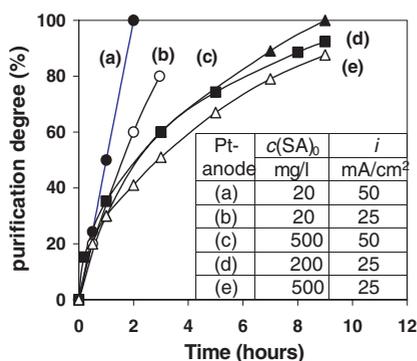


Figure 1 Purification degree *p* of SA solution vs. electrolysis duration time; Pt-anode

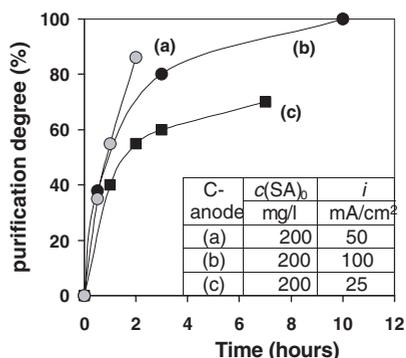


Figure 2 Purification degree *p* of SA solution vs. electrolysis duration time; C-anode

hours. In this case, the growth of the colony proceeds irregularly in time. In the growth curve, the induction period and three sections of the exponential growth of bacteria number can be isolated. The curves complying with the micro-organism growth in the presence of SA and the products of its electrochemical oxidation are, as a whole, of the same character as the reference line. However, some parameters of the above curves strongly depend on the SA electrolysis time. With ongoing electrolyses, the bacteria growth curves approach the reference line. This corresponds with the reduction of SA in the course of its electrolysis. After 3 hours of electrochemical treatment of SA, the induction period is just the same as in the absence of SA, and the value of X_{\max} reaches 92% of the initial value. The change of impedance curve parameters connected with SA oxidation can be observed particularly clearly in Figure 4, where the curves are presented in a differential form. It is apparent that the addition of SA into the nutrient medium inhibits the growth of the micro-organisms or leads to a remarkable adaptation phase, so that the peaks become less marked all the time and the development of the increase becomes less steep. This means that SA strongly decelerates the growth rate of the bacteria. At the subsequent SA electrochemical oxidation, the gradual regeneration of peak disposition and the induction period duration happen on the differential bacteria growth curves. The appearance of these curves for 3-hour experiments as good as completely coincides with RL.

The data received using MBT 2, luminescent bacteria acute and chronic test, also demonstrates a decreasing of the biocide effect of SA in the course of the electrochemical oxidation process. In the presence of SA itself, this value is close to zero; as fast as it oxidises, the intensity of bacteria glowing strongly increases up to 80–90% of the initial value. The increase of the light production by the bacteria can be observed for both periods, acute and chronically. Here, better results were observed with the C-anode than with the Pt-anode.

TC is a widely used antibiotic in livestock breeding, and unfortunately also found in soil and agriculture. Therefore, the electrochemical oxidation of TC was investigated under the same conditions, but with special analysis methods (Hamscher, 2002). The electrochemical oxidation of TC using Pt and C anodes has shown that for both electrodes the content of TC in a solution, determined using the mass spectrometric method, steadily decreases in time. In an acid solution, the process of electrochemical destruction proceeds rather faster than in an alkaline solution. The velocity of the TC concentration drop within the process of electro-oxidation over the range of current density of 25–50 mA/cm² does not depend on the current density. This can be connected with the low velocity of the transfer of large TC molecules to the electrode surface and, correspondingly, to the little limiting diffusion currents of the oxidation (Weichgrebe *et al.*, 2002).

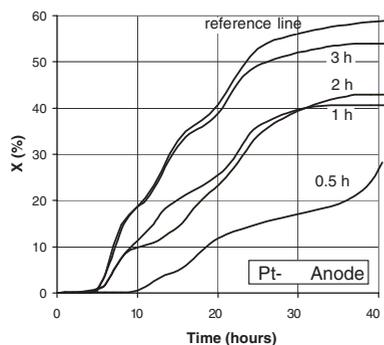


Figure 3 Percentage impedance with time of MBT3 in the presence of SA

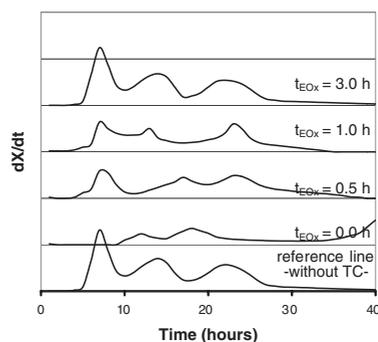


Figure 4 Differential impedance with time of MBT3 in the presence of SA

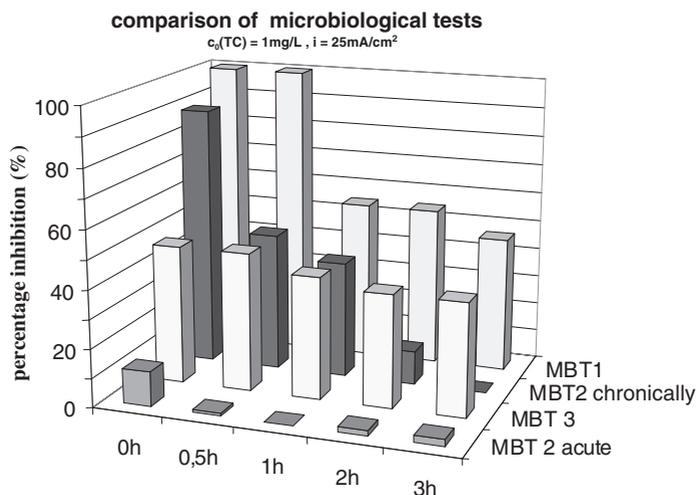


Figure 5 Comparison of microbiological test

In general, the biocide effects decrease with progressive oxidation of the antibiotics. Yet, the observed results of the three applied microbiological tests are inconsistent (Figure 5). This shows that the oxidation products have a different effect on the growth and the activity of the micro-organisms. Therefore, a single microbiological test is not sufficient to observe the variation of the biocide effect, and tests especially referring to different effect mechanisms have to be used in parallel.

Figure 5 shows the determined results with the Pt-anode in a model solution having an initial concentration of $c_0(\text{TC}) = 1 \text{ mg/l}$ and a current density of $i = 25 \text{ mA/cm}^2$. It is obvious that the electrolysis products do not offer such an inhibition as TC by itself, except for MBT 3. For the total liquidation of the biological activity of TC solution, it is sufficient to destroy its structure, especially its functional group, whereas it is not necessary to achieve its total oxidation.

Obviously, hardly any inhibition was detected with the MBT2 acute luminescent bacteria tests with *Vibrio fischeri*. This is in total disagreement with the chronic test. Thus, the acute test method is not suitable for the determination of the inhibition of TC. The strongest opposing effect of TC on these bacteria can be observed within the period of its chronic development. Within the period of the sharp reaction, this effect is apparent at a smaller degree. The TC electrochemical oxidation reduces its inhibition effect to zero after 3 h.

The use of the impedance test also indicates that the TC biological activity drops in the course of its electro-oxidation. Figure 6 shows that the curves of the bacteria colony growth of the activated sludge in the presence of TC and the products of its oxidation on Pt (dashed lines) are considerably lower than RL. Once the electro-oxidation on Pt has been run, the impedance curve comes slightly closer to RL. In this case, within 40 hours of growth the number of micro-organisms will be equal to 75% of those on RL. The presentation of curves in Figure 7 in a differential form turned out to be more informative. Figure 7 shows that the introduction of TC into the nutrient medium of the activated sludge reduces the bacteria colony growth at all stages to approximately half of the original velocity. The third peak on the differential reference curve in the presence of TC itself is displaced towards the high values of time. Apart from that, there appears one more small peak between the 2nd and the 3rd peaks. Once the TC electrolysis had been made, a curve is received within 0.5 hour whose form strongly differs from that of the RL. There are only two peaks on this curve, one of which apparently represents the sum of two or more peaks. The subsequent TC oxidation results in the fact that the form of the differential curves becomes similar to

that of the reference one. These data show that the intermediate products of TC oxidation affect the bacteria colony growth to a greater extent than the reference TC and the final products of its oxidation.

The bio-testing of TC electrolysis products on a C-anode (solid line) by means of the impedance method shows that, as a whole, the same picture as in the case of Pt-anode emerged: TC and the products of its electrolysis lead to the marked changes of the differential curves. However, as a result of the 2-hour oxidation, this curve becomes more similar to the appearance of the reference curve than after the oxidation of TC within 3 hours on Pt. Compared to RL the maximum number of micro-organisms within the 2-hour experiment on Pt are lower (78% vs. 73%). This confirms the greater efficiency of the C-anode in comparison to the Pt-anode.

The comparison of testing results of TC oxidation products made using MBT 1 and MBT 3 allows us to draw the following conclusions. The electro-catalytic oxidation as a whole makes it possible to drastically reduce its inhibition effect. In so doing, the products of SA electrochemical oxidation only slightly affect the vital activity of the micro-organism colony compared to the reference SA. At the same time, the primary products of SA oxidation retard the process of micro-organism colony growth to a greater extent than SA.

The results of the electrochemical oxidation of GM are given in Figure 8. While using the C-anode, the oxidation is more preferable than that of the Pt-electrode. The change of

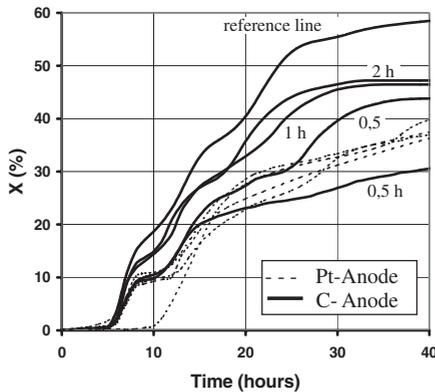


Figure 6 Percentage impedance with time of MBT3 in the presence of TC

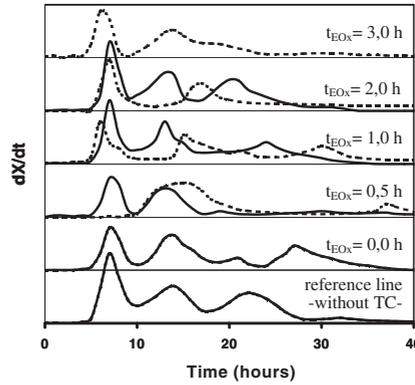


Figure 7 Differential impedance with time of MBT3 in the presence of TC

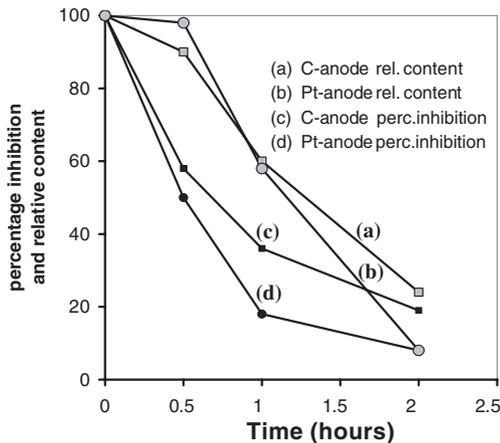


Figure 8 Progress of GM relative content and its inhibition

GM content in the solution in the course of the electrolysis was determined and verified by mass-spectroscopy.

The percentage inhibition was calculated using the MBT 2 chronic method. The relationship between the reduction of the percentage inhibition and the degree of the GM content in the course of electrolysis time is undoubted. It is obvious that within 2 hours the molecules of the reference GM are as good as totally destroyed and its biocide effect has almost completely disappeared.

This can be interpreted that the biocide effect of the GM solution only can be observed in the presence of its functional group, which must be oxidised very quickly. The electrolysis products have practically no inhibition. Such progress can be identified in the case when, at the initial stage of GM's electrochemical oxidation, a mono-saccharide fragment, carrying the GM biological activity, is destroyed. Using the impedance method, it was observed that in the presence of GM the micro-organism growth rate slows down considerably. The electrolysis of GM solution Pt does not actually reduce the extent of its influence on the process of colony formation. The obtained results differ fundamentally from those received when testing the biological activity of GM electrolysis products by means of MBT 1, according to which even the primary products of GM oxidation possess a slight biological activity. This can be explained by the fact that, as in the case of TC, the products of the electrochemical oxidation of these medicinal forms do not strongly affect the vital activities of the micro-organism colonies, but they can influence the process of colony formation to an even greater extent than the reference substrates.

Conclusions

The electrochemical oxidation is a sufficiently effective method of destroying drug residues like aspirin[®], tetracycline or gentamicine in water. Similar degradation results are achieved with C-anode (modified by manganese oxides) and Pt anode. The mechanism of aspirin[®] and tetracycline oxidation could be partially determined. Three different microbiological tests were applied to determine the biocide effects. In general, the biological availability increases with progressive oxidation of the antibiotics, but the applied microbiological test presents inconsistent results due to their different points of effect. Therefore the present work helps to understand the necessity of the validation of different microbiological test methods to determine and assess intermediates of oxidation processes which obviously have an negative impact on the environment.

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