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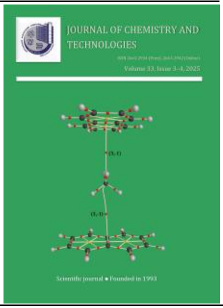

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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF Cu^+ MALEINATE COMPLEXES WITH INCREASED STABILITY

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Abstract

To improve the quality of the target product, the possibility of replacing zinc with ascorbic acid in the synthesis of maleic complexes of Cu^+ and copper-containing composites based on them was investigated. The analysis of the results of potentiometric titration of a solution of 1M $CuSO_4$, 1M maleic acid (H_2M) with a neutralised solution of 1M ascorbic acid (H_2Asc) showed that $HAsc^-$ anions behave exclusively as a one-electron reducing agent, and the existing conjugation of the redox systems $[Cu(HM)]^+/[Cu(HM)]$ and $HAsc/{}^*Asc^-$ contributes to a decrease in the yield of the target product, the maleate Cu^+ complex. It has been found that about 50% of ascorbic acid reacts with $[Cu(HM)]^+$ ions, and this figure is unchanged in the range of low reducing agent concentrations (up to 0,01M) and in the range of high concentrations (up to 1 M). A two-step scheme for the synthesis of the $\{[Cu(HM)(H_2O)]xCu\}$ composite was implemented, in which the reduction of copper ions was carried out exclusively with ascorbic acid. At the first stage, the $[Cu(HM)]^+$ complex was reduced to $[Cu(HM)]$. At the second stage, the required amount of 1M $CuSO_4$ solution was added to the suspension of $[Cu(HM)]$ in a 1M solution of neutralised ascorbic acid to obtain the composite $\{[Cu(HM)(H_2O)]0,5Cu\}$. It was found that the $[Cu(HM)]$ complex isolated from the ascorbate solution is a highly stable form with respect to atmospheric oxygen. Microbiological studies of the effect of the obtained substances on *Staphylococcus aureus* and *Escherichia coli* strains confirmed the high bactericidal activity of the maleinate complex compared to the composite.

Keywords: ascorbic acid; Cu^+ maleate; synthesis; bactericidal activity; Staphylococcus aureus; Escherichia coli.

СИНТЕЗ ТА АНТИБАКТЕРІАЛЬНА АКТИВНІСТЬ МАЛЕЇНАТНИХ КОМПЛЕКСІВ Cu^+ ПІДВИЩЕНОЇ СТІЙКОСТІ

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Анотація

Для поліпшення якості цільового продукту досліджена можливість заміни цинку на аскорбінову кислоту в синтезі малеїнатних комплексів Cu^+ та мідьвмісних композитів на їх основі. Аналіз результатів потенціометричного титрування розчину 1M $CuSO_4$, 1M малеїнової кислоти (H_2M) нейтралізованим розчином 1M аскорбінової кислоти (H_2Asc) показав, що аніони $HAsc^-$ ведуть себе виключно як одноелектронний відновник, а наявна спряженість окисно-відновних систем $[Cu(HM)]^+/[Cu(HM)]$ і $HAsc/{}^*Asc^-$ сприяє зменшенню виходу цільового продукту – малеїнатного комплекса Cu^+. Встановлено, що з йонами $[Cu(HM)]^+$ реагує близько 50 % аскорбінової кислоти, причому цей показник є незмінним і в області низьких концентрацій відновника (до 0.01 M), і в області високих – до 1M. Реалізовано двоступінчату схему синтезу композиту $\{[Cu(HM)(H_2O)]xCu\}$, в рамках якої відновлення йонів купруму велось виключно аскорбіновою кислотою. На першому етапі комплекс $[Cu(HM)]^+$ відновлювався до $[Cu(HM)]$. На другому – до суспензії $[Cu(HM)]$ в 1M розчині нейтралізованої аскорбінової кислоти додавали необхідну кількість 1M розчину $CuSO_4$ і отримували композит $\{[Cu(HM)(H_2O)]0,5Cu\}$. Встановлено, що виділений з аскорбінатного розчину комплекс $[Cu(HM)]$ являє собою високостабільну по відношенню до атмосферного кисню форму. Мікробіологічні дослідження дії отриманих речовин на штами *Staphylococcus aureus* та *Escherichia coli* підтвердили високу бактерицидність малеїнатного комплексу порівняно з композитом.

Ключові слова: аскорбінова кислота; малеїнат Cu^+; синтез; бактерицидність; стафілокок; кишкова паличка.

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Introduction

Copper complex compounds are known to have significant bioactivity potential [1]. It is noted that they are promising substances for targeted next-generation anticancer drugs [2–6], as well as an effective alternative to classical antibiotics [7–14]. A high level of bactericidal activity of Cu+ maleate complexes and copper-containing composites based on them with the general composition {[$Cu(C_4O_4H_3)(H_2O)$]xCu} was reported in [15]. Based on the established dependence between the inhibitory effect of the drug on microorganisms and the content of atomic copper in it, the possibility of programmable control of the bioactivity of this class of substances was considered. It was noted that the active bioactive component of the composites is the [$Cu(C_4O_4H_3)(H_2O)$] complex, the practical application of which is hampered by the ability to decompose under the influence of oxygen and moisture. Therefore, to stabilise the drug, the mononuclear complex [$Cu(C_4O_4H_3)(H_2O)$], which is active with respect to oxygen, was combined with an inert binuclear complex [$Cu_2(C_4O_4H_3)(H_2O)_2$]. Such composites indeed acquired the necessary stability, but at the same time lost bactericidal activity. It also turned out that the method used to synthesise {[$Cu(C_4O_4H_3)(H_2O)$]xCu} did not produce a substance of homogeneous chemical composition. The copper content within one sample varies from 47 wt. % to 74 wt. % [16]. The reason for such a wide range was the use of zinc as a reducing agent. After all, the reaction of reduction of Cu^+ ions and the addition of the resulting Cu^0 atoms to the complexes [$Cu(C_4O_4H_3)(H_2O)$]

$$Zn + 4[Cu(C_4O_4H_3)(H_2O)] = Zn^{2+} + 2[Cu_2(C_4O_4H_3)(H_2O)_2] + 2C_4O_4H_3^-$$

is heterogeneous and occurs only on the surface of zinc crystals, creating a high concentration of Cu atoms and, accordingly, binuclear complexes in this zone.

It takes time for the reaction products to move deeper into the solution, so the further away from the zinc surface, the lower the copper concentration becomes. Since the final product in the form of a composite is a water-insoluble substance, its composition was not averaged after the process.

An analysis of the literature has shown that the use of ascorbic acid, which is well soluble in water, is quite successful for the synthesis of nanodispersed copper [17]. However, an attempt to synthesisethe composite {[$Cu(C_4O_4H_3)(H_2O)$]xCu} by the action of ascorbic acid on the [$Cu(C_4O_4H_3)(H_2O)$] complex was unsuccessful. Obviously, due to the replacement of the water molecule in Cu^+ aqua complexes with an acidic maleinate ion, the redox potential of the [$Cu(C_4O_4H_3)(H_2O)$]/Cu system has changed significantly relative to the redox potential of the [$Cu(H_2O)_2$]$^+$/Cu system. Therefore, ascorbic acid is no longer a reducing agent for [$Cu(C_4O_4H_3)(H_2O)$] complexes.

We have investigated the possibility of implementing a two-step scheme for the homogeneous synthesis of a copper-containing composite, in which copper ions are reduced by ascorbic acid. We also studied the bactericidal properties of the resulting substances.

Experimental procedure

Distilled water was used to prepare the solutions. The reagents had the following qualifications by purity class: $CuSO_4 \cdot 5H_2O$ and NaOH – chemically clean; maleic acid – pure for analysis. Zinc powder (made in Germany) and ascorbic acid (made in China) contained 99.0 % and 99.7 % of the basic substance, respectively.

The acidity of the solutions was monitored with an EV-74 ionometer.

The redox potential was measured on a platinum electrode, which was previously cleaned with a suspension of MgO and thoroughly rinsed with distilled water. The comparison electrode was a silver chloride semi-element in a saturated KCl solution. The salt bridge was filled with a 1 M Na_2SO_4 solution.

The potential of the platinum electrode was recorded with a digital amperovoltmeter Sh-4313.

The temperature in all experiments was kept constant at 20 °C by a UTU-2/82 thermostat.

The total copper content in the composites was determined using the complexometric method. Details are given in [16].

The bactericidal effect of the synthesised substances was studied using clinical strains of *Staphylococcus aureus* (*St. aur.*) and *Escherichia coli* (*E. coli*). The methods of microbiological experiments are described in our previous work [18].

Results and discussion

Ascorbic acid anions ($HAsc^-$) in a slightly acidic environment (pH = 4–6) can be a two-electron reducing agent [19]

$$HAsc^- - \bar{e} \rightarrow {}^*HAsc \rightarrow H^+ + {}^*Asc^- \quad (1)$$

$$^*Asc^- - \bar{e} \rightarrow DHA, \quad (2)$$

here, *HAsc and $*Asc^-$ are the corresponding radical forms of oxidised ascorbic acid; DHA is dehydroascorbic acid.

Therefore, to determine the quantitative ratios of the reagents, we performed potentiometric measurements during the action of a neutralised solution of ascorbic acid on a solution of acidic maleate Cu^{2+}.

The solution of acidic maleate Cu^{2+} was prepared by adding to a 0.1 M $CuSO_4$ solution the corresponding weight of maleic acid (H_2M) in a molar ratio (Cu^{2+}) : (H_2M) = 1 : 1. Next, the pH value was raised to 2 with a concentrated NaOH solution (1 M). According to [20], under these conditions, the dominant form in solution is monosubstituted acidic maleate $[Cu(HM)]^+$.

Redox titration was performed with a neutralised (pH=6) solution of 1M H_2Asc. According to [19], both H_2Asc and DHA are highly soluble in water. The same applies to acidic Cu^{2+} maleate. However, the maleic complex Cu^+ has a limited solubility at the level of 0.006 M [15]. Therefore, we chose the range of 0.001–0.01 M as the working range of ascorbic acid concentrations.

The results of potentiometric measurements are shown in the figure.

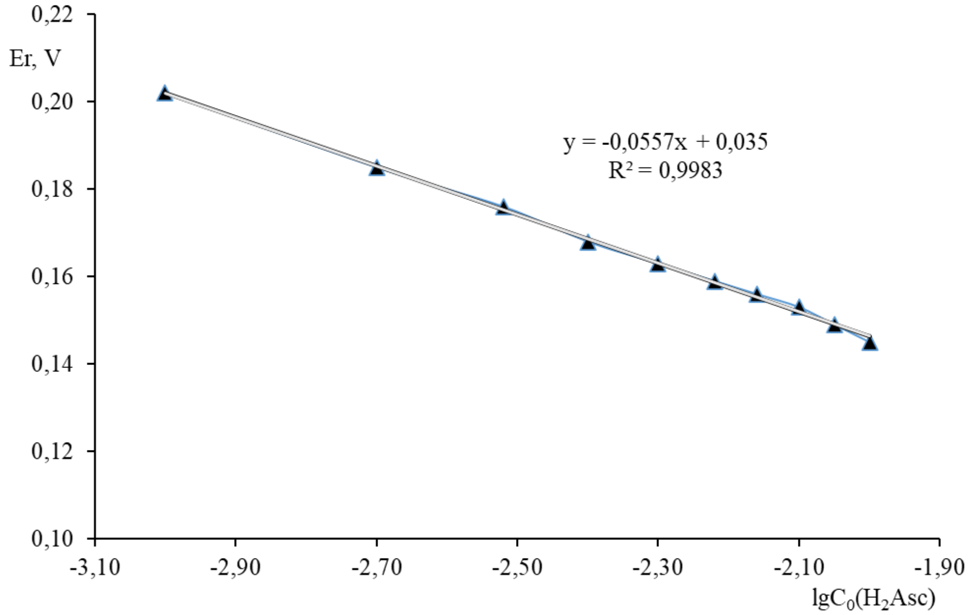

Figure. Dependence of the redox potential measured in a solution of 0.1M $CuSO_4$, 0.1M H_2M (pH=2) + xM H_2Asc (pH=6) on the amount of ascorbic acid $C_0(H_2Asc)$, M.

As can be seen from the figure, the experimental dependence of the redox potential on the logarithm of the ascorbic acid concentration is clearly linear and has an angular coefficient of 0.056 V, which practically coincides with the theoretical value (2.3RT/F)=0.058 V for the one-electron transition. Thus, the process under study is reduced to the reaction of

$$[Cu(HM)]^+ + HAsc^- \rightarrow [Cu(HM)] + {}^*Asc^- + H^+. \quad (3)$$

It was also of interest to calculate the completeness of the interaction of ascorbic acid with copper ions, i.e., the product yield. This parameter determines the absolute value of the compromise equilibrium potential (E_e) in accordance with the two conjugate redox equilibria $[Cu(HM)]^+/[Cu(HM)]$ and $HAsc^-/{}^*Asc^-$:

$$E_e = E_0(Cu^{2+}/Cu^+) + 0.058\lg(K_1/K_2) + 0.058\lg\{C(CuHM^+)/C(CuHM)\} \quad (4)$$

$$E_e = E_0({}^*Asc^- / HAsc^-) + 0.058\lg\{C({}^*Asc^-)C(H^+)/C(HAsc^-)\}. \quad (5)$$

Here, K_1 and K_2 are the stability constants of the $[Cu(HM)]$ and $[Cu(HM)]^+$ complexes, respectively; $C(CuHM^+)$ and $C(CuHM)$ are the equilibrium concentrations of $[Cu(HM)]^+$ ions and $[Cu(HM)]$ molecules in solution.

To estimate the completeness of the reduction of $[Cu(HM)]^+$ to $[Cu(HM)]$, we represent the ratio $C(CuHM^+)/C(CuHM)$ in equation (4) as follows: $\{C_0(CuHM^+) - C(CuHM)\}/C(CuHM)$, where $C_0(CuHM^+)$ is the initial concentration of $[Cu(HM)]^+$. As noted in [20], at pH=2, almost 100 % of Cu^{2+} ions are bound to the $[Cu(HM)]^+$ complex. That is, under our experimental conditions, we can assume that $C_0(CuHM^+)$ = 0.1 M.

In calculating the values of C(CuHM) according to equation (4), we used the reference value $E_0(Cu^{2+}/Cu^+)$ = 0.159 B (n.h.e.) = –0.042 B (s. sil.

chl. e.) and literature data on stability constants: $K_1=1.2\cdot10^4$ [21] and $K_2=1.6\cdot10^2$ [22].

Table 1 shows the results of the calculations. As can be seen from the data in Table 1, the product yield is indeed incomplete and amounts to about 50 %.

To determine the product yield by weight, the complex was synthesised by mixing 50 ml of a solution of 0.1 M $CuSO_4$, 0.1M H_2M (pH = 2) and 50 ml of 0.1 M H_2Asc (pH=6). After decantation, washing the precipitate with distilled water, and filtration, the complex was dried at 120 °C. According to [20], the complex obtained under these conditions corresponds to the formula $[Cu(C_4O_4H_3)(H_2O)]$. Weighing and comparing the obtained mass of the complex (1.008 g) with the calculated amount (1.965 g) showed that the gross yield of the target product was 51.3 %. This confirms the reliability of the calculations of redox equilibria in the studied system: acidic maleic acid Cu^{2+}, Cu^{+}-ascorbic acid.

Table 1

The results of equilibrium potential measurements when titrating a solution of 0.1 M $CuSO_4$, 0.1 M H_2M (pH = 2) with a solution of 1 M H_2Asc (pH = 6) and calculating the ratio between the amount of complex formed C(CuHM) and the amount of ascorbic acid added $C_0(H_2Asc)$: X=C(CuHM)/$C_0(H_2Asc)$

E_e, В (нас. х.с.е.)	$C_0(H_2Asc)$, M	C(CuHM), M	X, %
0.202	0.001	0.00055	55.0
0.185	0.002	0.00098	49.0
0.176	0.003	0.00152	50,7
0.168	0.004	0.00210	52.5
0.163	0.005	0.00251	50.2
0.159	0.006	0.00296	49.3
0.156	0.007	0.00331	47.3
0.153	0.008	0.00370	46.3
0.151	0.009	0.00431	47.9
0.149	0.010	0.00498	49.8

The information obtained made it possible to plan and successfully implement a two-stage scheme for the synthesis of a copper-containing composite based on acidic Cu^{+} maleate using ascorbic acid as a reducing agent.

At the first stage, an equimolar solution of neutralised ascorbic acid (pH = 6) was added to a solution of 0.1 M $CuSO_4$, 0.1 M H_2M (pH = 2). A yellow precipitate of the complex $[Cu(C_4O_4H_3)(H_2O)]$ was obtained.

At the second stage, the complex suspension obtained and washed with distilled water was mixed with a neutralised 1M ascorbic acid solution. Then, gradually, $CuSO_4$ was added as a 1M solution under vigorous stirring, maintaining the final molar ratio of $CuSO_4$: complex = 1 : 2. The colour change of the dispersed phase from yellow to light brown indicated the formation of the composite. The analysis of the copper content in the composite showed that the quantitative composition of the components corresponded to the formula $\{[Cu(C_4O_4H_3)(H_2O)]0.5Cu\}$ and was absolutely homogeneous.

The results of the study of the effect of the obtained samples of the complex and copper-containing composite on clinical strains of *Staphylococcus aureus* and *Escherichia coli* are presented in Table 2.

Table 2

The content of colony-forming units (CFU/ml) in the control sample and in the presence of the complex $[Cu(C_4O_4H_3)(H_2O)]$ or the composite $\{[Cu(C_4O_4H_3)(H_2O)]0.5Cu\}$ depending on the exposure time (t)

	t, hours	control	complex	composite
Strain *St. aur*	1	10^9	10^4	10^5
	2	10^9	10^3	10^4
	4	10^9	10^1	10^2
Strain *E. coli*	1	10^9	10^2	10^4
	2	10^9	10^1	10^3
	4	10^9	0	10^1

As can be seen from this table, the bactericidal activity of the Cu^{+} maleate complex is significantly higher than that of the copper-containing composite. Bactericidal ratios close to the above were also recorded in the case of products synthesized using zinc metal as a reducing agent [15]. However, it was noted that the practical use of Cu^{+} maleate complexes is hampered by their instability.

We have studied the behaviour of samples of the $[Cu(C_4O_4H_3)(H_2O)]$ complex obtained using ascorbic acid. It was found that the composition of the complex did not change during 6 months of observations – no traces of Cu^{2+} ions, which could have arisen as a result of oxidation of Cu^{2+} ions by air oxygen, were recorded. Obviously, the residues of ascorbic acid adsorbed on the crystals of the complex together with DHA prevent its degradation.

This allows us to consider the obtained highly stable form of the maleic acid complex Cu^+ as an effective bactericidal preparation.

Conclusions

The analysis of the results of potentiometric titration of a solution of 1M $CuSO_4$, 1M maleic acid (H_2M) with a neutralised solution of 1M ascorbic acid (H_2Asc) showed that $HAsc^-$ anions behave exclusively as a one-electron reducing agent, and the existing conjugation of the redox systems $[Cu(HM)]^+$/[Cu(HM)] and $HAsc^-$/$^*Asc^-$ contributes to a decrease in the yield of the target product – maleic complex Cu^+ . It has been found that about 50 % of ascorbic acid reacts with $[Cu(HM)]^+$ ions, and this figure is unchanged in the range of low reductant concentrations (up to 0.01 M) and in the range of high concentrations (up to 1 M).

A two-step scheme for the synthesis of the $\{[Cu(HM)(H_2O)]xCu\}$ composite was implemented, in which the reduction of Cu ions was carried out exclusively with ascorbic acid. At the first stage, the $[Cu(HM)]^+$ complex was reduced to [Cu(HM)]. At the second stage, the required amount of 1M $CuSO_4$ solution was added to the suspension of [Cu(HM)] in a 1M solution of neutralised ascorbic acid to obtain the composite $\{[Cu(HM)(H_2O)]0.5Cu\}$.

It was found that the [Cu(HM)] complex isolated from the ascorbate solution is a highly stable form with respect to atmospheric oxygen.

Microbiological studies of the effect of the obtained substances on *Staphylococcus aureus* and *Escherichia coli* strains confirmed the high bactericidal activity of the maleinate complex compared to the composite.

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