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ARTICLES

Determination of Urea and Creatinine by Chronoamperometry

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Abstracts—Methods are developed for the non-enzymatic chronoamperometric determination of urea in blood serum and creatinine in model solutions imitating the composition of blood serum. The selectivity of determination is ensured using columns packed with an anion-exchange resin for urea or synthesized molecularly imprinted polymers for creatinine.

Keywords: molecularly imprinted polymers, urea, creatinine, chronoamperometry, voltammetry, electrocatalysis, acrylic acid

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ìThe determination of urea and creatinine in biological fluids, plasma, blood serum, and urine, is used in clinical diagnostics for the evaluation of the kidney function and the efficiency of hemodialysis [1]. There are direct photometric methods for urea, based on its reaction with diacetyl monoxime [2] or catalytic reaction with urease [3]. The photometric determination of creatinine is usually based on the Jaffe reaction [4] or, quite rarely, on enzymatic hydrolysis with creatinine iminohydrolase and the use of automated analyzers [5].

It seems promising to determine urea and creatinine by the catalytic electrochemical oxidation of analytes with the chronoamperometric detection of the signal [6]. The major problem of the chronoamperometric analysis of urea and creatinine in multicomponent solutions and real samples refers to its insufficient selectivity toward the analytes. The problem can be solved using additional steps of the separation and preconcentration of the target components.

Since the major components of blood are acids, i.e., amino acids, ascorbic and uric acids, and the concentration of creatinine is lower by an order of magnitude, for the selective determination of urea is anion-exchange columns should be reasonably used.

Molecularly imprinted polymers (**MIPs**), which are used for the recognition and determination of organic compounds in solutions and biological fluids, can be used for creatinine. MIPs are structures with highly specific binding sites, or sites of molecular recognition, complementary to specific organic molecules, i.e., target molecules (**TMs**) or imprint molecules [7] in size, shape, and physicochemical properties. The unlimited possibility of the creation of adsorbents selective to organic compounds from various classes, mechanical and chemical stability of MIPs, and also the relative simplicity and low cost of their synthesis favored an enhancement of the theoretical and applied interest to this class of chemicals. Methods of TM isolation are based on their interaction with the functional monomer (**FM**) forming both at the step of complex formation prior to polymerization itself (prepolymerization complex, **PC**) and in the repeated binding of MIPs with the target molecule [8].

Now the most abundant application among the methods of MIP synthesis is provided by a simple and versatile technique of non-covalent binding. Among the main areas of application of molecularly imprinted polymers are the solid-phase extraction (**SPE**) of organic compounds from solutions and biological fluids [9] and the chromatographic separation of organic compounds [10]. Most often SPE is performed under dynamic conditions by passing or pumping a test solution through a microcolumn, preconcentration cartridge, or disc filled with a rather small amount of an sorbent; this requires a smaller amount of a solvent for the subsequent desorption of the concentrated species and eliminates the evaporation step.

In creatinine MIPs β-cyclodextrin and 4-vinylpyridine are used as functional monomers [11]; noteworthy, a better adsorption capacity is demonstrated by creatinine MIPs based on 4-vinylpyridine. In this case the formation of PC is due to intramolecular hydrogen bonds. If acrylic acid is used as the functional mono-

mer, intramolecular bonds with nitrogen- and oxygen-containing groups of creatinine are formed; therefore, in this work MIPs of creatinine were prepared using acrylic acid as a functional monomer.

The goal of this work was the development of an electrocatalytic method for determining urea and creatinine on electrodes modified with nickel-based catalytic systems and columns filled with an anion exchanger or a molecularly imprinted polymer.

EXPERIMENTAL

Reagents. Creatinine (Fluka, Germany) was used as an imprint molecule. The functional monomer was acrylic acid; the cross-linking agent, divinylbenzene (DVB, Fluka, Germany); and the initiator of polymerization, benzoyl peroxide (BP, Reakhim, Russia). Acetone (reagent grade, Russia) was used to purify MIPs from monomers. As components of model solutions for studying the adsorption capacity of creatinine MIP we used creatinine (Merck, Germany), α-L-alanine (Egis, Hungary), α-L-aspartic acid (Egis, Hungary), α-L-leucine (Reakhim, Russia), α-L-glycine (Reakhim, Russia), α-L-glutamic acid (Egis, Hungary), D-glucose (Reakhim, Russia), uric acid (Merck, Germany), L-ascorbic acid (Merck, Germany), urea (Fluka, Germany), and NaCl (reagent grade, Reakhim, Russia).

The samples of chemicals entitled were prepared by dissolving precisely weighed portions in deionized water. We used NaOH (analytical grade, Vekton, Russia), CH_3OOH (chemically pure grade, Russia), and ethanol (chemically pure grade, Russia). All substances in the work were used with no extra purification.

Apparatus. IKA RCT basic stirring plates with heating were used (Ika Werke, Germany). Samples of synthesized creatinine MIPs were sieved through U1-ESL-P laboratory sieves (Kraft, Russia) with particle size from 0.04 to 0.08 mm. For the selective extraction of urea from model solutions and blood serum samples we used a HyperSep IC-OH anion-exchange column (Metrohm, Switzerland). An IVA-5 stripping voltammetry analyzer with a three-electrode cell (IVA, Ekaterinburg, Russia) was used for electrochemical measurements. As a working electrode we used thick-film electrodes based on Metech carbon-containing inks and prepared by the screen-printing technology and modified with dinickel(II) 1,1,1,7,7,7-hexafluoroheptane-2,4,6-triketonate (IVA, Ekaterinburg, Russia). As reference and auxiliary electrodes we used a saturated silver–silver chloride electrode and a glassy carbon rod, respectively.

RESULTS AND DISCUSSION

As shown before, working electrodes modified with organic complexes of nickel(II) make possible the registration of an analytical signal in the heterogeneous electrocatalytic oxidation of electrically inactive urea or creatinine [6]. The linear relationships of the analytical signal to the analyte concentration with a correlation coefficient of 0.999 and detection limits of 8.7×10^{-6} and 2.7×10^{-5} M for urea and creatinine, respectively, confirm the possibility of use of these electrodes for the chronoamperometric determination of the target analytes in biological fluids. In the further studies we used electrodes modified according to the procedure [6].

Table 1. Composition of model solutions Ms1 and Ms2 imitating blood serum, mM

Components of model solution	Ms1	Ms2
Creatinine	1	1
α-L-alanine	0.314	0.314
α-L-aspartic acid	0.022	0.022
α-L-leucine	0.187	0.187
α-L-Glycine	0.320	0.320
α-L-glutamic acid	0.089	0.089
D-glucose	6.4	6.4
Uric acid	0.480	0.480
L-ascorbic acid	0.057	0.057
Urea	0	5

Determination of urea in model solutions. The model solutions are listed in Table 1; they contained the maximum concentration of substances potentially interfering with the determination of urea and creatinine, whose oxidation reactions are catalyzed by nickel(II) (amino acids, uric and ascorbic acids). Most of substances enlisted in Table 1 possess acidic properties and can thus be separated from urea by adsorption on an anion-exchanger.

Samples were analyzed as follows: 0.1 mL of sample (model solution) was mixed with 0.9 mL of a 0.9% NaCl solution and passed through an anion-exchange column. A 10-mL portion of a 0.25 M NaOH solution was placed into an electrochemical cell followed by the working electrode, and a chronoamperogram of nickel oxidation was recorded at 0.54 V. Then 0.1 mL of a 0.01 M urea solution was added and another chronoamperogram at 0.54 V was recorded. Then 0.1-mL portions of filtrate solutions Ms1 or Ms2 were added and a chronoamperogram at 0.54 V was recorded again. The concentration of urea in the sample, c_{sam}, M, was calculated as follows (1):

$$c_{sam} = c_{add}\left(\frac{\Delta I_{sam}}{\Delta I_{add}}\right)\left(\frac{V_{add}}{V_{sam}}\right), \quad (1)$$

where ΔI_{add} is the current of nickel(II) oxidation recorded after adding a standard addition of urea or creatinine, A; ΔI_{sam} is a difference between nickel(II) oxidation currents in the presence of a sample and a standard addition, A; c_{add} is the concentration of a standard addition of urea or creatinine, M; V_{add} is the

Table 2. Chronoamperometric determination of urea in model solutions (I) with an anion-exchange column and (II) with no column ($n = 5$, $P = 0.95$)

Model solution	Found, $c \pm \delta$, mM (RSD, %)	
	I	II
Ms1	0.92 ± 0.04 (5.2)	8.42 ± 0.76 (7.3)
Ms2	5.37 ± 0.45 (9.6)	14.73 ± 0.69 (3.8)

Table 3. Determination of urea in blood serum by the developed ($n = 5$, $P = 0.95$) and reference methods

Standard urease method (Vitros BUN/UREA Slide, Johnson&Johnson Clinical Diagnostics, Inc.), c_1	Method proposed, c_2	c_2/c_1, %
13.3 ± 0.2	12.4 ± 0.7	93
4.8 ± 0.1	5.3 ± 0.6	109
2.30 ± 0.04	2.3 ± 0.3	99
4.0 ± 0.1	4.0 ± 0.2	100

volume of a standard addition of urea or creatinine, mL; and V_{sam} is the volume of the sample injected, mL.

It can be seen from the data of Table 2 that the use of an anion-exchange column substantially enhances the selectivity of urea determination in model solutions imitating blood serum.

Determination of urea in blood serum. A 0.9-mL portion of a 0.9% NaCl aqueous solution was added to 0.1 mL of a sample (blood serum) and treated as described above. Chronoamperograms recorded with an electrode modified with the catalyst in the presence of urea and samples are presented in Fig. 1; the data of urea determination in blood serum are presented in Table 3. A comparison of the results of analysis of real samples by the proposed method and the standard urease method widely used in clinical practice showed the virtual absence of interferences and proved the accuracy of the results.

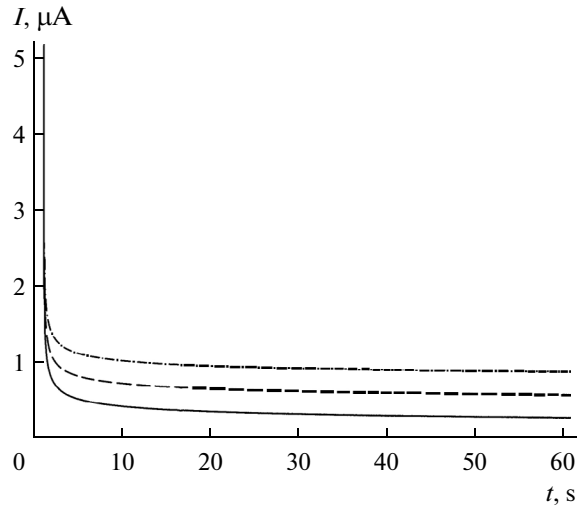

Fig. 1. Chronoamperograms recorded on an electrode modified with a catalyst. The composition of solutions is 0.25 M NaOH (—), as above + 1×10^{-4} M of urea (— —), as above + sample (– · –).

Synthesis of polymers with molecular imprints of creatinine (MIP-Cr). Acrylic acid (0.136 mL, 2 mmol) was added to 56.5 mg, or 0.5 mmol, of creatinine. The reaction mixture was heated to the complete dissolution of creatinine. Then 1.4 mL, or 10 mmol, of DVB, and 10 mg of BP were added to the solution prepared, and the mixture was kept in a water bath at 70°C for 1 h. The solid polymer obtained was ground, washed with 10 mL of acetone to remove monomers, filtered under vacuum, and dried at 70°C for 20 min.

The polymers prepared were ground in an agate mortar. The powder was sieved to collect the fraction 0.08–0.04 mm, which was used in the next experiments. The adsorbents ground were washed with 2 mL of a mixture of glacial acetic acid with deionized water (7 : 3) and then with 10 mL of deionized water and dried at 100°C.

Polymers with no molecular imprints (NMIP) were prepared similarly as MIP-Cr ones using no creatinine.

Determination of the adsorption capacity of polymers to creatinine and the analysis of filtrates. A 0.27-g portion of a polymer, MIP-Cr or NMIP, was charged into a 25-mL beaker and 10 mL of a 0.10 M creatinine solution were added, and mixture was stirred for 1 h. The mixture was filtered, and creatinine was determined in the filtrate (F1) by chronoamperometry [6]. Then the polymer, MIP-Cr or NMIP, with creatinine absorbed was washed with 10 mL of ethanol and dried at 80°C for 45 min to the complete evaporation of ethanol. Then the polymer was charged into a 25-mL beaker followed by 10 mL of deionized water and the mixture was stirred vigorously for 1 h. The mixture was filtered under vacuum, and creatinine was determined in the filtrate (F2) [6].

The adsorption capacity of MIP-Cr and NMIP was found by the equations:

$$\alpha = \frac{(c_0 - c_p)V}{m}, \quad (2)$$

$$R = \frac{(c_0 - c_p)}{c_0} \times 100, \quad (3)$$

$$D = \frac{RV}{(100 - R)m}, \quad (4)$$

where α is sorption, mmol/g; R is recovery, %; D is distribution coefficient, mL/g; c_0 is creatinine concen-

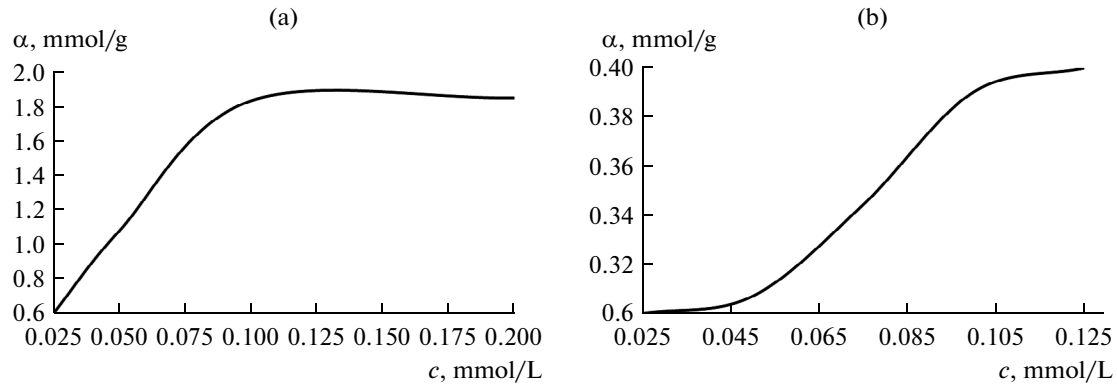

Fig. 2. Sorption isotherms of creatinine from aqueous solutions on (a) MIP-Cr and (b) NMIP.

tration in the starting solution, M; c_p is equilibrium concentration of creatinine in the filtrate (F2) after adsorption on MIP, M; V is volume of the starting solution, mL; m is weight of polymer in use, g.

The capacity of a polymer with molecular imprints of creatinine to recognize TM was evaluated by the imprinting factor:

$$IF = \frac{D_{\text{MIP-Cr}}}{D_{\text{NMIP}}}, \quad (5)$$

where $D_{\text{MIP-Cr}}$ is the distribution coefficient of an analyte on MIP-Cr, mL/g and D_{NMIP} is the distribution coefficient of creatinine on NMIP, mL/g.

It can be seen in Fig. 2 that, in contrast to NMIP, MIP-Cr better adsorbs creatinine. Moreover, for MIP-Cr only, the relation of creatinine adsorption to the concentration of analyte in solution under consideration follows the Langmuir equation.

The recoveries (R, %) and distribution coefficients (D, mL/g) to MIP-Cr and NMIP calculated by Eqs. (3) and (4) are presented in Table 4. The data indicate a higher adsorption capacity of the MIP-Cr synthesized in comparison to NMIP and indirectly confirm the presence of pores complimentary to creatinine in the polymer structure.

The imprinting factor of the MIP-Cr polymer calculated by Eq. (5) is equal to 52, which allows the extraction of creatinine from multicomponent solutions using MIP-Cr.

The chronoamperograms recorded on the electrode modified with the catalyst in the presence of creatinine and a sample are shown in Fig. 3. The results of the chronoamperometric determination of creatinine in filtrates F1 and F2 using polymers synthesized are listed in Table 5.

The data acquired show that the selected MIPs-Cr absorb no less than 70% of creatinine. This is, probably, due to the morphology of pores generated within MIPs-Cr. Their size in the polymers prepared and the arrangement of functional groups in pores may favor non-specific interactions with creatinine molecules.

Determination of the selectivity and adsorption capacity of polymers to creatinine in model solutions. A polymer, MIP-Cr or NMIP, was put into a beaker with a magnetic stirrer followed by 10 mL of a model solution; the mixture was stirred for 60 min. Then it was filtered under vacuum with simultaneously rinsing with 1 mL of deionized water and dried at 75°C for 20 min. The polymer was transferred into a beaker with a magnetic stirrer, 10 mL of deionized water was added; the mixture was stirred for 60 min and filtered under vacuum. The concentration of creatinine was found as described in [6].

Creatinine and interfering components of model solutions imitating the composition of blood serum were absorbed under static conditions from aqueous solutions Ms1 and Ms2 differed by concentrations of urea using periodically stirring at ambient temperature. The results of the chronoamperometric determi-

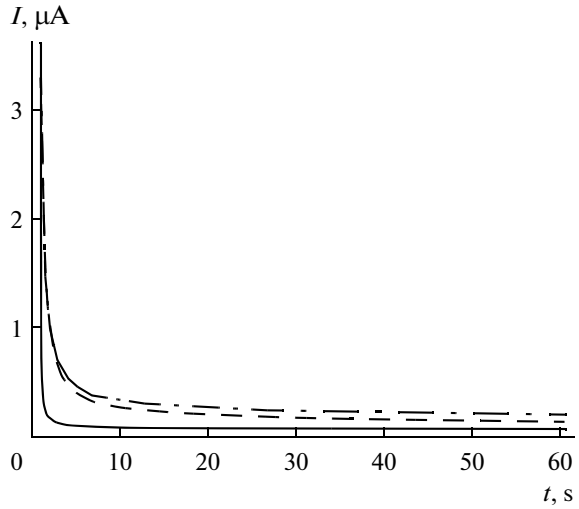

Fig. 3. Chronoamperograms recorded on an electrode modified with a catalyst. The composition of solutions is 0.25 M NaOH (—), as above + 1×10^{-4} M of creatinine (— —), as above + sample (– · –).

Table 4. Recovery and distribution coefficient of creatinine on MIP-Cr and NMIP (c_{Cr} = 0.1 M, V = 10 mL, m = 0.2705, t = 60 min)

Polymer	R, %	D, mL/g
MIP-Cr	69.5	36.3
NMIP	7.0	0.7

Table 5. Chronoamperometric determination of creatinine in filtrates F1 and F2 (n = 5, P = 0.95, 0.1 M creatinine added)

Test solution	Found, $c \pm \delta$, mM (RSD, %)	
	MIP-Cr	NMIP
F1	0.025 ± 0.004 (0.15)	0.096 ± 0.012 (0.14)
F2	0.076 ± 0.012 (0.15)	0.009 ± 0.001 (0.12)

Table 6. Chronoamperometric determination of creatinine after its extraction from model solutions by MIP-Cr (n = 5, P = 0.95, 1 mM creatinine added)

Model solution	Found, $c \pm \delta$, mM (RSD, %)
Ms1	1.11 ± 0.08 (6.16)
Ms2	1.17 ± 0.07 (4.59)

nation of creatinine after its extraction from model solutions by the polymers synthesized are presented in Table 6.

The results obtained are somewhat overestimated, probably, because of the non-specific adsorption of interfering components of model solutions Ms1 and Ms2. Nevertheless, polymer MIP-Cr is selective to creatinine molecules and can be used for its extraction from model solutions.

A column filled with the MIP of creatinine is also applicable to the extraction of creatinine from model solutions.

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