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AN IMPROVED VOLTAMMETRIC METHOD FOR THE DETERMINATION OF TRACE AMOUNTS OF URIC ACID WITH ELECTROCHEMICALLY PRETREATED CARBON PASTE ELECTRODES

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Summary—Carbon paste electrodes, preanodized in alkaline medium at 1 4 V *vs* SCE for a short period of time, exhibit a great shift of the oxidation potential of uric acid in cathodic direction and a marked enhancement of its current response, compared to unpretreated electrodes These effects are dependent on the preanodization potential and the time imposed on the electrodes as well as on the alkalinity of the supporting electrolyte. The enhanced voltammetric response can be used to determine uric acid in the concentration range of $5.0–4.0 \times 10^4$ μg/l ($3\,0 \times 10^{-8}$–$2\,4 \times 10^{-4} M$) with a detection limit (3σ) of 2 0 μg/l ($1\,2 \times 10^{-8} M$) Ascorbic acid in less than 30-fold excess does not interfere For multiple determinations (5 runs), the relative standard deviation is 2 1% at a concentration of 1 mg/l uric acid. The proposed procedure can be used to determine uric acid in human urine and serum without any preliminary treatment of the samples in an accurate, rapid and simple way

Uric acid as a primary end-product of purine metabolism is a constituent of many body fluids. It has been shown that extreme abnormalities of uric acid levels in these fluids are symptoms of several diseases.[1] Therefore, it is necessary to apply simple and rapid methods for its determination in routine analysis. As uric acid can be easily oxidized at carbon-based electrodes, its electrochemical detection seems to be one method of choice.[2–4] Earlier procedures were based on the oxidation of uric acid at glassy carbon electrodes (GCEs)[5] and carbon paste electrodes (CPEs)[6] in acidic solutions, which, however, suffer from more or less interference from ascorbic acid which can be oxidized at a potential close to that of uric acid. To solve this problem, various techniques were developed. Wang's group described an adsorption/medium exchange approach to the analysis of uric acid. The interference from ascorbic acid could be avoided, but the detection limit is only about $10^{-5} M$.[7] Doubtlessly, various enzyme-based techniques are promising due to their high selectivity. But these methods, generally, are inherently more expensive and the detection limits ($\sim 10^{-6} M$) reported in the literature still need to be improved.[4,8,9] More recently, polymer membranes, which can preconcentrate analytes with some selectivity, were used as coatings for carbon-based electrodes. Purdy *et al.* reported a glassy carbon electrode coated with quaternary ammonium functionalized polymers which yielded a linear voltammetric response to uric acid in the concentration range of 1–10 μM.[10] A highly sensitive dialysis membrane-covered carbon paste electrode was described by Kinoshita and Usui, which has a detection limit as low as $10^{-9} M$.[11]

In this paper, the influence of electrochemical pretreatment of carbon paste electrodes on the oxidation of uric acid has been investigated with respect to experimental conditions for the determination of uric acid and possible interferents, including ascorbic acid. Electrochemical pretreatment, a special modification method for carbon-based electrodes, has been studied by several authors.[12–16] It has been found that anodic pretreatment can improve the performance of carbon-based electrodes over freshly polished electrodes in some respects: increased

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electrochemical activity, lowered overpotential and increased wettability. Although the chemical and electrochemical reactions involved are not completely clear until now, the main effect may be due to the generation of hydrophilic electron-transfer mediating groups by oxidation of the electrode material. The improved electrochemical response, especially obtained for some irreversible systems, are very useful for the detection of the biologically important compounds such as reduced nicotinamide adenine dinucleotide (NADH), ascorbic acid, hydrazine, 6-mercapurine, *etc.*[17-21] However, there is no detailed report dealing with the effects of electrochemical pretreatment on the determination of uric acid up to now.

EXPERIMENTAL

Apparatus

For voltammetric measurements, a polarograph (Model PAR 264A, Princeton Applied Research) was used in combination with a self-constructed electrode assembly of Plexiglass.[22] The cell consisted of a titration vessel of glass (6.1415.220 from Metrohm) with a platinum wire as the counter electrode and a saturated calomel electrode (SCE, Ingold 30W-NS) as reference. The latter was in contact with the solution over a salt bridge with a vycor frit, filled with KCl solution (1*M*). Since dissolved oxygen did not interfere with the anodic voltammetry, no deaeration was performed.

Voltammetric curves were registered either on a two-channel recorder (Model RE 0089, Princeton Applied Research) or by using an appropriate interface for A/D-conversion of the data in combination with a personal computer.[23] The parameters for cyclic voltammetry (CV) were: equilibration time: 15 sec; scan rate: 50 mV/sec. The scan potential range was set according as stated in the text. Linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV) were performed with 15 sec equilibration at the beginning of each run with the initial potential applied; scan rates: 50 mV/sec (LSV) and 10 mV/sec (DPV); pulse height (DPV). 50 mV.

Working electrodes

The body of the carbon paste working electrode was a Teflon rod (11 mm o.d.) with a hole (7 mm diameter, 3 mm deep) bored at one end for the electrode filling. Contact was made with a platinum wire through the center of the rod. Carbon paste was prepared in conventional fashion by thoroughly hand-mixing spectral carbon powder (2.0 g RWB, Ringsdorffwerke) and paraffin oil (0.7 g, Uvasol, Merck) in a mortar with a pestle. The carbon paste was packed into the hole of the electrode body and smoothed off with a PTFE spatula.

Reagents

Deionized water was distilled twice in a quartz still and then purified by a cartridge deionization system (Nanopure, Barnstead). Unless stated otherwise all chemicals were of analytical grade (p a., Merck) and were used as received. Uric acid was purchased from Merck. All other purine derivatives were from Aldrich.

Procedure

The supporting electrolyte for the voltammetric measurements was a mixture of $NaClO_4$ (0.1*M*) and NaOH (0.01*M*). The same solution was also used for the electrochemical pretreatment of the CPEs. Before each measurement, preanodization was done by polarizing the electrode in the supporting electrolyte solution at 1.4 V *vs* SCE for 40 sec. Quantitative determinations were performed in the DPV mode. The potential range was set from −0.4 to 0.4 V in the anodic direction.

Analysis of samples. After the electrode was pretreated, 200 μl urine sample or 500 μl serum sample was added to the supporting electrolyte solution, and the DPV current response was recorded. The standard addition method was used to evaluate the content of uric acid in samples (addition of at least two standards).

RESULTS AND DISCUSSION

Effect of the electrochemical pretreatment on the voltammetric response

The electrochemical pretreatment was carried out as described by Engstrom.[13,14] The author used this method for the determination of hydroquinone, hydrazine, *etc.* In the work presented here, the effects such as peak potential shift and enhanced voltammetric response for some biologically important components, in particular for uric acid, are investigated in detail. Figure 1 shows the cyclic voltammograms obtained for uric acid with an unpretreated and a pretreated carbon paste electrode,

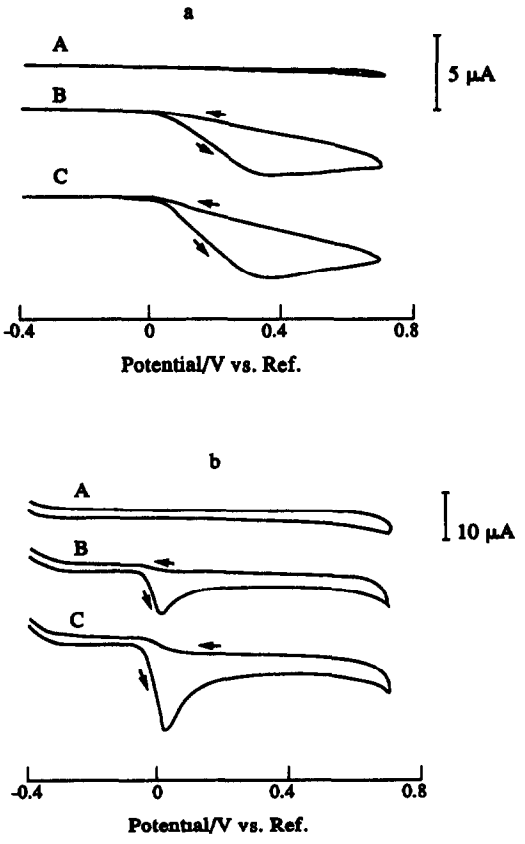

Fig 1 Cyclic voltammograms of uric acid with a non-treated (a) and a pretreated (b) CPE, supporting electrolyte for the preanodization and for the measurement 0 01*M* NaOH, 0 1*M* $NaClO_4$, scan rate 50 mV/sec, equilibration time 15 sec, preanodization potential 1 4 V, preanodization time 40 sec, (A) blank, (B) 10 mg/l, (C) 20 mg/l uric acid

respectively It is evident that the anodic polarization effects the following changes.

(1) a shift of the oxidation potential of uric acid by some 300 mV in cathodic direction to about 0 V *vs* SCE,
(2) enhanced current response,
(3) improvement of shape of the signal.

The current response at the unpretreated electrode is rather broad, while it is sharp and well defined at the pretreated one, indicating that the pretreatment accelerates the electron transfer reaction.[16] Similar effects were also reported for NADH and ascorbic acid, however, dramatically increased current response were not found for these compounds.[12]

Repeated scans in LSV mode after a single electrochemical activation at the beginning exhibit a decrease in peak current, whereas the peak potential remains unchanged, suggesting that a freshly pretreated surface exhibits higher activity than a used one. Therefore, for the repetitive use of the CPE, it is necessary to re-preanodize it before each scan, but not to renew the carbon paste for at least up to 20 measurements. It also should be noted that after more than 30 measurements with the same paste filling, the electrode material started to get swollen at the surface, due to the repetitive preanodizations, whereupon, the background current increased.

When using a glassy carbon electrode instead of a carbon paste electrode, the same effects of the electrochemical pretreatment could be observed. The potential shift is similar to that observed at a CPE, however, the enhancement of the peak current is not so pronounced. Although the rate of charge transfer at CPEs is generally slower than at GCEs due to layers of non-conducting pasting liquid at the electrode surface,[24,25] this disadvantage can be overcome by stripping them off by preanodization.[26,27]

Since it had been shown that uric acid could adsorb onto the CPE surface in acidic solution,[7] investigation was made to ascertain whether or not uric acid would also undergo adsorption at the pretreated CPE surface Figure 2 shows the dependence of the LSV current response on the scan rate and its square root value, obtained at a pretreated electrode The current is linearly proportional to the square root of scan rate, which illustrates that the peak current is diffusion-controlled. A similar behaviour of uric acid was also reported in acidic medium (pH 4.0) with a screen-printed electrode.[4] Increase of the preconcentration time under open or closed circuit conditions did not show any effect on the

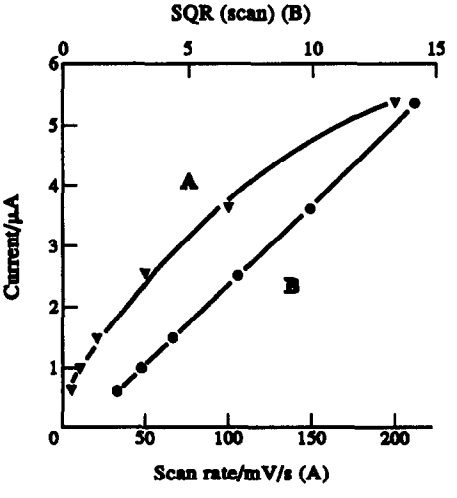

Fig 2 Dependences of the LSV current on the scan rate (A) and on the square root of the scan rate (B), obtained at the preanodized electrode, concentration of uric acid 1 mg/l, initial potential −0 4 V, other conditions as in Fig 1

current response. Further evidence for the non-adsorptive behavior of uric acid under these conditions was obtained by a medium exchange between preconcentration and measurement, which gave no voltammetric signal at all. The same experiments were also performed with an unpretreated CPE, and the results gave no evidence for adsorption, too. As uric acid is a binary acid (its dissociation constants, pK_a, are 4.17 and 11.57),[28] it exists in strongly alkaline solutions mainly as a hydrophilic, doubly negative anion which does not tend to adsorb onto the lipophilic carbon paste

Effect of chemical pretreatment

It has been shown that various oxygen-containing functional groups, such as carbonyl, quinoid, carboxylate and hydroxyl radical species are generated on the surface of carbon-based electrodes if they are polarized with reasonable positive potentials. These functional groups can accelerate or electrocatalyse irreversible oxidation of some organic compounds.[29,30] These catalysts are present in very small quantities even on the surface of freshly polished electrodes, and their amount can be increased by various physical and chemical methods. Among them, anodic polarization and chemical oxidation (including exposure to the air) are the most simple.[12,16,31]

In order to prove that oxidation of the electrode material by anodic polarization is the main reason for the improvement of signal response of uric acid, chemical oxidation was also performed with a solution of dichromate (1%) in perchloric acid (1*M*), and with a solution of hydrogen peroxide (15%) in perchloric acid (1*M*), respectively After the CPEs were treated with these oxidizing reagents at ambient temperature for 10 hr, they exhibited similar effects like the electrochemically pretreated ones· potential shifts of about 100 mV (with dichromate) and 150 mV (with hydrogen peroxide) in cathodic direction, combined with a small increase of the peak current compared to the unpretreated electrodes These results are quite in accordance with the behaviour of ferrocyanide at chemically oxidized electrodes as described by Adams.[16] Pretreatment by air was done by exposing the freshly prepared electrode to air for 24 hr; the resulting electrode was found to show very similar effects. Regardless of the results obtained with the chemical oxidation, the electrochemical pretreatment is much more convenient, faster and effective.

Effect of the pretreatment potential

In order to further characterize the effect of preanodization on the analytical performance of the electrode, the pretreatment potential was varied from 1.0 to 1.6 V, applied to a freshly prepared surface for 40 sec. The results are shown in Fig. 3. Compared to curve A, which was obtained with an unpretreated CPE, all the oxidation peaks were shifted to more negative potentials if the pretreatment potential became more positive. The peak current increased upon positively increasing the pretreatment potential. However, when the potentials were more positive than 1.5 V, the peak potential was not shifted anymore, and the peak current did not increase either. Instead, higher background current was observed. For practical use, a pretreatment potential of 1.4 V was chosen. If DPV was used for analytical purpose, the detection limit with the unpretreated electrode was only about 0.2 mg/l, which is higher than that with the pretreated one by nearly 100 times.

Apart from the anodic pretreatment, other methods were also checked for their effect on the oxidation of uric acid. Application of potentials of -1.0 to -1.4 V had no effect, whereas, pretreatments involving positive potentials of about 1.4 V (*e.g.* repetitive cyclic scanning, application of alternating positive and negative potentials) actually gave responses similar to the anodically treated CPEs. Therefore, only preanodization has substantial effect and was used in this work for simplicity.

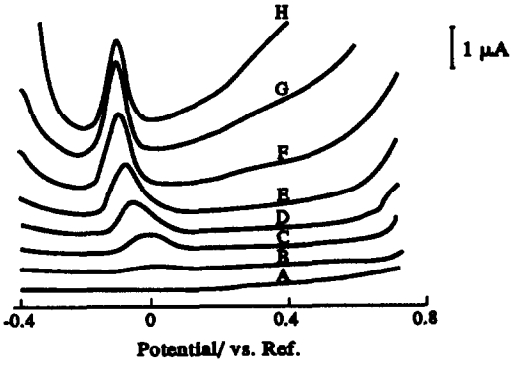

Fig. 3 Effect of the preanodization potential of the CPE on the differential pulse voltammetric response of 1 mg/l uric acid, equilibration time 15 sec, scan rate· 10 mV/sec, initial potential -0.4 V, supporting electrolyte for the preanodization and for the measurement 0 01*M* NaOH, 0 1*M* $NaClO_4$; preanodization time· 40 sec, preanodization potential. (A) without preanodization, (B) 1 0, (C) 1 1, (D) 1 2, (E) 1 3, (F) 1 4, (G) 1 5, (H) 1 6 V

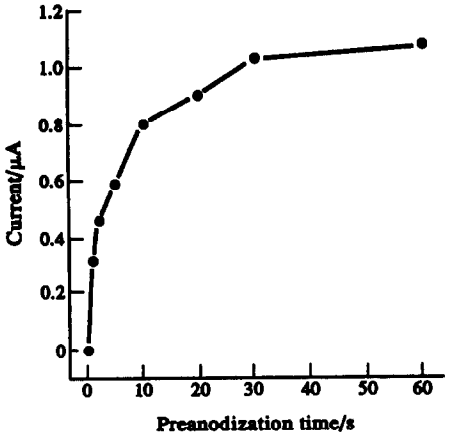

Fig 4 Effect of the preanodization time on the differential pulse voltammetric response of 1 mg/l uric acid, preanodization potential 1 4 V, other conditions as in Fig 3

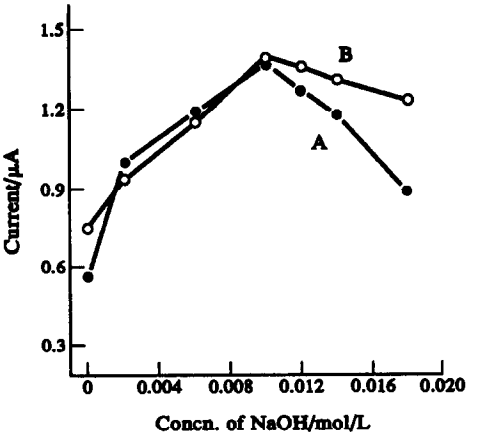

Fig 5 Effect of the concentration of sodium hydroxide in the supporting electrolyte for preanodization (A) and voltammetric measurement (B) on the peak current Concentration of uric acid 1 mg/l, pretreatment time 40 sec, pretreatment potential 1 4 V, other conditions as in Fig 3

Time dependence of anodization

It can be expected that an increase of the preanodization period should enhance the voltammetric response. Figure 4 shows the dependence of the peak current in DPV mode on the preanodization time. The current response increases enormously only within a few seconds. At the same time, the peak potential is also shifted to more negative values upon increasing the time as expected. After about 30 sec, the peak current reaches its maximum and the peak potential a most cathodic value, indicating that the electrode surface has become "saturated" with catalytically active species. Compared to other systems, this pretreatment time is very short,[12-16] which is doubtlessly advantageous for the practical use of this electrode. A preanodization time of 40 sec was used in this work

Effect of the medium

Various media were used for the preanodization of the CPE with respect to their effect on the oxidation of uric acid. It was found that only neutral and alkaline media were effective and that the concentration of hydroxide ions had a strong influence on the peak current, but, only little effect on the peak potential. As can be seen from curve A in Fig. 5, a dilute solution of sodium hydroxide (0.01*M*) is preferable for the pretreatment The same concentration in the supporting electrolyte of the voltammetric measurement gave also a maximum response (curve B). In order to attain a high ionic strength, 0 1*M* sodium perchlorate, which had no effect on the peak current, was used together with sodium hydroxide. It seemed that the additional component in the supporting

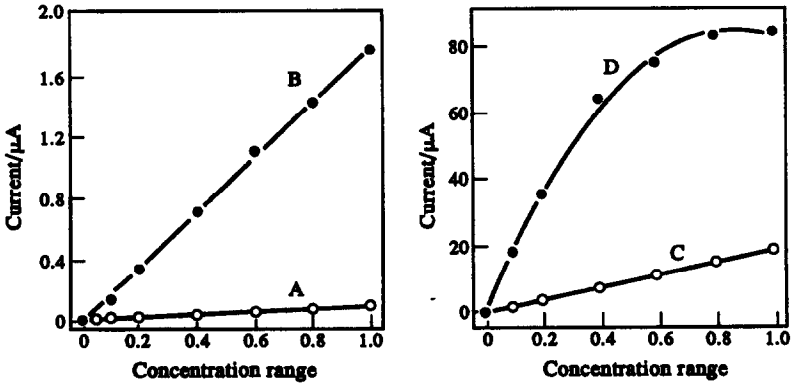

Fig 6 Dependence of the DPV peak current on the concentration of uric acid, Concentration range (A) 0–10, (B) 0–100, (C) 0–1000, (D) 0–10,000 μg/l, preanodization potential 1 4 V, other conditions as in Fig 3

electrolyte was not critical with respect to the peak current since different salts such as potassium nitrate or sodium chloride gave identical results. It was also found that there was no significant difference if the pretreatment was carried out with or without uric acid present during the pretreatment. A similar phenomenon was also reported by Engstrom.[13] Therefore, for repetitive measurements, the electrode can be re-preanodized without medium exchange.

Under optimum conditions, the DPV current response is linearly dependent on the concentration of uric acid between 5.0 and 4.0×10^4 μg/l (Fig. 6); the detection limit (3σ) is 2.0 μg/l. For multiple determinations (5 runs), *i.e* repeating preanodization and scan, the relative standard deviation is 2.1% for a concentration of 1 mg/l

Effect of interferents

Various interferents were examined for their effect on the determination of uric acid (Table 1) Most of these species do not interfere with the determination up to a 20–100 fold excess. These species are either electrochemically inactive, or, as in the case of ascorbic acid, their oxidation potentials are resolved sufficiently from the oxidation potential of uric acid.

Table 1 Change of the peak current (DPV) of 1 mg/l uric acid in the presence of interfering compounds, preanodization potential 1 4 V, preanodization time 40 sec, equilibration time 15 sec, scan rate 10 mV/sec, initial potential −0 4 V, final potential 0 4 V, supporting electrolyte for the preanodisation and the measurement 0 01*M* NaOH, 0 1*M* $NaClO_4$

Potential interferent	Concentration (*mg/l*)	Change of signal (%)
Ascorbic acid	20	0 0
	30	−3 4
Xanthine	10	0 0
	20	−2 4
Hypoxanthine	20	0 0
Purine	20	0 0
Thymine	20	−1 0
Thymidine	20	0 0
Uracil	20	0 0
Guanosin hydrate	10	0 0
	20	−3 4
Guanine	20	0 0
Adenine	10	−1 0
	20	−4 0
Cytosine	20	0 0
Glucose	100	−4 3
Hydrazine	10	−4 3
	20	−12 0
Oxalic acid	100	0 0
Hydroxylamine hydrochloride	20	−3 0
	50	−11 8
Thiourea	50	0 0
	100	−5 5
Cysteine	10	−5 5
	20	−8 0
Sulfite	50	0 0
	100	−3 5

It is well known that ascorbic acid co-exists with uric acid in lots of samples; therefore, its behaviour was also investigated in more detail under the same conditions as used for uric acid. The pretreated electrode also shifts the oxidation potential of ascorbic acid to about −0.3 V *vs* SCE and enhances the current response. Figure 7 shows differential pulse voltammograms of 1 mg/l uric acid in the presence of different concentrations of ascorbic acid Evidently, the current responses of uric acid and ascorbic acid are separated with a potential difference of 150 mV; the pretreated electrode actually responds much better to uric acid. Since the acceptable tolerance of concentration of ascorbic acid for the determination of uric acid is as high as a 30-fold excess, the method is applicable to various biological samples [32] Therefore, determination of uric acid in real samples without separation of interferents is possible. It can be seen in Fig 7 that simultaneous determination of uric acid and ascorbic acid is possible. Although the oxidation of ascorbic acid at preanodized carbon-based electrodes was studied thoroughly by several authors,[14,15,21] its determination in the presence of uric acid by using a preanodized CPE has not been reported until now; this will be a subject for further studies in this laboratory.

Samples

Two human urine samples from volunteers and two human sera were determined with the method presented above. The results are listed

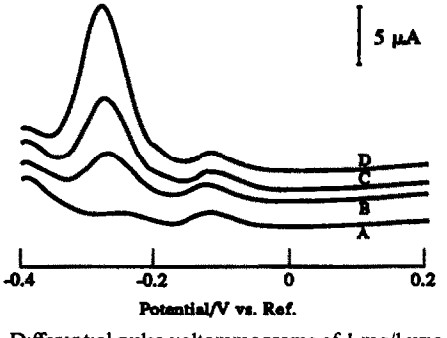

Fig 7 Differential pulse voltammograms of 1 mg/l uric acid in the presence of ascorbic acid, concentration of ascorbic acid (A) 5, (B) 10, (C) 20, (D) 30 mg/l, preanodization potential 1 4 V, other conditions as in Fig 3

Table 2 Determination of uric acid in real samples with the pretreated electrode, conditions as in Table 1

Sample	Original UA* found, (mg/l)	UA added (mg/l)	Total UA found, (mg/l)
Urine #1	450 5 ± 5.6	200 0	640 0 ± 12 0
Urine #2	412 6 ± 8 2	200 0	599 0 ± 18 0
Serum #1	12 0 ± 0 43	10 0	22.6 ± 0 79
Serum #2	10 9 ± 0 62	10 0	21 7 ± 0 91

*UA—uric acid

in Table 2. In order to ascertain the correctness of the results, the samples were spiked with certain amounts of uric acid in about the same concentration as found in the sample themselves; the recovery rates of the spiked samples were determined and ranged between 98.5 and 102.4% (mean values of two analyses with additions of two standards each). In conclusion, this method is very simple, accurate and rapid, and appears to be amenable to clinical analysis.

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