A new and simple DP polarographic method for the determination of uric acid in serum after elimination of the interference of ascorbic acid and dopamine

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Abstract: Determination of trace amounts of uric acid (UA) in serum is of great importance since its level in serum may show disorders in purine metabolism. With this method, trace amounts of UA in blood serum can be determined by a fast and simple procedure using differential pulse polarography (DPP). The limit of detection was estimated to be 2×10^{-7} M (S/N = 3), lower than that given in previous studies. Among the many electrolytes studied, the best electrolyte condition was found to be 0.05 M phosphate buffer, pH 10–12, in 0.1 M KNO_3. The peak for UA appeared at about –0.15 V in this medium, which responded well to standard additions and high reproducibility was obtained. The validity of the proposed method was tested in serum samples by spiking known concentrations of UA to serum samples and high recovery was obtained. The interference effect of dopamine (DA) and ascorbic acid (AA) was eliminated simply by the addition of Fe(III), in a very fast and quantitative reaction.

The proposed method was successfully applied to the determination of UA in serum using only very small quantities (0.5 mL) of serum. There is no need for long and tedious extraction or separation procedures, or to prepare modified electrodes in order to eliminate the interference by AA and DA.

Key words: Uric acid, determination, serum, differential pulse polarography, elimination of ascorbic acid and dopamine

1. Introduction

Uric acid (UA) is known as the principal end product of purine metabolism in the human body.[1] Abnormal levels of UA are a symptom of several diseases, including hyperuricemia, gout, and Lesch–Nyhan disease.[2] Therefore, its determination in blood serum and urine will serve as a marker for the detection of disorders associated with purine metabolism.[3,4] Gout occurs when sodium urate crystals are deposited in the joints, bursae, and tendons. Elevated concentrations of UA may also indicate some other medical conditions, such as leukemia, kidney injury, and pneumonia.[4] A high UA level can be lowered by using chemotherapeutic drugs and for this purpose its level has to be controlled continuously. According to a recent study, low serum/cerebrospinal fluid levels of UA are a biomarker of Parkinson's disease and high urate levels could slow this disease.[5] Thus, the determination of trace concentration of UA in human blood and urine is very important for the diagnosis and treatment of these kinds of diseases.

UA has been determined in the clinical laboratory by colorimetric, enzymatic, and electrochemical methods.[6−9] In the most popular colorimetric method, UA is oxidized by phosphotungstic acid and the absorbance of tungstic acid formed is measured. Interference by some reducing agents such as ascorbic acid (AA) restricts the analytical applications of this method.[10]

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Enzymatic methods[11−14] are more selective than colorimetric methods, but, because of readily oxidizable interferents, difficulties will be faced to eliminate the interferences, which make this procedure complex.

In electrochemical methods, AA and dopamine (DA) present in urine and blood serum are interfering constituents since they are oxidized close to the potential of UA. For the elimination of this interference, various modified electrodes have been developed. Fast scan voltammetry was applied with a bare carbon fiber electrode,[4] and the results obtained showed good selectivity in the determination of low concentrations of UA in the presence of AA. A mono-layer of aminophenol was grafted on a glassy carbon electrode, which demonstrated electrocatalytic activity towards the oxidation of ascorbate, so that AA and UA could be separated. Thus, simultaneous detection of UA and AA was possible and the limit of detection for UA was 1.4×10^{-5} M.[15] For the simultaneous determination of DA, AA, and UA, a carbon ionic liquid electrode was developed and it was used with cyclic voltammetry and differential pulse voltammetry (DPV). With this electrode, UA was determined with a detection limit of 4×10^{-4} M, but DA and AA could not be detected in human serum; their concentrations were below this limit. However, after increasing their concentrations by adding both DA and AA to the serum, it was shown that they could only be determined[16] at higher concentrations.

Simultaneous detection of UA and AA in urine and human serum could be achieved using a copper modified electrode. A linear calibration curve was obtained in the concentration range of $1.0–4.0 \times 10^{-5}$ M for UA.[17] Prior to every experiment, the glassy carbon electrodes had to be polished, rinsed, sonicated, and then activated to remove the charging currents.

For the determination of UA in the presence of AA, gold nanoclusters were electrodeposited on a glassy carbon electrode.[18] It was used for the determination of UA in urine in the presence of a large amount of AA. Using a Pt Au hybrid film modified electrode with DPV, DA, AA, and UA could be determined.[19] At pH 4 the calibration curve was obtained in the 2×10^{-5} to 3.3×10^{-4} M concentration range. AA, UA, and DA were detected using a pyrollytic graphite electrode modified in DA solution.[20] The detection limit obtained for UA was 1.4×10^{-6} M.

In most electrochemical studies where modified electrodes were used, AA and DA were given as the most interfering substances. For their elimination, various modified electrodes have been developed.[4,15−21] Although these modified electrodes show selectivity, most of them are based on adsorption phenomena and thus they need to be renewed after nearly each measurement. Their detection limit is also mostly not as low as expected from these methods.

Polarographic methods, using dropping mercury electrodes (DMEs), offer useful alternatives since they allow faster, cheaper, and safer analysis. Validation can be achieved by working with different electrolytes and at different pH values.[22−24] There is no need for long and tedious electrode preparation procedures after each use. The results obtained with differential pulse polarography (DPP) are very reproducible since with the use of a DME the behavior of the electrode is independent of its history. So far no work has been found in the literature that used DPP for the determination of UA.

The aim of this work was to establish a new and simple DPP method for the trace determination of UA in serum. In this method there is no need to prepare modified electrodes that need long and tedious electrode preparation procedures after each use.

2. Experimental

2.1. Apparatus

A polarographic analyzer (PAR 174 A) equipped with a PAR mercury drop timer was used. The drop time of the electrode was in the range 2–3 s (2.35 mg s^{-1}). A Kalousek electrolytic cell with a saturated calomel electrode (SCE), separated by a liquid junction, was used in the 3-electrode configuration. The counter electrode was platinum wire. The polarograms were recorded with a Linseis (LY 1600) X-Y recorder under the conditions of a drop life of 1 s, a scan rate of 2 mV s^{-1}, and a pulse amplitude of 50 mV. High purity nitrogen (99.999%) was used to expel oxygen present before each measurement. Cr(II) solution was used in gas washing bottles to reduce any trace oxygen in nitrogen gas.

2.2. Reagents

All chemicals used were of analytical-reagent grade (proanalysis). Triply distilled water was used in the preparation of all solutions. Solutions of 10^{-3} M and more dilute ones were prepared before every use in order to avoid aging of the solution.

1.0 M HAc/Ac buffer was prepared by adding 6 g of NaOH, washed with distilled water in order to remove the carbonate formed, to 57 mL of 1 M HAc and diluting to 1 L.

0.05 M phosphate buffer: 0.23 g of $Na_3PO_4.12H_2O$, 2.13 g of $Na_2HPO_4.2H_2O$, and 2.5 g of KNO_3 are dissolved in distilled water. The pH is adjusted to 10.5 by the addition of 0.1 M NaOH and then diluting to 250 mL.

10^{-3} M UA solution: 0.0168 g reagent grade uric acid ($C_5H_4O_3N_4$) (Merck) is shaken in about 30 mL of 0.1 M NaOH for 20 min and then it is diluted to 100 mL. It is prepared daily.

0.1 M AA solution: 1.76 g of ascorbic acid ($C_6H_8O_6$) is dissolved in 100 mL of deaerated distilled water and stored in darkness in an air-tight container. It can be used for only a few days.

0.1 M Fe(III) solution: 0.68 g of $FeCl_3.6H_2O$ is dissolved in 3 M HCl and diluted to 25 mL.

6.5×10^{-2} M DA solution: 10 mg of dopamine hydrochloride is dissolved in 10 mL of water.

The mercury used in the dropping mercury electrode was obtained from Merck (Darmstadt, Germany). Contaminated mercury was cleaned by passing it successively through dilute HNO_3 (3.0 M) and water columns in the form of fine droplets by using a platinum sieve.

This mercury was then washed in the same way until no acidic reaction was observed. The collected mercury was dried between sheets of filter paper and stored in a closed vessel covered with water. The mercury used was not lost since it was collected quantitatively, without causing any pollution. Thus, no mercury loss was possible and it can be used continuously. Before use, a DPP polarogram of this mercury was recorded in order to confirm the absence of impurities.

2.3. Procedure for the elimination of AA and DA in serum sample

To a beaker were added 0.5 mL of blood serum and 0.5 mL of 10^{-2} M Fe(III) and left for about 1 min; for the reaction of both AA and DA with Fe(III), pH has to be around 2–3. This sample was then added to a polarographic cell containing 0.05 M phosphate buffer (pH 10.5) and 0.1 M KNO_3.

3. Results and discussion

3.1. Polarographic determination of UA

Polarograms of UA were taken in acetate, phosphate, borate, and ammonium electrolytes at various pH levels. The best electrolyte condition was found to be 0.05 M phosphate buffer (pH range 10–12) in the presence of 0.1 M KNO_3. At higher pH values a mercury peak appeared at about –0.1 V, which caused an overlap with the UA peak in this medium. The UA peak in phosphate buffer appeared at about –0.15 V; it responded well to standard additions and high reproducibility was obtained (Figure 1). Scan rate was 2 mV/s (instead 5 mV/s) in order to obtain better separation of UA and Hg peaks. The limit of quantification was 1×10^{-6} M and the limit of detection (LOD) was estimated to be 2×10^{-7} M (S/N = 3), which is lower than the LOD values given in the literature.
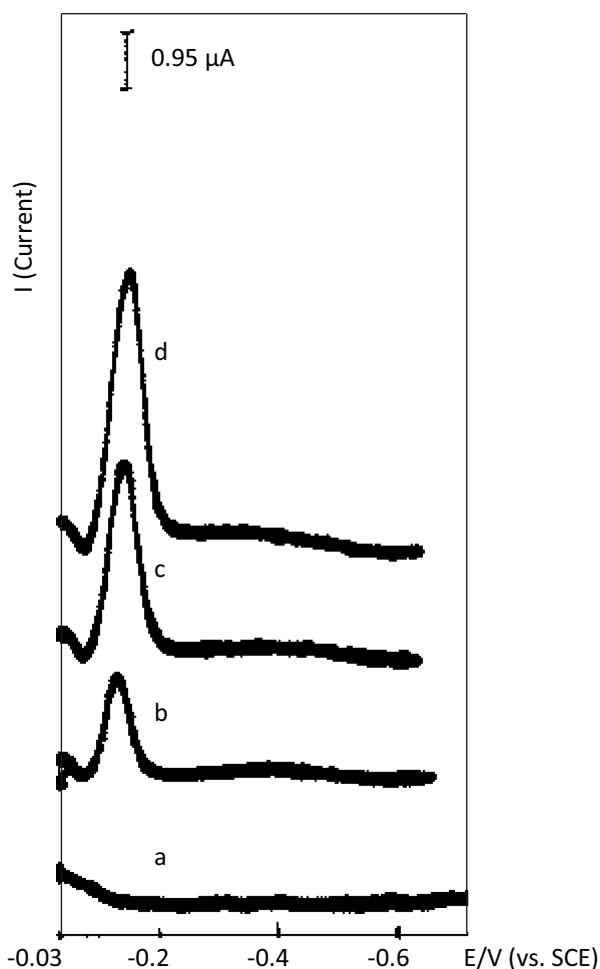

Figure 1. Determination of uric acid (UA) by standard additions using DPP a) 10 mL 0.05 M HPO_4^{2-}/PO_4^{3-} + 0.1 M KNO_3, pH 10.5 b) a + 0.1 mL 10^{-3} M UA c) b + 0.1 mL 10^{-3} M UA d) c + 0.1 mL 10^{-3} M UA.

3.2. Anodic peak of UA

As known, the nature (cathodic or anodic) of a polarographic peak cannot be evaluated by DPP. For this purpose, direct current (DC) polarography has to be used. The DC polarographic wave in phosphate buffer

at pH 11 for 10^{-4} M UA is observed. As can be seen in Figure 2, UA has an anodic wave on DME with a half-wave potential at about –0.2 V and it responds well to standard additions.
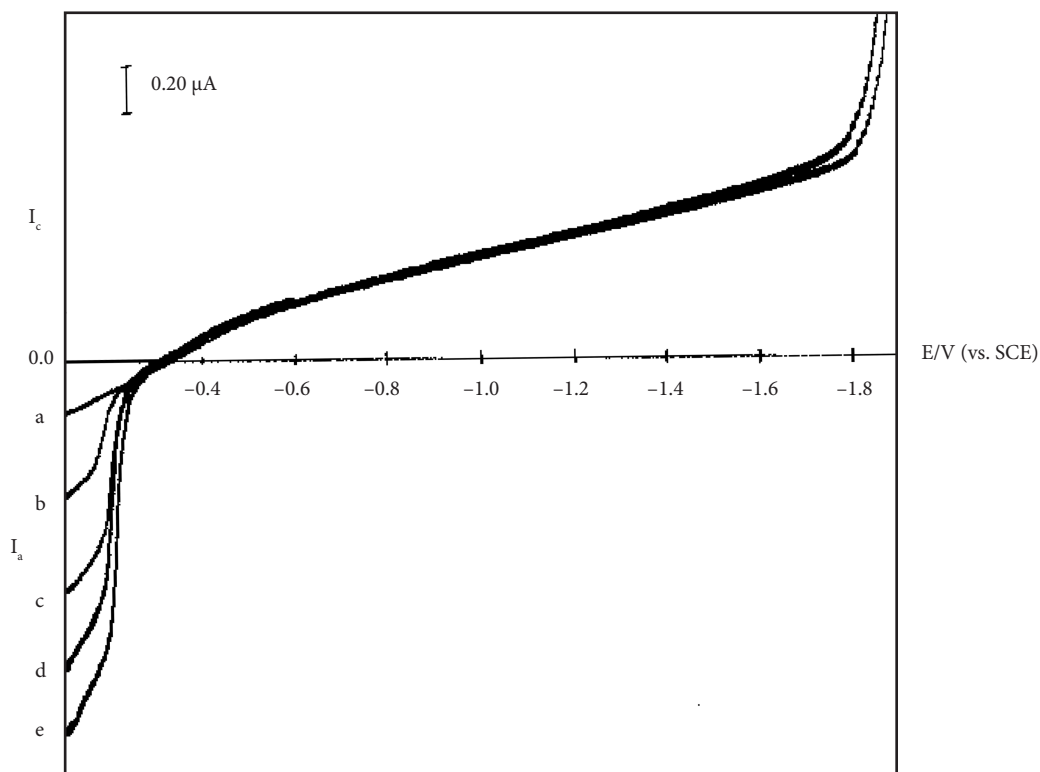

Figure 2. Direct current (DC) polarogram of UA a) 10 mL 0.05 M Phosphate buffer + 0.1 M KNO_3, pH 11.0 b) a + 0.1 mL 10^{-2} M UA c) b + 0.1 mL 10^{-2} M UA d) c + 0.1 mL 10^{-2} M UA e) d + 0.1 mL 10^{-2} M UA.

3.3. Interference studies

Interference of AA, DA, Cu(II), Fe(III), and Fe(II) has been studied. In most electrochemical studies for the determination of UA, AA and DA are given as the most interfering substances. For their elimination, various modified electrodes have been developed.[4,15−22]

3.3.1. Interference from AA and its elimination

It is well known that AA is found at about the same concentration as UA in blood serum and 100 to 1000 times higher than the concentration of DA. When AA was added to the polarographic cell containing phosphate buffer (pH 10–12) it had an anodic peak at about –0.20 V. Since the UA peak is at about –0.15 V there will be a peak overlap in the presence of high concentrations of AA. For the elimination of AA, Fe(III) is selected, since AA is a reducing agent and Fe(III) with a +0.78 V standard electrode potential will act as an oxidizing agent. We have shown experimentally that this reaction takes place very fast and quantitatively. For this purpose, first a synthetic sample was prepared containing AA and various quantities of Fe(III). It was found that when Fe(III) concentration was more than 2 times the AA concentration, AA was completely oxidized and no peak for AA was observed. It was also found that this reaction took place in a slightly acidic solution (pH 2–3). The acidity of Fe(III) added for AA oxidation was found to be sufficient. As known, because of the hydrolysis reaction, Fe(III) solutions have to be prepared with HCl (Section 2.2). The pH of an artificially

prepared sample containing 1 mL of 10^{-3} M UA, 1 mL of 10^{-3} M AA, and 2 mL of 10^{-3} M Fe(III) was about 3.0. In this medium Fe(III) had no peak and thus addition of excess iron(III) did not interfere. As can be seen from Figure 3, the 1×10^{-5} M UA content in the synthetic sample was determined in the presence of 1×10^{-5} M AA, as $(1.0 \pm 0.1) \times 10^{-5}$ M using standard addition.
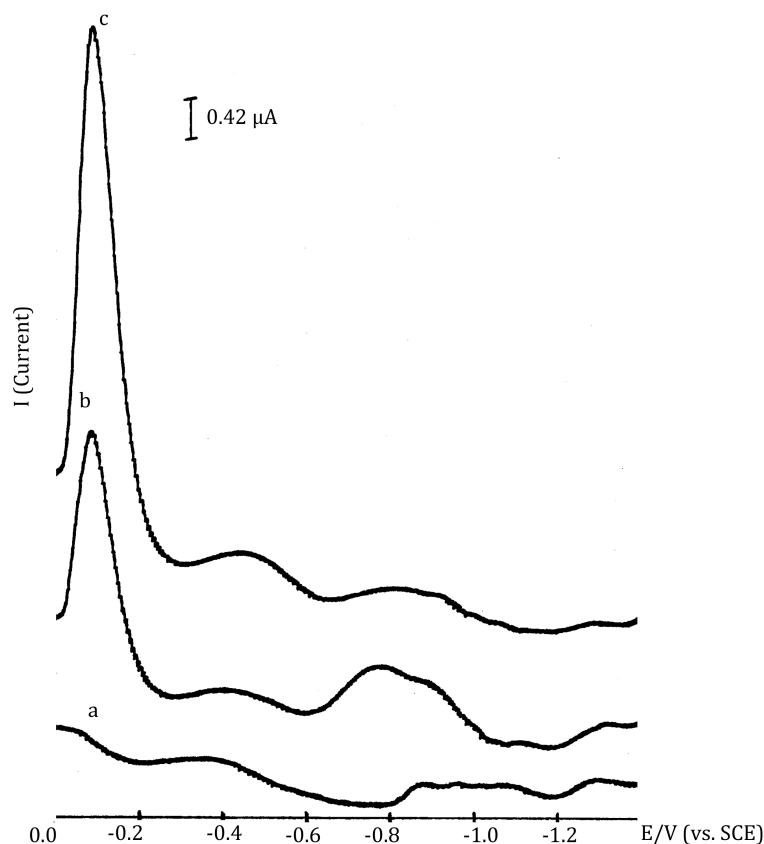

Figure 3. Determination of UA in a synthetic sample using DPP a) 10 mL 0.05 M Phosphate buffer + 0.1 M KNO_3, pH 10.9 b) a + 0.4 mL sample c) b + 0.1 mL 10^{-3} M UA

Sample: 1 mL 10^{-3} M UA + 1 mL 10^{-3} M AA + 2 mL 10^{-3} M Fe^{3+}.

3.3.2. Effect of copper

The interference of copper has also been studied since copper(II) has a peak at about 0 V and thus an overlap with the UA peak may take place. A polarogram of 2×10^{-5} M copper(II) in this medium had a peak at –0.2 V; with the addition of 0.1 mL of 1×10^{-3} M UA, the copper peak decreased and was lost with the second UA addition. With a reduction potential of +0.34 V it is expected that copper(II) will oxidize UA. Experimentally it was found that 1 mol of copper oxidized 1 mol of UA. Since UA and copper peaks overlap, in order to see whether copper was still in the solution, EDTA was added. When 1 mol UA per 1 mol copper(II) was added, no Cu-EDTA peak (at about –0.5 V) was observed, which indicates that UA and copper reacted quantitatively.

However, the presence of copper ion in serum will not create a problem, since it will react with UA and will be lost, there will be no overlapping copper peak, and the UA present in serum after the reaction can correctly be determined.

3.3.3. Effect of Fe(III) and Fe(II)

Since Fe(III) was used for the elimination of AA, its excess will be present in solution during the UA determination. There will be a possibility to react with UA; however, when Fe(III) in high concentration was added to a solution containing UA, no decrease in the UA peak was observed. During the oxidation of AA with Fe(III), the reduction product will be Fe(II) and it may have an overlapping peak, but addition of Fe(II) had no peak in this medium.

3.3.4. Interference of DA

It is known that DA is also present in blood serum; its concentration is 1000 times lower than that of UA present. To see its effect, a polarogram of 6.5×10^{-5} M DA solution is taken under the same conditions (0.05 M phosphate buffer) used for UA determination. DA had 2 peaks (Figure 4, curve b) in this medium, at –0.11 V and at –0.22 V. These 2 peaks would overlap with the peak of UA; however, with the addition of the same concentration of (6.5×10^{-5} M) iron(III), DA was oxidized and lost. As can be seen from Figure 4 (curve c), there was no peak for DA after addition of Fe(III) and the UA peak responded well to standard additions (curves d, e, and f) in this medium. Here we have to mention that the DA concentration used for this experiment was much higher than that normally present in serum.
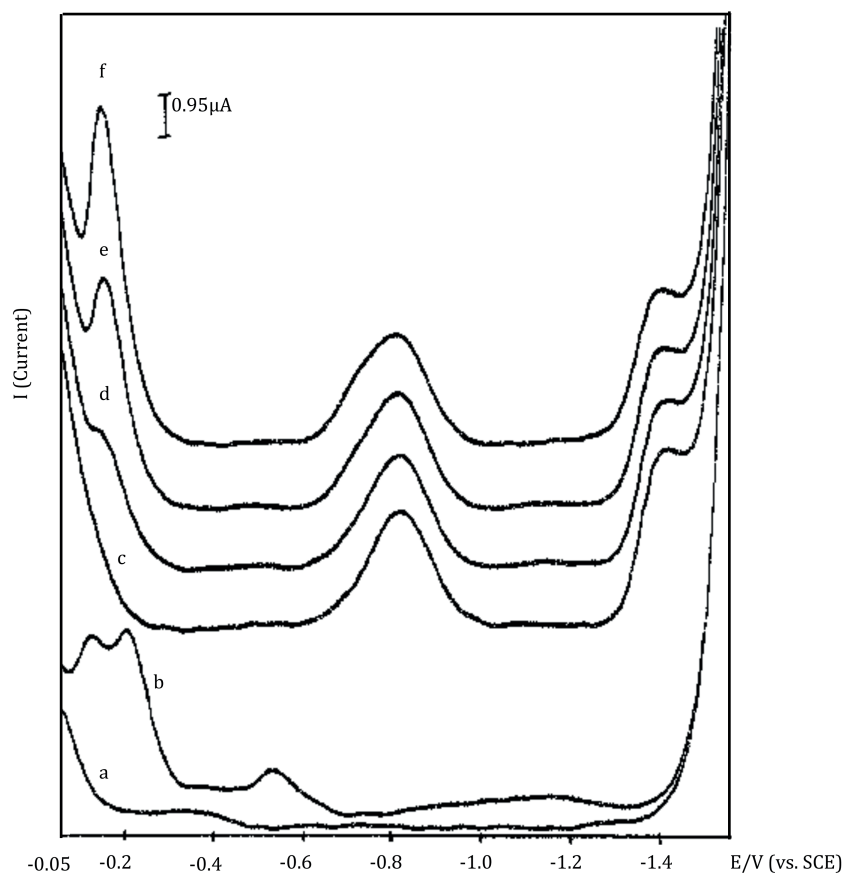

Figure 4. Elimination of dopamine interference using Fe^{3+} a) 10 mL (0.05 M HPO_4^{2-} /PO_4^{3-} + 0.1 M KNO_3), pH 10.8 b) a + 0.1 mL 6.5×10^{-3} M Dopamine c) b + 0.1 mL 6.5×10^{-3} M Fe^{3+} d) c + 0.1 mL 10^{-3} M UA e) d + 0.1 mL 10^{-3} M UA f) e + 0.1 mL 10^{-3} M UA

Since in normal cases DA concentration is 1000 times lower than that of UA in blood serum, its peak will not be observed. However, in the case of higher concentrations of DA in serum, Fe(III) added for the elimination of AA will oxidize DA also and thus no interference is expected.

3.4. Determination of UA in human serum in the presence of AA and DA

For this purpose, blood serum (centrifuged) was obtained from the Medical Center of Gazi University. To 0.5 mL of blood serum, 0.5 mL of 10^{-2} M Fe(III) was added and left for about 1 min for the reaction between AA and Fe(III). It was shown in our preliminary studies that this reaction was very fast. Then 0.5 mL was taken and added to a polarographic cell containing 9.5 mL of 0.05 M phosphate buffer (pH 10.5) and 0.1 M KNO_3. The quantity of UA was determined by standard additions as can be seen from Figure 5. The amount of UA
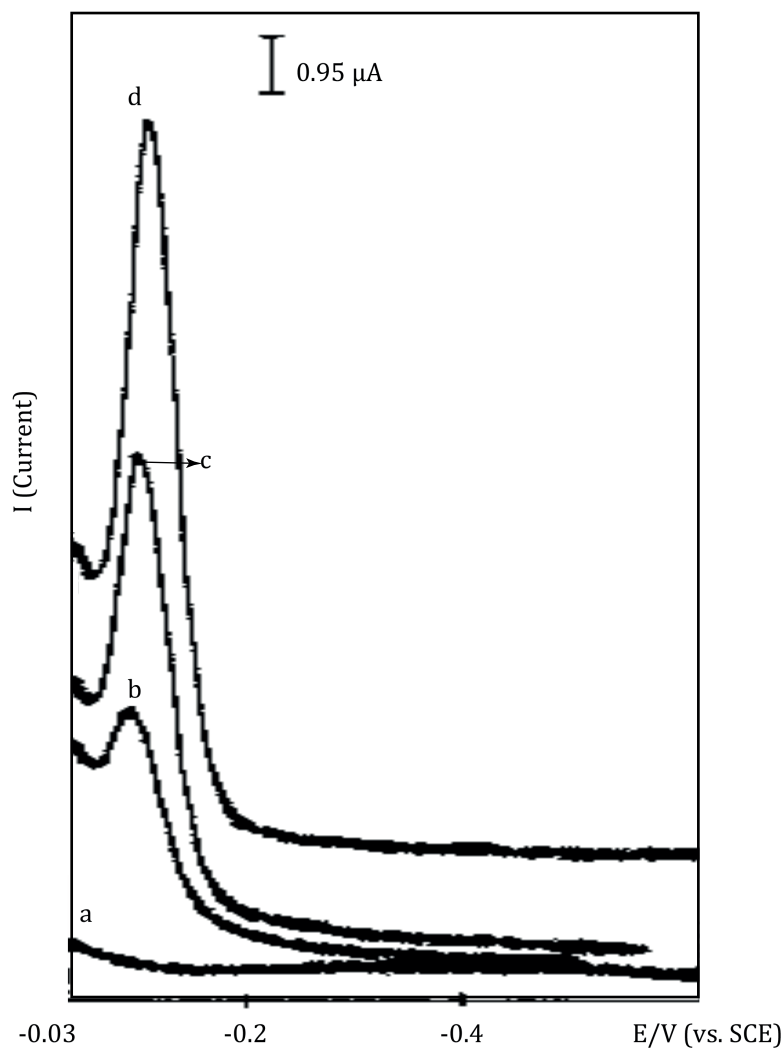

Figure 5. Determination of UA in human serum using DPP a) 9.5 mL (0.05 M HPO_4^{2-}/PO_4^{3-} + 0.1 M KNO_3), pH 10.5 b) a + 0.5 mL serum sample c) b + 0.1 mL 10^{-3} M UA d) c + 0.1 mL 10^{-3} M UA
Sample: 0.5 mL serum + 0.5 mL 10^{-2} M Fe^{3+}.

in this serum sample was 7.8 ± 0.6 mg/100 mL for N = 3, 90% confidence interval; it corresponds to about 4.6×10^{-4} M UA.

To authenticate the validity of the result, the sample was spiked with known concentrations of UA. Table 1 summarizes the results obtained for 4 different centrifuged serum samples taken from the Medical Center. The recoveries obtained varied from 99% to 115%.

Table 1. Determination of UA in various blood serum samples, before and after addition of known amount of UA (N = 3, 90%) 9.5 mL buffer (0.05 M HPO_4^{2-}/PO_4^{3-} + 0.1 M KNO_3) (pH 10.5).

Samples	UA determined using this method in blood serum (M)	Spike (to blood serum) (M)	UA determined (this method) after spike (present in blood serum) (M)
Blood Serum I	$(3.3 \pm 0.3) \times 10^{-4}$	4.0×10^{-4}	$(7.9 \pm 0.7) \times 10^{-4}$
Blood Serum II	$(3.1 \pm 0.4) \times 10^{-4}$	4.0×10^{-4}	$(8.1 \pm 0.5) \times 10^{-4}$
Blood Serum III	$(1.5 \pm 0.2) \times 10^{-3}$	0.5×10^{-3}	$(2.1 \pm 0.2) \times 10^{-3}$
Blood Serum IV	$(2.5 \pm 0.3) \times 10^{-4}$	5.0×10^{-4}	$(7.5 \pm 0.5) \times 10^{-4}$
Blood Serum V	$(2.8 \pm 0.3) \times 10^{-4}$	5.0×10^{-4}	$(8.1 \pm 0.5) \times 10^{-4}$

A calibration graph for UA in human serum was also prepared. For this purpose, first the UA content of a serum sample was determined after elimination of the interference of AA and DA by the addition of Fe(III). Then to another aliquot of the same serum sample the same procedure was applied after addition of known amounts of UA. After each addition the UA content was determined by standard addition. This procedure was repeated several times. As can be seen from this curve (Figure 6), standard additions showed linear dependence in serum medium. The results obtained are summarized in Table 2. It can be concluded that with this method it is possible to determine UA concentration in serum medium with high precision and accuracy.

Table 2. Response to UA additions in serum solutions 9.5 mL buffer (0.05 M HPO_4^{2-}/PO_4^{3-} + 0.1 M KNO_3) (pH 10.5).

[UA] originally present in serum* / M	[UA] added to serum / M	[UA] in serum present / M after addition (calculated)	Determined, using this method [UA] / M
4.0×10^{-4}	No addition	4.0×10^{-4}	$(4.0 \pm 0.3) \times 10^{-4}$
	5.0×10^{-4}	9.0×10^{-4}	$(9.6 \pm 0.3) \times 10^{-4}$
	10.0×10^{-4}	14.0×10^{-4}	$(13.5 \pm 0.5) \times 10^{-4}$
	15.0×10^{-4}	19.0×10^{-4}	$(20.1 \pm 0.3) \times 10^{-4}$

*UA originally present in serum: 4×10^{-4} M

Additions are made so that each time the concentration of UA in serum increased 5×10^{-4} M

3.5. Recommended procedure for the determination of UA in serum

To a small beaker are added 0.5 mL of blood serum (centrifuged) and 0.5 mL of 10^{-2} M Fe(III) and left for about 1 min; for the reaction of both AA and DA with Fe(III), pH has to be around 2–3. The acidity of Fe(III) added for AA oxidation is sufficient, since Fe(III) solutions have to be prepared with HCl. A 0.5 mL aliquot of this sample is then added to a polarographic cell containing 9.5 mL of 0.05 M phosphate buffer (pH 10.5) and 0.1 M KNO_3. The quantity of UA is then determined by standard additions.
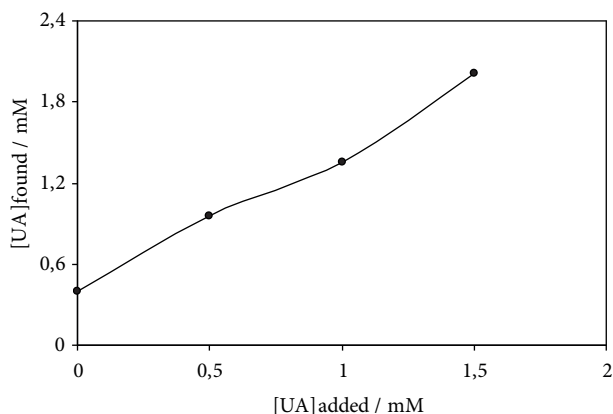
Figure 6. Calibration curve in serum medium 9.5 mL $(0.05$ M HPO_4^{2-}/PO_4^{3-} $+ 0.1$ M $KNO_3)$, pH 10.5 UA present in serum: 4×10^{-4} M. Additions are made so that each time the concentration of UA in serum increased 5×10^{-4} M. The figures on the abscissa and ordinate have to be multiplied with 10^{-4} to find the real concentration in M.

The determination of the trace concentration of UA in human blood and urine is very important for the diagnosis and treatment of many diseases. So far no work has been found in the literature that used DPP for the determination of UA. It is possible to determine low concentrations of UA with high precision and reproducibility using this method.

With the newly proposed DPP method, UA in serum could be determined in the presence of large quantities of AA and DA using very small quantities of serum. Interference of AA and DA was eliminated simply by the addition of Fe(III) to the sample solution. It was possible to determine UA in serum with high accuracy and very quickly using this method. The limit of detection was estimated to be 2×10^{-7} M (S/N $= 3$), which was lower than that given in previous studies. The reproducibility is very high since with the use of a DME the behavior of the electrode is independent of its history. The validity of the proposed method was tested in serum samples by spiking known concentrations of UA to serum samples and high recovery was obtained. This newly developed method can be applied to many biological materials directly. It is possible to determine small quantities of UA in serum by a fast and simple method. There is no need to prepare new modified electrodes in order to eliminate the interference of DA and AA. Although these modified electrodes show selectivity, besides their long and tedious preparation procedures, these electrodes need to be renewed after each measurement since most of them are based on adsorption phenomena. Their detection limit is also mostly not as low as expected from these methods.

References

1. Dryhurst, G.; Stocker, J. H. *Electrochemistry of Biological Molecules*; Academic Press: New York, NY, USA, 1977.

2. Zen, J. M.; Jou, J. J.; Ilangovan, G. *Analyst* **1998**, *123*, 1345–1350.

3. Harper, H. A. *Review of Physiological Chemistry*; 13th ed., Lange Medical Publications: Los Altos, CA, USA, 1977.

4. Bravo, R.; Hsueh, C. C.; Jaramillo, A.; Brajter-Toth, A. *Analyst* **1998**, *123*, 1625–1630.

5. Ascherio, A.; LeWitt, P.A.; Xu, K.; Eberly, S.; Watts, A.; Matson, W. R.; Marras, C.; Kieburtz, K.; Rudolph, A.; Bogdanov, M. B.; et al. *Arch. Neurol.* **2009**, *66*, 1460–1468.

6. Pilleggi, J. V.; Di Diorgio, J.; Wybenga, R. D. *Clin. Chim. Acta* **1972**, *37*, 141–146.

7. Benedict, R. J. *Biol. Chem.* **1930**, *86*, 179–187.

8. Jung, H. D.; Parekh, C. A. *Clin. Chem.* **1970**, *16*, 247–250.

9. Wyngaarden, B. J.; Kelly, N. W. *Gout and Hyperuricemia*; Grune and Stratton: New York, NY, USA, 1976.

10. Gilmartin, M. A. T.; Hart, J .P.; Birch, B. *Analyst* **1992**, *117*, 1299–1303.

11. Gilmartin, M. A. T.; Hart, J. P. *Analyst* **1994**, *119*, 833–840.

12. Gilmartin, M. A. T.; Hart, J. P.; Birch, B. *Analyst* **1994**, *119*, 243–251.

13. Miland, E.; Ordieres, M. J. A.; Blanco, T. P.; Smyth, N. R.; Fagain, C. O. *Talanta* **1996**, *43*, 785–796.

14. Nassef, H. M.; Radi, A. E.; O'Sullivan, C. *Anal. Chim. Acta* **2007**, *583*, 182–189.

15. Safavi, A.; Maleki, N.; Moradlou, O.; Tajabadi, F. *Anal. Biochem.* **2006**, *359*,224–229.

16. Selvaraju, T.; Ramaraj, R. *Electrochim. Acta* **2007**, *52*, 2998–3005.

17. Li, J.; Lin, X .Q. *Anal. Chim. Acta* **2007**, *596*, 222–230.

18. Thiagarajan, S.; Chen, S. M. *Talanta* **2007**, *74*, 212–222.

19. da Silva, R. P.; Lima, A.W. O.; Serrano, S .H. P. *Anal. Chim. Acta* **2008**, *612*, 89–98.

20. Ensafi, A. A.; Taei, M.; Khayamian, T.; Arabzadeh, A. *Sensors and Actuators* **2010**, *147*, 213–221.

21. Hadi Beitollahi, H.; Bakhsh Raoof, J.; Hosseinzadeh, R. *Electroanal.* 2011, *23*, 1934–1940.

22. Yılmaz, Ü. T.; Somer, G. *Anal. Chim. Acta* 2007, *603*, 30–35.

23. Somer, G.; Doğan, M. *Bioelectrochemistry* **2008**, *74*, 96–100.

24. Çalışkan, A. C.; Somer, G. *Turk. J. Chem.* **2007**, *31*, 411–422.