# The Influence of Ascorbic Acid, Creatinine and Urea on the Analysis of Uric Acid in the Blood Serum by Stripping Voltammetry using Graphite Electrode

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# The Influence of Ascorbic Acid, Creatinine and Urea on the Analysis of Uric Acid in the Blood Serum by Stripping Voltammetry using Graphite Electrode

Miratul Khasanah[a], Handoko Darmokusumo[a], Ganden Supriyanto[a], Ahmad Zaky Pulungan[a], Putut Satrio Dahono[a]

**Abstract**

The voltammetry method using bare electrode to analyse uric acid encountered a major problem concerning the interference from other organic compound which present together in the serum sample. This research studied the effect of ascorbic acid, creatinine and urea in the analysis of uric acid by stripping voltammetry using graphite electrodes. The deposition potential of uric acid on the surface of electrode was 0.3 V during 60 s at the pH 5. The analytical performance of the method were as follows: precision (RSD) of 0.17%-0.89% for concentrations of 0.1 µg/L – 0.5 µg/L, sensitivity of 1.331 µA.L/µg, detection limit of 0.036 µg/L, and accuracy of 97.0 % -105.6%. The creatinine was found not to interfere the uric acid analysis, but urea and ascorbic acid significantly interfere on the uric acid analysis by this method. Analysis of uric acid in the serum sample showed lower result as compared to that done by spectrophotometric method, with recovery of 88%.

Keywords: uric acid; creatinine;ascorbic acid; urea; graphite electrode

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## Introduction

Uric acid is the primary end product of purine metabolism that is commonly found in biological fluids of human, mainly in blood and urine. in the healthy human being, the typical concentration of uric acid in serum of male is 3,5-7 mg/dL and 2,6-6 mg/dL in female. Clinical studies have shown that the extreme abnormalities of uric acid levels in blood serum and urine are symptoms of several diseases like diabetes, hypertension, kidney disease and also a risk factor for cardiovascular. The method of uric acid determination which is ordinary done in biomedical field is by using spectrophotometry (Chen et al., 2005).

The weaknesses of this method are the large amount of sample needed, high detection limit (nM), and the complicated of the sample pretreatment, so that it need long time. Therefore, it is necessary to develop simple and rapid methods for the determination uric acid in routine analysis. The research of uric acid determination using reversed phase of HPLC have been reported (George et al., 2006). Result of the research showed that detection limit and recovery were 0.11 µg/mL and 94 – 104 %, respectively.

Various electrochemical methods were developed to solve the problem on uric acid analysis method. The determination of uric acid by voltammetry method have received much interest because of its higher selectivity, less expensive and less time consuming compared to colorimetric and enzymatic methods (Matos et al., 2000; Chen et al., 2005). However, a major problem encountered in this method to determine uric acid is the interference from other compounds in blood and urine which can be oxidized at the potentials close to the uric acid (Li et al., 2009).

This recent research studied influence of ascorbic acid, creatinine and urea on uric acid determination by voltammetry using graphite electrode. The parameters which be studied on this research were deposition potential, deposition time, and pH solution, and method validity. The influence of ascorbic acid, creatinine and urea was determined by adding each compound on the uric acid standard solution with the variation of molar ratio.

## Methodology

### Chemicals, Materials and Instrumentation

Chemical used were uric acid (Fluka), creatinine (Sigma-Aldrich), ascorbic acid, acetic acid, sodium acetate, sodium hydroxide trihydrate, sodium hydrogen phosphate hepta hydrate, sodium

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dihydrogenphosphate, sodium phosphate dodecahydrate, and ureum (Merck). All chemicals were analytical grade. The solvent used was ultra-high purewater. The stock solution of uric acid 1000 mg/L was prepared by dissolving 0.1000 g uric acid in about 10 mL sodium hydroxide 50% (b/b) and diluted with water until 100 mL in volumetric flask. Working uric acid solutions under 1 mg/L were prepared daily by diluting appropriate working solution, and their pH were adjusted with the addition of acetate and phosphate buffer. Sample used is a serum from a pathological clinic laboratory in Surabaya Indonesia.

The instrumentations used in this study were 797 Computrace Voltammetry (MVA system-1) which comprises a sample container, stirrer, processor units, PCs, working electrode of graphite, reference electrode of Ag/AgCl and Pt auxiliary electrodes. The other equipments were micropipet, pH meter, hot plate and glassware.

**Methods**

**Optimization of the research conditions**

The research conditions optimized were deposition potential, deposition time, and pH of solution. The measurement of test solution for each condition had been done three times. The optimization of research conditions used 25.0 mL uric acid 5 µg/L. The deposition potential was varied from -500 mV until 500 mV. Variation of deposition time started 30 s to 150 s using optimum potential. pH solution have been varied on pH 4, 5, 6 and 7.

**Calibration curve and method validation**

Each of the uric acid standard solution 0.1; 0.2; 0.3; 0.4; and 0.5 µg/L was analysed by stripping voltammetry using graphite electrode under the optimum conditions. The calibration curve which current versus concentration uric acid was made and the method validity including linearity of calibration curve, sensitivity, precision, recovery and detection limit was studied.

**Influence of ascorbic acid, creatinine and urea**

Influence of ascorbic acid, creatinine and urea was studied by adding each of the compound to uric acid solution 5 µg/L, so molar ratio of uric acid and ascorbic acid concentration was 1:0.5; 1:1; and 1:10. While the molar ratio of uric acid and creatinine was 1:0.25; 1:0.5; 1:1; 1:1.5 and 1:2.5. The molar ratio of uric acid and urea was 1:1; 1:5; 1:10; and 1:15. All of the solutions were analysed by stripping voltammetryusing graphite electrode. The current responses obtained from the measurement of uric acid without and with the addition of ascorbic acid, creatinine or urea were compared.

## Results and Discussion

**Optimization of deposition potential and time**

Deposition is the electrochemical pre-concentration of the analyte on the electrode surface. This step involved the deposition and adsorption of the analyte on the electrode surface, or electron transfer mechanism on modified electrode surface, depending on the interaction between the analyte and the electrode. in this research, the uric acid deposition process occur because of the surface-active character of uric acid thatcan be accumulated on the graphiteelectrode (Gandour et al., 1994). The results showed that the peak current of uric acid was detected when using potential deposition of 0.3 V. Analysis of uric acid at the deposition potential of 0.3 V did not produce the greatest current signal. However, the generated voltammogram better compared to other potential. Voltammogram of uric acid obtained from analysis at deposition potential of 0.3 V is shown in Figure 1. The type of voltammetric method of uric acid analysis in this study iscathodic stripping voltammetry because of deposition potential worth more positive than the peak potential (Wang, 2000). Reaction in the surface of electrode is explained in the Figure 2.

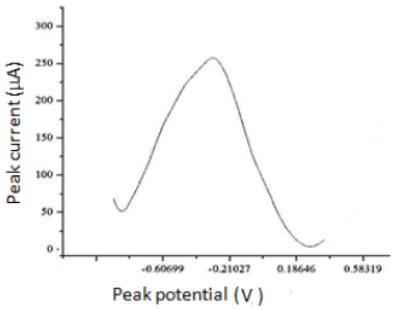


**Figure 1.** Voltammogram of uric acid 10 µg/L using deposition potential 0.3 V and deposition time 5 s.

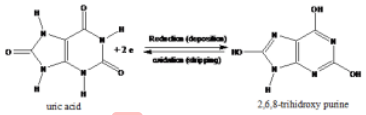


**Figure 2.** Reaction of uric acid at the electrode surface

The longer the deposition the higher the current produced. The relationship between accumulated analyteon the electrode surfaceversus deposition time explained by Faraday's law(Wang, 2000). Election of deposition time was done to obtain efficient in time of analysis and to prevent interferences that occur which was caused by saturation of electrode. Deposition time chosen in this research was 60 s.

**Effect of pH**

Determination of optimum pH was done by analysing the 5 µg/L uric acid with a pH of 4, 5, 6 and 7 on the deposition potential of 0.3 V and deposition time of 60 s. Selection of pH variation based on the results of research on the analysis of uric acid by adsorptive stripping voltammetry that has been conducted before (Gandour et al., 1994; Khasanah et al., 2009).Current data on the pH optimization is shown in Table 1. in this research has been acquired selected 5 as the optimum pH with consideration of pKa of uric acid is 5.75, so at pH 5 the uric acid is in the form of molecules, whereas at higher pH are in the form anionic and it is not to be accumulated on the electrode [(Zen and Hsu,1992; Zen et al., 1998).

**Table 1.** Data of the uric acid current signal on the various pH

| No. | pH | Peak potential (V) | Peak current*) (µA) |
|---|---|---|---|
| 1 | 4 | 0.02 | 12.98 |
| 2 | 5 | 0.03 | 13.31 |
| 3 | 6 | -0.08 | 6.06 |
| 4 | 7 | -0.12 | 7.32 |

*) n= 3

**Calibration curve and method validity**

The curve between uric acid concentration of 0.1; 0.2; 0.3; 0.4 and 0.5 µg/L at pH 5 and their current signal of each solution was explained by the equation of calibration curve ofy = 1.331x +18.527 with correlation coefficient (r) = 0.9978. Intercept obtained in the study was quite high, that was affected by the presence of non-faradic measurable current. The calibration curve and the standard solution current were then used to determine the method validity including linearity, sensitivity, precision, and limit of detection.

A very good linearity of response current toward concentration of uric acid expressed by the correlation coefficient of regression equation (r) of0.9978. Correlation coefficient is acceptable if $t_{crit} > t_{table}$. The calculation of t resulted the value of 26.3845, while the $t_{crit}$ is 2.353. This shows that there is a linear relationship between uric acid concentration and current signal. The sensitivity of the method was found to be 1.331 µA L/µg, that is higher than that obtained using hanging mercury drop electrode [Khasanah et al., 2009]. Relative standard deviation obtained from measurement of standard solution was 0.17 – 0.89% (n=3). According to the Horwit's trumpet, at the concentration level of part per billion (µg/L) the RSD of 32% is still statistically acceptable (Traverniers et al., 2004; Workman and Mark, 2006). The voltammetry method using graphite electrode to determine uric acid was very accurate. At the level of 0.1 to 0.5 µg/L, the accuracy of 40-120% is still statistically acceptable (Traverniers et al., 2004; Workman and Mark, 2006) and the studied method showed an accuracy range of 97.0-105.6%.

The detection limits obtained in this study is 0.036 µg/L, which is low enough for the analysis of uric acid in the natural sample. Compared to the previous studies which used a glassy carbon, bare HMD and modified HMD electrode, the use of graphite electrode produced the lowest detection limit (Khasanah et al., 2009; 2010[a,b]).

**Influence of the ascorbic acid, creatinine and urea on the analysis of uric acid by stripping voltammetry using graphite electrode**

One of the major problems on the determination of uric acid in the sample by the voltammetry method is the presence of ascorbic acid, a compound usually found together with uric acid in serum and urine samples (Luo et al., 2005; Wei et al., 2006). Using HMDE, the presence of ascorbic acid in equal concentration with uric acid decreased the current response of 63.61%(Khasanah et al., 2009). Using glassy carbon or carbon paste electrode, the voltammetric response of uric acid and ascorbic acid tends to occur at close potential and sometimes even overlapping (John, 2005). The high interference is caused by the competition of uric acid and ascorbic acid to the electrode surface during the deposition process.

In this study, the interference of ascorbic acid, creatinine and urea on determination of the uric acid by graphite electrode have been studied. Data of the influence of ascorbic acid, creatinine and urea on uric acid determination by stripping voltammetry is shown in Table 2. Concentration of ascorbic acid and urea in the real serum Sample are a half and 8-fold of uric acid concentration, respectively, whereas the creatinine concentration is a quarter of the concentration of uric acid. in this study, the significant influence of ascorbic acid and urea on uric acid determination has been observed. The influence of ascorbic acid with a half of concentration of uric acid is demonstrated by decreasing the current signal until 9.8%. Presence of urea with concentration five times of uric acid concentration caused the current deviation 6.9%, even if its concentration 10 times to the concentration of uric acid can cause the deviation current until 100%.Urea and uric acid competed with uric acid to reach the electrode surface during the diffusion process. Data in the Table 2 shows that the presence of creatinine in this research had not significantly interfere on the uric acid analysis using this method, was indicated by the current deviation of less than 5%. Although creatinine is an electroactive compound but the peak potential far adrift from uric acid. The creatinine was detected at a potential of 0.9 V, while the uric acid was detected at -0.32V.

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**Analysis of uric acid in serum sample by stripping voltammetry using graphite electrode and recovery test**

Application of voltammetry method using graphite electrode for the analysis of uric acid in a serumsample was performed at optimum conditions, namely at deposition potential 0.3 V during 60 s and a pH 5. The concentration of uric acid found in the sample was 3.18 mg/dL (n=3), whereas result from analysis using spectrophotometry in clinical laboratory was 5.43 mg/dL. This suggests that the presence of other matrices in the blood serum interfered the analysis of uric acid by stripping voltammetry using graphite electrode. Furthermore, recovery test have been done with the data obtained as shown in Table 3.

**Table 2.** The current deviation of uric acid at the various of matrices addition

| [uric acid]:[matrix] | Current deviation (%) |
|---|---|
| Ascorbic acid | |
| 1 : 0 | 0 |
| 1 : 0.5 | 9.80 |
| 1 : 1 | 14.20 |
| 1 : 10 | 17.61 |
| Creatinine | |
| 1 : 0 | 0 |
| 1 : 0.25 | 4.75 |
| 1 : 0.5 | 4.89 |
| 1 : 1 | 5.66 |
| 1 : 1.5 | 5.82 |
| Urea | |
| 1 : 0 | 0 |
| 1 : 1 | 3.49 |
| 1 : 5 | 6.19 |
| 1 : 10 | 100.00 |
| 1 : 15 | 100.00 |

Note. Concentration of uric acid was $6x10^{-10}$ M(0.1 µg/L)

**Table 3.** Data of the analysis of uric acid in serum sample

| Sample | Current*) (µA) | Concentration found (µg/dL) | Recovery (%) |
|---|---|---|---|
| Blood serum 1 | 18.716 | 0.1397 | 88 |
| Blood serum 1 + uric acid 0.25 µg/L | 18.820 | 0.3598 | |
| Uric acid 0.25 µg/L | 18.863 | 0.250 | |

*) n = 3

## Conclusions

Analysis of uric acid in the serum sample showed lower result as compared to that done by spectrophotometric method, with recovery of 88%. The creatinine was found not to interfere the uric acid analysis, but urea and ascorbic acid significantly interfere on the uric acid analysis by this method.

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