

Highly Selective Differential Pulse Voltammetric Determination of Uric Acid using Modified Glassy Carbon Electrode

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A nanocomposite was prepared by incorporating Fe₃O₄ onto graphene sheets, and then a layer of SiO₂ were deposited on the surface of the Fe₃O₄/graphene composites. This modified electrode was used for electrochemical determination of uric acid (UA). Cyclic voltammetry, differential pulse voltammetry and chronoamperometry were used to investigate the electrochemical behavior of uric acid at the chemically modified electrode. According to the results, Fe₃O₄@SiO₂/GO/GCE showed high electrocatalytic activity for uric acid oxidation, producing a sharp oxidation peak current at 330 mV vs. Ag/AgCl reference electrode at pH 7.0. The peak current was linearly dependent on uric acid concentration over the range of 0.5 to 250.0 μM with the detection limit (3σ) of 0.07 μM. The proposed method was successfully applied as a rapid, highly selective, simple, and precise one to determine uric acid in urine.

Keywords: Uric acid; Fe₃O₄@SiO₂/GO nanocomposite; Graphene; Glassy Carbon Electrode; Differential pulse voltammetry

1. INTRODUCTION

Uric acid (2,6,8-trihydroxypurine, UA) is the main end product of endogenous and dietary purine nucleotide metabolism in humans [1-3]. Clinical studies have shown that uric acid can stimulate

inflammation, oxidative stress, endothelial dysfunction and vasoconstriction. Experimental studies have illustrated that extreme abnormalities of uric acid levels in blood, known as Lesch-Nyhan syndrome or hyperuricemia, is linked with diseases, such as high cholesterol, gout, kidney stones, type 2 diabetes, high blood pressure, cardiovascular diseases and renal impairment [4-8]. Therefore, the quantitative determination of uric acid in human physiological fluids at various levels is of great significant in diagnosing of related diseases. Various analytical techniques, such as the ion chromatography, chemiluminescence, spectrophotometry, fluorescent biosensor and electrochemical methods, have been developed to determine uric acid concentrations [9-13]. Despite tremendous efforts was made to detection of uric acid with high sensitivity and selectivity, these methods still involved laborious, time-consuming, perform sophisticated equipment, and expensive, which make them unsuitable for rapid detection and restrain their wide applications [14].

Among these methods, electro-analytical techniques are a fast, simple and non-destructive operation method [15-19]. Although some electro-analytical sensors have been reported to detect uric acid, these sensors suffer from certain limitations such as high expense, weak electrochemical intensity. But the use of bare electrodes, for electrochemical detection of uric acid, have a number of limitations, such as slow electron transfer reaction, low reproducibility and sensitivity, low stability over a wide range of solution compositions and high overpotential at which the electron transfer process occurs [20-25]. Hence, it is significantly important to develop new materials with excellent properties and suitable designs to gain modified electrode owning superior performance [26-29]. Nanomaterials have been recently used in various applications [30-40].

Since discovered in 2004 [41], graphene, a novel two-dimensional (2D) carbon nanostructure, has garnered much attention due to its unique mechanical, physical and chemical properties. It is reported that graphene possesses a high theoretical specific surface area, excellent thermal and electrical conductivity [42-46]. These unique properties make graphene suitable for modified of surface electrode. The 2D structure, abundant surface functional groups and large surface area make graphene a great substrate for the synthesis of graphene based nanocomposites. The graphene based composite materials not only inherit the advantages of the graphene but also exhibit some other unique properties [47]. For example, decoration of Fe, Fe(OH)₃, γ -Fe₂O₃, Fe₃O₄ nanoparticles onto graphene sheets can endow them magnetic properties. Among them, due to their special magnetic properties and nontoxic nature, superparamagnetic iron oxide NPs have been widely used like many other fields, such as information storage, drug delivery and targeting, recoverable catalysts and magnetic separation. But, pure Fe₃O₄ NPs may not be very useful in technological applications because they are very likely to aggregate. Newly, Fe₃O₄/SiO₂ core/shell magnetic microspheres have attracted intense attention, because of their unique magnetic response, chemically modifiable surface and low cytotoxicity [48-63].

In the present work Fe₃O₄@SiO₂/GO nanocomposite was synthesized and used for the modification of glassy carbon electrode and applied for the sensitive determination of uric acid. Performance of this newly fabricated electrode was studied using various electrochemical methods. The proposed sensor showed good sensitivity, selectivity and satisfactory reproducibility for measurement of uric acid.

2. EXPERIMENTAL

2.1 Reagents and Apparatus

All electrochemical measurements were performed by an Autolab potentiostat/galvanostat (PGSTAT 302 N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrodes cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{GO}/\text{GCE}$ were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was employed for pH measurements.

All the solutions were prepared freshly with double distilled water. Uric acid and all other reagents were of analytical grade and were purchased from Merck chemical company (Darmstadt, Germany). Buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0.

2.2 Synthesis procedure for $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{GO}$ nanocomposite

An aqueous suspension (50 mL) of GO was diluted two times to give a concentration of 2 mg mL^{-1} of carboxylated GO, and then sonicated for 1 h to give a clear solution. NaOH (12 g) and chloroacetic acid ($\text{Cl}-\text{CH}_2-\text{COOH}$) (10 g) were added to the GO suspension and again sonicated for 2 h to convert the $-\text{OH}$ groups to $-\text{COOH}$ via conjugation of acetic acid moieties to form G-COOH. The resulting G-COOH solution was neutralized, and purified by repeated rinsing and filtration [64].

About 0.06 g of GO-COOH was dissolved in 42 mL of water by ultrasonic irradiation (Sono swiss SW3-H, 38 kHz, Switzerland) for 20 min. The mixture was further stirred vigorously for 30 min at 60 °C. Next, 106.2 mg of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was added while stirring. After the mixture was stirred vigorously for 30 min under N_2 atmosphere, 57 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was added while stirring under N_2 atmosphere for 30 min. At last 18 mL of 6% NH_4OH aqueous solution was added into the mixture drop by drop at 60 °C during 1 h and reacted for another 2 h. N_2 atmosphere was used during the reaction to prevent critical oxidation. The reaction mixture was then centrifuged, washed with double distilled water and dried. The obtained black precipitate was $\text{Fe}_3\text{O}_4/\text{GO}$ nanoparticles and was ready for use. Core-shell $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{GO}$ nanocomposites were prepared by growing silica layers onto the surface of the $\text{Fe}_3\text{O}_4/\text{GO}$. Fifteen milliliters of ethanol, 0.6 mL water, 0.6 mL ammonium hydroxide and 90 μL of TEOS were then added in a 250 mL three neck flask in a 40 °C water bath. $\text{Fe}_3\text{O}_4/\text{GO}$ were added to the above solution under mechanical stirring. Aliquots of the mixture were taken out after 12 h by centrifugation and washed using water and vacuum-dried at 60 °C overnight [65].

2.3 the electrode preparation

AGCE was polished mechanically with 0.05 μm Al_2O_3 in water slurry and then, it was electrochemically activated in a 0.1 M sodium bicarbonate solution, and pouring 5 μL of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{GO}$ nanocomposite suspension (0.01 g/1 mL) onto the activated GCE surface.

3. RESULTS AND DISCUSSION

3.1 Electrocatalytic oxidation of uric acid at $Fe_3O_4@SiO_2/GO/GCE$

The electrochemical behavior of uric acid is dependent on the pH value of the aqueous, therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of uric acid. Thus the electrochemical behavior of uric acid was studied in 0.1 M PBS in different pH values ($2.0 < \text{pH} < 9.0$) at the surface of $Fe_3O_4@SiO_2/GO/GCE$ by CV. It was found that the electrocatalytic oxidation of uric acid at the surface of $Fe_3O_4@SiO_2/GO/GCE$ was more favored under neutral conditions than in acidic or basic medium. This appears as a gradual growth in the anodic peak current. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of uric acid oxidation at the surface of $Fe_3O_4@SiO_2/GO/GCE$.

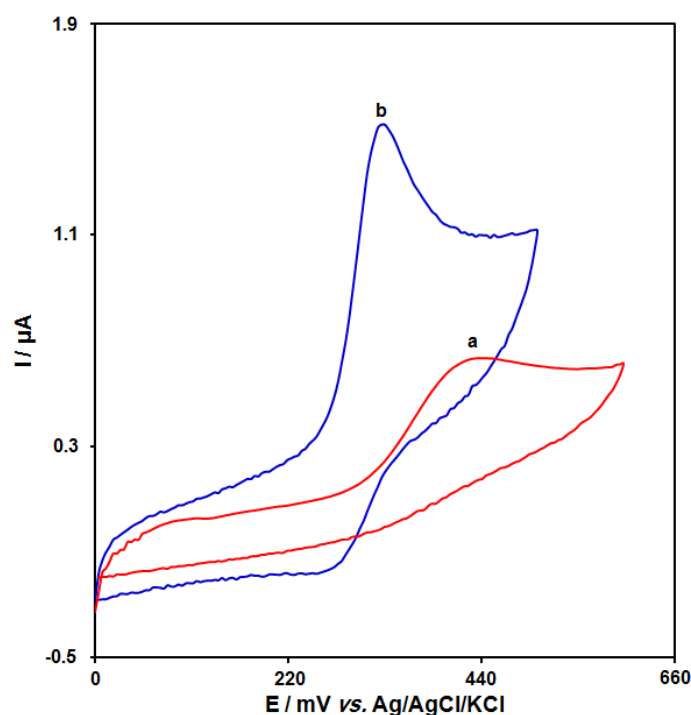


Figure 1. Cyclic voltammograms of (a) bare GCE and (b) $Fe_3O_4@SiO_2/GO/GCE$ in 0.1 M PBS (pH 7.0) in the presence of 30.0 μM uric acid at the scan rate 50 mVs^{-1} .

Fig. 1 depicts the cyclic voltammetric responses for the electrochemical oxidation of 30.0 μM uric acid at $Fe_3O_4@SiO_2/GO/GCE$ (curve b) and bare GCE (curve a). The anodic peak potential for the oxidation of uric acid at $Fe_3O_4@SiO_2/GO/GCE$ (curve a) is about 330 mV compared with 440 mV for that on the bare GCE (curve a).

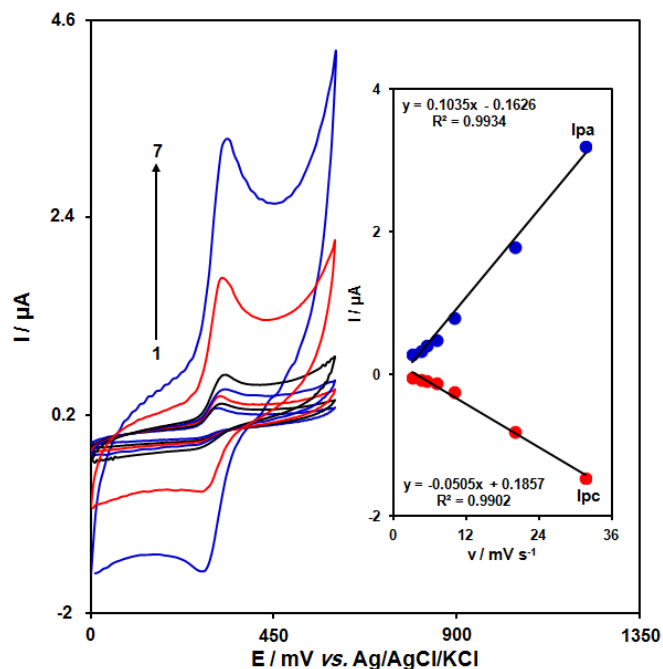


Figure 2. Cyclic voltammograms of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{GO}/\text{GCE}$ in 0.1 M PBS (pH 7.0) containing $7.0 \mu\text{M}$ uric acid at various scan rates; numbers 1-7 correspond to 10, 20, 30, 50, 100, 400 and 1000 mV s^{-1} , respectively. Inset: Variation of anodic and cathodic peak current vs. $v^{1/2}$.

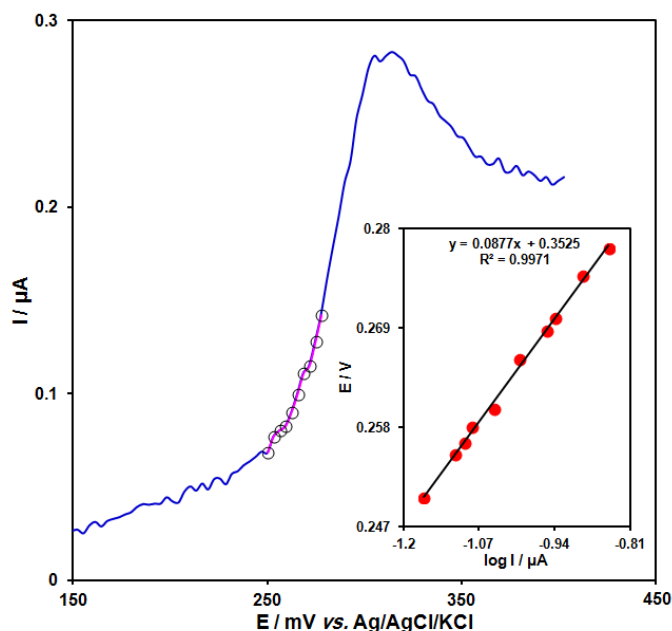


Figure 3. LSV (at 10 mV s^{-1}) of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{GO}/\text{GCE}$ in 0.1 M PBS (pH 7.0) containing $7.0 \mu\text{M}$ uric acid. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV.

Similarly, when the oxidation of uric acid at the $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{GO}/\text{GCE}$ (curve b) and bare GCE (curve a) are compared, an extensive enhancement of the anodic peak current at $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{GO}/\text{GCE}$ relative to the value obtained at the bare GCE is observed. In other words, the

results clearly indicate that the combination of graphene and Fe₃O₄@SiO₂ nanocomposites improve the uric acid oxidation signal.

Potential scan rates on the oxidation current of uric acid was optimized (Fig. 2). by increasing the scan rate, an increase in the peak current was observed. Furthermore, the oxidation process is a kind of diffusion controlled process as deduced from the linear dependence of the anodic peak current (I_p) on the square root of the potential scan rate (v^{1/2}) over a wide range of 10 to 1000 mV s⁻¹. Fig. 3 depicts the Tafel plot for the sharp rising part of the voltammogram at the scan rate of 10 mV s⁻¹. If deprotonation of uric acid is adequately fast, the Tafel plot can be used to estimation the number of electrons involved in the rate determining step. A Tafel slope of 0.0877 V was achieved which in good agreement with the involvement of one electron in the rate determining step of the electrode process [66], assuming a charge transfer coefficient, α of 0.33.

3.2 Chronoamperometric measurements

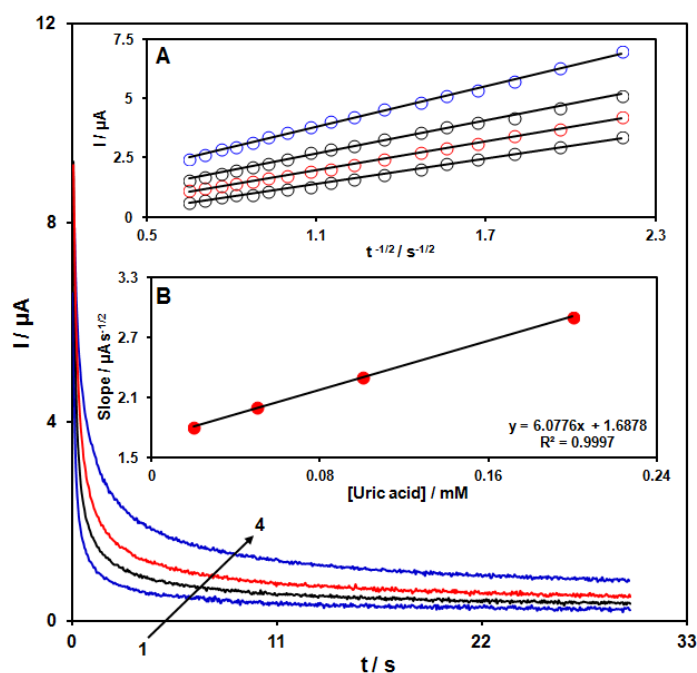


Figure 4. Chronoamperograms obtained at Fe₃O₄@SiO₂/GO/GCE in 0.1 M PBS (pH 7.0) for different concentration of uric acid. The numbers 1–4 correspond to 0.02, 0.05, 0.1 and 0.2 mM of uric acid. Insets: (A) Plots of I vs. t^{-1/2} obtained from chronoamperograms 1–4. (B) Plot of the slope of the straight lines against uric acid concentration.

Chronoamperometric measurements of uric acid at Fe₃O₄@SiO₂/GO/GCE were studied by setting the working electrode potential at 0.4 V for the various concentration of uric acid in PBS (pH 7.0) (Fig. 4). For an electroactive material (uric acid in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition which can be described by the Cottrell equation [66].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \tag{1}$$

Where D and C_b are the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of uric acid (Fig. 4A). The slopes of the resulting straight lines were then plotted vs. uric acid concentration (Fig. 4B). From the resulting slope and Cottrell equation the mean value of the D was found to be $3.15 \times 10^{-6} \text{ cm}^2/\text{s}$.

3.3 Calibration plot and limit of detection

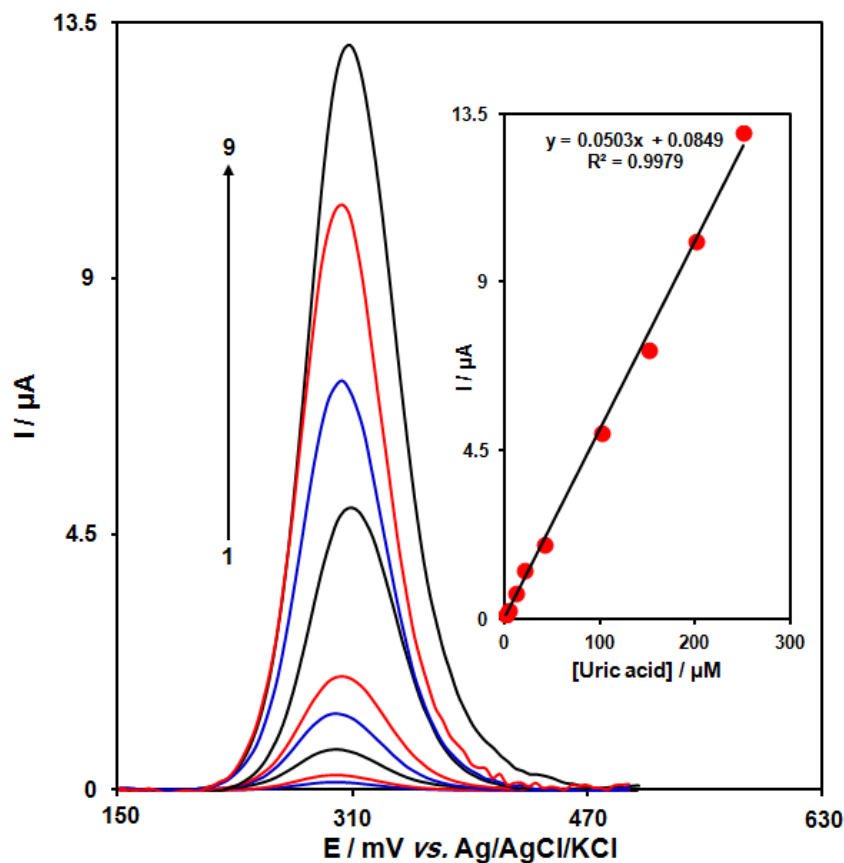


Figure 5. DPVs of $\text{Fe}_3\text{O}_4@/\text{SiO}_2/\text{GO}/\text{GCE}$ in 0.1 M (pH 7.0) containing different concentrations of uric acid. Numbers 1–9 correspond to 0.5, 3.0, 10.0, 20.0, 40.0, 100.0, 150.0, 200.0 and 250.0 μM of uric acid. Insets: (A) A plots of the electrocatalytic peak current as a function of uric acid concentration in the range of 0.5–250.0 μM .

The peak current of uric acid oxidation at the surface of the modified electrode can be applied for determination of uric acid in the solution. Therefore, differential pulse voltammetry (DPV) experiments were performed for different concentrations of uric acid (Fig. 5). The oxidation peak currents of uric acid at the surface of a modified electrode were proportional to the concentration of the uric acid within the ranges 0.5 to 250.0 μM . The detection limit (3σ) of uric acid was found to be $7.0 \times 10^{-8} \text{ M}$. These values are comparable with values reported by other research groups for electrocatalytic oxidation of uric acid at the surface of chemically modified electrodes (see Table 1).

Table 2. Comparison of the efficiency of some electrodes used in detection of uric acid.

Electrode	Modifier	LOD	LDR	Ref.
Glassy carbon electrode	Graphitic carbon nitride (g-C ₃ N ₄) nanosheets	0.5 μM	5.0-189.0 μM	67
Gold interdigitated microelectrodes	Graphene flakes and platinum nanoparticles	18.0 μM	60.0-288.0 μM	68
Glassy carbon electrode	Gold nanotube arrays	0.22 nM	0.14-464.0 μM	69
Glassy carbon electrode	Three-dimensional porous graphene	1.0 μM	0.14-464.0 μM	70
Glassy carbon electrode	poly(diallyldimethylammonium chloride) and graphene oxide	0.08 μM	0.25-1500.0 μM	71
Glassy carbon electrode	Quantum dots CdTe and graphene	1.0 μM	3.0-600.0 μM	72
Screen-printed electrode	β-cyclodextrin (β-CD) polymer and graphene oxide	0.026 μM	0.08-150.0 μM	73
Glassy carbon electrode	Graphene hydrogel and gold nanoparticles nanocomposite	5.0 nM	1.0-60.0 μM	74
Glassy carbon electrode	Fe ₃ O ₄ @SiO ₂ /graphene nanocomposites	0.07 μM	0.5-250.0 μM	This work

3.4 Urine sample analysis

To evaluate the analytical applicability of the proposed sensor, it was applied to the determination of uric acid in urine samples. The results for determination of uric acid in real sample are given in Table 2. Acceptable recovery of the results was obtained. The reproducibility of the method was showed by the mean relative standard deviation (R.S.D.).

Table 2. The application of Fe₃O₄@SiO₂/GO/GCE for simultaneous determination of uric acid in urine samples (n=5). All concentrations are in μM.

Sample	Original content (μM)	Added (μM)	Found	Recovery (%)	R.S.D. (%)
	uric acid	uric acid	uric acid	uric acid	uric acid
Urine	10.0	-	9.9	99.0	3.4
	10.0	10.0	20.5	102.5	1.7
	10.0	20.0	30.2	100.7	2.9
	10.0	30.0	39.5	98.7	2.4

4. CONCLUSIONS

The objective of the present work is to develop a sensor based on glassy carbon electrode modified with Fe₃O₄@SiO₂/GO nanocomposite for the determination of uric acid. The sensitive and stable detection of uric acid can be achieved at pH 7.0. The detection limit of the modified electrodes for uric acid was 70.0 nM. It provides a simple and reliable technique for uric acid detection in real samples.

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