Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(11):404-409

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Poly calcein modified electrode for the simultaneous determinations of uric acid, xanthine and hypoxanthine

Xiaoling Guo[a,b], Tiejun Hou[b], Jinlian Li[b], Jiwen Cui[b], Hongfu Gao[b], Sue Hao[a*] and Dongmei Wu[b]

[a]Department of Chemistry, Harbin Institute of Technology, Harbin, China
[b]College of Pharmacy, Jiamusi University, Jiamusi, China

ABSTRACT

Poly calcein modified glassy carbon electrode (PCA/GCE) has been fabricated by simple electropolymerization method. The electrochemical behaviors of uric acid (UA), xanthine (XA) and hypoxanthine (HX) at PCA/GCE were studied by cyclic voltammetry and linear sweep voltammetry. The results showed that PCA/GCE displays not only electrocatalytic activity toward the electrochemical oxidations of UA, XA and HX, but also good reproducibility, stability and long lifetime. Under the optimum conditions, the linear concentration dependences of LSV peak current responses were observed for UA, XA and HX in the concentration ranges of 0.2-80, 0.2-80 and 0.5-90 μM with the detection limits of 0.12, 0.12 and 0.3 μM, respectively. Moreover, PCA/GCE was applied to simultaneous detection of UA, XA and HX in human urine samples with satisfactory results.

Keywords: Calcein; Electrochemistry; Uric acid; Xanthine; Hypoxanthine

---

INTRODUCTION

Many physiological functions of organisms closely relate to purine nucleotide metabolism. Xanthine (XA) and hypoxanthine (HX) as intermediates and uric acid (UA) as the final product of purine nucleotides catabolism in the process catalyzed by xanthine oxidoreductase (XOR), can provide important information of the living system [1-3]. Thus their abnormal concentration levels in physiological fluids may serve as sensitive indicators of certain pathologic states, such as cardiovascular and cerebrovascular diseases, hypertension and metabolic syndrome, etc[4]. Therefore, developing an easy and accurate method for detection of UA, XA, and HX is critically important in study of diseases associated with altered purine metabolism [5].

Various techniques have been utilized to determine these biomolecules including high performance liquid chromatography (HPLC) [6, 7], capillary electrophoresis [8, 9], fluorimetry [10], enzymatic methods [11, 12] and electrochemical methods, etc.. Among various methods, the electrochemical method has attracted considerable attention because of its remarkable advantages such as label-free, high sensitivity, simplicity and rapid response [13-15].

Recently, many modified electrodes, such as poly (pyrocatechol violet)/functionalized multi-walled carbon nanotubes composite film modified electrode [16], $Ru(DMSO)_4Cl_2$ nano-aggregated Nafion membrane modified electrode [17], the surface enhancement effect of mesoporous silica [18] and preanodized nontronite-coated screen-printed electrode [19], have been used for the simultaneous determination of UA, XA and HX. However, construction of these electrode based on rare metal precursors or nanomaterial are always accompanied by the high cost and complicated operation. Polymer modified electrodes, prepared by electropolymerization method, have received extensive interest due to their simple, stable homogeneous film with controlled thickness [20]. It has been

demonstrated that electropolymerized film show excellent selectivity and sensitivity with stable voltammetric response towards several biomolecules [21-23].

Calcein (CA) is a cheap and widely used fluorescent dye. It has been used as a complexometric indicator for titration of Ca^{2+} with EDTA, and for fluorometric determination of calcium [24]. More recently, CA is also used as indicator for detection of DNA synthesis and as a fluorescent probe for determination of trace amounts of DNA[25]. However, to the best of our knowledge, no work related to the simultaneous determination of UA, XA and HX with poly calcein film modified electrode has been reported.

In this work, a new poly calcein modified glassy carbon electrode (PCA/GCE) was prepared by simple electropolymerization method for catalyzing the oxidation of UA, XA and HX and simultaneous determinations of them. PCA/GCE exhibited excellent electrocatalytic activity towards the oxidation of UA, XA and HX, and showed good sensitivity, high selectivity and remarkable reproducibility. Based on its excellent characteristics, the proposed modified electrode can be applied to simultaneous determination of UA, XA and HX in human urine samples.

EXPERIMENTAL SECTION

2.1. Reagents and chemicals

UA, XA and HX were purchased from Sigma (USA). Calcein (CA) and potassium nitrite (KNO_3) were obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical grade and were used without further purification. The 0.2 M phosphate buffer solutions (PBS) with various pH values were prepared by using the stock solutions of 0.2 M KH_2PO_4 and Na_2HPO_4. All solutions were prepared with doubly distilled water. The pH measurements of solutions were carried out on a pHs-25pH-meter (Leizi Instrumental Factory, Shanghai, China).

2.2. Preparation of the PCA/GCE

Prior to modification, the bare GCE was polished with 0.05 μm alumina slurry and sonicated in ethanol and distilled water continuously. After the electrode was pretreated electrochemically by scanning in a 0.5 M H_2SO_4 solution between -0.5 and 1.5 V at 100 mV s^{-1} for 10 cycles to get a stable background current, the polymer film was obtained by scanning between -1.4 and 1.8 V at 100 mV s^{-1} for 20 cycles in pH 7.4 PBS containing 0.01 M KNO_3 and 2.5×10^{-3} M CA. Then, the modified electrode (PCA/GCE) was washed with doubly distilled water and air-dried.

2.3. Electrochemical methods

Electrochemical experiments were carried out with a CHI 760B workstation (CH Instrumentation, Shanghai, China). The measurements were performed with a conventional three-electrode system comprising a Pt wire as the counter, an Ag/AgCl (Sat'd) electrode as reference, and PCA/GCE as the working electrode, respectively. The electrochemical behaviors of UA, XA and HX were characterized by cyclic voltammetry within the potential range from 0.0 to +1.2 V at a scan rate of 50 mV s^{-1}. After each measurement, the PCA/GCE was scanned for 5 cycles between 0.0 and +1.2 V in pH 7.4 PBS, and then rinsed thoroughly with double-distilled water. All experiments were accomplished at room temperature.

RESULTS AND DISCUSSION

3.1. Preparation of PCA/GCE

The cyclic voltammograms of 2.5×10^{-3} M CA in pH 7.4 PBS containing 0.01 M KNO_3 at bare glassy carbon electrode were shown in Fig.1. In the first cycle, oxidation peaks at 0.89 V and 0.52 V and a reduction peak at -0.48 V were observed. In the subsequent cycles, the peak current responses for the reduction peaks exhibited continuous increase with the potential scan progressing. It suggested not only the formation of polymer but also the gradual increase of the amount of electro-active polymer on GCE surface. Until the peak current responses remain unchanged after 18 scans, indicating that stable polymer film has been formed, so 20 scans were chosen for further experiments in the study.

3.2. Voltammetric behavior of UA, XA and HX on PCA/GCE

The electrochemical behaviors of the mixture solution containing 20 μM UA, 40 μM XA and 80 μM HX on bare GCE and modified electrode in pH 6.5 PBS were shown in Fig. 2. No oxidation peak was observed on PCA/GCE in pH 6.5 PBS (Inset). In mixture solution, three broad and poor peaks located at 0.3, 0.7 and 1.05 V, respectively, were observed on bare GCE. In contrast, the peak currents (i_{pa}) of UA, XA and HX on PCA/GCE (b) were increased greatly compared to bare GCE(a), respectively. In addition, all the three oxidation peak potentials on PCA/GCE shift slightly to more negative potential compared to bare GCE. The above result indicated that PCA/GCE can accelerate the rate of electron transfer and have excellent electrocatalytic activity towards the oxidation of UA, XA and HX.

PCA/GCE has high concentration of the negatively charged functional group (carboxyl) and the electron-rich oxygen atom, which could interact with the purine derivatives, such as UA, XA and HX. Therefore, the electrochemical responds of UA, XA and HX may be greatly improved due to synergistic effect in the presence of PCA film by accelerating the rate of electron transfer. UA, XA and HX could be identified entirely on PCA/GCE based on its high electrocatalytic activity.

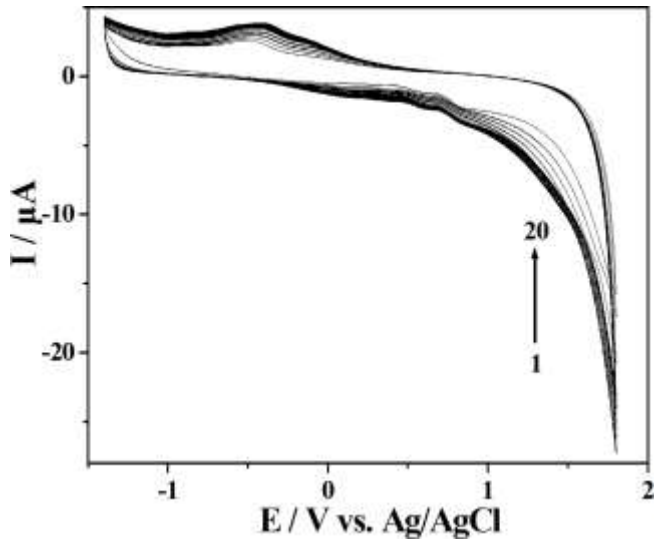

Fig. 1 CV of CA in electropolymerization process from 1 to 20 cycles. CA: 2.5×10^{-3} M; supporting electrolyte: pH 7.4 PBS containing 0.01 M KNO_3; scan rate: 100 mV s^{-1}

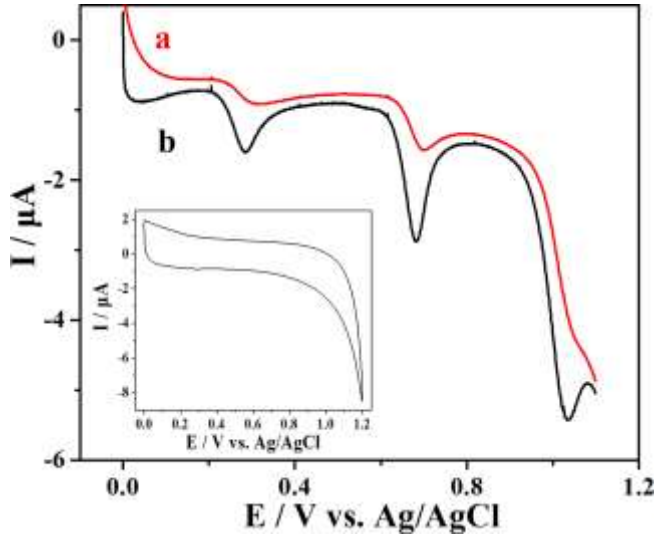

Fig. 2 LSVs of bare GCE (a), PCA/GCE (b), in pH 6.5 PBS containing 20 μM UA, 40μM XA and 80 μM HX with a scan rate of 50 mV s^{-1}. Inset: CV of PCA/GCE in pH 6.5 PBS

3.3. Effect of scan rate on the voltammetric behavior of UA, XA and HX on PCA/GCE

The voltammetric behaviors of 50 μM UA, 25 μM XA and 50 μM HX mixture solution on PCA/GCE at different scan rates were shown in Fig. 3A. With the increase of scan rate, the oxidation peak currents increased simultaneously. The plots of oxidation peak currents as a function of scan rate for three kinds of standard were shown in the Fig. 3B. In the range from 10 to 100 mV s^{-1}, the oxidation peak current linearly increase with the scan rate, suggesting that the system was an adsorption-controlled process for UA, XA and HX. The linear regression equation relating i_{pa} with the scan rate over the range of 10-100 mV s^{-1} was found to be: i_{pa} (UA) = 0.028 + 0.607 v (R^2 = 0.9987), i_{pa} (XA) = 0.015 + 0. 076 v (R^2 = 0.9970), and i_{pa} (HX) = 0.023 + 0.031 v (R^2 = 0.9859), respectively. In addition, it was observed that the catalytic oxidation peak potential (E_{pa}) all shifted to more positive potentials with increase of scan rate. The result showed that the electrocatalytic oxidation of UA, XA and HX on the modified electrode surface is irreversible.

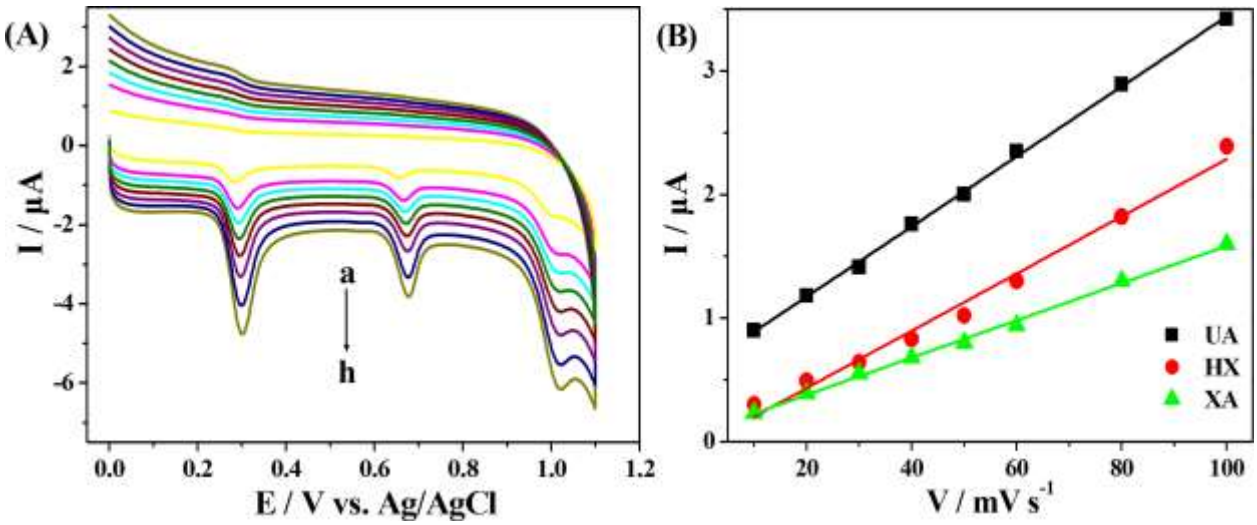

Fig. 3 (A) CVs of 50 μM UA, 25μM XA and 50μM HX mixture solution on PCA/GCE at with different scan rates (a-h: 10, 20, 30, 40, 50, 60, 80, 100 mV s^{-1}) in pH 6.5 PBS. (B) Plots of the oxidation peak current versus the scan rate

3.4. Influence of pH on the voltammetric behavior of UA, XA and HX on PCA/GCE

Fig. 4 illustrated the dependence of the anodic peak potential (E_{pa}) and anodic peak current (i_{pa}) for 50 μM UA, XA and HX on pH. The peak potentials (E_{pa}) for UA, XA and HX showed a same trend and shift almost linearly toward negative potentials when pH was increased in the range of 4.7-9.2, indicating that protons are directly involved in the rate determination step of the oxidation reaction of three compounds. The equation relating E_{pa} with pH was found to be: E_{pa} (V) = 1.465 - 0.0564 pH (R^2 = 0.9971) for HX, E_{pa} (V) =1.127 - 0.0581 pH (R^2 = 0.9948) for XA, and E_{pa} (V) = 0.712 - 0.056 pH (R^2 = 0.9969) for UA, respectively. The slopes of - 0.0564 V pH^{-1} for HX, - 0.0581 V pH^{-1} for XA, and - 0.056 V pH^{-1} for UA were close to the theoretical value of - 0.0600 V pH^{-1} which indicated that the numbers of electrons and protons participating in the electrochemical oxidation were equal over the studied pH range. The effect of pH on anodic peak current for UA, XA and HX was shown in Fig. 4B. It was seen that the maximum value almost appeared at pH 6.5 for the three compounds. Therefore, pH 6.5 PBS was chosen in this study.

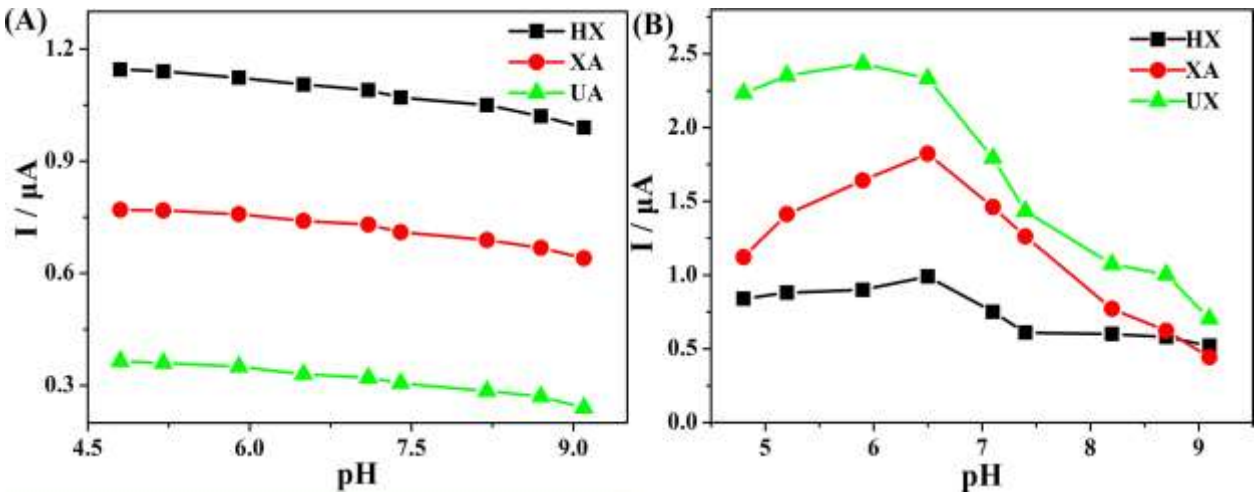

Fig. 4 Effects of pH on the anodic peak potential (A) and anodic peak current (B) of 50 μM UA, XA and HX on PCA/GCE. Scan rate: 50 mV s^{-1}

3.5 Selective determinations of UA, XA and HX

The voltammetric behaviors of a series of mixture solutions with pH 6.5 at a scan rate 50 mV s^{-1} were detected in order to confirm the availability of PCA/GCE for the separate determination of UA, XA and HX (Fig.5A-C). In a mixture of UA, XA and HX, the concentration of one compound changed while the concentrations of the other remained constant. The current of UA increased linearly with the increase of concentration of UA, while the currents of XA and HX keep nearly unchanged (Fig. 5A). Similarly, Fig. 5B and 5C showed that the peak currents of XA and HX increased linearly with the increase of concentrations of XA and HX, while the peak currents of the other compounds keep nearly stable. This indicated that oxidation processes of UA, XA, and HX at PCA/GCE are

independent from each other. Therefore, the PCA/GCE could be used for the selective determinations of UA, XA and HX.

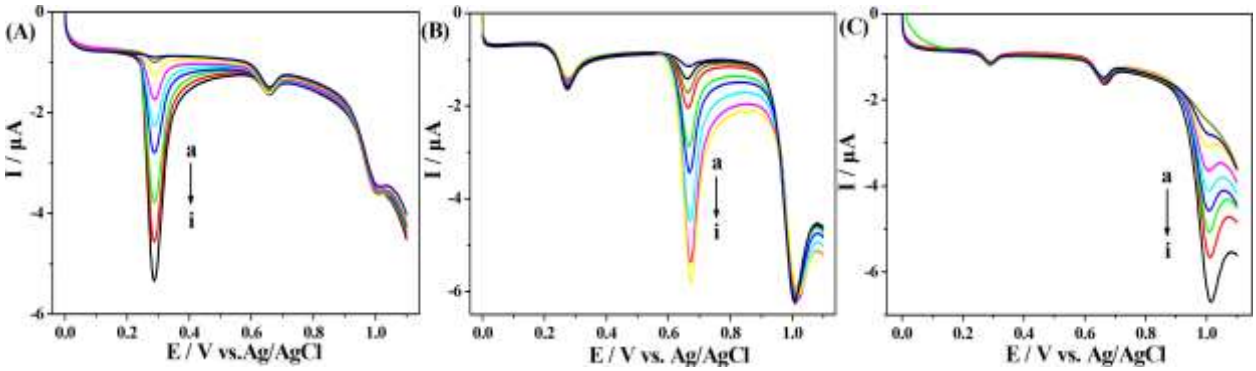

Fig.5 (A) LSVs of UA with different concentrations on PCA/GCE in the presence of 10 μM XA and 30 μM HX . UA concentrations (a-i): 0.1, 0.2, 2, 10, 20, 40, 60, 80 and 100 μM; (B) LSVs of XA with different concentrations on PCA/GCE in the presence of 20 μM UA and 100 μM HX. XA concentrations (a-i): 0.2, 2, 10, 20, 30 , 50, 70, 90 and 100 μM; (C) LSVs of HX with different concentrations on PCA/GCE in the presence of 10 μM UA and 10 μM XA . HX concentrations (a-i): 0.5, 5, 10, 30, 40, 50, 60, 80 and 100 μM; pH = 6.5 PBS

3.6. Simultaneous determination of UA, XA and HX

The LSV of UA, XA and HX was shown in Fig.6A, the peak currents of UA, XA and HX increased proportionally with their concentrations. The linear relationships between the peak currents i_{pa}(μA) and concentrations C (μmol L^{-1}) of UA, XA and HX are: i_{pa} = 0.036 + 0.040 C_{UA} (R^2 = 0.9997), i_{pa} = 0.042 + 0.034 C_{XA} (R^2 = 0.9988), and i_{pa} = 0.040 + 0.117 C_{HX} (R^2 = 0.9979), respectively (Fig.6B). The linear ranges for simultaneous detections of UA, XA and HX are 0.2-80 μM, 0.2-80 μM and 0.5-90 μM, respectively. The detection limits for determining UA, XA and HX are 0.12, 0.12 and 0.3 μM, respectively.

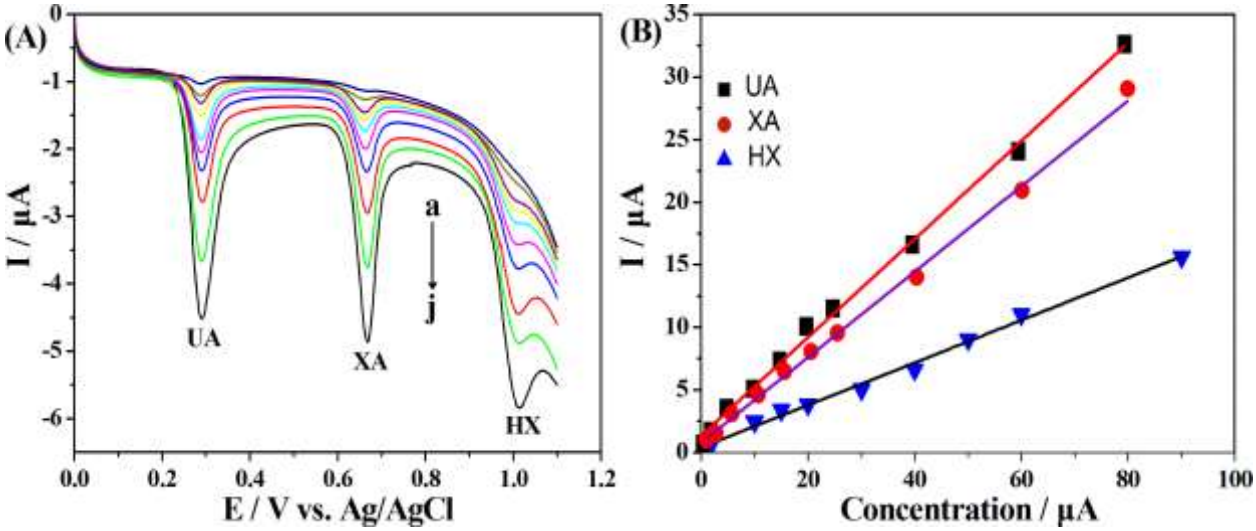

Fig. 6 (A) LSV of different concentrations of UA, XA (a-j: 0.2, 2, 5, 10, 15, 20, 25, 40, 60, 80 μM) and HX (a-j: 0.5, 2, 10, 15, 20, 30, 40, 50, 60, 90 μM) on PCA/GCE in pH 6.5 PBS. (B) Concentration calibration curve of the LSV current response for UA, XA and HX

3.7. Interferences, reproducibility and stability

For apply directly it to practical detection in the body fluid, a few possible interfering substances, such as urea, glucose, cysteine, dl-tyrosine, oxalic acid, caffeine and some inorganic compounds, such as NaCl, KNO_3, $Mg(NO_3)_2$ and so on was investigated. The results showed that no obvious change in current response was observed for 50 μM of UA, XA and HX in the presence of 10mM of these interferents , indicating that the present modified electrode is highly selective towards the determination of these analytes even in the presence of high concentrations common physiological interferents(figure not shown).

The reproducibility of the PCA/GCE was tested by 10 repetitive measurements for the mixture of 50μM of UA, XA and HX. The relative standard deviations (RSD) in peak currents for detecting UA, XA and HX were 3.3%, 3.6%, and 2.7%, respectively. Five electrodes were prepared independently and performed the measurements for UA, XA and HX with the relative standard deviations of 2.8%, 3.2% and 2.5%, respectively. Furthermore, PCA/GCE was

stored in 0.2 M PBS (pH = 6.5) at room temperature for 15 days in order to investigate its stability. The current response decreased to 94.0% of the initial response during the first week, after two weeks the response still maintained 90.5% compared with the initial current value. These results indicated that the PCA/GCE was reproducible and stable.

3.8. Sample Analysis

Human urine samples were selected as biological samples for analysis using standard addition method. All urine samples were detected by the LSV after they were diluted with pH 6.5 PBS, and then the urine samples were spiked with known concentrations of UA, XA and HX and detected (Table 1). The recoveries of the spiked samples were between 96.0% and 107.0%, indicating the proposed PCA/GCE could be effectively used for determinations of UA, XA, HX in real urine samples.

Table 1 Simultaneous determination of UA, XA and HX in human urine samples (n = 3)

Samples	Original (μM)			Added (μM)			Found (μM)			Recoveries (%)		
	UA	XA	HX	UA	XA	HX	UA	XA	HX	UA	XA	HX
Urine 1	6.7	1.2		1	1	1	8.0	2.17	0.96	103	98	96
Urine 2	7	1.1		1	1	1	7.92	2.25	0.98	99	107	98
Urine 3	5.9	0.80		1	1	1	7.05	1.85	1.01	102	103	101

CONCLUSION

A poly calcein modified electrode had been prepared by electropolymerization method, which exhibited good stability, sensitivity, selectivity and electrocatalytic activities for the oxidation of HX, XA and UA. This electrode was successfully applied for the determination of UA, XA and HX in human urine samples. It could be expected to use in clinical detection of UA, XA and HX.

Acknowledgements

We gratefully acknowledge the financial support of the Funds of NSFC from China (81273132), the Nature Science Foundation of Heilongjiang Province (B201310), Science Foundation of Heilongjiang province Ministry of Education (12541810) and the Foundation of Jiamusi University (L2010-122).

REFERENCES

[1] M.J. Downie, K. Kirk, and C.B. Mamoun, *Eukaryotic cell*, **2008**, 7, 1231-1237.
[2] Y. Kaminsky, and E. Kosenko, *Brain Res. Bull*, **2009**, 1294, 193-201.
[3] A.V. Sauer, I. Brigida, N. Carriglio, A. Aiuti, *Front. Immunol*, **2012**, 3, 265.
[4] P. Cekan, , S.T. Sigurdsson, *Journal of the American Chemical Society*, **2009**, 131, 18054-18056.
[5] M. Heinig, R.J. Johnson, *Clev. Clin. J.Med*, **2006**, 73, 1059-1064.
[6] S. Sumi, K. Kidouchi, S. Ohba, Y. Wada, *J. Chromatogr. B*, **1995**, 670, 376-378.
[7] T. Yamamoto, Y. Moriwaki, S. Takahashi, Y. Wada, *J. Chromatogr*.B, **1996**, 681, 395.
[8] Z. K. Shihabi, M. E. Hinsdale, A. J. Bleyer, *J. Chromatogr. B*, **1995**, 163, 669.
[9] F. Carlucci, A. Tabucchi, B. Biagioli, G. Sani, G. Lisi, M. Maccherini, F. Rosi, E.Marinello, *Electrophoresis*, **2000**, 21, 1552-1557 ()
[10] H. Y.Wang, Q. S. Hu, Xu L. X., J. G. Jiang, Y. Sun, *Anal. Chim. Acta*, **2003**, 497, 93-99 .
[11] M. A. Carsol, G. Volpe, M. Mascini, *Talanta*, **1997**, 44, 2151-2159.
[12] G. Cayuela, N. Pena, A. J. Reviejo, J. M. Pingarron, *Analyst*, **1998**, 123, 371 .
[13] J. Huang, X. Xing, X. Zhang, X. He, Q. Lin, W. Lian, H. Zhu, *Food Res. Int*, **2011**, 44,276-281.
[14] R.N. Goyal, V.K. Gupta, N. Bachheti, *Anal. Chim. Acta*, **2007**, 597, 82-89 .
[15] R.N. Goyal, V.K. Gupta, S. Chatterjee, *Biosens,Bioelectron*, **2009**, 24,1649-1654 .
[16] Y. Wang, *Colloids and Surfaces B: Biointerfaces*, **2011**, 88, 614-621.
[17] A.S. Kumar, P. Swetha, *Journal of Electroanalytical Chemistry*, **2010** , 642, 135-142 .
[18] D. Sun, Y. Zhang, F. Wang, K. Wu, J. Chen, Y. Zhou, *Sensors and Actuators B:Chemical*, **2009**, 14, 641-645.
[19] J.M. Zen, Y.Y. Lai, H.H. Yang, A.S. Kumar, *Sensors and Actuators B: Chemical*, **2002**, 84, 237-244.
[20] S. Cosnier, *Electroanal*, **2005**, 17, 1701-1715.
[21] L. Zhang, D. Yang, *Electrochimica Acta*, **2014**, 119, 106 -113.
[22] F. Zhang, Z. Wang, Y. Zhang, Zh. Zheng, Ch. Wang, Y. Du, W. Ye, *Talanta*, **2012** 93, 320 - 325.
[23] S.B. Revin, S.A. John, *Electrochimica Acta*, **2011**, 56, 8934- 8940.
[24] S. Kaehler, C. D. McQuaid, *Mar. Biol*, **1999**, 133, 455-460.
[25] A. L. Moran, *Mar. Biol*, **2000**, 137, 893-898.