Asian Journal of Chemistry; Vol. 26, No. 16 (2014), 5168-5170

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16599

Determination of DL-Methionine with Electro-chemiluminescence Molecularly Imprinting Method

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Received: 29 October 2013; *Accepted*: 17 January 2014; *Published online*: 28 July 2014; AJC-15654

In order to establish a novel EC-MIP method in the determination of DL-methionine in human urine, a carbon electrode of $[Ru(bpy)_3]^{2+}$ was modified with the molecular imprinting polymers electro-deposited with the sol-gel method. The abilities of the electrochemical and electro-chemiluminescence characteristics of the above electrode were then investigated with the quantitative determination of dL-methionine in human urine. The method exhibited the high sensitivity and excellent selectivity towards DL-methionine. With the optimized conditions, the linear range of dL-methionine was from 1×10^{-11} mol/L to 1×10^{-8} mol/L, with the LOD as 2.6×10^{-12} mol/L. This method could be successfully applied to the determination of dL-methionine in human urine samples for the precaution of certain diseases.

Keywords: Electro-chemiluminescence, Molecularly imprinting method, DL-Methionine.

INTRODUCTION

DL-Methionine (DL-Met) is one of the 8 essential amino acids in human body, closely related to the *in vivo* metabolism of a variety of sulfur-containing compounds. When the body is lack of methionine, the appetite would lose, body growth would be slow or does not increase, the kidney would enlarge and the phenomenon of liver iron accumulation would appear, leading to liver necrosis or fibrosis. The •O-caused lipid over-oxidation is a leading cause of various damages to the body, which would damage the primary and secondary lysosome membranes, resulting in the damages in such important organelles as cells and mitochondrial membranes, while methionine could combat the damage by various ways, therefore it exhibits the great significance in the diagnosis and treatment of various diseases and pathological studies[1-3]. Currently, many methods have been developed for the quantitative determination of DL-methionine, including HPLC[4], GC-MS[5] and electrochemistry, *etc*.[6]. However, not only the sensitivities of the above methods are not satisfactory, but also the complicated preparation procedures are necessary in some methods and certain equipment is also expensive.

The electro-chemiluminescence has such advantages as high sensitivity, simple instrument and rapid detection, *etc*., though the specificity of electro-chemiluminescence is not that much ideal, it has already been applied into the detection of several kinds of amino acids and successfully applied into the analysis of peptides, proteins, nucleic acids and other biological macromolecules, drugs and ultra-trace *in vivo* bioactive substances. It has also begun the usage in the fields of immuno-assay, PCR analysis and single molecule detection of DNA[7,8]. The terpyridine ruthenium $[Ru(bpy)_3]^{2+}$ is the most widely used luminescent reagent in present. Because the nature and structure of $[Ru(bpy)_3]^{2+}$ does not exhibit changes before and after the chemical reaction, it could be fixed onto the electrode and used repeatedly, which would not only save the detecting reagent, but also effectively reduce the testing costs and simplify the experimental instrument at the same time.

The molecularly imprinting polymer has the capability of highly specific recognition towards the target molecules, with specific combination action, molecularly imprinting polymer could efficiently eliminate the impurities, keep the target molecular onto, which equals to concentrate the trace, or ultra-trace substances and therefore increase the sensitivity, leading to its wide usage in the fields of chemistry, biology and many other studies. There are many developed methods to apply the molecularly imprinting polymer into different detectors towards the different detection purposes, among which the sol-gel method could make the prepared detector obtain the stable physical and chemical properties and therefore the testing abilities are quick and easy[9]. There has been the report about the application of the molecularly imprinting

polymer detector into the detection of other amino acids in human plasma,with the limit of detection (LOD) as 1×10^{-9} mol/L[10], indicating the molecularly imprinting polymer had the satisfactory sensitivity and specificity in these applications.

In this paper, the electro-chemiluminescence and molecularly imprinting polymer were combined for the detection of DL-methionine in human urine. The result showed that not only the specificity of electro-chemiluminescence could be solved, but also because of the isolation of sol-gel, the sensitivity of molecularly imprinting polymer was effectively improved. Thus, the method developed in this study could be used in the detection of DL-methionine in human urine, which could predict certain diseases in clinic when the disease was still in the early stage and thus improve the treatment efficacy of the diseases.

EXPERIMENTAL

The EC-MPI analytical system was purchased from Ruimai Analytical Co., Ltd. (Xi'an, China), with the high voltage of the photomultiplier tube as -700V; the WHS-CS350 electrochemical workstation was the product of Beijing Hengaode Instrument Co., Ltd. (Beijing, China); the electrode system had 3 electrodes: the working electrode was a 3 mm glassy carbon electrode (GCE), the counter electrode was the platinum wire,and the reference electrode was the Ag/AgCl electrode, with the saturated KCl solution as the filling solution.

The multi-wall carbon nanotube (ϕ20-40 nm, MWCNT) was the product of Beijing DekedaojinTechnology Co., Ltd. (Beijing, China). Nafion was the solution of 5 % lower fatty alcohol and water), the $[Ru(bpy)_3]^{2+}$ was purchased from the Sigma-Aldrich Corporation (98 %); the tetraethoxysilane was from the J&K Technology Co., Ltd. (98 %, TEOS). All the other reagents were purchased from the Sinopharm Chemical reagent Co., Ltd.; 0.05 mol/L phosphate buffer solution (PBS) was prepared with the double-distilled water.

Preparation of detector: Firstly, 0.8 g MWCNTs was accurately weighed and added into the mixture of 50 mL concentrated H_2SO_4 and 20 mL concentrated HNO_3 and stirred with the magnetic stirring for 48 h at room temperature. The mixture was washed with water to the neutral status, followed by the filtration with a 0.22 μm microporous membrane; dried the filtrate at 80 °C for 4 h and ground under the infrared lamp for 0.5 h for the future use. 3 mg acidified MWCNTs was accurately weighed and added by 1.5 mL isopropanol and 0.3 mL Nafion for the ultrasonic dispersion and then left until no more precipitation appeared. 5 μL prepared dispersed solution was pipetted and dropped onto the surface of the GCE and let it dried naturally. The GCE was then placed into the 1×10^{-3} mol/L $[Ru(bpy)_3]^{2+}$ solution for 0.5 h; removed the GCE, rinsed with water and dried naturally, which generated the solid electro-chemiluminescence electrode. The preparation of Nafion electrode followed the same procedure of electro-chemiluminescence electrode, except for the addition of MWCNTs.

1.5 mL TEOS, 2 mL ethanol, 0.3 mL PTMOS, 0.3 mL MTMOS, 0.1 mL HCl (0.01 mol/L) and 1 mL H_2O were mixed and sonicated for 2 h, generating a clear homogeneous electrodeposition base liquid. Half of the base liquid was added with 0.1 mL DL-methionine (0.01 mol/L) for the ultrasonic preparation for 0.5 h to obtain the molecularly imprinting polymer; then the electro-chemiluminescence electrode was placed into the molecularly imprinting polymer, scanned with 50mV/s cyclic voltammetry (CV) for 25 laps:the potential range was from -0.8 to 1.5 V; removed the electro-chemiluminescence electrode and rinsed with water,then dried at the room temperature. The electrode was then immersed with 40 % formic acid and water alternately for 4 h to remove the template molecule and generated the EC-Met-MIP electrode. The other half of the base liquid was used to prepare the EC-NIP electrode under the same conditions, without the template molecule.

RESULTS AND DISCUSSION

The immobilization of $[Ru(bpy)_3]^{2+}$ was performed with the carbon nanotube because of its excellent electrical properties[11]. Fig. 1a exhibited the cyclo-voltammogram curves of Nafion electrode (a) and electro-chemiluminescence electrode (b) in PBS, with the presence of 1×10^{-5} mol/L methionine, in which the peak current of curve b was significantly larger than that of curve a, indicating that the MWCNTs could improve the electron transferring rate on the electrode surface. Fig. 1b showed the luminous intensity of the two electrodes, in which the luminous intensity of electro-chemiluminescence electrode was about 8 times larger than that of the Nafion electrode, indicating that MWCNTs could significantly improve the sensitivity of the method.

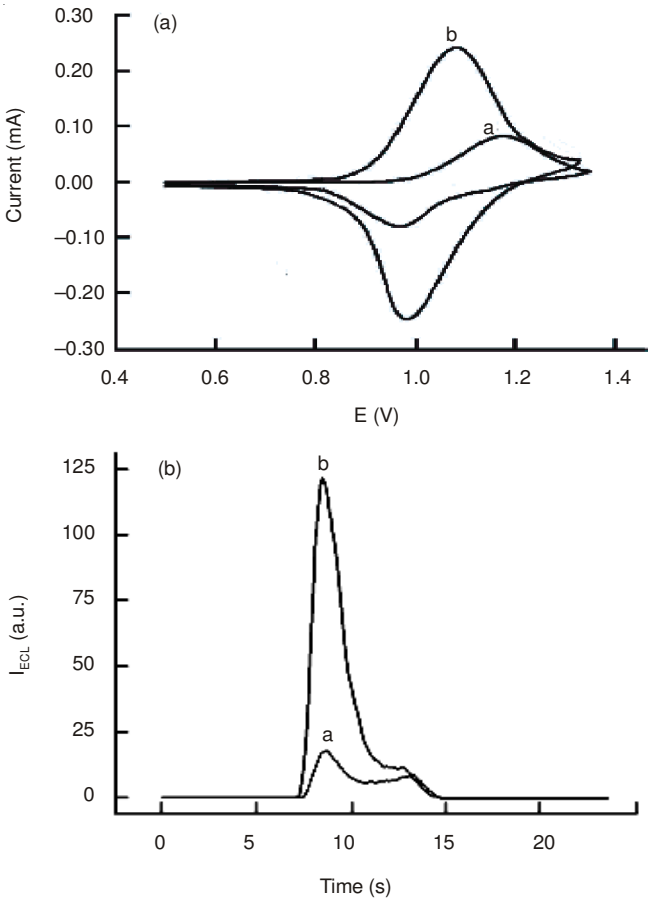

Fig. 1. Cyclo-voltammetry (a) and intensity curves (b) of Nafion electrode (a) and ECL electrode (b) in PBS, with the presence of 1×10^{-5} mol/L methionine, scan rate: 100 m V/s

Influence of scanning rate: The scanning rate 30-450 mV/s was tested for the optimization. From the results, it could be found that the oxidation peak current (i) of the $[Ru(bpy)_3]^{2+}$ was positively proportional to the square root of scanning rate ($\nu^{1/2}$) and the linear equation was $i = -0.132 + 0.035\ \nu^{1/2}$, with r = 0.999 3. As the scanning rate increased, the potential of the oxidation peak gradually shifted towards the positive direction, while the potential of the reduction peak gradually shifted negatively, in order to ensure the stability of the electrodes and prevent the too large charging current, the subsequent experiments selected the scanning rate as 100 mV/s.

Elution of template molecule: In this experiment, water, anhydrous alcohol, 0.01 mol/L NaOH and 40 % formic acid solution were tested the elusion abilities towards the methionine template molecule. Because the template molecules combined with the functional monomer and the crosslinking agents through the non-covalent bonds, which was some degree mild, the dehydrated alcohol and 0.01 mol/L NaOH would destroy the integrity of the template molecule; though the 40 % formic acid could rapidly elute the template molecule. This solution would also too strong to keep the whole film on the detector. Therefore, in this experiment, 40 % formic acid and water were used alternately to rinse the electrodes. With the elution time increased, the electro-chemiluminescence value decreased and after 4 h, the illumination approached the baseline value, indicating that the template molecule was renounced completely.

Optimization of determination conditions: The solution pH was tested about the influence on the electro-chemiluminescence intensity. When the solution was acidic, the electro-chemiluminescence value would increase with the pH increasing and reach the maximum at pH 7; when the solution was alkaline, the intensity value did not significantly decrease, while the blank value increased, which would then interfere the detection of methionine. Therefore, pH 7 was chosen in this experiment as the optimal pH.

Linear range and limit of detection: After the optimized determination pH was decided, the methionine concentration increased from 1×10^{-11} mol/L to 1×10^{-8} mol/L, the electro-chemiluminescence intensity also increased. When the methionine concentration was higher than 1×10^{-8} mol/L, the electro-chemiluminescence value increased slowly, indicating the molecularly imprinting polymer reached the adsorptive saturation. According to the results, the linear equation was calculated with the intensity of electro-chemiluminescence (IEC) to the logarithm of methionine concentration (log C), IEC = 73.2 + 3.7 log C, with r = 0.995 1 and the LOD was 2.6×10^{-12} mol/L.

Reproducibility and stability: The reproducibility and the stability of the method was also tested with the same concentration of methionine. The 1×10^{-9} mol/L of methionine was tested 6 times, with the relative standard deviation (RSD) as 3.1 %, indicating that the reproducibility of the method was good; after 3 weeks' detection, the detection results towards the same methionine solution revealed that the RSD was 5.2 %, indicating that stability of the method was also acceptable towards the long term determination.

Sample determination: The human urine samples were diluted with 0.05 mol/LPBS (pH 7) to the 50 folds for the determination. The results were shown in Table-1, with the average recovery as 92.3-96.3 % and the RSD as 7.7-4.3, indicating that the method had high sensitivity and could be applied to the analysis of trace methionine in the urine samples, providing the ultra-diagnosis towards many diseases.

TABLE-1
DETERMINATION OF DL-METHIONINE IN HUMAN URINE SAMPLES (n = 5)

No	Detected (mol/L)	Added (mol/L)	Recovery (%)	RSD (%)
1	3.13×10^{-9}	1.56×10^{-9}	93.1	6.2
2	4.24×10^{-8}	2.11×10^{-8}	96.2	4.5
3	7.06×10^{-8}	2.35×10^{-8}	96.3	4.3
4	9.27×10^{-9}	4.31×10^{-9}	92.9	7.2
5	32.72×10^{-10}	14.77×10^{-10}	92.3	7.7

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