Analytical Methods

Cite this: *Anal. Methods*, 2012, **4**, 482

www.rsc.org/methods

PAPER

Electrochemical sensor of 4-aminobutyric acid based on molecularly imprinted electropolymer

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Received 2nd October 2011, Accepted 7th December 2011
DOI: 10.1039/c2ay05629a

A simple and effective procedure based on a molecularly imprinted polymer (MIP) was developed for preparing a selective 4-aminobutyric acid (4-ABA) sensor. The sensitive layer was prepared by electropolymerization of *o*-phenylenediamine (*o*-PD) on a gold electrode in the presence of 4-ABA, which acts as a template. Cyclic voltammetry (CV), differential pulse voltammetry (DPV), linear sweep voltammetry (LSV) and electrochemical impedance spectroscopy (EIS) measurements were used to monitor the process of electropolymerization. The molecularly imprinted sensor was tested by CV as well as DPV to verify the changes of redox currents of hexacyanoferrate. The concentration of 4-ABA in the range of 0.2–20.0 μmol L^{-1} can be determined with a detection limit of 0.08 μmol L^{-1} (defined as S/N = 3) under the optimum conditions. The MIP sensor shows high selectivity, sensitivity and reproducibility. The results from sample analysis indicate that the MIP-4-ABA sensor can be used for quantitative analysis.

1 Introduction

4-aminobutyric acid (4-ABA) is a major inhibitory neurotransmitter in the central nervous system and is essential for brain metabolism and function.[1] It has been suggested that it is involved in the cortical reorganization that follows a limited sensory deprivation.[2–5]

Several techniques, such as magnetic resonance spectrometry,[6] electroanalysis,[7] fluorescence,[8] spectrophotometric,[9] capillary electrophoresis,[10] gas[11,12] and liquid chromatography,[13,14] have been developed for the determination of 4-ABA. The most common method employed for the determination of 4-ABA is liquid chromatography. But this method requires complex column derivatization[13–18] since 4-ABA does not show any characteristics of UV absorption. It is also difficult to detect directly because 4-ABA has deficiency of fluorimetry and electrochemical activity. Therefore the development of a simple, sensitive and reliable analytical method to detect 4-ABA is of great importance.

During the past decade, molecular imprinting techniques are becoming more commonly accepted as an analytical tool due to their high selectivity, low cost, and ease of preparation. Molecularly imprinted polymer (MIP) is made by the synthesis of highly crosslinked polymers in the presence of "imprint" molecules (template). The template molecule is removed after the polymerization of the monomer while its size, shape and chemical functions are reserved in the polymer, which can be used as a selective binding medium for the imprint molecule or other structurally related compounds.[19] The use of MIP as recognition elements in sensors has been reported in several articles.[20–24] As far as we know, there is no report on the detection of 4-ABA by molecularly imprinted sensors.

The aim of this work was to fabricate a highly selective and sensitive 4-ABA sensor based on *o*-phenylenediamine (*o*-PD), which was proved to be easily electropolymerized on various substrate materials and form films with good chemical and mechanical stability.[20,25–27] Considering that *o*-PD films and 4-ABA are both electro-inactive,[25] an electroactive substance should be introduced to the system to guarantee the conductivity of the imprinted electrode. Thus, hexacyanoferrate was chosen as the mediator between the imprinted electrodes and substrate solutions containing the analyte. A relationship between the signal produced by the reduction of hexacyanoferrate, and the concentration of analyte in supporting electrolytes can be obtained.[28]

By using the proposed sensor, a relatively high sensitivity and low limit of detection (LOD) for the analyte could be obtained. The MIP-4-ABA sensor has been successfully applied to analyze 4-ABA in germinated brown rice.

2 Experimental

2.1 Apparatus

Electropolymerization was carried out at 25 °C on a CHI660D electrochemical workstation (Shanghai Chenhua Instruments, Shanghai, China). The classical three-electrode system consists of a KCl saturated Ag/AgCl electrode (reference electrode),

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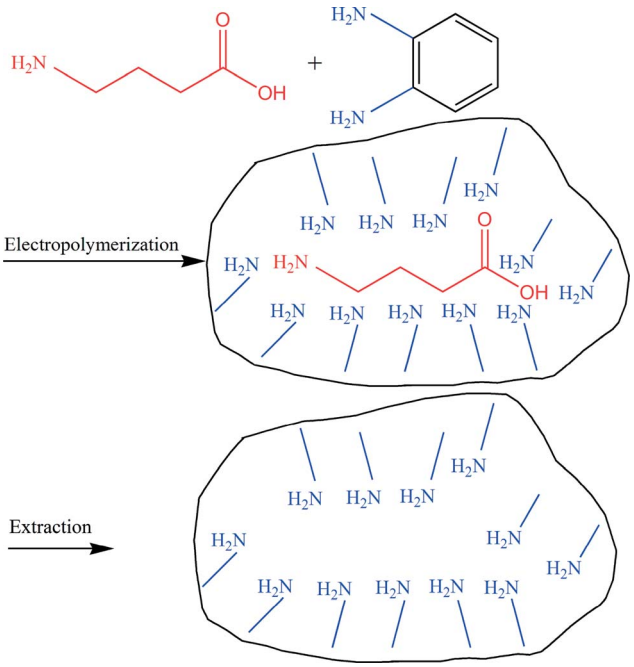

Fig. 1 Schematic diagram of MIP preparation.

a platinum electrode (auxiliary electrode), and a MIP-modified gold electrode with a diameter of 2.0 mm (working electrode).

2.2 Reagents and materials

4-ABA was obtained from the Chinese Institute of Biological Products Control (Beijing, China). Germinated brown rice was purchased from the Fuzhou Haomai food limited company. All the reagents involved were bought from local commercial sources and were analytical grade. Distilled water was used for preparation of all solutions and for washing.

2.3 Preparation of standard solution and buffer solution

Stock solution of 4-ABA (1.00 mmol L^{-1}) was prepared by distilled water, and diluted to desired concentration. Buffer solution was prepared by mixing 0.2 mol L^{-1} Na_2HPO_4 and 0.2 mol L^{-1} NaH_2PO_4 solutions to form a phosphate (PP) buffer solution (0.2 mol L^{-1}). Both standard solution and buffer solutions were kept in a 4 °C refrigerator.

2.4 Preparation of imprinted membrane

The MIP was constructed by electropolymerization of *o*-PD on the surface of the gold electrode by potentiodynamic conditions (10 cycles) in the potential range of 0 to 0.8 V at a scan rate of 50 mV s^{-1}, from a solution which contains 5 mmol L^{-1} *o*-PD and 0.2 mol L^{-1} PP buffer (pH = 5.8). Prior to the electropolymerization, the surface of gold electrodes was polished by 0.3 μm Al_2O_3, and then sonicated in distilled water for 5 min. The polished electrodes were electrochemically cleaned by cyclic voltammetry between −0.20 and 1.50 V in 0.5 mol L^{-1} H_2SO_4 until a stable cyclic voltammogram was obtained. 4-ABA with a concentration of 0.10 mmol L^{-1} was prepared

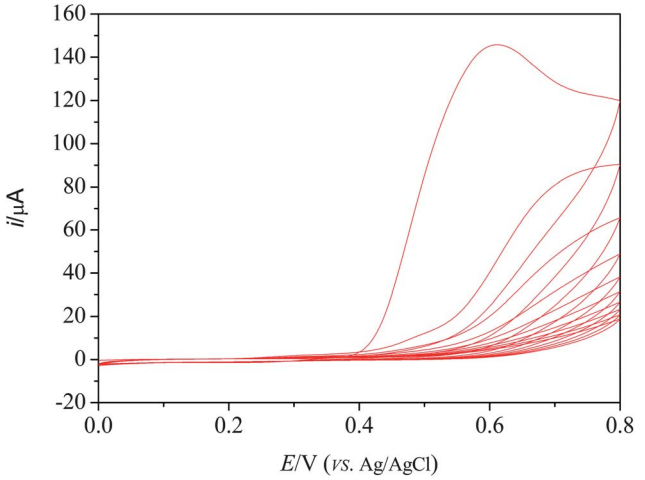

Fig. 2 Cyclic voltammograms for the electropolymerization of 5 mmol L^{-1} *o*-PD on a gold electrode in PP buffer solution (pH = 5.8). Scan rate: 50 mV s^{-1}; number of scans: 10; potential range: 0 to 0.8 V; concentration of 4-ABA: 0.10 mmol L^{-1}.

for imprinted polymerization. A non-imprinted polymer (NIP) electrode was also prepared in the same way without addition of the 4-ABA.

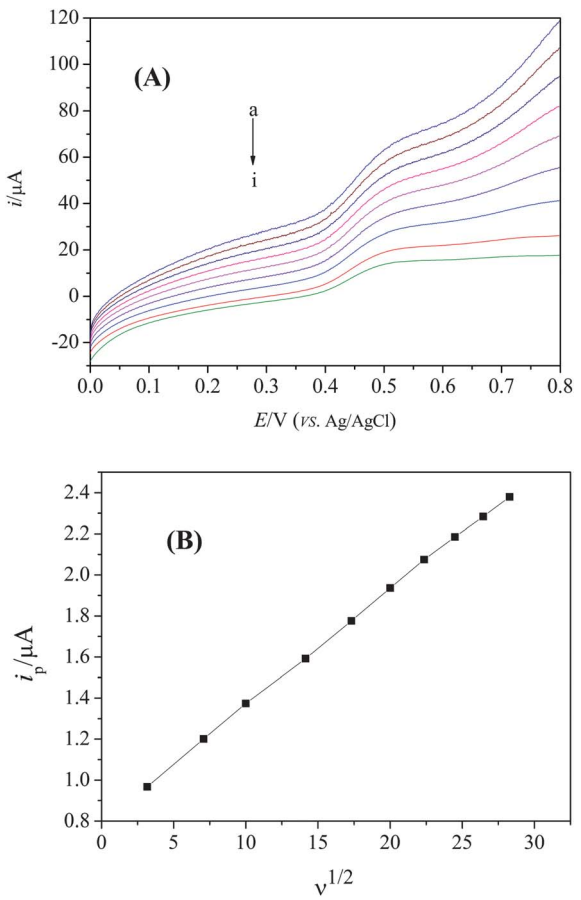

Fig. 3 Linear sweep voltammograms of hexacyanoferrate on a MIP/Au electrode, (A). Linear relationships of i_p *vs* $v^{1/2}$, (B). Scan rates: a-10mV s^{-1}, b-50mV s^{-1}, c-100mV s^{-1}, d-200mV s^{-1}, e-300mV s^{-1}, f-400mV s^{-1}, g-500mV s^{-1}, h-600mV s^{-1}, i-700mV s^{-1}.

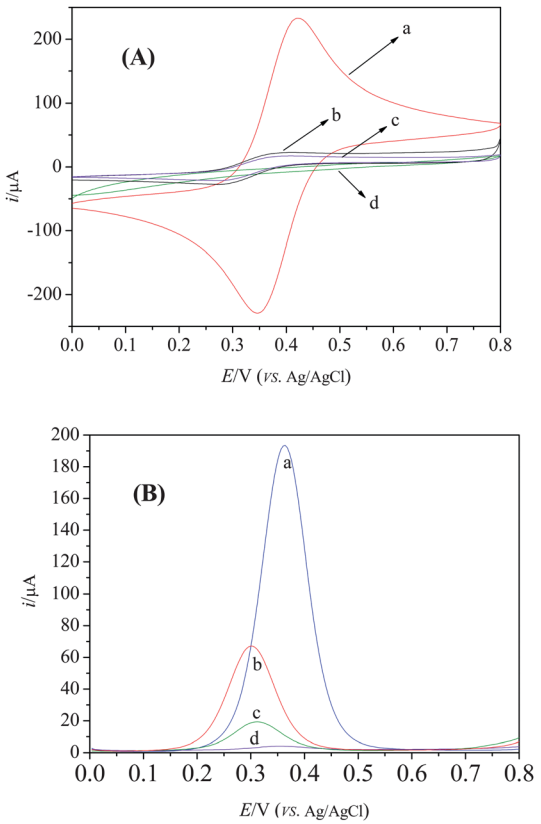

Fig. 4 (A) CVs of 5 mmol L^{-1} of hexacyanoferrate on different electrodes, scan rate = 50 mV s^{-1}. (B) DPVs of 5 mmol L^{-1} of hexacyanoferrate on different electrode, pulse amplitude = 50 mV, pulse width = 50 ms, scan range = 0 to 0.8 V, scan rate = 50 mV s^{-1}. a: Bare Au electrode, b: MIP/Au electrode after removal of the imprinted 4-ABA molecule, c: MIP/Au electrode after interaction with 10.0 μmol L^{-1} 4-ABA, d: NIP/Au electrode.

Finally, the MIP electrode was immersed in distilled water for 48 h, and then washed with distilled water. The template molecules can be removed from the modified electrode surface.

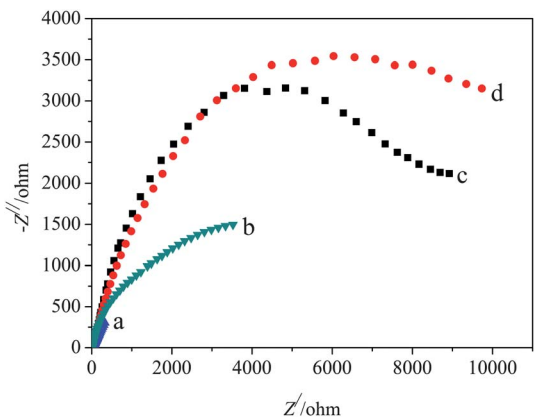

Fig. 5 AC impedance spectroscopy of different electrodes, a: bare Au electrode, b: MIP/Au electrode after removal of the imprinted 4-ABA molecule, c: MIP/Au electrode after interaction with 10.0 μmol L^{-1} 4-ABA, d: NIP/Au electrode.

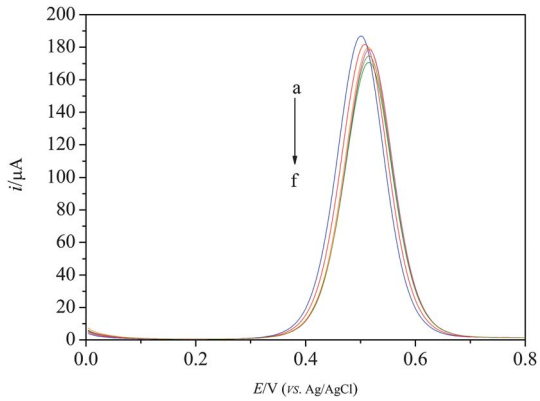

Fig. 6 DPVs of hexacyanoferrate at the MIP/Au after removing the template (a), a concentration of 0.2–20.0 μmol L^{-1} 4-ABA was added (b–f). All conditions are the same as Fig. 4B.

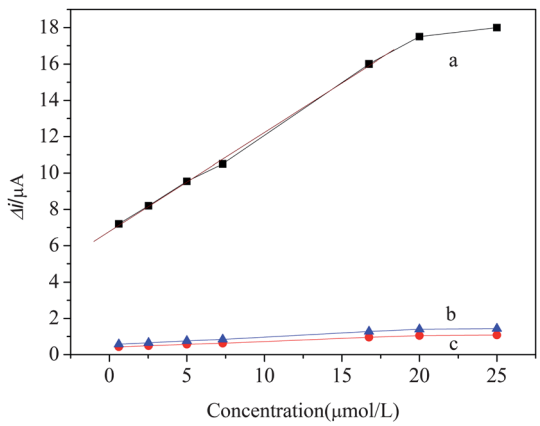

Fig. 7 Relative current changes in the MIP sensor for different concentrations of 4-ABA and some interferents, a: 4-ABA, b: glutamic acid, c: aminopropionic acid. Experimental conditions are the same as those in Fig. 4B.

2.5 Electroanalytical measurements

A typical three-electrode cell connected to a CHI660D was used for electrochemical measurements, in a supporting 5.0 mmol L^{-1} hexacyanoferrate mediator containing 0.5 mol L^{-1} KCl. CV measurements were performed over a potential range from 0 to 0.8 V at a scan rate of 50 mV s^{-1}. EIS measurements were carried out by applying an ac voltage of 5 mV in the frequency range from 0.1 Hz to 10 MHz. All experiments were carried out at room temperature (25 °C).

2.6 Sample preparation

Germinated brown rice was ground in a ceramic mortar to obtain a fine powder. Then five grams of the powder was placed into a 50 mL polypropylene centrifuge tube, to which 40 mL of the extraction solvent (mixture of ethanol and water in a volume ratio of 3 : 2) was added. After 1 h of sonicating, the mixture was centrifuged at 4000 rpm for 10 min, and the supernatant was filtered through a 0.22 μm filter membrane. The filtrate was diluted with extraction solvent to 50 mL to obtain the samples for detection.

Table 1 The selectivity of the electrochemical sensor

Interfering substance	Selectivity coefficient
Glutamic acid	0.06
Aminopropionic acid	0.08

3 Results and discussion

3.1 Preparation of MIP

Template molecule 4-ABA has a hydroxyl group and an amino group in its structure. Therefore, during polymerization, it would be able to interact with amino group in the *o*-PD network through hydrogen bonding. A schematic diagram of MIP preparation is shown in Fig. 1.

Fig. 2 shows a typical cyclic voltammogram recorded during the electropolymerization of *o*-PD in the presence of 0.10 mmol L^{-1} 4-ABA on a gold electrode in PP buffer (pH = 5.8) solution. The highest current was obtained in the first scan. Oxidation of *o*-PD was recorded as a distinct and irreversible peak at a peak potential of 0.611 V. Then the peak current dropped significantly with each scan. When the number of cycles was increased to 10, the redox peak disappeared completely, which indicated that the insulating polymer was formed and bound to the electrode surface.

3.2 Electrochemical properties of the MIP-modified electrodes

Fig. 3A shows the linear sweep voltammograms (LSV) of hexacyanoferrate on a MIP/Au electrode. The peak currents increased with scan rates, in agreement with the expected electrochemical behavior of a thin layer.[29] The plots of i_p *versus* $v^{1/2}$ are linear (Fig. 3B), indicating that the rate of the overall process was controlled by the rate of diffusion of hexacyanidoferrate to the electrode.

3.3 Molecular recognition by MIP-modified film

The sensor based on different modified electrodes (bare Au, MIP/Au, NIP/Au electrode) was investigated by CV methods in aqueous solution containing 5 mmol L^{-1} of hexacyanoferrate, which was used as the active probe (Fig. 4A). A couple of typical redox peaks of hexacyanoferrate appeared on the bare Au electrode (curve "a"). However, only a very small background response was observed on the NIP/Au electrode (curve "d") since the film coated on the electrode was compact and the active probe can not penetrate through the layer of polymer to arrive at the surface of the electrode. Curve "b" shows the CV using a MIP/Au electrode after removal of the imprinted 4-ABA molecules, The peak current in curve "b" is lower than that in curve "a". It suggests the low conductivity of film coated on Au electrode.[25] When MIP/Au electrode interacted with 10.0 μmol L^{-1} 4-ABA, the peak current (curve "c") decreased compared with that of curve "b", which indicates that electron transfer was blocked by the non-conducting 4-ABA molecules again. As we expected the same results can be obtained by DPV methods (Fig. 4B).

3.4 Impedance measurement

AC impedance spectroscopy is an effective method for studying the interface features of a modified electrode surface. In EIS, the semicircle diameter equals to the electron-transfer resistance (R_{et}). Fig. 5 shows the results of impedance spectroscopy on different electrodes. It is obvious that there is almost no heterogeneous charge-transfer resistance on the bare Au surface (curve "a"). A significant increase in R_{et} can be observed on MIP/Au after removing the template (curve "b"). Curve "c" shows that a further increase of R_{et} on the MIP/Au electrode after interaction with 10 μmol L^{-1} 4-ABA for 10 min, which suggests that imprinted molecular cavities exist in the film, and the cavities are occupied by 4-ABA molecules, preventing the electron transfer between the electrode and the electrolyte. However, the R_{et} of the NIP/Au is much larger (curve "d"), indicating that the compact film of low conductivity acts as a definite kinetic barrier for the charge transfer. These results are in accordance with CV assays as detailed above.

3.5 Linear range and limit of detection

In this study, DPV was employed for the quantitative determination of 4-ABA to elucidate its relative sensitivity. Different concentrations of 4-ABA from 0 to 20 μmol L^{-1} were added to hexacyanoferrate solution each time, when the template molecularly was removed from the MIP. As shown in Fig. 6, with the increase of the 4-ABA concentration, the current value was gradually decreased, and the peak potentials shifted to positive. The decrease of the current can be attributed to the number of binding sites in the film occupied by 4-ABA molecules from the hexacyanoferrate solution, and the peak potentials shifted to positive is because of the electrode polarization enhancement. A series of different concentrations of 4-ABA solutions were measured with the 4-ABA imprinted sensor. The regression equations and coefficient correlation (R^2) were $y = 0.5448x + 6.782$ ($R^2 = 0.9981$), where the y and x are the peak current reduction (μA) of hexacyanoferrate and the concentration of analytes (μmol L^{-1}), respectively. The linear response ranges by the imprinted sensing film was 0.2–20.0 μmol L^{-1} for 4-ABA with the detection limit of 0.08 μmol L^{-1}.

Table 2 Results of the determination of GABA in germinates unpolished rice and recovery ($n = 5$)[a]

Compound	GABA found (μmol L^{-1})	Added (μmol L^{-1})	Found (μmol L^{-1})	Recovery (%)	R.S.D. (%)
GABA	1.22	2.0	3.26	103.3	4.0
		10.0	11.18	96.7	4.6

[a] All conditions are the same as Fig. 4B.

3.6 Measurement of selectivity

To verify the selectivity of the 4-ABA-MIP sensor, glutamic acid, aminopropionic acid were used to carry out the selectivity test of obtained sensor. The results are shown in Fig. 7. As we can see, no significant effects were observed upon addition of these interferents. The selectivity coefficient was defined as $K = \triangle i_r / \triangle i_m$, where $\triangle i_r$ is the corresponding relative current change of 10.0 μmol L^{-1} interfering material solution and $\triangle i_m$ is the relative current change of sample solution containing 10.0 μmol L^{-1} 4-ABA. The selectivity of the sensor is shown in Table 1. The results show that this sensor provides an efficient way for eliminating interferences from compounds with similar structures to that of 4-ABA, and exhibits good selectivity toward 4-ABA.

3.7 Measurement of repeatability and stability

To investigate the repeatability of the developed sensor, five sensors were fabricated independently under the same conditions. They were examined in 5 mmol L^{-1} of hexacyanoferrate solution after interaction with 10.0 μmol L^{-1} 4-ABA for 10 min. The standard deviation of the response obtained did not exceed 5% for 10 μmol L^{-1} 4-ABA. The repeatability of a single electrode was also estimated by determining the response of 5 mmol L^{-1} hexacyanoferrate solution after interaction with 10.0 μmol L^{-1} 4-ABA for 10 min. After the first detection, the electrode was immersed in distilled water for 48 h, and then washed by distilled water to extract the templates. Then, the second detection by the regenerated electrode was carried out. Afterwards, the electrode was washed again and the third detection was processed likewise. The coefficients of variation were calculated to be 3.8%. The good repeatability revealed that 4-ABA molecule can be reversible with the binding sites, and the MIP sensor could be regenerated and used repeatedly.

The performance of sensor does not change very much even if it is stored in air at room temperature. The current of sensor interacted with 10 μmol L^{-1} 4-ABA decreased by about 10% after 1 month of storage.

3.8 Real samples analysis

The sensor was applied to the determination of 4-ABA in Germinated Brown Rice. The obtained results are shown in Table 2. The concentration of 4-ABA in germinated brown rice was found to be 1.22 μmol L^{-1} and the mass content of 4-ABA in germinated brown rice can be finally calculated as 22.32 mg kg^{-1}. The average recoveries were from 96.7% to 103.3%, with a RSD less than 4.6%. It was demonstrated that this method was suitable for the determination of the total content of 4-ABA in Germinated Brown Rice.

4. Conclusions

In this paper, the MIP-4-ABA sensor has been shown to be a highly selective and sensitive molecularly imprinted sensor for determination of 4-ABA. The performance of the imprinted sites was assured by the electropolymerization of the imprinted *o*-PD. The imprinted polymeric layer has a high selectivity for 4-ABA. This sensor has been applied to analyze 4-ABA in Germinated Brown Rice samples successfully. The developed sensor may find its application in the field of food analysis.

Acknowledgements

The authors are grateful for the key Program of Industry-University cooperation of Fujian Province, China (2010N5004), and the National University Student Innovative Experiment Project, China (091038901).

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