Study and Application of Molecularly Imprinted Electrochemical Sensor Based on AuNPs/N-GR@CS for Highly Selective Recognition of Trace Hyperoside

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Establishing a high-selectivity and rapid detection technology for trace index components in complex samples is of great significance for real-time and on-site drug quality evaluation. In this study, a molecularly imprinted electrochemical sensor with highly selective recognition and detection of trace hyperoside was prepared using chitosan functionalized Nitrogen-doped graphene composite coated with gold nanoparticles (AuNPs-N-GR@CS) as electrode substrate modiﬁcation material, and the deposition of AuNPs further improved the conductivity of the modiﬁed electrode. With the aid of molecular imprinting technology, polymer ﬁlms with high selectivity and identiﬁcation of hyperoside were successfully prepared on glassy carbon electrodes (GCE) by self-assembly using hyperoside as template molecule and acrylamide as functional monomer. Because the acrylamide can accept protons through the oleinic double bond and ﬁrmly polymerize with each other, while it binds with hyperoside through hydrogen bonds. Therefore, the hyperoside can be easily dissociated in the eluate, which offers a condition for forming a molecularly imprinted polymer ﬁlm to highly select hyperoside. The highly conductive N-GR@CS modiﬁed at the bottom of the polymer ﬁlm provides the possibility to electrocatalyze hyperoside, and facilitate electron transfer to amplify the response signal. Under the optimized experimental conditions, the sensor showed a detection limit was 6.42 × 10⁻⁸ mol l⁻¹ (S/N = 3) with a good linear relationship in the range of 2.15 × 10⁻⁷ to 2.15 × 10⁻⁵ mol l⁻¹. Moreover, it displayed good reproducibility and stability, and could realize the direct and highly selective detection of trace hyperoside in complex samples. In consequence, this study is expected to provide a convenient and reliable method for on-site real-time evaluation of traditional Chinese medicine (TCM) quality with reference to the index component.

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Hyperoside is an index component of the quality standard for Hypericum perforatum1,2 in Chinese Pharmacopoeia. Modern pharmacological studies show anti-inﬂammatory, anti-cancer, anti-depression, and other activities.3–7 Therefore, it is of great signiﬁcance to establish a rapid, real-time and accurate method for analysis of hyperoside content in herbal materials, herbal preparations and human blood with the minimum blood concentration, which is used to control the quality of traditional Chinese medicine (TCM), preparations, and to evaluate the effectiveness of containing these drugs in preventing and treating diseases based on hyperoside.

Hyperoside, as a typical ﬂavonol glycoside compound, is composed of quercetin as the aglycon, and the 3-O is connected with the galactose pyranose by a glycosidic bond. Hyperoside has a P–π conjugated system between B ring and C2=C3 double bond, 4-position carbonyl group and the A ring, which is prone to electron delocalization and causes the 3” and 4” hydroxyl groups (–OH) to be highly reactive, so that hyperoside is prone to be involved in oxidation and reduction reaction. Therefore, the hyperoside in the samples can be detected using the electrical signal generated by the REDOX reaction of 3” and 4” hydroxyl groups with the electrochemical method. By means of documented research, there are few reports on the electrochemical detection of hyperoside. Although the most commonly used methods can realize the accurate and reliable analysis of hyperoside, including chromatography and chromatography-mass spectrometry.8–14 Nevertheless, complex sample pretreatment, particular analysis laboratory, and the requirement of professional executors greatly restrict the development of technology with on-site, real-time, and rapid detection of the indicative component. Therefore, it is urgent to develop efﬁcient, convenient, accurate, and reliable detection technology to solve these problems.

In recent years, electrochemical techniques have received extensive attention, in-depth research, and application in drug analysis and other ﬁelds15–19 because of their high sensitivity, fast analysis speed, portable equipment, low cost, and no complex sample pretreatment.18–20 Zhang et al. developed an electrochemical sensor for sensitive detection of honokiol by using the prepared graphene nanosheets.21 Liu et al. used the prepared three-dimensional highly porous gold ﬁlm (hp-Au) to modify the gold electrode (Au) to construct an electrochemical sensor, which can be used for rapid and sensitive detection of quercetin in beverage, food, and pharmaceutical samples.22 Li et al. successfully designed and constructed a novel, highly sensitive electrochemical sensor (ZrO2-SDS-SWCNTs/GCE) using glass carbon electrode (GCE) as the carrier, and the proposed sensor had also worked well on sensitive hyperin determination in natural species Abelmoschus manihot.23 Thus, electrochemical techniques have unique advantages in the ﬁeld of index component detecting.

As one of the factors affecting the analytical performance of the sensor, the electrode material plays a key role in improving the detection performance of the sensors. Among many substrates for modification electrodes, Nitrogen-doped graphene (N-GR), as a graphene-based carbon material with N-type semiconductor properties,24 not only preserves the excellent conductivity of single-layer graphene, but also effectively overcomes the agglomeration phenomenon caused by strong π–π stacking between graphene sheets due to the Van der Waals force. At the same time, the free carrier density of graphene, the conductivity and stability of the N-GR are also effectively improved.25 When N-GR is functionalized by chitosan (N-GR@CS), it will carry a large number

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of carboxyl groups and amino groups on its surface, resulting in N-GR@CS showing good film formation and adsorption, etc., which provide conditions for the construction of high-performance sensors. Yang and coworkers constructed an electrochemical sensor for polymer recognition and detection of Rhodamine B, using chitosan functionalized reduction of graphene oxide and ferrocene nanocomposites. In the meantime, the results showed that the detection limit of the sensor could reach 0.5 nM.

However, it is difficult to guarantee the high selectivity of the sensor when it is used to detect the index components in complex samples only depending on high-performance materials. With the development of molecular imprinting technology (MIT), molecularly imprinted electrochemical sensors using molecularly imprinted polymers (MIPs) as substrate materials have become an essential point in the application of MIT. The molecularly imprinted polymer (MIP) membrane based on the lock-key model can significantly improve this problem. It uses template molecules and functional monomers to form MIP, and then removes template molecules by simple elution to form cavity structures that can specifically identify embedded template molecules. Therefore, it is very high that the matching degree between MIP and the target-molecule to be measured. Based on this principle, Meng et al. used 3,4-ethylenedioxythiophene (EDOT) and pyrrole (Py) as bifunctional monomers, rutin as template molecule, and a molecularly imprinted polymer (MIP) with 3D worm-like nanorod structures was constructed by cyclic voltammetry (CV) polymerization on a glassy carbon electrode (GCE). The optimal polymerization conditions of MIP were obtained by orthogonal experiment. Rutin was detected at concentrations ranging from 0.5 nM to 1 μM, and 5 to 50 μM with the low detection limit as 0.24 nM (S/N = 3). The rutin content in Flos Sophorae Immaturus (FSI) was 25.37% with good accuracy.

Therefore, the construction of molecularly imprinted electrochemical sensors with the aid of high-performance materials and MIP has become an essential issue to develop nanocomposites. In the meantime, the results showed that the functional monomers to form MIP, and then removes template molecules by simple elution to form cavity structures that can specifically identify embedded template molecules, which has a “grasper” to promote and improve the sensitivity and selectivity of index component analysis.

In the present study, a new type of molecularly imprinted electrochemical sensor based on AuNPs/N-GR@CS for highly selective and sensitive detection of hyperoside in complex samples was described. Chitosan functionalized N-doped graphene composite coated with gold nanoparticles (AuNPs/N-GR@CS) was modified on the surface of the glassy carbon electrode by electrochemical polymerization. Then, hyperoside was used as the template molecule and acrylamide as the functional monomer by self-assembly, a molecularly imprinted polymer membrane for specific selective recognition of hyperoside was prepared. Finally, an electrochemical molecularly imprinted sensor for highly selective recognition and detection of trace hyperoside was successfully constructed. In addition, the described convenient and reliable method is expected to provide ideas and methods for implementing technics with on-site, real-time, and rapid evaluating the quality of TCM based on the index components.

**Experimental**

**Apparatus.**—All electrochemical tests were carried out on the CHI660E electrochemical workstation (Shanghai Chenhua Instrument), which consists of a traditional three-electrode system: modified glassy carbon electrode (GCE) was the working electrode, saturated calomel electrode was used as reference electrode, and platinum wire electrode was used as the auxiliary electrode. SB-5200DTD Ultrasonic Cleaner (Ningbo Xinzhi Biological Technology) was used for ultrasonic cleaning. The sample solution was extracted by rotating evaporator RE-52A (Shanghai Bilang Instrument). The pH of the solutions was adjusted with the PHS-3D pH meter (Shanghai Precision Scientific Instrument). IFS66v/S infrared spectrometer (Bruker, Germany), LabRAM HR Evolution Micro Confocal Raman Spectrometer (HORIBA Jobin Yvon S.A.A., France), and JSM-6701F cold field emission scanning electron microscope (Japan Electro-Optics Corporation) were used to characterize the prepared modification material and electrodes.

**Chemicals and reagents.**—Hyperoside (Shanghai Aladdin Biochemical Technology), acrylamide (AA) (Shanghai guangnuo Chemical Technology), chloroauric acid HAuCl₄ (Tianjin kemio Chemical Reagent), chitosan (CS) (SANE Chemical Technology (Shanghai)), N-doped graphene (N-GR) was purchased from Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, China). Hypericum perforatum (picked from Shanmen Town, Qingshui County, Tianshui City, Gansu Province, China). Acetate buffer solution (ABS) was made by mixing 1.0 mol l⁻¹ gluconic acid (CH₂(OH)₄ ⩾99.5%, China Tianjin Fuyu Fine Chemical) with 1.0 mol l⁻¹ sodium acetate (CH₃COONa, ⩾99.0%, Tianjin Guangfu Science and Technology Development) was prepared by mixing the solutions in a certain proportion. The chemical reagents were of analytical grade and distilled water was used throughout the experiment.

**Preparation of N-GR@CS composite solution.**—20.00 mg chitosan was added to 100 ml 1% acetic acid solution, and stirred continuously until clear and transparent CS solution was produced without bubbles, then stored at 4 °C for use. Adding 10.00 mg N-GR into 10 ml distilled water, ultrasonic 2 h to get an evenly dispersed N-GR suspension. 5 ml N-GR suspension was added to 10 ml chitosan solution, ultrasonic dispersion for 30 min to make N-GR evenly distributed in the chitosan solution, the solution of N-GR@CS composite material was obtained.

**Preparation of N-GR@CS modified GCE (N-GR@CS/GCE).**—Before modification, exposed GCE was polished to mirror surface with 0.30 and 0.05 μm alumina powder on suede successively, then washed with methanol and distilled water for 10 min in an ultrasonic bath, and dried in air. The GCE modified by N-GR@CS (N-GR@CS/GCE) was obtained by carefully dripping 20 μl of the composite solution onto the GCE and drying it under an infrared lamp.

**Preparation of AuNPs/N-GR@CS/GCE.**—The modified electrode N-GR@CS/GCE was placed in 10 ml of 0.10 mol l⁻¹ H₂SO₄ solution containing 1.00 mM HAuCl₄, scanned for 15 cycles by cyclic voltammetry (CV) at the rate of 50 mV s⁻¹ in the potential range of −0.80 ~ 1.20 V, and Au nanoparticles were deposited to obtain AuNPs/N-GR@CS/GCE.

**Preparation of MIP/AuNPs/N-GR@CS/GCE.**—The assembly solution was prepared by simultaneously dissolving 6.00 mM acrylamide and 1.00 mM Hyperoside in 10 ml methanol. AuNPs/N-GR@CS/GCE self-assembled in the assembly solution for 12 h. CV was carried out in 13 cycles at −0.10 ~ 1.00 V and 50 mV s⁻¹, unbound and loosely bound templates and monomers were washed with distilled water. Subsequently, it was immersed in 9:1 (V/V) methanol/glacial acetic acid for 15 min to elute the template molecules and thoroughly dried in the open air to obtain MIP/AuNPs/N-GR@CS/GCE. Non-imprinted polymer (NIP) was synthesized by the same procedure without a template to obtain NIP/AuNPs/N-GR@CS/GCE.

**Preparation of sample solution.**—A certain amount of Hypericum perforatum (100.00 mg) was put into a volumetric flask and dissolved with 10 ml methanol. Hyperoside was extracted by ultrasound for 3 h and centrifuged at 5000 rpm for 10 min. Repeat 3 times to ensure complete extraction. All extracted solutions were put together and concentrated to 10 ml in a chemical hood for analysis. Before each measurement, the sample solution was diluted with a supporting electrolyte of 20 μl.
Results and Discussion

Characterization of modified materials.—Fourier transform infrared spectroscopy (FTIR) was utilized to characterize N-GR, CS, and N-GR@CS (Fig. 1A). The characteristic absorption peaks of N-GR in 1557 and 1182 cm\(^{-1}\) were ascribed to C=N and C–N bonds, respectively, due to the doping of N atoms into the graphene. The CS spectrum shows a typical characteristic absorption band. At about 3400 cm\(^{-1}\), it is multiple absorption peaks of \(-\text{OH}\) stretching vibration absorption peak forming hydrogen bond association and the \(-\text{NH}\) stretching vibration absorption peak overlapping and widening. C–H stretching vibration absorption peak at 2871 cm\(^{-1}\). At 1588 cm\(^{-1}\) is the characteristic absorption peak of \(-\text{NH}_2\) in amide \(\text{II}\) in CS. The characteristic peak band corresponding to 1375 cm\(^{-1}\) is the C–H bending and \(-\text{CH}_3\) symmetrical deformation vibration absorption peak. The firm absorption peaks of C–O stretching vibration are shown at 1149, 1024 and 893 cm\(^{-1}\). FT-IR spectra of N-GR@CS composites showed a strong absorption peak at 1543 cm\(^{-1}\) corresponding to the amide group (\(-\text{CONH}\))–. This unique characteristic absorption peak clearly indicated the formation of a new bond between CS and N-GR, and the results proved that N-GR@CS was successfully synthesized.

The Raman spectrum is often used to characterize some carbon materials, such as graphite, carbon nanotubes, graphene, etc. The variation of the Raman spectrum is very sensitive to the doping, intercalation, defects, and chemical modification of carbon materials. As shown in Fig. 1B, both N-GR and N-GR@CS electrode materials had characteristic peaks around 1350, 1360 and 1580, 1600 cm\(^{-1}\), respectively, corresponding to the D characteristic peak and G characteristic peak of the graphene material. The D band of graphene is caused by sp\(^2\) hybridized carbon atoms, and the G band is caused by sp\(^3\) hybridized carbon atoms. Raman spectra showed that CS was successfully inserted into the N-GR surface to form an N-GR@CS composite, and no damage was caused to N-GR materials. The result was consistent with the FTIR spectrum.

Scanning electron microscopy (SEM) was used to characterize the morphology of N-GR, N-GR@CS, MIP/AuNPs/N-GR@CS before and after elution. As shown in Fig. 1, N-GR (Fig. 1C) had an obviously thick folded two-dimensional sheet structure, which significantly increased the specific surface area of graphene and provided more active sites for the enrichment of small electroactive compounds and adsorption of biomolecules. N-GR@CS (Fig. 1D) could see many obvious rod-like structures, while the bright spots were rod-like. The surface of N-GR@CS was relatively smooth and wrinkles were significantly reduced, indicating that CS was wrapped around N-GR, and the two were successfully combined. The addition of chitosan could make N-doped graphene film better on the electrode and improve the stability of modified electrodes.

Figures 1E and 1F respectively showed SEM images of MIP/AuNPs/N-GR@CS before and after elution. In Fig. 1E, a large number of small dense particles could be clearly seen, and some particles were highlighted with bright spots. Different from Fig. 1E, the surface of MIP/AuNPs/N-GR@CS (Fig. 1F) after elution was smoother as if a film was formed. The granular material became less dense, and glimmering holes were revealed. The reason may be that in the elution process, only part of the template molecules and the combination of functional monomers so that some tiny pores stand out. Therefore, the specific porous structure of the MIP contributed to increasing the specific surface area of the electrode, which improved the selectivity and responsiveness of the sensor to hyperoside.

Electrochemical characterization of sensor preparation.—The characteristics of the preparation process of molecularly imprinted
electrochemical sensors were studied by CV and electrochemical impedance spectroscopy (EIS) (Figs. 2A and 2B). Figure 2A showed that the current response of N-GR@CS/GCE (curve b) was significantly improved compared to bare GCE (curve a), with a significant increase in peak current due to its excellent conductivity. Furthermore, when Au nanoparticles were deposited, the redox peak current of AuNPs/N-GR@CS/GCE (curve c) rose again, indicating the high specific surface area and affinity of Au nanoparticles and the high conductivity of the former occurred excellent combination.

When the MIP (curve d) was made on the modified electrode, the peak current was significantly reduced, the REDOX reaction that occurred through the polymer flowing to the electrode surface was partially blocked because of $[\text{Fe(CN)}_6]^{3-/-4-}$. When the template molecule was removed, the peak current increased again (curve e), indicating that specific pores were created in the polymer that facilitate the transfer of probe electrons to the electrode surface, increasing the peak current. Curve f was the case where no template molecule was added.

EIS was used to investigate further template formation and its interaction with hyperoside to characterize the progressive modification of MIP/AuNPs/N-GR@CS/GCE. In the Nyquist diagram of Fig. 2B, regular semicircles could be observed in the high-frequency region with diameters equal to the electron transfer resistance, in other words, depending on the conductivity of the interface between electrode and electrolyte. Compared with bare GCE (curve a), the modified electrode was coated with N-GR@CS composite material (curve b) and Au nanoparticles (curve c) to obtain a smaller diameter, indicating that AuNPs/N-GR@CS/GCE further improved the electron transport rate. Then, when the polymer (curve d) was formed on the decorative electrode, the diameter of the semicircle
increased significantly, because hyperoside was filled in the pores of the polymer, which hindered the electron transfer path and reduced the electron transfer rate. After elution of template molecules (curve e), the semicircle diameter was significantly reduced because many pores formed on the surface of the modified electrode were exposed again, which promoted the diffusion of ions to the electrode surface and reduced the charge transfer resistance. The results were in agreement with those obtained by CV measurement.

The electrode surface area is directly related to sensor response signal and detection sensitivity. The effective electrochemical surface areas of GCE, N-GR@CS/GCE, MIP/AuNPs/N-GR@CS/GCE before and after elution in $1.0 \times 10^{-4}$ mol l$^{-1}$ K$_3$[Fe(CN)$_6$] (containing 0.1 mol l$^{-1}$ KCl) were compared by the Chronocoulometric method (as shown in Fig. 2C). Setting of relevant experimental parameters: initial potential $= -0.20$ V, termination potential $= 0.70$ V, step count $= 1.00$, pulse width $= 0.062$ s, resting time $= 5.00$ s, sampling interval $= 2.0 \times 10^{-6}$ s. The corresponding $Q^{-1/2}$ curve was shown in Fig. 2D. According to Anson formula:

$$Q = \frac{2nFAc(Dt)^{1/2}}{\pi^{1/2}} + Q_{di} + Q_{ads}$$

Where A is the surface area of the electrode, c is the material concentration, F is the Faraday electrolysis constant, D is the diffusion coefficient, $Q_{di}$ is the double charge, $Q_{ads}$ is the Faraday charge, and n is the number of electron transfer. For $1.0 \times 10^{-4}$ mol l$^{-1}$ K$_3$[Fe(CN)$_6$] (containing 0.1 mol l$^{-1}$ KCl), n = 1, D = 7.6 $\times$ $10^{-6}$ cm$^2$ s$^{-1}$, the effective surface area of the electrode could be calculated. The A values of GCE(a), N-GR@CS/GCE(b), MIP/AuNPs/N-GR@CS/GCE before and after elution(c), MIP/AuNPs/N-GR@CS before elution(d) were 0.076 cm$^2$, 0.148 cm$^2$, 0.246 cm$^2$ and 0.281 cm$^2$, respectively. The results showed that compared with the bare electrode, the surface area of the modified MIP/AuNPs/N-GR@CS/GCE electrode was significantly increased, the electrochemical response signal was significantly enhanced, and the sensitivity of hyperoside detection was improved.

**Optimization of experimental conditions.— N-GR@CS selection of modification amount.**—The amount of modified material on the modified electrode will affect the electron transfer rate, and the current response will increase with the modification amount. When the modified N-GR@CS was 20 $\mu$l, the prepared sensor would generate the maximum peak current and had the best performance. The response signal decreases as more modifications were added, as shown in Fig. 3A. The reason may be that the high amount of modification makes the composite material accumulate, and the electron transfer rate is impeded. Therefore, the amount of 20 $\mu$l was selected in the subsequent experiment.

**Selection of template molecule/functional monomer molar ratio.**—Since the interaction between the template molecule and functional monomer is the main factor for site identification in the polymerization reaction, selecting of the molar ratio of functional monomer to template molecule is very important for the performance of the polymer. The molar ratio of functional monomer to
Figure 3. (A) The influence of N-GR@CS modification amount on peak current. (B) The influence of template molecule and functional monomer ratio on peak current. (C) The influence of different self-assembly time on peak current. (D) The influence of elution time on peak current. (E) CV curve of hyperoside in different buffer solution systems. (F) CV curve of hyperoside at different pH values (a to h: 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5). (G) The relationship between pH value and peak current. (H) The relationship between pH value and peak potential (Epa). (I) CV curves of hyperoside different scan rates. (J) The relationship between peak current and scanning rate. (K) The relationship between the peak current and the square root of the scan rate. (L) The relationship between the peak potential and the logarithm of the scan rate.
template molecule was optimized by preparing a series of MIP/AuNPs/N-GR@CS/GCE with different assembly solution ratios (1:1 ~ 10:1). As shown in Fig. 3B, the sensor prepared with the molar ratio of 6:1 functional monomer to template molecule had the maximum peak current. Other sensors prepared with more or less monomer had a weak current response. In other words, when the mole ratio was small, the polymer could not effectively form hyperoside binding sites on the electrode surface, and when the number of functional monomers gradually increases, it would lead to high cross-linking, low printing, template embedding, and other problems, thus affecting the electronic transmission rate and detection accuracy of the prepared sensor. Therefore, the functional monomer/template molecule ratio was selected as 6:1 for the experiment.

**Selection of self-assembly time.**—The proper density of the monomer layer is associated with the formation of MIP structure, and incubation time is another factor affecting the differential pulse method (DPV) response of MIP to the target molecule. Therefore, selecting an appropriate incubation time can effectively improve the response sensitivity of hyperoside on the molecularly imprinted film. As shown in Fig. 3C. With the increase of self-assembly time, the number of functional monomers attached to the electrode surface increases, which could be used to identify hyperoside and form a specific site for it. The peak current at the maximum incubation time was 12 h. However, as the incubation time continued to increase, the peak current showed a downward trend, which might include the recognition sites in the molecularly imprinted film had been basically occupied, and the adsorption tended to be saturated, hindering electron transfer. Therefore, 12 h was chosen as the best incubation time.

**Optimization of elution time.**—Completing elution of template molecules is essential to obtain a satisfactory sensor. A mixture of methanol and glacial acetic acid was used as the eluent, and the elution time was optimized from 1 to 20 min. The results were shown in Fig. 3D. By immersing the sensor in 1:9 (V/V) glacial acetic acid/methanol for 15 min, the template molecule could be effectively removed to obtain the maximum peak current, and the integrity and stability of the membrane could be maintained under such elution conditions. When the elution time was short, the template molecule was not easy to be completely elution removed, and there were few imprinting recognition sites left in the polymer, which was not conducive to the detection of hyperoside by MIP membrane. Similarly, a longer elution time might destroy the structure of the membrane itself, destroying the recognition site, which was also not conducive to the detection of hyperoside by the MIP membrane. Therefore, 15 min was selected for elution in subsequent experiments.

**Choosing a buffer system.**—The effect of 0.1 mol l$^{-1}$ acetate buffer (ABS), phosphate buffer (PBS), and citrate buffer (CPBS) on the electrochemical response of the sensor was studied. From the experimental results (Fig. 3E), it could be concluded that the sensor had a more stable baseline and relatively higher sensitivity in ABS. Finally, ABS was selected as the supporting electrolyte in this study.

**Influence of optimum pH value of buffer solution.**—After optimizing the buffer solution system, ABS buffer solution was selected for the experiment. The pH value mainly determines the existence of hyperoside bound to MIP in molecular form. Therefore, the electrochemical behavior of hyperoside was optimized in acetate buffer solutions (ABS) with different pH values. Figure 3G showed
the change of peak current between pH 3.0 and 6.5. The peak current reached its maximum value at pH 5.0. However, when pH continued to increase, hyperoside existed in ionic form and had weak hydrogen bond interaction with AA, which weakened the fixation effect gradually.

Therefore, pH 5.0 was selected as the best pH for subsequent experiments. The electrochemical behavior of hyperoside in ABS with different pH (3.0 ~ 6.5) was also studied by CV. As shown in Fig. 3F, the oxidation peak potential moved negatively with the increase of pH, indicating that H\(^+\) was involved in the REDOX reaction. There was a good linear relationship between peak potential (E\(_{pa}\)) and pH, as shown in Fig. 3H, which was E\(_{pa}\)(V) = -0.0658 pH + 3.169 (R\(^2\) = 0.9913). The slope was 0.0658. Comparison with theoretical slope: According to Nernst equation:\(^\text{16}\)

\[
dE_{pa}/d\text{pH} = 2.303mRT/nF
\]

Where R, T, and F are constants, m and n are the numbers of transferred protons and electrons, respectively, and m/n = 1.0943 \(\approx\) 1 (m is the number of transferred protons and n is the number of transferred electrons). In other words, the ratio of proton to the electron in the hyperoside electrode reaction was 1:1.

**Influence of scanning rate on modified sensor.**—Figure 3I showed the CV curves of hyperoside at different scanning rates (10 mV s\(^{-1}\) ~ 300 mV s\(^{-1}\)). With the increased scanning rate, the peak current of hyperoside on the modified electrode increases, and the peak potential gradually shifts. The relationship between peak current and peak potential and scanning rate can reflect the reaction process on the electrode surface. If the reaction on the electrode surface is controlled by adsorption, the peak current and sweep speed have a linear relationship, indicating that the reaction on the electrode surface is mainly controlled by adsorption. As shown in Fig. 3J, I_{pa} = 1.0189 \times 10^{-6}v + 8.0422 \times 10^{-5} (R^2 = 0.9701) and I_{pc} = -9.9760 \times 10^{-7}v + 7.7843 \times 10^{-5} (R^2 = 0.9688), this indicated that the redox reaction of hyperoside on manufactured sensors was controlled by adsorption. If the reaction on the electrode surface is controlled by diffusion, its peak current is proportional to the square root of the sweep speed, indicating that the reaction on the electrode surface is mainly controlled by diffusion. As shown in Fig. 3K, I_{pa} = 2.2794 \times 10^{-5}v^{1/2} - 3.0965 \times 10^{-5} (R^2 = 0.9997) and I_{pc} = -2.2329 \times 10^{-5}v^{1/2} + 3.1340 \times 10^{-5} (R^2 = 0.9999), this indicated that the redox reaction of hyperoside on manufactured sensors was controlled by diffusion. Meanwhile, as shown in Fig. 3L, oxidation peak potential and reduction peak potential were proportional to the logarithm of scanning rate within a certain range. The linear relationship was expressed as E_{pa} = -0.1991logv (mVs\(^{-1}\)) + 0.3804 (R\(^2\) = 0.9059) and E_{pc} = 0.2278logv (mVs\(^{-1}\)) + 0.0126 (R\(^2\) = 0.9567). According to Laviron equation,\(^\text{37}\) some parameters related to the electrochemical mechanism can be calculated:

\[
E_{pa} = E^\theta - \frac{2.303RT}{(1 - \alpha)F} \log v
\]

\[
E_{pc} = E^\theta - \frac{2.303RT}{\alpha F} \log v
\]

\[
\log k = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log \frac{RT}{nF} = \alpha(1 - \alpha) \frac{nF\Delta E_{pa}}{2.303RT}
\]

According to formula 1 and 2, the electron transfer coefficient \(\alpha = 0.50\), and the electron transfer rate constant \(k = 1.16\text{ s}^{-1}\) were calculated from Formula 3. Conjecture mechanism of hyperoside redox process of MIP/AuNPs/N-GR@CS/GCE (as shown in the Scheme 1). In summary, the redox reaction of hyperoside on manufactured sensors was controlled by both adsorption and diffusion.

**Linear range and detection limit.**—Under the optimized experimental conditions, the DPV response of hyperoside with different concentrations (2.15 \times 10^{-7} \sim 2.15 \times 10^{-5} mol l\(^{-1}\)) in 4.00 mmol l\(^{-1}\) K\(_2\)[Fe(CN)\(_6\)]/K\(_3\)[Fe(CN)\(_6\)] electrolyte solution containing 0.01 mmol l\(^{-1}\) KCl was determined by MIP using DPV with high sensitivity. Figure 4A showed the DPV distribution generated by adding hyperoside with the prepared sensor in the concentration range of 2.15 \times 10^{-7} to 2.15 \times 10^{-5} mol l\(^{-1}\). As shown in Fig. 4B, the linear equation was I_{pa} = -1.7324C + 3.9255 \times 10^{-5} (R^2 = 0.9904), the linear range was between 2.15 \times 10^{-7} and 2.15 \times 10^{-5} mol l\(^{-1}\), and the detection limit was 6.42 \times 10^{-8} mol l\(^{-1}\) (S/N = 3). By comparing this method with other reported methods (Table 1), it was found that this method had a wider linear range and a good detection limit.

**Selectivity, reproducibility and stability.**—Good selectivity is crucial for electrochemical sensors to study the electroactive constituents of medicinal plants. To investigate the interference of

![Figure 4](image-url) (A) DPV curves of with hyperoside different concentrations (a to k: 2.15 \times 10^{-7} \sim 2.15 \times 10^{-5} mol l\(^{-1}\)) on MIP/AuNPs/N-GR@CS/GCE in 4.00 mmol l\(^{-1}\) K\(_2\)[Fe(CN)\(_6\)]/K\(_3\)[Fe(CN)\(_6\)] electrolyte solution containing 0.01 mmol l\(^{-1}\) KCl. (B) The linear relationship between the peak current and the concentration of hyperoside on the manufactured sensor.
various inorganic ions and molecules on hyperoside determination, a certain amount of possible interference substances was added to hyperoside (20.00 μmol l⁻¹) for DPV determination. Including the mixture of rutin, glucose, vitamin C, quercetin, proanthocyanidins, carotene, tannins (200.00 μmol l⁻¹), and Cu²⁺, Na⁺, Ca²⁺ (2.00 mmol l⁻¹) were selected as the coexistence interference of hyperoside to study the selectivity of the sensor, the concentration of inorganic ions was 1000-fold higher than hyperoside, and the molecular concentration was 10-fold higher than hyperoside. As shown in Fig. 5, when hyperoside coexisted with each distractor, the RSD of peak current from each mixture was less than 5.00%, which was 3.58%, indicating that the determination of hyperoside was not significantly affected by the interferences with high concentration. Therefore, the MIP/AuNPs/N-GR@CS/GCE sensor has good selectivity and anti-interference ability.

Four MIP/AuNPs/N-GR@CS/GCE electrodes were prepared in parallel, and hyperoside (20.00 μmol l⁻¹) was determined by DPV. The RSD of peak current from the four electrodes was 3.41%, indicating that the prepared sensor had good reproducibility. The electrode was placed at 8 °C for 10 d and 20 d, and the peak current was measured by adding hyperoside (20.00 μmol l⁻¹) into the electrolyte solution, compared with the initial electrode, the electrochemical response decreased to 94.50% and 92.30%, respectively, indicating that the modified sensor has good stability.

Analysis of actual samples.—20 μl extract was accurately transferred to 4 mmol l⁻¹ 10 ml electrolytes containing 0.01 mmol l⁻¹ KCl in K₃[Fe(CN)₆]/K₄[Fe(CN)₆] and mixed evenly. After the system was stabilized, the content of hyperoside was determined by DPV and CV with the standard addition method. The results were shown in Table II. The sensor expressed a sensitive, fast and straightforward detection for hyperoside in biological samples with satisfactory recoveries from 98.70% to 101.20%, clarifying that the proposed method could accurately determine hyperoside in actual samples.

Conclusions

In this study, a simple and practical molecularly imprinted electrochemical sensor for highly selective recognition of trace hyperoside was successfully constructed. Chitosan functionalized Nitrogen-doped graphene composite coated with gold nanoparticles (AuNPs/N-GR@CS) was used as the electrode substrate to modify the glassy carbon electrode. Subsequently, the molecularly imprinted polymer films with hyperoside as template molecule and acrylamide as functional monomer for high selectivity and identification of hyperoside were prepared on AuNPs/N-GR@CS surface by self-assembly method, which was used to fabricate the hyperoside electrochemical molecularly imprinted sensor. According to the experimental results, the prepared MIP could ensure the selectivity.

Table I. Comparison of different analytical methods for the detection of hyperoside.

| Detection methods | Linear range (mol l⁻¹) | Detection limit (mol l⁻¹) | References |
|------------------|------------------------|---------------------------|------------|
| HPLC             | 2.15 × 10⁻⁵ ~ 2.15 × 10⁻⁴ | 0.15 × 10⁻⁶                   | 9          |
| HPLC             | 1.08 × 10⁻⁶ ~ 2.15 × 10⁻⁵ | 1.08 × 10⁻⁶                   | 38         |
| HPLC             | 2.15 × 10⁻⁸ ~ 1.94 × 10⁻³ | 7.11 × 10⁻⁷                   | 11         |
| Capillary electrophoresis | 2.76 × 10⁻⁶ ~ 1.12 × 10⁻⁵ | 2.76 × 10⁻⁶                   | 39         |
| ZrO₂-SDS-SWCNT/GCE | 1.0 × 10⁻⁶ ~ 3.0 × 10⁻⁷ | 5.0 × 10⁻¹⁰                   | 23         |
| c-MWCNT/CPGE     | 2.2 × 10⁻⁷ ~ 3.0 × 10⁻⁵ | 1.0 × 10⁻⁷                   | 40         |
| PDDA-Gr/GCE      | 7.0 × 10⁻⁹ ~ 7.0 × 10⁻⁸ | 5.0 × 10⁻⁹                   | 41         |
| α-Fe₂O₃-GR/GCE   | 1.0 × 10⁻⁹ ~ 3.0 × 10⁻⁷ | 5.0 × 10⁻¹⁰                   | 42         |
| MIP/AuNPs/N-GR@CS/GCE | 2.15 × 10⁻⁷ ~ 2.15 × 10⁻⁵ | 6.42 × 10⁻⁸                   | This work |

Figure 5. Comparison of the influence of peak current and different interfering substances on the determination of hyperoside.
of the sensor for detecting hyperoside. The electrochemical behavior of hyperoside was also studied. A large specific surface area and highly conductive of AuNPs/N-GR@CS gives hyperoside countless applications. Under the optimal experimental conditions, MIP/AuNPs/N-GR@CS/GCE showed satisfactory analytical performance with a wide linear range, low detection limit, and high stability during the storage and handling of the sensor. Furthermore, the application of this sensor to detect hyperoside in complex samples (Hypericum perforatum) exhibited satisfactory results. This study provided a technical reference for the development of on-site, real-time, and rapid quality evaluation of TCM based on index components.

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Table II. Determination of hyperoside in real samples (Hypericum perforatum).

| Sample | Added (μmol l⁻¹) | Found (μmol l⁻¹) | Recovery (%) | RSD (%) |
|--------|-----------------|-----------------|--------------|--------|
| 1      | 0               | 19.79           | 101.2        | 2.02   |
| 2      | 5               | 24.85           | 98.7         | 0.82   |
| 3      | 10              | 29.66           | 100.2        | 1.80   |
| 4      | 15              | 34.82           | 100.2        | 0.85   |

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