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Potentiometric Determination of Total Polyphenols in Green Tea Based on Complexation-Reaction-Induced Response

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A novel potentiometric approach for the determination of total tea polyphenols is described in this paper. The proposed approach is based on the complexation reactions of polyphenols with metal ions (e.g., Cu^{2+}) and the depletion of copper ions is monitored by a solid-contact Cu^{2+}-selective polymer membrane electrode. The potential change induced by the complexation reaction can be used for reliable detection of polyphenols. By using epigallocatechin gallate (EGCG) as a model of tea polyphenols, the potentiometric sensor shows a linear response over the concentration range of 0.01 g/L to 0.1 g/L with a low detection limit of 3.2 mg/L. It has also been found that the detection system is not susceptible to interference by other organic substances associated with polyphenols analysis such as amino acid, organic acid, caffeine and vitamin C at their usual levels in green tea. The practical application of the proposed system has been successfully applied to the determination of polyphenols in real green tea samples.

Keywords: Cu^{2+}-selective polymer membrane electrode; complexation reaction; polyphenols; determination; green tea

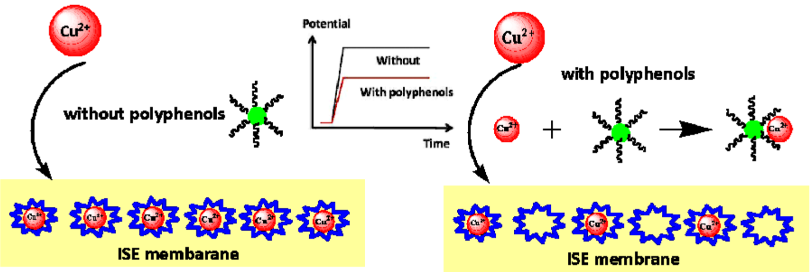
1. INTRODUCTION

During recent years, green tea has attracted broad attention since the consumption of green tea may help prevent several chronic diseases such as cardiovascular disease and cancers [1, 2]. It is believed that polyphenols in green tea are attributed to these health benefits [1]. Therefore, the content of total polyphenols has been considered as one of the most important quality indexes of green tea [3, 4]. In this regard, it is important to develop efficient analytical methods for the determination of total polyphenols in green teas.

The traditional technique for polyphenols detection is colorimetric Folin–Ciocalteu method [5]. However, this approach usually yields an overestimation of polyphenols because Folin–Ciocalteu reagent interacts with other different reducing nonphenolic substances in green tea. The accurate determination of polyphenols can be achieved by chromatography coupled with various detection systems, or near infrared spectroscopy [6-8]. Nevertheless, these analytical systems require expensive instrumentation and time-consuming sample pretreatment. Highly sensitive, cost-effective and miniature electrochemical sensors have been used as alternative methods to determine the total tea polyphenols. Electrochemical sensors based on voltammetry have been prepared for the rapid and sensitive detection of polyphenols [9, 10]. As generic and highly successful approaches to chemical sensing, potentiometric sensors have shown to be very promising for trace-level environmental and biological measurements. Unfortunately, to date, none of potentiometric sensors has been exploited in the analysis of polyphenols in green tea [11, 12].

A few potentiometric sensors have been developed for the determination of phenolic compounds. Polymeric membrane ion-selective electrodes (ISE) containing lipophilic quaternary ammonium salt shows anionic responses to neutral and deprotonated phenols [13, 14]. However, such sensors show rather poor selectivities and limited sensitivities because the potential response is governed dominatedly by the lipophilicity of phenols. This poses serious limits to their use for the analysis of polyphenols in green tea. Although we have developed a potentiometric sensor based on a molecularly imprinted polymer (MIP) as a receptor for sensitive and selective determination of neutral bisphenol A [15], this sensing methodology is still unsuitable for the analysis of polyphenols because only one of polyphenols in tea sample can be detected owing to the presence of the selective MIP receptor. So the aim of this work is to provide an indirect potentiometric technique for analysis of tea polyphenols.

It has been well established that tea polyphenols form stable complexes with metal ions (e.g., Cu^{2+}, Al^{3+} and Fe^{3+}) [16-18]. Inspired by this, a new potentiometric detection system based on complexation-reaction-induced response is proposed in this work. In this system, epigallocatechin gallate (EGCG) has been chosen as a model of green tea polyphenols since it is the key active component in tea for chemoprevention against cancer and the main polyphenolic constituent of extracts of green tea. Copper ion is used as the indicator ion to indicate the potential change. When the complexation reaction between EGCG and Cu^{2+} occurs, the consumption of copper ions is monitored by a solid-contact Cu^{2+}-selective polymer membrane electrode. The resultant potential changes can be utilized for the determination of total polyphenols in green tea. The representation of the potentiometric detection of polyphenols is shown in Scheme 1.



Scheme 1

2. EXPERIMENTAL

2.1 Materials and reagents

High molecular weight poly (vinyl chloride) (PVC), *o*-nitrophenyl octylether (*o*-NPOE), tetradodecylammonium tetrakis (4-chlorophenyl) borate (ETH 500), poly (3-octylthiophene) (POT), epigallocatechin gallate (EGCG, from green tea) were purchased from Sigma-Aldrich. Cu^{2+} ionophore N,N,N’,N’-tetradodecyl-3,6-dioxaoctane-dithioamide (ETH 1062) and sodium tetrakis [3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB) were Selectophore from Fluka AG (Buchs, Switzerland). Tetrahydrofuran (THF) was freshly distilled prior to use. All other reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. Aqueous solutions were prepared with freshly deionized water (18.2 MΩ cm specific resistance) obtained with a Pall Cascada laboratory water system.

2.2 Preparation of solid-contact Cu^{2+}-ISE

Cu^{2+}-selective membrane was prepared by dissolving 200 mg of components (in wt %) in 2.0 mL of THF: ionophore (ETH 1062) (1), lipophilic cation-exchanger NaTFPB (0.5), PVC (33) and *o*-NPOE (65.5). The membrane cocktail was degassed by sonication for 10 min before use.

The glass carbon electrodes were carefully polished using alumina nanoparticles (500 nm), thoroughly rinsed with water and then bathed for 5 min in acetone. After that, they were completely air-dried. Then the conducting polymer POT was deposited on glass carbon surface by drop-casting 50 μL of a 25 mM chloroform solution and the solvent was left to evaporate at least 5 min. The electrode was then introduced into a fitting PVC tube at a depth of 1 mm, allowing the casting of ∼100 μL of membrane cocktail on the top of the POT layer, and left to dry at room temperature. Before use, the electrodes were conditioned in 10^{-3} M $CuCl_2$ solution for 1 d.

2.3 EMF measurements

All electromotive force measurements (EMF) were carried out at 25 °C using a CHI 760D electrochemical workstation (Shanghai, China) in the following galvanic cell: SCE/1 M LiOAC/sample solution/Cu^{2+}-sensing membrane/POT/glass carbon. The EMF values were corrected for liquid junction potentials using the Henderson equation, and ion activities were calculated according to the Debye-Hückel approximation [19].

2.4 Procedures for real sample analysis

The green tea samples were ground and sieved with a mesh width of 500 μm before analysis. The tea powder (0.1 g) was infused in 500 mL boiling water and for 20 min with N_2 protection. After cooling to ambient temperature**,** the infusion was filtered with 0.22 μm PVDF filter disk. Then, 10^{-4} M $CuCl_2$ was added into the extracts solution and the concentration of Cu^{2+} was determined by using the fabricated potentiometric sensor under N_2 atmosphere.

3. RESULTS AND DISCUSSION

3.1 Response characteristics of solid-contact Cu^{2+}-ISE

Solid-contact ion-selective polymer membrane electrodes, which have an electrically conducting polymer as the ion-to-electron transducer between the polymeric membrane and electrode interface, are attractive for application in clinical chemistry, industrial analysis and environmental monitoring [20-23]. Due to the elimination of the internal reference aqueous solution, the solid-contact potentiometric sensor is easy to be miniaturized and commercialized; in addition, its instrument is simple, reliable and cheap [24, 25]. In this work, a solid-contact solvent polymeric membrane Cu^{2+}-selective electrode was fabricated as the indicator for tea polyphenols determination using POT as the ion-to-electron transducer. Since many Cu^{2+}-selective polymer membrane electrodes have been successfully developed [26-29], the membrane composition of the proposed electrode was not optimized and used as described before. The potential responses of solid-contact Cu^{2+}-ISE are shown in Figure 1. As can be seen, the potentiometric sensor exhibits a Nernstian response of 29.1±0.3 mV/decade over the concentration range of 1.0×10^{-6} to 1.0×10^{-2} M with a detection limit of the 2.9×10^{-7} M. Additionally, the sensor exhibits a fast response time (less than 20 s).

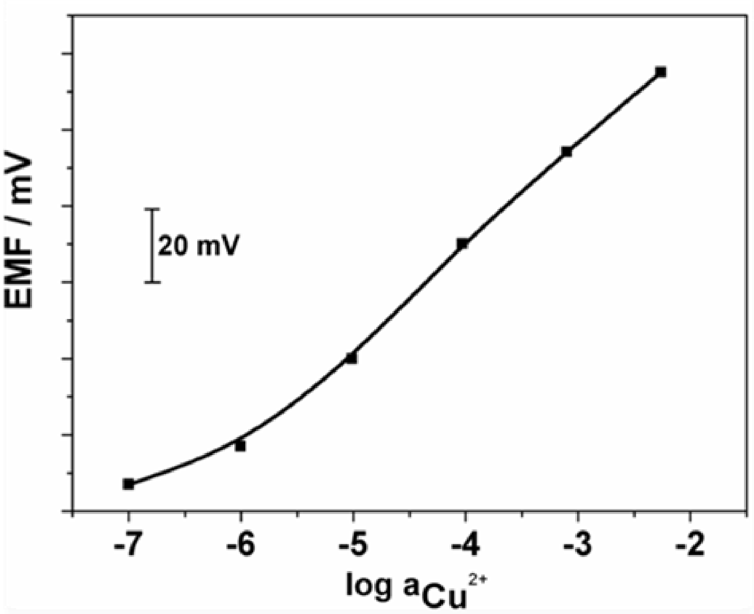


Figure 1. Potential response of the proposed solid-contact Cu^{2+}-ISE.

3.2 Optimization of the proposed potentiometric detection system

In order to obtain a sensitive potentiometric detection system for the determination of total tea polyphenols, the influence of experimental parameters on the detection sensitivity was investigated. Since the concentration of indicator Cu^{2+} plays important role in the complexation reaction of EGCG with Cu^{2+}, the effect of the Cu^{2+} concentration was tested, and the result is shown in Figure 2. The concentration of Cu^{2+} was varied from 10^{-5} to 10^{-3} M, while that of EGCG was fixed at 0.1 g/L. It can be seen that the potential change after complexation increases with a decrease of the concentration of Cu^{2+}. Considering the sensitivities of both of the electrode and the detection system, 10^{-4} M Cu^{2+} was chosen for all further experiments. As a control experiment, the potential response of the proposed electrode to 0.1 g/L EGCG was examined. Experiment showed that no obvious potential response was observed (data not shown), which is probably due to the electrically neutral form of EGCG. It should be noted that the potentiometric detection of EGCG in the presence of Cu^{2+} was performed under neutral pH conditions. Such pH can effectively prevent the autoxidation of EGCG during the complexation reaction since Cu^{2+} functions as a catalyst of the initial oxidation step of EGCG and enhances the autoxidation at pH > 7.0 [30]. In addition, the detection solution was deaerated by bubbling nitrogen through it, which further precludes the possibility of EGCG autoxidation.

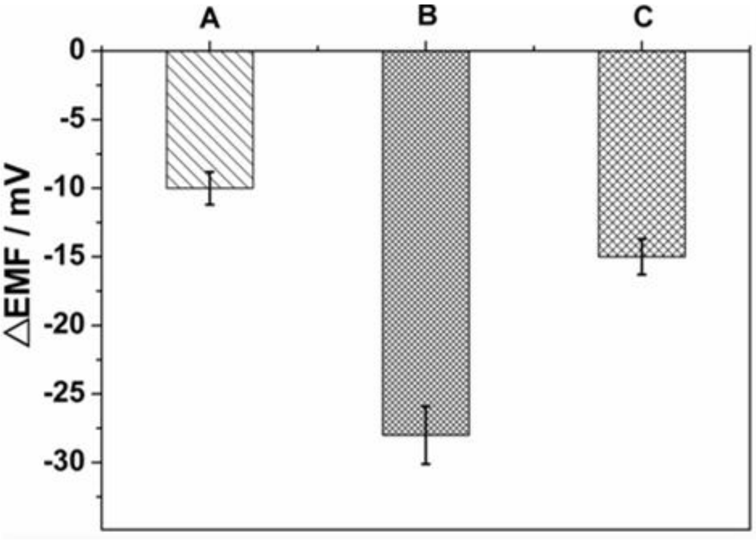


Figure 2. Potential changes to Cu^{2+} of 10^{-3} M (A), 10^{-4} M (B) and 10^{-5} M (C) after complexing with EGCG of 0.1 g/L.

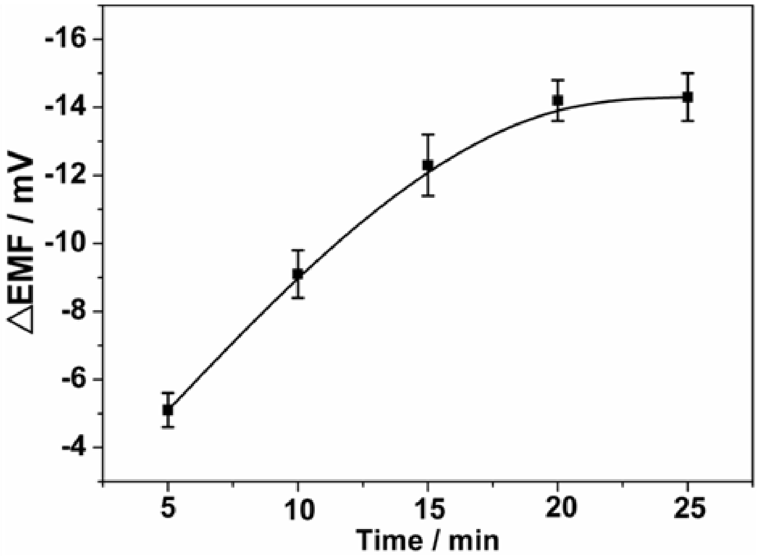


Figure 3. Effect of tea polyphenols with different extraction time on potential changes to 10^{-4} M Cu^{2+}. Polyphenols was extracted using boiling water of 500 mL from green tea of 0.1 g.

Different solvents have been used for the extraction of polyphenols from tea samples such as water, aqueous mixtures of ethanol and methanol. Since the potential detection is generally carried out in aqueous medium, so boiling water was selected as extraction solvent in this work. It has been known that the amount of polyphenols in tea extracts, which determines the detection sensitivity, depends on the extraction time of green tea samples. The influence of the extraction time on the detection sensitivity was tested and the results are shown in Figure 3. It can be seen that the potential change increases with increasing extraction time, but at times longer than 20 min, the increase is no longer significant. On the basis of these results, an extraction time of 20 min was chosen for subsequent experiments.

3.3 Characteristics of the proposed potentiometric detection system

Under optimized conditions, a calibration curve of the potentiometric detection of EGCG based on solid-contact Cu^{2+}-ISE as indicator electrode was obtained. Figure 4 shows the potential response of the proposed electrode for measuring EGCG at concentrations ranging from 0.01 to 0.1 g/L. As can be seen, there is a linear dependence of the potential change on the concentration of EGCG in the range of 0.01 to 0.1 g/L ($\gamma = 0.99$) with a detection limit of 3.2 mg L^{-1} (3σ). The proposed potentiometric detection system has a good repeatability and reproducibility for EGCG determination. For 0.075 g/L EGCG, the relative standard deviation (RSD) was found to be 4.4% for one electrode with six measurements. After each measurement, the potentiometric sensor was washed with an aqueous solution for 30 s to remove EGCG adsorbed onto the surface of polymeric membrane and then immersed in the 10^{-3} M Cu^{2+} to obtain the original baseline potential value. The stability of the potentiometric system was also studied at room temperature, and the results indicated that no significant change in sensitivity was observed for 10 days.

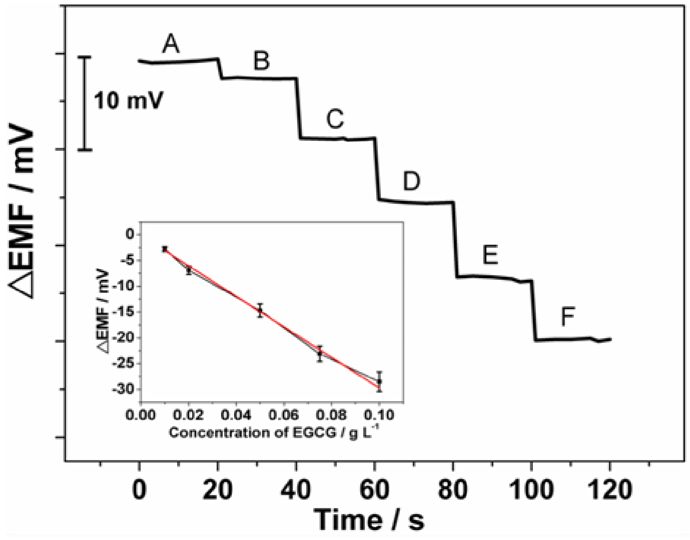


Figure 4. Potential responses to the indicator Cu^{2+} of 10^{-4} M after complexation with EGCG with different concentrations (A: 0 g/L; B: 0. 01 g/L; C: 0.02 g/L; D: 0.05 g/L; E: 0.075; F: 0.1 g/L). Inset shows the calibration curve of the proposed detection system for EGCG. Each data point represents an average ± standard deviation for three measurements.

3.4 Interference study

Green tea samples usually contain relatively high concentrations of inorganic interfering ions such as Na^+, K^+, Ca^{2+}, and Mg^{2+} and some organic interfering compounds amino acids, organic acids, caffeine and water soluble vitamin which can form complexes with Cu^{2+}, therefore it is necessary to evaluate the interference of interfering substances co-existing in green tea samples. According to the results previously reported by our recent paper, the logarithmic potentiometric selectivity coefficients of polymeric membrane Cu^{2+}-selective electrode using ETH 1062 as ionophore for Cu^{2+} over Na^+, K^+, Ca^{2+}, and Mg^{2+} are -10.8, -8.9, -11.4 and -13.9, respectively. On the other hand, the concentrations of inorganic ions are normally present below 10^{-3} M levels in the tea aqueous extracts. Thus, it can be demonstrated that the inorganic interfering ions co-existing in tea hardly show any interference to EGCG detection. For the further experiments, the influences of organic compounds were investigated. In this work, glutamic acid, malic acid, caffeine and vitamin C were selected as the models, and the results are summarized in Figure 5. Compared with the response to EGEG, neglectable potential changes can be observed for these organic compounds at their usual levels in green tea, which is probably due to the much lower concentration of these compounds than that of EGCG. These results suggested that the proposed method offers promising potential for reliable detection of polyphenols in green tea.

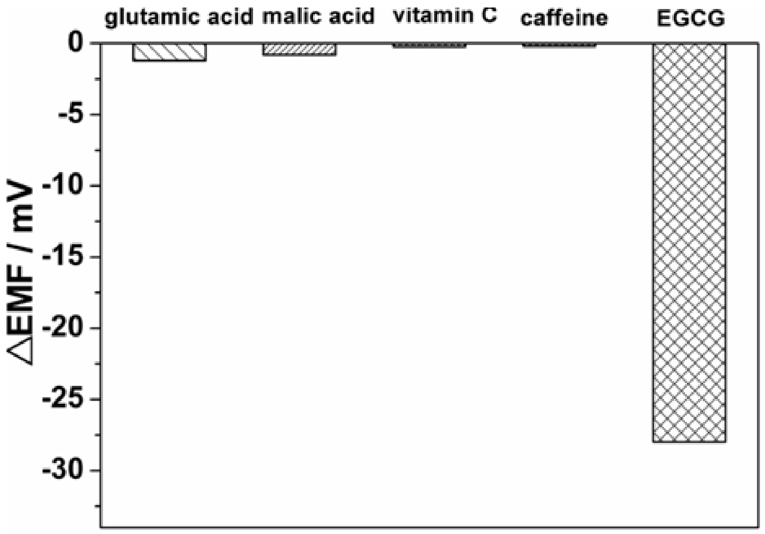


Figure 5. Potential changes with respect to different interfering organic compounds. The concentrations of Cu^{2+} and EGCG are 10^{-4} M and 0.1 g/L, respectively, while those of glutamic acid, malic acid, vitamin C and caffeine are 10^{-5} M.

3.5 Analysis of green tea samples

To evaluate the reliability of the present methodology, the proposed detection system based on potentiometric sensor as indictaor was applied to detect the tea polyphenols in real green tea samples. The tea samples were collected from local markets. Polyphenols were extracted by boiling water for 20 min [31], then, 10^{-4} M Cu^{2+} was added to the tea abstracts of 500 mL which contained 0.1 g green tea samples and spiked EGCG. The content of tea polyphenols was calculated and the results are summarized in Table 1. It can be seen that the recoveries of the spiked sample varies from 89.5% to 95.7%, indicating that the proposed detection system based potentiometric sensor has promising feasibility for determination of polyphenols in green tea samples. The results also show that the concentration of the total polyphenols in the collected green tea samples varies from 188.5 mg/g~283.4 mg/g. In addition, the content of polyphenols in green tea samples was also estimated by Folin–Ciocalteu method. It is shown that the results obtained from Folin–Ciocalteu method are slightly higher than that of the fabricated system, which can be ascribed to the fact that Folin–Ciocalteu reagent interacts with other different reducing nonphenolic substances in green tea and thus an overestimation of polyphenols content is obtained. Compared with other techniques, the proposed method offers potential advantages of simplicity, high accurancy and high selectivity for polyphenols determination in green tea.

Table 1. Application of the proposed method to determination of polyphenols in green tea samples

Sample	Present method （mg/g）	Folin–Ciocalteu method （mg/g）	Added（mg/g）	Recovery（%）
1	188.5±10.5[a]	202.1±10.1	200	90.5
2	201.1±12.1	225.1±9.7	100	92.1
3	223.1±15.1	249.1±16.1	200	95.7
4	283.4±22.3	300.2±22.9	100	89.5

[a] Average value of three determinations ± standard deviation.

4. CONCLUSIONS

In summary, we have demonstrated the use of a promising potentiometric detection system based on the complexation reaction between polyphenols and Cu^{2+} for the determination of polyphenols in green tea for the first time. The proposed system offers a low detection limit of 3.2 mg/L for EGCG and is not susceptible to interference by other organic substances associated with polyphenols analysis. Since many potentiometric sensors have been successfully exploited in analytical chemistry and some of them are even commercially available, such a sensing strategy has promising potential for fabrication of various potentiometric sensing systems based on reaction-induced response for the determination of anions, cations and neutral species.

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