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Article

Voltammetric Behavior of Sodium 7-Methoxyl-4'-hydroxylisoflavone-3'-sulfonate and Its Application[†]

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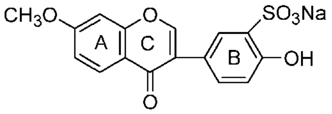
Voltammetric behavior of sodium 7-methoxyl-4'-hydroxylisoflavone-3'-sulfonate (SMHS) in the aqueous solution from pH 1 to 5 was studied by linear sweep voltammetry, cyclic voltammetry and normal pulse voltammetry. Experimental results showed that in 0.2 mol•L^{-1} sodium citrate-hydrochloric acid buffer solution (pH＝4.65), SMHS caused only one reduction wave at －1.34 V (vs. saturated calomel electrode, SCE), which was an irreversible adsorptive wave of SMHS protonized involving one electron and one proton. The peak current of SMHS on linear sweep voltammogram was proportional to its concentration in the range of 8.0×10^{-6}—8.0×10^{-5} mol•L^{-1} (r＝0.995), and the detection limit was 5.0×10^{-6} mol•L^{-1}. The method was applied to determination of SMHS in synthetic samples. In addition, its scavenging effect on superoxide anion radical was studied by the auto-oxidation of pyrogallol in HCl-tris buffer solution (pH＝8.2) in order to explain its peculiar biological effects. The experimental results proved that SMHS has antioxidant quality, and it is an efficient free radical scavenger of superoxide anion radical.

Keywords　voltammetric behavior, reduction, sodium 7-methoxyl-4'-hydroxylisoflavone-3'-sulfonate, superoxide anion radical

Introduction

Daidzein (7,4'-bihydroxylisoflavone) is a main activity constituent of the soybean isoflavones. It has activities of hyperkinesias,[1—4] inhibiting cancer cell growth,[5,6] accelerating the formation of bone cells and playing a role of female hormone.[7,8] Daidzein has been a medicine and used to treat coronary heart disease, hypertension, cerebral block, cerebral hemorrhage sequela and cerebral thrombosis.[9] However, because the solubility of daidzein is poor, its biological utilization rate is lower and the dose is more.[10] In order to improve the solubility of daidzein, we have synthesized sodium 7-methoxyl-4'-hydroxylisoflavone-3'-sulfonate (SMHS).[11] Its molecular structure is shown in Scheme 1.

Scheme 1　Molecular structure of sodium 7-methoxyl-4'-hydroxylisoflavone-3'-sulfonate (SMHS)



Up to present, nothing concerning electrochemical reactivity of SMHS has been published. As a result, the aim of this study was, firstly, to carry out a detailed investigation on the electrochemical behavior of SMHS in the aqueous medium with linear sweep voltammetry, cyclic voltammetry and normal pulse voltammetry; secondly, to propose an electrode reaction mechanism consistent with the experimental results. In addition, linear sweep voltammetry was applied to determination of SMHS in synthetic samples, and its scavenging effect on superoxide anion radical caused by the auto-oxidation of pyrogallol in HCl-tris buffer solution (pH＝8.2) was studied in order to explain its peculiar biological effects.

Experimental

Materials and apparatus

Pure SMHS (98%) was prepared with daidzein according to the method reported in Ref. 11, and was confirmed by infrared, NMR, mass spectroscopies and HPLC. A 2.5×10^{-3} mol•L^{-1} standard stock solution of SMHS was made up by dissolving it in aqueous solution. Other SMHS standard working solutions were prepared by diluting a certain amount of the stock solution with

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[†] Dedicated to Professor Chengye Yuan on the occasion of his 80th birthday.

water to the desired concentration. 0.2 mol•L^{-1} sodium citrate-hydrochloric acid buffer solution was prepared. All chemicals were of analytical-reagent grade. Double glass-distilled water was used throughout the whole experiments.

A Model CHI660A electrochemistry workstation (Chenhua Instruments, Inc. in Shanghai, China) was employed for all the electrochemical techniques. A three-electrode system was used, where a standard saturated calomel electrode (SCE) served as a reference electrode, a platinum wire electrode as the auxiliary electrode, and a PAR303 dropping mercury electrode as the working electrode (EG & G. U.S.A.). The workstation was controlled by CHI660A software and operated under Windows 98 environment. A pH meter with a combined glass electrode was used for pH measurements.

Methods

Transfer appropriate amount of SMHS standard working solution into 25 mL of volumetric flask, and dilute it to the mark with the supporting electrolytes. Take about 10 mL of the solution into the 303 cell. Linear sweep voltammograms, cyclic voltammograms and normal pulse voltammograms of SMHS were recorded with a Model CHI660A electrochemical workstation after deaeration with high-purity nitrogen (99%) for 10 min. According to the method reported in literature,[12] the experiment of SMHS precluding superoxide anion radical was done.

All experiments were performed at room temperature.

Results and discussion

Voltammetric behavior of SMHS

SMHS can be reduced on the mercury electrode, causing one wave in 0.05 mol•L^{-1} H_2SO_4, 0.2 mol•L^{-1} B-R, 0.2 mol•L^{-1} sodium citratehydrochloric acid, 0.2 mol•L^{-1} potassium acid phthalate+0.1 mol•L^{-1} H_2SO_4 and 0.05 mol•L^{-1} H_2SO_4+0.1 mol•L^{-1} KCl and other acidic medium. Because there is higher and more stable peak current of SMHS in 0.2 mol•L^{-1} sodium citrate-HCl solution than in other base solution, the buffer solution was selected as base solution. Linear sweep voltammograms of SMHS in the 0.2 mol•L^{-1} sodium citrate-HCl medium was shown in Figure 1, and the effect of pH value on both peak potentials (E_p) and peak currents (i_p) (insert) of the reduction waves was shown in Figure 2, where it is obvious that E_p shifts to more negative potential with increases of pH values, and E_p-pH relationship equation is E_p (V) = − 1.05 − 0.062pH (r=0.997). The slope in the equation is − 0.062, indicating that the number of proton involved is equal to the electron transfer number.[13] On the other hand, the peak current of reduction wave of SMHS decreased in shape of a dissociation curve with increasing pH, indicating that SMHS in the base solution is in a protonated form.[14]

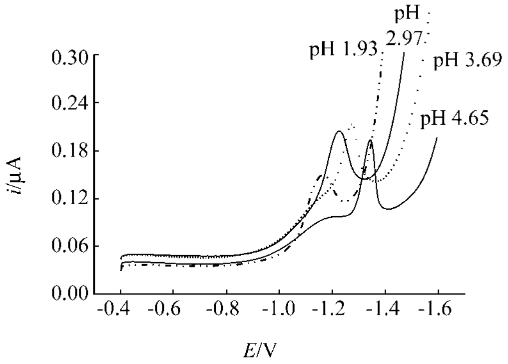


Figure 1 Linear sweep voltammograms for 4.0×10^{-5} mol•L^{-1} SMHS in 0.2 mol•L^{-1} sodium citrate-HCl solution, v=300 mV•s^{-1}, t=2 s.

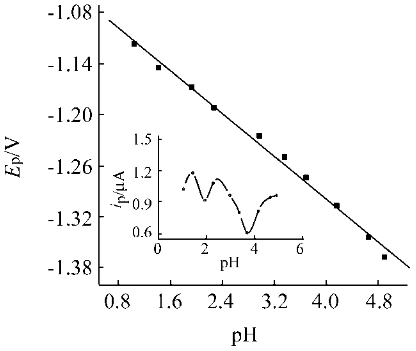


Figure 2 Effect of pH on the peak potentials and currents (inset). c=4.0×10^{-5} mol•L^{-1}, v=300 mV•s^{-1}, t=2 s.

The effect of the potential scan rate (v) on peak potentials (E_p) of the reduction waves and peak currents (i_p) in the sodium citrate-hydrochloric acid medium of pH 1.0—4.9 was investigated by linear sweep voltammetry (Figure 3), where it is obvious that E_p shifts to more negative potential with increases of v, indicating an irreversibility of the reduction waves. The E_p-log v curve equation is E_p (V)=−1.36−0.054 log v (V•s^{-1}) (r=0.994). Assuming the transfer coefficient α=0.5,[15] the electron transfer number was calculated to be n=1 from the slope of E_p-log v curve. On the other hand, i_p increased with increasing of v, and the log i_p-log v curve equation is log i_p (μA)=0.426+0.994 log v (V•s^{-1}) (r =0.9998), log i_p-log v slope is close to 1 which indicated that the adsorption of SMHS at the mercury electrode is ideally adsorptive characteristic.[15,16]

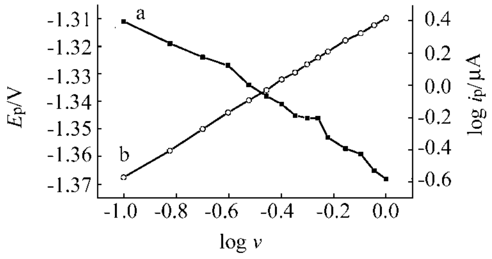


Figure 3 Effect of scan rate on the peak potentials and peak currents. c_{SMHS}=1.0×10^{-4} mol•L^{-1}, t=20 s.

In order to further check the mechanism of the reduction process, the repetitive cyclic voltammetric behavior and normal pulse voltammetric behavior of 6.0×10^{-5} mol•L^{-1} SMHS in 0.2 mol•L^{-1} sodium citrate—hydrochloric acid buffer solution (pH=4.65) was investigated (Figures 4 and 5). In Figure 4, SMHS yielded one reduction wave at −1.34 V on cathodic scan and no oxidation wave on anodic scan. It was obvious that a large reductive current peak is observed at the first scan curve. Subsequently the current peak decreased with the increase of the potential scan times, and reached to a stable value. This phenomenon illustrates that the reduction wave of SMHS had adsorptive characteristic, further confirming that the reduction process is an irreversible process. In Figure 5, a reductive peak (curve a) was observed and no peak occurred on the anodic branch (curve b), indicating that the reaction product of SMHS had adsorptive characteristic on the mercury electrode.

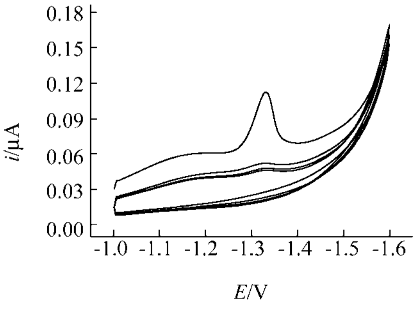


Figure 4 Repetitive cyclic voltammograms of SMHS in 0.2 mol•L^{-1} sodium citrate-HCl solution (pH=4.65). $c_{SMHS}=6.0\times 10^{-5}$ mol•L^{-1}, v=300 mV• s^{-1}, t=2 s, scan time=6.

The effect of various surfactants on the peak current of 6.0×10^{-5} mol•L^{-1} SMHS in 0.2 mol•L^{-1} sodium citrate-hydrochloric acid buffer solution (pH=4.65) was examined to investigate the adsorptive property of SMHS on the electrode. The cationic surfactant, cetyltrimethyl ammonium bromid (CTAB), decreased the peak current (i_p). However, neutral surfactant gelatin and anionic surfactant, polyvinyl sulfonate and sodium dodecylbenzene sulfonat, had little effect on the peak currents (i_p). This indicated that the reaction species were adsorptive and positively charged.

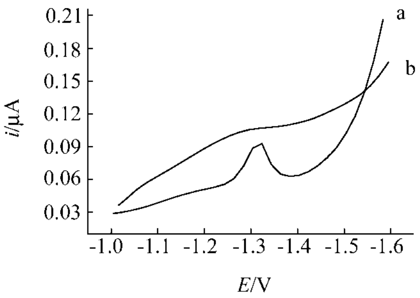


Figure 5 Normal pulse voltammograms of SMHS in 0.2 mol• L^{-1} sodium citrate-HCl solution (pH=4.65). $c_{SMHS}=6.0\times 10^{-5}$ mol•L^{-1}, v=300 mV•s^{-1}, pulse width=0.05 s, sample width=0.0167, pulse period=0.2 s, quiet time=5 s.

Electrode reaction mechanism

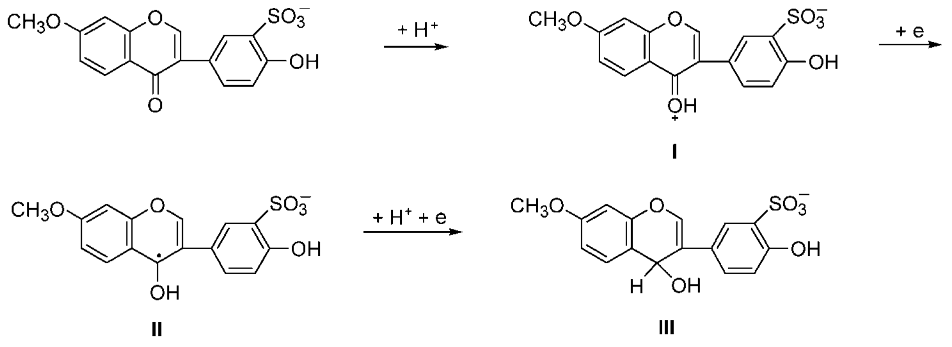
As mentioned above, the number of electrons transferred per SMHS molecule is n=1 in the acidic media. However, according to the structure of SMHS, its reduction process should be a two-step one-electron process. In order to explore the redox behavior, cyclic voltammetric behavior in different rest time before reverse scan was studied, and there is no oxidation wave at −1.58 V before reverse scan. But the peak current of oxidation wave (P_a) which peak potential being −0.99 V rose with the increasing of rest time at −1.70 V before reverse scan. Thus it is obvious that P_a wave does not correspond to the reduction wave at −1.34 V. P_a wave should correspond to the wave which is overlapped because of a discharge of hydrogen ion into the electrolyte solution. As a result, from the experimental phenomena and results described above, the reduction mechanism of SMHS on the mercury electrode was proposed as Scheme 2.

Analytical application

Determination of SMHS content in synthetic sample

The effect of quiet time (t) on i_p of of 6.0×10^{-5} mol•L^{-1} SMHS in the medium 0.2 mol•L^{-1} sodium citrate- hydrochloric acid was tested (Figure 6). Obviously, i_p was enhanced with increasing of quiet time at the same concentration of SMHS, and the time in which SMHS reached to an adsorption equilibrium between

Scheme 2 Reduction mechanism of SMHS on the mercury electrode



the electrode and the solution interface was different at different concentration of SMHS. Considering the determination of sensitivity and linearity range, a quiet time of 20 s was used for further studies.

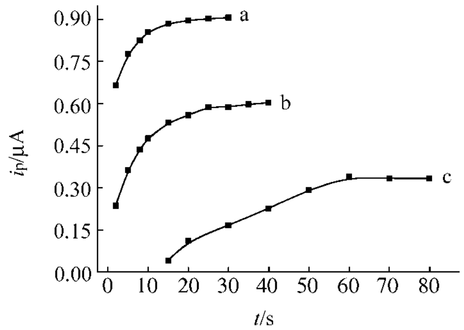


Figure 6 Effect of quiet time on the peak current of SMHS c_{SMHS}: a, 1.0×10^{-5} mol•L^{-1}; b, 3.0×10^{-5} mol•L^{-1}; c, 6.0×10^{-5} mol•L^{-1} in 0.2 mol•L^{-1} sodium citrate-hydrochloric acid (pH=4.65), scan rate=0.3 V•s^{-1}.

In order to assess the possibility of applying the proposed method to the assay of SMHS, the influence of common solvent and coexisting drugs brought about in the process of medicine preparation on the determination of SMHS was studied. When relative error of analytical results was less than ±5% for the determination of 5.0×10^{-5} mol•L^{-1} SMHS, the experimental results showed that 10% excess of ethanol, 30-fold excess of starch, 10-fold excess of daidzein, 1-fold excess of sodium daidzein-3'-sulfonate and 1-fold excess of 4',7-dimethoxyisoflavone did not interfere.

Under optimized experimental conditions, the first-order derivative peak current (i_p) of the reduction wave of SMHS was linearly proportional to the concentration of SMHS in the range of 8.0×10^{-6}—8.0×10^{-5} mol•L^{-1} and there was a linear regression equation i_p (μA)=$0.0148+1.483\times10^4$ c (mol•L^{-1}) (r=0.995). The detection limit was 5.0×10^{-6} mol•L^{-1}. The peak current could remain nearly unchanged for more than 3 h. Reproducibility was evaluated by performing 10 independent measurements for 3.0×10^{-5} mol•L^{-1} SMHS solution with the relative standard deviation (*RSD*) of 2.9%. Synthetic samples contained 1.0×10^{-7} mol•L^{-1} daidzein besides SMHS of different concentration. Their measurement process was the same as the experimental procedure described above. The calibration curve was constructed by plotting the first-order derivative peak current (i_p) of the reduction wave of SMHS against its concentration. The SMHS concentration in synthetic samples was obtained according to the calibration curve. The results are listed in Table 1.

Scavenging ability of SMHS on superoxide anion

The pharmacological researches in recent years indicate that many diseases such as senium, carcinomatous change of cells and cardiovascular system disease are related to free radicals.[17] Under the normal metabolization, the active oxygen radicals produced by cells and scavenged by cells themselves are in stable equilibrium. If they are maladjusted, many kinds of diseases may occur due to overabundance of free radicals *in vivo*. As a result, how to prevent radicals and active oxygen from the harm to organism tissue has become a very interesting study for many chemists. In this paper, the ability of scavenging active oxygen radicals using SMHS was studied through the autoxidation of pyrogallol. In Tris-HCl buffer solution (pH=8.2), pyrogallol can generate O_2^{-}• by autoxidation and its oxidation products are deoxidized at the potential of −0.96 V.[18,19] When SMHS was added to the solution contained pyrogallol, the peak current of pyrogallol decreased with increasing concentration of SMHS (Figure 7), indicating that SMHS could scavenge the active oxygen radicals and inhibit the production of active oxygen radicals yielded by the autoxidation of pyrogallol. The relationship of inhibitory rates (defined as the percentage of the ratio of the difference of the peak current yielded by pyrogallol before and after adding SMHS to the peak current yielded by pyrogallol before adding SMHS) with the concentrations of SMHS was shown in Figure 8. When the inhibitory rate reached 50%, IC_{50} value of SMHS obtained from Figure 8 was 2.46×10^{-5} mol•L^{-1}.

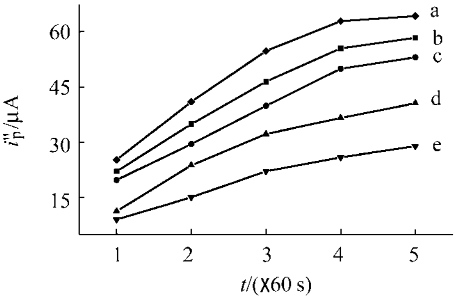


Figure 7 The inhibitory action of SMHS to pyrogallol autoxidation. a, 8.0×10^{-4} mol•L^{-1} pyrogallol+HCl+tris buffer solution (pH=8.3), b, a+5.0×10^{-6} mol•L^{-1} SMHS; c, a+1.0×10^{-5} mol•L^{-1} SMHS; d, a+2.0×10^{-4} mol•L^{-1} SMHS; e, a+3.0×10^{-4} mol•L^{-1} SMHS.

Table 1 Analytical results of SMHS in synthetic samples

No.	Concentration/ ($\times10^7$ mol•L^{-1})	Found/ ($\times10^5$ mol•L^{-1})	Average/ ($\times10^5$ mol•L^{-1})	RSD/ %	Added/ ($\times10^5$ mol•L^{-1})	Found/ ($\times10^5$ mol•L^{-1})	Recovery/ %
1	2.00	1.92, 2.05, 1.87	1.95	4.7	2.00	2.07	103.5
2	2.50	2.41, 2.53, 2.39	2.44	3.1	2.50	2.45	98.0

Experimental results indicated that SMHS had the scavenging ability on O_2^-•. This was due to the nucleophilic reaction taking place between unsaturated carbonyl group in the SMHS and O_2^-• as well as the single electron transforming between phenolic hydroxyl group and O_2^-•.

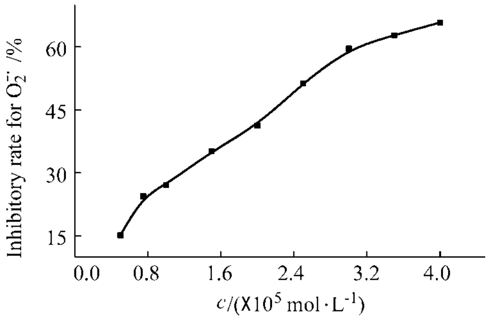


Figure 8 The relationship of inhibitory rates with the concentrations of SMHS.

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