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Spectrophotometric and Voltammetric Studies on the Interaction of Heparin with Phenosafranine

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Abstract: The interaction of phenosafranine (PSF) with a glycosaminoglycans of heparin (Hep) in aqueous solution has been characterized by UV-Vis absorption spectrophotometry and cyclic voltammetry in pH 1.5 Britton-Robinson (B-R) buffer solution. The addition of Hep caused decrease of the absorbance of PSF at 532 nm and the redox peak current of PSF. The study showed that an supramolecular complex of PSF-Hep was formed because of the electrostatic attraction of negatively charged Hep with the positively charged PSF, which resulted in the decrease of the equilibrium concentration of PSF in solutions, and the decrease of the absorbance or the peak current of PSF. The stoichiometry of the Hep/PSF complex was further calculated by voltammetric data with the result of 1:1 complex.

Keywords: Heparin, phenosafranine, interaction, voltammetry, spectrophotometry.

Heparin (Hep) is a kind of glycosaminoglycans (GAGs) containing glucosamine-N-sulfate and uronic acid with a variable number of sulfate, carboxyl and acetyl residues, which has many important biological functions. Hep belongs to anti-coagulants agent and can be found in blood vessels, liver capsule, lung, skin, intestine and peritoneal wall with the function as to avoid coagulation during haemodialysis and extra corporeal blood circulation. Many methods have been proposed for Hep determination, such as biological method[1], spectrophotometry[2], chromatography[3], electrophoretic method[4], light scattering technique[5] and electrochemical method[6].

In this paper, a cationic dye of phenosafranine (PSF) was selected to investigate the interaction with Hep with UV-Vis absorption spectrophotometry and cyclic voltammetry. PSF is a thiazine dye and has been used as the spectrophotometric probe for DNA[7]. The experimental results showed that it could strongly interact with Hep to form a supramolecular complex, which resulted in the decrease of spectrophotometric and voltammetric response of PSF solution.

The absorption spectra of PSF before and after the addition of different amount of Hep were recorded and shown in **Figure 1**. In pH 1.5 B-R buffer solution, PSF showed an absorption band at 532 nm, with the increase of the amount of Hep solution, the absor-

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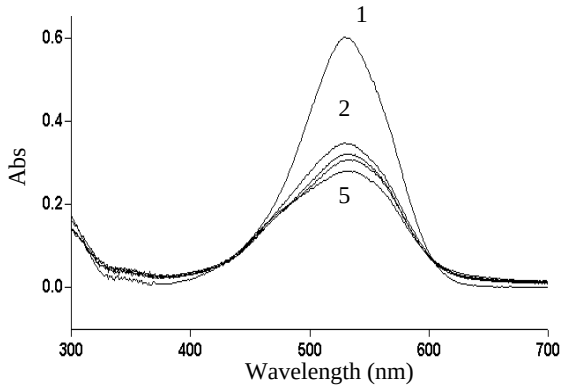
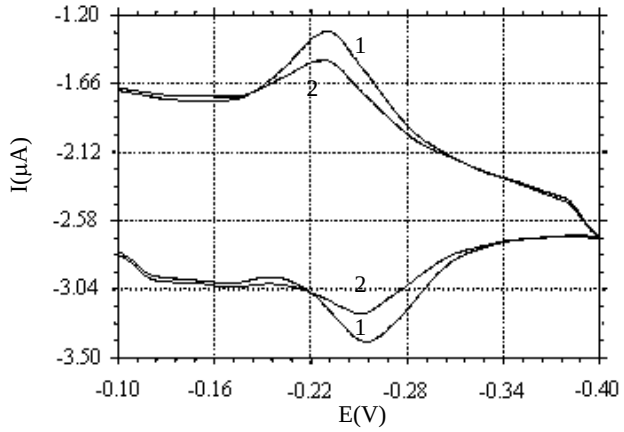
Figure 1 UV-Vis absorption spectra of PSF-Hep reaction solution



1.pH 1.5 B-R buffer+3.0×10^{-5} mol/L PSF; 2→5.1+80.0, 100.0, 200.0, 400.0 mg/L Hep

Figure 2 Cyclic voltammograms of PSF-Hep reaction solution



1. pH 1.5 B-R buffer+3.0×10^{-5} mol/L PSF; 2.1+2.0 mg/L Hep; scan rate: 200.0 mV/s

bance of PSF at 532 nm decreased gradually. The spectral changes indicated that PSF and Hep interacted with each other. Because in the selected buffer pH of 1.5, PSF is in positively charged and Hep in negatively charged, so electrostatic forces might have been involved in the interaction reaction as driving forces.

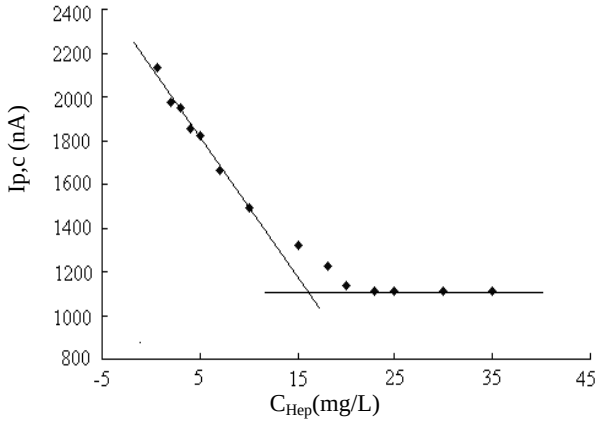
Cyclic voltammetric experiments were carried out using a DS model 2004 electrochemical analyzer (Shandong Dongsheng Electronic Instrument, China) with a DS-991 hanging mercury electrode (Shandong Dongsheng Electronic Instrument, China) as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as counter electrode. The cyclic voltammograms of PSF and its mixture with Hep were recorded and shown in **Figure 2**. PSF is an electroactive dye and under the experimental conditions, PSF had a pair of redox peak (curve 1) in the potential range of -0.1 V—-0.4 V. When Hep was added into PSF solution with subsequent scanning over the same potential range, the peak current in both anodic and cathodic waves (curve

2) were decreased without the movement of the peak potentials and appearance of new redox peaks. So these results also indicated that interactions between PSF and Hep took place in the mixture solutions.

The electrochemical behaviors of PSF and PSF-Hep reaction solution were discussed. As for PSF solution, PSF has a pair of redox peak (curve 1) with Ep,a=-256 mV and Ep,c=-231 mV (*vs*. SCE) and Ip,a/Ip,c≈1. So the redox process of PSF on hanging mercury drop electrode (HMDE) was reversible. According to the formula of ΔEp=58/n (25℃) (ΔEp=25 mV), the electron transfer number n was calculated to be 2. The relationship between the peak potential and pH was directly investigated in the pH range from 1.5-5.0 with a linear regression equation as Ep,c (mV)=-0.057 pH-0.12 (correlation coefficient γ=0.997). According to the equation -0.059x/n=-0.057, where n is the number of electron transfer and x is the number of hydrogen ion participating in the reaction, so the uptake of electron is accompanied by an equal number of hydrogen ion and x=2. As for the PSF-Hep interaction solution, it also can be seen from the curve 2 of **Figure 2** that the reaction solution had a pair of redox peak with ΔEp=25 mV and Ip, a/Ip, c≈1. The addition of Hep only induced the decrease of the peak current, without producing new peaks or causing shift of the peak potential. The relation between the reductive peak potential and pH was directly investigated and a regression equation of Ep,c (mV)=-0.060 pH-0.10 (correlation coefficient γ=0.995) was obtained. Then the electron transfer number (n) and the hydrogen ion (x) participating in the electrode reaction can also be calculated as x=n=2. The conclusion can be drawn that no matter whether Hep was present or not the electrode reaction process were both 2 electrons transfer with 2 protons uptake. So the interaction of Hep with PSF did not change the electrochemical behavior of PSF solution. The formation of PSF-Hep supramolecular complex results in the decrease of the equilibrium concentration of PSF in the reaction solution, therefore the decrease of the redox peak current of PSF was observed.

Keeping the PSF concentration as 3.0×10^{-5} mol/L and changing the concentration of Hep, the stoichiometry of Hep-PSF was obtained by the commonly used molar ratio method. The relationship of the reductive peak current with the Hep concentration was

Figure 3 Determination of coordination number of Hep-PSF complex



plotted in **Figure 3**. It can be seen that when the concentration of Hep was over certain value, the peak current kept stable, which indicated that the interaction reached to equilibrium. The intersection point was obtained from the two linear curves with the turning point calculated at 16.5 mg/L Hep. As one mole of Hep was defined as the mass of the repeating disaccharides unit of Hep (wt. 665). So the stoichiometry of PSF/Hep in the metachromatic complex was calculated as 1:1.

Since Heparin has three O-sulfates, two N-sulfate groups and two carboxyl groups per tetrasaccharide unit. The O-sulfates and N-sulfate groups are completely dissociated even below pH 3.0. The carboxyl groups is weakly acidic, and the pKa of D-glucuronic acid in Hep is 3.6. Under the selected pH value of 1.5, Hep is negatively charged and the positively charged free PSF species can be easily aggregated on Hep to form a supramolecular complex, which resulted in the decrease of the equilibrium concentration of PSF in solution, and the decrease of the absorbance, the peak current response, respectively.

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