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A SPECTROFLUORIMETRIC SENSOR BASED ON GRAPE SKIN TISSUE FOR DETERMINATION OF IRON(III)

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ABSTRACT. A spectrofluorimetric method based on the grape skin has been developed for the determination of Fe^{3+} at pH 5.0. The emission wavelength of the grape skin sensor occurs at 680 nm and the excitation wavelength at 421 nm. The fluorescence of sensor could be quenched by Fe^{3+} due to the complexing ability of anthocyanin with the metal ions. Anthocyanin, the main pigment in the grape skin, has been found fluorescence sensing material. The sensor based on the grape skin exhibited a calibration response for Fe^{3+} in two concentration ranges of 1.0×10^{-8} - 1.0×10^{-5} M ($r^2 = 0.9888$) and 3.2×10^{-5} - 3.2×10^{-4} ($r^2 = 0.9856$) M at 60 ^oC. The detection limit was found to be 7.5×10^{-9} M, and the other common ions did not interfere.

KEY WORDS: Grape skin, Fluorescence, Iron(III)

INTRODUCTION

Spectrofluorimetry has received considerable attention because of its rapid, simple, highly sensitive and selective advantages [1]. At present, some fluorimetric methods for the determination of Fe^{3+} based on quenching phenomena have been reported. Cha *et al.* proposed a sensitive procedure for the determination of Fe^{3+} using salicylic acid as fluorescent reagent. Unfortunately, Fe^{2+}, Co^{2+}, Sm^{3+} and Y^{3+} ions could cause interference [2]. A selective fluorescent chemosensor for Fe^{3+} which is composed of water-soluble β-cyclodextrin and 2,6-bis(benzoxazoly)pyridine, was developed by Feng *et al.* [3]. Askeland *et al.* isolated a fluorescent pigment from *Pseudomonas fluorescens* to selectively measure Fe^{3+} [4]. For the first time, however, we wish to report on the use of grape skin tissue based on fluorescent method for the determination of Fe^{3+}.

Grape is one of the major fruit crops and about 80% of the produced grape is used in wine making. Wine industry generates about 5-9 million tones grape waste per year [5]. Anthocyanins in grape skins, which are responsible for the red colour of grape and wine, include delphinidin, cyanidin, petunidin, peonidin, malvidin 3-glucosides, 3-(6-acetyl)-glucosides, 3-(6-*p*-coumaroyl)-glucosides, peonidin, malvidin 3-(6-caffeoyl)-glucosides and some pyruvates [6, 7]. Anthocyanins are currently being associated with health benefits, such as antioxidant and anti-cancer activity [8]. Two hydroxyl groups or hydroxyl and methoxyl groups in the ortho position can improve the combining capacity of anthocyanins with metallic ions [9, 10].

In this work, a natural fluorescent material, the grape skin, was used as a sensor for determination of Fe^{3+}. The spectral characteristics of the sensor and the sensor response to Fe^{3+} were investigated. In addition, the effects of pH, temperature and foreign ions on the fluorescence intensity were studied. The results reported in the present paper indicated that the spectrofluorimetric sensor based on the grape skin worked well for the determination of Fe^{3+}.

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EXPERIMENTAL

Apparatus and reagents

Fluorescence measurements of the grape skin were carried out on a spectrofluorimeter (LS-55, Perkin-Elmer, USA) with a spectrophotometric 1.0 cm × 1.0 cm quartz cuvette. The scanning rate of monochromators was fixed at 400 nm min^{-1} and the excitation and emission slit widths were set at 10.0 nm and 15.0 nm, respectively. Morphology of the grape skin was examined by the scanning electron microscopy (SEM, S-4800, Hitachi, Japan).

The standard solution of Fe^{3+} was prepared by dissolving $FeNH_4(SO_4)_2$ in distilled water. $FeNH_4(SO_4)_2$ was purchased from Kelong Chemical Co. (Chengdu, China). Working solutions were obtained from appropriate dilution of the stock solution. The grape skin pigments ($E_{1cm}^{1\%} 525nm \geq 10$, Pengyuan Co. Qingdao, China) was dissolved in water. HCl and NaOH were used to adjust pH of solutions. The red grape skin used in the sensor construction was purchased from a local market and stored after dryness. All chemicals were of analytical reagent grade and the redistilled water was used throughout.

Sample processing and experimental method

The pulps of fresh grape were removed by hand, and the grape skins were cleaned with distilled water before drying. Air-dried grape skins were washed with distilled water until they became soft, and then they were cut into smaller pieces using scissors. The coat of the grape skin was mounted on glass slide (6.0 cm × 1.2 cm). The glass slide with grape skin was winded by Teflon tape, and then fixed to a quartz cuvette filled with 2.0 mL distilled water. Different concentrations of Fe^{3+} were added into the cuvette gradually. The volume of Fe^{3+} solutions added was less than 50 μL to keep the total volume of testing solution without obvious change. The fluorescence intensity of the sensor, at an excitation wavelength of 421 nm, was measured at the wavelength of 680 nm.

RESULTS AND DISCUSSION

The spectral characteristics and main fluorophore of the sensor

After drying at room temperature, the morphological characteristics of the grape skin were examined by scanning electron microscopy (SEM). Figure 1 shows the SEM micrograph for the inner section of the grape skin, which was cleaned with distilled water. The inner surface of grape skin consisted of cross-linked fibrous materials and had many irregular small pores. The thickness, moisture content, fibre content of grape skin would affect the fluorescence intensity of sensor. To obviate this problem, the same kind of grapes and the same section of grape skin were selected to fabricate the sensor. Fortunately, the sensors have the same fluorescence quenching behavior. The excitation and emission spectra of the grape skin-based sensor in the presence of Fe^{3+} and of sensor alone were also shown in the insert of Figure 1. It was found that the maximal fluorescence excitation and emission wavelengths occurred at 421 nm and 680 nm, respectively. Therefore, the excitation wavelength at 421 nm and the emission wavelength at 680 nm were selected as operating wavelengths for the further studies. The presence of 5.0×10^{-5} M Fe^{3+} can cause obvious decrease of the fluorescence intensity of the sensor.

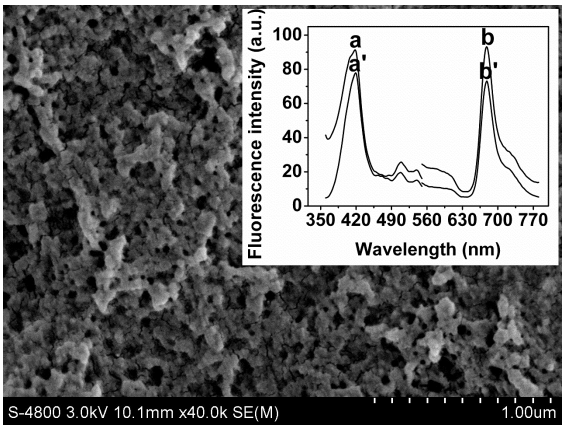


Figure 1. SEM micrograph of the inner surface of grape skin. Inset: fluorescence excitation (a) and emission (b) spectra; a, b: the grape skin-based sensor blank; a', b': adding 5.0×10^{-5} M Fe^{3+} to the system.

Effect of pH

The pH dependence of the sensor in the presence of 5.0×10^{-5} M Fe^{3+} solution was studied by using different solutions with pH range from 5.0 to 7.5. As shown in Figure 2, the fluorescence intensity of the sensor increased with the increase in pH value from 5.0 to 6.5 and then decreased at pH value higher than pH 6.5. Anthocyanins have the different chemical forms depending on the pH of the solution. At pH values between 4.0 and 6.0, four forms of the anthocyanins, flavylium cation, anhydrous quinoidal base, colourless carbinol base and the pale yellow chalcone coexist. When pH value is higher than 7.0, the anthocyanins are degraded depending on their substituent groups [11]. The degradation of the anthocyanins would affect the fluorescence intensity of grape skin. Since minimum fluorescence intensity occurred at pH 5.0, pH 5.0 was recommended for further studies.

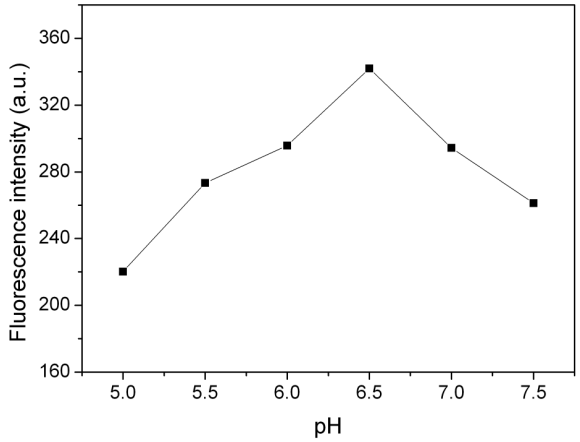


Figure 2. Effect of pH on the fluorescence intensity of the sensor at 20 °C.

Effect of temperature

As the temperature is an important factor in the measuring process, the effect of temperature on the fluorescence intensity of the sensor was studied at various temperatures ranged from 20 to 90 ^{o}C. Although the fluorescence quantum yields inherently decreased with the increase in temperature, the ratio of the fluorescence quenching (I_0/I) of the sensor in a given concentration of Fe^{3+} (2.0×10^{-4} M) increased with increasing temperature (Figure 3). When the temperature was higher than 60 ^{o}C, the presence of gas bubbles could affect the measurement. Therefore, 60 ^{o}C was chosen for further experiments.

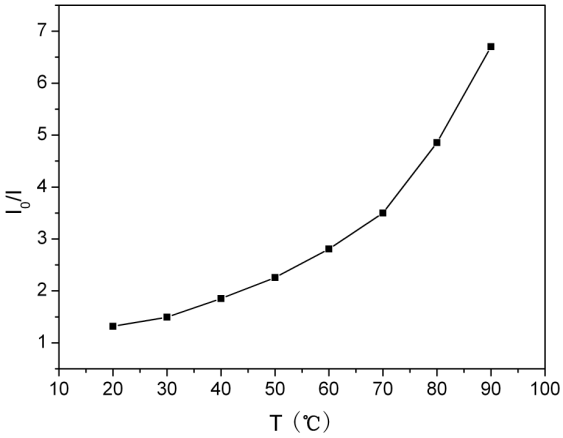


Figure 3. Effect of temperature on the fluorescence intensity of the sensor.

The sensor responses toward Fe^{3+}

Red fluorescence emission can be observed with naked eyes when the grape skin is illuminated by a UV lamp. Anthocyanin is the well known fluorophore which can recognize and bind Fe^{3+} because of its special structure. It was thought to be that the fluorescent quenching occurred in a given concentration range of Fe^{3+} as Fe^{3+}-anthocyanin complex was formed. The fluorescence emission spectra of the grape skin pigments and the sensor based on grape skin in the presence of different concentrations of Fe^{3+} were shown in Figure 4. It could be seen that the maximum emission wavelength of the grape skin pigments was at 680 nm. When 1.0×10^{-3} M Fe^{3+} was present, the fluorescence of grape skin pigments was completely quenched (see the insert of Figure 4). The emission wavelength of the grape skin also occurred at 680 nm and the fluorescence emission intensities of grape skin decreased considerably with the increase in the concentration of Fe^{3+}, and no measurable spectral position shift is observed.

Calibration curve

The calibration curve can be established based on the linear relationship between fluorescence quenching (*Io/I*) of the sensor and the logarithm of Fe^{3+} concentration in water, and the results are shown in Figure 5. The fluorescence intensity of the grape skin-based sensor increased linearly with increase of the Fe^{3+} concentration in the ranges of 1.0×10^{-8} - 1.0×10^{-5} M and 3.2×10^{-5} - 3.2×10^{-4} M, respectively. The corresponding linear equation can be expressed as $I_0/I = 1.79 + 0.0954$ logC(M) ($r^2 = 0.9888$) and $I_0/I = 5.97 + 0.9917$ logC(M) ($r^2 = 0.9856$), and the

detection limit (LOD) for Fe^{3+} was found to be 5.3×10^{-9} M. A more obvious change in fluorescence intensity was observed when the concentration of Fe^{3+} solution was higher than 1.0×10^{-5} M. This might be caused by the change of Fe^{3+} ligands number [Scheme 1]. At the beginning of the interaction between Fe^{3+} and the grape skin pigments, the complex might be expressed as $Fe(anthocyanin)_3$ because the number of ligands was much more than that of Fe^{3+}. However, with the increase of the concentration of Fe^{3+}, the number of ligands was less than that of Fe^{3+}, the molecular formula of complex was then changed to be $Fe(anthocyanin)_2.½\ SO_4$ [12, 13].

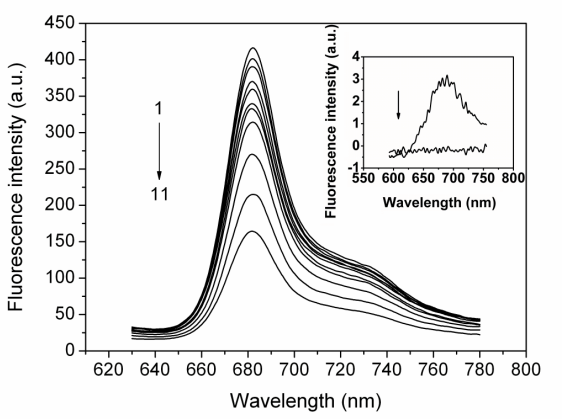


Figure 4. Fluorescence emission spectra of the grape skin-based sensor in the presence of different concentrations of Fe^{3+}, and the Fe^{3+} concentration of curves 1 - 11: (1) blank solution; (2) 1.0×10^{-8} M; (3) 3.2×10^{-8} M; (4) 1.0×10^{-7} M; (5) 3.2×10^{-7} M; (6) 1.0×10^{-6} M; (7) 3.2×10^{-6} M; (8)1.0×10^{-5} M; (9) 3.2×10^{-5} M; (10) 1.0×10^{-4} M; (11) 3.2×10^{-4} M. The temperature was 60 ^{o}C. Insert: fluorescence spectra of the grape skin pigments upon addition of Fe^{3+} (0 and 1.0×10^{-3} M).

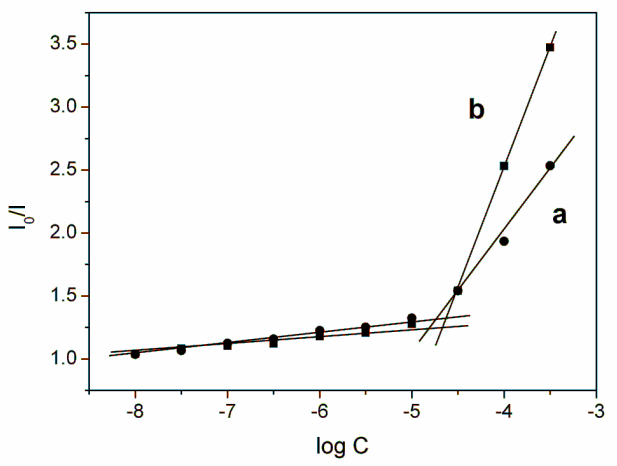
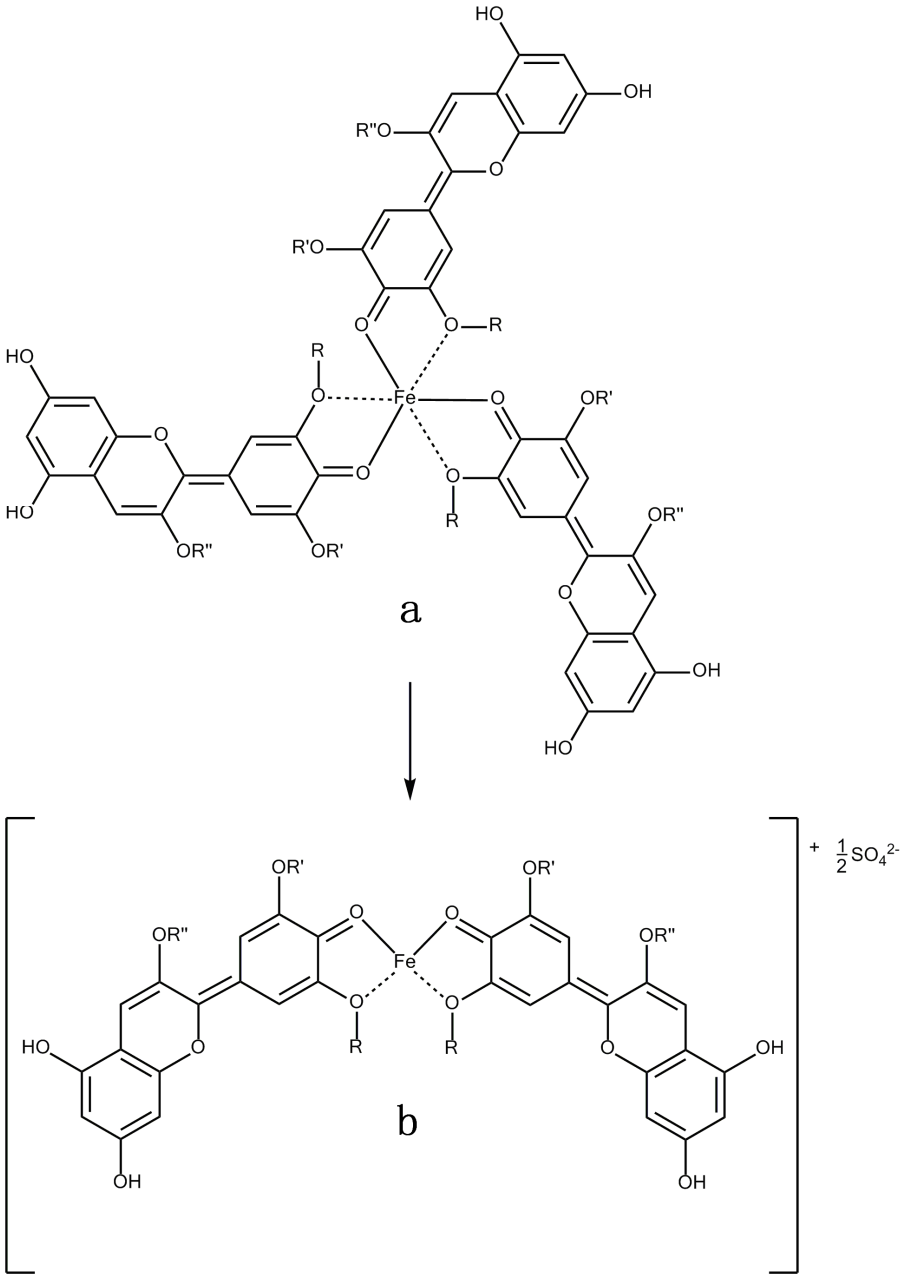


Figure 5. The calibration curve for Fe^{3+} determination (a) the sensor used in this experiment and (b) the sensor fabricated by another grape skin.



Scheme 1. The interaction between anthocyanin and Fe^{3+} when the concentration of Fe^{3+} was (a) below 1.0×10^{-5} M and (b) above 1.0×10^{-5} M.

In addition, it is worth mentioning that the working curve must be re-established if a new grape skin is used. The sensor is irreversible in this experiment. Fortunately, the fluorescence quenching behavior under the optimal conditions is the same (Figure 5).

Interferences studies

For the complexing capacity of the anthocyanin, the common cations and anions were chosen to determine their effect on the determination of iron(III). The tolerance limit was taken as the maximum concentration of foreign ions that caused a 5% deviation in the fluorescence intensity of the sensor when Fe^{3+} was present and its concentration was fixed at 1.0×10^{-5} M. Cd^{2+}, Sr^{2+}, Mn^{2+}, Ni^{2+}, Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, Ba^{2+}, Al^{3+}, Cl^{-}, NO_3^{-}, SO_4^{2-}, CO_3^{2-}, H_2O_2, citrate at 200-fold concentration and Cr^{3+}, Pb^{2+}, Hg^{2+}, Co^{2+}, NH_4^{+} at 50-fold concentration of Fe^{3+} ions did not interfere.

CONCLUSIONS

The proposed method, based on the grape skin, is found to be useful for the determination of Fe^{3+} with high sensitivity and selectivity, and it does not require complex pre-treatment or rigorous conditions. Therefore, the grape skin-based sensor has a great potential for the practical application.

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REFERENCES

1. Fan, L.J.; Jones, W.E. *J. Am. Chem. Soc.* **2006**, 128, 6784.
2. Cha, K.W.; Park, K.W. *Talanta* **1998**, 46, 1567.
3. Feng, L.H.; Chen, Z.B.; Wang, D.S. *Spectrochim. Acta A* **2007**, 66, 599.
4. Askeland, R.A.; Skogerboe, R.K. *Anal. Chim. Acta* **1987**, 192, 133.
5. Lafka, T.I.; Sinanoglou, V.; Lazos, E.S. *Food Chem.* **2007**, 104, 1206.
6. Monagas, M.; Garrido, I.; Bartolomé, B.B.; Gómez-Cordovés, C. *Anal. Chim. Acta* **2006**, 563, 401.
7. Revilla, I.; Pérez-Magariño, S.; González-SanJosé, M.L.; Beltrán, S. *J. Chromatogr. A* **1999**, 847, 83.
8. McCallum, J.L.; Yang, R.; Young, C.; Strommer, J.N.; Tsao, R. *J. Chromatogr. A* **2007**, 1148, 38.
9. Corrales, M.; García, A.F.; Butz, P.; Tauscher, B. *J. Food. Eng.* **2009**, 90, 415.
10. Esparza, I.; Salinas, I.; Caballero, I.; Santamaría, C.; Calvo, I.; García-Mina, J.M.; Fernández, J.M. *Anal. Chim. Acta* **2004**, 524, 215.
11. Castañeda-Ovando, A.; Pacheco-Hernández, M.L.; Páez-Hernández, M.E. *Food Chem.* **2009**, 113, 859.
12. Hajji, H.E.; Nkhili, E.; Tomao, V.; Dangles, O. *Free Radical Res*. **2006**, 40, 303.
13. Smyk, B.; Pliszka, B.; Drabent, R. *Food Chem.* **2008**, 107, 1616.