Study of fluorescence spectra of starch suspension excited by 260-nm UV light

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To achieve detection, monitoring, and automation of starch graft copolymerzation at any time, the investigation is being made according to luminescence of free radicals produced in the reaction. The investigations on the fluorescence spectra of starch-water suspension excited by ultraviolet (UV) light and its characteristics contribute to study the characteristics and mechanisms of free radicals coming into being, emitting, and disappearing. Fluorescence spectra of starch, dextrose, sucrose, and maltose excited by 260-nm light are compared and their similarities further verify that this fluorescence comes from the transition from nonbonding, namely n electrons in the hetero-atom (O) of the functional group (C–O–C) called ether linkage, to the antibonding orbital σ^*. The functional group is a part of six-membered ring structure in starch molecule. Meanwhile, the experimental results indicate that relative peak intensity of fluorescence emitted by 1% starch suspension comes to climax when starch suspensions with different concentrations are excited by the same wavelength.

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Molecule fluorescent spectroscopy is a prevalent and future spectrographic analysis technique. It makes use of some substance that can emit its characteristic fluorescence when excited by ultraviolet (UV) light or visual light to analyze qualitatively or quantitatively[1,2]. Molecule fluorescent spectroscopy is of a few merits such as high sensitivity, high selectivity, few samples, and simple technique, and is one of the important methods for chemists, biochemists, medicament analyzers or food analyzers. There are a few fluorescent analytic methods. Different methods have different characteistiscrs and merits, used for detecting and analyzing different substances[3]. A normal method is used in our work.

Starch is a common part of advanced plants. The structure of starch granule is complex. Its real structure isnot clearly understood until today. Studying its structure is an advanced subject on fundamental research field of starch science[4]. Modified starch is widely used for adhesives, paper, foods, textiles, water treatment, resin, rubber, etc.[5]. Measuring microelements mixed in starch also adopts fluorescent spectroscopy[6,7]. A study on room temperature phosphorescence spectra of starch and its derivatives has been reported, but not about fluorescence spectra of starch itself. In this paper, the authors declared that many kinds of starch and their derivatives have the room temperature phosphorescence phenomenon that their maximal excitation light ranges from 292 to 405 nm, and their maximal emission light ranges from 447 to 523 nm. Dextrose residues are chained to form starch molecule. Many electrochemical methods or photochemical methods of dextrose determination have been reported and are used for analyzing different samples. Although the detection methods are different, they almost belong to indirect methods[9−11]. Meanwhile to our knowledge, a paper about fluorescence spectra of dextrose itself has not been reported. Free radicals produced in the reaction of natural high polymer graft copolymerzation are of the luminescence characteristic[12], which may make it realistic to achieve detection, monitoring and automation of starch graft copolymerzation. The investigation on the fluorescence spectra of starch-water suspension contributes to study the characteristics and mechanisms of free radicals coming into being, emitting and disappearing.

SP-2558 multifunctional spectrometer system (Roper Scientific Inc., America) is used to get fluorescence spectra of starch suspension. Its light source is a xenon lamp. The given light beam chosen by a monochromatic system irradiates the desired sample filled in the quartz colorimetric cuvette placed in the sample cell. Fluorescence emitted by the sample goes through another monochromatic system and is collected by a charge-coupled device (CCD). Then a computer acquires the real signals and outputs the experimental results after dealing with them. The used grating in excited monochromatic system is 1200 grooves/mm blazed grate whose blazed wavelength is 300 nm. While 150 grooves/mm blazed grating whose blazed wavelength is 500 nm is used in emitted monochromatic system.

The reagents used in our experiment are maize starch (Zhu Chen Xin Mao Corn Developing Inc.) dextrose and maltose (Sinopharm Chemical Reagent Co., Ltd.), and sucrose (Chemical Reagent Inc.) Reagents are diluted with super-pure water ($\geq$ 12 MΩ) (Wuxi Huajing Co., Ltd.) to form different concentrations according to their mass ratio. Determine the exposure time of CCD according to different samples and keep the scanning range from 150 to 800 nm in our whole work. Then excite samples with a series of UV-light wavelength and scan them to get corresponding fluorescence spectra.

Because starch does not dissolve in water, starch suspension is not stable and a deposition will come after short time[13]. To decrease measurement error for it, take out the quartz colorimetric cuvette filled with a sample in the sample cell, shake the sample up, place it back after scanning and get a fluorescence spectrum, immediately change exciting wavelength and scan to get another spectrum during the whole course of experiments.

Table 1. Fluorescence Peak value and its Relative Intensity of 0.01% Starch Suspension Induced by Different UV-Light

Exciting Wavelength (nm)	230	240	250	260	270	280
Fluorescence Peak Wavelength (nm)	No Obvious Fluorescence	585.24	610.79	631.65	653.82	684.33
Relative Fluorescence Peak Intensity (a.u.)		4821.14	11202.22	12150.68	9834.46	8645.56
Exciting Wavelength (nm)	290	300	310	320	330	340
Fluorescence Peak Wavelength (nm)	694.76	713.80	736.49	755.52	772.47	795.07
Relative Fluorescence Peak Intensity (a.u.)	10352.95	6104.21	4198.04	2967.73	2553.27	841.31

Exciting light from 220 to 340 nm irradiates 0.01% starch suspension at intervals of 10 nm to get fluorescence spectra. The determined exposure time of CCD is one second. Because its concentration is lower, the deposition performance is less. As Table. 1 shows, starch suspension excited by UV-light from 240 to 340 nm can all emit obvious fluorescence that exhibits red-shift with the increasing of exciting wavelength, and relative fluorescence intensity of starch suspension excited by 260 nm is the strongest. The wide band of fluorescence from 570 to 780 nm which starch suspension emits after it absorbs UV-light from 240 to 330 nm is due to the transition from nonbonding namely n electrons in the hetero-atom (O) of the functional group (C–O–C) called ether linkage, to the antibonding orbital σ^*. The functional group is a part of six-membered ring structure in starch molecule.

We chose fluorescence spectra of starch, dextrose, sucrose and maltose excited by 260-nm UV-light to compare. Dextrose, sucrose, and maltose are diluted with super-pure water to form 1% concentration and their exposure time is determined to be 5 s. While starch is diluted to form 0.01% concentration and its exposure time is 1 s. The comparing results are shown in Fig. 1. In the figure, the first two peaks are primary and secondary diffraction peak of exciting light and the last peak is fluorescence peak.

As we can know from Fig. 1, dextrose, sucrose, maltose, and starch excited by 260-nm UV-light have similar fluorescence spectra, but only their fluorescence intensities are different. Starch's concentration is the lowest and its exposure time is the shortest, but its relative fluorescence intensity is the strongest, which indicates starch may be of strong fluorescence and great fluorescent quantum yield. In balanceable dextrose-water solution, there are three different forms, i.e., open chain, alpha pyranose, and beta pyranose. α-D-glucose is about 36% and β-D-glucose is about 64%, while open chain form is less than 0.01%. A maltose molecule has a glucoside hydroxy that can become open chain and is of reducibility of carbonyl, while a sucrose molecule is not of this character for the absence of a glucoside hydroxy[14]. But the four kinds of molecules also have a six-membered ring structure where there is an ether linkage (C–O–C). So these similarities can further verify this fluorescence comes from the transition from nonbonding, namely n electrons in the hetero-atom (O) of the functional group (C–O–C), to the antibonding orbital σ^*.

Next, we compared fluorescence spectra of starch suspension with different concentration excited by 260 nm. Relative fluorescence intensity increases with increasing concentration, so exposure time of CCD is determined to be 50 ms in order to avoid exceeding the systematic measurement maximum. A few fluorescence spectra are shown in Fig. 2. Values of relative fluorescence peak intensities are listed in Table 2.

As we can know from Fig. 2, starch suspensions with different concentrations excited by the same UV-light have similar spectra and the fluorescence peak wavelength doesnot move with changing concentration. Meanwhile, we can know from Table 2 that relative fluorescence peak intensity isnot proportional to concentration and it is strongest at 1% concentration. The absorbance of 0.001% starch suspension is equal to 0.0377, which we can know from its absorbance experiment. Only at very low concentration, that is to say, the absorbing ratio of exciting energy isnot more than 5% or the absorbance is not more than 0.05, can fluorescence intensity be proportional to concentration[15]. That is the

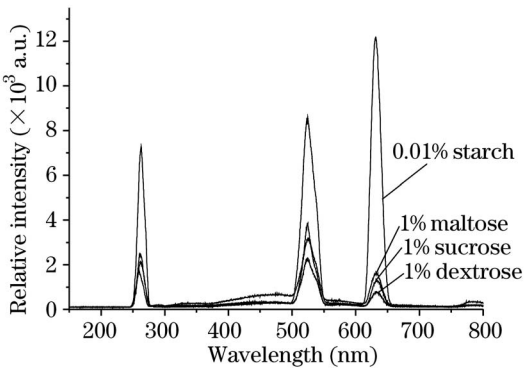

Fig. 1. Fluorescence spectra of starch, dextrose, sucrose and maltose excited by 260 nm UV-light.

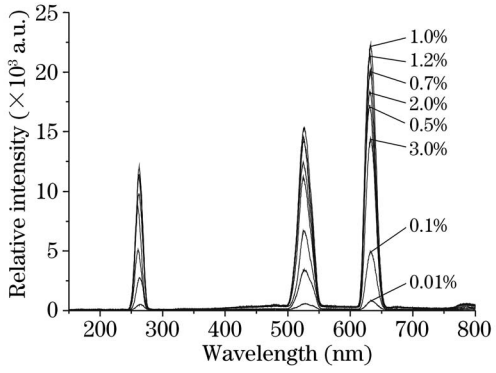

Fig. 2. Fluorescence spectra of starch suspension with different concentration excited by 260 nm.

Table 2. Fluorescence Spectra of Starch Suspension with Different Concentrations Excited by 260 nm

Concentration (%)	0.01	0.1	0.2	0.3	0.4	0.5	0.6
Relative Fluorescence Peak Intensity (a.u.)	832.51	4971.95	8665.18	11856.75	14604.57	17231.86	18991.39
Concentration (%)	0.7	0.8	0.9	1	1.1	1.2	1.3
Relative Fluorescence Peak Intensity (a.u.)	20125.10	20890.91	21463.16	22269.50	21486.76	21490.76	21082.63
Concentration (%)	1.4	2	3	4	100 (solid)		
Relative Fluorescence Peak Intensity (a.u.)	20750.85	18351.66	14447.83	12499.17	410.48		

reason why fluorescence intensity is not proportional to concentration in our experiments. While concentration of starch suspension is high, the front part of suspension in absorbance cell absorbs strongly and emits strong fluorescence, but its back part emits lower fluorescence for lower exciting light. That results in the whole fluorescence intensity declining. But if the concentration is too low, fluorescence intensity is not strong as well for absence of fluorescent substances[1]. So there must be a best concentration.

Comparing fluorescence spectra of starch, dextrose, sucrose, and maltose excited by 260-nm UV-light further verifies that this fluorescence comes from the transition from nonbonding, namely n electrons in the hetero-atom (O) of the functional group (C–O–C) called ether linkage, to the antibonding orbital σ^*. The functional group is a part of six-membered ring structure in starch molecule. 1% starch suspension excited by the same wavelength emits the strongest fluorescence. The research results contribute to study the characteristics and mechanisms of free radicals coming into being, emitting, and disappearing and to establish bases for detection, monitoring, and automation of starch graft copolymerzation at any time.

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