# Effects of alcohol on fluorescence spectra of erythrocyte solution

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Based on the experimental researches on the fluorescence spectra of 1% erythrocyte solution with various concentration of alcohol excited by violet light-emitting diode LED light at 407 nm, the mechanism of alcohol on the fluorescence spectra of erythrocyte solution is investigated theoretically. The experiment results indicate that induced by the LED light at 407 nm, erythrocyte solution with the concentration of 1% can emitting striking spectra with two fluorescence regions. One is the short-wave fluorescence region from 430 to 525 nm, and the other is the long-wave fluorescence region from 580 to 750 nm. When the concentration of alcohol in erythrocyte solution increasing, the fluorescence intensity of short-wave area decrease while the fluorescence intensity of long-wave area increases. Combining the blood absorb spectra to the experiment results, it is shown that the formation of the short-wave fluorescence area is because the solution transmits the fluorescence spectra and the self-absorption of erythrocyte. The long-wave fluorescence region comes from porphyrin such as protoporphyrin, zinc porphyrin etc.. And there is resonance energy transmission between the short-wave fluorophores and the long-wave ones. According to the experiment results and the physical theory in erythrocyte fluorescence, it is found that alcohol make higher self-absorption ratio of the erythrocyte which improves the resonance energy transmission between fluorophores in the two wave bands. The result will offer experimental and theoretical reference for examining the alcohol content in blood.

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Alcohol intake is harmful to human's health, especially for heavy alcohol drinkers. Recently, more and more researchers study the interaction of alcohol and bio-tissues. Monte[1] said that chronic gestational exposure to alcohol has profound adverse effects on brain development. Insulin-stimulated central nervous systems are significantly impaired by chronic gestational to alcohol, and that the abnormalities in insulin signaling mechanisms persist in the early postnatal period, which is critical for brain development. Gdovinová[2] reported that heavy drink could affect bio-activity of mosaic protein in erythrocyte membrane and the conformation of membrane protein, which decreased deformability of erythrocytes. Scientists also said that when the alcohol concentration in blood raise to 0.1%, human's action, sense of sight and linguistic function would be injured; when the alcohol concentration raise to 0.5%, the neural balance would be injured heavily, even lose his consciousness, which is very dangerous to drivers. Hillbom[3] had reported that stroke is the third most common cause of death in developed counties and the alcohol is recognized as a possible risk factor for stroke. Therefore, it is important to detect the alcohol concentration in human blood. In this paper, we present our fluorescence spectroscopy research on erythrocyte solution with different content of alcohol.

All the erythrocyte solution is obtained from an albino mouse. Firstly, the albino mouse blood is centrifuged at 3000 rpm for 5 min to separate plasma. The pelleted elements washed with 0.9% saline and centrifuged at 3000 rpm, and the procedure was repeated thrice to separate erythrocytes. The erythrocyte is diluted by 0.9% saline and made erythrocyte solution of 1%. The absolute ethyl alcohol (>99.9%) is diluted by distilled water and made solution with concentration of 50%. Dropping 0.01–0.1 mL alcohol solution of 50% to 1 mL erythrocyte solution of 1% to make erythrocyte-alcohol solution for fluorescence detection.

The fluorescence spectra of erythrocyte-alcohol solution are measured by WGD-8 grating spectrometer (1200 $mm^{-1}$, Tianjing Guangdong Corp., China) with a photomultiplier (PM, R955, Hamamatsu Corp., Japan) measuring the fluorescence intensity. The excitation light is light-emitting diode (LED) light with wavelength at about 407 nm. The emission spectrum of each sample was measured more than three times for reproducibility. The measured spectra were stable in time.

The autofluorescence spectra of erythrocyte solution with different content of alcohol are obtained. Figures 1(a)–(c) show the spectrum of erythrocyte solution of 1% and the spectra of erythrocyte solution of 1% with 50% alcohol of 0.05 and 0.1 mL. In order to comparison, the three spectra are merged into Fig. 1(d). It can be seen from our experimental results that, erythrocyte solution can emit two striking spectral band induced by LED light at 407 nm (Fig. 1(a)). One spectral band is from 430 to 525 nm, which is called short-wave band. And the other is from 580 to 750 nm, which is called long-wave band. In addition, there are two smaller peaks in the profile, locating at 556 and 694 nm, respectively.

With alcohol increasing in the erythrocyte solution, the fluorescence intensity of the short-wave band deceasing, and the long-wave band is just the reverse. In addition, the peak of the long-wave band is about 4 nm red-shift, i.e. the peak is shifted from 608 to 612 nm. And the peak locating at 556 nm disappears little by little (Figs. 1(b)–(d)).

Autofluorophors or fluorophors of native tissues are characteristics of a given tissue and any alteration in the pathological status can be sensitively detected using fluorescence spectroscopy[4]. Boulnois[5] and Chen[6] reported that the wavelength range from 400 to 500 nm is the strongest absorption range of blood and its components. Kessel[7] and Perkin-Elmer Ltd.[8]

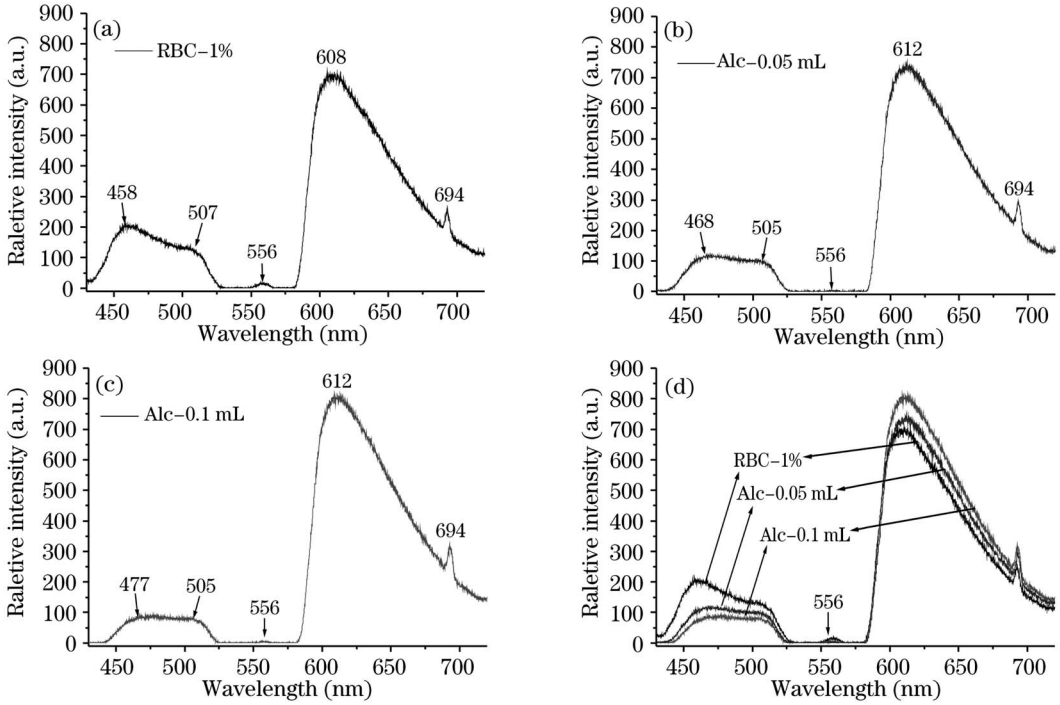


Fig. 1. The fluorescence spectra of erythrocyte solution (1%) with different content of alcohol solution (50%).

reported that the fluorescence spectrum of protoporphyrin is characterized by two peaks at 620 and 680 nm, which stem from the transition of $^1S_{1,0} \rightarrow {}^0S_{0,0}$ and $^1S_{1,0} \rightarrow {}^0S_{1,0}$; and zinc protoporphyrin emits the fluorescence at 594 and 690 nm and the former peak stems from the transition of $^1S_{1,0} \rightarrow {}^0S_{0,0}$. In our previous researches on spectroscopy of blood and its components[9,10], there is a correlation between two fluorescence bands when their intensity changes with the erythrocyte concentration. According to our experimental results and the knowledge mentioned above, we conclude that the short-wave band results from autofluorescence and self-absorption of blood. The self-absorption of blood excites the porphyrin fluorophores and results in the long-wave band. In a word, there is resonance energy transition between the two bands. As for the small peak at 556 nm, it might result form the self-absorption of erythrocyte because both erythrocyte and hemoglobin have rather high absorption at 540 and 580 nm. Between them, there is a lowest absorption point at about 556 nm.

With the alcohol concentration increasing, the fluorescence intensity of short-wave band decreases while that of long-short wave band increases. The main reason might be that the alcohol could enhance the absorptivity of erythrocyte among the wavelength range from 400 to 500 nm. As a result, the fluorescence intensity of short-wave band decreases with the increasing of alcohol concentration. On the other hand, the resonance energy transition is enhanced because the absorptivity of erythrocyte among the wavelength range from 400 to 500 nm increases. Therefore, the fluorescence of long-wave band is increasing.

In summary, upon the excitation of LED light at 407 nm, erythrocyte solution can emit striking spectra with short-wave fluorescence region from 430 to 525 nm and the long-wave fluorescence region from 580 to 750 nm. With the concentration of alcohol in erythrocyte increasing, the fluorescence intensity of short-wave band decreases while that of long-short wave band increases. Consequently, it can judge whether a human drink or not by measuring the fluorescence of his erythrocyte solution.

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