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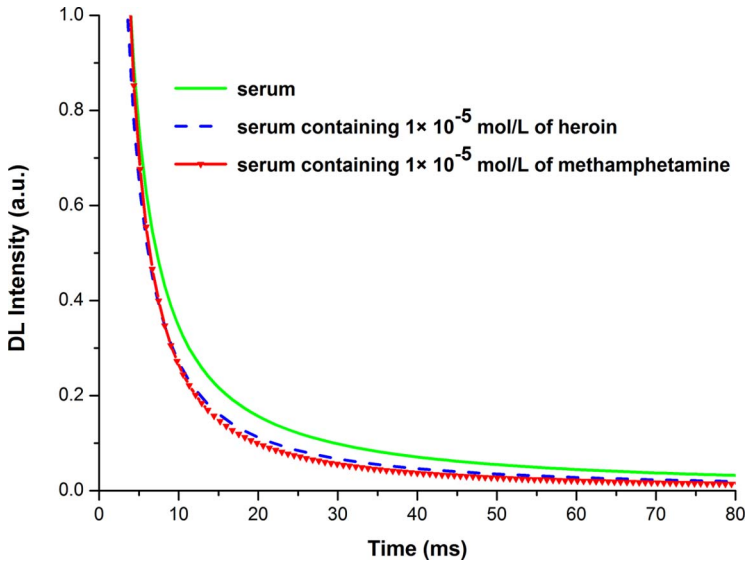

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Detecting Trace Amounts of Narcotics in Serum by Delayed Luminescence

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Abstract: This paper was designed to test the feasibility of optical identification of trace amounts of narcotics in serum using photoinduced delayed luminescence (DL). Comparative investigation of control serum and contaminated samples with methamphetamine and heroin was conducted. For the control serum without chemical contamination, the delayed photon emission decay shows an intrinsic lifetime of about 38.4 ms, whereas DL decay curves of the serum containing 1×10^{-5} mol/L of methamphetamine and 1×10^{-5} mol/L of heroin reveal shortened characteristic lifetimes of 19.3 and 24.6 ms, respectively. Experimental results demonstrate that the lifetime can be used as an indicator of containing narcotics in serum, promising a new method for detecting trace amounts of narcotics in serum.

Index Terms: Narcotics, serum, delayed luminescence, lifetime.

1. Introduction

Because narcotics-related crimes pose critical risks to public safety and national security, narcotics control has the drawn attention of the international community. A rapid and sensitive detection of narcotics is the first step of narcotics control. Currently, a number of methods including the conventional chemical-detection method [1], [2], immunoassay [3]–[5], chromatography [6]–[8], as well as spectroscopic technique have been widely used for narcotics detection. Among a variety of detection methods, spectroscopic technique which observes the structural or chemical compositions of samples through characteristic spectra such as fluorescence spectrum, Raman spectrum and infrared spectrum, takes advantage of no pretreatment, minor sample consumption, no direct contact with the samples, etc. Pioneering studies have reported a wealthy of fluorescent probes based on nanostructures for narcotics detection, and analyzed the fluorescence spectra of cannabinoid [9], MDMA [10], and methamphetamine precursors [11], with the concentration of about 2.8×10^{-3} mol/L, 2.5×10^{-3} mol/L and 3.3×10^{-3} mol/L, respectively. Moreover, infrared spectra have been employed for successful detection of narcotics, which indicates that vibrational detection is possible [12], [13]. Raman spectra have been widely

utilized for narcotics identification. Ryder *et al.* detected the cocaine dispersed in glucose powder with the quality fraction of 10% by Raman spectroscopy, and they proposed a possible application of this method in forensic science [14]. A successful application of Ramon spectroscopy has been conducted by Taplin *et al.* to detect phenylethylamine and ephedrine with the concentration of about 8.3×10^{-3} mol/L and 6.1×10^{-3} mol/L, respectively [15]. Raman spectra of saliva containing 1.3×10^{-2} mol/L of cocaine applying the gold and silver doped sol-gels immobilized in glass capillaries have been further investigated by Inscore *et al.* [16]. Based on these work, it is worth noticing that these established spectroscopy techniques is applicable to identify above the magnitude of 10^{-3} mol/L of narcotics. Therefore, a more sensitive technique with the advantages of optical method to detect trace narcotics is a hot topic in the area of narcotics detection.

The technology of photoinduced delayed luminescence (DL), which involves induced characteristic luminescent phenomenon that persists for a while, has drawn more and more attention nowadays. The DL process is highly sensitive to the fingerprint of the biological molecule and its surrounding environment, thus benefitting the inference of the biological effect [17]. DL technique is as sensitive as chromatography and takes advantage of the convenient preparation, the feasibility of online detection and the fast and nondestructive examination. The main applications of DL are distinguishing different biological states of bio-samples or detecting trace substance in bio-samples. For instance, Kim *et al.* measured the DL of human lung cancer tissue and found that the DL characteristics of the tissues was significantly different from that of normal lung tissue, which indicated the DL from the biological samples was not a random but characteristic phenomenon [18]. A nondestructive evaluation of photosynthesis by DL in Arabidopsis under the different illumination conditions provided a new method for evaluating photosynthesis in higher plants without interrupting plant culture [19]. Also, the characteristic properties of DL of normal and leukemic human serum were investigated by Chen *et al.*, and they pointed out that the initial luminescence intensity and decay rate of the two serums were of notable discrepancy [20]. In addition, it has been reported that the DL sensor was used to detect serum containing 5×10^{-4} mol/L of potassium, which would play a positive role in diagnosis of hypertension, stroke and seizures [21]. Furthermore, Peterson *et al.*'s work has demonstrated the detection of the 9.7×10^{-5} mol/L of hydroxyl radical by lanthanide complexes in water using DL [22]. Based on these encouraging efforts, DL technique has shown its potential to improve the sensitivity of narcotics detention. In this paper, we report the feasibility of DL technique to detect trace amounts of methamphetamine and heroin in serum. The intrinsic lifetime of delayed luminescence τ of each sample was quantitatively analyzed, promising a novel biomarker for detecting the trace narcotics in biological samples.

2. Experimental Details

DL of all control serum and contaminated samples with methamphetamine and heroin were detected by a homebuilt ultra-weak luminescence detection system [23]. Briefly, a xenon flash lamp (L7685, Hamamatsu Photonics, Japan) was chosen as the excitation light source. The detection system consisted of a highly sensitive PMT (R943-02, Hamamatsu Photonics, Japan), a preamplifier (SR445, SRS, America.), a photon counter (Multichannel Scaler/Averager, SR430, SRS, America), two shutters and a sequential control unit. The two shutters were located in the excitation and signal light paths and were able to complete open and close actions respectively one after the other within 1.5 ms interval, which can eliminate the disturbance by the afterglow of light source and ensure the safe measurement of the luminescence signal by the PMT. All the experimental process were carried out under the same excitation conditions (mean power: 30 mW, illumination time: 16 s), at constant temperature (22 °C) and humidity (40% RH).

Absorption spectra of control serum and contaminated samples with methamphetamine and heroin were recorded on a Shimadzu UV-2101 spectrometer.

Methamphetamine and heroin were acquired from Narcotics Control Bureau of Tianjin Public Security Bureau, and the fetal bovine serum was purchased from Sigma, America. All materials were used without further purification.

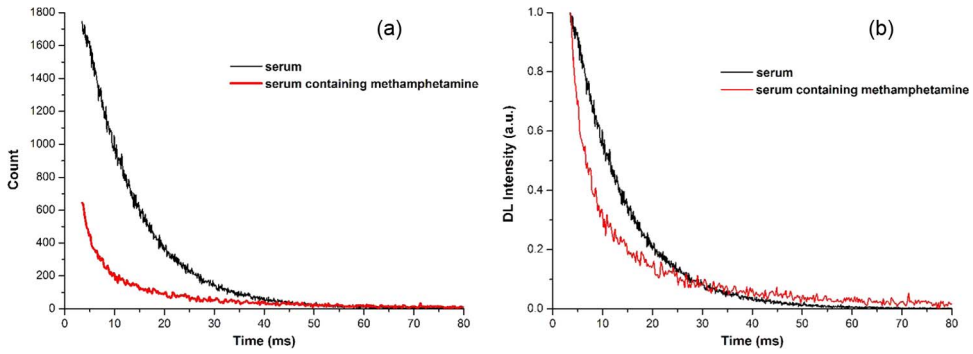

Fig. 1. (a) DL decay curves of (black) serum and (red) serum containing 1×10^{-3} mol/L of methamphetamine. (b) Normalized DL decay curves of (black) serum and (red) serum containing 1×10^{-3} mol/L of methamphetamine.

3. Results

Fig. 1(a) shows the DL decay curves of serum and serum containing 1×10^{-3} mol/L of methamphetamine. Initially, the luminescence photon number of the serum containing methamphetamine reduced significantly, compared to that of the control serum sample. Also, we can see, the luminescence photon number of the two samples decreased in a non-linear way as a function of time. After about 60 seconds of the excitation light delivery, the luminescence of the two samples tended to be stable. In order to express the decay kinetics process of these two samples, the experimental data shown in Fig. 1(a) were normalized according to the intensity at 3.5 ms. As shown in Fig. 1(b), the red curve is found to decrease more rapidly than the black one, which visually indicates that methamphetamine affects the luminescence of serum significantly. According to Fig. 1, serum and serum containing methamphetamine can be distinguished qualitatively, and we further expressed the decay characteristics of the two samples quantitatively.

As shown in Fig. 1(b), the luminescence decay conforms to the law of first-order curves as an exponential distribution, thus the relative luminescence intensity $I(t)$ can be expressed as the following equation:

$$I(t) = I_0 e^{-vt} = e^{-vt} \tag{1}$$

where t is the luminescence duration after shutoff of excitation light, v is the decay rate coefficient. Since the DL of serum is a process of composite luminescence, (1) can be further modified as

$$I(t) = \int f(v) e^{-vt} dv \tag{2}$$

where $f(v)$ is the probability distribution of the decay rate coefficients. The form of equation (2) accords with the Laplace transformation in mathematics, in which $I(t)$ is the Laplace transformation of $f(v)$. As a result, we can calculate $f(v)$ by the anti-Laplace transform of $I(t)$ and $f(v)$ can be written as

$$f(v) = L^{-1}\{I(t)\}. \tag{3}$$

Some research results have shown that the decay process of DL conformed to a hyperbolic function [24], as expressed by

$$I(t) = \frac{1}{(1 + t/t_0)^m}. \tag{4}$$

Based on equations (3) and (4), we have

$$f(v) = \frac{t_0^m v^{m-1} e^{-vt_0}}{\Gamma(m)}. \tag{5}$$

Here $\Gamma(m) = (m-1)!$ is the Gamma function. Equation (5) is the Gamma distribution in mathematics while the mathematical expectation of v is the average decay rate coefficient. Therefore, v can be calculated as

$$\begin{aligned} v_m &= \int_0^\infty v f(v) dv \\ &= \frac{\int_0^\infty t_0^m v^m e^{-vt_0} dv}{\Gamma(m)} \\ &= \frac{m}{t_0}. \end{aligned} \tag{6}$$

The lifetime refers to the average retention time of molecules in the excited state. It not only represents the parameter of decay characteristics but demonstrates the intrinsic physical parameter of samples as well. The lifetime depends on sample structure, the polarity and viscosity of the micro-environment, and so on. Therefore we can research on the change of the structure of serum or the micro-environment of serum by measuring the lifetime. The lifetime is the numeric equivalent of the reciprocal of decay rate coefficient; thus, it can be written as

$$\tau = \frac{1}{v_m} = \frac{t_0}{m}. \tag{7}$$

The parameter t_0 is

$$t_0 = m\tau. \tag{8}$$

Based on (4) and (8), we have

$$I(t) = \frac{1}{(1 + t/m\tau)^m}. \tag{9}$$

On this basis, the DL decay curves of serum and serum containing 1×10^{-3} mol/L methamphetamine were fitted according to function (9) and the lifetime τ were calculated as 38.4 ms and 14.2 ms, respectively, as can be shown in Fig. 2. The results show that the luminescence lifetime of serum containing methamphetamine reduced about 63 percent, compared to that of serum. It turned out that methamphetamine makes the luminescence of serum decay more quickly.

We thereafter measured the DL of the serum containing 1×10^{-5} mol/L of methamphetamine and the serum containing 1×10^{-5} mol/L of heroin, and the normalized data were fitted through function (9), as shown in Fig. 3. Table 1 shows the luminescence lifetime of the three samples. It is shown that the lifetime of the serum containing methamphetamine (19.3 ms) and the serum containing heroin (24.6 ms) was reduced about 49.7% and 36.2%, compared to the pure serum (38.4 ms), respectively. These results show that the intrinsic lifetime of DL can be used as an indicator of serum containing narcotics. The DL is capable of distinguishing and detecting trace amounts of different kinds of narcotics in serum.

4. Discussions

This work has demonstrated that the capability and potential of DL to distinguish different kinds of narcotics in serum and to realize the trace detection of narcotics in serum. In the enforcement of narcotics suppression, the identification of narcotics addiction requires urgently the detection

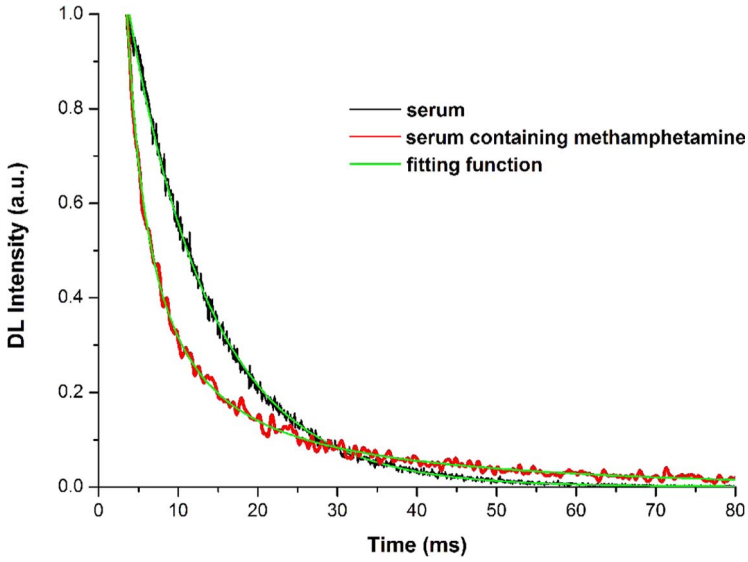

Fig. 2. Normalized DL decay curves of (black) serum and (red) serum containing 1×10^{-3} mol/L of methamphetamine. The data are fitted using function (9) (green).

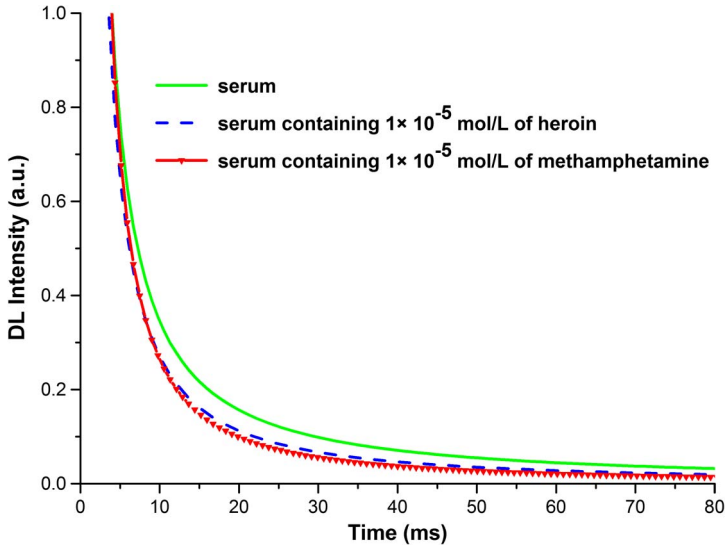

Fig. 3. Normalized DL decay curves of (green) serum, serum containing 1×10^{-5} mol/L of (red) methamphetamine, and serum containing 1×10^{-5} mol/L of (blue) heroin. The date are fitted using function (9).

of narcotics in biological specimens, such as blood, urine, hair, and so on. The accuracy of the detection results are affected by the selection of specimens directly. The concentration of narcotics and metabolites in the blood can reflect the physiological effects of narcotics abusers directly, and the toxic dose and the fatal dose are calibrated by the concentration of narcotics in the blood. Besides, blood is less susceptible to external contamination and has become an important specimen of narcotics certification and the behaviors of abusers by narcotics. Blood is made up of blood cells and plasma and serum is the plasma without fibrinogen, which is derived from cell secretion and the degradation or seepage of cell tissue. In the medical

TABLE 1

Luminescence Lifetime of Serum, Serum Containing 1×10^{-5} mol/L of Methamphetamine, and Serum Containing 1×10^{-5} mol/L of Heroin

	Serum	Serum containing methamphetamine	Serum containing heroin
T (ms)	38.4	19.3	24.6

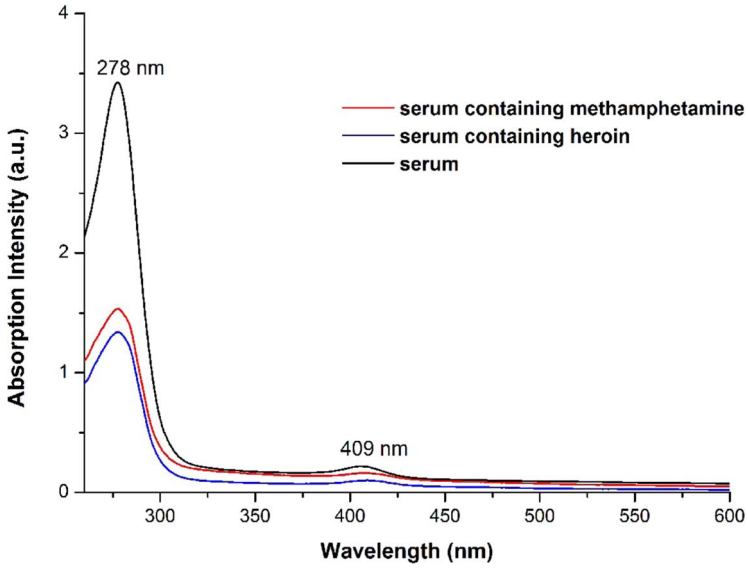

Fig. 4. Absorption spectra of (black) serum, (red) serum containing 1×10^{-3} mol/L of methamphetamine, and (blue) serum containing 1×10^{-3} mol/L of heroin.

community, it has been common knowledge that most narcotics will metabolize and degrade rapidly in serum, and only a few narcotics could affect the central nervous system through blood circulation, which makes it difficult to detect narcotics. It has also been previously been demonstrated that narcotics could weaken the luminescence intensity and decrease the luminescence lifetime. What's more, the lifetime increased from 19.3 ms to 14.2 ms when the concentration of methamphetamine rose from 1×10^{-5} mol/L to 1×10^{-3} mol/L. According to our analysis, the serum could be associated with narcotics, and these interactions lead to the change of luminescence characteristics of serum. Therefore, the interaction between serum and narcotics were elaborated preliminarily.

The absorption spectra of serum, serum containing 1×10^{-3} mol/L of methamphetamine and serum containing 1×10^{-3} mol/L of heroin are shown in Fig. 4. It indicates the absorption peaks of serum were located at 278 nm and 409 nm, which mainly arise from tryptophan and tyrosine [25], [26]. Compared to serum, the location of absorption peaks of serum containing narcotics change almost noting, while the intensity weaken observably. It is noteworthy that the intensity of serum containing methamphetamine and that of serum containing heroin at 278 nm reduced about 53 percent and 59 percent, respectively. We infer this distinct decrease to the changed solvent polarity resulting from the amino group of methamphetamine which influences the molar absorption coefficient of the whole system and weakens the light absorption of tryptophan and tyrosine. In addition, the weakened absorbance of serum containing heroin could be resulted

from the association of heroin molecule and certain residue of serum, such as tryptophan residues and tyrosine residues. The analysis shows that narcotics could be associate with certain bio-macromolecules such as protein and amino, or the narcotics could change the physicochemical property of serum. Revealing the mechanism of interactions between serum with narcotics will play a positive role in improving the sensitivity of DL in trace detection of narcotics, which are under further investigation of our research group.

5. Conclusion

In this work, DL was used to distinguish samples of serum and serum containing narcotics. The DL decay curves of pure serum, the serum containing 1×10^{-5} mol/L of methamphetamine and the serum containing 1×10^{-5} mol/L of heroin were measured using a homebuilt ultra-weak detection system. The results show that the intrinsic lifetime of the three samples were 38.4 ms, 19.3 ms, and 24.6 ms, respectively. It can be concluded that the intrinsic lifetime of DL can be used as an indicator of serum containing narcotics, and DL is promising to not only detect but also distinguish trace amounts of different kinds of narcotics in serum. The absorption spectra were utilized to study on the interactions between serum and narcotics preliminarily which implicated that the amino group of methamphetamine changed the solvent polarity and heroin molecule could be associated with tryptophan residues and tyrosine residues. In general, these two different modes of action weaken the absorbance of serum. We have been cooperating with the Narcotics Control Bureau of the Tianjin Public Security Bureau to put DL into practice. Further investigation will be followed up for a better understanding of the mechanism of interactions between serum and narcotics, and it will improve the sensitivity of DL and promote the practical application of DL technology in trace detection of narcotics.

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