DETECTION EXPERIMENT OF SIX VITAMINS BY LASER-INDUCED FLUORESCENCE SPECTRA

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Laser-induced fluorescence (LIF) is a potential technology for the rapid, sensitive, and selective detection of vitamins. In this study, the LIF spectra (LIFS) of vitamins A, B1, C, D, E, and K1, and their mixtures were investigated under a 266-nm laser excitation via an Nd:YAG laser. The experimental results showed that the LIFS of each vitamin solution had its unique profile. The six vitamins can be distinguished to some extent by the characteristic wavelength region: 320–380 nm for vitamins A and C, 350–440 nm for vitamins D and E, and 400–470 nm for vitamins B1 and K1. Additionally, it can be used for accurate diagnosis by characteristics of LIFS (starting wavelength, spectral range, peak wavelength, maximum intensity, and extinction coefficient) as a complement. Moreover, the features of LIFS can reflect the main vitamin components in the mixture of vitamins to a certain extent when several vitamins coexist. It was found that the spectral range, maximum intensity, and extinction coefficient could report the benzene ring number, double bonds, and OH group in molecules of components in mixtures, which is a discrimination-assisted method for vitamins in a mixture. This paper also proposes a technique to identify individual components in mixtures quantitatively by using LIFS and LIFS parameters. The present study will offer technical support in clinical diagnosis and targeted therapy using vitamins via experimental exploration on detection.

Keywords: *Laser-induced fluorescence, spectrum, vitamin, molecule, wavelength range, intensity.*

ЭКСПЕРИМЕНТАЛЬНОЕ ОБНАРУЖЕНИЕ ШЕСТИ ВИТАМИНОВ С ПОМОЩЬЮ ЛАЗЕРНО-ИНДУЦИРОВАННОЙ ФЛУОРЕСЦЕНЦИИ

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Спектры лазерно-индуцированной флуоресценции (LIFS) витаминов A, B1, C, D, E и K1 и их смесей исследованы при возбуждении излучением Nd:YAG-лазера с длиной волны 266 нм. Показано, что LIFS раствора каждого витамина имеет свой уникальный профиль. Витамины различаются по характерным диапазонам длин волн: 320–380 нм для витаминов A и C, 350–440 нм для витаминов D и E, 400–470 нм для витаминов B1 и K1. Это можно использовать в качестве дополнения для точной диагностики по характеристикам LIFS (начальной длине волны, спектральному диапазону, длине волны и интенсивности максимума, коэффициенту экстинкции). Особенности LIFS могут в определенной степени характеризовать основные компоненты в смеси витаминов. По спектральному диапазону, максимальной интенсивности и коэффициенту экстинкции можно определять количество бензольных колец, двойных связей и групп OH в молекулах компонентов в смесях, по которым различаются витамины. Разработан метод количественной идентификации отдельных компонентов

в смесяхс использованием параметров LIFS, а также предложена техническая поддержка в клинической диагностике и таргетной терапии с использованием витаминов.

Ключевые слова: *лазерно-индуцированная флуоресценция, спектр, витамин, молекула, диапазон длин волн, интенсивность.*

Introduction. Vitamins are essential micronutrients for the human body [1]. It is estimated that at least 1/3 of people all over the world suffer from hunger for vitamins [2–4], and vitamin deficiency contributes substantially to the incidence of disease, mortality of the disease, and the decline in the quality of life [4]. Vitamins have been divided into fat-soluble vitamins and water-soluble vitamins [1, 3, 5–8], they have dramatic impacts on the global burden of disease through their effect on cell function, metabolic status, immune function, anti-inflammation function, blood system and antioxidative function [8]. Interest in vitamins has increased greatly in medicine, drugs, and supplements for targeted therapy, or food and supplements for the prevention of diseases and health over the last few years [1, 8–10]. Therefore, the detection of vitamin levels is very important in cases of clinical assay and diagnosis, and food, drugs, and supplements survey.

There is an ever-increasing demand for analytical methods that can reliably detect and quantify vitamins. In the fields of qualitative and quantitative analysis in human serum and urine, cells suspension, food, plant, drugs, medicine and biological samples, liquid chromatography-mass spectrometry (LC-MS) has been successfully applied as a routine analytic tool for its advantages of stability, cueing capability, analysis range, linearity, accuracy, and precision [11–14]. Although the measurement accuracy of LC-MS was 40% higher than classical microbiological methods [15], there are still some existing hindering factors for LC-MS application, such as the pretreatment techniques and preparation time of samples, the ability of analysts on mass spectrometry estimation, signal processing and filtering, data processing, explanation of fragment peak and annotation of fragment structure, matrix effects and interference, structural damage of samples, and structure elucidation of unknown substances [16, 17]. However, there are some other analytical methods that also have been exploited and applied for vitamin detection—for instance, enzyme-linked immunosorbent assay (ELISA) and immunochromatographic (ICT) were used for vitamin B1 detection [18], and fluorescent sensing was used for vitamin C detection [19]. Based on fluorescent sensing advantages of simple handling, economical, rapid, ultrasensitive, and selective [20], laser-induced fluorescence (LIF) is a potential underlying method for vitamin detection.

LIF is a spectroscopic technique of molecularly targeted excitation and has been widely used in the field of medical diagnosis, pollutant monitoring, combustion products diagnosis and food detection, etc. [21]. It has wide application potential in the field of detection and diagnosis, due to its advantages of high efficiency, specificity, sensitivity, amplified ability and selectivity, rapid, reliability, small detection volume, acquiring information without ionization or dissociation damage, simple and easy-to-learn, non-intrusive, and online measuring [22, 23]. It should be emphasized that the LIF concentration detection limit is near 10−13 mol/L, which is a huge advantage over other methods [24]). In addition, LIF spectroscopy (LIFS) can be used for the detection and analysis of unknown substances based on time-dependent density functional theory (TD-DFT), and for simultaneous detection of multispecies with interleaved excitation and multiplexing technology [25, 26]. For the aforementioned advantages, LIF and LIFS have been used for medical diagnosis, such as dentistry, the immunological response of mice, and gastric inflammation and gastric cancer [27–29].

At present, LIF is a mature analysis technology for the optical diagnosis of polycyclic aromatic hydrocarbons (PAHs) [25, 30] under 266-nm laser exciting. Most vitamins, such as Vitamin D3 (VitD), have a benzene or benzene-like ring structure [31, 32]. Thus, LIFS can be used for rapid and selective detection of vitamins. It has been used to detect folic acid, vitamin A, vitamin B, and some other vitamins with a benzene ring structure [33–35]. There are many kinds of vitamins in human blood or tissues [36], and these vitamins have been proven to be closely related to the occurrence and development of diseases [4]. Additionally, multivitamins are usually used as drugs or food for targeted prevention or treatment [2]. Vitamin excess may raise some serious problems, such as acute or chronic toxicity [37]; therefore, this study is designed to investigate the possibility of the detection of vitamins A, B1, C, D, E, and K1 using LIF implementation with the aim that it can provide technical support for clinical diagnosis and targeted therapy.

Materials and methods. All chemical materials were obtained from Macklin Co., Shanghai, China and used without further processing or purification: vitamin A (VitA) (100 mg, 95%), vitamin B1 (VitB) (1 g, 98%), vitamin C (VitC): L-ascorbic acid (250 mg AR), vitamin D (VitD: 25-hydroxyvitamin D3 (5 mg 98%), vitamin E (VitE) (20mg ≥99% HPLC), vitamin K1 (VitK) (1 g, 98%) and ethanol (500 mL, 99.5%).

The concentration of the standard solutions was set to 120 μg/mL. VitB and VitC were prepared in distilled water, while the stock standard solution of VitA, VitD, VitE and VitK were prepared in ethanol. These stock solutions are stored in amber glasses.

A LIF measurement system, consisting of a sample cell, a laser-excitable system, and a fluorescence acquisition system, was used for vitamin LIFS detection (Fig. 1) [38]. The sample cell was a quartz cuvette (LAFELON, Beijing) for laser propagation and fluorescence detection, which was fixed on a lifting plate to guarantee effective and consistent measurements. In the laser excitable system, a 266-nm laser with a frequency of 10 Hz and energy of 0.085 J/cm^2 was provided by a Nd:YAG laser (Spectra Physics, Pro 250). A cylinder lens group converted the laser spot to a 50×0.8-mm laser sheet for analysing by going through sample cells. The fluorescence signals were captured by fiber bundles placed at a right angle with respect to the laser propagation and conveyed to a spectrometer (Bruker, 250is). In order to improve the signal-to-noise ratio of the fluorescence spectrum, a convex lens with a focal length of 100 mm was used to focus light on fiber bundles. An intensified charge-coupled device (ICCD) (Andor, DH720i) gathered the fluorescence spectra from the spectrometer. The grating of the spectrometer was set as 150 lines/mm, which guarantees spectral resolution and measurable spectral range. The acquisition time was 10ns after laser excitation by a delay trigger (DG535 Stanford Research, USA), the gate width was 40 ns with a gain of 200, and the number of scans was 200 with 10 repeating times.

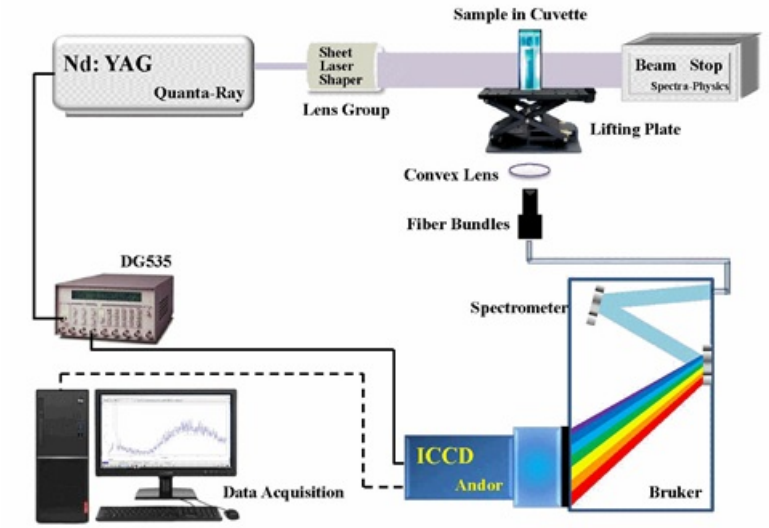

Fig. 1. LIF measurement system.

The laser extinction coefficient can be simply expressed as the sum of particulate matter scattering, particulate matter absorption, matters absorption in solution and Rayleigh scattering [39]. As there are no particulates in solutions, the molecular extinction coefficient was only the vitamin absorption with near zero laser absorption of water and ethanol.

According to Beer–Bouguer's Law, the mole extinction coefficient ε was computed by the following formula:

$$\varepsilon = AM / bc\,, \tag{1}$$

where A is laser absorbance at 266 and 532 nm, which was detected by spectrometry instead of laser energy meter in this paper; b is the length of optical paths whose value was 1 cm; c is the solution concentration whose value was 120 μg/mL; M is the molar mass of each vitamins with units in g/mol. The result of the mole extinction coefficient of VitK at 266 and 532 nm were shown in Fig. 2b. It can be seen that the mole extinction coefficient at 266 nm is more suitable as the main characteristic of the fluorescence than that at 532 nm. Thus, the mole extinction coefficient at 266 nm will be a characteristic for subsequent analysis.

The repetition times for all detections were 10 times. The fluorescence spectra were analyzed using Andor Solis (version 4.14.30001.0) and OriginLab (version Pro 8.1). Burrs in the original data of the fluorescence spectra were smoothed as shown in Fig. 2. Then the LIFS results were shown as averages of 10 repeated times of experimental data, and the characteristics of the fluorescence were shown with errors and confidence intervals.

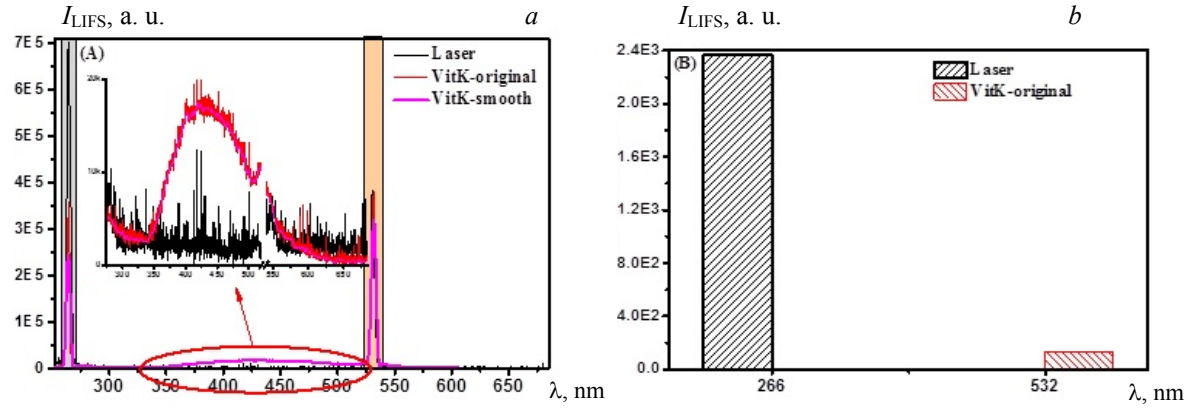

Fig. 2. Denoising laser interference and complete fluorescence spectra of six vitamins.

Results and discussion. *LIFS of different vitamins.* LIFS has been used as a standard technique to estimate the molecular structure feature of samples to a certain extent. In order to get rid of the effect of noise from the laser and obtain the real information of vitamins that the fluorescence spectra is carrying, the starting wavelength for results analysis is 276 nm, and the break region of the horizontal axis is from 520 to 540 nm. Figure 3a shows the UV-visible fluorescence emission spectra of sample solutions of six vitamins at 20°C. After the interferential signals from the laser at 266 and 532 nm were wiped off and the background intensity was removed, the LIFS of each vitamin solution showed their unique profile in Fig. 2a. It can be seen that the LIFS range of VitA was from 304 to 650 nm with two peaks at 369 and 417 nm. The LIFS range of VitB was from 339 to 568 nm with one peak at 433nm, while the LIFS range of VitC was from 310 to 632 nm with one peak at 366 nm. The LIFS range of VitD was from 292 to 570 nm with two peaks at 372 and 435 nm, whereas the LIFS range of VitE was from 303 to 621 nm with two peaks at 370 and 435 nm, and the LIFS range of VitK was from 340 to 550 nm with one peak at 419 nm. It can be noted that the vitamin solutions showed very strong LIFS emission peaks when they were excited at 266 nm, which is indicative of very good luminescent properties; however, the difference in LIFS intensity for every sample was obvious. The intensity of VitA LIFS peak was only 20% of that of VitK, mainly due to the following two aspects. One aspect is that the absorption efficiency at 266 nm is different for different molecular structures of vitamins. Some structures need more energy for electron transition from a ground state to an excited state. The other was that the number of electron transitions was different for different molecular structures. As described by Zheng [33] and Zhang [25], aromatic rings, five-membered rings, unsaturated six-membered rings, rich radicals and unsaturated aliphatic-branched chains were conducive to the formation of fluorescent quantum dots. For example, with the increasing aromatic ring number, the fluorescence emission wavelength increased, and the fluorescence intensity also strengthened. Compared to unsaturated five-membered rings, the quantum efficiency of saturated five-membered rings for UV excitation was quite low. Zhang [25] pointed out that the reason was caused by different electron transitions in the highest and lowest unoccupied molecular orbitals.

The results in Fig. 3 also indicated that the characteristic spectra of VitA and VitC were chiefly located in the UV region alongside that of VitD and VitE in the range from near UV to visible. That is to say, the identification range of VitA and VitC LIFS was from 320 to 380 nm. Meanwhile, the best identification range of VitD and VitE LIFS was from 350 to 440 nm with that of VitB and VitK in 400–470 nm. This can be used to simply identify the vitamin components, which were similar to the previous results [33]. This result undoubtedly provides important evidence and strong support for our proposed rapid, sensitive and selective detection of vitamin components. Further analysis showed that the LIFS intensities of VitA and VitC were lower than the other four due to the absence of an aromatic ring. The redshift of the cut-off and peak wavelengths of VitA to VitC were 18 and 51 nm, respectively (Fig. 3a). According to the molecule structure, there were four C=C bonds in branched olefin at 2-positions of cyclohexenyl in VitA. These five C=C bonds will cause vibrational spectrum congestion in the process of transitioning from an excited state to a ground state, further leading to a wider LIFS range by the doppler effect. In the VitC molecule, there was a C=O bond at the 2-position of the oxygenated pentatomic ring with dienol and a propylene glycol at the 5-position. The four OH groups in VitC had similar benefits regarding a widening range as the five C=C bonds

in VitA (Fig. 3a). Based on energy theory, the LIFS intensities of VitC were higher than that of VitA due to the C=C bond and the C=O bond in the pentatomic ring.

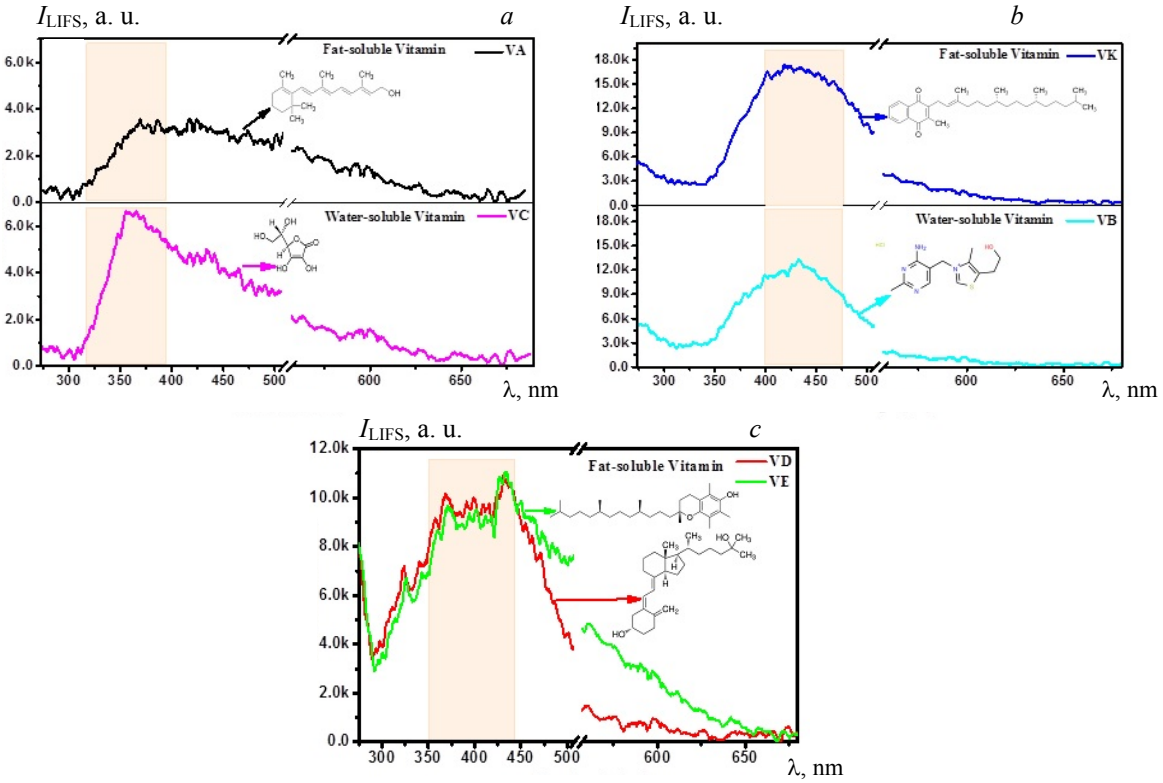

Fig. 3. LIFS of vitamin A and C (a), B and K (b), D and E (c).

The shape of VitK LIFS was similar to that of VitB LIFS with higher fluorescence intensity than the other four. This is mainly because of the aromatic ring in the molecule, which has a very attractive LIF emission property. As there were two C=O bonds at 1- and 4-positions of cyclohexenyl and a C=C bonds in branched hydrocarbons at 2-positions of cyclohexenyl in the VitK molecule, and there were two C=C bonds included in an unsaturated five-membered ring in the VitB molecule, the blueshift of the cut-off and peak wavelengths of VitK to VitB were 18 and 14 nm (Fig. 3b). This can be explained by the presence of more energy being released in the process of going from an excited state atom down to a ground state. It was also the reason that the VitK LIFS intensity was higher than that of VitB. The shape of VitE LIFS was very coincident with that of VitD LIFS, while the redshift values of the starting and cut-off wavelengths were 11 and 52 nm (Fig. 3c). Comparison and analysis of the molecular character showed that VitE and VitD both have three C=C bonds. The three C=C bonds in VitE were from an aromatic ring, and the three C=C bonds in VitD were from three unsaturated double bond at the 1-, 2- and 4-positions of cyclohexyl. Thus, the LIFS range of VitE with redshift was wider than that of VitD according to the energy theory. In brief, the results show that the effect of the unsaturated double bond in vitamin molecular on LIFS intensity was as follows: aromatic ring > 1–6 position of six-membered ring > cyclohexenyl > cyclopentenyl > pentatomic ring with dienol > branched olefin. Figure 3 showed that the six vitamins can be distinguished to some extent by LIFS range. However, unlike distinguishing vitamins by LIF color [33], it was still difficult to distinguish vitamins by LIFS range owing to existing regions of coincidence and the nondisjunction of similar regions. Therefore, in order to distinguish vitamins, the characteristic LIFS should be used rather than LIF image.

Figure 4 showed statistical results of the LIFS spectral range, peak wavelength, starting wavelength, maximum LIFS intensity, and mole extinction coefficient at 266 nm of the six vitamin solutions. It can be seen that the spectral range decreased with the increase of fluorescence intensity alongside redshifts of peak wavelength and starting wavelength. The redshifts of peak wavelength and starting wavelength were due to higher energy-level gaps, which release higher energy in electron transition. The decreased LIFS range was caused by two factors. One was the decrement of returned electrons to the ground state, congested molecular

vibration was eased, and the doppler effect was weakened [40]. The other was that electrons in the ground state need more energy to jump to an excited state, which in turn led to photons being emitted at shorter emission wavelengths [25]. In addition, combined Figs. 3 and 4, characteristics of the fluorescence can aid LIFS in the determination of vitamin composition. The starting wavelength, maximum LIFS intensity and mole extinction coefficient at 266 nm can be used to auxiliarily discriminate VitB and VitK. Starting wavelength, spectral range, peak wavelength, maximum LIFS intensity, and mole extinction coefficient at 266 nm can be used to auxiliarily discriminate VitA and Vit C. Spectral range can be used to auxiliarily discriminate VitD and VitE. It is noteworthy that maximum LIFS intensity is in inverse proportion to the mole extinction

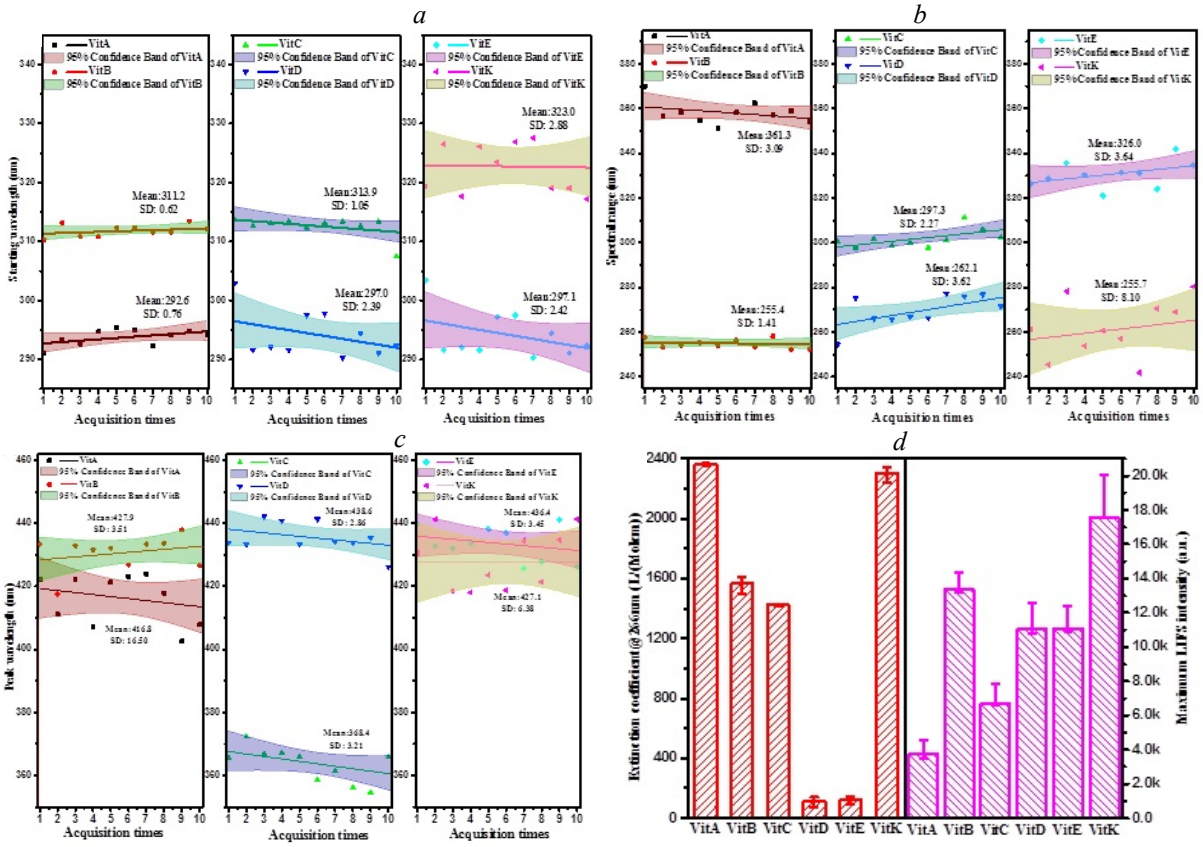

Fig. 4. Characteristics of the fluorescence of starting wavelength (a), spectral range (b), peak wavelength (c), extinction coefficient and maximum LIFS intensity (d).

coefficient at 266 nm except for VitB and VitK. This is mainly due to the different contributions of chemical structure [41]. The extinction coefficient at 266 nm contributions of vitamins' chemical structure was on the order of C=C bonds > C=N bonds > C=O bonds > OH bonds. The maximum LIFS intensity contributions of vitamins chemical structures were decided by the molecular structure and unsaturated bond in the molecular structure on the basis of the preceding analysis shown in Fig. 3. This finding suggests that molecular structure and unsaturated bonds were the key factors for vita-mins detection by LIF.

LIFS with different vitamin mixtures. Vitamins are usually present in multiple components at the same time. Therefore, the preceding experimental results may not be entirely suitable for the diagnosis of multiple vitamin components. This part will further analyse the LIFS of fat-soluble vitamin mixture (1 mL VitA solution+1 mL VitD solution +1 mL VitE solution +1 mL VitK solution, named VitMixFat), water-soluble vitamin mixture (1 mL VitB solution+1 mL VitC solution, named VitMixWater) and all mixtures (1 mL fat-soluble mixture+1 mL water-soluble mixture, named VitMixAll).

For a comparison of the fat-soluble mixture, water-soluble mixture, and all six vitamin mixtures, LIFS and fluorescence characteristics are shown in Fig. 5 and Table 1. It was found that the LIFS of VitMixFat mainly came from the contribution of VitD and VitK in the UV-visible range. VitK mainly contributed to the LIFS peak; thus, it can be seen that the LIFS range of VitMixFat was basically consistent with that of the medium LIF intensities vitamins (VitD and VitE), and the LIF intensity of the mixture took into account the

characteristics of the medium and high LIF intensities vitamins (VitD, VitE, and VitK). In addition, the transmission mechanism of radiation energy showed that there was a light loss in UV due to the complex influence of the aromatic ring on the molecule mixture [42]. In Fig.5, the LIFS range and LIFS peaks of VitMixWater reflected characteristics of both VitB and VitC. VitMixAll embodied the fluorescence intensity feature bringing attention to both the VitMixFat and VitMixWater (Fig. 5). The LIFS range of VitMixAll was slightly wider than that of VitMixFat or VitMixWater because of the congestion of the vibration. Besides, the maximum LIFS intensity and mole extinction coefficient at 266 nm of VitMixFat and VitMixWater were mainly dependent on the corresponding vitamin components and the amounts of vitamins. The maximum LIFS intensity and mole extinction coefficient at 266 nm of VitMixAll are lower than that of VitMixFat and VitMixWater in various degrees due to interference among the different molecular structures of six vitamins.

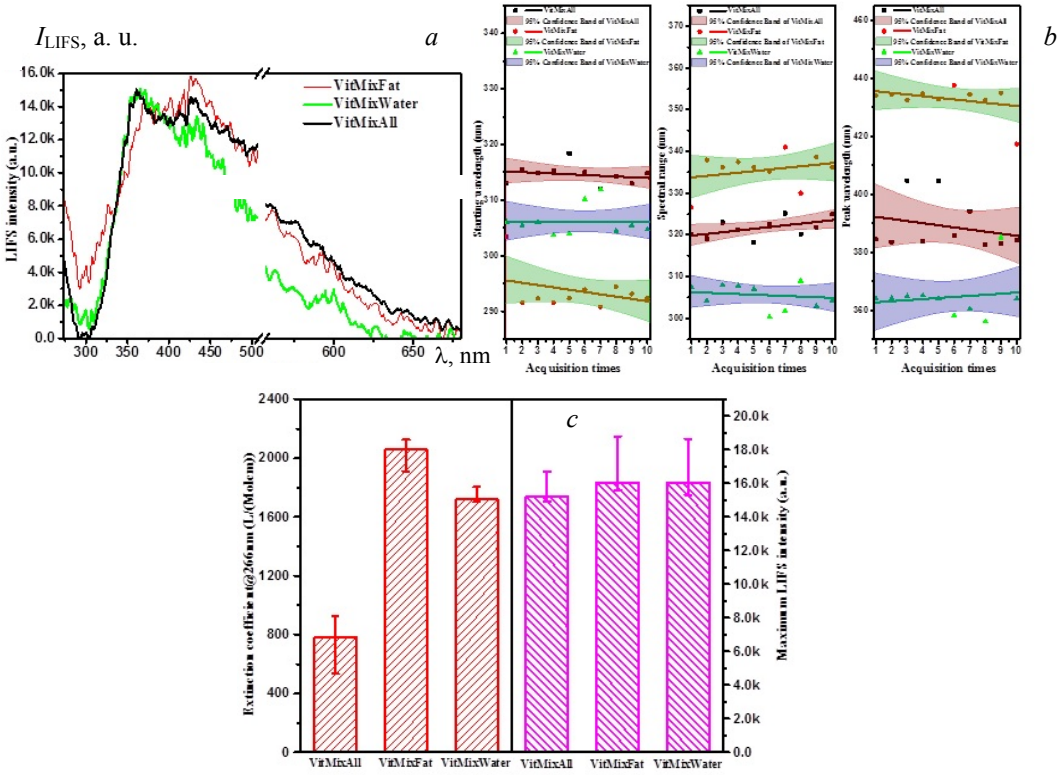

Fig. 5. LIFS (a) and characteristics of the fluorescence of starting wavelength, spectral range and peak wavelength (b), extinction coefficient and maximum LIFS intensity (c) of different mixture.

TABLE 1. Characteristics of the Studied Vitamins Mixtures' Fluorescence

Characteristics of LIFS	VitMixAll	VitMixFat	VitMixWater
Starting wavelength, nm [mean (SD)]	315.3 (1.14)	306.1 (1.80)	295.9 (2.16)
Spectral range, nm [mean (SD)]	319.4 (1.29)	333.4 (2.61)	306.4 (1.95)
Peak wavelength, nm [mean (SD)]	392.9 (5.58)	435.9 (3.37)	362.3 (4.98)
MEC @ 266 nm, L/(mol · cm) [mean (error)]	783.0 (+142.90,–247.24)	2056.9 (+68.07, –145.39)	1719.3 (+85.26, –17.57)
Maximum intensity a.u., L/(mol · cm) [mean (error)]	15229.8 (+1453.99, –305.69)	16038.2 (+2967.92, –443.17)	16012.6 (+2628.85, –715.73)

N o t e. MEC: Mole extinction coefficient.

Combined with the results in Figs. 3 and 4, when several vitamins coexist, the spectral range and peak wavelength, mole extinction coefficient at 266 nm, and maximum LIFS intensity can reflect the main vita-

min components in the mixture of vitamins to a certain extent. Simultaneously, spectral range and peak wavelength, extinction coefficient, and maximum LIFS intensity could also, to some extent, reflect the quantity with or without a benzene ring, double bonds, and OH group in the molecule. Compared with the LIFS analysis of the single pure vitamin, the LIFS analysis of vitamin mixtures had limitations in discrimination. It is necessary to further improve spectral resolution properties of LIFs of different vitamin components in mixtures by complemental methods in the separation of vitamins or the characterization of each vita-min LIFS. Therefore, this paper proposes a technique to identify individual components in model mixtures quantitatively by using specific fluorescence parameters as follows. First, the LIFS of mixtures are collected. Then the extinction coefficient at 266 nm and maximum LIFS intensities from the data analysis are used to deduce the molecular feature and the bonds. Second, the LIFS of VitMixFat and VitMixWater are collected after fat-water separation, and the extinction coefficient and maximum intensities are used to do the assistant analysis. Third, the purified possible components are purified by particular solvents. Then the spectral range, peak wavelength, starting wavelength, extinction coefficient, and maximum intensity are used to determine quantitative components, and the LIFS of residual mixtures are used to close loopholes.

Conclusions. This study aimed to investigate the possibility of detectin vitamins A, B1, C, D, E, K1 and their mixtures by using LIF, and to explore the technical support for the detection of vitamins in blood, tissues, drugs or food via LIFS for clinical diagnosis and targeted therapy. The conclusions can be summarized in the following.

When excited at 266 nm, six vitamin solutions have their own unique LIFS. The results indicated that LIFS can be used to distinguish vitamins via spectral range to some extent—e.g., VA and VC were in 320–380 nm, and VD and VE were in 350–440 nm with VB and VK in 400–470 nm. Starting wavelength, spectral range, peak wavelength, maximum intensity and extinction coefficient can auxiliarily discriminate between VitB and VitK, VitA and Vit C, VitD, and VitE. Molecular structure and unsaturated bonds were the key factors for vitamin detection by LIF. LIFS intensity can be used to identify vitamins by molecular structure as follows: aromatic ring > 1–6 position of six-membered ring > cyclohexenyl > cyclopentenyl > pentatomic ring with dienol > branched olefin. The extinction coefficient was a discrimination factor for vitamin identification by chemical structure in the order of C=C bonds > C=N bonds > C=O bonds > OH bonds. The number of the similar double bonds or radicals can be used to help with the identification starting with the spectral range. The starting wavelength and peak wavelength of LIFS can be used as complements for pure vitamin component identification. This undoubtedly provides important evidence and strong support for our proposed LIFS detection of vitamins, and might offer us one or more clues for technical support in clinical diagnosis and targeted therapy using vitamins.

Then, we explored the influence of vitamin mixtures on the LIFS characteristics. The experimental results showed that LIFS can reflect the main vitamin components in the mixture of vitamins to a certain extent when several vitamins coexist. The characteristics of LIFS (starting wavelength, spectral range, peak wavelength, maximum intensity and extinction coefficient) could also, to some extent, report the number with or without a benzene ring, double bonds and OH group in the molecule.

According to the analysis presented in this paper, it is a challenge to distinguish vitamins in mixture solutions based on the LIFS and its' characteristics, owing to existing coincidence regions and the nondisjunction of similar regions. Thus, it is suggested that characteristic LIFS should be used for distinguishing vitamins rather than LIF images. In order to resolve limitations in the discrimination of vitamin components in vitamin mixtures by LIFS analysis and allow the quantitative identification of individual components in mixtures, it is necessary to improve the spectral resolution properties of the LIFS of different vitamin components in mixtures. This paper proposes a technique to identify individual components in mixtures quantitatively by using LIFS and LIFS parameters in this order: LIFS identification of mixture, definition of possible components after fat-water separation, quantitative identification of individual components after targeted purification, and closing loopholes from residual mixtures. All told, LIFS is an appropriate, advanced, and developing technology for the detection of vitamins.

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