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# Determination of Palmitate on the Basis of the Fluorescence Enhancement of an $Eu^{3+}$–TTA Complex

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A simple, sensitive, and selective method for the determination of palmitate has been investigated on the basis of the fluorescence enhancement of $Eu^{3+}$–TTA complex in the form of $Eu^{3+}$–TTA–palmitate. An emission peak of $Eu^{3+}$–TTA, which increased linearly with the addition of palmitate, occurs at 610 nm in aqueous solution with excitation at 306 nm. The linear curve of calibration ranges is from $1.0 \times 10^{-6}$ to $6.0 \times 10^{-6}$ M, and the detection limit is $5.0 \times 10^{-7}$ M. The effects of foreign ions were studied. The method was applied to the determination of palmitate content in synthetic and beef fat samples.

Palmitic acid is widely distributed in most animal and vegetable oils and fats as the glycerol esters. The acid is obtained by hydrolysis and saponification from the esters which are mostly used as sources of olechemicals, surfactants, and edible oils.[1–3]

The optimal composition of vegetable oils for human consumption is a compromise between nutritional physiology and food technology. From a nutritional aspect, the content of saturated fatty acids such as palmitic acid should be as low as possible. From this perspective, oil-seed rapes yield the most valuable oil among major oil crops.[4] Whether or not the oils and fats contain palmitic acid can be ascertained through gas chromatography subsequent to methylation.[5]

Other methods of determining fatty acids such as spectrophotometry[6] and spectrofluorimetry[7] have been developed as well. However, they lack sensitivity. HPLC[8] and MALDI-TOFMS[9] (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) are also used in the determination of palmitate. However, they require special instruments with higher costs. Therefore, simple, sensitive, and inexpensive methods are required. We have found that the fluorescence intensity of the $Eu^{3+}$–TTA complex changes when some organic compounds[10,11] are added. We also investigated a determination method for palmitate by using the increasing fluorescence intensity of the $Eu^{3+}$–TTA complex added to palmitate.

## Experimental

**Apparatus and Reagents.** All the measurements were performed with a Shimadzu RF-5301PC Spectrofluorophotometer with a 150 W xenon lamp using 1 cm quartz cell. A Mettler Toledo MP 220 pH meter was used in measuring pH. Gas chromatography experiments were performed using a Shimadzu GC-2010 with a DB-5MS capillary column (length: 30 m, i.d.: 0.32 mm, thickness of coated film: 25 m) and Part No. US2472185H equipped with a mass spectrometer (Shimadzu MS-2010) as the detector. Europium oxide (99.95%), TTA (99.95%), and palmitic acid (99.9%) were obtained from Aldrich. An $Eu^{3+}$ solution was obtained by dissolving europium (III) oxide ($Eu_2O_3$) in hydrochloric acid. TTA and palmitate were dissolved in 50% ethyl alcohol. Acetic acid–sodium acetate was used as a buffer solution. Analytical chemicals and deionized distilled water were used throughout the whole experimental process.

**Procedure.** 1 mL of $1.0 \times 10^{-4}$ M $Eu^{3+}$, 3 mL of $1.0 \times 10^{-4}$ M TTA, and 5.0 mL of $1.0 \times 10^{-4}$ M palmitate were added to a 50 mL volumetric flask and diluted to the mark with water. The fluorescence intensity was corrected with a blank solution ($Eu^{3+}$–TTA). The overall experiments were conducted at 25 °C.

## Results and Discussion

**Excitation and Emission Spectra.** The excitation and emission spectra of the $Eu^{3+}$–TTA–palmitate complex are shown in Fig. 1. For practical purposes, it is necessary to use shorter excitation and longer emission wavelengths.[12] The maximum excitation and emission wavelengths were between 306 and 610 nm, respectively. The a and a′ curves are the excitation spectra of $Eu^{3+}$–TTA and $Eu^{3+}$–TTA–palmitate solutions, and the b and b′ curves are the emission spectra of the same solutions, respectively. The presence of palmitate caused on increase in the absorbance and emission intensity, but no changes in the maximum wavelength.

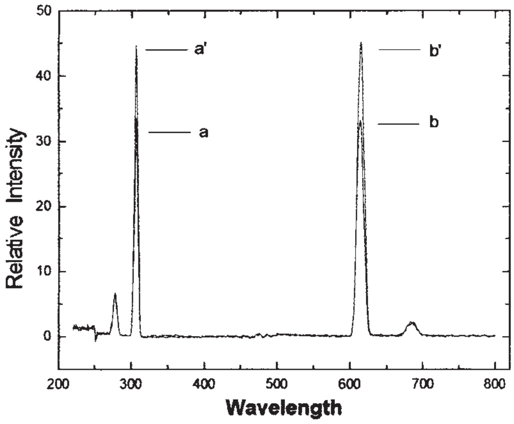

Fig. 1. Excitation (a and a′) and emission (b and b′) spectra in the absence (a, b) and presence (a′, b′) of palmitate. $Eu^{3+}$: $2 \times 10^{-6}$ M, TTA: $6 \times 10^{-6}$ M, Palmitate: $1 \times 10^{-6}$ M.

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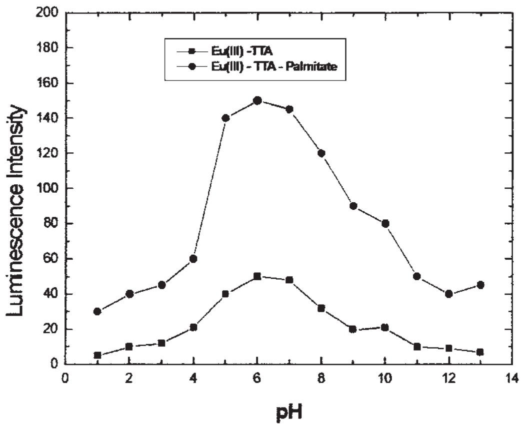

Fig. 2. pH effects on the fluorescence intensity of $Eu^{3+}$–TTA and $Eu^{3+}$–TTA–palmitate. $Eu^{3+}$: $2 \times 10^{-6}$ M, TTA: $6 \times 10^{-6}$ M, Palmitate: $1 \times 10^{-6}$ M.

The change in pH ranged from 3 to 9 (see Fig. 2). The highest fluorescence intensity appeared at pH 6.5 due to improved stability of the complex. Therefore, the pH 6.5 buffer solution of acetic acid–sodium acetate was used for all experiments. The concentration effect of $Eu^{3+}$ and TTA on the fluorescence intensity of the $Eu^{3+}$–TTA–palmitate system was also studied. The fluorescence intensity showed a progressive increase when the TTA concentration went up to $6 \times 10^{-6}$ M in the solution of $2 \times 10^{-6}$ M $Eu^{3+}$, but it decreased at higher concentrations. The mole ratio of $Eu^{3+}$ to TTA was 1:3 and that of $Eu^{3+}$ to palmitate was 1:2. The results indicate that the composition of the complex is $Eu^{3+}(TTA)_3(palmitate)_2$. The intensity of the triple complex was influenced by the concentration.

**Enhancement Mechanism of Fluorescence Intensity.** The fluorescence intensity of $Eu^{3+}$ is weak because of low oscillatory of its absorption.[13] However, a fluorescence enhancement of $Eu^{3+}$ could be achieved by transferring energy from the triple state of an organic ligand such as diketones to the $Eu^{3+}$ in the complex.[14,15] The composition of the $Eu^{3+}$–TTA chelate is $Eu^{3+}(TTA)_3(H_2O)_2$.[16] In the presence of palmitate ion, the structure of the chelate includes two palmitate ions instead of two water molecules. The palmitate ions prevent water molecules from quenching, which probably activates the vibration of the O–H bond.[17] Therefore, the fluorescence intensity of $Eu^{3+}$–TTA is increased with the addition of palmitate.

**Calibration Curve of Palmitate.** The increase in the fluorescence intensity of the $Eu^{3+}$–TTA–palmitate was linear with respect to palmitate concentration. The linear palmitate concentration ranged from $1 \times 10^{-5}$ to $6 \times 10^{-5}$ M when the concentrations of $Eu^{3+}$ and TTA were $2 \times 10^{-5}$ and $6 \times 10^{-5}$ M, respectively (see Fig. 3). It ranged from $1 \times 10^{-6}$ to $6 \times 10^{-6}$ M when the concentrations of $Eu^{3+}$ and TTA were $2 \times 10^{-6}$ and $6 \times 10^{-6}$ M, respectively (see Fig. 4). The detection limit was $5.0 \times 10^{-7}$ M (S/N = 3).

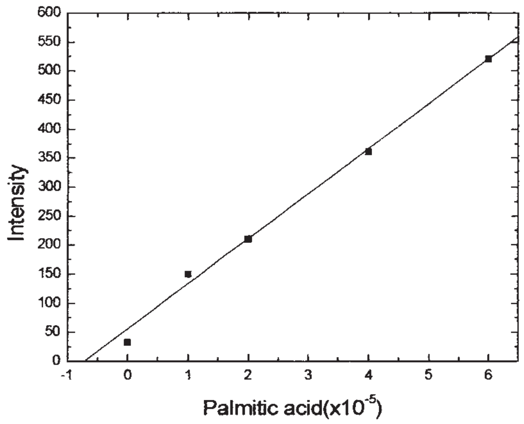

Fig. 3. Calibration curve of palmitate. $Eu^{3+}$: $2 \times 10^{-5}$ M, TTA: $6 \times 10^{-5}$ M.

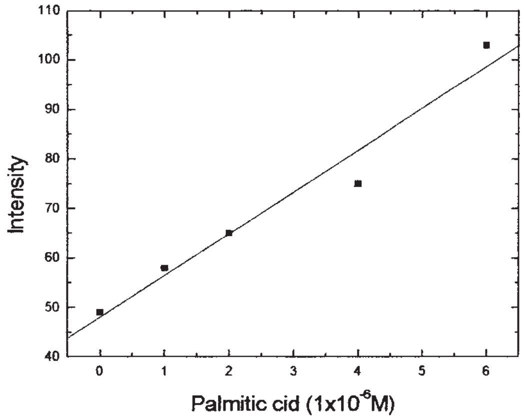

Fig. 4. Calibration curve of palmitate. $Eu^{3+}$: $2 \times 10^{-6}$ M, TTA: $6 \times 10^{-6}$ M.

Table 1. Tolerance Limits of Foreign Ions

| Ions | Tolerance/M |
|---|---|
| Citrate | $1 \times 10^{-4}$ |
| Benzoate | $1 \times 10^{-4}$ |
| Acetate | $1 \times 10^{-4}$ |
| Oleate | $5 \times 10^{-4}$ |
| Stearate | $1 \times 10^{-4}$ |
| Laurate | $2 \times 10^{-4}$ |
| $CO_3^{2-}$ | $5 \times 10^{-4}$ |
| $SO_4^{2-}$ | $5 \times 10^{-4}$ |
| $Ca^{2+}$ | $4 \times 10^{-4}$ |
| $Mg^{2+}$ | $6 \times 10^{-4}$ |

**Interference.** The effects of some anions and cations on the fluorescence intensity of the $Eu^{3+}$–TTA–palmitate complex were investigated in the presence of $5 \times 10^{-5}$ M palmitate (Table 1). The tolerance limits were calculated by the concentration of foreign ions, resulting in less than a 5% deviation in the fluorescence intensity of the $Eu^{3+}$–TTA–palmitate. As shown in Table 1, the interference factors of organic acids such as acetic, benzoic, citric, and lauric acids hardly affect the determination of palmitate when the concentration of these organic acids was less than $1 \times 10^{-4}$ M.

## Application

In order to test the validity of the current method, two synthetic and beef fat samples were prepared and the palmitate content was determined by gas chromatography. The analytical data obtained by the standard addition of the present method

Table 2. Samples and Analytical Data

| Composition | Present (RSD, $n = 3$) | Gas chromatography |
|---|---|---|
| Sample 1: $5 \times 10^{-6}$ M of oleic, lauric, stearic, acetic, and citric acids in $1.0 \times 10^{-5}$ M palmitic acid | $1.1 \times 10^{-5}$ M (3.1%) | $1.1 \times 10^{-5}$ M |
| Sample 2: $1 \times 10^{-5}$ M of oleic, lauric, stearic, acetic, and citric acids in $5.0 \times 10^{-5}$ M palmitic acid | $5.1 \times 10^{-5}$ M (2.4%) | $5.1 \times 10^{-5}$ M |
| Sample 3: Beef fat | 31.5% (4.2%) | 32.7% |

Table 3. Experimental Conditions of Gas Chromatography

| | |
|---|---|
| Carrier gas | He |
| Flow control mode | pressure |
| Pressure | 81.1 kPa |
| Total flow | 60.3 mL/min |
| Column flow | 0.89 mL/min |
| Linear velocity | 35.8 cm/s |
| Purge flow | 3.0 mL/min |
| Split ratio | 63.6 |
| Ion source temp. | 200 °C |
| Interface | 280 °C |
| Solvent cut time | 5.0 min |
| Detector mode (mass) | Scan |
| Start time (mass) | 5.0 min |
| End time (mass) | 19.0 min |
| Interval (mass) | 0.5 s |
| Scan speed (mass) | 1111 |
| Start $m/z$ (mass) | 50.0 |
| End $m/z$ (mass) | 550.0 |

was compared with the results from gas chromatography (Table 2). The experimental conditions in the gas chromatography used are shown in Table 3. From the data, a good agreement was reached. The analysis of beef fat samples by gas chromatography requires a saponification and methylation of the fatty sample into fatty acid methyl esters (FAME),[18] while the present method only requires a saponification of the fat.

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