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Investigation on a host–guest inclusion system by β-cyclodextrin derivative and its analytical application

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ABSTRACT

The host–guest inclusion system of ethyl substituted β-cyclodextrin (DE-β-CD) with mangiferin (MA) was investigated by fluorescence spectra in solution. The results showed that the MA was encapsulated in the DE-β-CD's cavity to form a 2:1 stoichiometry host–guest inclusion complex (DE-β-CD/MA) and the inclusion constant ($K = 3.04 \times 10^6$ L^2/mol^2) was confirmed by the typical double reciprocal plots. Furthermore, several experimental conditions were optimized in order to obtain the maximum fluorescence signal. In addition, the thermodynamic parameters, Gibbs free energy (ΔG°), enthalpy change (ΔH°) and entropy change (ΔS°) of DE-β-CD/MA were obtained by the Van't Hoff equation. A spectrofluorimetric method for the determination of MA in solution in the presence of DE-β-CD was developed based on the remarkable enhancement of the fluorescence intensity of MA. The linear range was 2.00×10^{-8}–7.00×10^{-6} mol/L and the detection limit was 4.05×10^{-9} mol/L. The proposed method was successfully applied to the analysis of MA in serum with the satisfactory result.

β-Cyclodextrin (β-CD), the macrocyclic compound with seven D-glucopyranose units linked by α-1,4-glycosidic bonds, which possess a hydrophilic exterior and an interior hydrophobic cavity. β-CD has been extensively investigated in host–guest chemistry for construction of versatile supramolecular aggregations owing to its special hydrophobic cavities and ability to improve physicochemical properties and chemical stability of drugs.[1–5] Nevertheless, the natural β-CD, whose solubility is poor.[6] It is well known that any of the hydrogen bond-forming hydroxyl groups of β-CD are substituted, even by lipophilic functions, the water solubility of β-CD will be greatly enhanced.[7] So far, native and modified β-CD have been extensive application in many fields such as medicinal chemistry and supramolecular chemistry.[8–10]

Mangiferin (MA, 1,3,6,7-tetrahydroxyxanthone-C-2-β-D-glucoside, Fig. 1) is a naturally occurring glucosyl xanthone derived from barks, leaves, and fruits of mango tree (*Mangifera indica*). It has a wide range of pharmacological activities such as anti-diabetic, anti-HIV, immunomodulatory activities, anti-cancer, anti-inflammatory, and antibone resorption effects.[11–16] However, MA is a low water-soluble drug, whose scarce absorption is well known.[17] These aspects limit the application and determination of MA. Thus, it is quite meaningful to develop new methods for determination of MA in aqueous media. Until now, several methods, such as UPLC–MS/MS,[17] reversed-phase HPLC,[18] spectrophotometry,[19,20] TLC,[21,22] NIRS,[23] and capillary electrophoresis method,[24] have been reported in the literatures for determination of MA. Among these studies, there was no spectrofluorimetric method for MA determination in the presence of DE-β-CD. This proposed method's dominance displayed high sensitivity, wide linear range and low analytical cost.

In this study, the host–guest inclusion interaction between DE-β-CD and MA were explored by spectrofluorimetry. A series of conditions during the formation of the inclusion complex was investigated. Based on the great enhancement of the fluorescence

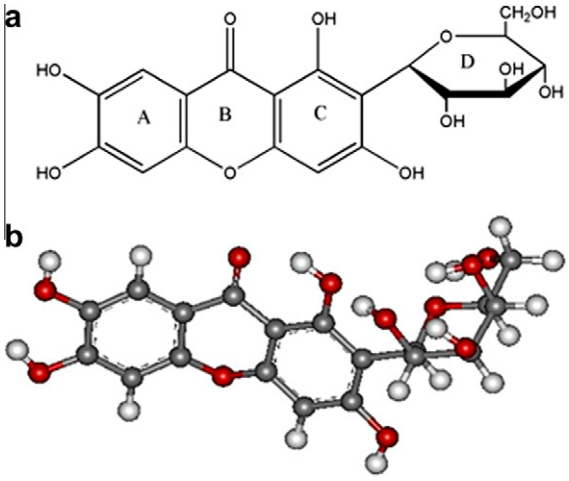

Figure 1. The structure and sketch of MA.

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intensity of MA, a novel method was developed to determine MA in serum. The proposed method for MA determination is fairly simple, rapid and has high selectivity and wide linear range, which is of significance for analytical determination, it will provide some basis for developing new methods for determination of MA in biological fluids.

The experiment procedure was carried out as follows: DE-β-CD was synthesized according to the reference[25] and its stock solution (1.0×10^{-3} mol/L) was conducted in doubly distilled water. In a 10 mL color comparison tube, 1.0 mL of 2.0×10^{-5} mol/L MA, 1.0 mL H_3BO_3–KCl–NaOH (pH 8.0) buffer solution, and the varied amounts of DE-β-CD (0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 mL of 1.0×10^{-3} mol/L) were added in this order. Then the mixed solution was diluted to the mark with doubly distilled water and ultrasonically oscillated for 30 min at room temperature. The fluorescence spectra was measured at $\lambda_{ex}/\lambda_{em}$ = 406 nm/505 nm, $slit_{ex/em}$ = 5/10 nm. The stoichiometry and inclusion constant of DE-β-CD/MA was gained from the double reciprocal plots.[26]

Calibration graph of MA: an aliquot of solutions containing 0.0–2.0×10^{-5} mol/L of MA were added in colorimetric tube, respectively, then 1.0 mL pH 8.0 H_3BO_3–KCl–NaOH buffer solution and 6.0 mL of 1.0×10^{-3} mol/L DE-β-CD were added sequentially. The mixture was diluted to 10.0 mL with doubly distilled water and ultrasonically oscillated for 30 min at room temperature. The fluorescence intensities at 505 nm were measured.

The pH of solution system greatly affects the formation of inclusion complex between DE-β-CD and MA. The concentrations of DE-β-CD and MA were held constant: 6.0×10^{-4} and 2.0×10^{-6} mol/L, respectively. The pH was changed from 4.0 to 10.0 with the Britton–Robinson buffer solutions. Figure 2 depicts the fluorescence intensity was relatively high and remained constant in the range of 7.0–8.6. Among Tris–HCl, H_3BO_3–KCl–NaOH, Britton–Robinson, and KH_2PO_4–NaOH buffer solution systems (pH 8.0), it was more sensitive for the reaction system in H_3BO_3–KCl–NaOH buffer solution. Therefore, the acidity of solution was adjusted to pH 8.0 with H_3BO_3–KCl–NaOH buffer solution in subsequent experiments.

The influence of DE-β-CD concentration on the fluorescence intensity of MA was also investigated by keeping its concentration constant at 2.0×10^{-6} mol/L and varying the DE-β-CD concentration from 0.0 to 8.0×10^{-4} mol/L. The gradually increased fluorescence signals of MA in solution with the stepwise addition of DE-β-CD are shown in Figure 3. The fluorescence intensity reached its maximum when the concentration of DE-β-CD up to 6.0×10^{-4} mol/L, and there is slight change in fluorescence intensity by further addition of DE-β-CD. So 6.0×10^{-4} mol/L of DE-β-CD was chosen.

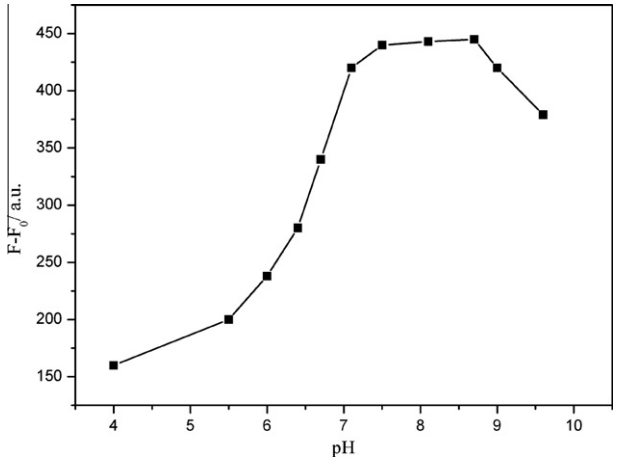

Figure 2. Effect of pH value on the fluorescence intensity of the complex. $C_{MA} = 2.0 \times 10^{-6}$ mol/L; $C_{DE\text{-}\beta\text{-}CD} = 6.0 \times 10^{-4}$ mol/L.

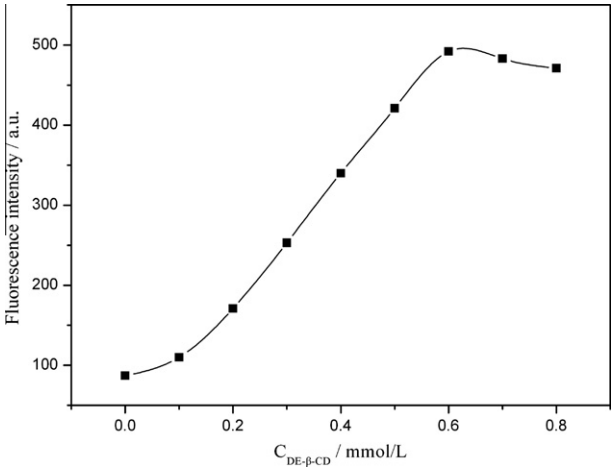

Figure 3. Fluorescence intensity variation at 406 nm are plotted against concentrations of DE-β-CD ($C_{MA} = 2.0 \times 10^{-6}$ mol/L).

The effect sequence of adding reagents on the fluorescence intensity of MA were also investigated. The order: MA, H_3BO_3–KCl–NaOH buffer solution, DE-β-CD was proved to be best. The effect of reaction time was also tested. The fluorescence intensity of the complex reached a maximum after the mixture of reagent solutions had been ultrasonically oscillated for 30 min, and then, the luminescence intensity could remain constant for at least 1 h. Hence, the reaction time of 30 min was selected.

In optimum conditions, the fluorescence spectra of MA in solutions in the absence and presence of DE-β-CD was performed and displayed in Figure 4, which exhibited emission maxima at 505 nm, the intensity of fluorescence increased with a concomitant increase concentration of DE-β-CD. The enhancement of fluorescence intensity could be rationalized by the increased micro environmental hydrophobicity and/or steric shielding around the fluorophore arising from the cooperative interactions between the host and guest. It is well known that fluorophores were partially or wholly encapsulated within the CD's cavity could be better protection from quenching and other processes that occur in the bulk solvent.[27,28] It would be the hindered rotation of the guest molecules as well as a considerable decrease in the relaxation of the solvent molecules. The effects can result in a decreased vibrational deactivation of the excited guest molecules and,

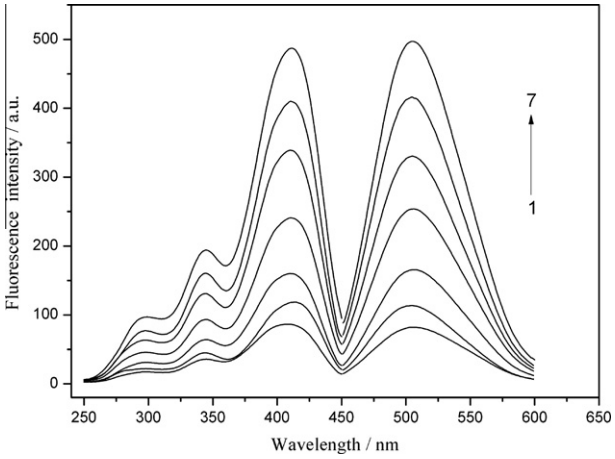

Figure 4. Fluorescence excitation (left hand) and emission (right hand) spectra of MA (2.0×10^{-6} mol/L) containing various concentrations of DE-β-CD (**1**→**7**: 0.0×10^{-4}, 1.0×10^{-4}, 2.0×10^{-4}, 3.0×10^{-4}, 4.0×10^{-4}, 5.0×10^{-4}, 6.0×10^{-4} mol/L of DE-β-CD).

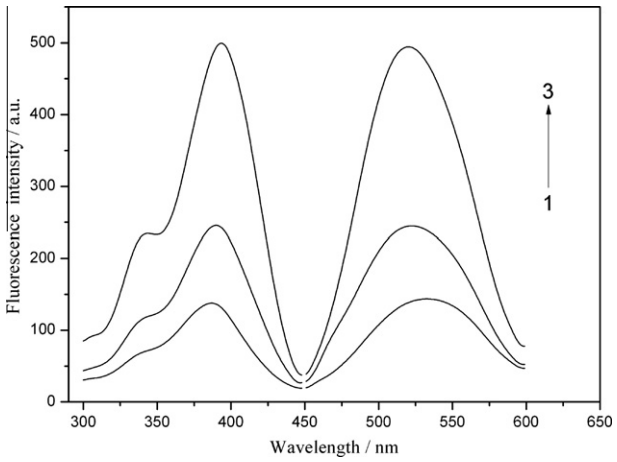

Figure 5. Fluorescence excitation (left hand) and emission (right hand) spectra of MA (2.0×10^{-6} mol/L) in different media at pH 8.0: (1) without β-CD; (2) native β-CD; (3) DE-β-CD. ($C_{β\text{-}CD} = 6.0 \times 10^{-4}$ mol/L; $C_{DE\text{-}β\text{-}CD} = 6.0 \times 10^{-4}$ mol/L).

consequently, in increased fluorescence intensity of the system. Thus, the phenomena revealed that MA molecule was moved into DE-β-CD's cavities, an obvious inclusion process occurred.

Figure 5 shows the fluorescence spectra of MA in the presence of β-CD and DE-β-CD, respectively. MA has weak fluorescence and there was an obvious advantage in the presence of DE-β-CD. Experimental results implied that DE-β-CD exhibits more forceful inclusive ability than native β-CD. It may be attributed to the cavity of DE-β-CD provides a better protective microenvironment, the substitution groups leads to the enlargement of the bigger opening, and destroy the strong hydrogen bond network, which makes it easier for guest molecules to gain access to DE-β-CD's cavity with excellent fluorescence property.

It is desirable for considering the formation of inclusion complex with a ratio of 1:1 or 1:2. From the fluorescence spectra, inclusion constants of DE-β-CD/MA could be ascertained by the typical double reciprocal plots.[26]

$$\frac{1}{F-F_0} = \frac{1}{(F_\infty - F_0)KC_{β-CD}} + \frac{1}{F_\infty - F_0} \quad (1)$$

$$\frac{1}{F-F_0} = \frac{1}{(F_\infty - F_0)K'C^2_{β-CD}} + \frac{1}{F_\infty - F_0} \quad (2)$$

where F denotes the fluorescence intensity of the MA solution at each DE-β-CD concentration tested. F_0 and F_∞ are the fluorescence intensity in the absence of DE-β-CD and when all the MA molecules were complexed, respectively. K and K' are the inclusion constants. It was taken into account as follows: (a) comparing with MA, DE-β-CD was in a large excess to assure its free concentrations similar to its analytical concentrations, (b) the variations in the fluorescence signals are proportional to the complex concentration and all of the MA molecules were absolutely complexed at high DE-β-CD concentration.[26]

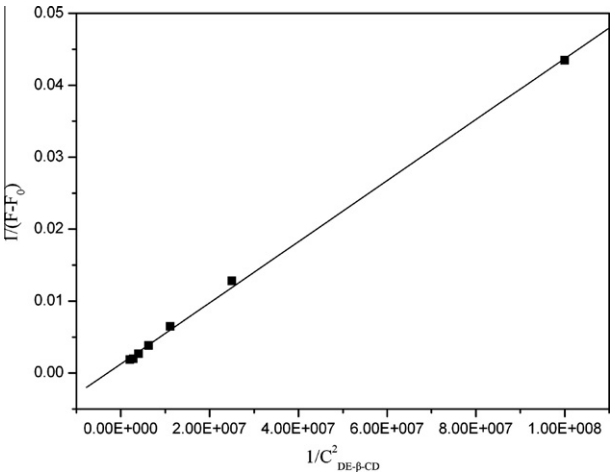

Figure 7. Double reciprocal plot obtained from $1/(F - F_0)$ plotted against $1/C^2_{DE-β-CD}$.

$1/(F - F_0)$ against $1/C_{DE\text{-}β\text{-}CD}$, and $1/(F - F_0)$ against $1/C^2_{DE-β-CD}$ were plotted through the experimental data. No linear relationship was gained when $1/(F - F_0)$ was plotted against $1/C_{DE\text{-}β\text{-}CD}$ in Figure 6. While making a plot of $1/(F - F_0)$ against $/C^2_{DE-β-CD}$, good linear relationship ($r = 0.9992$) was observed in Figure 7, which confirmed that DE-β-CD and MA formed host–guest complex in 2:1 stoichiometry. It can be shown that these the inclusion constant (K) was determined to be 3.04×10^6 L^2/mol^2 and the relative standard deviation (RSD) was 2.32% ($n = 5$).

The thermodynamic parameters, Gibbs free energy ($\Delta G°$), enthalpy change ($\Delta H°$) and entropy change ($\Delta S°$) for the inclusion complex of DE-β-CD/MA were obtained from the Van't Hoff equation: $\ln K = -\Delta H°/RT + \Delta S°/R$. The corresponding $\Delta H°$ and $\Delta S°$ can be obtained from the slope and intercept by plotting $\ln K$ versus $1/T$ (Figure 8), $\Delta G°$ was obtained according to the equation: $\Delta G° = \Delta H° - T\Delta S°$. The results of the analysis were collected in Table 1.

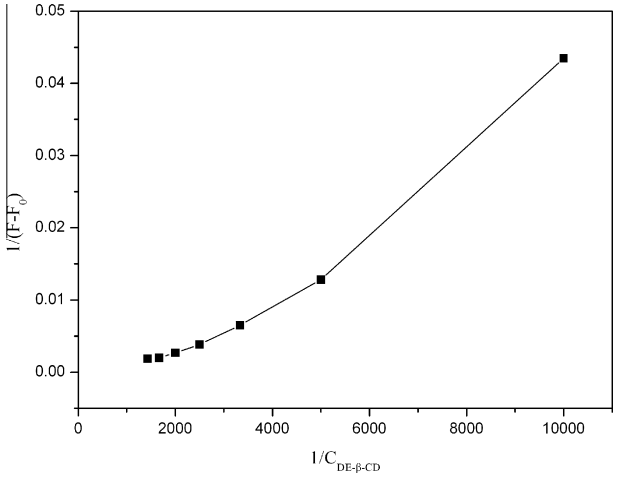

Figure 6. Double reciprocal plot obtained from $1/(F - F_0)$ plotted against$1/C_{DE\text{-}β\text{-}CD}$.

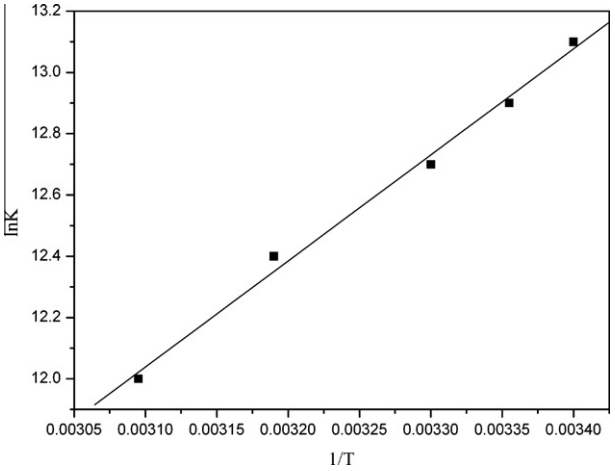

Figure 8. Plot of $\ln K$ versus $1/T$.

As can be seen from Table 1, The negative value of ΔG° displayed that the inclusion process was a spontaneous process and thermodynamically favored at experimental temperature. The negative ΔH° indicated that the complex dissociates when the temperature increases. ΔH° plays a predominant role in the free energy change, it is mainly affected by temperature and the size-fit degree of host–guest. ΔS° depends on the confused degree of system. The entropy increases is mainly due to the disorder caused by the breakage of the ordered solvation shell around the guest or inside the CD cavity, which only partially takes place when such a guest hardly enters into it.[29] Usually, the enthalpy decrease is beneficial to the reduction of the free energy, while the entropy increase is also in favor of the reduction of free energy.

A systematic study was carried out on the effects of the foreign interferences on the determination of 1.0×10^{-6} mol/L of MA. A 3000-fold mass excess of each interference over MA was investigated first, if interferences occurred, the ratio was reduced gradually until the interferences ceased. The criterion for interference was fixed at a ±5% variation of the average fluorescence intensity calculated for the established level of MA. The results are summarized in Table 2, it can be seen the proposed method had outstanding selectivity.

A simple and rapid spectrofluorimetric method for MA determination in solution in the presence of DE-β-CD was developed. At the optimum experimental conditions, there was a linear relationship ($r = 0.9975$) between the fluorescence intensity and the concentration of MA in the range of 2.00×10^{-8}–7.00×10^{-6} mol/L. The detection limit, as defined by IUPAC[30] was calculated to be 4.05×10^{-9} mol/L and RSD was 1.87%. The proposed method had high sensitivity, selectivity, and reproducibility at the same temperature.

The proposed method was also employed to determine MA in samples of human serum. The samples of human serum, obtained from the hospital of Lanzhou University, were stored below 4 °C and diluted appropriately to be within the linear range of determination of MA using the standard calibration method. Serum sample was treated according to the literature.[31] The standard solution containing 0.84 mg was added to 2.00 mL serum of adult man. This solution was divided into two same parts. One was transferred into 100 mL volumetric flask and diluted to the mark with doubly distilled water (sample 1). The other was mixed with acetonitrile in the volume ratio of 1:3 order to remove protein completely. Then, the mixture was centrifugated for 10 min. After that, the centrifugate was diverted into a 100 mL volumetric flask and diluted to volume with doubly distilled water (sample 2). The determination results were gained in Table 3 and displayed there was no interference from the serum compositions.

The host–guest inclusion system of DE-β-CD with MA was reported, which revealed that the inclusion compound was form in host–guest ratio 2:1 with excellent fluorescence property and MA enter the DE-β-CD's cavity. Moreover, thermodynamic parameters for inclusion complex were calculated. A spectrofluorimetric method with good accuracy and practicability for determination of MA in the presence of DE-β-CD was established on the basis of the significant enhancement of fluorescence intensity of MA. All these information will provide some theoretical and practical methods for determination of MA in biological fluids.

Table 1
The thermodynamic parameters

	Temperature (K)				
	293 K	298 K	303 K	313 K	323 K
ln K [a]	13.1	12.9	12.7	12.4	12.0
ΔG° [b] (KJ mol^{-1})	−31.911	−31.960	−31.993	−32.268	−32.225
ΔH° [c] (KJ mol^{-1})	−28.777				
ΔS° [d] (J mol^{-1} K^{-1})	10.800				

[a] Apparent formation constant (K).
[b] Standard free energy (ΔG°).
[c] Enthalpy (ΔH°).
[d] Entropy (ΔS°).

Table 2
Effect of interference (tolerance error ± 5.0%)

Tolerance ratio in mass	Interference
3000	$K^+(Cl^-)$, $Na^+(Cl^-)$, $Cl^-(Na^+)$, $CO_3^{2-}(K^+)$, $SO_4^{2-}(Na^+)$
2500	$HPO_4^-(K^+)$, $NO_3^-(Na^+)$, $Ca^{2+}(Cl^-)$, $Mg^{2+}(Cl^-)$
2000	Sucrose, polyethylene glycol
1500	Sodium acetate
1000	Carbamide, glucose, tryptophan
800	Glycine, polyethylene glycol
600	Polyvinyl pyrrolidone, corn starch, L-histidine
500	Lysine, lactose
300	β-Alanine, uric acid, L-cysteine

Table 3
Determination of MA in human serum ($n = 5$, $p = 95\%$)

Sample No.	MA added (10^{-6} mol/L)	MA found (10^{-6} mol/L)	Recovery (%)	RSD ($n = 5$)
1	2.00	2.02	101	1.32
	4.00	3.97	99	2.21
	8.00	8.25	103	0.95
	10.00	9.83	98	1.98
2	2.00	2.04	100	1.36
	4.00	3.96	99	3.64
	8.00	3.89	97	0.87
	10.00	10.18	102	2.87

Acknowledgments

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