

RSC Advances



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This *Accepted Manuscript* will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

A study on the interaction between emodin and ethyl violet by Resonance Rayleigh scattering technique

Ernv Wang^a, Jidong Yang^{a,b,c*}, Donghai Yu^a

^a School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China

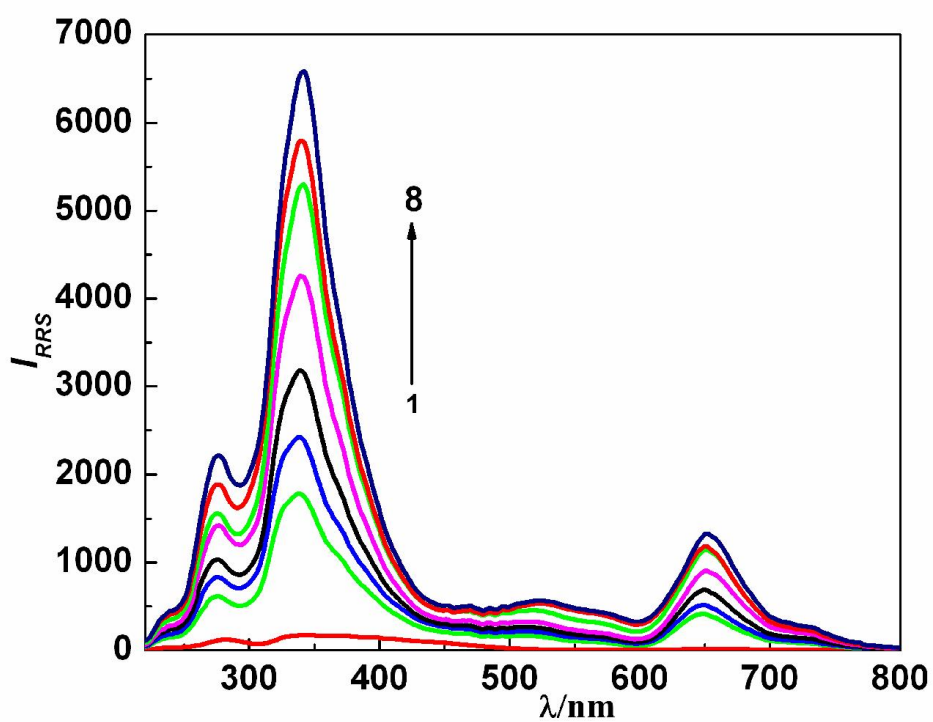
^b The Key Laboratory of Inorganic Special Functional Materials, Chongqing & Technology Commission, School of Chemistry and Chemical Engineerin Yangtze Normal University, Fuling, Chongqing 408100, China

^c School of Chemistry and Environment Engineering, Chongqing Three Groges University, Wanzhou, Chongqing 404000, China

Abstract

A novel resonance Rayleigh scattering method was developed for the determination of emodin (EMO). In pH 7.0 Britton-Robinson (BR) buffer medium, the scattering signal of ethyl violet was remarkably enhanced after adding trace amount of EMO and forming a 1:1 ion-association complex, which not only resulted in the change of absorption spectra, but also led to a significant enhancement of resonance Rayleigh scattering (RRS), frequency doubling scattering (FDS) and second order scattering (SOS). The maximum RRS, SOS and FDS wavelengths of the ion-association complex were located at 340nm, 528nm and 341nm, respectively. The linear ranges and detection limits for RRS, SOS and FDS were $0.4 \sim 4.2 \mu\text{g}\cdot\text{mL}^{-1}$, $0.4 \sim 4.8 \mu\text{g}\cdot\text{mL}^{-1}$, $0.4 \sim 4.8 \mu\text{g}\cdot\text{mL}^{-1}$ and $3.8 \text{ng}\cdot\text{mL}^{-1}$, $14.2 \text{ng}\cdot\text{mL}^{-1}$, $17.7 \text{ng}\cdot\text{mL}^{-1}$, separately. In this work, the optimum conditions, the influencing factors and the effects of coexisting substances on the reaction were investigated. The method can be applied to the determination of EMO in serum and urine sample and the results were satisfactory. Moreover, the reaction mechanism and reasons of the enhancement of resonance light scattering were discussed.

Keywords resonance Rayleigh scattering; frequency doubling scattering; second order scattering; emodin; ethyl violet



EV-EMO system. $C_{\text{EMO}}(1-6)$ 0,0.4,1.0,1.5,2.3,3.2,3.8,4.2 $\mu\text{g} \cdot \text{mL}^{-1}$; C_{EV} : $1.2 \times 10^{-5} \text{mol} \cdot \text{L}^{-1}$; pH7.0

Study on the Interaction between Emodin and Ethyl Violet by Resonance Rayleigh Scattering Technique

Ernv Wang^a, Jidong Yang^{a,b,c*}, Donghai Yu^a

a. School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P.R.China. **b.** School of Chemistry and Environment Engineering, Chongqing Three Groves University, Wanzhou, Chongqing 404000, P.R.China. **c.** School of Chemistry and Chemical Engineering, Yangtze Normal University, Fuling, Chongqing 408100, P.R. China

Abstract

A novel resonance Rayleigh scattering method was developed for the determination of emodin (EMO). In pH 7.0 Britton-Robinson (BR) buffer medium, the scattering signal of ethyl violet was remarkably enhanced after adding trace amount of EMO and forming a 1:1 ion-association complex, which not only resulted in the change of absorption spectra, but also led to a significant enhancement of resonance Rayleigh scattering (RRS), frequency doubling scattering (FDS) and second order scattering (SOS). The maximum RRS, SOS and FDS wavelengths of the ion-association complex were located at 340nm, 528nm and 341nm, respectively. The linear ranges and detection limits for RRS, SOS and FDS were $0.4\sim 4.2\mu\text{g}\cdot\text{mL}^{-1}$, $0.4\sim 4.8\mu\text{g}\cdot\text{mL}^{-1}$, $0.4\sim 4.8\mu\text{g}\cdot\text{mL}^{-1}$ and $3.8\text{ng}\cdot\text{mL}^{-1}$, $14.2\text{ng}\cdot\text{mL}^{-1}$, $17.7\text{ng}\cdot\text{mL}^{-1}$, separately. In this work, the optimum conditions, the influencing factors and the effects of coexisting substances on the reaction were investigated. The method can be applied to the determination of EMO in serum and urine sample and the results were satisfactory. Moreover, the reaction mechanism and reasons of the enhancement of resonance light scattering were discussed.

Keywords resonance Rayleigh scattering; frequency doubling scattering; second order scattering; emodin; ethyl violet

1. Introduction

Emodin (EMO), widely exists in plants cathartics such as rhubarb roots, buckthorn's root and root bark, and Cassia seeds, is a kind of anthraquinone compounds. EMO inhibits the synthesis DNA by interfering with the replication of DNA template^[1]. Based on that information it has the effects of anti-tumor^[2-4], anti-microbial growth^[5], immunosuppression,

etc. Clinically, pure EMO is mainly used to treatment leukemia^[6], gastric cancer and its most commonly used is the bacteriostasis. In the meantime, EMO also has antitussive, diuretic, choleric, antispasmodic, lower blood pressure, and so on. Although toxicity of emodin is small, it is worth noting that pregnant woman is forbidden because it may lead to miscarriage. Due to the wide application of EMO, the determination of EMO and its metabolites become especially interesting.

At present, the methods for the determination of EMO consist of fluorescence spectrometry^[7], micellar electrokinetic capillary chromatography^[8], spectrophotometry^[9], supercritical fluid extraction^[10], thin layer chromatography scanning^[11], reversed phase high performance liquid chromatography^[12], high-performance liquid chromatography(HPLC)^[13], and resonance light scattering technique^[14]. Among them, HPLC needs some complex pretreatments and 0.48~2.40 $\mu\text{g/mL}$ is a little narrow. Spectrophotometric method is simple to operate, but it has a low sensitivity. Fluorescence spectrometry method based on the fluorescence intensity of derivatives of calix [4] arene was quenched by EMO as the result of the formation of a weaker fluorescent inclusion complex sensitized in hexadecyl trimethyl ammonium bromide. However, its detection limit is not high enough^[7]. Therefore, to develop new, sensitive, simple, and rapid methods for trace determination of EMO make sense.

Resonance Rayleigh scattering (RRS) and resonance nonlinear scattering (RNLS) have got extensive application^[15-22] because of their sensitivity, simplicity and rapidity, and can be achieved in a common fluorescence spectrometer as new analytical methods. In recent years, these techniques have been increasing applied to determine DNA sequence^[15], ozone^[16], DNA^[17], metal ions^[18, 19] and polysaccharide such as hyaluronic acid^[20] and chondroitin sulfate A^[21], and pharmaceuticals^[22].

In the experiment, the interaction of EMO with some basic triphenylmethane dyes including ethyl violet(EV), crystal violet(CV), malachite green(MG), light green (LG)and methyl green(MeG), which have a similar structure and exist as cationic in aqueous solution have been investigated. The results show that in the CV and MeG systems, the intensities of resonance Rayleigh scattering was much smaller. As for MG and LG, the intensities of EMO alone are too large. The 1:1 ion-association complex of EMO with EV produced a significant enhancement of RRS, frequency doubling scattering (FDS) and second order scattering

(SOS), and the color fading of EV. The scattering intensities are proportional to the concentration of EMO in a wide range. The optimum conditions, the influencing factors and the effects of coexisting substances on the reaction were discussed by RRS method for its lowest detect limit of $3.8\text{ng}\cdot\text{mL}^{-1}$. The method can be applied to the determination of EMO in serum and urine sample and the results were satisfactory.

2. Experimental

2.1. Apparatus

A Hitachi F-2500 spectrofluorophotometer (Tokyo, Japan) was used to record the RRS, SOS and FDS spectra and to measure the scattering intensities with the slits (EX/EM) of $5.0/5.0\text{nm}$ for RRS and $10.0/10.0\text{nm}$ for SOS and FDS. A UV-3010 UV/VIS spectrophotometer (Tokyo, Japan) was employed for noting absorption spectra and measuring absorbance. A pH-S20K meter (Shanghai, Mettler-Toledo Instruments Co., LTD.) was used for adjusting pH values.

2.2. Reagents

The working solution of ethyl violet (EV) was $1.0\times 10^{-4}\text{mol}\cdot\text{L}^{-1}$.

A standard solution of emodin (EMO, Aladdin) at a concentration of $100\mu\text{g}\cdot\text{mL}^{-1}$ were prepared by weighing and dissolving suitable amount of emodin (EMO) reagent in ethanol. Then was further diluted with water to $20\mu\text{g}\cdot\text{mL}^{-1}$ as working solution.

Britton-Robinson (BR) buffer solutions with different pH were prepared by mixing the $0.2\text{mol}\cdot\text{L}^{-1}\text{NaOH}$ and the mixture of $0.04\text{mol}\cdot\text{L}^{-1}\text{H}_3\text{PO}_4$, H_3BO_3 and HAc according to suitable proportion. The pH value was adjusted by pH meter.

All reagents were of analytical reagent grade, doubly distilled water was used throughout.

2.3. Procedure

Into a 10mL calibrated flask were added 1.8mL of pH 7.0 BR buffer solution, 1.2mL EV and suitable amounts of EMO. The mixture was diluted to the mark with water and mixed thoroughly. After waiting for 10min, the RRS spectra of the system were recorded with synchronous scanning at $\lambda_{em}=\lambda_{ex}$, and the SOS and FDS spectra were recorded by scanning at $\lambda_{em}=2\lambda_{ex}$ and $\lambda_{em}=1/2\lambda_{ex}$, respectively. Then, the scattering intensity I_{RRS} , I_{SOS} and I_{FDS} for the

reaction product and I_{RRS}^0 , I_{SOS}^0 and I_{FDS}^0 for the reagent blank at their maximum wavelengths were measured, $\Delta I_{RRS} = I_{RRS} - I_{RRS}^0$, $\Delta I_{SOS} = I_{SOS} - I_{SOS}^0$ and $\Delta I_{FDS} = I_{FDS} - I_{FDS}^0$. The absorption spectra were recorded simultaneously.

3. Results and discussion

3.1. RRS spectra

Fig.1 shown the RRS spectra of EMO, EV and EMO-EV system at pH 7.0 (the pH value of the assay solution is 7.63). As shown in Fig.1, it can be seen that the RRS intensities of separate EMO and EV was very weak under optimum conditions. However, when a trace amount of EMO was added to the system of EV, the new spectra of RRS appeared and their intensities were enhanced remarkably with three peaks located at 276nm, 340nm and 657nm. The enhancement of

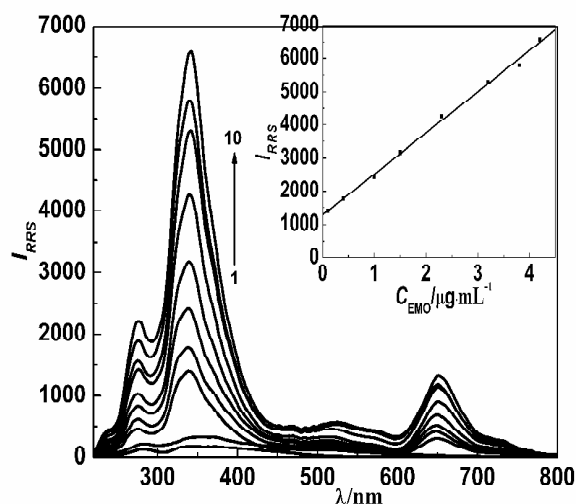


Fig.1. RRS spectra of EMO-EV system 1, EV ($1.2 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$); 2, EMO ($1.0 \mu\text{g} \cdot \text{mL}^{-1}$); 3-10, EV-EMO system. C_{EMO} : 0.1, 0.4, 1.0, 1.5, 2.3, 3.2, 3.8, 4.2 $\mu\text{g} \cdot \text{mL}^{-1}$; C_{EV} : $1.2 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$; pH=7.0

RRS intensities was directly proportional to the concentration of EMO and the maximum RRS wavelength was located at 340nm. So 340nm was selected as the analytical wavelength.

3.2. SOS spectra and FDS spectra

The SOS and FDS spectra of EMO-EV system were investigated (see SI, Fig.1 and Fig.2) and the results shown that the SOS and FDS intensities of EMO and EV themselves were very weak. After binding of EMO with EV to form binary complexes, SOS and FDS intensities were enhanced greatly and their maximum wavelengths ($\lambda_{\text{ex}}/\lambda_{\text{em}}$) were located at 260nm/520nm and 680 nm/340nm, respectively. In addition, there was also a little small SOS at 270nm/540nm. Due to the high signal-to-noise, we select 260nm/520nm and 680nm/340nm as detection wavelengths. The enhancement of SOS and FDS intensities were directly proportional to the concentration of

EMO. We all known RRS is a special elastic scattering produced when the wavelength of Raleigh scattering (RS) is located at or close to the molecular absorption band ^[23]. As RRS occurs, SOS and FDS may be possible. The scattering values and sensitivities of FDS and SOS are much lower than that of RRS.

3.3. Absorption spectra

Under the optimum conditions, the absorption spectra of EMO, EV and their complexes were researched (see SI, Fig.3). The results demonstrated that the absorption of EMO is located at 305nm and the maximum absorption wavelength of EV was at 590nm. When EV reacted with EMO to generate ion-association, its maximum absorption at 590nm decreased and the absorption intensity was directly proportional to the concentration of EMO in certain ranges (Fig.2). A new method for the determination of trace EMO based on fading reaction of EV was advanced.

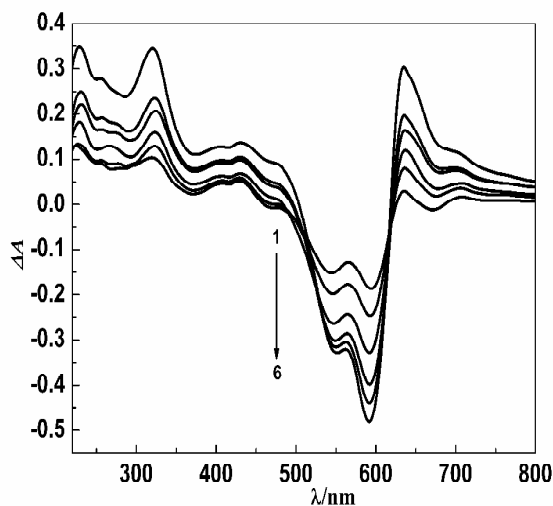


Fig.2. Absorption spectra EV-EMO system. $C_{\text{EMO}}(1-6)$ 0.8, 1.6, 2.4, 3.2, 4.0, $4.8 \mu\text{g} \cdot \text{mL}^{-1}$; $C_{\text{EV}}: 1.2 \times 10^{-5} \text{mol} \cdot \text{L}^{-1}$; pH=7.0

3.4. Optimum reaction conditions

3.4.1. Effect of acidity

The influences of solution acidity on the RRS intensity of the reaction system were tested. The results showed that the optimum pH range was 6.6~7.6. If pH was lower than 6.6, the RRS intensity of EMO increased rapidly; if pH was higher than 7.6, the RRS intensity of EMO-EV decreased significantly (see SI, Fig.4). So pH 7.0 was chosen as reaction acidity and the appropriate volume of buffer solution was 1.8mL.

3.4.2. Effect of the EV concentration

The experiment results indicated that the concentration of EV had little effect on the

determination of EMO. RRS intensity arrived the maximum when EV concentration is $1.2 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ (see SI, Fig.5). If EV is not enough, the reaction would be incomplete. Hence, we chosen $1.2 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ as a suitable EV concentration.

3.4.3. Effect of ionic intensity

The effect of ionic strength on the intensity of RRS was investigated using $1.0 \text{ mol} \cdot \text{L}^{-1}$ NaCl solution (see SI, Fig.6). The experiment results showed with the concentration of NaCl increasing, the intensities of RRS remained almost stable. Therefore, the reaction between EMO and EV, which maybe electrostatic attraction, hydrogen bond and hydrophobic force play important role together.

3.4.4. Effect of ethanol concentration

The effect of different volume ratio of ethanol on the RRS spectra of the EV and EMO-EV system was studied, and the results showed with the increasing ethanol concentration, ΔI_{RRS} was slightly affected, and decreased dramatically when the volume ratio of ethanol was beyond 0.04% (see SI, Fig.7). Too much ethanol would reduce the hydrophobic interface between EMO anion and EV cation or even make the interface between water molecules and association complexes disappear, which results in RRS decreased rapidly [24,25]. Therefore, ethanol should be controlled in a low concentration in the determination system.

3.4.5. Reaction speed and the stability

At room temperature, the reaction could complete in 10min and RRS intensity was stable in an hour.

4. Reaction mechanism and reasons for RRS enhancement

4.1. Formation of ion-association reaction

In the experiment, the composition ratio of EMO with EV in ion-association was researched by using Job's method of continuous variation and molar ratio method. When both the concentration of EMO and EV was $7.4 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$, the total volume of EMO and EV was 3.0mL in the Job's method of continuous variation, and the volume of EMO was constant 1.0mL in the molar ratio method. The RRS intensity was determined at $\lambda_{em} = \lambda_{ex} = 340 \text{ nm}$ and the results showed the ratio of EMO: EV was 1: 1 (see SI, Fig.8).

Using Conductor-Like Polarizable Continuum Model(CPCM)^[26] processing solution and Mo62X/CCPVCTZ level of density functional theory (DFT) method^[27], total energy and charge distribution of EMO were optimized to define the binding site of EMO and EV. The results showed EMO has three conformational isomers and the most stable conformation of EMO can be seen in Fig.3. As shown in Fig.6, the charge density of 1,8 α O and 3 β O of EMO were -0.711, -0.710 and -0.704. But α -OH interact with =O group to form a stable intramolecular hydrogen bond and make its acid

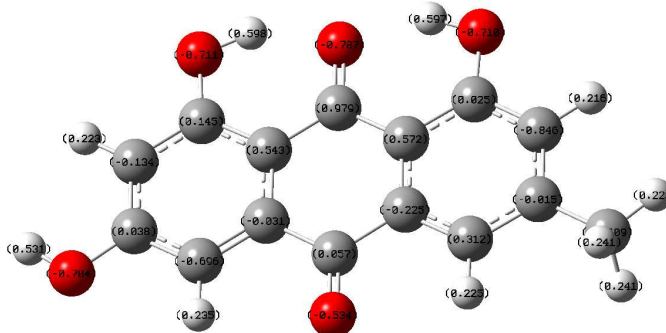


Fig.3. Optimized conformation and its electronic structure

weakened, so the most acidic phenolic hydroxyl group of emodin is at the 3 β position of the molecule. For instance, in sodium hydroxide solution, its balance is reversible. With the change of pH, 3-OH firstly dissociates and then followed by 1, 8-OH^[28]. The structure of the ion-association complex was shown in Fig.4.

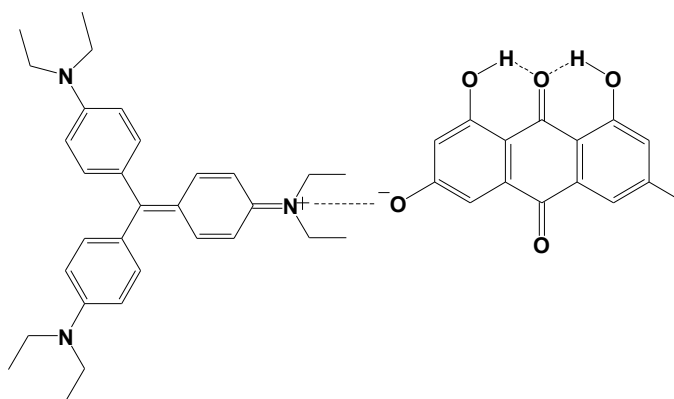


Fig.4.The structure of binary ion-association

4.2. Reasons for RRS enhancement

4.2.1. Effect of absorption spectra on RRS

When Raleigh scattering spectrum is located at or close to the molecular absorption band, the scattering can resonance with absorption light leading to the significant enhancement of RRS intensity and therefore RRS spectra should be closely related to the absorption spectra^[29]. From the comparison of RRS and absorption spectra (Fig.5) that three RRS peaks at

276nm, 340nm and 657nm are close to corresponding absorption peaks at 255nm, 305nm and 590nm of the EMO-EV system. Thus RRS intensity is remarkably increased. So, the resonance enhanced Raleigh scattering effect is a critical reason of scattering enlargement.

4.2.2. Enlargement of molecular volume

According to the simplified formula of Raleigh scattering $I = KCM/I_0$ ^[30], where molecular volume is replaced by molecular weight, RRS intensity is proportional to the molecular weight of aggregation of the particle. When EMO reacts with EV to form ion-association complex resulting in the increase of molecular weight, which is in favor of RRS enhancement. Also, the aggregation of the cationic dye on EMO is another important reason for the RRS enhancement.

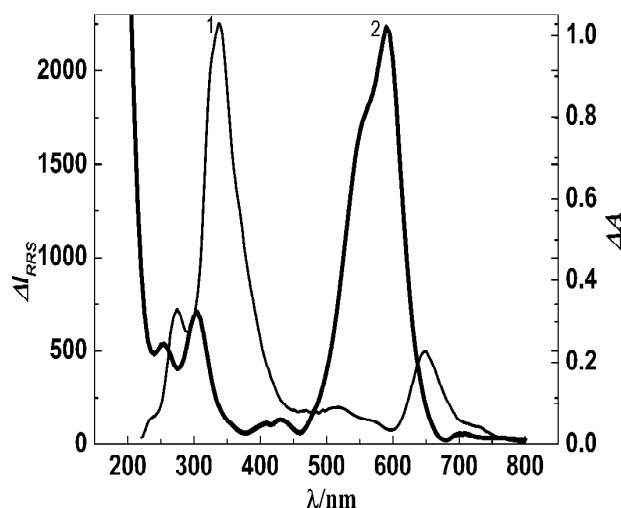


Fig.5. Comparison of RRS and absorption spectra
1, RRS spectrum ; 2, Absorption spectrum;
 $C_{\text{EMO}}: 1.0 \mu\text{g} \cdot \text{mL}^{-1}$, $C_{\text{EV}}: 1.2 \times 10^{-5} \text{mol} \cdot \text{L}^{-1}$ pH=7.0

4.2.3. Formation of the hydrophobic interface

Under the experimental conditions, EMO and EV exist in the form of anion and cation, respectively. They are well water-soluble, and can form hydrates easily in water, and the intensity of RRS is relatively low. When EMO and EV reacted with each other to form a neutralized ion-association complex, a hydrophobic liquid-solid interface appeared owing to the presence of the hydrophobic aryl framework of the binary complex. The formation of the hydrophobic interface is conducive to the enhancement of RRS signal^[31].

5. Selectivity, sensitivity and analytical application

5.1. Sensitivity

Under the optimum conditions, the RRS, SOS, FDS and absorbance spectral intensities of the system were measured at their own maximum wavelengths. The calibration graphs of ΔI_{RRS} , ΔI_{SOS} , ΔI_{FDS} and ΔA versus the concentration of EMO were constructed. The regression equation, linear range, correlation coefficient(r) and detection limit are listed in table1. It can be seen from the table that RRS method had highest sensitivity, and spectrophotometry sensitivity was far below the RRS, SOS and FDS method. The sensitivity of RRS method was higher than one to two orders of magnitude than those of common spectrophotometry, fluorimetry, micellar electrokinetic and capillary chromatography (shown in table2). So RRS method can be applied to the trace determination of EMO.

Table1 Related parameters of the calibration graphs for the determination of EMO

Method	Linear regression equation ($\mu\text{g}\cdot\text{mL}^{-1}$)	Linear range ($\mu\text{g}\cdot\text{mL}^{-1}$)	Correlation coefficient (r)	Detection limit ($3\sigma, \text{ng}\cdot\text{mL}^{-1}$)
RRS	$\Delta I=1272.38+1241.12c$	0.1~4.2	0.9986	3.8
SOS	$\Delta I=214.37+123.07c$	0.4~4.8	0.9930	14.2
FDS	$\Delta I=31.65+16.7675c$	0.4~4.8	0.9951	17.7
SP	$\Delta A=-0.1327-0.07682c$	0.8~4.8	0.9920	125.5

Table 2 Comparison of the sensitivities of some methods for the determination of EMO

Method	Medium condition	Detection Wavelength ($\lambda_{\text{ex}} / \lambda_{\text{em}}, \text{nm}$)	Linear range ($\mu\text{g}\cdot\text{mL}^{-1}$)	Detection limit ($\text{ng}\cdot\text{mL}^{-1}$)	Reference
HPLC		254	0.48~2.40		[8]
FL	pH 6.0	$\lambda_{\text{ex}}=270$	1.17~23.40	340	[2]
SP	pH>12.00	530	1~200	500	[4]
TLCS		445	0.1084~1.084		[6]
MECC	pH 9.5		5.2~260	290	[3]
RP-HPLC		254	0.01584~0.1584 ^a		[7]
RRS	pH 6.5	350	0.54~9.72	10.3	[9]
RRS	pH 7.0	340	0.4~4.2	3.8	This work
SOS	pH 7.0	528	0.4~4.8	14.2	This work
FDS	pH 7.0	341	0.4~4.8	17.7	This work

SP: spectrophotometry; FL: fluorimetry; TLCS: thin-layer chromatography scarmLng; MECC: micellar electrokinetic capillary chromatography.

^a μg .

5.2. Selectivity

Based on the potential interference composition in human urine and serum, the effects of common metal ions, common inorganic anions, proteins, amino acids and sugars on the

determination of $1.0\mu\text{g}\cdot\text{mL}^{-1}$ EMO were investigated and the results were given in Table 3. It showed that (the relative standard deviation was within $\pm 5\%$) the larger amounts of common metal ions NH_4^+ and Na^+ , inorganic anions Cl^- and NO_3^- , urea, surfactants and saccharides, as well as the large numbers of some amino acids were allowed in high concentrations, whereas the tolerate amounts of divalent metal ions may be small. The interferences can be diminished by diluting with doubly distilled water. Therefore, the method had a good selectivity and could be applied to real samples.

Table 3 Effects of coexisting substances (EMO was $1.0\mu\text{g}\cdot\text{mL}^{-1}$)

Coexisting substance	Times	Relative error (%)	Coexisting substance	Times	Relative error (%)
CoCl_2	10	4.6	SDBS	348.5	0.7
CuCl_2	5	-4.9	SDS	288.4	-2.8
CaCl_2	8	-4.0	CTAB	218.7	0.9
NaCl	2435	2.1	Cellulose	400	4.3
NH_4NO_3	2000	0.4	Glucose	200	3.0
MnSO_4	10	4.5	HSA	40	4.8
ZnSO_4	6	-4.8	L- Aspartic acid	160	5.0
Mg SO_4	10	2.1	L- cysteine	100	3.8
BSA	100	3.6	L- histidine	600	4.2
D- tyrosine	40	4.9	Glycine	200	3.9
D- phenylalanine	100	1.5	Sucrose	100	4.4
D- fructose	100	-4.7	Starch	200	2.3
SLS	544.8	-3.4	Urea	500	4.9

SLS: Sodium dodecyl sulfate; SDBS: Sodium dodecyl benzene sulfonate; SDS: Sodium dodecyl sulfate
CTAB: cetyl trimethyl ammonium bromide

5.3 Analytical application

5.3.1. Determination of EMO in human urine

Fresh urine sample (healthy human) centrifuged at 6,000rpm for 10min then 1.0mL aliquot supernatant fluid was pipette and was diluted to 10mL. A 1.0mL aliquot of this solution was added into a 10.0mL volumetric flask and EMO was determined according to the general procedure. The recovery and relative standard deviation (R.S.D) were tested by using the standard addition method and the results were listed in Table 4.

5.3.2. Determination of EMO in human serum

Fresh serum sample (healthy human) was treated with suitable amounts of trichloroacetic acid and centrifuged 10min at 6,000rpm to exclude proteins. A 1.0mL aliquot of the

supernatant fluid was diluted to 20mL and 1.0mL of this solution was pipetted into a 10.0mL volumetric flask and EMO was determined according to the general procedure. R.S.D. and recovery were examined by using the standard addition method and the results were listed in Table 4.

Table4 Results for the determination of trace EMO in serum and urine sample

Sample	Found ($\mu\text{g}\cdot\text{mL}^{-1}$)	Added ($\mu\text{g}\cdot\text{mL}^{-1}$)	Total found ($\mu\text{g}\cdot\text{mL}^{-1}$, n=5)	Recovery (%)	RSD (%)
Serum 1	ND	0.8	0.812,0.817,0.786,0.821, 0.772	101.7	1.5
Serum 2	ND	1.6	1.66, 1.58,1.65,1.61, 1.63	101.9	4.7
Serum 3	ND	2.4	2.45,2.33,2.43,2.35,2.48	101.2	3.8
Urine 1	ND	0.8	0.809,0.812,0.792, 0.822,0.831	101.5	4.5
Urine 2	ND	1.6	1.64,1.62,1.56,1.64,1.69	102.6	5.0
Urine 3	ND	2.4	2.45,2.44,2.36,2.42,2.47	101.8	5.6

6. Conclusion

In the paper, EMO can interact with EV via electrostatic attraction, hydrogen bond and hydrophobic force to form a 1:1 ion-association complex, resulting in the fading of absorption and the significant enhancement of RRS, FDS and SOS. Based on this, a novel and simple RRS assay with high sensitivity and good selectivity for the determination of trace EMO had been developed. The proposed method was successfully applied to the determination of EMO in serum and urine sample.

Acknowledgements

This work was financially supported with the National Science Foundation of China (NO.21175015) and all authors here express their deep gratitude.

References

- [1] M.Ionov, I.Tukfatullina, B.Salakhutdinov, N.Baram, M.Bryszewska, T.Aripov. Cellular & molecular biology letters, 2010, 15(1): 98-117.
- [2]Chen, Y. C., Shen, S. C., Lee, W. R., Hsu, F. L., Lin, H. Y., Ko, C. H., & Tseng, S. W. Biochemical pharmacology, 2002,64(12), 1713-1724.
- [3]Jing, X., Ueki, N., Cheng, J., Imanishi, H., & Hada, T. Cancer Science, 2002,93(8), 874-882.
- [4]Srinivas, G., Anto, R. J., Srinivas, P., Vidhyalakshmi, S., Senan, V. P., & Karunakaran, D. (). European journal of pharmacology, 2003,473(2), 117-125.
- [5]Wang H H, Chung J G. Current microbiology, 1997, 35(5): 262-266.

- [6] Chun-Guang, W., Jun-Qing, Y., Bei-Zhong, L., Dan-Ting, J., Chong, W., Liang, Z., ... & Yan, W.. *European journal of pharmacology*, 2010, 627(1), 33-41.
- [7] L. Ma, X. Zhu. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2012, 95: 246-251.
- [8] X. Wu, G. H. Chen, K. Wang, J. Shi, C. Q. Wu. *Journal of Hebei University of Science and Technology*, 2011, 32(5): 426-430, 481.
- [9] X. M. Sun, C. Ma. *Journal of South-Central University for Nationalities (Natural Science Edition)*, 2001, 20(4): 13-16.
- [10] K. B. Gou, H. L. Yuan, X. Y. Li, C. Zhang, Z. L. Liu, C. Guo. *Chinese Pharmaceutical Journal*, 1999, 34(9): 619-621.
- [11] L. B. Wei, M. Meng, L. Z. Xia. *Chinese Journal of Information on Traditional Chinese Medicine*, 2009, 16(10): 47-8.
- [12] X. Xia. *Chinese Journal of Pharmaceutical Analysis*, 2008, 28(8): 1310-1312.
- [13] D. Guo, N. N. Chen, Y. Yan, L. B. Hou. *China Pharmacy Publishing House*, 2003, 14(5): 296-296.
- [14] H. Xiang, Q. Luo, K. Dai, W. Duan, Y. Fan, Y. Xie. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2012, 96: 874-881.
- [15] Z. G. Chen, S. H. Qian, X. Chen, J. H. Chen, Y. J. Lin and J. B. Liu, *RSC Adv.*, 2012, 2, 2562.
- [16] Lin, C., Wen, G., Liang, A., & Jiang, Z. (2013). *RSC Adv.*, 3(18), 6627-6630.
- [17] L. Kong, Z. Liu, S. Liu, D. Wang. *Analytical Methods*, 2012, 4(12): 4346-4352.
- [18] Luo, Y., Xu, L., Liang, A., Deng, A., & Jiang, Z. *RSC Adv.*, 2014, 4(37): 19234-19237.
- [19] S. P. Liu, Z. F. Liu, H. Q. Luo. *Analytica chimica acta*, 2000, 407(1): 255-260.
- [20] T. Li, S. Liu, Z. Liu, X. Hu. *Archiv der pharmazie*, 2005, 338(9): 427-432.
- [21] H. Q. Luo, N. B. Li, S. P. Liu. *Biosensors and Bioelectronics*, 2006, 21(7): 1186-1194.
- [22] F. Tian, W. Huang, J. Yang, Q. Li. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2014, 126: 135-141.
- [23] Y. Cui, F. Cui, L. Wang, Q. Zhang, W. Xue, F. Jing, J. Sun. *Journal of Luminescence*, 2008, 128(10): 1719-1724.
- [24] S. P. Liu, S. Chen, Z. F. Liu, X. Hu, T. Li. *Analytica chimica acta*, 2005, 535(1): 169-175.
- [25] J. D. PENG, S. P. LIU, Z. F. LIU, Y. SHI. *Acta Chim Sin*, 2005, 63(8): 745-751.
- [26] Y. Takano, K. N. Houk. *Journal of Chemical Theory and Computation*, 2005, 1(1): 70-77.

- [27] M. Orio, D. A. Pantazis, F. Neese. Density functional theory[J]. Photosynthesis research, 2009, 102(2-3): 443-453.
- [28] X. D. Hou, R. C. Shi, L. P. Ye. China Tropical Medicine, 2005, 5(8): 1738-1740.
- [29] J. Anglister, I. Z. Steinberg. The Journal of Chemical Physics, 1983, 78: 5358.
- [30] Edition of Editorial Board of Chinese Macropaedia, Chinese Macropaedia Biology (II), Chinese Macropaedia Press, Beijing, 1991, p. 1374.
- [31] S. LIU, L. KONG. Analytical sciences, 2003, 19(7): 1055-1060.