Interaction of $[\text{HgX}_4]^{2-}$ with Berberine by Absorption and Resonance Rayleigh-Scattering Spectra and Their Analytical Applications

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In a diluted H_2SO_4 solution, $\text{Hg}(\text{II})$ reacts with halide anions X^- (including Cl^- , Br^- and I^-) to form anionic complexes $[\text{HgX}_4]^{2-}$ that can further react with berberine to form ion-association complexes of $[\text{Ber}]_2[\text{HgX}_4]$. As a result, the absorption spectra change, their maximum absorption wavelengths are at 230 nm for $[\text{Ber}]_2[\text{HgCl}_4]$, 278 nm for $[\text{Ber}]_2[\text{HgBr}_4]$ and 300 nm for $[\text{Ber}]_2[\text{HgI}_4]$. However, among the three complexes, only $[\text{Ber}]_2[\text{HgI}_4]$ can lead to distinctly enhanced resonance Rayleigh scattering (RRS), and a new RRS spectrum appears. The maximum RRS wavelength is located at 397 nm, and the RRS intensity is proportional to the concentration of berberine in the range of 0 – 2.5 $\mu\text{g mL}^{-1}$. The optimum conditions, the influence factors for the reaction and the effects of coexisting substances have been investigated. A new, simple and fast RRS method for the determination of berberine based on the ion-association reaction of $[\text{HgI}_4]^{2-}$ with Ber^+ was developed. The method has high sensitivity and good selectivity; the detection limit for berberine ($3\sigma/K$) is 7.22 ng mL^{-1} . The method can be applied to the determination of berberine in some Chinese patent drug and the extracts of *Coptis Chinensis*. Furthermore, the mechanism of the reaction and the reasons for RRS enhancement have been discussed.

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Berberine (Ber) is an isoquinoline alkaloid, which is mainly present in various berberis plants, and usually exists as a hydrochloride or sulfate. It is a medicine used for antibiosis and diminishing inflammation. It is also a nature drug with a low toxic level and few side effects. It is thus an alkaloid for wide studies and applications in the field of biochemistry, pharmacology and clinical medicine.

For the determination of berberine in plant extracts and in pharmaceutical preparations, a number of methods have been reported, such as titrimetry,^{1,2} spectrophotometry,³⁻⁶ fluorometry,⁷⁻⁹ HPLC,¹⁰⁻¹⁴ TLC,^{15,16} polarography¹⁷ and the chemiluminescence method.¹⁸ Each method has its own advantages and disadvantages. Many methods, however, have suffered from drawbacks; for instance, the titrimetric methods are unsuitable for microanalysis. Although spectrophotometry is simple, its sensitivity is low and only suitable for the determination of berberine in samples with simple components. For complicated samples, HPLC methods are used, but the instrument is expensive and the operating procedures are not sufficiently simple. Therefore, it is necessary to develop other new methods that are simple and fast, and have high sensitivity for the determination of berberine.

As a new analytical technique, resonance Rayleigh scattering has been given much attention owing to its high sensitivity, simplicity and speed, and is being increasingly studied. It has been applied to the study and determination of some biological macromolecules, such as nucleic acids,^{19,20} proteins,^{21,22} heparin,^{23,24} organic compounds,²⁵ as well as some metal²⁶ and nonmetal²⁷ ions. Furthermore, it has been proved that the RRS

method is also useful for pharmaceutical analysis, such as some antibiotics^{28,29} and vitamins.³⁰

We had studied the RRS spectra for the interaction for berberine with some acid xanthene fluorescent dye³¹ and some colorless inorganic anions, such as isopoly-tungstic acid anion.³² When berberine reacts with isopoly-tungstic acid anion, a greatly increased RRS and new RRS spectra appear. It had thus been used as highly sensitive method to determine berberine. Our experiment found that when berberine reacts with $[\text{HgX}_4]^{2-}$ (X is Cl, Br and I, respectively) to form ion-association complexes $[\text{Ber}]_2[\text{HgX}_4]$, the absorption spectra changes and the absorbance increases. The molar absorptivity (ϵ) is between 5.0×10^3 and 1.07×10^4 $\text{L mol}^{-1} \text{cm}^{-1}$, depending on different $[\text{HgX}_4]^{2-}$. Therefore, it can lay a foundation for the spectrophotometric determination of berberine. However only $[\text{Ber}]_2[\text{HgI}_4]$ can cause a remarkably enhanced RRS and a new RRS spectrum. Under suitable conditions, because the RRS intensity (ΔI_{RRS}) is proportional to the concentration of berberine, the method can be applied to the determination of berberine. The method has high sensitivity, since its detection limit (3σ) is 7.22 ng mL^{-1} . The method also has good selectivity, so, it has been used for the determination of berberine in some Chinese patent drugs and extracts of *Coptis Chinensis* with satisfactory results.

Experimental

Apparatus

A Hitachi F-2500 spectrofluorophotometer (Hitachi Ltd., Tokyo, Japan) is used to record the RRS spectra and to measure the RRS intensity at a given wavelength. The measurement

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Fig. 1 Absorption spectra of the reaction products for $[\text{Ber}]_2[\text{HgX}_4]$ systems. 1, $[\text{Ber}]_2[\text{HgCl}_4]$; 2, $[\text{Ber}]_2[\text{HgBr}_4]$; 3, $[\text{Ber}]_2[\text{HgI}_4]$. $[\text{Ber}] = 1.5 \mu\text{g mL}^{-1}$; $[\text{HgCl}_2] = 5.0 \mu\text{g mL}^{-1}$; $[\text{X}^-] = 0.03 \text{ mol L}^{-1}$; $[\text{H}_2\text{SO}_4] = 1 \times 10^{-3} \text{ mol L}^{-1}$.

parameters are: slit, 10 nm; PMT voltage, 400 V. A UV-8500 spectrophotometer (Tianmei Company, Shanghai) is used to record the absorption spectra.

Reagents

A berberine hydrochloride standard solution ($10.0 \mu\text{g mL}^{-1}$) was prepared by dissolving 25.0 mg of berberine hydrochloride (biochemical reagent, Shanghai Third Chemical Reagent Plant) in 100 mL of water. It was then transferred to a 250 mL calibrated flask, and diluted to the mark with water, and mixed. Then 10.0 mL of this solution was pipetted into a 100 mL calibrated flask, diluted to the mark with water and mixed. The concentration of berberine hydrochloride was $10.0 \mu\text{g mL}^{-1}$.

A mercuric chloride (HgCl_2) solution was $2.0 \times 10^{-4} \text{ mol L}^{-1}$; potassium halide (KX) solutions (including KCl, KBr and KI) were all 0.3 mol L^{-1} ; H_2SO_4 solution was $1.0 \times 10^{-2} \text{ mol L}^{-1}$.

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

General procedure

After adding 1.0 mL of a $2.0 \times 10^{-4} \text{ mol L}^{-1} \text{ HgCl}_2$ solution and 1.0 mL of a $0.3 \text{ mol L}^{-1} \text{ KI}$ (or KBr, KCl) solution into a 10 mL calibrated flask, 1.0 mL of a $1.0 \times 10^{-2} \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ solution was added and mixed. Finally, certain amounts of berberine were added and diluted to the mark with water and mixed.

RRS spectra of the solution were recorded with synchronous scanning at $\lambda_{\text{ex}} = \lambda_{\text{em}}$ and the RRS intensity (I_{RRS}) was measured for the ion-association complex and I_0 for the reagent blank at the maximum RRS wavelength, $\Delta I_{\text{RRS}} = I_{\text{RRS}} - I_0$. Simultaneously, corresponding absorption spectra were recorded.

Results and Discussion

Absorption spectra

Figure 1 shows the absorption spectra of the reaction products of berberine with $[\text{HgX}_4]^{2-}$. From Fig. 1, one can see that the absorption spectra will change along with the formation of ion-association complexes of $[\text{Ber}]_2[\text{HgX}_4]^{2-}$. The crystal ion radii of Cl^- , Br^- and I^- are 0.181, 0.196 and 0.216 nm,³³ respectively, and the maximum wavelengths of $[\text{Ber}]_2[\text{HgCl}_4]$, $[\text{Ber}]_2[\text{HgBr}_4]$ and $[\text{Ber}]_2[\text{HgI}_4]$ are 230, 278 and 300 nm, respectively. The maximum absorption wavelengths display a bathchromic shift with an increase of the ion radii.

In a weak acid medium, excess levels of Cl^- , Br^- and I^- can

Fig. 2 Absorption spectra of X^- and $[\text{HgX}_4]^{2-}$. 1, Cl^- ; 2, $[\text{HgCl}_4]^{2-}$; 3, Br^- ; 4, $[\text{HgBr}_4]^{2-}$; 5, I^- ; 6, $[\text{HgI}_4]^{2-}$. $[\text{X}^-] = 0.01 \text{ mol L}^{-1}$; $[\text{Hg}^{2+}] = 5.0 \mu\text{g mL}^{-1}$; $[\text{Ber}] = 1.5 \mu\text{g mL}^{-1}$. Measured against water.

react with Hg(II) to form anionic complexes of $[\text{HgCl}_4]^{2-}$, $[\text{HgBr}_4]^{2-}$ and $[\text{HgI}_4]^{2-}$, respectively.

The maximum absorption wavelength (λ_{max}) is located at 194 nm for $[\text{HgCl}_4]^{2-}$, 214 nm for $[\text{HgBr}_4]^{2-}$ and 240 nm for $[\text{HgI}_4]^{2-}$, and the molar absorptivity (ϵ) are 6.70×10^2 , 1.49×10^3 and $3.38 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, respectively (Fig. 2). In addition, λ_{max} of $[\text{HgX}_4]^{2-}$ are bathchromic shift ($\Delta\lambda$), 2, 2 and 6 nm than that of Cl^- , Br^- and I^- , respectively.

In a weak acid medium, berberine exists as a univalent cationic species (Ber^+):

Berberine can react with the above anionic complexes to form ion-association complexes and lead to changes of the absorption spectra. The maximum absorption wavelength shifts to 230 nm for $[\text{Ber}]_2[\text{HgCl}_4]$, 278 nm for $[\text{Ber}]_2[\text{HgBr}_4]$ and 300 nm for $[\text{Ber}]_2[\text{HgI}_4]$; bathchromic shifts by 36, 64 and 60 nm composed to $[\text{HgCl}_4]^{2-}$, $[\text{HgBr}_4]^{2-}$ and $[\text{HgI}_4]^{2-}$, respectively. The molar absorptivities are between 5.0×10^3 and $1.07 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, depending on different anionic complexes, and are all greatly increased compared to corresponding anionic complexes.

RRS spectra

The RRS spectra of the reaction system are shown in Fig. 3. It can be seen from the figure that (1) under the experimental conditions, the RRS intensities of berberine or $[\text{HgX}_4]^{2-}$ are very weak; (2) when Cl^- , Br^- and I^- react with Hg(II) to form complexes $[\text{HgCl}_4]^{2-}$, $[\text{HgBr}_4]^{2-}$ and $[\text{HgI}_4]^{2-}$, respectively, and the complexes further react with berberine, only $[\text{Ber}]_2[\text{HgI}_4]$ can lead to a greatly enhanced RRS and the appearance of a new RRS spectrum. However, $[\text{Ber}]_2[\text{HgCl}_4]$ and $[\text{Ber}]_2[\text{HgBr}_4]$ do not have this effect; (3) the maximum RRS peak of $[\text{Ber}]_2[\text{HgI}_4]$

Table 1 Effect of some coexisting substances ([Ber] = 1.0 µg mL⁻¹)

Coexisting substance	Concentration added/µg	Error, %
Glucose	14700	0.545
Lactose	19000	-6.84
Soluble starch	12000	-2.21
Cane sugar	20400	-1.15
Maltose	43200	-2.4
Sodium citric	20000	-5.1
Sodium tartaric	60000	-2.5
SiO ₄ ²⁻	640	3.9
Cl ⁻	12800	2.3
Br ⁻	7260	0.4
H ₂ PO ₄ ⁻	6000	2.0
SO ₄ ²⁻	10000	-3.7
CO ₃ ²⁻	5000	3.3
Ac ⁻	7000	1.9
Cd ²⁺	100	-2.7
Cu ²⁺	200	-0.2
Pb ²⁺	100	-5.5
V(V)	600	2.5
Mn ²⁺	6000	2.5
Cd ²⁺	2386	3.5
Zn ²⁺	1180	3.6
Al ³⁺	1700	1.4
NH ₄ ⁺	1680	4.2
Ca ²⁺	3600	-1.4
Co ²⁺	1420	-4.8
K ⁺	2600	3.9
Ni ²⁺	1340	6.5
Fe ²⁺	3740	3.9
Fe ³⁺	2.4	7.0

is at 397 nm, and there are three smaller RRS peaks at 450, 466 and 495 nm, respectively.

Because the RRS intensity is proportional to the concentration of berberine in a certain range, RRS method can be used for the determination of berberine. As the sensitivity was highest at 397 nm, this value was selected as the determination wavelength. Absorptiometry can also be used for the determination of berberine, while the sensitivity is still much lower than that of the RRS method. Thus the RRS method was used to study the reaction system of berberine with [HgI₄]²⁻ in the present work.

Optimum conditions of the reaction

Effect of acidity. The effects of the reaction media, such as H₂SO₄, H₃PO₄, BR, HCl-NaAc and HAc-NaAc buffer solution on the RRS intensity were investigated. A diluted H₂SO₄ solution is best for the reaction. Therefore, diluted H₂SO₄ solution was selected as the reaction medium.

The effect of the concentration of the H₂SO₄ solution on the RRS intensity was studied. The RRS intensities were the highest, and stable in the range of 2.5×10^{-4} – 2.0×10^{-3} mol L⁻¹ H₂SO₄ solution. If the acidity was lower than the optimum range, Hg²⁺ was likely to hydrolyze, which resulted in a decrease in the available concentration of [HgI₄]²⁻. The RRS intensity also decreased. When the acidity is too high, it will cause HI to disassociate with I⁻, and will be disadvantageous to the formation of [HgI₄]²⁻, which will also lead to a decrease of the RRS intensity. We thus selected a 1.0×10^{-3} mol L⁻¹ H₂SO₄ solution as the reaction medium.

Effect of the concentration of Hg(II). The effect of the Hg(II) concentration on RRS was studied. It was shown that in the range of 1.8×10^{-4} – 2.2×10^{-4} mol L⁻¹, the RRS intensity is the highest and stable. Thus 2.0×10^{-4} mol L⁻¹ was selected in the

experiment.

If the concentration of Hg(II) is lower than the above suitable range, the RRS intensity is also lower. However, if the concentration of Hg(II) is too high, the RRS intensity is also lower due to a decrease in the relative concentration of I⁻ to Hg(II), which can lead to the formation of some other complexes, such as [HgI₃]⁻, [HgI₂] and [HgI]⁺. These complexes can not react with berberine.

Effect of the I⁻ concentration. The effect of the I⁻ concentration on the RRS intensity was studied. It can be seen that the optimum concentration of I⁻ anion is 0.02 – 0.04 mol L⁻¹. When the concentration is lower than this range, the RRS intensity is lower, owing to incomplete formation of [HgI₄]²⁻ anionic complex. However, if the concentration of KI is higher than the optimum concentration range, the RRS intensity also decreases due to a competing reaction between [HgI₄]²⁻ and I⁻ with berberine.

Stability of the RRS intensity. At room temperature (10 – 20°C), 5 min is needed to complete the reaction, and the RRS intensity can remain stable for at least 3 h.

The calibration graph

In the spectrophotometric method, the molar absorptivity (ϵ) are 5.0×10^3 L mol⁻¹ cm⁻¹ for the [Ber]₂[HgCl₄] system, 5.80×10^3 L mol⁻¹ cm⁻¹ for the [Ber]₂[HgBr₄] system and 1.07×10^4 L mol⁻¹ cm⁻¹ for the [Ber]₂[HgI₄] system, respectively. Among them, the sensitivity for the [Ber]₂[HgI₄] system is highest. The linear regression equation and the correlation coefficient are $A = 0.084C + 0.081$ and 0.9998, respectively for the [Ber]₂[HgI₄] system.

Under the optimum reaction conditions, the RRS intensity is measured at 397 nm. The calibration graph is constructed with ΔI versus the concentration of berberine. The results show that the concentration of berberine is proportional to the intensity of RRS in the range of 0 – 2.5 µg mL⁻¹; the linear-regression equation and the correlation coefficient are $\Delta I = -162.9 + 7893.4C$ ($C = 1 \times 10^{-6}$ mol L⁻¹) and 0.9946, respectively. The detection limit ($3\sigma/K$) for berberine is 7.22 ng mL⁻¹ (1.76×10^{-8} mol L⁻¹).

For the determination of berberine, because the RRS method is much more sensitive than the spectrophotometric method, the experimental selectivity and the analytical application for the determination of berberine were studied by the RRS method.

Selectivity of the method

The effects of some common metal ions, nonmetal ions, acid radicals, and sugars as coexisting substances on the determination of berberine by RRS method were investigated. The results (Table 1) showed that large amounts of metal ions, nonmetal ions, acid radicals, and sugars were tolerable, except for Fe(III). Even if Pb(II) and Cd(II) could react with I⁻ to form anionic complexes, they would not interfere with the determination with 100-time amounts to berberine. The major interference comes from Fe(III), whose tolerable concentration was only 2.4 µg mL⁻¹, but the tolerance amounts of Fe(II) were larger than Fe(III). Thus adding suitable amounts of ascorbic acid to reduce Fe(III) to Fe(II) could avoid the interference of 3.74 mg mL⁻¹ of Fe(III). Therefore, the method has fairly good selectivity, and could be applied to the determination of

Fig. 3 RRS spectra of the reaction products for $[\text{HgX}_4]^{2-}$ and berberine. 1, Ber; 2, $[\text{HgCl}_4]^{2-}$ -Ber system; 3, $[\text{HgBr}_4]^{2-}$ -Ber system; 4-6, $[\text{HgI}_4]^{2-}$ -Ber system; 7, $[\text{HgCl}_4]^{2-}$; 8, $[\text{HgBr}_4]^{2-}$; 9, $[\text{HgI}_4]^{2-}$; 4-6, the concentration of berberine are 0.25, 0.5, 1.0 $\mu\text{g mL}^{-1}$, respectively; $[\text{Hg(II)}] = 2.0 \times 10^{-4} \text{ mol L}^{-1}$; $[\text{X}^-] = 0.03 \text{ mol L}^{-1}$, $[\text{H}_2\text{SO}_4] = 1 \times 10^{-3} \text{ mol L}^{-1}$. Measured against reagent blank.

Fig. 4 Comparison of RRS and the absorption spectra. 1-3, Absorption spectra, $[\text{Ber}] = 1.5 \mu\text{g mL}^{-1}$; 4, RRS spectrum, $[\text{Ber}] = 1.0 \mu\text{g mL}^{-1}$. 1, $[\text{Ber}]_2[\text{HgCl}_4]$; 2, $[\text{Ber}]_2[\text{HgBr}_4]$; 3, $[\text{Ber}]_2[\text{HgI}_4]$. $[\text{Hg}^{2+}] = 5.0 \mu\text{g mL}^{-1}$; $[\text{X}^-] = 0.03 \text{ mol L}^{-1}$; $[\text{H}_2\text{SO}_4] = 1 \times 10^{-3} \text{ mol L}^{-1}$.

berberine in some Chinese patent drugs and extracts of *Coptis Chinensis*.

Analytical applications

Determination of berberine in Chinese patent drugs. Twenty pieces of compound berberine tablet and Sanhuang tablet were treated according to the pharmacopoeia method of the People's Republic of China.³⁴ After the sugar coat was carefully peeled off, they were ground to powder. An appropriate amount of powder was then dissolved with 150 mL of boiling water. After being cooled, it was transferred into a 250 mL calibrated flask and then diluted to the mark with water. After 1.0 mL of this solution was pipetted to a 10 mL calibrated flask, the berberine was determined by the RRS method according to the general procedure. A comparison of the results with the pharmacopoeia method³⁴ is shown in Table 2.

Determination of berberine in goldthread extracts of *Coptis Chinensis*. According to Ref. 35, 1.0000 g of different *Coptis Chinensis* powders were precisely weighed and then soaked with 50 mL of 0.05 mol L^{-1} H_2SO_4 throughout the whole night; they were then filtrated and soaked with 40 mL of 0.05 mol L^{-1} H_2SO_4 over 24 h; the residues were filtrated and washed with small amounts of water. After collecting the filtration, it was diluted 100.0 mL with water. The samples had been diluted and determined according to the general procedure. The recovery

Table 2 Results for the determination of Chinese patent drugs

Sample	RRS method/ mg g^{-1}	Average/ mg g^{-1}	RSD % ($n = 6$)	Pharmacopoeia method
Compound berberine tablet	110.6, 97.7, 122.9, 125.7, 119.2, 116.8	115.5	8.79	128.3
Sanhuang tablet	12.58, 13.47, 10.73, 13.66, 12.79, 14.23	12.91	9.45	14.27

was between 92.2% and 104.0%. A comparison of the results for the RRS method with the pharmacopoeia method³⁴ is given in Table 3. Both methods are identified on the whole.

Formation of Ion-Association Complexes and Enhancement Effects of RRS

The composition ratios of $[\text{HgX}_4]^{2-}$ to Ber^+ in the ion-association complexes are all 1:2, as established by Job's method of continuous variation and molar-ratio method. Thus the composition of the ion-association complexes is $[\text{Ber}]_2[\text{HgX}_4]$.

From Figs. 1 and 3, it can be seen that (1) all three $[\text{HgX}_4]^{2-}$ complexes can form ion-association complexes with berberine, resulting in the change of the absorption spectra; (2) among the three ion-association complexes, only $[\text{Ber}]_2[\text{HgI}_4]$ exhibited an enhanced RRS, and the appearance of a new RRS spectrum, while $[\text{Ber}]_2[\text{HgCl}_4]$ and $[\text{Ber}]_2[\text{HgBr}_4]$ did not show this enhancing effect. The reasons for RRS enhancement of $[\text{Ber}]_2[\text{HgI}_4]$ are described as follows.

Resonance scattering enhancement effect

Resonance Rayleigh scattering (RRS) is an absorption-rescattering process produced by the resonance between Rayleigh scattering and light absorption. RRS should thus be close to the absorption band. It can be seen from Fig. 4 that the RRS is located near the absorption band, so a resonance-enhanced Rayleigh scattering may produced.

The absorption spectra change along with the formation of ion-association complexes for $[\text{Ber}]_2[\text{HgX}_4]$ systems, and the absorbance proportions to the concentration of berberine. They can be used for the determination of berberine. Among them, because the sensitivity for $[\text{Ber}]_2[\text{HgI}_4]$ is highest, it may be used for the determination of berberine, but the sensitivity is still much lower than that of the RRS method.

Increase of the volume of scattering particles

In general, the intensity of scattering light observed from the vertical direction of the incident light for a nonabsorbing spherical scattering particle could be calculated

$$I_{\text{RRS}} = \frac{24A^2\pi Nv^2}{\lambda^4} \left(\frac{n'^2 - 1}{n'^2 + 2} \right)^2,$$

where, λ is the incident light wavelength in the medium, A is the amplitude of the incident light, N is the number of scattering particles per unit volume, v is the volume of one particle and n' is the ratio of the refractive index of the particle to that of the surrounding medium. When other factors are constant, the square of v for the scattering particle is proportional to I_{RRS} . Owing to the molecular volume of $[\text{Ber}]_2[\text{HgI}_4]$ being larger than that of berberine or $[\text{HgI}_4]^{2-}$, the scattering intensity of $[\text{Ber}]_2[\text{HgI}_4]$ should be higher than berberine.

Table 3 Results for the determination of Chinese goldthread extracts ($n = 6$)

Sample No.	Present method		Pharmacopoeia method	Recovery experimental of RRS method		
	Ber found, % ($n = 6$)	RSD, % ($n = 6$)	Ber found, % ($n = 6$)	Ber added ($\mu\text{g}/10\text{ mL}$)	Ber recovered ($\mu\text{g}/10\text{ mL}$)	Recovery, %
1	5.50	3.1	5.47	10.0	10.4	104.0
2	5.46	4.8	5.53	10.0	9.50	95.0
3	5.63	2.4	5.57	15.0	13.83	92.2

The interface enhancement effect for RRS

Before the reaction, both berberine and $[\text{HgI}_4]^{2-}$ have good water solubility, and there is no interface between $[\text{Ber}]_2[\text{HgI}_4]$ and water, the RRS is very weak. However, after the formation of an ion-association complex, owing to neutralization of their charges, the hydrophobicity greatly increases, through which the ion-association complexes can self-aggregate to nanoparticle species. When the diameters of the nanoparticle congeries are very small, they are homogeneously dispersed in the solution, and can give rise to an interface between it and water. The formation of an interface is also advantageous to the enhancement of RRS.

In addition, the effect of some polar organic solvents, such as ethanol and acetone, on the RRS intensities has been investigated. The results show that with an increase in the levels of organic solvents, the RRS intensities decrease rapidly. The experimental results indicate that ethanol and acetone can dissolve the hydrophobic complexes, and the interface between the complex and water disappears, so the interface enhancement effect of RRS also disappears. Furthermore, the hydrophobic interaction between $[\text{HgI}_4]^{2-}$ and Ber^+ in the organic solvent is reduced. Therefore, the ion-association ability is low, which results in the scattering signal being greatly decreased.

Thus, the formation of a hydrophobic interface is an important reason for RRS enhancement.

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