Resonance Rayleigh-Scattering Method for the Determination of Vitamin B₁ with Methyl Orange

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In a pH 2.3 – 3.0 acid medium, the resonance Rayleigh scattering (RRS) intensity is greatly enhanced when vitamin B₁ reacts with Methyl Orange to form an ion-association complex. The maximum RRS peak appears at 588 nm, another higher peak is at 403 nm and there are two smaller RRS peaks at 800 nm and 288 nm. The RRS intensity is directly proportional to the concentration of vitamin B₁ in the range of 0 – 400 ng ml⁻¹. The method has high sensitivity and the detection limit (3 σ) for vitamin B₁ is 7.2 ng ml⁻¹. The effects of coexisting substances on the determination of vitamin B₁ were investigated, and the results show that this method has good selectivity and can be applied to the direct determination of vitamin B₁ in composite vitamin B tablets and multivitamin tablet samples.

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Vitamin B₁ is an important member in the vitamin B family and a quaternary ammonium compound that consists of an aminopyrimidine and a thiazole ring connected by a methylene. Vitamin B₁ takes part in glycometabolism in the body and is a coenzyme of the oxidative decarboxylation enzyme system of α -keto acids. It can maintain normal glycometabolism and the functions of the nervous and digestive systems. Its deficiency can cause various diseases. Therefore, it is very important for pharmaceutical analyses and clinical tests to determine vitamin B₁. At present, the methods for determining vitamin B₁ are mainly a weight method,¹ titrimetric methods,^{2,3} spectrophotometric methods,^{4–7} a fluorescence method,⁸ high-performance liquid chromatography (HPLC) methods,^{5,9,10} and electrochemical analytical methods.^{11–13} The sensitivities of the weight method, titrimetric method, spectrophotometric method and some electrochemical analytical methods are not sufficiently high to satisfy the requirement of trace analysis. Although fluorescence and HPLC (fluorescence detection) methods have higher sensitivities, before determinations, some pretreatments (for example, oxidation, acid hydrolysis and enzyme hydrolysis) or separation using organic solvent extraction are usually needed. Therefore, both methods are not simple.

In recent years, resonance Rayleigh scattering (RRS) as an analytical method with high sensitivity, simplicity and rapidness has been received increasing attention. It has been applied to the determination and study of biological molecules, such as nucleic acids,^{14–17} proteins^{18–21} and heparin.^{22,23} Pasternack *et al.*²⁴ thought that chromophore aggregation on biological macromolecules was the main reason to produce intensive RRS. However, our experiments show that when two small molecules with opposite charges form an ion-association complex, great enhancement of RRS can be observed and a new RRS spectrum appears. Based on the formation of ion-association complexes, the RRS method has been applied to the determination of inorganic ions such as trace mercury(II),²⁵ selenium(IV),²⁶

chromium(VI),²⁷ molybdenum(V)²⁸ and cadmium(II)^{29,30}; and the detection limits (3 σ) can usually obtain a grade of ng ml⁻¹ and sometimes even lower. We also found that when a pharmaceutical exists as an anion or cation, it can react with a counter ion with a big conjugated system (counter ion chromophore) to form an ion-association complex which can produce strong RRS. Therefore, the RRS technique can be applied to pharmaceutical analysis, which can be considered as a new way to develop pharmaceutical analysis with high sensitivity, simplicity and rapidness.

Our experiment discovered that in a pH 2.3 – 3.0 HCl-CH₃COONa (HCl-NaAc, hereafter) buffer solution, the RRS intensity of vitamin B₁, existing as a big cation species, was very weak. In this case, although Methyl Orange as a single charged anion species had a definite RRS, its RRS intensity was not strong. When both of them reacted with each other to form an ion-association complex by virtue of electrostatic and hydrophobic interaction forces, the RRS intensity of the solution was greatly enhanced. The maximum RRS peak was at 588 nm, another higher peak was at 403 nm and there were two smaller scattering peaks at 800 nm and 288 nm. The RRS intensity is directly proportional to the concentration of vitamin B₁ in the range of 0 – 400 ng ml⁻¹. The method has very high sensitivity and the detection limit (3 σ) for vitamin B₁ is 7.2 ng ml⁻¹, which is lower by several orders of magnitude than that of the usual spectrophotometry. The method has good selectivity and was applied to the direct determination of vitamin B₁ in composite vitamin B tablet and multivitamin tablet samples with satisfactory results.

Experimental

Reagents

A vitamin B₁ stock solution was prepared by dissolving 0.025 g of vitamin B₁ (biochemical reagent, Shanghai Chemical Reagent Company) in water and diluting to the mark in a 50 ml calibrated flask. The concentration of this solution was 500 μ g ml⁻¹. The working solution was further diluted with water to 10

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$\mu\text{g ml}^{-1}$. Owing to its instability in neutral solution, the vitamin B₁ solution should be prepared when needed. The Methyl Orange (Shanghai Third Chemical Reagent Plant) solution was 0.05% (70% ethanol-water solution). HCl-NaAC buffer solutions with different pH were prepared by mixing 0.2 mol l⁻¹ HCl with 0.2 mol l⁻¹ NaAC in proportions, and were revised by a pH meter. Solubilizing agent solutions were 0.2% polyvinyl alcohol (PVA) (Shanghai Third Chemical Reagent Plant), Tween-20 (Shanghai Chemical Reagent Stock Station), Triton X-100 (Shanghai Chemical Reagent Company) aqueous solutions.

All other reagents were of analytical reagent grade and were used without further purification. Doubly distilled water was used throughout.

Apparatus

A Shimadzu RF-540 spectrofluorophotometer (Kyoto, Japan) was used for recording and measuring the RRS spectra and the intensities at a given wavelength using a 1-cm path length. The determination parameters were low sensitivity, an ordinate scale of $\times 1$ and a slit (EX/EM) of 10 nm/10 nm. A pH-S-3 acidimeter (Shanghai Analytical Instrument Plant) was used for measuring the pH of the solutions.

General procedure

Into a 10 ml volumetric flask were added 2.0 ml of a pH 2.6 HCl-NaAC buffer solution, 2.0 μg of vitamin B₁ and 1.0 ml of a Triton X-100 solution; the solution was diluted to 8 ml with water. Subsequently, 0.6 ml of a Methyl Orange solution was added; this solution was then diluted to the mark with water and thoroughly mixed. After 30 min at room temperature, the RRS spectra of the system were recorded with synchronous scanning at $\lambda_{\text{ex}} = \lambda_{\text{em}}$ (i.e., $\Delta\lambda = 0$ nm), and the RRS intensity (I) for the ion-association complex and I_0 for the reagent blank at the maximum RRS wavelength were measured, $\Delta I = I - I_0$.

Results and Discussion

RRS spectra

Figure 1 shows the RRS spectra of the Methyl Orange-vitamin B₁ system, which were recorded by synchronous scanning at $\lambda_{\text{ex}} = \lambda_{\text{em}}$. It can be seen from Fig. 1 that: (1) the RRS intensity of the vitamin B₁ solution is very weak and (2) the Methyl Orange solution has a definite RRS; however, its RRS intensity is not strong. The maximum RRS peak is at 588 nm, another higher peak is at 403 nm and there are two smaller peaks at 800 nm and 288 nm. (3) When vitamin B₁ reacts with Methyl Orange to form an ion-association complex, the RRS intensity of the solution can be greatly enhanced. However, the locations of the RRS peaks do not change compared to that of the Methyl Orange solution.

Effects of the acidity and properties and amounts of the buffer solutions on RRS

Four buffer solutions, such as HCl-NaAC, Clark-Lubs (HCl-KHC₈H₄), HCl-KCl and (H₃PO₄, HCl, H₃BO₃)-NaOH, were used to adjust the pH of the solutions; the results show that the sensitivity and stability of the HCl-NaAC buffer solution were the best. Therefore, the HCl-NaAC buffer solution was selected to control the pH of the solutions.

When the HCl-NaAC buffer solution was used, the effects of the pH value on the RRS intensity were tested; the results are shown in Fig. 2. It can be seen from Fig. 2 that the optimum acidity range is pH 2.3 – 3.0. If the acidity is higher or lower

Fig. 1 Resonance Rayleigh scattering spectra of a vitamin B₁-Methyl Orange system. 1) vitamin B₁, [V-B₁] = 0.20 $\mu\text{g/ml}$; 2) Methyl Orange, [Methyl Orange] = 9.2×10^{-5} mol/l; 3) vitamin B₁-Methyl Orange, [V-B₁] = 0.10 $\mu\text{g/ml}$; 4) vitamin B₁-Methyl Orange, [V-B₁] = 0.20 $\mu\text{g/ml}$.

Fig. 2 Effect of the pH on the RRS intensity: 1) Methyl Orange; 2) vitamin B₁-Methyl Orange, [V-B₁] = 0.20 $\mu\text{g/ml}$; 3) ΔI ($I - I_0$).

than the optimum range mentioned above, the relative RRS intensity (ΔI) of the vitamin B₁ reaction with Methyl Orange decreases. Therefore, pH 2.6 of the HCl-NaAC buffer solution was used.

The effect of the addition amounts of pH 2.6 HCl-NaAC buffer solution on RRS was tested; the results showed that the optimum addition amounts were 1.4 – 2.4 ml. Therefore, 2.0 ml of the HCl-NaAC buffer solution was used.

Effects of solubilizing agents

Because the ion-association complex formed by the reaction of Methyl Orange with vitamin B₁ has hydrophobicity, it can easily precipitate out from aqueous solution, which can result in a decrease of the RRS intensity of the solution and the instability of the scattering signals. When a suitable amount of a solubilizing agent is added to the solution, the ion-association complex can exist stably in the aqueous solution. Non-ionic surfactants, such as Triton X-100 and Tween-20 and PVA, were tested; the results showed that the effect of Tween-20 was the

Fig. 3 Effect of the solubilizing agents: 1) PVA, 1.0 ml of 0.2% PVA; 2) Triton X-100, 1.0 ml of 0.2% Triton X-100; $t = 20^{\circ}\text{C}$.

worst and that of Triton X-100 was the best. The next was PVA. The experimental results showed that when 0.8 – 3.0 ml of 0.2% Triton X-100 solution or 1.0 – 4.0 ml of 0.2% PVA solution were added, the RRS intensities of the solution were the highest and consistent. Figure 3 shows the stability of the RRS intensity depending on time when 1.0 ml of Triton X-100 and PVA were added to the solution, respectively. It can be seen from Fig. 3 that: (1) the ion-association complex forms slowly and that the highest RRS intensity can be obtained by setting aside for about 30 min, after which it can be kept stable for about 20 min. Then, the RRS intensity gradually decreases. (2) The stability of a solution to which was added Triton X-100 was similar to that of a solution to which was added PVA, but the sensitivity of the former was much higher than that of the latter. Therefore, Triton X-100 was used as a solubilizing agent and the addition amount of 0.2% Triton X-100 solution was 1.0 ml. If an ionic surfactant (for example, cationic surfactant such as cetyltrimethylammonium bromide (CTMAB) or anionic surfactant such as sodium lauryl sulfate (SLS)) is added, the RRS intensity would be decreased.

Under the above-mentioned conditions, the effect of the reaction temperature on the RRS intensity was tested. It was found that the optimal temperature range was $10 - 26^{\circ}\text{C}$. When the temperature was lower than 10°C , the reaction became much slower, and the RRS intensity of the solution was lower when detected after 30 min. When temperature was higher than 26°C , because the enhancement of the molecular thermal motion weakens the binding force of ion-association complexes, which causes the dissociation of some ion-association complexes, the RRS intensity also decreased. Therefore, the reaction temperature of the system should be maintained between 10 and 26°C .

Effect of the concentration of Methyl Orange on RRS

The effect of the concentration of Methyl Orange on the RRS intensity was investigated. The results showed that the optimum concentration range was $0.77 \times 10^{-4} - 1.10 \times 10^{-4} \text{ mol l}^{-1}$. When the concentration of Methyl Orange was lower than the above concentration range, the reaction of Methyl Orange with vitamin B₁ was not complete and the RRS intensity of the solution was weaker. When the concentration of Methyl Orange was higher than this range, the RRS intensity of blank reagent increased, resulting in a decrease in ΔI . Therefore, in the experiment, $0.92 \times 10^{-4} \text{ mol l}^{-1}$ Methyl Orange solution was used, namely, 0.6 ml of 0.05% Methyl Orange solution was selected.

Fig. 4 Absorption spectra of the vitamin B₁-Methyl Orange system: Vitamin B₁; --- Methyl Orange; — Vitamin B₁-Methyl Orange; in pH 2.6 aqueous solution in the presence of Triton X-100.

Relation between the RRS intensity and the concentration of vitamin B₁

Under optimum conditions, different amounts of vitamin B₁ were used to react with Methyl Orange to form the ion-association complex, of which the RRS intensities were measured at the maximum RRS wavelength of 588 nm. The calibration graph of ΔI against the concentration of vitamin B₁ was constructed and the linear range of vitamin B₁ was from 0 to 400 ng ml^{-1} . The linear regression equation is $\Delta I = 19.4 + 16.1C$ ($C: \mu\text{g}/10 \text{ ml}$). The correlative coefficient (r) is 0.995. The detection limit for vitamin B₁, calculated as the concentration giving a signal tripling the background noise, is 7.2 ng ml^{-1} . The detection limit is lower by 2 – 3 orders of magnitude than that from UV-spectrophotometry⁴ and other usual spectrophotometric methods, such as Bromothymol Blue and *p*-methylaminophenol sulfate,⁵ diazo salt of 6-aminothymol,⁶ diazotized 4-hydroazino benzenesulfonic acid⁷ and tetrachlorobenzoquinone.³¹ Thus, the RRS method has high sensitivity.

Ion-association reaction of vitamin B₁ with Methyl Orange

The structure of vitamin B₁ (thiamine hydrochloride, abbreviated as TA·Cl) is

In aqueous solution, it was dissociated as a big quaternary ammonium cation and a Cl⁻ anion. Because the pyrimidine and thiazole rings are separated by a methylene, the conjugated system becomes small and there is only an absorption band in the ultraviolet region. From Fig. 4, it can be seen that in a pH 2.6 HCl-NaAC buffer solution, the maximum absorption wavelength (λ_{max}) is located at 226 nm and the molar absorptivity (ϵ) is $1.32 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$. While under this condition, Methyl Orange exists mainly as a red molecule (HR, which they may exist as an amphoteric quinoid-type molecule), of which the maximum wavelength is located at 502 nm and molar absorptivity (ϵ) is $2.4 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ (see Fig. 4). Moreover, parts of the Methyl Orange molecules are dissociated as single-charged anions (R⁻). When vitamin B₁ and Methyl Orange coexist, the big quaternary ammonium cation can react

Fig. 5 Comparison of the spectral characteristics for RRS and absorption: —, RRS spectrum: vitamin B₁-Methyl Orange, [V-B₁] = 0.10 µg/ml, [Methyl Orange] = 9.2×10^{-5} mol/l, Triton X-100, 3.3×10^{-4} mol/l, pH 2.6 HCl-NaAc buffer solution; --- absorption spectrum (against the reagent blank): vitamin B₁-Methyl Orange, [V-B₁] = 2.0 µg/ml, [Methyl Orange] = 9.2×10^{-5} mol/l, Triton X-100, 3.3×10^{-4} mol/l, pH 2.6 HCl-NaAc buffer solution.

with the dye anion (R⁻) to form an ion-association complex by virtue of electrostatic attraction and hydrophobicity,³² and the dissociation balance of the Methyl Orange shifts to the right, which results in a reduction of red-type molecules (HR), followed by a decrease of the absorbance at 502 nm and the production of a wider absorption band of the ion-association complex. Against the reagent blank, new absorption peaks were located at 598 nm and 408 nm (see Fig. 5). Using a fading spectrophotometric method (Job's method of continuous variation and the equilibrium shift method) at 502 nm, we determined that the composition ratio of vitamin B₁ to Methyl Orange in the ion-association complex was 1:1. Therefore, the ion-association reaction is as follows:

Obviously, a too low pH value is of a disadvantage to the formation of R⁻ and the ion-association reaction. Though an increase in the pH value is an advantage to formula (1) to shift to the right, vitamin B₁ can remain stable only in the range of pH 2 – 4.³³ An increase in the pH value would cause a ring-opening reaction of the thiazole ring, and be a disadvantage to the formation of an ion-association complex. Our experiments showed that the optimum pH value range is 2 – 3. The formation of the ion-association complex neutralizes the charges of the two organic counter ions, which causes an enhancement of the hydrophobicity. Therefore, a suitable solubilizing reagent (for example, Triton X-100) should be added in order to keep the ion-association complex stable in aqueous solution, and to obvert its precipitating out, which would further keep the stability of the RRS intensity. Our experiments showed that ionic surfactants (such as anion or cation surfactants) would compete with the ion-association

reaction and greatly decrease the RRS intensity. Thus, they cannot be used.

Characteristics of RRS spectra

Resonance Rayleigh scattering is a special elastic scattering produced when the wavelength of Rayleigh scattering is located at the molecular absorption band. It is an absorption rescattering process produced after light energy is absorbed by Rayleigh scattering. It is no longer pure Rayleigh scattering and no longer obeys the Rayleigh law, $I \propto 1/\lambda^4$. Moreover, its spectral characteristics are significantly affected by the electronic absorption spectrum. Figure 5 is a comparison of the spectral characteristics for RRS and absorption, from which it can be seen that 1) the RRS spectrum is located in the absorption spectrum and 2) the main RRS peaks at 588 nm and 403 nm are located near to the main absorption peaks at 598 nm and 408 nm. Obviously, there is a good correspondence between the absorption and scattering spectra (the RRS peak near 800 nm may be the second scattering peak of the scattering peak at 403 nm. Therefore, it shows no correspondence with the absorption spectrum.). Thus, it can be seen that the RRS spectrum is closely related to the absorption spectrum.

Enhancement of RRS

When the apparatus and the reaction conditions are fixed, RRS is mainly affected by two aspects:^{22,34,35} 1) the volume of the scattering molecule (the larger is the molecular volume, the stronger is the RRS) and 2) the molar absorptivity of the scattering molecule (the higher is the ϵ value, the stronger is the absorption intensity of the molecule, the more does the imaginary part of the molecular polarizability increase, and the greater is the contribution to the RRS intensity). When TA⁺ reacts with the dye anion R⁻ to form an ion-association complex, on the one hand, the volume of the scattering molecule enlarges; on the other hand, the conjugated system of the scattering molecule enlarges and the molar absorptivity increases. What is more, the hydrophobic ion-association complex can further aggregate to form bigger scattering molecules (i.e. $[(\text{TA}^+)(\text{R}^-)]_n$) by virtue of the Van der Waals force, by which the RRS intensity is greatly enhanced.

Selectivity of the method and its analytical application

The selectivity of the method was investigated using 2.0 µg of vitamin B₁ in the presence of a series of substances. The tolerance limit was taken as the maximum concentration of the foreign substances that caused an approximately $\pm 5\%$ relative error in the determination. The results are presented in Table 1. From Table 1, it can be seen that certain amounts of metal ions, inorganic anions, vitamins and glucides do not interfere with the determination of vitamin B₁; therefore, the method has good selectivity and can be applied to the direct determination of vitamin B₁ in some pharmaceuticals.

Determination of vitamin B₁ in composite vitamin B tablet samples

Five composite vitamin B tablets were finely powdered. After an accurate weight of the powder 0.0700 g was put into a 100 ml volumetric flask, water was added to the mark. Subsequently, it was mixed well and completely dissolved. The solution was then filtered through a dry and clean filter; 5.0 ml of the filtrate was added into another 100 ml volumetric flask and the solution was diluted to the mark with water and mixed thoroughly. Then, a 1.0 ml amount of this solution was pipetted into a 10 ml calibrated flask; the following procedure was the same as the experimental procedure in order to determine the

Table 1 Effects of foreign substances (vitamin B₁, 2 µg)

Foreign substance	Amount tolerated/µg	Coexisting multiple	Foreign substance	Amount tolerated/µg	Coexisting multiple
Mg(II)	100	50	Zn(II)	50	25
Al(III)	20	10	Cd(II)	200	100
Sr(II)	20	10	Cr(III)	100	50
Sn(II)	50	25	Sn(IV)	6	3
Co(II)	20	10	Fe(II)	10	5
Mo(VI)	10	5	Ca(II)	200	100
Ba(II)	10	5	Fe(III)	10	5
NO ₂ ⁻	500	250	Mn(II)	2	1
NH ₄ ⁺	10	5	C ₂ O ₄ ²⁻	50	25
S ₂ O ₃ ²⁻	4	2	K ⁺	1000	500
Cl ⁻	2000	1000	SO ₄ ²⁻	1000	500
Na ⁺	1200	600	NO ₃ ⁻	1200	600
Vitamin B ₆	200	100	Inositol	200	100
Nicotinamide	100	50	Folic acid	30	15
Riboflavin	5	2.5	Vitamin B ₁₂	500	250
Vitamin C	120	60	Maltose	1720	860
Lactose	3430	1715	Glucose	3680	1840

Table 2 Results for the determination of vitamin B₁ in a composite vitamin B tablet and multivitamin tablet samples

Sample	VB ₁ found (VB ₁ %)	Average value (VB ₁ %)	RSD, %	VB ₁ added (VB ₁ %)	Total VB ₁ found ^a (VB ₁ %)	Recovery, %
Composite vitamin B tablet	6.58, 6.71, 6.64, 6.84, 6.6	6.69	1.45	3.18	9.76	96.6
Multivitamin tablet	0.364, 0.352, 0.357, 0.359, 0.349	0.356	1.65	0.172	0.521	95.9

a. n = 5.

concentration of vitamin B₁. A recovery test was made by the standard addition method; the results are shown in Table 2.

Determination of vitamin B₁ in multivitamin tablet samples

Three multivitamin tablets were finely powdered. After an accurate weight of the powder 0.1500 g was put into a 50 ml volumetric flask, and water was added to the mark. It was mixed well and completely dissolved. The solution was then filtered through a dry and clean filter. The filtrate (10.0 ml) was taken into another 50 ml volumetric flask and diluted to the mark with water. A 1.0 ml amount of this solution was taken for determining the concentration of vitamin B₁ by the experimental method. The standard addition method was used to measure the recovery of the method; the results are listed in Table 2.

Acknowledgements

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