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ORIGINAL PAPER

Study on the Interaction Between Verapamil Hydrochloride and Eosin Y by Absorption, Fluorescence and Resonance Rayleigh Scattering Spectra and Their Analytical Applications

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Abstract In pH 2.8~3.6 HCl-NaAc buffer solution, eosin Y (EY) can react with verapamil hydrochloride(VP) to form a 1:1 ion-association complex, which not only causes the change of absorption spectra and the quenching of fluorescence, but also results in the great enhancement of resonance Rayleigh scattering (RRS). Furthermore, a new RRS spectrum with the maximum wavelength at 324 nm will appear. In this work, the spectral characteristics of absorption, fluorescence and resonance Rayleigh scattering spectra, the optimum conditions for the reaction, the influencing factors and the analytical properties have been investigated. Thereby, a sensitive, simple, rapid and new method for the determination of VP by using eosin Y as a probe has been developed. The detection limit is 0.95 ng/mL for RRS method, 6.4 ng/mL for fluorophotometry and 0.18 μg/mL for spectrophotometric method. The absorbance, RRS and fluorescence intensity is proportional to the concentration of VP in the range of 0.6036~4.0 μg/mL, 0.0032~4.5 μg/mL and 0.0213~4.0 μg/mL, respectively. The effects of the reaction of verapamil hydrochloride and eosin Y on the absorption, fluorescence and resonance Rayleigh scattering spectra have been investigated. Meanwhile, the influences of coexisting substances are tested by RRS method and the results show that this method can be satisfactorily applied to the determination of VP in tablet and human serum samples. The composition and structure of the ion-association complex and the reaction mechanism are discussed. Moreover, the energy transfer among absorption, fluorescence and RRS was investigated briefly in this work.

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Introduction

Verapamil hydrochloride (α-[3-[[2-(3, 4-dimethoxyphenyl) ethyl]-3, 4-dimethoxy]-α-isopropyl] benzene acetonitrile hydrochloride), a derivative of papaverine, belongs to the class IV anti-arrhythmic agent. In medical treatment practice, VP is a calcium-channel blocking agent [1]. Its principal physiological action is adjusted to the trans-membrane influx of calcium ions into the cardiac conduction cells, myocardial contractile cells and vascular smooth muscle membrane when the concentrations of serum calcium do not change. In addition, it plays a role in inhibiting the platelet aggregation. Recently, verapamil hydrochloride has been widely used in clinically, and mainly used in the treatment of hypertension, hypertrophic cardiomyopathy, supraventricular tachyarrhythmias, variant angina, cardiomyopathy and nephrotic syndrome, etc. [2]. Therefore, it is significant to further research and develop a new method for the determination of verapamil hydrochloride.

To take full advantage of VP and to weaken its toxicity, it is very necessary to quantitatively determine VP in both pharmaceutical analysis and clinical medicine. At present, various methods have been developed for the determination of VP, including spectrophotometry [3–6], capillary electrophoresis method [7, 8], spectrofluorometric method [9, 10], high performance liquid chromatography (HPLC) [11–14], liquid chromatography–mass spectrometry (LC–MS) [15], atomic emission spectrometry [16], electrochemistry method [17] and Flow injection-resonance Rayleigh scattering method

[18], etc. Among them, spectrophotometric methods have advantages of simplicity and cheapness, but they are not sensitive enough for the determination of trace VP. Some methods are complicated in the pre-processing and operations (such as HPLC and CE method), and other method, such as the LC-MS, has higher sensitivity and its analytical performance is the best, while the instrument is so expensive that it can not be applied to the routine analysis. Therefore, it is still significant to develop a highly sensitive and selective, convenient, rapid and new method for the determination of trace VP.

Rayleigh scattering (RS) is an elastic scattering with the scattering wavelength being equal to the incident wavelength and the scattering particle (e.g. a molecule) being much smaller than the incident wavelength (λ). In this case, when the scattering wavelength is far from molecular absorption, the intensity of scattered Rayleigh light is proportional to $1/\lambda^4$, and the signal level of Rayleigh scattering in a transparent and homogeneous aqueous solution is low. However, when the wavelength of Rayleigh scattering (RS) is located at or close to its molecular absorption band, Resonance Rayleigh scattering (RRS) which is a special elastic scattering produced. In this case, the frequency of the electromagnetic wave absorbed by the electron is equal to its scattering frequency. Because of the intensive absorption of light energy of the electron, re-scattering takes place. Therefore, the scattering intensity is enhanced by several orders of magnitude compared with single RS, and no longer obeys the Rayleigh law of $I \propto 1/\lambda^4$. RRS is not only related to forced vibration caused by the action of the electromagnetic field of the incident light in a molecule, but also affected by energy level transitions of the electrons. It shows as well the characteristics of the scattering spectrum as that of the electronic absorption spectrum. It provides new information concerning molecular structure, size, form, charge distribution, state of combination and so on. Further, different chromophore systems can produce different RRS spectra [19, 20]. This phenomenon is known as resonance Rayleigh scattering (RRS), or as resonance-enhanced Rayleigh scattering. Resonance Rayleigh scattering (RRS) spectra, as a new analytical technique, has received much attention because of its sensitivity and simplicity. At present, it has been successfully applied to the analysis of macromolecules such as heparin [21], nucleic acids [22] and proteins [23] and also used for the determination of some trace inorganic ions [24], organic compounds [25] and drugs [26–30].

In this work, the effects of the reaction of verapamil hydrochloride and eosin Y on the absorption, fluorescence and resonance Rayleigh scattering spectra have been investigated. Meanwhile, the optimum reactions and the influencing factors have also been examined. Moreover, the influences of coexisting substances were tested by RRS method and the results indicated that this method had a good selectivity. Therefore, this method could be satisfactorily applied to the determination of VP in tablet and human serum samples.

Experimental

Reagents

Verapamil hydrochloride (Shanghai Harvest Pharmaceutical Co. Ltd. China) standard solution: 10.0 μg/mL. Working solution of eosin Y (E. Merck Co. Ltd.): 2.0×10^{-4} mol/L. NaCl solution: 0.5 mol/L. HCl-NaAc buffer solutions were prepared by mixing 1.0 mol/L NaAc and 1.0 mol/L HCl, and the pH values were adjusted with pH meter. All reagents were analytical reagent grade and doubly distilled water was used throughout.

Apparatus

A Hitachi F-2500 spectrofluorophotometer (Tokyo, Japan) was used to record the RRS and fluorescence spectra and to measure the scattering and fluorescence intensity with the slits (EX/EM) of 2.5/2.5 nm for the RRS and 10.0/10.0 nm for the fluorescence spectra. A UV-8500 spectrophotometer (Tianmei, Shanghai, China) was used to record the absorption spectra and to measure its intensity. A PHS-3C pH meter (Shanghai Dazhong Analytical Instrument Plant, China) was used to adjust pH values.

General Procedure

Into a 10 mL calibrated volumetric flask were added 1.0 mL of EY solution, suitable amounts of VP solution and 0.5 mL HCl-NaAc buffer solution (pH 3.2). The mixture solution was diluted to the mark with water and mixed thoroughly. After reaction for 15 min, the RRS spectra were recorded with synchronous scanning at $\lambda_{ex} = \lambda_{em}$ and the RRS intensity (I_{RRS}) for ion-association complexes and I^0_{RRS} for reagent blank at their own maximum wavelengths (λ_{max}) were measured, $\Delta I_{RRS} = I_{RRS} - I^0_{RRS}$. Simultaneously, the spectra of absorption and fluorescence were recorded, respectively.

Results and Discussion

Spectral Characteristics

Absorption Spectra

The absorption spectra of verapamil hydrochloride, eosin Y and the complex are shown in Fig. 1. Figure 1a shows

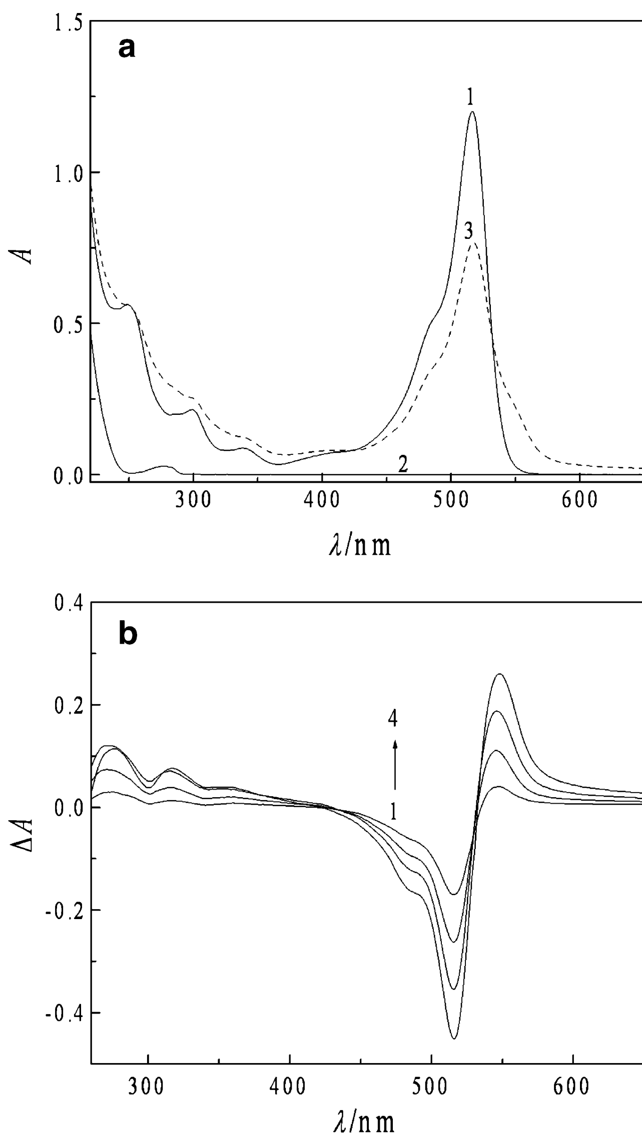

Fig. 1 **a** Absorption spectra. 1, EY; 2, VP; 3, VP-EY; EY, 2.0×10^{-5} mol/L; VP, 4.0 μg/mL using water blank as the reference solution, pH 3.2. **b** Absorption spectra. c_{VP} (1–4): 1.0, 2.0, 3.0 and 4.0 μg/mL; EY, 2.0×10^{-5} mol/L, using regent blank as the reference solution, pH 3.2

that the maximum absorption wavelength of eosin Y is located at 515 nm and that verapamil hydrochloride has little absorption in 300–700 nm range. While Fig. 1b demonstrates that, upon reaction with verapamil hydrochloride to form an ion-association complex, the absorption spectra of eosin Y will change. The absorbance intensity at about 515 nm drop significantly, followed by the appearance of a new absorption band with the maximum absorption wavelength at 546 nm. The absorption spectra of eosin Y has a 31 nm red shift before and after reaction. There is a linear relationship between the drug concentration in a certain range and the absorbance intensity at 515 nm and 546 nm so that it can be applied to determine the content of VP. And the molar absorptivities (ε) are 8.25×10^4 L/(mol·cm) and 1.92×10^4 L/(mol·cm) respectively. If the dual wavelength spectrophotometry is used in the study, the molar absorptivity increases to 1.02×10^5 L/(mol·cm) and the detection limit is 0.18 μg/mL. The sensitivity is higher than those of common spectrophotometry [3–5].

Fluorescence Spectra

The fluorescence spectra of EY-VP system are shown in Fig. 2. As shown in Fig. 2, verapamil hydrochloride itself does not exhibit fluorescence, but eosin Y can emit stronger fluorescence with the maximum excitation (λ_{ex}) and emission (λ_{em}) wavelength at 520 nm and 546 nm, respectively. Upon reaction with verapamil hydrochloride, the characteristics of fluorescence spectra of eosin Y do not change, but the fluorescence was quenched and the quenched intensity is directly proportional to the concentration of verapamil hydrochloride in a certain range. Therefore, it can be applied to the determination of verapamil hydrochloride. The detection limit is 6.39 ng/mL, and the sensitivity is higher than that of spectrophotometry.

RRS Spectra

The RRS spectra of the EY-VP system are showed in Fig. 3. It can be seen that RRS intensities of both Verapamil Hydrochloride and Eosin Y are very weak. But when verapamil hydrochloride reacts with eosin Y to form a

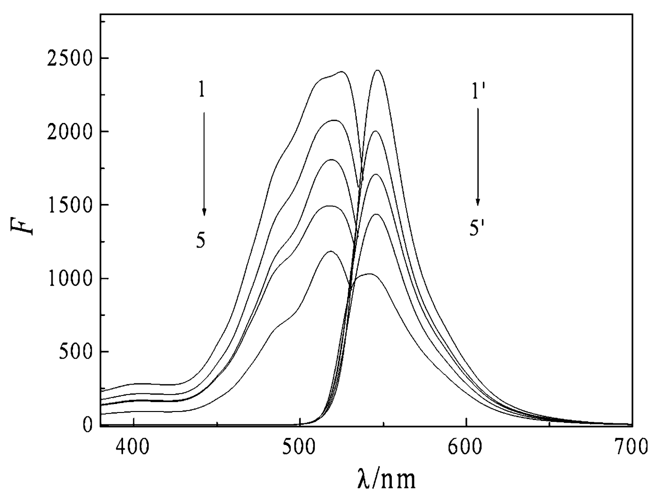

Fig. 2 Fluorescence spectra.1–5: Exitation spectra of EY-VP system: c_{VP} (1–5): 0, 1.0, 2.0, 3.0 and 4.0 μg/mL; EY, 2.0×10^{-5} mol/L, emission wavelength: 546 nm, slit: 10.0 nm, pH 3.2, 1′–5′: Emission spectra of EY-VP system: c_{VP} (1′–5′): 0, 1.0, 2.0, 3.0 and 4.0 μg/mL; EY, 2.0×10^{-5} mol/L, excitation wavelength: 520 nm, slit: 10.0 nm, pH 3.2

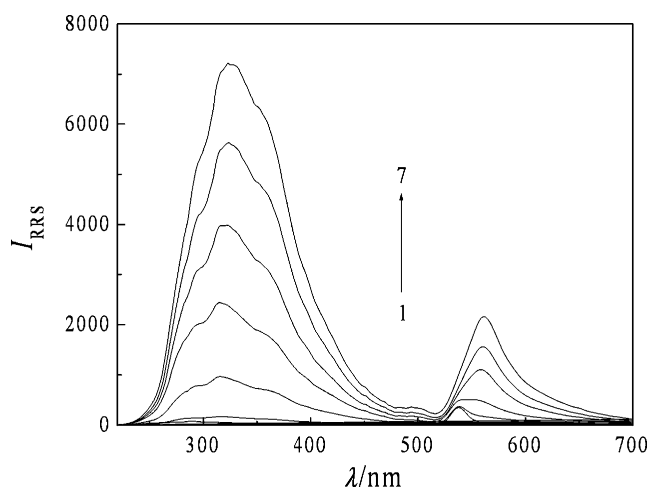

Fig. 3 RRS spectra.1, VP, 4.0 μg/mL; 2, EY, 2.0×10^{-5} mol/L; VP-EY: c_{VP} (3–7): 0.5, 1.5, 2.5, 3.5, 4.5 μg/mL; EY, 2.0×10^{-5} mol/L; Slit: 5.0 nm; pH 3.2

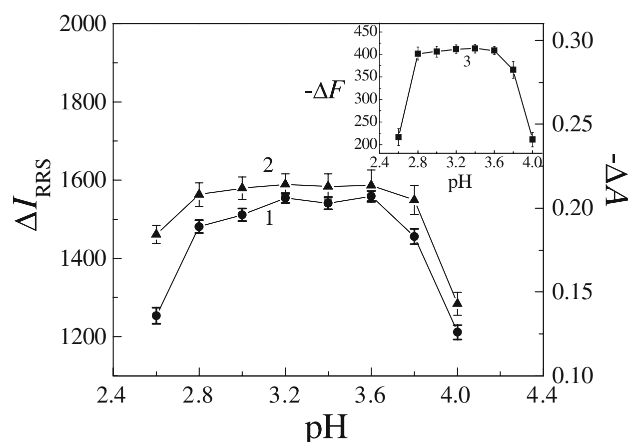

Fig. 4 Effect of acidity. 1, Absorption spectra (measured against the reagent blank); 2, RRS spectra; 3, Fluorescence spectra; VP, 1.0 μg/mL; EY, 2.0×10^{-5} mol/L; The error bars represent standard errors (*SE*) of the means. The date in the figure were means ± *SE* (n=6)

binary complex, the intensities of RRS at its maximum RRS wavelength (324 nm) are greatly enhanced, followed by the appearance of another weak RRS band with the maximum scattering wavelength at about 560 nm. And 0.0032–4.5 μg/mL of verapamil hydrochloride is directly proportional to the enhanced intensity of scattering at 324 nm and the detection limit is 0.95 ng/mL. For the same system, the sensitivity of this method is 190 times and seven times higher than those of the absorption spectrophotometry and fluorescence quenching method, respectively. Therefore, this method is more suitable for the determination of trace amounts of verapamil hydrochloride.

Optimum Reaction Conditions

Effect of Acidity

Different kinds of buffer solutions, such as BR, HCl-$C_6H_8O_7$-Na_2HPO_4, HAc-NaAc and HCl-NaAc, were used to investigate the effect of acidity on the RRS, absorbance and fluorescence intensities of the reaction system. The results showed that HCl-NaAc buffer solution had the best for the reaction. Therefore, the HCl-NaAc buffer solution was selected to control the pH of solution. In the range of pH 2.8–3.6, the enhanced intensity of scattering (ΔI_{RRS}), overlap value of the absorbance and the quenched intensity of fluorescence reached the maximum (Fig. 4). Therefore, pH 3.2 was chosen as the optimal acidity for the assay. In addition, the effect of the volume of buffer solution on the RRS, absorbance and fluorescence intensities were also examined and the results showed that too much buffer solution would result the decrease of ΔI_{RRS}, ΔA, ΔF. Thereby the suitable amount of the buffer solution was 0.5 mL.

Effect of the Concentration of Eosin Y

The effect of the concentration of eosin Y on ΔI_{RRS}, ΔA, ΔF was studied. The results showed that verapamil hydrochloride reacted with eosin Y gradually with the increase of dye concentration. When the concentration of eosin Y was between 0.8×10^{-5} mol/L and 2.8×10^{-5} mol/L, ΔI_{RRS}, ΔA, ΔF reached the maximum. Excessive or inadequate eosin Y would result in the decrease of the intensity. Thus, the EY concentration of 2.0×10^{-5} mol/L was chosen for the assay.

Effect of Ionic Strength

The effect of ionic strength on ΔI_{RRS}, ΔA, ΔF was investigated by NaCl solution. The result showed when the concentration of NaCl was lower than 0.1 mol/L, ΔI_{RRS}, ΔA, ΔF kept constant, and with the increase of the concentration of NaCl, ΔI_{RRS}, ΔA, ΔF decreased gradually. Thus, the assay should be performed under a low ionic strength condition.

Incubation Time and the Stability

At room temperature, the reaction could complete in 5 min and RRS, overlap value of absorbance and fluorescence intensity remained constant for 6 h. Therefore, the reaction system spectral signals could keep stable relatively.

Discussion of the Reaction Mechanism of EY with VP

The Ion-association Reaction

The composition ratio of EY with VP in an ion-association was determined by using Job's method and molar ratio

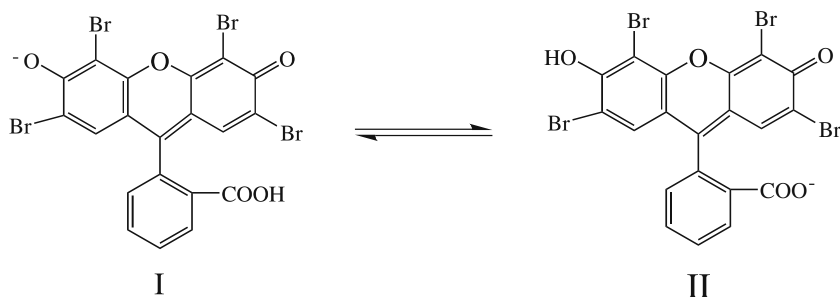

Fig. 5 The equilibrium associated with the two prototropic forms of EY^{-}

method, and the result showed that eosin Y reacted with verapamil hydrochloride to form 1:1 neutral ion-association complex. Eosin Y was a weak acid and its pka_1 and pka_2 is 2.6 [31] and 3.6 [32], respectively. In pH 3.2 weak acidic medium, the distribution fraction of EY^{-} and EY^{2-} are 60.7% and 24.1%, respectively. Although, theoretically, there will be two kinds of enantiotropic isomers produced by the dissociation of hydroxyl and carboxyl, the hydroxyl dissociation is predominant. As for fluorescein without halogen substituent group, –COOH firstly dissociates and then followed by –OH. Fluorescein mainly exists as the type II. Eosin Y (tetrabromofluorescein), as a halogenated fluorescein, has two electron-withdrawing groups of Br being close to –OH, which reduces the charge density of oxygen atom on –OH, therefore, –OH tends to dissociate more easily and exists as type I [31]. We use the quantum chemistry AM1 method to calculate the system's enthalpy change when –OH on xanthen and –COOH on phenyl dissociated. The results were −472.6 KJ/mol and −355.6 KJ/mol, respectively. The former emitted 117.0 KJ/mol was more than the latter did, which indicated that the system was more stable when –OH dissociated, namely, that eosin Y mainly existed as the type I (Fig. 5).

Therefore, in a certain acidic condition, –NH of diphenhydramine is easily protonated to form one positively charged cation and EY^{-} and VP^{+} will form a 1:1 neutral ion-association complex by the electrostatic attraction and the hydrophobic force. The reaction is shown as follows (Fig. 6):

Effect of the Ion-association Reaction on RRS

It could be seen from RRS spectra (Fig. 3) that the formation of the ion association complexes could result in great enhancement of RRS and the appearance of a new RRS spectrum. The possible reasons for RRS enhancement were as follows:

(1) Resonance enhanced scattering effect

Since RRS is a scattering-absorbing-rescattering process produced by the resonance of the scattering and absorption, RRS band should be closely related to the absorption band. To avoid the distortion of RRS spectrum which result from the difference of instrumental factor (light source and detector), we correct the resonance Rayleigh scattering profiles according to the methods proposed by McPhie [33]. The results showed that the spectral characteristics of RRS of the studied systems in the manuscript were little changed after the correction. The maximum RRS wavelength and the shoulder scattering wavelength shifted from 324 nm and 546 nm to 317 nm and 541 nm, respectively. And the profile of the RRS spectra had not obvious change as well. Figure 7 is the comparison of corrected RRS spectra according to the methods proposed by Collings and

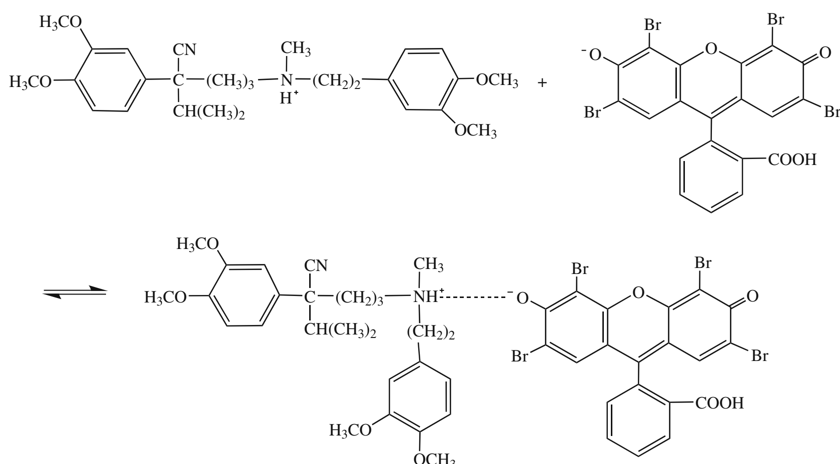

Fig. 6 The ion-association reactions of EY with VP

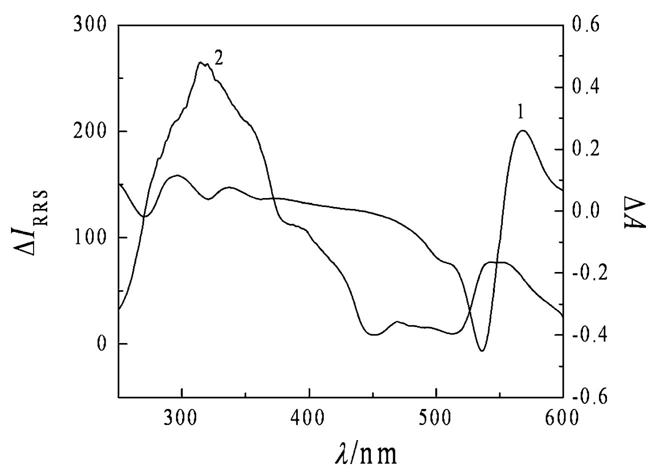

Fig. 7 The comparison of absorption spectra with corrected RRS spectra of EY-VP system. 1, Absorption spectra (using reagent blank as a reference solution) and 2, corrected RRS spectra. VP, 1.5 μg/mL; EY, 2.0×10^{-5} mol/L

McPhie with absorption spectra (using reagent blank as a reference solution) of the EY-VP system. It can be seen from Fig. 7 that the RRS spectra is situated in its absorption band and two RRS peaks (317 nm and 546 nm) are close to corresponding absorption peaks (301 nm and 536 nm and 566 nm). Therefore, the intensity of Rayleigh scattering is remarkably increased due to the absorption of light and a re-scattering process [19, 34].Therefore, the Resonance-enhanced effect is an important reason for scattering enhancement of the ion-association complex system.

(2) Effect of molecular volume

It is known that the increase of the volume of the scattering molecule is advantageous to the enhancement of scattering intensity. Before forming ion-association complexes, the molecular weight (491.07) of verapamil hydrochloride was not big. However, when it formed into ion-association complexes with EY, the total molecular weight increased to 1,200. According to the Rayleigh scattering formula, if the molecular volume was difficult to estimate, the formula could be simplified as $I = kI_0Mc$ [35], that was, when the incident light intensity (I_0), the concentration of the solution (c) and k was constant, the scattering intensity (I) was proportional to the molecular weight of the particle. Therefore, the increase of the molecular volume (or weight) is a significant factor to the enhancement of RRS intensity.

(3) The formation of hydrophobic interface

Both EY^- and VP^+ have good hydrophility and can easily dissolve in aqueous solution so that they can not form an interface with water. When EY^- reacts with VP^+ to form the neutral ion-association complex, their charges are neutralized and they will lose hydrophility. Therefore, hydrophobic interface forms between the hydrophobic aryl framework of the ion-association complex and aqueous phase, which is advantageous to the enhancement of RRS [36, 37].

Affected by all above factors, the RRS intensities of the productions enhanced notably.

The Energy Transfer Between Absorption, Fluorescence and RRS

It can be seen from Fig. 8 that when eosin Y reacts with verapamil hydrochloride to form the ion-association complex, the absorbance decreases and the fluorescence is quenched, but RRS enhances notably. There was a relationship of "As one falls, another rises" between the absorbance, fluorescence and RRS intensity. And resonance Rayleigh scattering is a resonance enhanced scattering produced by the resonance between Rayleigh scattering and absorption with the same frequency. During this process, scattering enhances because it absorbs light energy; namely, part of light energy absorbed by the molecule is transferred to the scattering through the resonance effect. Therefore, as RRS enhances, the absorbance decreases. In the same way, the relation between the released energy and the absorbed energy should be that the absorbed light energy (E_A) is equal to the sum of the energy of light emission(fluorescence)(E_L), resonance scattering (E_{RLS}) and nonradiation (E_N), viz, $E_A = E_L + E_{RLS} + E_N$. The resonance scattering in the transparent solution of small molecule systems has been always ignored. Therefore, the reason of fluorescence quenching is that the energy of fluorescence is transferred to nonradiation (E_N) energy. However, the existence of resonance scattering in the big molecule or ion-association systems could not be ignored. While the RRS spectrum is situated in its fluorescence band, there would be an energy transfer between fluores-

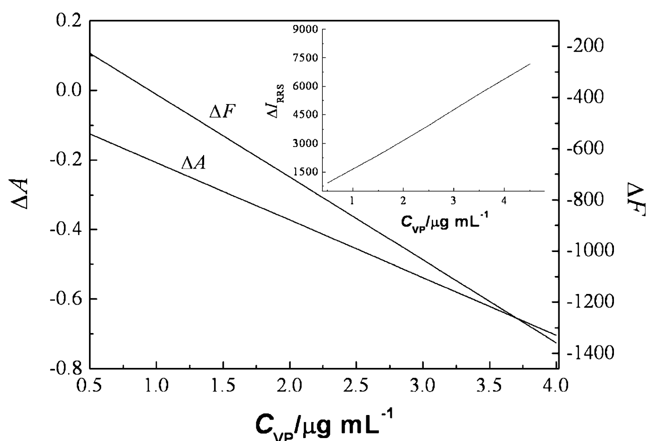

Fig. 8 The relationship between the absorbance, fluorescence and RRS spectrum

Table 1 Correlation coefficients, linear ranges and detection limits for calibration graphs

Method	Linear regression equation (c, μg/mL)	Linear range (μg/mL)	Correlation coefficient	Detection limit (c,ng/mL)
Spectrophotometry	$-\Delta A$=0.04149+0.1657 c	0.6036~4.0	0.9999	181.1
Fluorescence quenching method	$-\Delta F$=65+323.3 c	0.0213~4.0	0.9950	6.39
RRS	ΔI=9.2+1572.6 c	0.0032~4.5	0.9998	0.95

cence and resonance scattering. The fluorescence quenching could not be only considered as the transfer of the radiation energy to the nonradiation energy, but the transfer between fluorescence and resonance scattering. That is to say, part of radiant fluorescence is transferred to resonance scattering through the resonance effect to produce a resonance light scattering. This phenomena has been confirmed by many literatures already which based on known physical phenomena [38, 39]. The synchronous change of fluorescence quenching and scattering enhancement can be seen from Fig. 8. It is a very common phenomenon that fluorescence quenching can be observed in the study of RRS of fluorescence systems.

Therefore, it can be considered that the enhancement of RRS is the result of the energy transfer from light absorption and light emission to the scattering.

Sensitivity of Method

Under optimum conditions, the VP solution of different concentration reacted with EY solution and the ΔI_{RRS}, ΔA and ΔF values were measured at their respective maximum wavelengths. The calibration graphs of ΔI_{RRS}, ΔA and ΔF versus the concentrations of VP were constructed and the linear regression equations, correlation coefficients, linear ranges and detection limits (3σ) for the calibration curves were shown in Table 1. It can be seen from Table 1 that the sensitivity of the spectrophotometry method was too low and fluorescence quenching method has a high sensitivity. For these two methods, their sensitivity was higher than those of some common spectrophotometry and fluorospectrophotometry, but lower one order of magnitude than that of RRS method and the RRS method had the highest sensitivity, The detection limit for VP by RRS method was 0.95 ng/mL, which was several times or even several orders of magnitude higher than those of common spectrophotometry, capillary electrophoresis and high performance liquid chromatography (HPLC) method, etc. [3, 7, 10]. Therefore, a new method which is more suitable for the determination of trace amounts of VP has been developed.

Selectivity of RRS Method

Under optimum conditions, the effects of some coexisting substances on the determination of VP by RRS method were tested and the results were given in Table 2. As shown in Table 2, when the relative error was lower than ±5% and the concentration of VP was 1.0 μg/mL, the common metal

Table 2 Effects of coexisting substances (VP=1.0 μg/mL)

Coexisting substance	Multiple tolerant	Relative error (%)	Coexisting substance	Multiple tolerant	Relative error (%)
NH_4^+, NO_3^-	100	1.9	Potassium sodium tartrate	200	3.8
Al^{3+}, SO_4^{2-}	500	−2.1	HSA	10	3.1
Zn^{2+}, SO_4^{2-}	170	0.5	Threonine	200	−1.8
Ni^{2+}, SO_4^{2-}	32	3.0	Glycine	200	−2.5
Mn^{2+},SO_4^{2-}	56	1.1	L-Phenylalanine	200	−3.4
Na^+, NO_3^-	460	−2.6	DL-arginine	200	3.0
Na^+, Cl^-	1,200	−2.3	L-arginine	200	3.0
Mg^{2+},SO_4^{2-}	800	1.2	Uric acid	36	−1.4
Pb^{2+}, Ac^-	16	−2.0	Carbamide	120	2.6
Co^{2+}, NO_3^-	4	−3.0	Glucose	500	−5.0
Fe^{3+}, SO_4^{2-}	10	3.0	Lactose	500	−1.8
K^+, $H_2PO_4^-$	100	1.7	Maltose	500	−4.2
Cu^{2+}, SO_4^{2-}	80	2.8	Starch	120	−2.0
EDTA	676	1.0	VB_1	40	1.4
Ca^{2+}, Cl^-	400	2.7	VC	50	−2.0

Table 3 Results for the determination of VP in tablets

Method	Specified amount (mg/one tablet)	Average found (n=5, mg/one tablet)	Added (mg/piece)	Total found (n=5, mg/one tablet)	Recovery (%)	R.S.D (%)
RRS	40.0	39.96	5.0	44.95	99.8	1.2
Pharmacopoeia method	40.0	40.34	5.0	45.43	101.8	1.3

ions, inorganic acid radicals, anions, carbohydrate and amino acids hardly interfered with the determination of VP, 200 times of amino acids such as threonine, glycine and arginine had no interference, as well as the large amounts of some vitamins and urea did not interfere with determination of VP. Therefore, the method has a good selectivity and could be applied to the determination of trace VP.

Analytical Application

Determination of VP in Tablets

Two tablets (40 mg per tablet) were weighed accurately and ground into a fine powder. A suitable amount of the powder (containing about one quarter of the sample) was accurately weighed and dissolved in water, then leached and the filtrate was transferred into a 100 mL volumetric flask, diluted to the mark with water and mixed thoroughly. Then 10.0 mL of this solution was piped into 100 mL volumetric flask and diluted to the mark as working solution for the assay. The procedure described above was applied to the determination of VP in pharmaceutical formulations. The results are listed in Table 3 and are in good agreement with those obtained by the standard method of The Pharmacopeia of People's Republic of China [40].

Determination of VP in Human Serum Samples

A fresh serum sample (taken from healthy people) was treated with suitable amounts of 2.0 mol·L^{-1} trichloroacetic acid and centrifuged at 3,000 rpm for 30 min to separate proteins. Then, 1.0 mL of EY solution and 0.5 mL HCl-NaAc buffer solution were added for the determination of VP after a 1.0 ml of supernatant fluid was diluted into 10.0 mL calibrated flasks. The R.S.D. and recovery were tested by using the standard addition method and the results are listed in Table 4.

It can be seen from Table 4 that the method has a good repeatability (RSD are from 4.0% to 4.9%) and accuracy (recovery are from 96.4% to 102.7%) and can be applied to the determination of VP in human serum. This assay was a novel method for monitoring hemic-medical concentration of verapamil hydrochloride in clinical pharmacology and for the pharmacokinetics study, as well as provided valuable criterion for use of verapamil hydrochloride rationally, safely and effectively.

Conclusions

In pH 3.2 HCl-NaAc buffer solutions, eosin Y (EY) can react with verapamil hydrochloride (VP) to form a 1:1 ion-association complex, which not only results in the change of the absorption spectra and the quenching of fluorescence, but also results in the great enhancement of resonance Rayleigh scattering (RRS). Furthermore, a new RRS spectrum will appear. In this work, the absorbance, RRS and fluorescence intensities were proportional to the concentration of VP in the range of 0.6036~4.0 μg/mL, 0.0032~4.5 μg/mL and 0.0213~4.0 μg/mL, respectively. Based on this fact, a highly sensitive, simple, and rapid and new method for the determination of trace amounts of VP by resonance Rayleigh scattering technique has been proposed. Compared with other methods, it has obvious advantages not only in the operation but also in the sensitivity. The method may be satisfactorily applied to the determination of VP in tablets and human serum samples. The results are in good agreement with those obtained by the standard method, which promises this method a good analytical application prospect.

Table 4 The results for the determination of VP in serum samples

Sample	Found (μg/mL)	Added (μg/mL)	Total found (n=5, μg/mL)	Recovery (%,n=5)	RSD (%, n=5)
Serum 1	ND	1.0	1.02 0.97 0.89 0.98 0.96	96.4	4.9
Serum 2	ND	2.0	2.17 1.98 2.06 2.1 1.96	102.7	4.2
Serum 3	ND	3.0	3.12 3.09 3.21 2.96 2.89	101.8	4.0

ND not detected

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