

# Resonance Rayleigh Scattering Method for the Determination of Raloxifene with Evans Blue

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The intensity of resonance Rayleigh scattering (RRS) of Evans blue (EB) or raloxifene hydrochloride (Ralo) is very weak, but it can be enhanced significantly and a new RRS spectrum appears when both of them interact to form an ion-association complex in sodium acetate-hydrochloric acid buffer solution at pH 1.8. The intensity of RRS is directly proportional to the concentration of Ralo in the range of 0—8.3  $\mu\text{g} \cdot \text{mL}^{-1}$ , and the detection limit for Ralo ( $\sigma = 3$ ) is 18.9  $\text{ng} \cdot \text{mL}^{-1}$ . The method has high sensitivity and fairly good selectivity. Based on the above evidences, a new facile method for the determination of trace amount of Ralo has been established with satisfactory results.

**Keywords** resonance Rayleigh scattering, raloxifene, Evans blue, ion-association complex

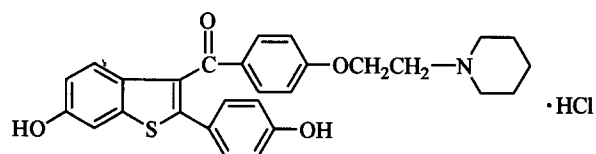
## Introduction

In recent years, resonance Rayleigh scattering (RRS) method as a new analytical technique has been extensively studied and widely used to the determination of some biological macromolecules, such as nucleic acid,<sup>1-4</sup> protein<sup>5-8</sup> and heparin,<sup>9</sup> as well as inorganic ion,<sup>10-14</sup> due to its simplicity, quickness and high sensitivity. However, up till now, very few reports on the RRS method utilized in pharmaceutical analysis were found in literature.

Raloxifene hydrochloride (Ralo) is a 3-aryl-2-arylbenzo[*b*]thiophene derivative with triarylethylene non-steroidal structure. The first time that Ralo marketed in American is on March 1998. Its commodity name is Evista, its formula is  $\text{C}_{28}\text{H}_{27}\text{NO}_4\text{S} \cdot \text{HCl}$ , and its structure

is illustrated in Scheme 1:

Scheme 1



Ralo belongs to selective estrogen receptor modulators (SERMs). At present, it has been applied to the prevention of osteoporosis,<sup>15</sup> the inhibition of breast cancer,<sup>16</sup> the therapy of cardiovascular disease,<sup>17</sup> etc. without stimulating reproductive tissues. Moreover, it has anticancer effect on uterus and ovary.<sup>18</sup>

The method for determination of Ralo is very few in literature. Its HPLC method is fussy and the sensitivity is not high enough, and the sample need to be derivatized and separated before use.<sup>19</sup>

It was demonstrated that though the intensity of RRS for Ralo existing as a large cation or for Evans blue (EB) existing as a large anion of acid bisazo dye was very low in an acidic medium, the intensity of RRS was enhanced significantly and a new RRS spectrum appeared simultaneously when Ralo reacted with EB to form an ion-association complex by virtue of electrostatic forces and hydrophobic interaction. In this work, the RRS spectral characteristics, influence factors, the optimum conditions of the ion-association reaction and the properties of analytical chemistry were investigated, and a sensitive, rapid and simple method was established.

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Received February 25, 2002; revised May 27, 2002; accepted July 1, 2002.

Project supported by the National Natural Science Foundation of China (No. 29875019).

## Experimental

### Apparatus and reagents

A UV-8500 ultraviolet-visible spectrophotometer (Tianmei Company, Hongkong) and a Shimadzu RF-540 spectrofluorophotometer (Kyoto, Japan) were used. The parameters of spectrofluorophotometer were: slit (EX/EM), 10.0 nm/10.0 nm; low sensitivity; ordinate, 6.

Ralo solution: raloxifene hydrochloride (offered by West China School of Pharmacy, Sichuan University. purity is 99.9%) was dissolved directly in water to prepare a stock solution ( $1 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ ). The working solution ( $1 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ) was obtained by diluting the stock solution with water. EB (Fluka A. G) solution:  $1 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$  and  $1 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ . Sodium acetate-hydrochloric acid buffer solution:  $1.0 \text{ mol} \cdot \text{L}^{-1}$  (pH 0.8—3.5).

All the reagents were of analytical grade and water was doubly distilled.

### General procedure

To a 10 mL of dried calibrated flask was added 0.5 mL of EB solution, 0.9 mL of sodium acetate-hydrochloric acid buffer solution (pH 1.8) and Ralo solution, and the mixture was diluted to the mark with water. After mixing and setting the solution aside for 10 min, the RRS spectra were recorded with synchronous scanning at  $\lambda_{\text{ex}} = \lambda_{\text{em}}$  (i. e.  $\Delta\lambda = 0 \text{ nm}$ ) and the RRS intensity  $I$  for the reaction product and  $I_0$  for the reagent blank at  $\lambda$  were measured,  $\Delta I = I - I_0$ .

## Results and discussion

### RRS spectra

Fig. 1 shows the RRS spectra of EB-Ralo system. It can be seen that: (1) the RRS of EB or Ralo is faint under the measurement conditions. (2) When Ralo reacted with EB to form an ion-association complex, a new RRS spectrum appeared and the intensity of RRS is enhanced remarkably. The maximum RRS wavelength is at 400 nm, another RRS peak at 470 nm. (3) The RRS intensity increases with the growth of Ralo concentration in the range of 0—8.3  $\mu\text{g} \cdot \text{mL}^{-1}$ .

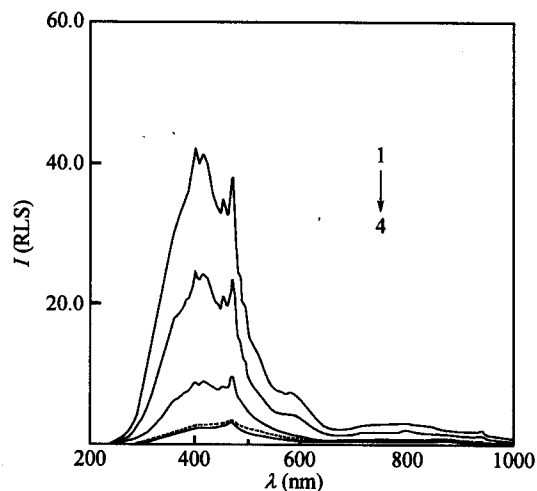


Fig. 1 RRS spectra of Evans blue-raloxifene system (pH 1.8). — EB-Ralo ( $\times 5.2 \mu\text{g} \cdot \text{mL}^{-1}$ ): (1) 1.0; (2) 0.6; (3) 0.2; (4) 0. --- Ralo.

### Optimum conditions of the reaction

**Effect of solution acidity** Fig. 2 shows the effect of solution acidity on RRS intensity. It can be seen that: (1) the RRS intensity of EB solution is not only very low but also nearly constant in buffer solution at pH 0.8—3.5. (2) The optimum pH value is in the range of 1.2—2.0. When higher or lower than this range, the RRS intensity of ion-associate of EB-Ralo will decrease. Based on the results, pH 1.8 of sodium acetate-hydrochloric

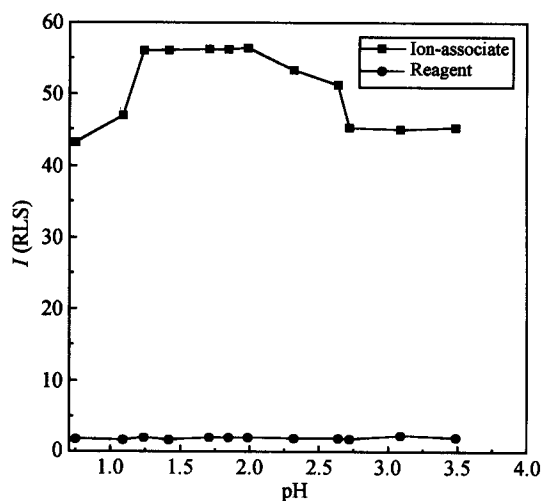
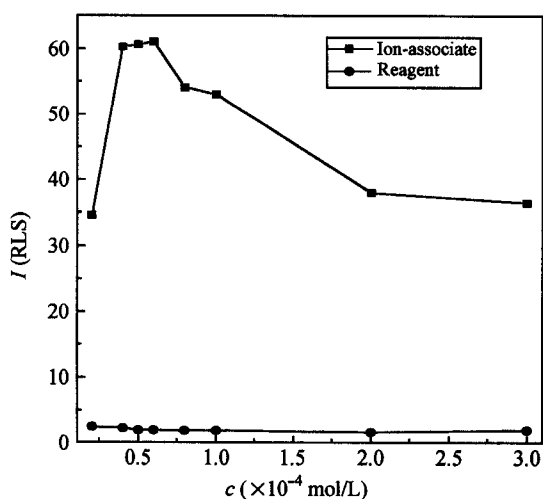


Fig. 2 Effect of solution acidity. EB:  $1 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ ; Ralo:  $7.8 \mu\text{g} \cdot \text{mL}^{-1}$ ; buffer solution (pH 1.8): 1.0 mL.

acid buffer solution was adopted in this work, and its optimum added amount is between 0.8 and 1.0 mL.

**Effect of EB concentration** The influence of EB concentration on RRS intensity is shown in Fig. 3. The change of EB concentrations has no effect on the RRS intensity of EB solution itself, but it affects notably that of the ion-associate. The optimum range is  $(4.0\text{--}6.0) \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}$ . If the concentration is lower than  $4.0 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}$ , the reaction of EB with Ralo will not perform completely and the scattering intensity gets lower. But if EB concentrations are too high, on the other hand, aggregation of EB may happen, the surface of ion-associate will absorb excessive molecules, thus the intensity of RRS will also reduce because the size of particles become too big to produce Rayleigh scattering (the fundamental element of producing Rayleigh scattering is that scattering wavelength equals to incident wavelength, and the diameter of scattering particles is far smaller than incident wavelength<sup>1</sup>).



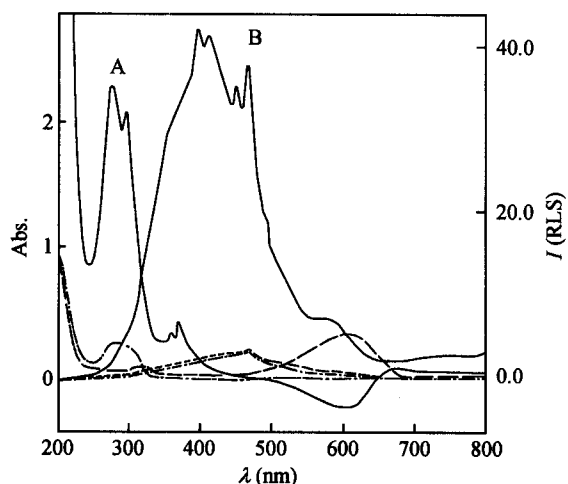
**Fig. 3** Effect of EB concentration. Ralo:  $7.8 \mu\text{g} \cdot \text{mL}^{-1}$ ; EB:  $1 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ; buffer solution (pH 1.8): 1.0 mL.

**Reaction time and stability** The reaction finishes rapidly at room temperature. The  $\Delta I$  value reaches the highest in 10 min and remains constant for at least 3 h. The influence of temperature is not obvious between 10—35 °C.

**Comparison of RRS spectra and absorption spectra**

The RRS spectrum (B) and absorption spectrum (A) of EB-Ralo system are shown in Fig. 4. It can be seen that: (1) in acidic medium at pH 1.2—2.0, the maxi-

imum absorption of EB is located at 585 nm, and another slightly weak absorption peak is located at 309 nm. Under the same conditions, Ralo itself has only one maximum absorption peak at 280 nm in ultraviolet region. In contrast, RRS intensities of them are very feeble respectively, and only one extreme weak RRS signal is observed at 470 nm. (2) After EB reacted with Ralo and then formed an ion-association complex, changes of the absorption spectrum took place: color faded reaction happened at 585 nm. Moreover, the absorbance is enhanced at 280 nm, and some new absorption spectra appear at 292, 351, 358 and 649 nm. It hints that the new product is produced. Although the sensitivity is still low, rapid changes occur in RRS spectrum, the maximum RRS peak shifts to 400 nm, and the scattering band is located at 300—550 nm. It is believed that there exists a correlation between the scattering band and the absorption band at 358 nm.



**Fig. 4** Comparison of RRS spectra (A) and absorption spectra (B) of EB-Ralo system. — EB-Ralo; --- EB; ·— Ralo. Ralo:  $5.2 \mu\text{g} \cdot \text{mL}^{-1}$ ; EB:  $5 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}$ ; buffer solution (pH 1.8): 0.9 mL.

As known, Ralo exists as protonated univalent cation in acidic medium at pH 1—2:



Meanwhile, the four  $\text{SO}_3\text{H}$  groups bearing on naphthalene ring of EB dissociate partially and EB exists as a large organic anion. Ralo can combine with EB by virtue of electrostatic forces and hydrophobic interaction, and

further form an ion-associate.

Usually, the reactant molar ratio of ion-associate is determined by the Job's method of spectrophotometry. From the results, Ralo:EB = 2:1, the possible structure of the complex is supposed as Scheme 2.

Thus it can be concluded that one of the important factors leading to RRS enhanced is the further augmentations of molecular volume and the resonance Rayleigh scattering produced from Rayleigh scattering located at the absorption band.

**Selectivity of the method** Under the optimum conditions, the influence of coexisting substances on the determination of Ralo was investigated and the results are shown in Table 1.

It can be seen that 100-fold of Amylogen and amino acid, 25-fold of carbohydrate along with 10-fold of nucleic acid and Ca(II), Mg(II), Fe(III), Cu(II), Zn(II), as well as a vast amount of K<sup>+</sup>, Na<sup>+</sup> has no effect on the determination of Ralo. So the method has fairly good selectivity.

**Relation between the RRS intensity and the concentration of Ralo** According to the general procedure

with different concentration of Ralo,  $I$  and  $I_0$  at  $\lambda_{\max}^{\text{RRS}}$  were measured respectively, and the calibrated graphs of  $\Delta I$  against concentration of Ralo were established. When the concentration is in the range of 0–8.3  $\mu\text{g}\cdot\text{mL}^{-1}$ , the linear regression equation is:  $\Delta I = 7.09c - 0.29$  ( $c$ :  $\mu\text{g}\cdot\text{mL}^{-1}$ ). Its correlation coefficient is 0.9989, and the detection limit ( $3\sigma$ ) is 18.9  $\text{ng}\cdot\text{mL}^{-1}$ .

## Analytical application

To a 10 mL of dried calibrated flask were added 0.5 mL of EB solution, 0.9 mL of sodium acetate-hydrochloride buffer solution (pH 1.8) and suitable amount of Ralo solution sequentially. Then appropriate amount of some metal ions, nucleic acid, amino acid and carbohydrate were added and the mixture was diluted to the mark with water. After mixed, the solution was set aside for 10 min. The RRS intensity of the sample was recorded as mentioned above. Every sample was determined for five times. The recovery of Ralo of three samples with the standard addition method was between 99.5% and 104.9%. The results are shown in Table 2.

Scheme 2

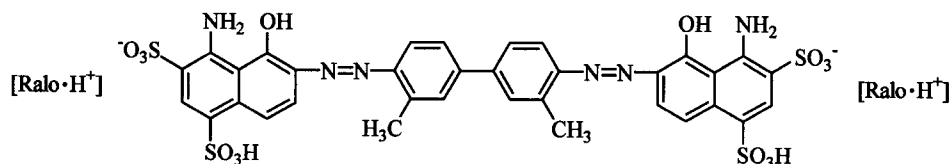


Table 1 Effect of coexisting substances (Ralo: 2.0  $\mu\text{g}\cdot\text{mL}^{-1}$ )

Coexisting substance	Concentration tolerated ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	Coexisting substance	Concentration tolerated ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	Coexisting substance	Concentration tolerated ( $\mu\text{g}\cdot\text{mL}^{-1}$ )
Fe(III)	11	Pb(II)	100	Amylogen	200
Co(II)	20	Ca(II)	20	Maltose	50
Mn(II)	50	Zn(II)	14	Glucose	50
Mg(II)	50	Al(III)	20	Fructose	50
Cd(II)	100	Cu(II)	100	hsDNA	20
K <sup>+</sup>	750	Na <sup>+</sup>	2900	yRNA	30
Glycine	100	L-Histidine	100	L-Leucine	100

Table 2 Determination of Ralo in artificial samples

Sample	Ralo concentration ( $\mu\text{g}\cdot\text{mL}^{-1}$ )		Other coexisting substances ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	RSD (%) ( $n = 5$ )	Recovery (%)
	Amount added	Amount determined <sup>a</sup>			
1	2.6	2.6	MgSO <sub>4</sub> 30, yRNA 14, Amylogen 100, Glucose 30	6.00	99.5
2	2.6	2.7	KCl 380, CuCl <sub>2</sub> 50, Glycine 50, Fructose 30	5.31	104.9
3	2.6	2.5	FeCl <sub>3</sub> 5, NaCl 1800, hsDNA 8, L-Histidine 50	4.14	103.8

<sup>a</sup> An average value determined for five times.

## References

- 1 Pasternack, P. F.; Collings, P. F.; Giannetto, A.; Gibbs, E. J. *J. Am. Chem. Soc.* **1993**, *115*, 5393.
- 2 Huang, C. Z.; Li, K. A.; Tong, S. Y. *Anal. Chem.* **1996**, *68*, 2259.
- 3 Huang, C. Z.; Li, Y. F.; Liu, X. D. *Anal. Chim. Acta* **1998**, *375*, 89.
- 4 Huang, C. Z.; Li, Y. F.; Li, N. B.; Li, K. A.; Tong, S. Y. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1791.
- 5 (a) Ma, C. Q.; Li, K. A.; Tong, S. Y. *Anal. Biochem.* **1996**, *239*, 86.  
(b) Ma, C. Q.; Li, K. A.; Tong, S. Y. *Anal. Chim. Acta* **1997**, *338*, 255.
- 6 Yang, R.; Liu, S. P. *Chin. J. Anal. Chem.* **2001**, *29*, 232 (in Chinese).
- 7 Liu, S. P.; Liu, Q.; Yang, R. *Anal. Sci.* **2001**, *17*, 239.
- 8 Liu, S. P.; Yang, R.; Liu, Q. *Anal. Sci.* **2001**, *17*, 243.
- 9 Liu, S. P.; Luo, H. Q.; Liu, Z. F.; Li, N. B. *Anal. Chem.* **2001**, *73*, 3907.
- 10 Liu, S. P.; Liu, Z. F. *Spectrochim. Acta* **1995**, *51A*, 1497.
- 11 Liu, S. P.; Liu, Z. F.; Zhou, G. M. *Anal. Lett.* **1998**, *31*, 1247.
- 12 Liu, S. P.; Liu, Q.; Liu, Z. F.; Li, M.; Huang, C. Z. *Anal. Chim. Acta* **1999**, *379*, 56.
- 13 Liu, S. P.; Zhou, G. M.; Liu, Z. F.; Li, M. *Chem. J. Chin. Univ.* **1998**, *19*, 1040 (in Chinese).
- 14 Liu, S. P.; Zhou, G. M.; Huang, C. Z. *Anal. Sci.* **1998**, *14*, 799.
- 15 Bryant, H. U.; Glasebrook, A. L.; Yang, N. N.; Nakazawa, T. *J. Steroid Biochem. Mol. Biol.* **1999**, *69*, 37.
- 16 Jordan, V. C. *J. Lab. Clin. Med.* **1999**, *133*, 408.
- 17 Kanda, I. *US 5968918*, **1999** [*Chem. Abstr.* **1999**, *131*, 282020d].
- 18 Singh, J. P.; Wood, D. L. *WO 9744029*, **1997** [*Chem. Abstr.* **1998**, *128*, 53242].
- 19 Lim, H. K.; Stelling, W. S.; Sisen, W. S.; Chan, K. W. *J. Chromatogr., A* **1999**, *831*, 227.

(E0202256 LI, L. T.; ZHENG, G. C.)