Original Paper

Resonance Rayleigh Scattering for the Determination of Berberine in Tablet Form with some Acidic Xanthene Fluorescent Dyes

Shaopu Liu* and Ping Feng

Institute of Environmental Chemistry, Southwest Normal University, Chongqing 400715, P.R. China

Received October 22, 2001; accepted May 22, 2002; published online October 15, 2002

© Springer-Verlag 2002

Abstract. A simple determination method of berberine with a limit of determination at the nanogram level is proposed under the use of a common spectrofluorometer to detect the intensity of resonance Rayleigh scattering (RRS). In aqueous solution at pH 4–5, berberine reacts with acidic xanthene fluorescent dyes such as eosine Y, erythrosine, ethyl eosin, phloxin and Rose Bengal to form an ion-associate. This results in a significant enhancement of RRS intensity and the appearance of new RRS spectra. The characteristics of RRS spectra of the ion-associates and the optimum conditions of these reactions have been investigated. The intensity of RRS is directly proportional to the concentration of berberine in the range of 0–5.0 × 10⁻⁶ mol/L. The procedures described have a very high sensitivity and the detection limits for berberine are 14–215 ng/ml, and the sensitivity order is eosine Y > erythrosine > ethyl eosin > phloxin > rose Bengal. The results of analysis for synthetic samples were in good agreement with the desired values, and the ones for tablets were identical with those obtained with the procedure described in the Chinese Pharmacopoeia.

Key words: Resonance Rayleigh scattering; berberine; acidic xanthene fluorescent dyes.

Berberine is an alkaloid present in various species of berberis and is widely used as a stomach and bowel medicine [1]. There are many methods for its assay in plant extracts and in pharmaceutical preparations such as titrimetry [1, 2], spectrophotometry [3–6], fluorometry [7–9], TLC [10] and HPLC [11]. These methods, however, have suffered from drawbacks such as tedious, time-consuming and of low sensitivity. Therefore, it is necessary to develop a simple assay for the analysis of berberine with high sensitivity and simplicity.

In recent years, resonance Rayleigh scattering (RRS) has become known for its sensitivity, simplicity and speed. As a new analytical method it can be applied to the study and determination of some biological macromolecules such as nucleic acid [12, 13], proteins [14, 15] and heparin [16, 17]. These studies are mainly based on the fact that the aggregation of chromophore on the biological macromolecule can lead to the enhancement of RRS. However, the ion-association compound of a dye monomer with a counter ion smaller than a biological macromolecule can also enhance the RRS effect greatly with the appearance of new spectrum [18]. We have already reported on the use of RRS for the determination of trace amounts of mercury(II) [19], selenium(IV) [20], chromium(VI) [21], molybdenum(V) [22], cadmium(II) [23], and cationic surfactant [24]. So, we suppose that RRS method can also be used to determine some pharmaceuticals after forming ion-associates. In the work presented, we establish a sensitive and simple method of berberine using RRS. It makes use of the fact that in weakly acidic aqueous solution, berberine
can react with an acidic xanthene dye such as eosine Y, erythrosine, ethyl eosin, phloxin and rose bengal to form an ion-associate which results in the significant enhancement of RRS intensity and the appearance of new RRS spectra. The scattering intensity is directly proportional to the concentration of berberine in the range of $0.5 \times 10^{-6}$ mol/L. For the eosine Y system, the detection limit for berberine is $14 \text{ ng ml}^{-1}$, with which RRS is the most sensitive method investigated. As an example, the procedure using eosine Y was applied to the determination of berberine in tablets.

**Experimental**

**Reagents**

A standard solution ($1 \times 10^{-3}$ mol/L) was prepared by dissolving berberine chloride (B.D.H.), and solutions of lower concentrations were prepared by successive dilution with doubly distilled water. Acidic xanthene fluorescent dyes including eosine Y (analytical-reagent grade, Guangzhou Chemical Reagent Plant), erythrosine (E. Merck, Darmstadt), ethyl eosin (chemically pure reagent, Beijing Chemical Plant), phloxin (chemically pure reagent, Shanghai Third Chemical Reagent Plant) and Rose Bengal (chemically pure reagent, Shanghai Third Chemical Reagent Plant) were used, and all working concentrations were 0.025% (m/v). The citric acid–potassium hydrogen phosphate buffer solution had a concentration of 0.1 mol/L (pH 3.6–5.5). All the other reagents were of analytical reagent grade and doubly distilled water was used throughout.

**Apparatus**

A Shimadzu RF-540 spectrofluorometer (Kyoto, Japan) and Hitachi U-3400 spectrophotometer (Tokyo, Japan) were used.

**General Procedure**

The volume of 1.0 ml of berberine solution was filled into a 25 ml calibrated flask, 2.5 ml of citric acid–potassium hydrogen phosphate buffer solution were added as well as 1.0 ml of the dyes solution, then diluted with water and mixed thoroughly. The resonance Rayleigh scattering spectra were measured by synchronous scanning with the same wavelength settings. The RRS intensity for the ion-associate is $I$ and $I_0$ is the intensity for the reagent blank at maximum scattered wavelength, $\Delta I = I - I_0$.

**Analysis of Tablets**

20 tablets were finely powdered and the powder thoroughly mixed. An accurately weighed quantity of the powder equivalent to 0.100 g of berberine was transferred to a 500 ml standard flask, and shaken with about 200 ml of hot water for 5 min to ensure complete dissolution. The solution was then made up to volume with water and filtered; 10.00 ml of filtrate were diluted in a 100 ml standard flask and this solution was analysed as the general procedure.

**Results and Discussion**

**Spectral Characteristics**

In Figure 1 the RRS spectra of eosine Y, berberine and the mixture of eosine Y with berberine are shown. The RRS effect of berberine is very faint and eosin displays only one narrow peak, which the maximum at a wavelength of 536 nm. However, the new RRS spectra can be observed for the mixture of berberine and eosin with the maximum intensity of resonance Rayleigh scattering being red shifted to 576 nm, which indicates an interaction between berberine and eosin. Two other scattering peaks at 322 nm and 378 nm can be observed, while the RRS intensity of eosin decreased slightly. The RRS spectral characteristics of the reaction products for eosine Y and four other dyes with berberine are listed in Table 1. As Table 1 shows, a RRS intensity increase in varying degrees and different RRS spectra can be observed depending on the dye used. The maximum RRS intensity is found at 572 nm for erythrosine, at 578 nm for ethyl eosin, at 384 nm for phloxin and at 464 nm for Rose Bengal, and each has two or three other scattering peaks of smaller intensity. The enhanced intensities ($\Delta I$) of RRS for the ion-associate are in the following order: eosine Y > erythrosine > ethyl eosin > phloxin > Rose Bengal.

**The Optimum Reaction Conditions**

It was found that the citric acid–potassium hydrogen phosphate buffer solution is the most suitable reaction

![Fig. 1. RRS spectra of berberine–eosine Y system: 1 eosine Y; 2 berberine–eosine Y](https://example.com/figure.png)

$\Delta I / 4 \times 10^{-6}$ mol/L

<table>
<thead>
<tr>
<th>Dye</th>
<th>$\lambda_{max}$ (nm)</th>
<th>Other peaks of RRS (nm)</th>
<th>$\Delta I / 4 \times 10^{-6}$ mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosine Y</td>
<td>576</td>
<td>378, 322</td>
<td>77.1</td>
</tr>
<tr>
<td>Erythrosine</td>
<td>585</td>
<td>381, 288</td>
<td>27.7</td>
</tr>
<tr>
<td>Ethyl eosin</td>
<td>578</td>
<td>470, 400, 328</td>
<td>17.5</td>
</tr>
<tr>
<td>Phloxin</td>
<td>583</td>
<td>384, 470, 332</td>
<td>12.3</td>
</tr>
<tr>
<td>Rose Bengal</td>
<td>590</td>
<td>580, 396</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* $\lambda_{max}$ present the wavelength at maximum intensity.