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Resonance Rayleigh Scattering Method for the Determination of Dextran Sulfate Sodium with Diantipyrylmethane①

HAO Xiu-li, LI Nian-bing, LUO Hong-qun

School of Chemistry and Chemical Engineering, Southwest University, Chongqing Municipal Key Laboratory of Luminescence and Real-Time Analysis, Chongqing 400715, China

Abstract: A new method for the determination of dextran sulfate sodium (DSS) with diantipyrylmethane (DAM) by resonance Rayleigh scattering (RRS) technique has been proposed. In pH 10.0 Britton-Robinson buffer medium, the interaction of DSS with DAM occurred, which greatly enhanced the RRS intensity with a maximum scattering peak at 352 nm. The interaction conditions were optimized. The affecting factors and characteristics of RRS for the interaction of DSS with DAM were investigated. Under the optimum condition, the enhancement of the RRS signal is directly proportional to the concentration of DSS in the range of 5.0×10^{-2}－1.5 $\mu g \cdot mL^{-1}$ and its detection limit is 17.0 $ng \cdot mL^{-1}$.

Key words: resonance Rayleigh scattering; dextran sulfate sodium, diantipyrylmethane

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1 Introduction

Dextran sulfate sodium (DSS) is a heparin-like long-chain polysaccharide containing approximately 17% sulfur and up to three sulfate groups per glucose molecule. As an investigational drug, DSS is now being evaluated as a microbicide to prevent the sexual transmission of HIV－1[1]. In addition, DSS is an anticoagulant and antihyperlipidemic agent and has also been evaluated in vitro and in vivo as a microbicide inhibitor of herpes simplex virus(HSV)－1, HSV－2. However, DSS is toxic if overcommit and could produce profound but reversible thrombocytopenia and extensive but reversible alopecia because of inappropriate IV infusion[2]. Therefore, it is important to study the characteristic of DSS and determine its concentration; however, there is scarcely report about the determination of DSS up to the present.

The resonance Rayleigh scattering (RRS) technique is a promising method developed in recent years. With high sensitivity, rapidity and simplicity, it has been widely applied to the determination of biological macromolecules[3-5], trace amounts of metals and non-metals[6-11], and some cationic surfactants[12-13]. In

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作者简介：郝秀利 (1981－)，女，河北邢台人，硕士研究生，主要从事环境分析化学方面的研究.
通讯作者：罗红群，教授，博士生导师.

addition, it also has been used to the study of some physicochemical parameters[14]. However, there was no study for the determination of DSS by RRS technique up to now.

In this paper, the interaction between DSS and DAM and its optimum reaction conditions were studied and the sample analysis was described. The results showed that this method was effective, simple and fast for the determination of DSS.

2 Experimental

2.1 Reagents

The stock solution of DSS (1.0 g·L^{-1}) was prepared by dissolving 100 mg of DSS reagent (Sigma) in water and diluting to the mark in a 100 mL calibrated flask. It was diluted to 1.0×10^{-2} g·L^{-1} for the working solution. DAM(1.0 g·L^{-1}) solution was prepared by dissolving DAM($C_{23}H_{24}N_4O_2$, Kelong Chemical Reagent Factory) in dilute hydrochloric acid (1.0 mol·L^{-1}); Britton-Robinson buffer solution with different pH was prepared by mixing the mixed acid (composed of 0.04 mol·L^{-1}, H_3PO_4, HAc and H_3BO_3) with 0.2 mol·L^{-1} NaOH in proportion. All other reagents were of analytical-reagent grade and doubly distilled water was used throughout.

2.2 Apparatus

A Hitachi F－4500 fluorescence spectrophotometer (Hitachi Ltd., Tokyo, Japan), which was equipped with a 150 W xenon lamp, was used for recording the spectra and measuring the intensity at a given wavelength using a 1 cm path length. The slit (EX/EM) was 10.0 nm/10.0 nm, the PMT voltage was 400 V. A UV－VIS 8500 spectrophotometer (Tianmei Co., Shanghai, China) was used for measuring absorption spectra. A pHS－3D pH meter (Shanghai Analytical Instrument Factory, Shanghai, China) was used for pH measurement.

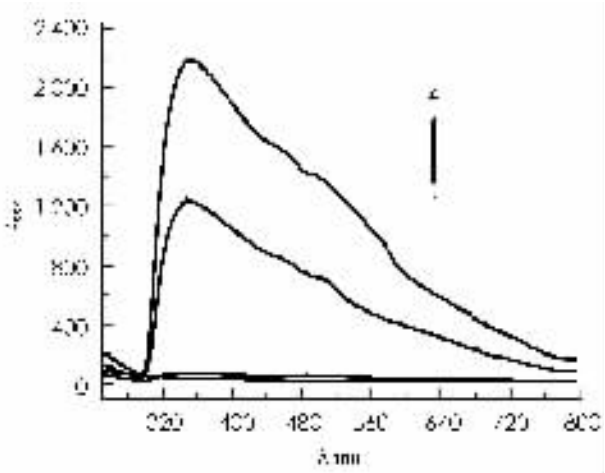
2.3 General Procedure

1.0 mL of BR buffer (pH 10.0) was placed in a 10 mL calibrated flask, and then appropriate volume of sample or working solution of DSS was added; at last 1.0 mL of DAM solution (1.0 g·L^{-1}) was added. The solution was diluted to the mark with water and mixed thoroughly. After 15 min, the spectra of the system were recorded with synchronous scanning at $\lambda_{ex}=\lambda_{em}$, and the intensity ($I$) for the DSS-DAM complex and I_0 for the reagent blank at the maximum wavelength, $\Delta I=I-I_0$, were measured. All measurements were performed at room temperature. (10～25 ℃).

3 Results and Discussion

3.1 RRS Spectra

Fig. 1 shows the RRS spectra of DSS, DAM and their complex at pH 10.0 from 250 to 800 nm. It can be seen that the intensity for either DSS or DAM in the whole scanning wavelength region was very weak. However, when they reacted with each other to form a complex, the RRS intensity enhanced greatly and increased linearly with the increase of DSS concentration at 352 nm and this can be served as a basis for the determination of DSS. In this system, DSS has a hydrophobic framework and DAM has a hydrophobic multiphenyl body. When DSS binded with DAM, they could form hydrophobic supermolecular complex,



(1) DSS (0.3 μg·mL^{-1}); (2) DAM (0.1 g·L^{-1}); (3) DSS-DAM: C_{DAM}, 0.1 g·L^{-1}; C_{DSS}, 1.7 μg·mL^{-1}1; (4) DSS-DAM: C_{DAM}, 0.1g·L^{-1}; C_{DSS}, 3.0 μg·mL^{-1}

Fig. 1 RRS spectra of the DSS-DAM system

which tended to form an interface between the complex and the water phase which greatly enhanced the RRS intensity.

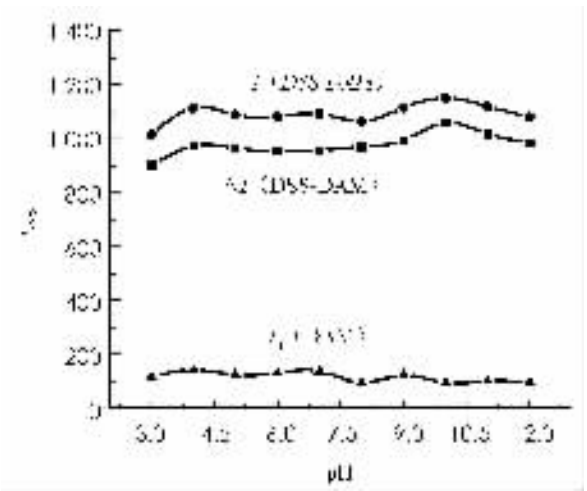
3.2 Optimal Conditions of the Assay

3.2.1 Effect of Acidity

The effect of the solution acidity on the scattering intensity of the system has been studied. We investigated the influence of different buffer types, such as BR buffer, phosphate buffer, NaAc-HAc buffer and Kolthoff buffer. The results showed that BR buffer was the best. It can be seen from Fig. 2 that the effect of the acidity is not serious in a wide pH range. Considering the stability and sensitivity, we selected 1.0 mL of pH 10.0 BR buffer for further studies.

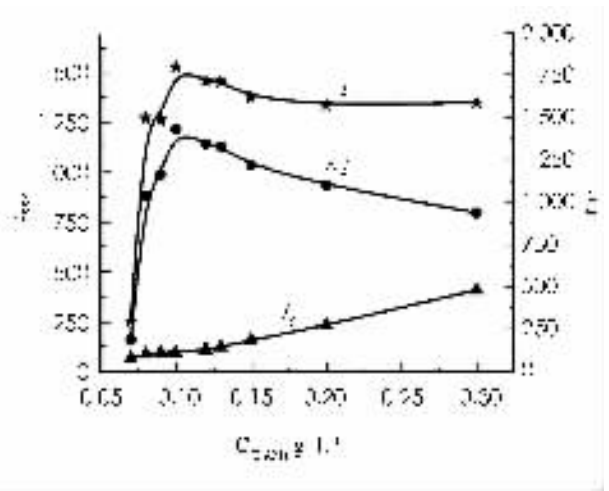
3.2.2 Effect of the DAM Concentration

The effect of DAM concentration on the RRS intensity was studied and the result is presented in Fig. 3. It was found that the concentration of DAM affected greatly the RRS intensity of the system. When the DAM concentration was low, the RRS intensity of DSS-DAM system was faint due to the incomplete reaction. With the increase in DAM concentration, the scattering intensity of the system enhanced gradually and reached a maximum at the concentration of 0.1 g · L^{-1} and then if the amounts of DAM increased further, the scattering intensity decreased gradually. Therefore, 0.1 g · L^{-1} DAM was selected as the best concentration for the further study.



(C_{DAM} = 0.1 g · L^{-1}, C_{DSS} = 1.0 μg · mL^{-1})

Fig. 2　Effect of pH on the RRS intensity



pH = 10.0, C_{DSS} = 1.0 μg · mL^{-1}

Fig. 3　Effect of DAM concentration on the RRS intensity at 352 nm

3.2.3 Effect of Ionic Strength

The effect of ionic strength on the RRS intensity of the DSS-DAM system was carried out by adding different concentration of NaCl into the system and the results revealed that the addition of NaCl caused a significant decrease of RRS intensity. Therefore, the concentration of NaCl should be strictly controlled and in this study no extra NaCl solution was added.

3.2.4 Effect of Addition Sequence

The effect of the reagent addition order on the intensity was studied. The result showed that mixing buffer and DSS solution first and then adding DAM solution could result in a higher RRS intensity than any other adding sequences of the reagents. So the best addition order for the system is to add buffer solution, DSS and DAM in succession.

3.2.5 Effect of Temperature and Stability of the System

The effect of temperature on the intensity of the system was also studied. According to the results of the ex-

periment, the temperature in the range of 10～25 ℃ is optimum for the system. Under the temperature of this range the reaction of DSS and DAM will be complete within 15 min and the RRS intensity remains stable for 120 min. With the increase in temperature (>25 ℃) the RRS intensity will decrease gradually. Therefore, our experiment was performed in the room temperature, which is between 10 ℃ and 25 ℃.

3.3 Selectivity of the Method

The influences of foreign coexistent substances on the determination of 1.0 $\mu g \cdot mL^{-1}$ DSS were investigated by pre-mixing DSS with foreign substances. The tolerance limit was taken as the maximum concentration of the foreign substances which caused an approximately ±5% relative error in the determination and the results are listed as follows ($\mu g \cdot mL^{-1}$): urea (90), sucrose (25), fructose (200), glucose (200), cellobiose (25), galactose (50), tyrosine (150), L-cystine (10 000), L—glutamic acid (25), L-lysine (50), ascorbic acid (10), BSA (300), β-CD (100), NH_4^+ (300), Mg^{2+} (10), K^+ (14), Sn^{2+} (4), Al^{3+} (3), $C_2O_4^{2-}$ (10), NO_2^- (10), and dopamine (5).

3.4 Relation Between the RRS Intensity and the DSS Concentration

Under the optimum conditions, the enhanced relative scattering intensities, ΔI, of the system were measured at 352 nm. The calibration graph of ΔI against the DSS concentration was constructed. The linear regression equation is $\Delta I = -7.02 + 739.74\ C$($C$, $\mu g \cdot mL^{-1}$) over the range of 0.05～1.5 $\mu g \cdot mL^{-1}$. The correlation coefficient is 0.999 2 with the detection limit (3σ) of 17 $ng \cdot mL^{-1}$. So the proposed method is highly sensitive for the determination of trace amount of DSS.

3.5 Analytical Application of the Method

Three synthetical samples were finely measured by the method. Based on the tolerance amounts of the foreign substances presented in Table 1, some substances were mixed thoroughly with DSS before the measure. The detailed data and results are listed in Table 1.

Table **1** Analytical Results of the Synthetical Samples

Sample	DSS Added /$\mu g \cdot mL^{-1}$	Coexistent Substance	DSS Found /$\mu g \cdot mL^{-1}$	Recovery /%	RSD /%
No. 1	1.0	A	0.98,1.02,1.03,1.01,1.04	101.6	2.3
No. 2	1.0	B	0.97,1.00,0.99,1.01,0.98	99.0	1.5
No. 3	1.0	C	0.96,1.02,0.97,0.98,1.05	99.6	2.0

A. Fructose(1.0×10^{-4} $mol \cdot L^{-1}$), urea(5.0×10^{-5} $mol \cdot L^{-1}$), galactose (4.0 $\mu g \cdot mL^{-1}$), KNO_3(2.5×10^{-5} $mol \cdot L^{-1}$), NaCl (2.5×10^{-5} $mol \cdot L^{-1}$), and tyrosine (15.0 $\mu g \cdot mL^{-1}$).

B. $AlCl_3$(0.15 $\mu g \cdot mL^{-1}$), sucrose(3.0×10^{-5} $mol \cdot L^{-1}$), $Bi(NO_3)_3$(0.3 $\mu g \cdot mL^{-1}$), L-glutamic acid (0.3 $\mu g \cdot mL^{-1}$), and dopamine(1.0×10^{-6} $mol \cdot L^{-1}$).

C. Glucose(2.0×10^{-4} $mol \cdot L^{-1}$), glycin (15.0 $\mu g \cdot mL^{-1}$), and $SnCl_2$(0.4 $\mu g \cdot mL^{-1}$)

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二安替比林甲烷共振瑞利散射法测定硫酸葡聚糖钠

郝秀利，　李念兵，　罗红群

西南大学化学化工学院，发光与实时分析重庆市重点实验室，重庆 400715

摘要： 采用共振瑞利散射法研究了二安替比林甲烷与硫酸葡聚糖钠的相互作用及其分析应用. 在 pH 为 10.0 的 Britton-Robinson 缓冲溶液中，二安替比林甲烷与硫酸葡聚糖钠结合后共振瑞利散射急剧增强，最大散射峰在 352 nm 处. 研究了此反应体系的最佳实验条件以及共振瑞利散射光谱的特征，在最佳条件下，共振瑞利散射信号的增强与硫酸葡聚糖钠的浓度在 0.05～1.5 $\mu g \cdot mL^{-1}$ 范围内成正比，检出限为 17.0 $ng \cdot mL^{-1}$，该法用于痕量硫酸葡聚糖钠的测定，灵敏，简便.

关键词： 共振瑞利散射；二安替比林甲烷；硫酸葡聚糖钠

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