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Determination of sodium hyaluronate with some basic bisphenylnaphthylmethane dyes by resonance Rayleigh scattering method

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Abstract

In an acetic acid–sodium acetate buffer solution of pH 3.6–6.8, a compound complex was formed between sodium hyaluronate (abbreviated as SH) and some basic bisphenylnaphthylmethane dyes, leading to a great enhancement of the intensity of resonance Rayleigh scattering (RRS) and giving a new RRS spectrum, with its maximum scattering peak near 280 nm. It was also found that the intensity of RRS was directly proportional to the concentration of SH near the range between 0 and 3.0 mg/L. Based on these facts, a sensitive method for the determination of SH has been established. The method had good selectivity, and has been used for the determination of total amounts of SH in samples with satisfactory results. For the NB–SH system, the detection limit of SH was down to 13.7 ng/mL.

Keywords: Resonance reyleigh scattering (RRS); Sodium hyaluronate; Basic bisphenylnaphthylmethane dyes; Determination

Hyaluronic acid (HA) and its' sodium (SH) with the favorable biocompatibility, high viscoelasticity, plasticity and permeability can be used widely in cosmetics, ophthalmology, joint disease [1], gynaecology and obstetrics, surgery, soft tissue repair [2], cosmetology and so on.

The main methods for determining SH are HPLC method, CE method, spectrophotometric method [3] and immunity method such as RiA, ELISA [4]. As a new analytical method, resonance Rayleigh scattering (RRS) has attracted more attention for its sensitivity, simplicity and speed in recent years, it has been applied to the study and determination of some biological macromolecules [5,6], trace amounts of metal cationic [7] and cationic surfactant [8] and so on. However, up to now, the RRS method used in determining SH by RRS technique is seldom reported. We found that some basic bisphenylnaphthylmethane dyes can react with SH 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 SH in a certain range. Thus, a new method to measure SH have been built up and applied to the determination of SH in samples.

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1. Experimental

A Hitachi F-2500 spectrofluorophotometer (Kyoto, Japan), a UV-8500 spectrophotometer (Shanghai, China) and a PHS-3C meter (Shanghai, China) were used.

The SH solution was prepared by dissolving 10 mg SH in 1000 mL water. 0.01% (m/v) Night Blue (NB), Victoria Blue B (VBB) and Victoria Blue 4R (VB4R) solution was prepared by diluting the stock solution with water. Buffer solution: pH 3.6–6.8, prepared by mixing 0.2 mol/L acetic acid and 0.2 mol/L solution acetate in a certain ratio and adjusting pH value with pH meter. All the other reagents were of analytical reagent grade and doubly distilled water was used throughout.

An appropriate amount of SH was place in 10 mL dry calibrated flask, appropriate amount of acetic acid–sodium acetate buffer solution were added as well as appropriate 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, $\Delta I = I - I_0$. One millilitre moisten eye drop, mioclear eye drops and sodium hyaluronate injection were finely transferred to a 100, 100 and 1000 mL standard flask, then were diluted to the mark with water. The solutions were analyzed as the general procedure.

2. Results and discussion

Fig. 1 shows the RRS spectra of (1) SH, (2) VBB, (3) NB, (4) VB4R, (5) SH-VB4B, (6) SH–NB and (7) SH–VBB solution. It can be seen, the scattering intensity of SH, VBB, NB and VB4R are very faint under the measurement conditions; the intensity of RRS increases obviously when SH coexist to form the SH-dye complex; the spectra of SH–VBB system is similar to that of SH–VB4R system, but different with that of SH–NB system. 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 285.5 nm for VBB–SH system, at 276.5 nm for VB4R–SH and at 411.5 nm for NB–SH, and each has two or three other scattering peaks of smaller intensity. The enhanced intensities (ΔI) of RRS for the ion-associate are in the following order: NB > VBB > VB4R.

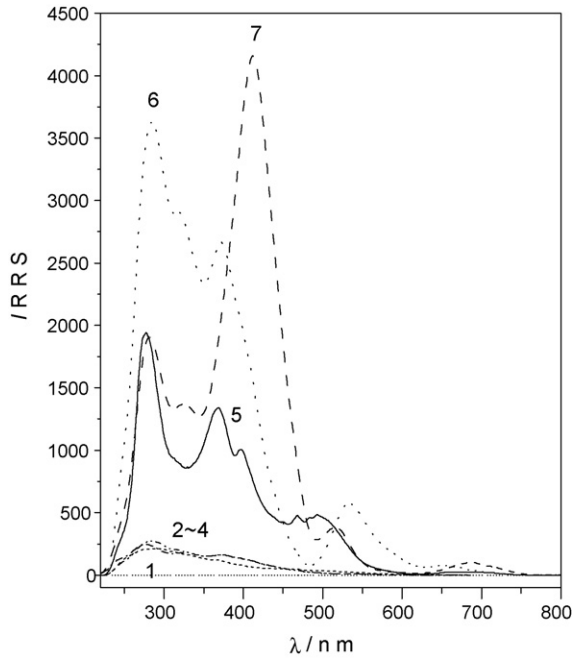

Fig. 1. RRS spectra of three system.

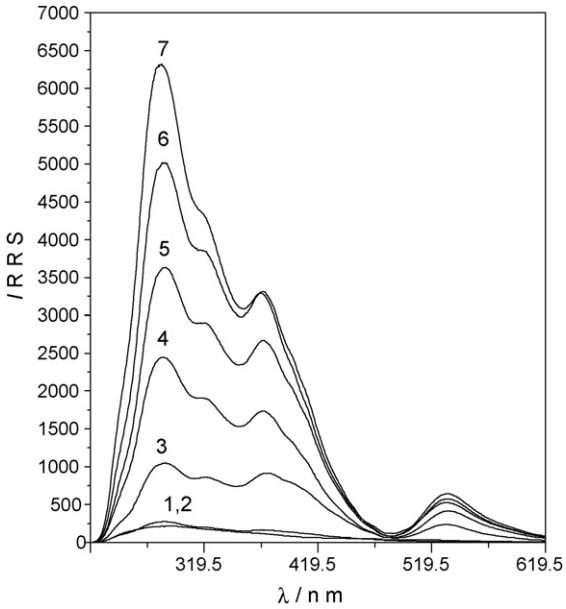

Fig. 2. RRS spectra (pH3.6) of SH-VBB.

Fig. 2 shows the RRS spectra of SH–VBB systems with a series SH concentrations. c_{SH} are 1, 2, 3, 4, and 5 μg/mL separately in 3,4,5,6, and 7 line, respectively. It tells us clearly the scattering intensity is directly proportional to the concentration of SH in a certain range.

The most suitable reaction medium is the acetic acid–sodium acetate buffer solution and the optimum pH ranges are 3.0–4.5 for VBB–SH system, 3.0–3.9 for NB–SH system and 4.9–6.8 for VB4R–SH system. The effect of dyes on the RRS' intensities was examined at various dyes concentrations, the amounts of SH and pH being kept constant. The optimum added amounts of dyes solution were 0.5–1.5 mL for VBB, 0.5–1.8 mL for NB and 0.5–3.5 mL for VB4R, the optimum concentrations were (0.99–2.96) $\times 10^{-5}$ mol/L for VBB, (0.87–3.12) $\times 10^{-5}$ mol/L for NB and (0.96–6.73) $\times 10^{-5}$ mol/L for VB4R, separately.

Under the optimum conditions, the formation time of all reaction products was 5 min and the RRS intensity remained constant for at least 40 min.

The common surfactant like OP and Triton X-100 will increase the I_0 and decrease the I_{RRS}, which cause ΔI became small. ΔI is reduced with the increasing of ion-strength.

The common metallic such as Mn^{2+}, K^+, Ca^{2+} and Mg^{2+} can be allowed at high concentrations in these systems, whereas ions, such as V (δ) and Bi^{3+} can be allowed only at low concentration levels. Organic compounds, including glucose, lactose, amino acids and inositol did not interfere at high concentration levels, but the concentration of starch and yRNA must be controlled.

According to the general procedures, the calibration relationship of the RRS intensity in dependence of the concentration of SH was obtained. As shown in Table 1, different dyes give different response, and the linear range for SH is different for different dyes. The method developed was applied to the determination of SH in samples. The results are shown in Table 2.

Table 1
Some parameters of calibration graphs for the determination of SH

Dye	Linear range (μg mL^{-1})	λ (nm)	Correlation coefficient ($n = 6$)	Regression equation (c, μg mL^{-1})	Limit of determination (3σ, ng mL^{-1})
VBB	0–5.0	$\lambda_{ex} = \lambda_{em} = 285.5$	0.9998	$\Delta I = 1296.3C - 486.2$	14.9
NB	0–1.5	$\lambda_{ex} = \lambda_{em} = 408.0$	0.9959	$\Delta I = 1656.4C - 345.0$	13.7
VB4R	0–3.0	$\lambda_{ex} = \lambda_{em} = 276.5$	0.9998	$\Delta I = 971.1C + 414.8$	22.1

Table 2
Determination results of SH in samples and recovery

Sample	c (RRS method) ($\mu g\ mL^{-1}$)	c (marked) ($\mu g\ mL^{-1}$)	W (Added) (μg)	W (Found)/ μg	R.S.D. (%)	Recovery (%)
Moisten eye drops	2.08×10^3	2×10^3	10.00	9.54	1.4	95.4
Mioclear eye drops	1.02×10^3	–	10.00	9.69	0.9	96.9
SH injections	1.01×10^4	1.00×10^4	10.00	10.09	3.1	100.9

3. Conclusion

SH can be determined in synthetic samples with satisfactory results by RRS. RRS method is simpler, faster and more sensitive than the official method. Hence, it is possible to adapt the proposed method for routine determination.

Acknowledgments

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