Study on Frequency Doubling Scattering and Second-Order Scattering Spectra of Heparin-Methylene Blue System

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Binding of heparin with methylene blue (MB) in pH 5.7 Britton-Robinson buffer can result in a significant enhancement of frequency doubling scattering (FDS) and second-order scattering (SOS). Their maximum scattering wavelengths (λ_{max}) appear at 350 nm for FDS and 700 nm for SOS, respectively. The optimum conditions of the reaction, the influencing factors and the relationship between the two scattering intensities and the concentration of heparin have been investigated. The new methods for the determination of trace amounts of heparin based on the FDS and SOS methods have been developed, which exhibit high sensitivities. The detection limits of heparin are 4.36 ng/mL for the FDS method and 3.55 ng/mL for the SOS method, respectively. Both of the methods have fairly good selectivity and were applied to the determination of heparin in sodium heparinate injection samples with satisfactory results. Moreover, the relative mechanisms have also been discussed.

Keywords frequency doubling scattering, second-order scattering, heparin, methylene blue

Introduction

Heparin is the sodium salt of heteropolysaccharides containing glucosamine-*N*-sulfate and uronic acid with a variable number of sulfate, carboxyl and acetyl residues. It exists in the blood excreted by hypertrophy cells of the body. It has antithrombotic, anticoagulant, immunoregulatory, antiphlogistic, antianaphylactic, *etc*. activities.[1] The biological method is commonly used for heparin determination.[2] Spectrophotometric,[3-6] capillary electrophoretic and chromatographic,[7-13] resonance Rayleigh scattering,[14] ion-selective electrode,[15] ion-channel sensor,[16] and electromechanical transducer[17] methods have also been reported.

Our experiment showed that in a Britton-Robinson buffer of pH 5.0—11.2, FDS and SOS of methylene blue (MB) or heparin are very weak. However, when MB reacts with heparin to form a heparin-MB complex by virtue of electrostatic and hydrophobic interaction forces, the FDS and SOS intensities of the solution are enhanced greatly. The maximum scattering peak for FDS is at 350 nm. The maximum scattering peak for SOS is at 700 nm. Furthermore, the enhancement of the FDS and SOS signals is directly proportional to the concentration of heparin in the range of 4.36×10^{-3}—2.4 μg/mL for the FDS method and 3.55×10^{-3}—2.0 μg/mL for the SOS method. It is shown that the methods have very high sensitivities, and the detection limit (3σ/s) of heparin is 4.36 ng/mL for the FDS method and 3.55 ng/mL for the SOS method. The methods with simple operation and fairly good selectivity can be directly applied to the determination of heparin in sodium heparinate injection samples. Moreover, the primary reaction mechanism and the reasons of enhancement for FDS and SOS have been discussed.

Experimental

Reagents

Heparin stock solution (1.00 mg/mL) was prepared by dissolving 0.1000 g of sodium heparinate (160 IU/mg, Shanghai Chemical Reagent Plant) in water and diluting to the mark in a 100-mL calibrated flask. The working solution was further diluted with water to 10.0 μg/mL. Methylene blue (Beijing Chemical Reagent Company) solution (0.01%) was prepared by dissolving 0.1000 g of MB in water and diluting with water to the mark in a 1000-mL calibrated flask. Britton-Robinson buffer (pH 5.7) was prepared by mixing 100 mL of the mixed acid (composed of 0.04 mol/L H_3PO_4, HAc and H_3BO_3) with 40 mL of 0.20 mol/L NaOH. All of the other reagents were of analytical reagent grade, and were used without further purification. Doubly distilled water was used throughout.

Apparatus

A Shimadzu RF-540 spectrofluorometer (Tyoto, Japan) was used for recording and measuring the FDS and SOS spectra and the intensities at a given wavelength using a 1-cm path in length. The slit (EX/EM) was 10.0 nm/10.0 nm. A Hitachi U-3400 spectrophotometer (Tokyo, Japan) was used for recording the absorption spectra.

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General procedure

To 20 μg of sodium heparinate in a 25-mL calibrated flask, 1.0 mL of Britton-Robinson buffer (pH 5.7) was added and the solution was diluted to about 17 mL with water. Subsequently, 5.0 mL of MB solution (0.01%) was added, and this solution was then diluted to the mark with water and mixed thoroughly. After 20 min, the FDS intensity, I_{FDS}, and the SOS intensity, I_{SOS}, of the system produced in different incident light were recorded at $\lambda_{em} = 1/2\lambda_{ex}$ and $\lambda_{em} = 2\lambda_{ex}$, separately. The FDS and SOS spectra were obtained by plotting the different wavelength against I_{FDS} and I_{SOS}. Then I_{FDS} and I_{SOS} for the heparin-MB complex and the scattering intensities, I^0_{FDS} and I^0_{SOS}, for the reagent blank at their own maximum FDS and SOS wavelengths were measured, $\Delta I_{FDS} = I_{FDS} - I^0_{FDS}$ and $\Delta I_{SOS} = I_{SOS} - I^0_{SOS}$.

Results and discussion

FDS *and* SOS *spectra*

When the incident light (λ_{ex}) with longer wavelength (500—1000 nm) gets across the solution, it can be seen that FDS appears at $\lambda_{em} = 1/2\lambda_{ex}$. When the incident light with shorter wavelength (250—500 nm) gets across the solution, SOS appears at $\lambda_{em} = 2\lambda_{ex}$. The FDS scanning spectra were obtained in the emission wavelength region from 250 nm to 500 nm with $\Delta\lambda_{ex} = 10$ nm from 500 nm to 1000 nm. Similarly, the SOS scanning spectra were obtained in the emission wavelength region from 500 nm to 1000 nm with $\Delta\lambda_{ex} = 10$ nm from 250 nm to 500 nm. The results are shown in Figs. 1 and 2. It can be seen that the FDS and SOS intensities produced at different incident wavelength are different.

When the FDS and SOS intensities produced at different incident wavelength were measured and plotted against the correspondent wavelength, the FDS and SOS spectra were obtained, separately. The results show that: (1) The FDS and SOS intensities of the MB and heparin solutions alone are all very weak under optimum conditions. (2) In coexistence of MB and trace amounts of heparin, heparin-MB complex is formed and its FDS and SOS intensities are greatly enhanced. The maximum peak of FDS is at 350 nm, and a smaller peak of FDS appears at 470 nm. The maximum peak of SOS is at 700 nm, and three smaller peaks of SOS appear at 540, 900 and 940 nm. (3) The FDS spectrum is symmetrical with the SOS spectrum. Their maximum scattering wavelengths have correspondence of $\lambda_{FDS} = 1/2\lambda_{SOS}$. The relative intensity of SOS is slightly higher than that of FDS. Both of the scatterings can be applied to the quantitative analysis of trace amounts of heparin. Particularly because FDS is based on the long incident wavelength ($\lambda_{ex} = 2\lambda_{FDS}$), its incident energy is lower than that of SOS. Therefore, the FDS method is conducive to the systems that are not stable and their photochemical reactions easily take place.

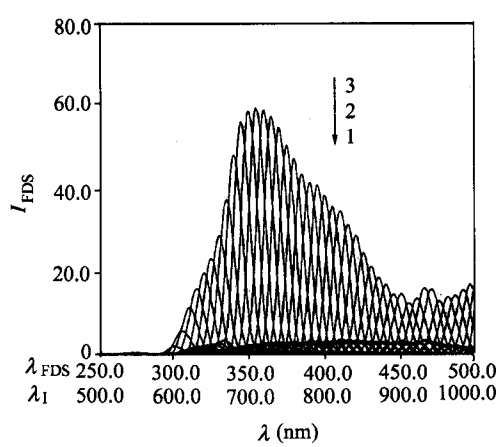

Fig. 1 Frequency doubling scattering (FDS) scanning spectra of heparin-MB system. λ_I represents the wavelength of the incident light. (1) heparin 20.0 μg/25 mL, pH 5.7; (2) MB 6.25×10^{-5} mol/L, pH 5.7; (3) heparin-MB: heparin 20.0 μg/25 mL, MB 6.25×10^{-5} mol/L, pH 5.7.

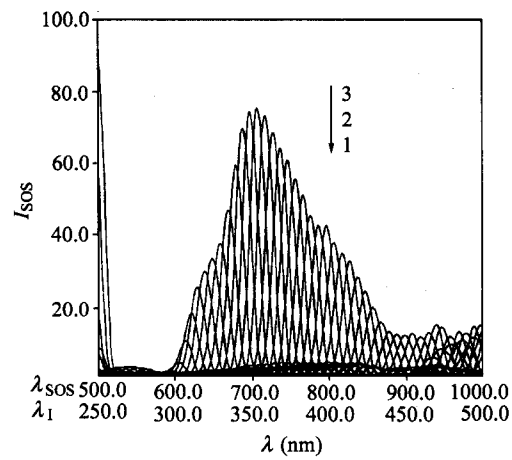

Fig. 2 Second-order scattering (SOS) scanning spectra of heparin-MB system. λ_I represents the wavelength of the incident light. (1) heparin 20.0 μg/25 mL, pH 5.7; (2) MB 6.25×10^{-5} mol/L, pH 5.7; (3) heparin-MB: heparin 20.0 μg/25 mL, MB 6.25×10^{-5} mol/L, pH 5.7.

Optimum conditions for the reactions

Effect of the acidity

Fig. 3 shows the dependence of the two scattering intensities of the system on the pH of the solution. It can be seen that the optimum pH ranges of FDS and SOS are the same and wide as pH from 5.0 to 11.2. If the acidity is higher than the optimum ranges above, the relative scattering intensities (ΔI_{FDS} and ΔI_{SOS}) of the system decrease.

Effect of the MB *concentration*

The added optimum amounts of the MB solution (0.01%) were found experimentally as follows: 4.5—6.0 mL of MB for two kinds of scattering. That is, the optimized concentration range of MB is 5.63×10^{-5} mol/L to 7.50×10^{-5} mol/L. When the MB concentration is lower, the intensities of

FDS and SOS are lower. When the MB concentration is higher than the optimum concentration range, the FDS and SOS intensities of reagent blanks have little change, while those of the heparin-MB complex decrease. Under optimum conditions, the intensities of FDS and SOS for the system can be stable for over 2 h.

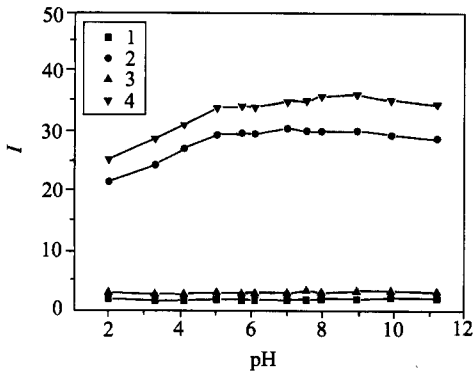

Fig. 3 Effect of pH on the FDS and SOS intensities. FDS: (1) reagent blank; (2) heparin-MB system, heparin 10.0 μg/25 mL, MB 6.25×10^{-5} mol/L. SOS: (3) reagent blank; (4) heparin-MB system, heparin 10.0 μg/25 mL, MB 6.25×10^{-5} mol/L.

Effects of ionic strength

The effects of ionic strength on MB and the heparin-MB complex have been tested. They were obtained by keeping heparin and MB concentrations, the pH constant and changing the NaCl concentration, and the results showed that ΔI_{FDS} and ΔI_{SOS} decreased slowly with increasing ionic strength. It revealed that an increase in salt concentration caused the combination of heparin with MB to decrease. This effect may be explained as a competition between Cl^- anion and heparin for the same binding sites on dye species.[18] The higher the concentration of the anion was, the fewer the binding numbers of heparin with MB. Therefore, large amounts of Cl^- anion shielded the combination of the cationic dye with heparin, causing ΔI_{FDS} and ΔI_{SOS} to decrease. However, when the NaCl concentration was lower than 2.73×10^{-2} mol/L, the interaction of MB with heparin almost was not affected by ionic strength of the medium.

Relation between the concentration of heparin and the FDS *and* SOS *intensities*

Under optimum conditions, the relative scattering intensities ΔI_{FDS} and ΔI_{SOS} of the heparin-MB complex were measured at their maximum scattering wavelengths, 350 and 700 nm. Calibration graphs of ΔI_{FDS} and ΔI_{SOS} against concentration of heparin were constructed. The results showed that the relative scattering intensity was directly proportional to the heparin concentration of 4.36×10^{-3}—2.4 μg/mL for the FDS method and 3.55×10^{-3}—2.0 μg/mL for the SOS method. If the concentration of heparin is larger than the linear range, the linearity begins to break down right. The two methods have high sensitivity. The detection limit for heparin, calculated as the concentration giving a signal tripling the background noise, is 4.36 ng/mL for the FDS method and 3.55 ng/mL for the SOS method. The results are listed in Table 1.

Reaction mechanism and the enhancement reason of FDS *and* SOS

Binding sites of heparin with MB

The charge distribution of cation MB has been calculated using the AM1 method of quantum chemistry. The result is shown in Fig. 4. It can be seen that the biggest positive charge density is 0.708 at S atom. Therefore, the binding location of MB with heparin is at S atom of MB.

Heparin has three *O*-sulfate groups, two *N*-sulfate groups and two carboxyl groups per tetrasaccharide unit. The

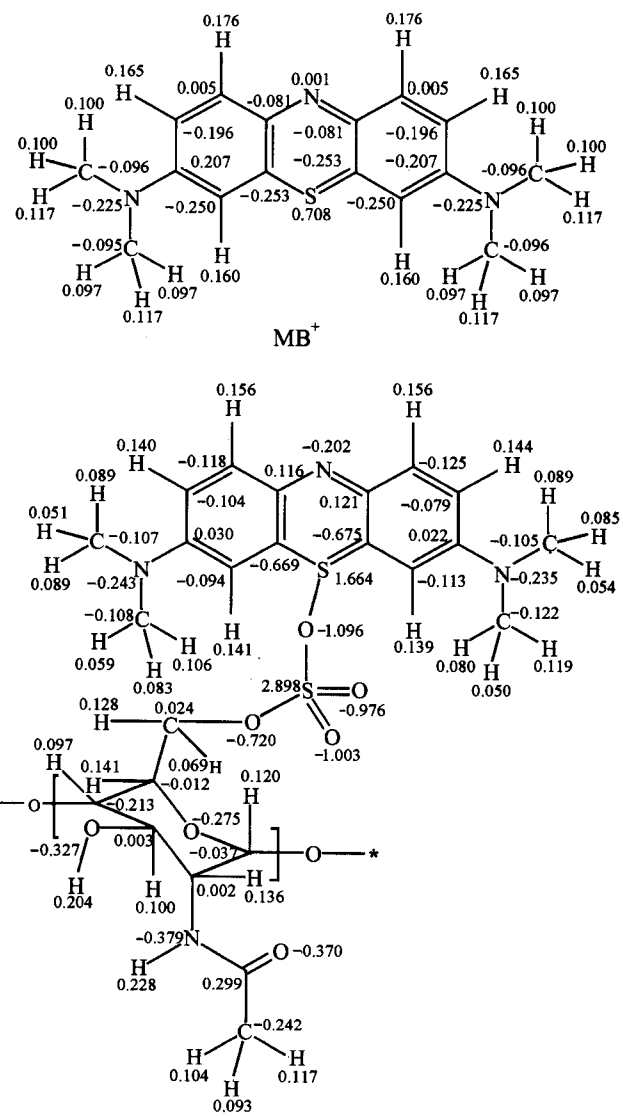

Fig. 4 Charge distribution of MB^+ and heparin-MB at ground state.

Table 1 Linear ranges and correlation coefficients of the calibration graphs, and the detection limits for heparin

Method	Determination wavelength (nm)	Linear range (μg/mL)	Linear regression equation (C, μg/25 mL)	r^a	Detection limit ($n=9$) (3σ, ng/mL)
FDS	350	4.36×10^{-3}—2.4	$\Delta I = 2.752C + 3.432$	0.9998	4.36
SOS	700	3.55×10^{-3}—2.0	$\Delta I = 3.581C + 4.487$	0.9996	3.55

[a] Correlation coefficient ($n=7$).

O-sulfate and *N*-sulfate groups completely dissociate, even below pH 3.0. The carboxyl group is weakly acidic, and the pK_a of *D*-glucuronic acid in heparin is 3.6. The carboxyl groups can not dissociate completely until above pH 5.0.[19] From the relationship of the FDS and SOS intensities of the heparin-MB complex with pH (Fig. 3), it can been seen that the FDS and SOS intensities are very weak when the acidity is higher. When pH is above 5.0, the FDS and SOS intensities reach the maximum value and no longer increase with an increase in the pH value. Therefore, it can be said that the binding sites of heparin with MB are *O*-sulfate groups, *N*-sulfate groups and carboxyl groups.

*Formation of the heparin-*MB *charge transfer complex*

Under the experimental conditions, the absorption spectrum of 5.63×10^{-5}—7.50×10^{-5} mol/L MB solution in visible has two characteristic absorption peaks at 610 and 666 nm. When MB combines with heparin, the two peaks decrease with the increase of heparin concentration, while a new absorption peak appears at 550 nm. This peak illustrates the formation of a new substance, heparin-MB complex, which will result in the change of the energy needed in the energy level transition of the electron in the MB molecule. This energy change mainly results from the change of π charge distribution occurred in the MB molecule. The charge distribution and relative parameters of the MB molecule before and after combination with heparin with the AM1 method of quantum chemistry were calculated. The results showed that: (1) When the cation MB (MB^+) reacts with heparin to form the complex, the negative charge of heparin anion is partly transferred to MB^+. The net charge of MB^+ changes from +1.00 to +0.68 at ground state. This result illustrated that heparin reacted with MB^+ to form a charge transfer complex structure (Fig. 4). (2) The combination of MB with heparin results in the decrease of the π electron energy of MB and the increase of transition energy ΔE to 2.304 eV, whose corresponding λ_{max} is at 538 nm, corresponding with the experimental value (550 nm) closely. Therefore, it proved the presumption that the combination product should be heparin-MB complex with λ_{max} at 550 nm. It is considered that owing to the combination of heparin with MB, the π charge distribution of MB molecule changed and the charge transfer complex of heparin with MB was formed.

Relation between resonance Rayleigh scattering (RRS) *and the two kinds of scattering* (FDS and SOS)

Under the experimental conditions, binding of heparin with MB can result in a significant enhancement of RRS spectrum recorded with synchronous scanning at $\lambda_{ex} = \lambda_{em}$ (*i.e.*, $\Delta\lambda = 0$ nm), and the maximum RRS peak appears at 540 nm. The next peak is at 350 nm. Moreover, there are five smaller RRS peaks at 270, 450, 470, 700 and 940 nm (Fig. 5). Therefore, when the RRS peak wavelengths, 270, 350, 450 and 470 nm, are chosen as the incident light (*i.e.*, the exciting light), the SOS peaks can be observed in the wavelength range of the spectrofluorometor, and the corresponding peaks are at 540, 700, 900 and 940 nm. When the RRS peak wavelengths, 700 and 940 nm, are taken as the incident light, the FDS peaks at half incident wavelengths, 350 and 470 nm, can be observed. Therefore, it can be concluded that FDS and SOS are a series of nonlinear scattering phenomena produced by RRS and it is called as "resonance nonlinear scattering".[20]

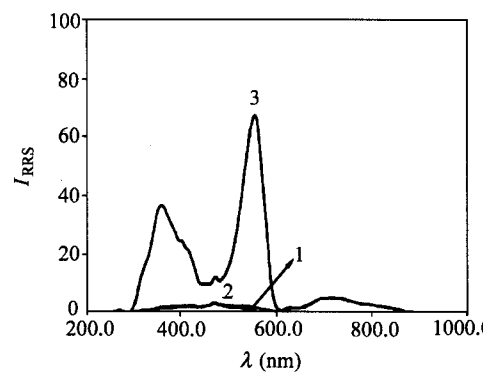

Fig. 5 Resonance Rayleigh scattering spectra of heparin-MB system. (1) MB 6.25×10^{-5} mol/L, pH 5.7; (2) heparin 20.0 μg/25 mL, pH 5.7; (3) heparin-MB: heparin 20 μg/25 mL, MB 6.25×10^{-5} mol/L, pH 5.7.

The experimental results show that many ion-association systems with strong RRS can produce strong FDS and SOS, illustrating that FDS and SOS are closely related to RRS. The reasons are as follows: (1) FDS and SOS synchronously appear and are changed along with the appearance and change of RRS. There exists an obvious reliable relationship among them.[20-23] (2) In the action of a light wave, a substance produces an induced electric polarization. When the light intensity is weak, this effect is linear. Because the intensity of single Rayleigh scattering is weak, in this case, FDS can not be observed. However, when Rayleigh scattering is located near the absorption band, RRS will be produced and its intensity can be enhanced by several orders of magnitude.[24] As the light intensity is enhanced sharply, the item that is proportional to the upwards of the quadratic component of the electric field may appear in the polarizability, namely, non-

linear polarization appears,[25] which provides a possibility to produce FDS. Furthermore, the optical double frequency phenomenon of resonance Raman scattering in which its mechanism is similar to that of RRS was also observed.[26] This also holds out this issue from other point of view. (3) Because the MB molecule has a big conjugate system, the combination of MB with heparin forms the charge transfer structure. All these encourage the form of a big hyperpolarizability, and the increase of the hyperpolarizability is the important condition that the nonlinear scattering such as FDS is produced.[27] However, sometimes the nonlinear scattering of the systems that do not produce intensive RRS can not be observed.

Therefore, it is concluded that FDS and SOS are produced by RRS. Owing to the formation of an ion-association complex, intensive RRS is produced, which results in the appearance of strong FDS and SOS. The three kinds of scattering are dependent on each other and change synchronously. It can be presumed that any system which can produce strong RRS can also be observed the obvious FDS and SOS.

Effects of foreign substances and analytical application

Effects of foreign substances

The effects of foreign substances on the reaction of 20 μg of heparin with MB have been tested. The results showed that the effects of foreign substances on the FDS intensity were the same as that on the SOS intensity. The resultsabout the effects of foreign substances on the FDS intensity are listed in Table 2. The tolerance limit was taken as the maximum concentration of the foreign substances that caused approximately a ± 5% error in the determination. It could be seen that many kinds of substances did not interfere at 10-1000-fold concentration of heparin. However, Fe(III), Al(III), HSA and BSA have lower tolerances. The tolerance limits of Fe (III) and Al(III) can reach 50 and 100 μg by adding 3.0-mL of 2% EDTA. Therefore, the method can be applied to the determination of heparin in heparin sodium injection samples directly which do not contain interfering substances such as HSA and BSA.

Determination of heparin in sodium heparinate injection samples

A 1.0-mL portion of sodium heparinate injection was pipetted into a 100-mL calibrated flask and was diluted to the mark with water. A 2.0-mL portion of this solution of heparin was pipetted into a 100-mL volumetric flask, and then diluted to the mark with water. A 2.0 mL of this solution was pipetted into a 25-mL calibrated flask; the following procedure is the same as the general procedure. The results are listed in Table 3. The determination results are satisfactory.

Table 2 Effects of foreign substances on the determination of heparin (FDS method)[a]

Foreign substance	Amount tolerated (μg)	Foreign substance	Amount tolerated (μg)
Glucose	10000	KCl	40000
Maltose	10000	NaCl	40000
Starch	5000	$NaHCO_3$	40000
Glycin	1000	$NaNO_3$	40000
L-Tryptophan	1000	Na_2SO_4	40000
L-Histidine	300	NH_4Cl	20000
Pepsin	30	$MgSO_4$	10000
HSA[b]	10	$CaCl_2$	4000
BSA[c]	10	$Ba(NO_3)_2$	3000
BTC[d]	150	Cu(II)	300
TPB[e]	100	Fe(III)	10
CDBAC[f]	10	Al(III)	2
CPB[g]	5	EDTA[i]	80000
SDS[h]	100	Fe(III)[j]	50
Triton X-100	1000	Al(III)[j]	100

[a] [heparin] = 20 μg/25mL. [b] Human serum albumin. [c] Bovine serum albumin. [d] Benzyl trimethylamine chloride. [e] Tetradecane pyridinium bromide. [f] Cetyldimethyl benzylammonium chloride. [g] Cetylpyridinium bromide. [h] Sodium dodecyl sulfonate. [i] Disodium ethylene diamine tetraacetate. [j] In the presence of 3.0 mL of 2% EDTA.

Table 3 Results for the determination of heparin in sodium heparinate injections

Sample number	Heparin specified (IU/2 mL)	SOS method (n = 5)		FDS method (n = 5)	
		Average (IU/2 mL)	RSD (%)	Average (IU/2 mL)	RSD (%)
980815[a]	12500	12480	3.75	12475	3.49
991218[b]	12500	12232	4.30	12208	3.98
980723-2[b]	12500	12570	1.78	12491	2.27

[a] Shanghai Biochemical Pharmaceutical Factory of China. [b] Changzhou Qianhong Biochemical Pharmaceutical Co. Ltd. of China.

References

1 Casu, B. In *Heparin and Related Polysaccharides, Structure and Activities*, Eds.: Ofosu, F. A.; Danishefsky, I.; Hirsh, J., The New York Academy of Sciences, New York, **1989**, p. 556.

2 *Pharmacopoeia of the People's Republic of China (Second part)*, People Wealth Press, Beijing, **1985**, Appendix 98 (in Chinese).

3 Johnson, E. A.; Mulloy, B. *J. Pharm. Pharmacol.* **1976**, *28*, 836.

4 Band, P.; Lukton, A. *Anal. Biochem.* **1982**, *120*, 19.

5 Powell, R. K.; Hendley, J. O.; Pohl, K. E.; Friedberg, A.; Valk, W. A. *Anal. Biochem.* **1982**, *119*, 31.

6 Nemcova, I.; Rychlovsky, P.; Havelcova, M.; Brabcova, M.

Anal. Chim. Acta **1999**, *401*, 223.
7 Zhou, X. M.; Liu, J. W.; Zhang, M.; Chen, S. J. *Talanta* **1998**, *46*, 757.
8 Toida, T.; Shima, M.; Azumaya, S.; Maruyama, T.; Toyoda, H.; Imanari, T.; Linhardt, R. J. *J. Chromatogr., A* **1997**, *787*, 266.
9 Takagaki, K.; Munakata, H.; Nakamura, T.; Endo, M. *Japan Analyst* **1985**, *44*, 789 (in Japanese).
10 Malsch, R.; Harenberg, J.; Piazolo, C.; Huhle, G.; Heene, D. L. *J. Chromatogr.* **1996**, *685*, 223.
11 Toyoda, H.; Nagashima, T.; Hirata, R.; Toida, T.; Imanari, T. *J. Chromatogr., B* **1997**, *704*, 19.
12 Mao, P.; Huang, X. L; Li, C. Z.; Chen, F.; Guan, M. M.; Cai, X. Y.; Huang, R. Y.; Chen, Y. H. *Chin. J. Med. Lab. Sci.* **1995**, *18*, 358 (in Chinese).
13 Karamanos, N. K.; Vanky, P.; Tzanakis, G. N.; Tsegenidis, T.; Hjerpe, A. *J. Chromatogr., A* **1997**, *765*, 169.
14 Liu, S. P.; Luo, H. Q.; Li, N. B.; Liu, Z. F.; Zheng, W. X. *Anal. Chem.* **2001**, *76*, 3907.
15 Ma, S. C.; Yang, V. C.; Meyerhoff, M. E. *Anal. Chem.* **1992**, *64*, 694.
16 Gadzekpo, V. P. Y.; Bühlmann, P.; Xiao, K. P.; Aoki, H.; Umezawa, Y. *Anal. Chim. Acta* **2000**, *411*, 163.
17 Cheng, T. J.; Lin, T. M.; Wu, T. H.; Chang, H. C. *Anal. Chim. Acta* **2001**, *432*, 101.
18 Jiao, Q. C.; Liu, Q. *Anal. Lett.* **1998**, *31*, 1311.
19 Katayama, T.; Takai, E. I.; Kariyama, R.; Kanemasa, Y. *Anal. Biochem.* **1978**, *88*, 382.
20 Liu, S. P. *Analytical Chemistry: Achievements and Challenges*, Eds.: Huang, B. L.; Zhang, Z. J., The Press of Southwest China Normal University, Chongqing, **2000**, p. 180 (in Chinese).
21 Liu, S. P.; Liu, Z. F. *Chem. J. Chin. Univ.* **1996**, *17*, 887 (in Chinese).
22 Liu, S. P.; Yang, R.; Liu, Z. F. *Chin. J. Anal. Chem.* **1998**, *26*, 1432 (in Chinese).
23 Liu, S. P. *Advances in Analytical Chemistry*, The Science and Technique Press of Shanxi, Taiyuan, **1997**, p. 34 (in Chinese).
24 Pasternack, R. F.; Collings, P. J. *Science* **1995**, *269*, 935.
25 *Chinese Macropaedia Physics (I)*, Edition of Editorial Board of Chinese Macropaedia, Chinese Macropaedia Press, Beijing, **1987**, p. 334 (in Chinese).
26 Edition of Editorial Board of Chinese Macropaedia *Chinese Macrpaedia Chemistry (II)*, Chinese Macropaedia Press, Beijing, **1989**, p. 643 (in Chinese).
27 Yamaguchi, K.; Nakano, M.; Fueno, T. *Chemistry* **1987**, *42*, 757 (in Japanese).

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