Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(6):1695-1698

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Spectrophotometric method for the determination of sodium hyaluronate with basic bisphenylnaphthylmethane dyes

Lianhui Chen[1], Shaopu Liu[2], Hongqun Luo[2] and Xiaoli Hu[2]

[1]Department of Chemistry, North-Sichuan Medical College, Nanchong
[2]School of Chemistry and Chemical Engineering, Southwest-China University, Chongqing

---

ABSTRACT

In weak acidic medium, bisphenylnaphthylmethane dyes, such as night blue (NB),Victoria blue B (VBB) and Victoria blue 4R (VB4R) , react rapidly with sodium hyaluronate to form complexes, which causes the change of absorption spectra, the decrease of absorbance near 612nm(NB),616nm(VBB),585nm(VB4R), separately, and ΔA(=A-A_0) values are proportional to the concentration of sodium hyaluronate, Beer's law is obeyed in the range between 0.5~2.0 mg/L(NB) , 0.1~2.5mg/L(VBB) and 0.1~0.6 mg/L(VB4R) for sodium hyaluronate. The molar absorptivities of different systems are between 1.51×10^8~ 5.52×10^8 (the molecular weight of SH is 5×10^5). Based on these, we developed a new spectrophotometric method for the determination of sodium hyaluronate. The method is simple, rapid, repeatable, and it has good selectivity. It has been used for the determination of total amounts of sodium hyaluronate in samples with satisfactory results.

Keywords: Sodium Hyaluronate, Spectrophotometry, Bisphenylnaphthylmethane dyes

---

INTRODUCTION

Hyaluronic acid(HA) is a kind of polar-carbonhydrate, with different lengthes of chain, the molecular weight is between 2×10^5 and 7×10^6 g/moL. For its strong viscosity and elasticity, HA is an important content in many kinds of tissues. Because its poor water-soluble, we changed it as Sodium Hyaluranate (SH). Now, SH has been used in many fields including cosmetics[1,2] , clinical treatment, diagnosis[3-7]（such as ophthalmology operation, remedy of joint disease, reparation tissue, prevention coherence after operations and so on）, so its quantitive analysis is very important. Now, it is usually determined by biochemical method[8] , immune method[9]（such as radioactive immunization(RiA), enzyme league immunization analysis(ELISA) and immunity fluorescent method） and polarigraphy method[10].

We find that in weak acidic medium, bisphenylnaphthylmethane dyes such as night blue (NB), Victoria blue B (VBB) and Victoria blue 4R (VB4R), react rapidly with SH to form complexes, which causes the change of absorption spectra, the decrease of absorbance near 612nm(NB),616nm(VBB),585nm(VB4R), separately, and ΔA(=A-A_0) values are proportional to the concentration of SH, Beer's law is obeyed in the range between 0.5~2.0 mg/L(NB) , 0.1~2.5mg/L(VBB) and 0.1~0.6 mg/L(VB4R) for SH. The molar absorptivities of different systems are between 1.51×10^8~ 5.52×10^8 (while the molar absorptivity of SH is 5×10^5). Based on these, we developed a new spectrophotometric method for the determination of SH. In this work, we mainly study its absorption spectrum, optimum reaction conditions, properties of analytical chemistry and its analytical application, The method is simple, rapid, repeatable, and it has good selectivity. It has been used for the determination of total amounts of SH in samples with satisfactory results.

EXPERIMENTAL SECTION

Reagents

Night Blue(NB, Shanghai Medical Industrial Plant, China), Victoria blue B (VBB, Shanghai Medical Industrial Plant, China) and Victoria blue 4R (VB4R, CHROMA): 0.01%, SodiumHyaluranate (SH,SIGMA Corporation)standard solution: the solution of SH were kept in refrigerator between 1 and 4 ℃. The working solution is prepared by diluting the stock solution to 10 μg/ml; Buffer solutions (pH1.8 ～ 6.0) were prepared by mixing 0.2 mol/L acetic acid and 0.2 mol/L sodium acetate according to certain ratioes and the pH values were adjusted by pH meter.

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

Apparatus

A UV-8500 spectrophotometer (Shanghai, China), a 721-A spectrophotometer (Chongqing, China), and a PHS-3C pH meter (Shanghai, China) were used.

Procedure

Into a 10mL of calibrated flask were added appropriate amounts of SH solution, 2.00 mL of pH3.6 buffer solution, 2.0mL of NB (or 1.5mL of VBB, or 3.0mL of VB4R) solution. Then the resulting solution was mixed and diluted to the mark with water and mixed completely again. The absorption spectra were recorded with UV-8500 spectrophotometer against the reagent blank. The absorbance (ΔA) were measured at the same time.

RESULTS AND DISCUSSION

The absorption spectra

Among the NB-SH, VB-SH and VB4R-SH systems, the VB4R-SH system has the most strong absorption. Fig.1 and fig.2 show the absorption spectra of VB4R and thebinding product of VB4R-SH. It can be seen that VB4R has strong absorption, when SH is added into VB4R solution, it can cause a significant fading of absorption, near 585nm; besides, against the reagent blank, new absorption peaks at 528nm and 676 nm appears, but their sensitivities are relative lower. NB and VBB have similar interaction with SH, but their sensitivities are much lower than that of VB4R-SH.

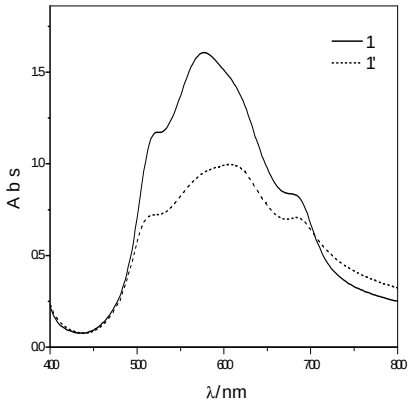

fig.1 Absorption spectra
1. Victoria blue 4R, 1'.Victoria blue 4R-Sodium Hyaluranate system (against water)
c_{Dye}=0.003%, c_{SH}=0.5mg/L,pH2.1

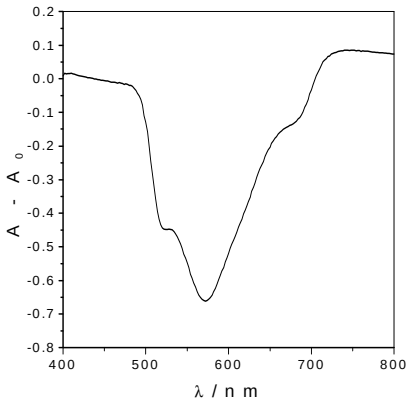

fig.2 Absorption spectra of Victoria blue 4R-Sodium Hyaluranate system (against the reagent blank)
c_{VB4R}=0.003%, c_{SH}=0.5mg/L, pH2.1

Optimum reaction conditions

The effect of pH on the absorption is shown in Fig.3. We can see that the best reaction medium is acetic acid-sodium acetate buffer solution and their optimum range of acidity are pH2.8～6.0 for NB-SH system, 2.6~4.0 for VBB-SH system, and 1.8~3.0 for VB4R-SH system separately. If the acidity is lower or higher than those pH ranges, ΔA will decrease. So we use 1.0mL of pH3.6 acetic acid-sodium acetate buffer solution in NB-SH system, 2.0mL of pH3.6 buffer solution in VBB-SH system and 2.0mL of pH2.1 buffer solution in VB4R-SH system.

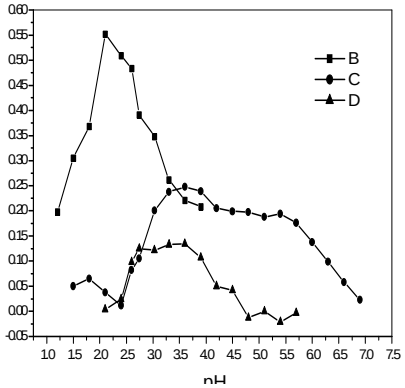

Fig.3 Effect of pH
B.VB4R, C.NB, D.VBB; c_{Dye}=0.0015%, c_{SH}=0.5 mg/L
(against reagent blank)

The effect of the concentrations of dyes on ΔA (=A-A_0) were investigated. At first, ΔA increases with the increase of the concentration of all kinds of dyes. It shows that the maximum concentration ranges are 1.0 ~ 4.0mL for NB, 0.5 ~ 2.5mL for VBB, 1.0 ~ 5.0mL for VB4R . When the concentration is higher or lower than these ranges, the absorbance will decrease. Therefore, in the experiment, 2.0ml of 0.1% NB, 1.5ml of 0.1% VBB and 3.0ml of 0.1% VB4R were added.

Under room temperature, the reaction can complete in 5 min. The absorbance become the maximum and can keep stable at least for 2h.

The effect of ionic strength adjusted by NaCl on the ΔA was investigated. The results show that the ΔA decreases with the increase of ionic strength. The reason may be that the metal cation and inorganic anion cause a competive reaction with SH large cation with the anionic chelate of dye-SH. So we control the ionic strength smaller than 0.03 g/L.

Calibration graphs

Calibration graphs of three systems were constructed with ΔA versus the concertration of SH under the optimum conditions. The results are listed in Table 1.

Table 1 Some parameters of standard curves

Dye	λ (nm)	Linear range (mg/L)	Correlation coefficient(n=6)	Regression equation (c/ mg/L)	molar absorptivity (L/mol/cm)
VBB	616	0~2.5	0.9991	A=-0.305c+0.00579	1.51×10^8
VB4R	585	0~0.6	0.9982	A=-1.092c+0.00579	5.52×10^8
NB	612	0.5~2.0	0.9996	A=-0.492c-0.00243	2.48×10^8

Effects of coexisting substances

The interference of coexisting substances such as metal ions, amino acids, EDTA and D(+)-glucose were tested in VB4R-SH system. When the concentration of SH is 15.0 μg/10mL and the relation error is within ±5%, the foreign substances with the following times did not interfere with the determination::. D.L-Asp（1800）、L-Try（3000）、D.L-Thr（1200）、D.L-Cys（2400）、L-Ile（1000）、L-Tyr（1000）、urea（1500）、glucose（1500）、maltose（1000）、starch（150）、α-starch enzyme（2000）、pepsin（2400）、cellulose enzyme（1200）、yRNA(1000)、Ca^{2+}(600)、KCl(600)、Mg^{2+}(600)、Al（Ⅲ）(780)、V(V)(30)、Mo(Ⅵ)(50)、Mn（Ⅱ） (1100)、Bi（Ⅲ） (1000)、$NaNO_3$(1000)、$Na_2S_2O_3$(200)、NaBr(3000)、Na_3MoO_4(20)、Ni^{2+}(1400)、$Si_2O_3^{2-}$(800)。Effects of coexisting foreign substances on VBB-SH and NB-SH system are simiar with VB4R-Shsystem. We can see that most of the substance can be tolerated except V(V) and Na_3MoO_4. Therefore, the method has fairly good selectivity.

Determination of total SH in samples

Moister eye drops (RUNSHU), moiclear eye drops (RUNJIE) and sodium hyaluronate injection (SHIPEITE) from Shandong Freda company were measured. 1.0 mL of moister eye drops and moiclear eye drops were diluted to 100.00 mL and 1.0mL of sodium hyaluronate injection was diluted to 1000.00 mL. Then according to the experimental procedure, the concentration of SH were determined by VB4R-SH method. The recovery was tested by

the standard addition method and the results are shown in Table 2.

Table 2 Results for the determination of sodium hyaluronate in SH samples

Sample	Found (present method) mg•L^{-1}	RSD (n=5,%)	Labeled mg•L^{-1}	Added μg	Found μg	Recovery %
RUNSHU Moisten eye drops	1.98×10^3	6.3	2.00×10^3	10.00	9.75,9.78, 9.99,9.86,9.87	98.5
RUNJIE Mioclear eye drops	1.01×10^3	5.9	—	10.00	10.10,10.08, 10.05,9.93,10.09	100.5
SHIPEITE SH injections	9.98×10^5	7.6	1.00×10^6	10.00	10.11,10.06, 10.21,10.28,10.05	101.4

Acknowledgments
This project is supported by Scientific Research Fund of Sichuan Provincial Education Department (No. 12ZA056) and Scientific Research Fund of North Sichuan Medical College (No. CBY11-A-ZP13).

REFERENCES

[1] Du Pingzhong. *Chinese Journal of Biochemical Pharmaceutics*, **1998**, 19（5）：278-279
[2] Lu Nianci, Tan Tianwei. *Journal of Functional Polymers*, **2001**, 14（3）：370-376
[3] Karen L G, Paul B. *Drugs*, **1994**, 47(3): 536-566
[4] Balazs E A, Miller D, Stegmann R. Viscosurgery and the use of Na-hyaluronate in introcular lens implantation[R]. Cannes: The international congress and fist film festival in intraocular implantion, **1979**, 5:56-59
[5] Onok, Nakahara H, Yonenobn K, et al. *Jpn Pharmacol Ther*, **1993**, 21(Suppl.2): 333-354
[6] Clark R A F. *J Am Acad Dermat*, **1985**,13: 701-725
[7] Yang Xiaohong, Ling Peixue, Wang Fengshan. *Chinese Journal of Biochemical Pharmaceutics*, **1998**, 19（4）：205-208
[8] Zhou Rongqing, Guo Siyuan, Li Lin，Hu Songqing. *Journal of South China University of Technology*(Natural Science Edition), **2001**, 29（11）：55-58
[9] Li Jinglan, Zhao Chunping, Lin Zhiku, Xi Dongyan, Cao Jinli. *Clinical Medicine of China*, **2002**, 18（2）：126-127
[10] Sun Wei, Gao Ruifang, Ding Yaqin, Jiao Kui. *Chinese Journal of Analytical Chemistry*, **2006**, 34(9): 1265-1268