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Spectrophotometric determination of vitamin B_1 in a pharmaceutical formulation using triphenylmethane acid dyes

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Abstract

A highly sensitive colour reaction has been developed, based on the fact that vitamin B_1 reacted with a triphenylmethane acid dye such as thymol blue, bromothymol blue, bromophenol blue, bromocresol green, phenol red or cresol red to form an ion-association complex in a weak-base aqueous solution in the presence of some solubilization agents e.g. polyvinyl alcohol, emulgent OP, Triton X-100 or Tween-20. The wavelengths of maximum absorbance of the six ion-association complexes were between 420 and 450 nm, and fading reaction appeared at the longer wavelength and the maximum fading wavelengths were between 550 and 620 nm. The reactions had highly sensitivities and their apparent molar absorptivities of the colour reactions were $(0.82–1.65) \times 10^5$ l mol^{-1} cm^{-1} and those of fading reactions were $(1.26–3.92) \times 10^5$ l mol^{-1} cm^{-1} depending on the different dye systems. The composition ratio of the ion-association complex was vitamin B_1:dye = 1:1 as established by Job's and equilibrium shift methods. The method had good selectivity and could be applied to direct spectrophotometric determination of vitamin B_1 in aqueous phase without using organic solvent extraction. Therefore, the method was simple and rapid. The colour reaction mechanism was discussed with the quantum chemistry method.

Keywords: Vitamin B_1; Spectrophotometry; Triphenylmethane acid dyes; Solubilization agent

1. Introduction

Vitamin B_1 (VB_1) is a quaternary ammonium compound that is made from the methylene junction between aminopyrimidine ring and thiazole ring. It participates in the normal sugar metabolic process of body, maintains the normal function of nerve, heart and digestion, and it is mainly used to cure beriberi and varies of polyneuritis in clinical. Therefore, the determination of VB_1 is one of the important contents in food and clinical analysis.

There were many methods for the determination of VB_1, including the classical gravimetric [1,2] and titration methods [3,4], electrochemical analysis method [5–10], thin-layer chromatography [11,12], gas chromatography [13,14], capillary

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electrophoresis [15–17], high performance liquid chromatography [18–24], fluorometry [25–29] and spectrophotometry [30–40] etc. Among them, the gravimetric method, titration and many electrochemical analysis methods were suitable for the determination of macro VB_1. There were small applications for the determination of VB_1 by using the thin-layer chromatography, gas chromatography and capillary electrophoresis methods. At present, the most common methods used are high performance liquid chromatography, fluorometry and spectrophotometry. The high performance liquid chromatography method used to determine VB_1 has high sensitivity, good selectivity and the ability of simultaneous multicomponent determination. Therefore, this method has been widely used to the study and application, particularly applied to the separation and determination of the complex samples. Fluorometry used to determine VB_1 was mainly based on the fluorescent substance thiochrome obtained by oxidizing VB_1 with different oxidants. This method had high sensitivity and was suitable for the determination of trace VB_1. However, its operation was more rigorous and there are only a few other fluorescence reaction systems used to the determination of VB_1 up to now. Spectrophotometry is still one of the common methods used to the determination of VB_1 owing to its simplicity and high accuracy. At present, the most method studied and applied is the ultraviolet spectrophotometry [30–34]. In recent years, the main improvement is the multicomponent determination using differential and derivative spectrophotometry [40–43] and chemometrics [44–47]. Another new development for the determination of VB_1 is the use of the solid-phase UV-spectrophotometry which its sensitivity and selectivity are increased largely [48]. However, there were a few methods for the determination of VB_1 by using the visual spectrophotometry, and most of them had not high sensitivity or had not good selectivity. Therefore, it is important to develop a simple, rapid and highly sensitive visual spectrophotometric method for the determination of VB_1. An extraction-spectrophotometric determination of VB_1 was reported, based on the ion-association reaction of VB_1 with bromothymol blue (BTB) [49], but the method had the following defects: (1) the reaction product was difficult to dissolve in water, which must be extracted using poisonous organic solvent. Therefore, the method was troublesome. (2) The calibration graph did not pass through original point, so the method did not obey the Beer's law when the concentration of VB_1 was lower than 4 μg ml^{-1}; (3) the sensitivity was also lower and the method was only suitable for the determination of high level of VB_1 [40].

In the experiment, we found that VB_1 reacted with a triphenylmethane acid dye such as BTB, thymol blue (TB), bromophenol blue (BPB), bromocresol green (BCG), phenol red (PR) and cresol red (CR) to form a 1:1 ion-association complex in the presence of solubilization agent such as polyvinyl alcohol (PVA), emulgent OP, Triton X-100 or Tween-20 etc. When apparent change of the colour reactions took place and the solution remained clear, the maximum absorption wavelengths of the ion-association complexes were between 420 and 450 nm, and fading reaction occured in a longer wavelength and the maximum fading wavelengths were between 550 and 620 nm depending on the different dye systems. The apparent molar absorptivities of the colour reactions (ε_c) of the former were $(0.82–1.65) \times 10^5$ l mol^{-1} cm^{-1} and those of fading reactions (ε_f) of the latter were $(1.26–3.92) \times 10^5$ l mol^{-1} cm^{-1}. Both of them can be applied to spectrophotometric determination of VB_1. The BTB system was the most sensitive among them and using fading reaction method, its ε_f was several 10-fold of that of the extraction-spectrophotometry with the same system. The linear ranges of the systems were 0–15 (BTB system), 0–9 (PR system) and 0–10 μg/25 ml (the other systems), respectively. So low concentration of VB_1 also can be determined. The methods eliminated the step of solvent extraction and can directly determine VB_1 in aqueous solution. Therefore, they were quiet simple and rapid.

In this paper, the optimum conditions of the ion-association reactions of VB_1 with six triphenylmethane acid dyes such as BTB, TB, BPB, BCG, PR or CR, the spectral characteristics of the reaction products, the influencing factors

and sensitivities of the methods were investigated. As an example, the selectivity of the method using the VB_1–BTB system was studied and the results showed that most of coexistent substances did not interfere the determination of VB_1. A new spectrophotometric method for determination of VB_1 in aqueous solution has been developed. It was applied to the determination of VB_1 in synthetic samples and VB_1 tablets with satisfactory results. The colour reaction mechanism was discussed with the quantum chemistry method.

2. Experimental

2.1. Apparatus and reagents

A Hitachi U-3400 spectrophotometer (Tokyo, Japan) and SA 720 pH meter were used.

2.1.1. Triphenylmethane acid dyes:
0.05% for 40% alcohol–aqueous solution, BTB, TB, BPB, BCG, PR and CR, which were all produced by Shanghai Third Chemical Reagent Plant. The solubinization agent solutions: 1%, polyvinyl alcohol (PVA-124, manufacture of Japan), Tween-20 (analytical-reagent grade, Shanghai Chemical Reagent Company), emulgent OP (chemical-reagent grade, Shanghai Chemical Reagent Company) and Triton-X-100 (manufacture of the United States). NH_4Cl–$NH_3{\cdot}H_2O$ buffer (pH 7.6, 7.7, and 7.9) which were made up with 0.2 mol l^{-1} of NH_4Cl and 0.2 mol l^{-1} $NH_3{\cdot}H_2O$ at the rates of 49:1, 39:1, and 24:1, v/v, respectively.

2.1.2. Vitamin B_1 standard solution:
A stock solution containing 500 μg ml^{-1} of vitamin $B_1$was prepared by dissolving 0.025 g of vitamin B_1 (biochemical reagent, 99.9%, Shanghai Chemical Reagent Company) in doubly distilled water, then transferring into a 50 ml calibrated flask and diluting to the mark with water. Then it was stored in the refrigerator and can be used in 1 month. The working concentration of VB_1 was 10 μg ml^{-1} prepared daily from the stock solution by appropriate dilution. All the other reagents were of analytical-reagent grade and doubly distilled water was used throughout.

2.2. General procedure

Place suitable amounts of vitamin B_1 in a 25 ml calibrated flask, add 1.5 ml of NH_4Cl–$NH_3{\cdot}H_2O$ buffer, then add solubilization agent (1%) (1.0 ml of polyvinyl alcohol for BTB system, 1.0 and 2.0 ml of Tween-20 for TB and BPB systems, respectively, 1.5 ml of Triton X-100 for CR system. One milliliter of Triton X-100 for PR and BCG systems) and dilute to about 15 ml with water. Add dye solution (0.05%) 3 ml for BTB and BCG systems, or 3.5 ml for BPB and TB systems or 1.5 ml for PR and CR systems, and dilute to the mark with water. Mix and set the solution aside for 2 min for BTB, BCG, BPB and TB systems, 10 min for PR and CR systems, the absorbances were measured in a 1-cm cell at their own maximum absorption wavelength and maximum fading wavelength against the reagent blank.

3. Results and discussion

3.1. Absorption spectra

Experiments showed that obvious change took place in the colour of solution when VB_1 reacted with a triphenylmethane acid dye such as BTB, TB, BPB, BCG, PR or CR. The colour of four systems of BTB, TB, BPB and BCG changed from green to yellowish-green and that of the PR and CR systems from salmon pink to orange. Fig. 1 shows the absorption spectra of the ion-association complexes and the spectral characteristics for the six ion-association complexes are listed in Table 1.

3.2. Optimum conditions for the reactions

3.2.1. Effects of acidity and buffer solution
For the triphenylmethane acid dyes are acid–base indicators, the solution acidity has a great effect upon the color reactions. Acidity therefore must be strictly controlled and buffers should be used. The experiments showed that the optimum pH ranges were 7.6–7.8 for BTB system, 7.8–8.0 for TB system and 7.5–7.7 for BPB, BCG, PR and CR systems respectively. The higher or lower

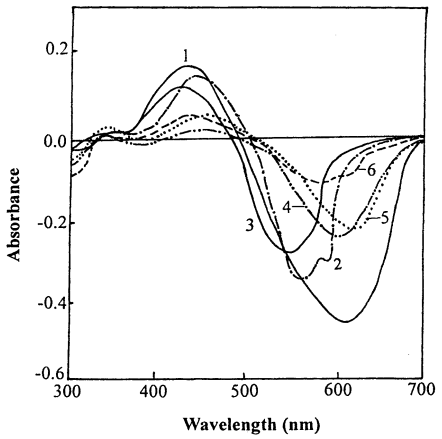


Fig. 1. Absorption spectra of the six systems. BTB system; 2, BCG system; 3, TB system; 4, BPB system; 5, PR system; 6, CR system. $[VB_1] = 10$ (BTB system) and 8 μg/25 ml (other five systems), against reagent blank, 1 cm cell.

pH value caused a marked decrease in the ΔA value.

Experiments showed that the ΔA was low when using the buffer such as Na_2HPO_4–NaOH, NH_2CH_2COOH–NaOH, or hexamethylenamine. While using NH_4Cl–$NH_3{\cdot}H_2O$ buffer, the sensitivity was the highest. So NH_4Cl–$NH_3{\cdot}H_2O$ buffer was recommended.

Addition amounts of NH_4Cl–$NH_3{\cdot}H_2O$ buffer depended on different dyes. The optimum amount was 1.4–1.7 ml for BTB system, 1.0–1.8 ml for BPB and TB systems, 1.3–1.7 ml for PR and BCG systems and 1.2–1.6 ml for CR system, respectively. Therefore, 1.5 ml of NH_4Cl–$NH_3{\cdot}H_2O$ buffer was added in all systems.

3.2.2. Effect of dye concentrations

The optimum concentrations of dyes were 0.86×10^{-4}–1.06×10^{-4} mol l^{-1} for BTB, 1.29×10^{-4}–1.71×10^{-4} mol l^{-1} for TB, 0.89×10^{-4}–1.28×10^{-4} mol l^{-1} for BPB, 0.74×10^{-4}–1.26×10^{-4} mol l^{-1} for BCG, 0.73×10^{-4}–0.96×10^{-4} mol l^{-1} for PR and $0.63 \times 10^{-4} - 0.84 \times 10^{-4}$ mol l^{-1} for CR, respectively. If the concentrations of dyes were above or below these ranges, the ΔA decreased.

3.2.3. Effects of property and amounts of the solubilization agents

If the solubilization agent was absent from the solution, the ion-association complex would gradually separate out as a precipitate because of its hydrophobility, which would affect the spectrophotometric measurement. If PVA or the non-ionic surfactant such as emulgent OP, Triton-X-100 or Tween-20 was added, the ion-association complex would be solubilized in the micellar solution so as to keep the solution clear and the obvious change of colour would be observed [50]. Of course, for the sake of the competing action on the reaction of ion-association reaction, the ionic surfactant cannot be used as the solubilization agent. The effects of different solubilization agents on the sensitivity and the stability of the six reaction systems were different. The optimum solubilization agent was PVA for BTB system, Tween 20 for BPB and TB system, and Triton-X-100 for BCG, PR and CR systems, respectively. The suitable addition amounts of the solubilization agent solution were as follows: 0.5–1.8 ml of PVA (BTB system), 0.5–3.0 ml of Triton-X-100 (BCG system), 0.5–2.0 ml of Tween-20 (TB system), 1.5–5.0 ml of Tween-20 (BPB system), 0.5–2.2 ml of Triton-X-100 (PR system) and 1.0–1.6 ml of Triton-X-100 (CR system).

3.2.4. Effect of the temperature and the stability of absorbances for the ion-association complexes

The effects of temperature on the colour reactions have been tested. It was shown that the

Table 1
Spectral characteristics for the ion-associates

System	BTB	TB	BPB	BCG	PR	CR
Wavelength of maximum absorption (nm)	440	438	433	445	427	424
Wavelength of maximum fading (nm)	618	596	568	613	554	560

Table 2
Optimum conditions of the ion-association reactions

System	pH	Solubilization agent and its add amount (ml)	Dye concentration (1×10^{-4} mol l^{-1})	Suitable reaction temperature (°C)	Stable time (min)
BTB	7.6–7.8	PVA, 0.5–1.8	0.86–1.06	14–28	30
BCG	7.5–7.7	Triton X-100, 0.5–3.0	0.74–1.26	14–28	30
TB	7.8–8.0	Tween-20, 0.5–2.0	1.29–1.71	14–28	35
BPB	7.5–7.7	Tween-20, 1.5–5.0	0.89–1.28	14–28	30
PR	7.5–7.7	Triton X-100, 0.5–2.2	0.73–0.96	14–28	20
CR	7.5–7.7	Triton X-100, 1.0–1.6	0.63–0.84	14–28	20

appropriate temperature range was from 14 to 28 °C and the absorbance would decrease when temperature was higher or lower. If the room temperature was lower than 14 °C, the solution should keep warm for 2 min in 20 °C water-bath for BTB, BCG, BPB and TB systems, and 10 min for PR and CR systems. If the room temperature was higher, suitably reducing the temperature of reaction and measurement was necessary.

The ion-association complexes were formed completely and their absorbances reached the maximum value only in 1–2 min for BTB, BCG, BPB and TB systems, and their stabilization times were 30–35 min. The colour reactions for PR and CR systems took 10 min to reach the maximum absorbance and their stabilization times were 20 min. The optimum conditions of the ion-association reactions are listed in Table 2.

3.3. Calibration graphs

Different amounts of VB_1 were used to form the ion-association complexes under the optimum conditions. The calibration graphs were constructed with absorbance versus the concentration of the VB_1. Linear regression equations, correlation coefficients (r), molor absorptivities (ε) and linear ranges have been obtained based on calibration graphs and the results are listed in Table 3. From Table 3 it can been found that: (1) both of the colouration at shorter wavelength and the colour fading at longer wavelength could be applied to the spectrophotometric determination of VB_1. They all had highly sensitivities and the ε values of the former were between 0.82×10^5 and 1.65×10^5 l mol^{-1} cm^{-1}, the latter were between 1.26×10^5 and 3.92×10^5 l mol^{-1} cm^{-1}. When VB_1 was determined by colour fading method, their sensitivities were higher by one order of magnitude compared with the extraction-spectrophotometric methods with the same dyes systems and were also higher by one order of magnitude compared with the ultraviolet spectrophotometry ($\varepsilon_{325} = 1.35 \times 10^4$ l mol^{-1} cm^{-1}, $\varepsilon_{246} = 1.16 \times 10^4$ l mol^{-1} cm^{-1}) [33], the phenylhydrazine hydrachloride–$NaNO_2$ method ($\varepsilon = 1.10 \times 10^4$ l mol^{-1} cm^{-1}) [36], and 6-amonothymol method ($\varepsilon = 1.34 \times 10^4$ l mol^{-1} cm^{-1} calculated according to the data of the literature [35]). The limits of detection ($\sigma = 3$) for VB_1 are between 22.4 and 84.6 ng ml^{-1}, depending on the different systems, of which the BTB system is the most sensitive and its limit of detection is even lower than that of the thiochrome fluorometric method (0.17 μg ml^{-1}) [29]; (2) All calibration graphs passed original point and obeyed Beer's law within 9 μg/25 ml for PR system, 10 μg/25 ml for TB, BPB, BCG and CR systems and 15 μg/25 ml for BTB system, respectively; (3) the sensitivity, linear range and the stability of absorbance of BTB system were better, so this system was selected to study the selectivity and analytical application.

3.4. Selectivity of the method and analytical application

3.4.1. Selectivity of the method

The selectivity of the BTB method was investigated in the determination of 10 μg of VB_1 in the

Table 3
Linear ranges and correlation coefficients of the calibration graphs and their molar absorptivities

System	Measurement wavelength (nm)	Linear regression equation (c: μg/25 ml)	Correlation coefficient (r)	Linear range (μg/25 ml)	Molar absorptivity (ε: 10^5 l mol^{-1} cm^{-1})	Limit of detection ($\sigma = 3$, ng ml^{-1})
BTB	440	$A = 0.0135 + 0.0166C$	0.996	0–15	1.65	47.0
	618	$A = 0.036 + 0.0429$	0.995	0–15	3.92	22.4
TB	438	$A = 0.0009 + 0.0093C$	0.996	0–10	0.82	84.6
	596	$A = 0.0036 + 0.0330C$	0.995	0–10	2.88	30.4
BPB	433	$A = 0.0323 + 0.0072C$	0.995	0–10	0.88	65.6
	568	$A = 0.0333 + 0.0119C$	0.996	0–10	1.26	61.5
BCG	445	$A = 0.0008 + 0.0132C$	0.995	0–10	1.15	45.6
	613	$A = 0.0059 + 0.0321C$	0.996	0–10	2.76	35.4
PR	427	$A = 0.0027 + 0.00146C$	0.994	0–9	1.29	42.5
	554	$A = 0.0041 + 0.0375C$	0.993	0–9	3.24	24.0
CR	424	$A = 0.0022 + 0.0109C$	0.996	0–10	0.97	70.5
	560	$A = 0.0006 + 0.0344C$	0.995	0–10	3.00	26.2

presence of a series of other substances. The results are summarized in Table 4. It can be seen that the main interfering substances were vitamin B_2, vitamin B_6, vitamin C, cupric chloride, nicotinic acid and theobromine. The interference of other substances was smaller.

3.4.2. Analytical application of the method

As an example, BTB was used to test the determination of VB_1 in synthetic samples, and the VB_1 tablets.

3.4.2.1. Determination of VB_1 in synthetic samples. Place 5 μg of VB_1 in a 25 ml dried standard flask, add amounts of other substances to obtain the synthetic solution, and measure it in accordance with the procedure, the results are listed in Table 5.

Five recovery tests were taken by using 1–3 μg of VB_1 separately and the recovery of VB_1 was 98.8–101.4%.

3.4.2.2. Determination of VB_1 in tablets. Grind several medicinal tablets of VB_1 into powder, weigh a portion of the powder and dissolve it in water and transfer it in a 50 ml calibrated flask and dilute to the mark with water. Then filter it with dry filter paper. Take 1.5 ml of the filtrate in a 50 ml calibrated flask, dilute to the mark with water. Take 1 ml of this solution to determine the content of VB_1 according to the procedure. Meanwhile, the Chinese pharmaceutical method edited in 1995 was used as contrast to determine VB_1 and the results are listed in Table 6. It is shown that the results obtained by this method agree well with those obtained by the Chinese pharmaceutical method [4].

3.5. Discussion of reaction mechanism

The six triphenylmethane acid dyes have the structures as follows:

Table 4
Selectivity of the method (BTB system, $VB_1 = 10$ μg)

Foreign substance (FS)	Amount tolerated (FS/VB_1)	Foreign substance (FS)	Amount tolerated (FS/VB_1)
D-calcium pantothenas	120	Nicotinic acid	2.5
Vitamin B_2	2	Folic acid	88
Inositolum	16	Nicotinamidum	12
Vitamin B_6	2.5	Vitamin B_{12}	50
Vitamin C	2	Sulphuric chinine	15
Starch	60	Theobromine	4
Glucose	46	Maltose	17
Lactose	17	Zinc sulphate	114
Cupric chloride	3	Maganese sulphate	136
Sodium sulphate	173	Magnesium nitrate	105

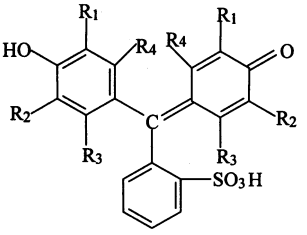
Table 5
Results for the determination of VB_1 in synthetic samples

No.	VB_1 (μg/25 ml)	Coexistent matter and content (μg/25 ml)	Found (μg/25 ml)	RSD (%) ($n = 5$)	Recovery (%)
1	5.00	D-calcium pantothenas 400, vitamin B_6 10, iosilum 50, vitamin B_{12} 200, sodium sulphate 300	4.95	3.69	99.0
2	5.00	Folic acid 300, vitamin B_2 5, ncotinamidum 50, vitamin B_{12} 200, glucose 150	4.89	3.98	97.8
3	5.00	Vitamin B_{12} 200, vitamin C 5, nicotinic acid 5, maltose 50, inositolum 50	5.22	4.72	104.4

Table 6
Results for the determination of VB_1 in tablets

Method	Found (VB_1%)[a]					Average (VB_1%)	RSD (%)
	1	2	3	4	5		
This method	11.38	11.49	11.36	11.45	11.34	11.41	2.80
China pharmaceutical method	10.84	11.35	11.66	11.57	11.66	11.43	2.70

[a] VB_1% represents the percent by quality of VB_1 in each VB_1 tablet.



Dye	BTB	TB	BPB	BCG	PR	CR
R_1	$CH(CH_3)_2$	$CH(CH_3)_2$	Br	Br	H	H
R_2	Br	H	Br	Br	H	CH_3
R_3	CH_3	CH_3	H	H	H	H
R_4	H	H	H	CH_3	H	H

The main existent species of the triphenylmethane acid dyes were HL^- and L^{2-} in neutral and weak basic media, which showed that all the HL^- were yellow, their λ_{max} were among 424–445 nm, while L^{2-} were red, green and blue, their λ_{max} were among 554–618 nm depending on different dyes. The results were identical with the report of Refat et al. [51].

For an example, we studied the reaction of VB_1 with BTB. From the absorption spectra of BTB in different pH media (Fig. 2), it was shown that BTB mainly existent species was yellow HL^- (λ_{max} 440 nm) in pH 6.2. pH value increased to 7.7, the colour of the solution changed from yellow to green, and two absorption peeks appeared at 440 and 618 nm. It was shown that the two species i.e. yellow HL^- and blue L^{2-} coexisted. When pH value was further increased to 10, λ_{max} shifted to 618 nm and the solution colour changed from green to blue. It was shown that BTB mainly existent species was L^{2-}. Therefore, under the experiment condition (pH 7.6–7.7), BTB had the following balance:

$$\underset{\text{yellow, } \lambda_{max}=440 \text{ nm}}{HL^-} \overset{pK_a\ 7.49}{\rightleftharpoons} \underset{\text{blue, } \lambda_{max}=618 \text{ nm}}{L^{2-}} + H^+$$

The composition of the ion-associate for VB_1^+ with BTB was established by the equilibrium shift method and Job's method. The results showed that $[VB_1^+]$:BTB = 1:1.

In pH 7.6–7.7, when BTB reacted with VB_1^+, the colour of the solution changed from green to yellow. At this time, fading occurred at 618 nm, and the absorbance increased at 440 nm. Therefore, it was proposed that the combination of VB_1^+ with L^{2-} formed the yellow ion-association complex whose λ_{max} was at 440 nm. It is owing to the change of blue L^{2-} into yellow ion-association complex that the absorbance at 618 nm decreased, namely, the ion-association reaction was as follows:

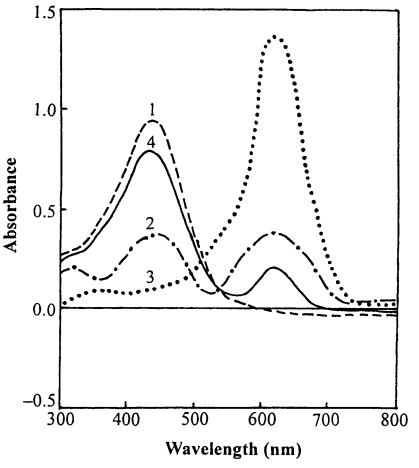


Fig. 2. Absorption spectra of BTB and its ion-associate with VB_1. 1, BTB, pH 6.2; 2, BTB, pH 7.7; 3, BTB, pH 10.0; 4,VB_1–BTB, pH 7.7. $[BTB] = 3.2 \times 10^{-5}$ mol l^{-1}, $[VB_1] =$ 500 μg/25 ml against reagent blank, 1 cm cell.

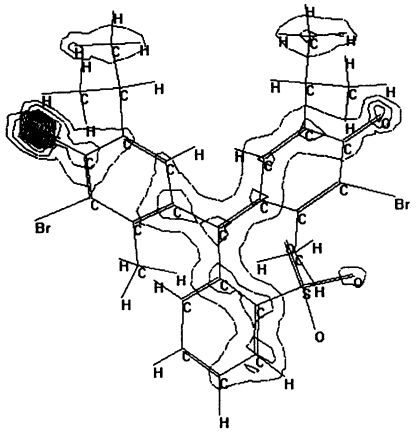


Fig. 3. Charge density distribution of L^{2-} (BTB).

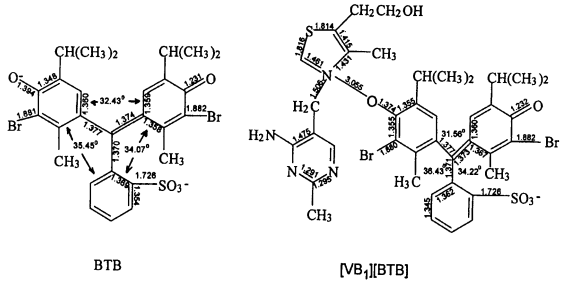


Fig. 4. The change of the related parameters of L^{2-} (BTB) before and after reaction with VB_1^+.

$$\underset{\text{colourless}}{VB_1^+} + \underset{\text{blue } (\lambda_{max}\ 618\ \text{nm})}{L^{2-}} = \underset{\text{yellow } (\lambda_{max}\ 440\ \text{nm})}{[VB_1][L^{2-}]}$$

We discussed theoretically the accuracy of the above presumption. First we calculated with AM1 method the dye anionic L^{2-} charge distribution and the $\pi-\pi^*$ transition energy. The result showed that the transition energy ΔE was 1.9742 eV, and its corresponding λ_{max} was at 626 nm, closing to the experimental value (618 nm). It was proved that the blue style should be L^{2-}.

The charge density distribution of L^{2-} is shown in Fig. 3. From Fig. 3, it can be seen that the maximum negative charge density of L^{2-} was at dissociated phenolic hydroxyl, while the charge density of $-SO_3^-$ was lower. Therefore, VB_1^+ should react with the dye at the phenolic hydroxyl other than at $-SO_3^-$. Moreover, only when VB_1^+ reacted with the dye at the phenolic hydroxyl could it produce intensive effect on $\pi-\pi^*$ transition of the conjugate system and result in the change of absorption spectra. However, the effect of VB_1^+ combining at non-conjugated $-SO_3^-$ on the colour was very small. The change of the related parameters of L^{2-} before and after reaction with VB_1^+ is shown in Fig. 4. From Fig. 4, it can be seen that the combination of L^{2-} with VB_1^+ had little effect on the bond length of L^{2-} and its twist angles among the three phenyls. However, it could decrease the π electron energy of the dye and result in the increase of $\pi-\pi^*$ transition energy ΔE to 2.837 eV, whose corresponding λ_{max} was at 437 nm, corresponding with the experimental value (440 nm) closely. Therefore, it proved the presumption that the combination product should be $[VB_1^+][L^{2-}]$ whose λ_{max} was at 440 nm. It is also shown from Fig. 4 that the distance between the quarterly nitrogen atom of the positive charge and oxygen atom of phenolic hydroxyl of VB_1^+ in combination product was 3.055, which was much longer than the distance in the covalent bond (including coordinate bond) and ionic bond. Therefore, it was impossible for them to form chemical bond. The ion-association complex was formed mainly in virtue of the weak combination forces such as electrostatic attraction and hydrophobic interaction.

References

[1] Y.L. Shao, Pharm. Bull. 4 (1956) 549.
[2] D.K. An, Z.X. Zhang, L.S. Sheng, Pharmaceutical Analysis, Jinan Press of China, Jinan, 1992, p. 1342.
[3] K. Sachiko, T. Yoshihisa, Bunseki Kagaku 32 (1983) T1.
[4] Pharmacopoeia Committee for the Ministry of Health of the People's Republic of China, Pharmacopoeia of the People's Republic of China, Chemical Industry Press of China, Beijing, 1995 pp. 817.
[5] S.S. Hassam, E. Eman, Talanta 36 (1989) 1011.
[6] P. Seagopaul, A.R. Garry, Anal. Chem. 55 (1983) 1929.
[7] B. Huang, B.H. Yu, P.B. Li, M. Jiang, Y.S. Bi, S.F. Wu, Anal. Chim. Acta 312 (1995) 329.
[8] D.H. Chaston, C.P. Jones, D.E. Williams, Talanta 38 (1991) 17.
[9] G.H. Zhang, T. Imato, Y. Asano, T. Sonoda, H. Kobayashi, N. Ishibashi, Anal. Chem 62 (1990) 1644.
[10] R.C. Martinez, F.B. Dominguez, I.M.S. Garcia, J.H. Méndez, R.C. Orellana, R.S. Guzmán, Anal. Chim. Acta 336 (1996) 47.
[11] S.A. Ismaiel, D.A. Yassa, Analyst 98 (1973) 5.
[12] T.A. Kouimtzis, I.N. Papadoyannis, Mikrochim. Acta 1 (1979) 145.

[13] R.E. Echols, J. Harris, R.H. Miller, J. Chromatogr. 193 (1980) 470.
[14] D.M. Hilker, J.M.L. Mee, J. Chromatogr. 76 (1973) 239.
[15] M.M. Gladdines, J.C. Reijenga, R.G. Trieling, M.J.S. Van Thiel, F.M. Everaerts, J. Chromatogr. 470 (1989) 105.
[16] S. Fanali, M. Cristalli, J. Chromatogr. 472 (1989) 441.
[17] Y. Mrestani, R.H.H. Neubert, J. Chromatogr. A 871 (2000) 351.
[18] J. Bontemps, L. Bettendorff, J. Lombet, C. Grandfils, G. Dandrifosse, E. Shoffeniels, F. Nevejans, J. Crommen, J. Chromatogr. 295 (1984) 486.
[19] P.L.M. Lynch, I.S. Young, J. Chromatogr. 881 (2000) 267.
[20] C.M. Cho, J.H. Ko, W.J. Cheng, Talanta 51 (2000) 799.
[21] M. Amin, J. Reusch, Analyst 112 (1987) 989.
[22] K.O. Chu, K.C. Tin, Anal. Lett. 51 (1998) 2707.
[23] P.C.H. Hollman, J.H. Slangen, P.J. Wagstaffe, V. Faure, D.A.T. Southgate, P.M. Finglas, Analyst 118 (1993) 481.
[24] P. Moreno, V. Salvadó, J. Chromatogr. A 870 (2000) 207.
[25] M. Fujiwara, K. Matsui, Anal. Chem. 25 (1953) 810.
[26] A.K. Christine, V.M. Barry, Analyst 106 (1981) 344.
[27] V. González, S. Rubio, A. Gómez-Heus, D. Perez-Bendito, Anal. Lett. 21 (1988) 993.
[28] F.P. Garcia, C. Diaz, A. Hardisson, Anal. Lett. 26 (1993) 1977.
[29] T. Perez-Ruiz, C. Martinez-Lozano, V. Tomas, I. Ibarra, Talanta 39 (1992) 907.
[30] F.T. Bandelin, J.V. Tuschhoff, Anal. Chem. 25 (1953) 1198.
[31] P.D. Sethi, P.K. Chatterjee, Y. Kumar, Indian J. Pharm. Sci. 5–6 (1985) 118.
[32] A.M. Wahbi, S. Belal, H. Abdine, M. Bedair, Analyst 106 (1981) 960–967.
[33] X.Z. Li, G.H. Li, J. Chin. Hosp. Pharm. 14 (1994) 267.
[34] R.T. Sane, U.M. Vaidya, Indian J. Pharm. Sci. 40 (1978) 72.
[35] K.J. Hayden, Analyst 52 (1957) 61.
[36] G.Z. Fang, M. Cheng, M. Peng, J. Pharm. Anal. Chin. 14 (1994) 19.
[37] V. Nirmalchnadar, N. Balasubramanian, Indian Drugs 26 (1989) 233.
[38] V. Nirmalchandar, N. Balasubraniam, Indian Drugs 26 (1989) 233.
[39] K.K. Viswanath, R.A. Sivaprasad, Indian J. Pharm. Sci. 48 (1986) 80.
[40] J.Y. Park, Anal. Chem. 47 (1975) 452–458.
[41] B. Morelli, J. Pharm. Sci. 84 (1995) 34.
[42] B. Morelli, Anal. Lett. 27 (1994) 2751.
[43] I.I. Hewala, Anal. Lett. 26 (1993) 2217.
[44] L.B. Yin, Z. Li, X. Li, Z.N. Chen, Acta Chim. Sinica 51 (1993) 379.
[45] C.S. Yin, Y. Shen, S.S. Liu, Z.L. Li, Z.X. Pan, Chem. J. Chin. Univ. 21 (2000) 49.
[46] H.X. Shen, S.W. Cai, Chin. J. Anal. Chem. 22 (1994) 716.
[47] L.M. Lin, C.S. Wang, Chin. J. Anal. Chem. 20 (1992) 545.
[48] P. Ortega-Barrales, M.L. Femández-de Córdova, A. Molina-Diaz, Anal. Chem. 70 (1998) 271.
[49] V.D. Gupta, D.E. Cadwallader, J. Pharm. Sci. 57 (1968) 112.
[50] S.P. Liu, Science 5 (1993) 45 Scientific American in Chinese.
[51] A.H. Refat, A.G. Samer, R.M. Mohamed, Spectrochim. Acta 44A (1988) 93.