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RESEARCH ARTICLE

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Study on the interaction of aminoglycoside antibiotics with titan yellow by spectrophotometric method and their analytical applications

Abstract In a weak acidic medium, the reaction of some aminoglycoside antibiotics (AGs) such as kanamycin sulfate (KANA), gentamycin sulfate (GEN), and tobramycin sulfate (TOB) with acid thiazolyl bisazo dye Titan Yellow (TY) can result in the fading of TY. The maximum fading wavelength was located at 409 nm. The molar absorptivities ($\varepsilon/\times10^4$ l mol^{-1} cm^{-1}) were 2.0, 1.5 and 2.5 for KANA, GEN and TOB, respectively. The spectral characteristics, effect factors, optimum conditions of the reaction and the influence of foreign substances were also investigated. The procedure is easy and fast. This method has high sensitivity and can be applied to the determination of commercial aminoglycoside antibiotics and serum samples with satisfactory results.

Keywords aminoglycoside antibiotics, titan yellow (TY), spectrophotometry

1 Introduction

Kanamycin sulfate (KANA), gentamycin sulfate (GEN) and tobramycin sulfate (TOB) all belong to the aminoglycoside group of antibiotics. They have similar chemical properties and antibiotic characteristics, good water solubility, wide antimicrobial spectrum, powerful sterilization, low cost, and are convenient to use without skin examination [1]. These advantages make them important clinical antibacterial drugs. At present, these aminoglycoside antibiotics are determined by microbiological assay (MA) [2, 3]. However, MA is too trivial, time-consuming and expensive to satisfy rapid determination. High-performance liquid chromatography (HPLC) [4], fluorescence method [5], ultraviolet visual spectrophotometry (UV–Vis) [6–8], immune assay (IA) [9], colorimetry [10], electrochemical method [11], polarimetry [12] and resonance Rayleigh scattering method [13] have all been used for the determination of aminoglycoside antibiotics. Owing to the simplicity, high accuracy, good repeatability, wide determination range and low cost of the instrument, UV–Vis has been widely used to test pharmaceutical products. The determination of aminoglycoside antibiotics by UV–Vis has been previously based on the formation of a complex with metal ions [14] or on the color reaction with organic reagents [15], with sensitivities between 1.8×10^3 and 1.8×10^4 l mol^{-1} cm^{-1}. In this work, the fading reaction between the acid thiazolyl bisazo dye Titan Yellow (TY) and some aminoglycoside antibiotics (AGs) such as kanamycin sulfate, gentamycin sulfate and tobramycin sulfate were studied. Their maximum fading wavelengths were located at 409 nm and there were new absorption peaks at 490 nm. However, the fading reaction at 409 nm was more sensitive and the molar absorptivities, ε, were between 1.5×10^4 l mol^{-1} cm^{-1} and 2.5×10^4 l mol^{-1} cm^{-1}. The method has high sensitivity, good selectivity, wide pH range, stable color, is simple and fast. It was also used for the determination of the commercial aminoglycoside antibiotics and serum samples with satisfactory results.

2 Experimental

2.1 Apparatus and reagents

UV-VIS 8500 spectrophotometer (Tianmei Company, Shanghai, China), pHS-3C meter (Shanghai Dazhong Analytical Instrumental Plant, China), AL-204 electronic balance (Shanghai Meitele-Tolido Instrument Co. Ltd, China)

The stock concentrations of kanamycin sulfate (KANA,

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Sigma Co.), gentamycin sulfate (GEN, Sigma Co.) and tobramycin sulfate (TOB, Chongqing Daxin Medicine Plant) were 500.0 μg ml^{-1}, and the working concentrations were 50.0 μg ml^{-1}. The concentration of Titan Yellow (TY, Shanghai Tingxin Chemical Reagent Plant, China) used was 1.0×10^{-3} mol l^{-1}. Britton-Robinson buffer solutions with different pH were prepared by mixing the mixed acid (composed of 0.04 mol l^{-1} H_3PO_4, H_3BO_3 and HAc) with 0.2 mol l^{-1} NaOH proportionally. pH values were adjusted with a pH meter.

All the above reagents were of analytical-reagent grade. Double-distilled water was used throughout the experiment.

2.2 Experimental method

In a 10ml volumetric flask, 1.0 ml of TY solution and 0.25 ml of Britton-Robinson buffer, with the corresponding pH according to different systems, were mixed. Then 1.0 ml of the antibiotic solution was added into the flask. Finally, the mixture was diluted to the mark with water and mixed thoroughly. After 15 minutes, the absorbance was measured against a water blank at the maximum fading wavelength using a 1cm cell.

3 Results and discussion

3 .1 Absorption Spectra

KANA, GEN and TOB are all saturated substitution derivates. Their molecular structures contain only σ-bond and n-bond electrons. The sigma bonding orbital to sigma antibonding orbital (σ → σ ∗) and honbonding obital to the sigma antibonding orbital(n→ σ ∗) transitions need high energy, so the absorption spectra are located at the ultraviolet zone. The experiments showed that the three antibiotics almost had no absorption between 200–800 nm. TY, as an acid thiazolyl bisazo dye**,** has a bigger conjugated system and has strong absorption in the UV–Vis zone. Its maximum absorption wavelength was located at 406 nm. When the three antibiotics react with TY, it results in the fading reactions. The maximum fading wavelengths were all located at 409 nm and a new absorption peak appeared at 490 nm (Fig.1). The fading reaction is more sensitive and at a certain range, the concentration of AGs is proportional to the absorbance (ΔA). Therefore, the fading reaction can be used for the spectrophotometric determination of AGs such as KANA, GEN and TOB. Figure 2 shows the absorption spectra of the system of TY and KANA with different concentrations.

3.2 Optimum reactive conditions

3.2.1 Effects of acidity

TY can react with AGs in most buffer solutions (BR, CL, HAc–NaAc and Na_2HPO_4–$C_6H_8O_7$), but because it has the highest sensitivity in a BR buffer solution, we selected BR as the reactive medium. Effects of acidity on the reactive system were investigated. The results showed that the optimum pH range was 3.0–7.0. In the experiment, 0.25 ml of pH 5.0 BR buffer solution was used.

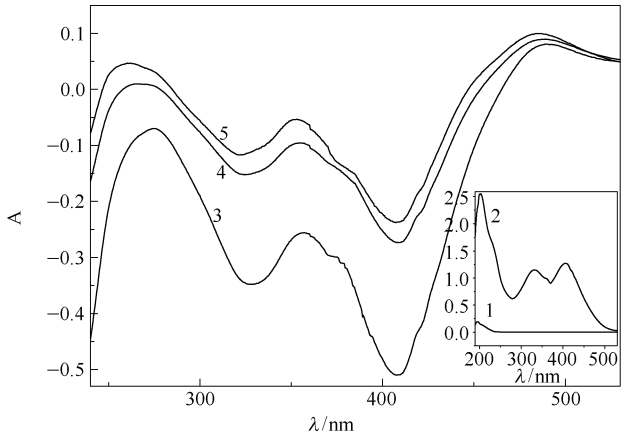

Fig.1 Absorption Spectra of AGs-TY system
1.KANA,[KANA]=10.0μg/mL 2.Reagent blank,[TY]=10^{-4}mol/L(1~2against water) 3. GEN-TY 4. KANA-TY5.TOB-TY (against reagent) [KANA] =[GEN] =[TOB]= 10.0 mg/mL pH 5.0

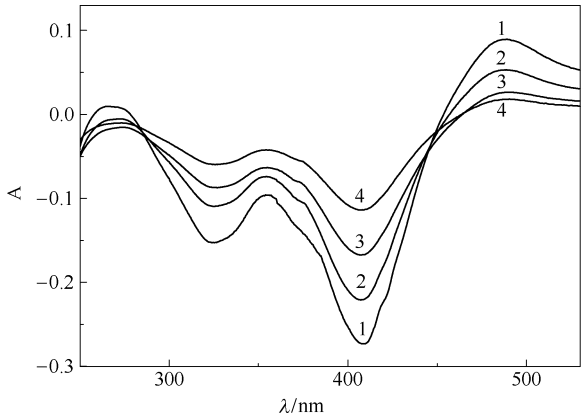

Fig.2 Absorption Spectra of KANA-TY system（against reagent black）
1~4:the concentrations of KANA are 10.0, 8.0, 6.0, 4.0 mg/ml, [TY]=1.0×10^{-4} mol/L pH 5.0

3.2.2 Effect of the concentration of TY

Effects of the concentration of TY on the absorbance at 409 nm were investigated and the results showed that when the concentration of TY was between 0.1×10^{-4} and 0.6×10^{-4} mol/l, the absorbance was very weak and the association reaction was incomplete. When the concentration was too high (>1.2×10^{-4} mol/l), the absorbance decreased sharply because the enhancement of self-aggregation between the dyes was disadvantageous to the association reaction. For KANA, TOB, and GEN systems, the optimum concentration ranges were 0.6～1×10^{-4} mol/l, 0.6～1×10^{-4} mol/l, and 0.8～

1×10^{-4} mol/l, respectively. In this work, 1.0×10^{-4} mol/l of TY was selected.

3.2.3 Stability of the absorbance of the three systems.

The stability of the absorbance of three systems was tested and the results showed that, under room temperature, the absorbance of the systems became maximum in 15 minutes and was stable for more than 6 h. This means that the experiment should be done in 15 minutes.

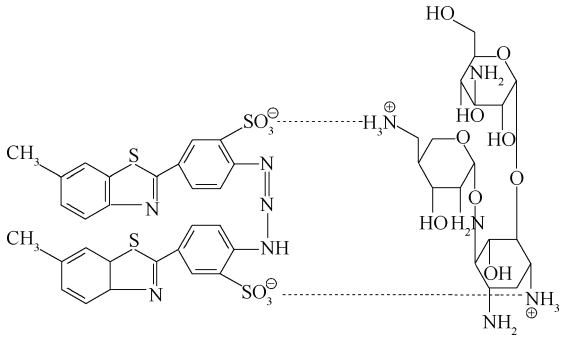

Fig.3 Diagrammatic sketch of ion association complex of TY^{2-}-$KANA^{2+}$

3.3 Composition of the ion-association

The composition ratio of TY with KANA in an ion-association was determined by using Job's method. The result showed that the ratio of TY:KANA is 1:1. Two Na^+ on the $-SO_3^+$ of TY all dissociate, so TY exists as an organic anion of TY^{2-}. In a weak acidic medium, KANA can be easily protonated, become positively charged and exists as a cation, $KANA^{2+}$. TY^{2-} and $KANA^{2+}$ can combine by electrostatic force and hydrophobic effect to form an ion-association. Based on the AM1 quantum chemistry method, the protonated position should be on two amino groups [13]. Figure 3 shows the diagrammatic sketch of the ion-association of TY^{2-} and $KANA^{2+}$.

3.4 Standard curve

Under optimum conditions, the absorbance of the reagent blank and the three systems were measured. The absorbance (ΔA) was plotted versus the concentration of the aminoglycoside antibiotics (C). The linear ranges, regression equations, correlation coefficients and the molar absorption absorptivity are listed in Table1. The method has high sensitivities for the three aminoglycoside antibiotics and, among them, the TOB system has the highest sensitivity and the GEN system has the lowest.

Table 1 Calibration graphs of AGs-TY systems and their some parameters

System	Determined wavelengthnm	Regression equation	Linear range (μg/ml)	Correlation coeffcient (n=6)	Molar absorption absorptivity, ε $(10^4\,L\cdot mol^{-1}\cdot cm^{-1})$
KANA	409	ΔA=0.0071+0.0266C	0～16.0	R=0.9966	2.0
TOB	409	ΔA=-0.0083+0.0518C	0～10.0	R=0.9998	2.5
GEN	409	ΔA=0.0081+0.0229C	0～10.0	R=0.9968	1.5

3.5 Effects of coexisting substances

Under the selected conditions, the effects of more than 30 foreign substances on the absorbance of 5 μg/l kanamycin sulfate were investigated. When the relative error was less than ±5%, all of the following substances did not interfere with the reaction: Cl^-, K^+ (500 times), Ca^{2+} (200 times), SO_3^{2-} (150 times), Na^+ (60 times), Trionx-100, EDTA (50 times), Br^-, sodium dodecyl sulfate (SDS) (30 times), sodium citrate, Ba^{2+} (20 times), NH_4^+, saccharose, maltose (15 times), $H_2PO_4^-$, SiO_3^{2-}, sodium tartaric, starch, urea, sodium dodecylbenzene sulfonate (SDBS) (10 times), NO_2^-, NO_3^{2-}, glucose, SDS (5 times), Ni^{2+} (3 times), Co^{2+}, Nicotinic acid (2 times), V_C, L-tyrosine, Pb^{2+}, Fe^{3+}, Cu^{2+}, Mg^{2+}, Zn^{2+}, V(v) (0.5 times), hydrochloric thiamine, BSA, pepsin (0.2 times), Al^{3+}, D-threonine, and Globin (0.1 times). It can be seen that most of these coexisting substances do not interfere with the determination except proteins, parts of metal ions, and amino acids. Therefore, the method has good selectivity.

3.6 Analytical application

3.6.1 Analysis of commercial antibiotics injections

To investigate the practicability of the method, the concentration of KANA, GEN and TOB were determined using the standard addition method and the results are listed in Table 2. The method has good recovery and the results are satisfactory, and can therefore be used for the analysis of infections.

3.6.2 Determination of medicine concentration in serum

Trichloroacetic acid (0.25 ml of 2.0 mol l^{-1}) was added to ten serum samples (each 1.00 ml of sample was taken from the hospital of Southwest China University). After mixing thoroughly, each solution was centrifuged to let proteins

deposit completely [16]. Then, 0.5 ml of the supernatant fluid was pipetted into a 10-ml volumetric flask and TY, BR and TOB were added in turn. The mixture was then diluted to the mark. The samples were tested according to the experimental method and the results are listed in Table 3. It can be seen from Table 3 that, in the secure concentration range of 2 to 12 μg/ml of TOB [17], the method has good repetition and the RSDs are 1.8% and 2.2%. The method also has high accuracy with mean recoveries of 95.6% and 97.0%. The method can be used for the effective clinical determination of aminoglycoside antibiotics.

Table 2 Results for the determination of commercial amino glycoside antibiotics

Samples	Labeled amount (mg/ml)	Prepared concentration (μg/ml)	Determined concentration (μg/ml)	RSD % (*n*=5)
KANA	250	5.0	4.8 5.0 4.9 5.0 5.0	1.8
GEN	40	5.0	5.1 5.3 5.0 5.2 5.3	2.5
TOB	40	5.0	4.9 4.7 4.9 5.1 4.8	2.9

Table3 Results for the determination of TOB in serum samples

Samples	Found μg/ml (*n*=5)	Added concentration of TOB (μg/ml)	Determined concentration of TOB (μg/ml) (*n*=5)	RSD % (*n*=5)	Recovery % (*n*=5)
1	0	5.0	4.9 4.8 4.7 4.8 4.7	1.8	95.6
2	0	4.0	4.0 3..8 3.9 3.9 3.8	2.2	97.0

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