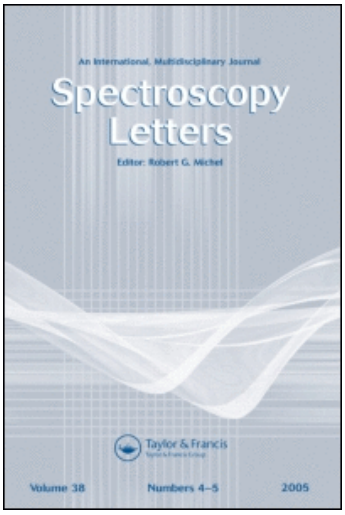
This article was downloaded by:
On: *30 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Resonance Rayleigh Scattering Spectra of the Interaction Between Alizarin Green and Tolterodine Tartrate

Zong Hui Qin[a]; Xiao Hui Zhao[a]; Wei Fen Niu[a]
[a] Department of Chemistry and Environmental Science, Yangtze Normal University, Fuling, China

To cite this Article Qin, Zong Hui , Zhao, Xiao Hui and Niu, Wei Fen(2009) 'Resonance Rayleigh Scattering Spectra of the Interaction Between Alizarin Green and Tolterodine Tartrate', Spectroscopy Letters, 42: 2, 100 — 107
To link to this Article: DOI: 10.1080/00387010802428856
URL: http://dx.doi.org/10.1080/00387010802428856

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Spectroscopy Letters, 42:100–107, 2009
Copyright © Taylor & Francis Group, LLC
ISSN: 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010802428856

Taylor & Francis
Taylor & Francis Group

Resonance Rayleigh Scattering Spectra of the Interaction Between Alizarin Green and Tolterodine Tartrate

Zong Hui Qin, Xiao Hui Zhao, and Wei Fen Niu

Department of Chemistry and Environmental Science, Yangtze Normal University, Fuling, China

Downloaded At: 02:49 30 January 2011

ABSTRACT In HCl–NaAc buffer medium of pH 3.25 ~ 3.85, when tolterodine tartrate (TDT) reacted with alizarin green (AG) by electrostatic force and hydrophobic force to form an ion-association complex, the resonance Rayleigh scattering (RRS) intensity could be enhanced greatly, and a new RRS spectrum appeared. The maximum RRS peak was at 344 nm, and the other RRS peak was located at 454 nm. Under optimum conditions, there is a linear relationship between the RRS intensity and TDT concentration in the range of 7.06×10^{-7} to about 3.12×10^{-5} mol/L. The method is very sensitive, and the detection limit ($3\sigma/K$) for tolterodine tartrate is 3.31×10^{-7} mol/L. The optimum reaction conditions and influence factors were investigated. A highly sensitive, simple, and fast method is proposed for the determination of TDT.

KEYWORDS alizarin green, analytical application, resonance Rayleigh scattering, tolterodine tartrate

INTRODUCTION

In overactive bladder (OAB), patients have uncontrollable urges to pass urine and urinary incontinence.[1–3] In recent years, the study of drugs for urinary incontinence is now leading to the development of new drugs.

Tolterodine tartrate (TDT), (+)-(*R*)-2-[2-(Diisopropylamino)ethyl]benzyl]-*p*-cresol tartrate, is a type of medicine called an anticholinergic (or antimuscarinic) muscle relaxant. It works by relaxing the involuntary muscle that is found in the wall of the bladder.[4] Now tolterodine tartrate is considered the most efficacious drug to treat urinary frequency, urinary urgency, and incontinence in people with unstable bladders.

The most commonly employed analytical techniques for TDT determination are high performance liquid chromatography (HPLC),[5–7] gas chromatography-mass spectrometry (GC-MS),[8] liquid chromatography-mass spectrometry (LC-MS),[9,10] capillary electrophoresis (CE),[11] and solid phase extraction-mass spectrometry (SPE-MS),[12] but the method of resonance Rayleigh seattering (RRS), for TDT determination was not found by us after a search of the literature. In this paper, based on the

Received 28 February 2008; accepted 13 August 2008.

Address correspondence to Zong Hui Qin, Department of Chemistry and Environmental Science, Yangtze Normal University, Fuling 408100, China. E-mail: qinzonghui654321@163.com

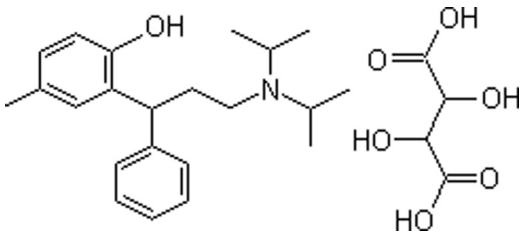


FIGURE 1 **Structure of tolterodine tartrate.**

strong binding of TDT to alizarin green (AG), we have developed a new, selective method for determination of trace amounts of TDT based on the enhancement of the RRS intensity. In the experiment, we discovered that in hydrochloric acid/sodium acetate (HCl–NaAc) buffer medium of pH $3.25 \sim 3.85$, the reaction of AG and TDT would result in great enhancement of RRS intensity, and its maximum scattering peak is located at 344 nm, while the other RRS peak is located at 454 nm. Under optimum conditions, there is a linear relationship between the RRS intensity and tolterodine tartrate concentration in the range of 7.06×10^{-7} to about 3.12×10^{-5} mol/L. The method is very sensitive, and the detection limit ($3\sigma/K$) for tolterodine tartrate is 3.31×10^{-7} mol/L depending on the dye. The methods are simple, rapid, and have good selectivity and can be applied to the direct determination of TDT in practical samples.

MATERIALS AND METHODS

Materials and Apparatus

Tolterodine tartrate 1.0×10^{-4} mol/L (product purity > 99.0%; Sichun Chengdu Yuyang High-tech Developing Co., Sichuan, China) stock solution was prepared by dissolving its sodium salt in water. Alizarin green 1.0×10^{-4} mol/L stock solution (Tianjin Jinke Fine Chemical Study Institute, Tianjin, China) was prepared by dissolving its crystal in water for use. HCl–NaAC buffer solution (pH 3.60, composed of a proper proportion between 1.0 mol/L HCl and 1.0 mol/L NaAc) was prepared and used to control the acidity. All other reagents were of analytical-reagent grade, and doubly distilled water was used throughout.

The RRS spectra were recorded and measured with a Hitachi F-4500 spectrofluorometer (Tokyo, Japan), while the absorption spectra were obtained by using a Hitachi U-4100 spectrophotometer. A HI9024 laboratory instrument (HANNA Co.) was used to measure the pH values of the solutions.

Procedures

A 2.0 mL AG working solution was pipetted in a 10.0 mL flask, and 0.45 mL HCl–NaAc buffer (pH = 3.60) and an appropriate TDT stock solution was subsequently added. The mixture was then diluted to the mark and mixed thoroughly. Ten minutes later, the mixture was used for RRS or absorption measurements.

The RRS spectra were scanned throughout by adjusting the excitation and emission monochromators of the the Hitachi F-4500 spectrofluorometer with $\Delta\lambda = 0$ nm. The RRS intensity was measured at 344.0 nm.

RESULTS AND DISCUSSION

Spectroscopic Characteristics of the Interaction Between TDT and AG

Figure 2a shows the RRS spectra of the interaction of TDT with AG in acidic medium. It can be seen that the RRS signals of both TDT and AG are very weak when they exist separately in the buffer solution over the scanning wavelength range 220.0–700.0 nm. However, strong RRS signals can be observed for the mixture of AG and TDT with the maximum RRS peak being located at 344.0 nm. This indicated that interactions between AG and TDT have occurred. Enhanced RRS shoulder peaks at 246.0, 270.2, and 454.0 nm can be observed.

Compared with the absorption features of the interaction of AG with TDT, the enhanced RRS

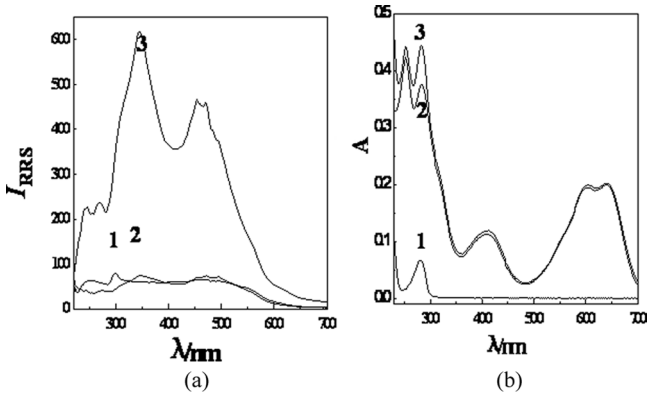


FIGURE 2 **(a) RRS spectra 1. 2.0×10^{-5} mol/L AG; 2. 2.0×10^{-5} mol/L TDT; 3. 2.0×10^{-5} mol/L AG + 2.0×10^{-5} mol/L TDT; (b) absorption spectra. 1. 2.0×10^{-5} mol/L TDT; 2. 2.0×10^{-5} mol/L AG; 3. 2.0×10^{-5} mol/L AG + 2.0×10^{-5} mol/L TDT.**

Downloaded At: 02:49 30 January 2011

signals are very significant. As Fig. 2b shows, AG has five absorption bands characterized at 252.0, 282.0, 410.0, 604.0, and 640.0 nm. The addition of TDT does not lead to any wavelength shift for the characteristic absorption band and only displays a weak hyperchromic effect. It is obvious that the weak increase of the absorption in the 240.0-470.0 nm regions cannot be used for determining TDT with high sensitivity. Comparatively, enhanced RRS signals, as Fig. 2a shows, can be used for a TDT determination with high sensitivity.

Resonance Rayleigh scattering is a special scattering produced when the wavelength of Rayleigh scattering (RS) is located at or close to its molecular absorption band. In this case, the frequency of the electromagnetic wave absorbed by the electron is equal to its scattering frequency. Therefore, the scattering intensity is enhanced several orders of magnitude compared with single RS.[13–16]. Comparing the RRS spectra to the absorption spectra, we can find the RS peaks of the ion-association complex are located close to its molecular absorption band. Owing to the intensive absorption of light energy of the electron, rescattering takes place. Therefore, the scattering intensity is enhanced several orders of magnitude compared with single RS.

Optimization of the General Procedure

Effect of Acidity

As stated above, the enhancement RRS signals are due to the small particles produced by the interaction of AG with TDT. The interactions, however, were found to strongly depend on the pH of the aqueous solutions. As Fig. 3a shows, the RRS intensities (ΔI_{RRS}) reach maximum and hold stabilization when the pH is in the range of 3.25 to about 3.85. While pH value is higher than the range, there exists probably AG negative ion in the solution, but it is not in favor of TDT moleculer existing with positive ion. On the other hand, while the pH is lower than the range, AG negative ion will bind H^+ to form a neutral molecule, which therefore lessens the electrostatic interaction between AG and TDT. In order to obtain stable and high RRS data in this study, we should control the acidity of the interaction system at pH 3.60. In addition, we investigated the dose of HCl–NaAc buffer solution (Fig. 3b). The result shows that 0.45 mL HCl–NaAc buffer solution is the optimum dose.

Effect of AG Concentration

The experimental results show that the RRS intensities (ΔI_{RRS}) reach maximum and hold stabilization when the concentration of AG is 1.9×10^{-5} to about 2.25×10^{-5} mol/L (Fig. 4). If the concentration of AG is lower than 1.9×10^{-5} mol/L, the reaction would be incomplete. On the contrary, if the concentration is higher than 2.25×10^{-5} mol/L, the RRS signals would decrease because of the formation of dye dimers by self-aggregation. This is unfavorable to the ion-association reactions. Thus the experimental concentration is 2.0×10^{-5} mol/L for AG.

Effect of Organic Solvent and Surfactant

From the results of experiments, it can be seen that organic solvent such as methanol, ethanol,

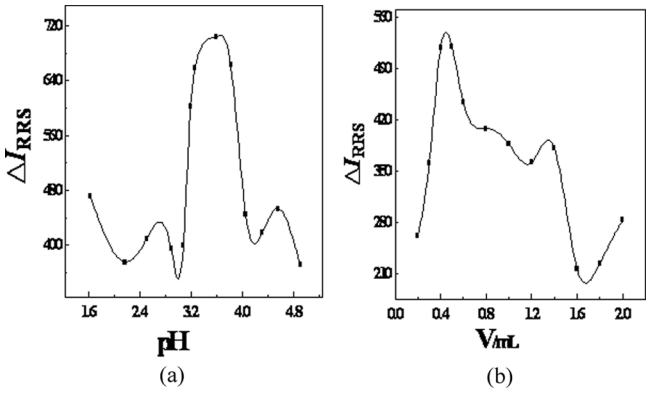


FIGURE 3 (a) Effects of different pH; (b) effects of dosage of pH 3.60.

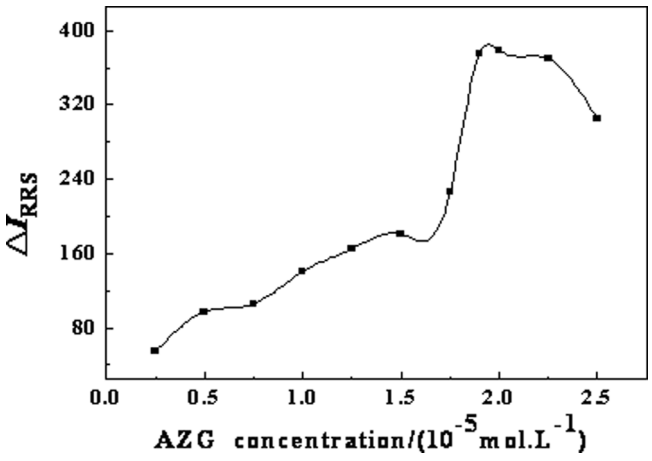


FIGURE 4 Effect of alizarin green concentration.

Downloaded At: 02:49 30 January 2011

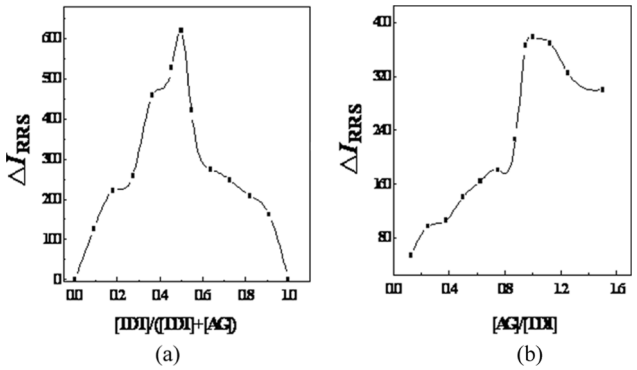


FIGURE 5 **(a) Determination of the composition ratio of AG and TDT by Job's method. (b) Determination of the composition ratio of AG and TDT by molar ratio method.**

acetone, isopropyl alcohol added to the solution can make ΔI_{RRS} decrease significantly. Thus in our experiment, we did not choose organic solvent as an enhancing agent.

Simultaneously, the effects of some surfactants such as nonionic surfactant, cationic surfactant, anionic surfactant on ΔI_{RRS} of TDT–AG systems were tested; the result shows that nonionic surfactant (Tween-80, surfactant OP, etc.) and anionic surfactant (sodium dodecyl sulfate (SDS), sodium lauryl sulfonate (SLS), and sodium dodecyl benzene sulfonate (SDBS) etc.) had no enhanced sensitivity function on TDT–AG system. However, cationic surfactant (cetylpyridinium bromide (CPB), cetyltrimethylammonium bromide (CTMAB), etc.) reduced the sensitivity of TDT–AG system greatly. Thus we added no surfactant in our experiment.

Effect of Ionic Strength

The effect of ionic strength on the RRS intensity was investigated with 1.0 mol/L NaCl solution. The RRS intensities decrease as the ionic strength increases. When [NaCl] <0.01 mol/L, as the concentration of NaCl increases, ΔI_{RRS} holds stability nearly; when [NaCl] >0.01 mol/L, ΔI_{RRS} decreases along with the increase of NaCl.

The Sequence of the Reagents and the Stability of the System

The sequence of addition of the reagents affects the intensity of RRS. We investigated the effect of addition order of the reagents. The result shows that mixing AG and buffer solution first and then adding TDT solution can give a higher RRS intensity than do other sequences of addition of the reagents. Under the optimal conditions of the reaction, the formation time of all reaction products is 5 min, and the RRS intensity will keep constant in 4 h. In addition, we investigated the influence of temperature range room temperature to 303 K and found the effect of temperature was very faint. Thus we selected AG → buffer → TDT solution as the optimum addition sequence of the reagents and stored at room temperature for 10 min.

Effect of Ion-Association Complex on RRS

Exploration of the Mechanism of Ion-Association Complex

The composition of TDT with AG in ion association was established by using molar ratio method and Job's method. When the concentration of TDT and AG were 1.0×10^{-4} mol/L, the total volume of TDT and AG was 4.0 mL in Job's method, and the volume of TDT was a constant 1.5 mL in the molar ratio method. The results were showed in Fig. 6. It can be seen from Fig. 5a (Job's method) and Fig. 5b (molar ratio method) that the ratio of n(TDT): n(AG) = 1:1. That is to say, the ion-association complex of TDT–AG system is TDT·AG.

In pH 3.60 HCl–NaAc acid medium, the N atom of TDT dissociated, and TDT existed with

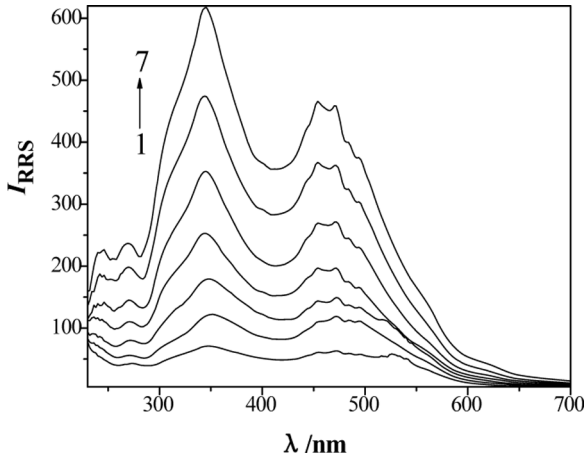


FIGURE 6 **RRS spectra of linearity. (1) 2.0×10^{-5} mol/L AG + 0.25×10^{-5} mol/L TDT; (2) 2.0×10^{-5} mol/L AG + 0.50×10^{-5} mol/L TDT; (3) 2.0×10^{-5} mol/L AG + 0.75×10^{-5} mol/L TDT; (4) 2.0×10^{-5} mol/L AG + 1.00×10^{-5} mol/L TDT; (5) 2.0×10^{-5} mol/L AG + 1.25×10^{-5} mol/L TDT; (6) 2.0×10^{-5} mol/L AG + 1.50×10^{-5} mol/L TDT; (7) 2.0×10^{-5} mol/L AG + 1.75×10^{-5} mol/L TDT.**

Downloaded At: 02:49 30 January 2011

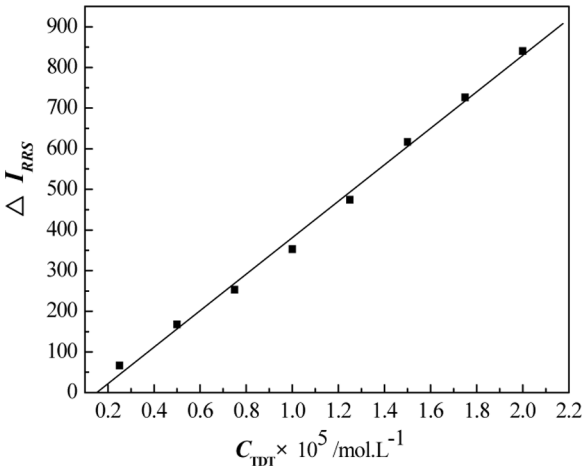


FIGURE 7 The calibration graph of tolterodine tartrate.

protonated molecule [$(TDT)^+$]. The Na atom binding of sulfonic acid group in AG dissociated in aqueous solution, and then the AG molecular existed with negative ion [$(AG)^-$]. [$(TDT)^+$] and [$(AG)^-$] form a 1:1 neutral ion-association complex TDT·AG by the electrostatic attraction and the hydrophobic force.

Reasons for Enhancement of RRS

It is shown in our experiment that the RRS produced merely by the AG solution or TDT solution is very faint. However, the RRS would be enhanced greatly when TDT anion combined with the AG cation to form the reaction product. The main reasons probably lie in the following three points: (1) When AG and TDT form the ion-association complex, we can find the Rayleigh scattering peaks of ion-association complex are located close to its molecular absorption band. Owing to the intensive absorption of light energy of the electron, rescattering takes place. Therefore, the scattering intensity is enhanced several orders of magnitude compared with single RS. (2) According to the simplified equation of Rayleigh scattering $I_{RRS} = KCMI_{0,}$[15,17], the I_{RRS} is direct proportion to intensity of incident light (I_0), the molecular weight (M), and the concentration (C) of scattering particles. It is known that the bigger the molecular volume,[18] the higher the RRS intensity. When the molecular volume is calculated, it can be estimated generally according to the variation of molecular weight. When TDT reacts with AG to form the ion-association complex, the molecular weight increases from 622.58 (AG) and 475.58 (TDT) to 1098.16 (TDT·AG), which would result in great enhancement of RRS intensity. (3) Under experimental conditions, AG molecule is dissociated and takes negative charge [$(AG)^-$], while TDT exists as the form of protonation molecule [$(TDT)^+$]. They both have a high hydrophilic property and easily form hydrate. When [$(TDT)^+$] and [$(AG)^-$] form a 1:1 neutral ion-association complex, the complex exists in the hydrophobic state and easily forms liquid–solid interface, and then the surface enhancement scattering effect takes place.

Sensitivity and Selectivity

Sensitivities of the Method

Under the optimum conditions, the RRS intensities of binding products between TDT and AG were measured at different RRS peaks (Fig. 6). The calibration graph of ΔI_{RRS} against concentration of TDT was constructed. The calibration graph (Fig. 7). Linear regression equations, quantitative determination range, correlation coefficient (r), and detection limits are listed in Table 1. It can be seen that the range of quantitative determination for TDT is 0.00071-0.03 μmol/mL. It also shows that RRS intensities of binding products at 344 nm is larger than ones at 454 nm, similarly, the detection limit at 344 nm is lower, so we chose 344 nm as measurement wavelength.

The sensitivity for the proposed RRS method was compared with that of many reported methods, as shown in Table 2. The detection limit of RRS is lower than that of the method of CE, and partly higher than that of the method of HPLC. Although the detection

TABLE 1 Correlation Coefficient, Linear Ranges, and Detection Limits for Standard Curves

No.	λ (nm)	Linear regression equation (C/mol·L^{-1})	r	Linear ranges C/(×10^{-7}mol·L^{-1})	Detection limits $3\sigma/K$ (×10^{-7}mol·L^{-1})
1	344	$\Delta I = 448.6 \times 10^5 C - 67.58$	0.9988	7.06 ~ 312	3.31
2	454	$\Delta I = 312.44 \times 10^5 C - 29.59$	0.9957	11.5 ~ 240	5.28

Downloaded At: 02:49 30 January 2011

TABLE 2 Comparison of the Sensitivities for Some Methods

Method	Reagent	Detection limit	Linear range	Reference
HPLC	*n*-hexane–isopropyl alcohol–diethylamine–trifluoroaceticacid	$0.1\,\mu g \cdot mL^{-1}$	$0.1 \sim 0.6\ mg \cdot mL^{-1}$	[5]
HPLC	Ammonium formate–methanol (pH 3.0)	—	$5.072 \sim 40.567\,\mu g \cdot mL^{-1}$	[6]
HPLC	Triethylamine–Acetonitrile–mixedphosphate (pH 3.5)	$0.\ 15\,\mu g \cdot mL^{-1}$	$0.\ 0050 \sim 0.050\ mg \cdot mL^{-1}$	[7]
GC-MS	Di-sodium hydrogen phosphatebuffer (pH 11.2)–diethylether-*n*-pentane (1:3)–phosphate buffer (pH 7.5)	$0.3\,ng \cdot mL^{-1}$	$0.5 \sim 50.0\,ng \cdot mL^{-1}$	[8]
LC-MS	*n*-hexane–isopropyl alcohol–acetonitrile–water–ammoniumacetate (pH 3.0)	$0.05\,ng \cdot mL^{-1}$	$0.1 \sim 30.0\,ng \cdot mL^{-1}$	[9]
CE	HP-β-CD-trishydroxymethylaminomethane–phosphateacid (pH 3.0)	$0.5\,\mu g \cdot mL^{-1}$	$0.001 \sim 1.5\ mg \cdot L^{-1}$	[11]
SPE-MS	Methanol–Triethylamine-formicacid (pH 4.8)	$50\ pg \cdot mL^{-1}$	$0.05 \sim 1000\,ng \cdot mL^{-1}$	[12]
RRS	Alizarin green–hydrochloric acid–sodium acetate (pH 3.60)	$3.31 \times 10^{-7} mol \cdot L^{-1}$ ($0.108\,\mu g \cdot mL^{-1}$)	$7.06 \times 10^{-7} \sim 3.12 \times 10^{-5}\ mol \cdot L^{-1}$ ($0.230 \sim 10.16\,\mu g \cdot mL^{-1}$)	This work

Downloaded At: 02:49 30 January 2011

limit of MS is much lower than that of the method of RRS, the apparatus is expensive and the solvents used are harmful for the analyzer. Therefore, the method of RRS has very high sensitivity and is more suitable for the determination of trace amounts of TDT.

Tolerance of Foreign Substances

We tested the effect of foreign substances on the determination by premixing TDT with interfering substances, including metal ions, proteins, amino

TABLE 3 Effect of Foreign Substances ($C_{TDT=}$ 3.26 mg/L)

Foreign substance	Concentration ($\times mg \cdot L^{-1)}$	Change of RRS intensity (%)	Foreign substance	Concentration ($\times mg \cdot L^{-1}$)	Change of RRS intensity (%)
NH_4^+,F^-	90	0.62	Cu^{2+}, SO_4^{2-}	50	1.52
NH_4^+,Cl^-	95	−1.29	Zn^{2+}, SO_4^{2-}	100	2.59
Na^+, CO_3^{2-}	70	−4.31	Al^{3+}, Cl^-	10	3.64
Na^+, SO_3^{2-}	95	2.48	Bi^{3+}, NO_3^-	15	2.69
Na^+, SO_4^{2-}	100	−1.03	Fe^{3+}, Cl^-	1	3.94
Na^+, PO_4^{3-}	90	−0.84	Ce^{4+}, SO_4^{2-}	3	2.61
Na^+, Br^-	90	0.83	Lactose	100	2.91
Na^+, $C_2O_4^{2-}$	65	1.69	Starch	55	3.15
K^+, I^-	100	−0.98	Urea	50	1.83
Hg^{2+}, NO_3^-	0.6	3.15	Sucrose	95	0.82
Mg^{2+}, SO_4^{2-}	90	1.23	Glucose	80	1.57
Ca^{2+}, Cl^-	85	2.34	Maltose	100	0.78
Sr^{2+}, NO_3^-	100	2.74	d-Mannose	40	1.32
Ba^{2+}, Cl^-	80	4.21	d-*Fructose*	90	1.18
Zn^{2+}, Cl^-	100	1.72	Oxalic acid	50	−2.10
Sn^{2+}, Cl^-	20	3.10	Vitamin C	80	3.66
Pb^{2+}, NO_3^-	75	1.47	l-Tryptophan	10	−4.03
Cd^{2+}, Cl^-	85	1.46	dl-Malic acid	45	−2.75
Ag^+, NO_3^-	0.6	2.07	Citric acid	20	−2.41

TABLE 4 Results of the Determination of Tolterodine Tartrate Medicine Samples (n = 6)

Samples	Nominal	Found	RSD %	Contrast experiment[3]
Tablet 1	2 mg/tablet	2.029 mg/tablet	2.16	2.033 mg/tablet
Tablet 2	2 mg/tablet	1.961 mg/tablet	1.78	1.982 mg/tablet
Capsule	2 mg/granule	2.016 mg/granule	1.09	2.004 mg/granule

acids, and carbohydrates. As Table 3 shows, such substances as starch, urea, vitamin C, and carbohydrates can be allowed at relatively high concentrations, whereas such metal ions as Fe^{3+}, Al^{3+}, Ce^{4+}, Bi^{3+}, Sn^{2+}, Hg^{2+}, and Ag^{+} can be allowed only at very low concentrations. The effect of acids on the system is stronger than the effect of carbohydrates. We speculated that the stronger influence of acids was because the scope of pH was narrow, and the adding of acids can lead to the change of pH. However, the effect of Hg^{2+} and Ag^{+} may be because the sulfonic acid group of AG can ionize SO_4^{2-} and then produce water-fast sulfate deposition.

Analytical Application

Sample Determination

We took 20 tolterodine tartrate tablets (trade name: Ning Tong; 2 mg/tablet; Lunan Pharmaceutical Group Corporation) (Table 1) and 20 tolterodine tartrate tablets (trade name: Tolterodine; 2 mg/tablet; Nanjing MeiRui Pharma Co., Ltd.) (Table 2) separately and removed their sugar-coats and then weighed accurate dosage of TDT and placed in a 100 mL volumetric flasks (about 1.0×10^{-4}mol/L) as tablet sample solution.

We took 20 tolterodine tartrate capsules (trade name: Mei Peng; 2 mg/grain; Anhui Huanqiu Pharmaceutical Co., Ltd.) (Table 2) removed their capsules, and then weighed accurately dosage of TDT and placed in 100 mL volumetric flasks (about 1.0×10^{-4}mol/L) as capsule sample solution.

Under optimum conditions, we took the abovementioned capsule and tablet sample solutions separately to scan the RRS spectra. The RRS intensity was measured at 344.0 nm, and the results are listed in Table 4. The parallel determination results (n = 6) showed the recovery ranged from 97.5% to 101.8%, and their relative standard deviation was in the range of 1.09% to about 2.16%. Because the drug is not indexed by the Chinese Pharmacopoeia, the literature[3] conditions were compared with the experimental samples, and the results are consistent with those obtained by our experiment.

TDT Determination in Human Serum and Urine Samples

First, we tested 0.5 mL of fresh urine sample (analyzer provided) and 1.0 mL of human serum (Provided by Fuling Central Hospital) 6 times respectively, and no TDT was found. Next, 1.5 mL of TDT (1.0×10^{-4} mol/L) and 0.5 mL of fresh urine sample (or 1.0 mL of human serum) was added into a 10.0 mL volumetric flask and then tested 6 times in parallel according to the general procedure. The parallel determination results showed the recovery ranged from 99.1% to 101.5%, and the relative standard deviation was in the range of 0.83% to about 2.01%. At the same time, the limit of detection (LOD) and the limit of quantitation (LOQ) of TDT in the human serum and urine were also determined. The results are listed in Table 5.

TABLE 5 Results of the Determination of Tolterodine Tartrate in Human Serum and Urine Samples (n = 6)

Samples	Found (μg)	Added (μg)	Total found (μg)	Recovery (%)	RSD (%)	LOD ($\times 10^{-7}$mol·L^{-1})	LOQ ($\times 10^{-7}$mol·L^{-1})
Human serum	ND	4.76	4.83	101.5	2.01	5.05	11.6
		7.04	7.11	101.0	1.64		
Human urine	ND	4.76	4.73	99.4	1.97	5.02	9.87
		7.04	6.98	99.1	0.83		

ND, not detected.

Downloaded At: 02:49 30 January 2011

CONCLUSIONS

In weak acidic medium, TDT reacts with AG by virtue of electrostatic attraction and hydrophobic force to form 1:1 ion-association complexes. As a result, the RRS intensities of complexes enhance remarkably. The increments (ΔI) are directly proportional to the concentrations of TDT in a certain range. A new RRS method for determination of TDT has been developed. This highly sensitive, simple, and fast method can be applied to determine trace amounts of TDT in serum and urine samples, and the results obtained by this method are satisfactory.

ACKNOWLEDGMENTS

This project was supported by Chongqing Education Commission Scientific Research Foundation (no. KJ081301), "Chunhui Projects" of the State Education Ministry of China (no. z2005-1-55005), Chongqing University Academy of Outstanding Person with Ability Grant [no. (2005)2], and Fuling Normal University Scientific Research Foundation (no. flsyk 2006-25), and all authors here express their deep thanks.

Downloaded At: 02:49 30 January 2011

REFERENCES

1. Hu, T.; Wagner, T. H.; Bentkover, J. D. et al.; Costs of urinary incontinence and overactive bladder in the United States: A comparative study. *Urology* **2004**, *63*(3), 461–465.
2. Andersson, K. E. Antimuscarinics for treatment of overactive bladder. *Lancet Neurology* **2004**, *3*, 46–53.
3. Rovner, E. S. Trospium chloride in the management of overactive bladder. *Drugs* **2004**, *64*(21), 2433–2446.
4. Gillberg, P. G.; Sundquist, S.; Nilvebrant, L. Comparison of the in vitro and in vivo profiles of tolterodine with those of subtype-selective muscarinic receptors antagonists. *Eur. J. Pharmacol.* **1998**, *349*, 285–292.
5. Zhang, B. B.; Zhang, Z. J.; Tian, Y. et al.; High performance liquid chromatography-electrospray ionization mass spectrometric determination of tolterodine tartrate in human plasma. *J. Chromatogr. B.* **2005**, *824*(1-2), 92–98.
6. Saxen, V.; Zaheer, Z.; Farooqui, M. Stability-indicating HPLC determination of tolterodine tartrate in pharmaceutical dosage form. *Indian J. Chem. Technol.* **2006**, *13*(3), 242–246.
7. Xia, Z. L.; Chen, Z. Y.; Yao, T. W. An enantiospecific HPLC method for the determination of (S)-enantiomer impurities in (R)-tolterodine tartarate. *Pharmazie* **2007**, *62*, 170–173.
8. Palmer, L.; Andersson, L.; Andersson, T. et al.; Determination of tolterodine and the 5-hydroxymethyl metabolite in plasma, serum and urine using gas chromatography-mass spectrometry. *J. Pharm. Biomed. Anal.* **1997**, *16*, 155–165.
9. Zhang, B. B.; Zhang, Z. J.; Tian, Y. et al.; High performance liquid chromatography-electrospray ionization mass spectrometric determination of tolterodine tartrate in human plasma. *J. Chromatogr. B.* **2005**, *824*, 92–98.
10. Teng, C. H.; Alan, P.; Clark, C. et al.; Effect of duloxetine on tolterodine pharmacokinetics in healthy volunteers. *Br. J. Clin. Pharmacol.* **2004**; *57*(5), 652–656.
11. Huang, Z. Y.; Du, J. Q.; Cai, W. Determination of the levo-enantiomer in tolterodine tartrate by HPCE. *Chin. J. Pharm. Anal.* [in Chinese] **2001**, *21*(5), 351–354.
12. Swart, R.; Koivisto, P.; Markides, K. E. Capillary solid phase extraction - tandem mass spectrometry for fast quantification of free concentration of tolteridine and two metabolites in ultrafiltrated plasma sample. *J. Chromatogr. B* **1999**, *736*, 247–253.
13. Long, X. F.; Miao, Q.; Bi, S. P. et al.; Resonance Rayleigh scattering method for the recognition and determination of double-stranded DNA using amikacin. *Talanta* **2004**, *64*(2), 366–372.
14. Liu, S. P.; Zhou, G. M.; Liu, Z. F. Resonance Rayleigh scattering for the determination of trace amounts of mercury (II) with thiocyanate and basic triphenylmethane dyes. *Anal. Lett.* **1998**, *37*, 1247–1259.
15. He, Y. Q.; Liu, S. P.; Kong, L. et al.; A study on the sizes and concentrations of gold nanoparticles by spectra of absorption, resonance Rayleigh scattering and resonance non-linear scattering. *Spectrochim. Acta A* **2005**, *61*, 2861–2866.
16. Huang, C. Z.; Li, Y. F.; Pu, Q. H. et al.; Interactions of nile blue sulphate with nucleic acids as studied by resonance light-scattering measurements and determination of nucleic acids at nanogram levels. *Anal. Lett.* **1999**, *32*(12), 2395–2415.
17. The Editorial Committee of China Cyclopaedia (eds.). *China Cyclopaedia, Biology*; China Cyclopaedia Press: Beijing, [in Chinese] 1991.
18. Zhang, Y. H. *Physical Chemistry*; Shanghai Traffic University Press: Shanghai, [in Chinese] 1988.