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Short communication

Determination of antibacterial quaternary ammonium compound in lozenges and human serum by resonance light scattering technique

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ABSTRACT

A method for the specific determination of an antibacterial quaternary ammonium compound Dequalinium chloride (DQC) was described in this paper. At pH 0.5, the resonance light scattering (RLS) intensity of sodium dodecyl benzene sulfonate (SDBS) remarkably was enhanced by adding DQC. A RLS peak at 392.0 nm was found, and the enhanced intensity of RLS at this wavelength was proportional to the concentration of DQC in the range of 0.096–2.88 μg/mL. The detection limit was 2.98 ng/mL and the correlation coefficient was $r = 0.9988$ ($n = 9$). The method was applied to the analysis of DQC in lozenges and human serum. The results indicated that the method was sensitive, simple, practical and useful in the clinical assay.

1. Introduction

Dequalinium chloride (DQC), chemically decamethylenebis-(4-aminoquinaldinium)-chloride (Fig. 1), is a quaternary ammonium compound with antimicrobial and antifungal activity. DQC molecule consists of two positively charged quinolinium rings which are connected by an alkylene chain. DQC has been investigated as a safe and effective medicine for the treatment of chronic, pharyngitis, laryngitis and stomatitis. DQC is also an antibacterial and antifungal drug in pharmaceutical products for the treatment of G^+ bacteria, G^- bacteria, antacid bacteria and anaerobic bacteria. It has been reported that, as a kind of antimicrobial and antifungal drug, DQC was applied in the clinical practice without any side effect [1].

During the last decade, different methods to analyze DQC in pharmaceutical and body-fluid have been published including high performance liquid chromatography (HPLC) [2–4], capillary electrophoresis (CE) [5], and fluorescence analysis (FL) [6–8]. However, these methods have some disadvantages. The HPLC and CE used as separation technique coupled with different detection systems require large amounts of high purity organic solvents, long system stabilization time and special sample preparation. Because the intrinsic fluorescence of DQC is very weak, the technique of FL is not sensitive enough to detect the trace mount of DQC in lozenges and body-fluid. To be of simple practical analytical utility, we proposed a sensitive and selective method to determine the DQC with SDBS as a probe by the resonance light scattering (RLS) technique.

In recent years, the RLS technique is arisen as a simple, highly sensitive and selective method in the modern analytical investigation. Pasternack et al. established the RLS technique to study the extended aggregates of chromophores, the biological macromolecules and the assembly molecules [9,10]. Huang et al. first successfully used this technique in quantitative analysis, and reported the investigation in the determination of the biological macromolecules [11–13]. Subsequently, the RLS studies have been applied in the analytical investigation fields with a great progress [14–16]. Many researchers pay great attention to determining proteins [17], DNA [18], metal [19], drugs [20,21], and the immunoassay [22] by the RLS technique. We have also done amounts of correlative work by this novel method. Previously, we have successfully determined some biological macromolecules such as DNA [23,24], proteins [25–27] and small biomolecules such as amino acid [28].

In the acidic medium, two nitrogen atoms of the DQC quinolinium rings can be protonated, which results in reaction between negative SDBS and positive DQC by the electrostatic force. Furthermore, the π–π interaction between quinolinium rings of DQC and aroma ring of SDBS strengthened the combination. The stable particle of SDBS-DQC was formed, which resulted in the enhanced RLS signal. The enhanced RLS intensity was proportional to the DQC concentration in a certain range. Based on the linear relationship, a

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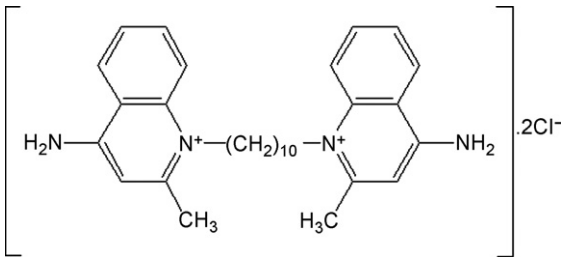

Fig. 1. The structure of DQC.

novel method for the determination of DQC was proposed. The real samples were studied and the present method proved to be simple, sensitive, selective and practical.

2. Materials and methods

2.1. Materials

The stock solution of DQC was prepared by dissolving DQC (Beijing Double-crane Pharmaceutical Chemical Co. Ltd.) with appropriate ethanol. Then diluted and the concentration was 240 μg/mL with the doubly distilled water. The concentrations of the working solutions were 24 and 0.96 μg/mL by diluting the stock solution of DQC in the water. The stock solution of SDBS was prepared by dissolving SDBS (obtained from Shanghai Shengzhong fine Chemical Co. Ltd.) in the doubly distilled water. The working solution concentration was 1.1×10^{-3} M by diluting the stock solution of SDBS. They were all stored at 4 °C in fridge.

The HCl–KCl buffer solution was used to control the acidities of different solutions. The HCl–KCl buffer solutions were prepared by 1MHCl, 0.2MHCl and 0.2MKCl. pH = 0.1–1.0 of the buffer solutions were made of different proportions of 1 M HCl and 0.2 M KCl solutions. And pH 1.0–2.2 buffer solutions were made of different proportions of 0.2 M HCl and 0.2 M KCl solutions. The ionic strength of the system was adjusted by 0.5 M solution.

All chemicals used were of analytical grade without further purification, and the water used throughout was doubly distilled water.

2.2. Apparatus

The RLS spectra and intensity were measured with LS-55 spectrofluorometer (PerkinElmer, USA) with a 1 cm × 1 cm quartz cell. The pHS-3C acidity meter (Shanghai Optical Instrument Factory) was used to measure the pH of the solutions.

2.3. Standard procedure

Into a 10-mL calibrated colorimetric tube were added 1.0 mL HCl–KCl buffer solution, appropriate volume of DQC or samples, and 0.9 mL SDBS working solution. Mixed thoroughly after each addition of the above additives. Then diluted to the mark with the doubly distilled water.

The RLS spectra was recorded on the LS-55 spectrofluorometer by scanning simultaneously the excitation and emission monochromators from 250.0 to 700.0 nm with $\lambda_{ex} = \lambda_{em}$ ($\Delta\lambda = 0$). The RLS intensity was maximum at $\lambda_{ex} = \lambda_{em} = 392.0$ nm and $\lambda_{max} = 392.0$ nm was selected to determine the RLS intensity in the further study. The both optimal excitation and emission slit widths were 5.0 nm. The RLS intensity of blank reagents without DQC was also recorded according to the above method. The enhanced RLS intensity of DQC-SDBS system was presented as $\Delta I_{RLS} = I_{RLS} - I^0_{RLS}$,

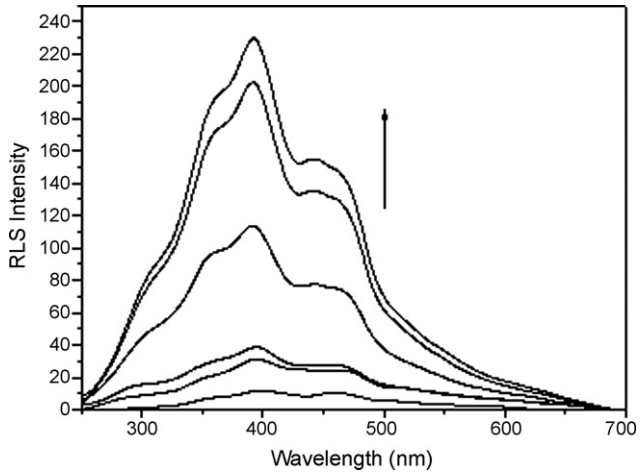

Fig. 2. The RLS spectra of the SDBS-DQC system, Conditions: SDBS 9.9×10^{-4} M. DQC (μg/mL) in the direction of arrow: (1) 0; (2) 0.12, (3) 0.24; (4) 1.2; (5) 2.4; (6) 2.88. pH = 0.5.

where I_{RLS} and I^0_{RLS} were the RLS intensities of the system in the presence and absence of DQC, respectively.

3. Results

3.1. Features of spectra

The RLS spectra of SDBS and SDBS-DQC systems were shown in Fig. 2. It can be seen that there was no RLS signal in the SDBS system without DQC. However, the RLS intensity was observed obviously with a peak located at 392.0 nm when the trace amount of DQC was added. The intensity of RLS at 392.0 nm increased intensely with the DQC increase. The phenomenon above illuminated the interaction occurred between SDBS and DQC and new substance was produced. The new substance had good particle radius which can produce significant RLS signal. The enhanced RLS intensity of SDBS system with increasing DQC was clearly observed and was proportional to the concentration of DQC in a certain range. The novel method was established to determine the DQC in the samples.

3.2. Effect of pH

The HCl–KCl buffer was used to adjust the acidity of the assay solutions. The RLS intensity of the assay system affected by different pH values was studied and the results were shown in Fig. 3. It can be seen that variational pH values had great effect on the RLS intensity. The RLS intensity was maximum at pH 0.5 and kept stable in the pH value of 0.3–1.0. At pH lower 0.3 and higher 1.0, the RLS intensity decreased sharply. So the optimal pH was selected at 0.5 for the further study.

3.3. Effect of SDBS concentration

The effect of SDBS concentration on the RLS intensity of assay system was studied and the result was shown in Fig. 4. It can be seen that the RLS intensity of the blank reagent kept steady with the increasing of SDBS. Under the lower concentration of SDBS in the assay system, there was not enough SDBS to react with the DQC resulting in weak RLS intensity. However, the enhanced RLS intensity of SDBS-DQC increased obviously with the SDBS increasing and reached maximum when the concentration of SDBS was 9.9×10^{-4} M.When the SDBS concentration increased in the assay system higher 9.9×10^{-4} M, the enhanced RLS intensity tended to

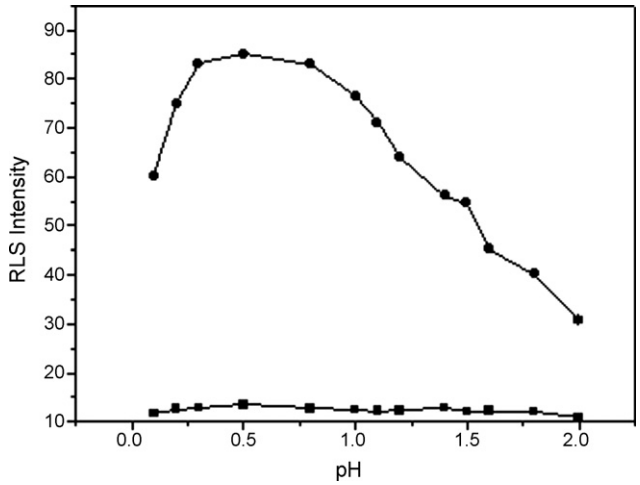

Fig. 3. Effect of pH on the RLS intensity. The symbols (■) and (●) represent I^0_{RLS} and ΔI_{RLS}, respectively. Conditions: DQC 0.96 μg/mL, SDBS 9.9×10^{-4} M.

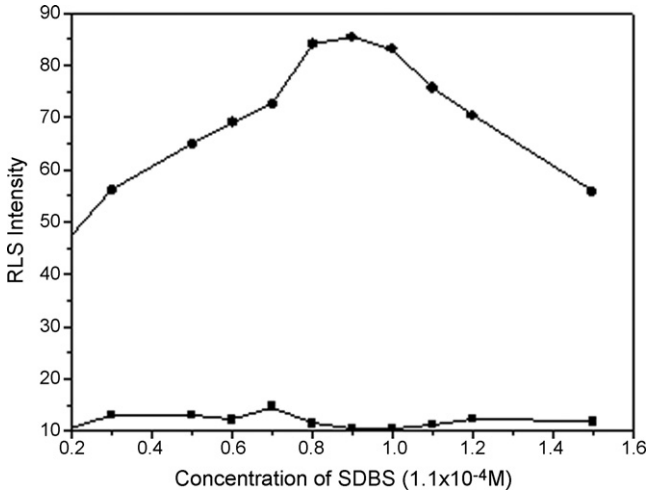

Fig. 4. Effect of SDBS concentrations on the RLS intensity. The symbols (■) and (●) represent I^0_{RLS} and ΔI_{RLS}, respectively. Conditions: DQC 0.96 μg/mL, pH 0.5.

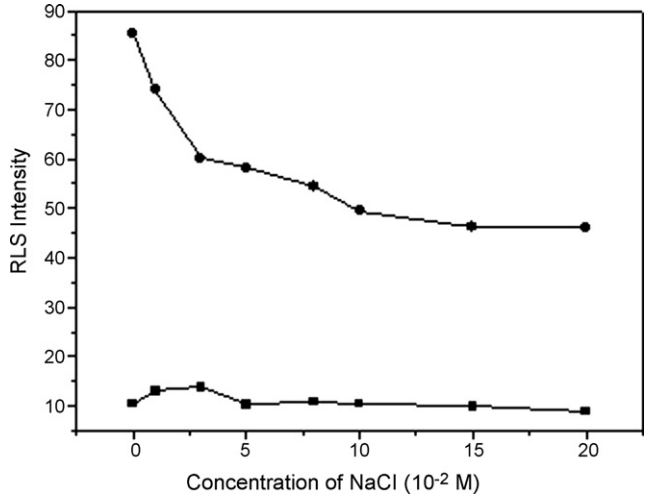

Fig. 5. Effect of ionic strength on the RLS intensity. The symbols (■) and (●) represent I^0_{RLS} and ΔI_{RLS}, respectively. Conditions: DQC 0.96 μg/mL, SDBS 9.9×10^{-4} M, pH 0.5.

Table 1
Effects of the potentially interfering substances on the determination of DQC (0.96 μg/mL) under the optimum conditions

Tolerance (μg/mL)	Level foreign substances
≥100	$CuSO_4$, $BaCl_2$, $CaCl_2$
≥50	Mannitol, NaCl
≥10	tartaric acid, D-fructose, tyrosine acid, KCl, [a]PEG, citric acid,
≥1	Glutamic acid, $ZnSO_4$, [b]fsDNA, histidine, lysine, glucose
≥0.1	$FeCl_3$, [c]BSA, pepsin, sodium acetate, cinnamic
0.05	[d]ctDNA, gelatin
1:100	Ethanol

[a] polyethylene glycol.
[b] bfish sperm DNA, fsDNA, fish sperm DNA.
[c] bovine serum albumin.
[d] calf thymus DNA.

decrease. Therefore, 9.9×10^{-4} M SDBS is considered suitable for this assay.

3.4. Effect of ionic strength and the addition order of reagents

The influence of the ionic strength on the RLS intensity of the assay system was studied by adding 0.5 M NaCl and the result was shown in Fig. 5. It can be observed that the enhanced RLS intensity of SDBS-DQC system reduced with the increasing of the ionic strength. The effects of electrostatic shielding of charges, including the shielding of DCQ molecules from SDBS or the shielding of SDBS from DCQ molecules increased with the concentration of the NaCl increasing, which reduced the combination of SDBS with DCQ and resulted in a decreased RLS signal. For the optimal binding of SDBS and DQC, the low ionic strength must be maintained. Assay systems were not including NaCl and the real samples were diluted approximate 1000-fold in order to eliminate the effect of ionic strength.

The effect of the addition order of reagents on the RLS intensity was tested. In the strong acid medium, DQC containing two positive charges can strongly bind to SDBS which contains negative charges by electrostatic force. However, in the addition orders of Buffer-SDBS-DQC and SDBS-DQC-Buffer systems, the DQC cannot be protonated and positively charged enough to combine with SDBS readily which resulted in the decreasing of the enhanced RLS intensity. Thus, the Buffer-DQC-SDBS was selected for the further study.

3.5. Incubation time and stability

Under the optimal conditions, the time required to react completely was studied by measuring the RLS intensity of SDBS-DQC systems per 2 min after mixing. The results showed that the reaction of SDBS and DQC occurred rapidly at room temperature and

Table 2
Analytical characteristics compared with other methods of determination DQC

Methods	Linear range	LOD
HPLC [2]	–	2 ng/mL
HPLC [3]	5–25 μg/mL	1.5 μg/mL
HPLC [4]	8–32 μg/mL	–
CE [5]	2.5–12.5 μg/mL	2 μg/mL
Spectrofluorimetric [7]	$6.38–1.60 \times 10^3$ ng/mL	1.9 ng/mL
Spectrofluorimetric [8]	0.041–30.0 μg/mL	12.3 ng/mL
Spectrofluorimetric [6]	0.024–4.00 μg/mL	8.11 ng/mL
The proposed method	0.096–2.88 μg/mL	2.98 ng/mL

Table 3
Analytical results of compound DQC in Dequalinium chloride lozenges

Samples (No.)	Found value (n = 3, μg/mL)	Standard added (μg/mL)	Total found value (μg/mL)	Recovery (n = 3, %)	R.S.D. (%)
1	0.69 ± 0.01	1.44	2.14, 2.09, 2.14	99.3	1.4
2	0.91 ± 0.01	1.44	2.48, 2.45, 2.50	108.3	1.2
3	1.29 ± 0.01	1.44	2.70, 2.71, 2.69	97.9	0.4

Table 4
Analytical results of compound DQC in human serum samples

Samples (No.)	Standard added (μg/mL)	Found value (μg/mL)	Recovery (n = 3, %)	R.S.D. (%)
1	0.96	0.95, 0.94, 1.01	101.0	3.9
2	1.44	1.56, 1.58, 1.42	105.6	5.1
3	1.92	1.99, 1.93, 1.89	101.0	2.6

reacted completely in the 10 min. The RLS intensity remained stable at least 2 h. Therefore, this method is practical for analytical application.

3.6. Selectivity and interference

Under the optimum conditions, effect of potentially interfering substances on RLS intensity in the assay system was detected and the results were shown in Table 1. It can be seen that the auxiliary materials in candy-based lozenges such as mannitol, tartaric acid, D-fructose, citric acid, glucose, sodium acetate can be tolerated at a high level. However, the other kind of auxiliary materials gelatin can be only tolerated at level of 0.05 μg/mL. The real samples of the candy-based lozenges containing DQC were pretreated with ethanol to remove the gelatin. Then the real samples were diluted 1000-fold to eliminate the interference of gelatin. At the same time, the concentration of DQC was in the linear range. In the human serum, most potentially interfering substances can be tolerated at high levels except DNA. However, DNA was not presented in the human serum which was pretreated before determination. Therefore, the selectivity of this proposed method was acceptable for the determination of DQC in the real samples.

3.7. Calibration and detection limit

Under the optimum conditions, the calibration curve was constructed according to above standard procedure. The linear regression equation was presented as $\Delta I_{RLS} = 8.01 + 176.57c(\times 2.4\ \mu g/mL)$ with regression coefficient $r = 0.9988$ ($n = 9$) and the linear range was 0.096–2.88 μg/mL. The detection limit was 2.98 ng/mL. The detection limit was given by $3S_0/K$, where 3 is the factor at 99% confidence level, S_0 is the standard deviation of the blank measurements ($n = 16$) and the K is the slope of the calibration curve.

The analytical characteristics of the established method compared with other methods of determination DQC was shown in Table 2. It can be seen that this method provided a sufficient sensitivity for the one-step determination of the trace amount of DQC in the real samples. Compared to the other complex methods, the proposed method is simple and sensitive for the quantification of the trace DQC in practice.

3.8. Analytical applications

3.8.1. Dequalinium chloride lozenges

6 units of Dequalinium chloride lozenges (purchased from Zhuhai Special Economic Zone biochemical pharmaceutical factory in China) were weighed and ground in a mortar. The adequate amount of the powder was taken and dissolved in an appropriate volume of ethanol by immersion in an ultrasonic bath for 10 min. Then filtered through a fine filter paper, transferred the filtrate into 100 mL volumetric flask and diluted to the mark with doubly distilled water. The variable volumes of the samples were taken for assay. The standard addition method was used to determine the recovery of the DQC and the results were shown in Table 3. From Table 3, it showed that the present method was acceptable for the determination of DQC in the Dequalinium chloride lozenges.

3.8.2. Human serum

5.0 mL human serum was taken from the healthy people who had not taken any kinds of medicines for 3 days before test. The proteins in the human serum were precipitated by adding approximate 10 mL acetonitrile and centrifuging (40 min at 4000 rpm) at the room temperature. Then the acetonitrile was removed under reduced pressure on a rotary evaporator with a water bath at a temperature of 60 °C. The human serum without proteins was dissolved in a 100 mL volumetric flask and diluted to the mark with doubly distilled water. For the analysis of DQC, the determination of the DQC recovery in the human serum samples was performed by the standard addition method and the results were shown in Table 4. It can be noticed that the present method had a good recovery ratio and was suitable for the assay of DQC in clinical detection.

4. Discussion

In this study, due to the electrostatic force and the π–π interaction between SDBS and DQC, the large particles were produced. The large particles resulted in the enhancement of the RLS intensity which was proportional to the concentration of the DQC in a certain range [11,16]. Based on the linear relationship, a novel method to determine the trace of DQC in the candy-based lozenges and human serum was established. The proposed method proved to be rapid, sensitive and selective; the time required to react completely was short; the experiment procedure was very simple and the operation was just performed on a common spectrofluorometer; the probe SDBS was very cheap and easy to get.

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