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# Assay of Niclosamide by Resonance Light Scattering Technique with Cetyltrimethylammonium Bromide

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A sensitive assay of niclosamide was developed based on the measurement of the enhanced resonance light scattering signals of cetyltrimethylammonium bromide by the addition of trace amount of niclosamide. Under the acid conditions, the interaction of niclosamide with cetyltrimethylammonium bromide results in great enhanced resonance light scattering signals, owing to the interaction between niclosamide and the cationic surfactants. It was found that the enhanced resonance light scattering intensity was proportional to the concentration of niclosamide in the range of $2.5 \times 10^{-7}$ to $1.0 \times 10^{-5}$ mol/L with a detection limit of $4.7 \times 10^{-8}$ mol/L. This method was simple, rapid and economical. The developed method was applied to the determination of niclosamide in real samples with satisfactory results.

**Key Words: Resonance light scattering, Cetyltrimethylammonium bromide, Niclosamide, Surfactant.**

## INTRODUCTION

Niclosamide (NIC, 2',5-dichloro-4'-nitrosalicylanilide; Fig. 1) is the only commercially available molluscicide recommended by WHO for largescale use in schistosomiasis control program. However, niclosamide is known to cause undesirable effects in several biological systems. At molluscicidal concentrations, niclosamide has been reported to induce harmful effects on several aquatic plants as well on phytoplankton[1]. In addition, an increase in the percentage of sperm anomalies in orally exposed mice to niclosamide was recorded[2]. It is therefore of great importance to develop sensitive methods for the determination of niclosamide. Methods for analysis of niclosamide include spectrophotometric methods[3-5] and liquid chromatography[6,7] and gas chromatography[8,9]. However, some of the procedures require indirect analysis of the hydrolysis product 2-chloro-4-nitroaniline and the treatment procedures are also very complicated. In this paper, a simple and sensitive method has been developed by using cetyltrimethylammonium bromide (CTMAB) as a probe coupled with a sensitive light scattering detection.

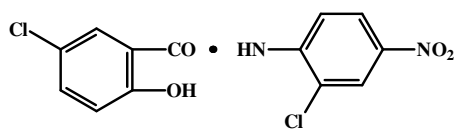


Fig. 1. Structure of niclosamide

Resonance light scattering (RLS) technique is an extremely sensitive and selective technique for monitoring molecular assemblies. Especially, resonance light scattering with the distinct advantages of speed, convenience and sensitivity, resonance light scattering is a very attractive technique to provide exact size and shape information of the supramolecular assemblies[10]. A number of excellent works have been reported with resonance light scattering technique for the determination of macromolecules such as DNA[11-15] and proteins,[16-20] only a few of works for the determination of small molecules have been reported[21,22]. However the using of resonance light scattering technique for the determination of niclosamide, a small molecule, has not yet been reported. With resonance light scattering measurements, it is found that under the appropriate conditions, a supramolecular complex is formed between niclosamide and cetyltrimethylammonium bromide (CTMAB) and result in great enhancement of the resonance light scattering intensities of the niclosamide-CTMAB system.

The experimental results show that the light scattering background of the CTMAB is very low. The great enhancement of light scattering originated from the aggregate formation by the interaction between niclosamide and CTMAB can be easily measured by using a common spectrofluorimeter equipped with a 150W high pressure Xenon lamp. Since the variation in enhanced resonance light scattering intensity is linearly related to the concentration of niclosamide added, we investigated the possibility of employing such a system in a simple

and rapid method for the quantitative determination of niclosamide in solution.

## EXPERIMENTAL

Cetyltrimethylammonium bromide (CTMAB) was procured from (Beijing Chemical Reagent Company) and its stock solution ($1.0 \times 10^{-2}$ mol/L) was prepared by dissolving 0.3644 g CTMAB in 100 mL volumetric flask with deionized water. Niclosamide (NIC) was purchased from Sigma Chemical Company (St. Louis, MO, USA). The stock solution was prepared by dissolving the product in N,N-dimethyl formamide solution. Its working solution was $1.0 \times 10^{-4}$ mol/L. The Britton-Robinson (B-R) buffer solution, being made up of 0.04 mol $L^{-1}$ phosphoric acid, 0.04 mol/L acetic acid, 0.04 mol/L boric acid, 0.2 mol/L sodium hydroxide, was used to control the acidity of the solution. 2.0 mol/L NaCl solution was used to adjust the ionic strength of the aqueous solution. All chemicals used were of analytical grade or the best grade commercially available and doubly deionized water was used throughout.

The light scattering spectra and the intensity of light scattering were recorded and measured by a Shimadzu Model RF-5301 spectrofluorimeter (Kyoto, Japan) with a quartz cuvette (1 × 1 cm). All absorbance measurements were measured with an UV-4501S ultraviolet spectrophotometer (Gandong Science Instrument Company, Tianjing). A PB-10 instrument (Sartorius, Beijing) was used to measure the pH of the solution.

**General procedure:** Into a 10 mL calibrated flask were added 0.5 mL DMF solution, 1.0 mL B-R buffer, 0.2 mL CTMAB working solution and appropriate niclosamide (or samples) and then diluted to 5.0 mL with water and stirred thoroughly. All resonance light scattering spectra were obtained by scanning simultaneously the excitation and emission monochromators ($\Delta\lambda = 0.0$ nm) from 250 to 700 nm with the excitation and emission slits 3.0 nm. The resonance light scattering intensities were measured at 385 nm. The enhanced resonance light scattering intensity of the system by proteins was represented as $\Delta I_{\text{resonance light scattering}} = I_{\text{resonance light scattering}} - I^0_{\text{resonance light scattering}}$ ($I_{\text{resonance light scattering}}$ and $I^0_{\text{resonance light scattering}}$ were the intensities of the systems with and without niclosamide).

## RESULTS AND DISCUSSION

The resonance light scattering spectra of the CTMAB-niclosamide system measured at different niclosamide concentrations are presented in Fig. 2. It can be seen that there are two weak resonance light scattering peaks for the CTMAB-niclosamide located at 385 and 556 nm. The weak resonance light scattering intensity of CTMAB increases greatly in the presence of niclosamide. And the intensity of the resonance light scattering signal, located at 385 nm, is the strongest. Therefore, 385 nm was selected as the analytical wavelength. Moreover, the enhanced resonance light scattering intensity is proportional to the concentration of niclosamide. Thus a novel and sensitive niclosamide assay was developed.

The absorption spectrum of niclosamide and niclosamide-CTMAB system are displayed in Fig. 3. The spectrum of niclosamide consists of one strong absorption peak at 338 nm. An addition of CTMAB causes a decrease in the intensity of the absorption spectra and a red-shift in the position of the band. The results also demonstrate that there is an intermolecular interaction between CTMAB and niclosamide.

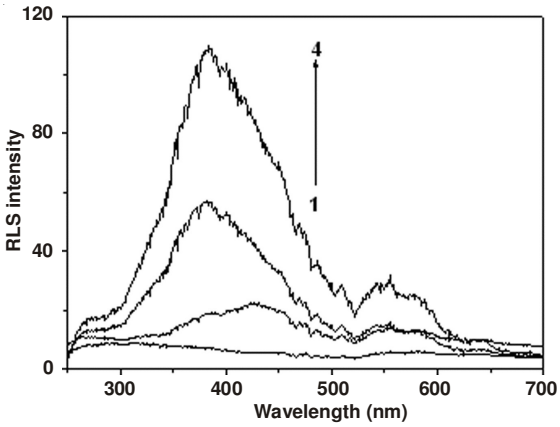


Fig. 2. resonance light scattering spectra of the CTMAB (line 1), niclosamide (line 2) and CTMAB-niclosamide system (lines 3-4). Conditions: $C_{CTMAB} = 4.0 \times 10^{-4}$ mol/L, $C_{niclosamide}$ (mol/L): 1, 0.0; 2, $4.0 \times 10^{-6}$ (without CTMAB); 3, $2.0 \times 10^{-6}$; 4, $4.0 \times 10^{-6}$, pH 2.5

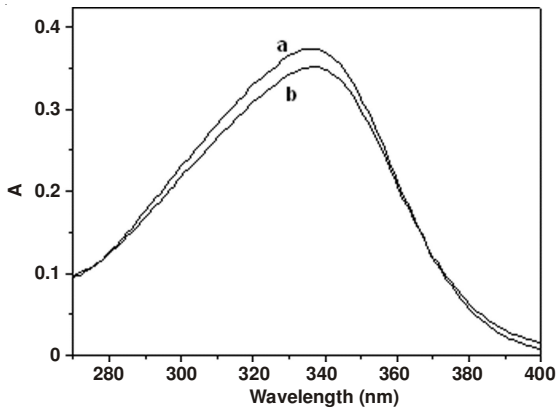


Fig. 3. Absorption spectra of niclosamide (a) and CTMAB-niclosamide system (b). Conditions: $C_{CTMAB} = 4.0 \times 10^{-4}$ mol/L, $C_{niclosamide} = 4.0 \times 10^{-6}$ mol/L, pH 2.5

**Optimization of the general procedures:** The pH of solution plays an important role in the aggregation of CTMAB with niclosamide. We adjusted the pH of the medium that decides the charge status of the niclosamide molecules and thereby affect the interaction. As shown in Fig. 4, acidity of solution has great influence on both the resonance light scattering intensity of the system and the formation of CTMAB-niclosamide complex. The resonance light scattering intensity of the system is increasing strengthened with increasing pH value. However, the enhanced resonance light scattering intensity reaches its maximum at pH 2.5. When pH value is greater than 2.5, decreased resonance light scattering intensity could be observed. So pH 2.5 was chosen for further studies.

Effect of CTMAB concentration is shown in Fig. 5. It can be seen that the enhanced resonance light scattering intensity increases with the increase of CTMAB concentration and reaches a maximum when the concentration of CTMAB is $4.0 \times 10^{-4}$ mol/L. While the concentration of CTMAB is less than $4.0 \times 10^{-4}$ mol/L, the resonance light scattering intesity of the system increases with the increasing of CTMAB concentration. It can be seen that the maximum $\Delta I_{\text{resonance light scattering}}$ of the system is reached in the $4.0 \times 10^{-4}$ mol/L CTMAB. There-

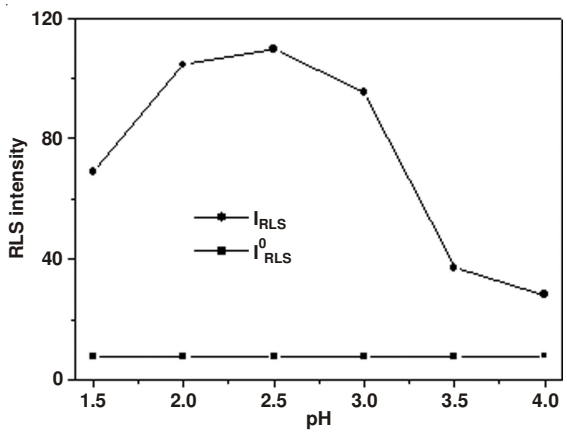


Fig. 4. Effect of pH on the resonance light scattering intensity. Conditions: $C_{CTMAB} = 4.0 \times 10^{-4}$ mol/L, $C_{niclosamide} = 4.0 \times 10^{-6}$ mol/L

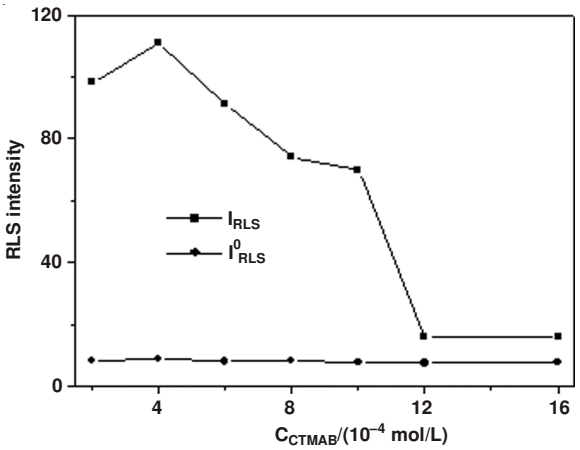


Fig. 5. Effect of CTMAB concentration on the resonance light scattering intensity. Conditions: $C_{niclosamide} = 4.0 \times 10^{-6}$ mol/L, pH 2.5

fore, a CTMAB concentration of $4.0 \times 10^{-4}$ mol/L was recommended for the subsequent research.

In order to verify the role of electrostatic interaction in the interaction process, the effect of ionic strength was examined by the addition of NaCl. As shown in Fig. 6, with the increase of the ionic strength of the aqueous solution, electrostatic factors prevented the interaction between them. Thus, a decreased resonance light scattering intensity was observed. As a result, the ionic strength of the aqueous medium should be kept relatively low in the assay in order to obtain strong resonance light scattering signals.

The time required to react completely was studied by measuring the resonance light scattering intensity values at a set niclosamide concentration every 5 min for at least 90 min immediately after mixing. As shown in Fig. 7, the reaction between CTMAB and niclosamide occurred rapidly at room temperature (< 5 min) and the resonance light scattering intensity remained stable at least 90 min. Thus, this assay does not require crucial timing and the stability of this assay is reasonable and acceptable for analytical application.

**Selectivity and interference:** Tolerance of foreign substance is very important for the analytical application of a proposed method. The effect of the foreign substance on the determination was tested by pre-mixing niclosamide with other foreign substances such as various common anions, under the

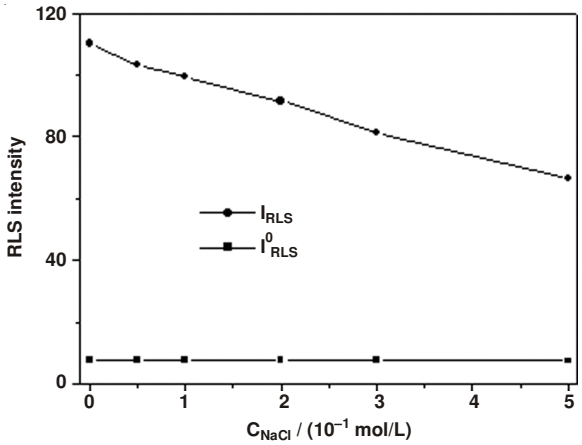


Fig. 6. Effect of ionic strength on the resonance light scattering intensity. Conditions: $C_{CTMAB} = 4.0 \times 10^{-4}$ mol/L, $C_{niclosamide} = 4.0 \times 10^{-6}$ mol/L, pH 2.5

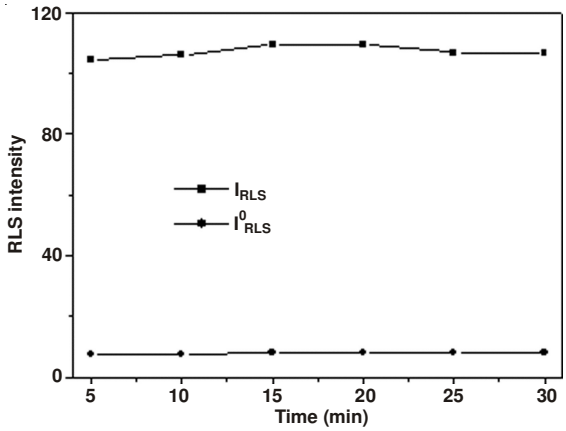


Fig. 7. Effect of incubation time on the resonance light scattering intensity. Conditions: $C_{CTMAB} = 4.0 \times 10^{-4}$ mol/L, $C_{niclosamide} = 4.0 \times 10^{-6}$ mol/L, pH 2.5

optimum conditions of the general procedure. The criterion for an interference was an $I_{resonance\ light\ scattering}$ value varying by more than 5 % from the expected value for niclosamide alone. There was no interference from the following common ions: 8.0 mmol/L of $Cl^-$ and $SO_4^{2-}$, 4.0 mmol/L of $Ca^{2+}$ and $Mg^{2+}$, 0.8 mmol/L of $Fe^{3+}$, 0.4 mmol/L of $Ba^{2+}$, 0.04 mmol/L of $Mn^{2+}$, 0.02 mmol/L of $Cu^{2+}$ and 1.2 mmol/L of ethanol. On the contrary, some other ions such as $Hg^{2+}$ only can be tolerated at relative low concentration levels (less than 0.02 mmol/L). Dilution with water can minimize all these interferences, which offers this method with the possibility of determination of trace amount of niclosamide in real samples.

**Calibration curve and detection limit:** Under optimal conditions according to the standard procedures, the calibration curve for niclosamide was constructed by increasing the niclosamide concentrations. As shown in Fig. 8, there is a good linear relationship between the resonance light scattering intensity and the concentration of niclosamide in the range $2.5 \times 10^{-7}$ to $1.0 \times 10^{-5}$ mol/L and the linear regression equation was calculated as $\Delta I_{resonance\ light\ scattering} = 26.3\ C\ (\times 10^{-6}$ mol/L)-1.5, with regression coefficient r = 0.9991 (n = 7). The detection limit was $4.7 \times 10^{-8}$ mol/L. The limit of detection (LOD) is given by $3S_0/S$, where 3 is the factor at the 99 % confidence

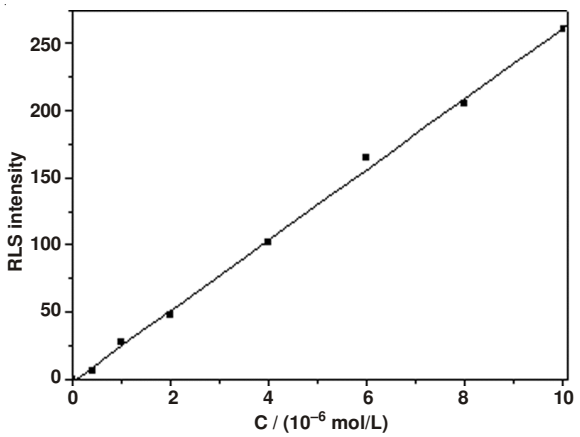


Fig. 8. Relationship between concentration of niclosamide and $\Delta I_{\text{resonance light scattering}}$

level, $S_0$ is the standard deviation of the blank measurements (n = 10) and S is the slope of the calibration curve.

**Sample determination:** To demonstrate the usefulness of CTMAB as a probe for the niclosamide analysis in real samples, the present method was applied to determine niclosamide in lake water samples. The results are listed in Table-1. The excellent recovery results (96-107 %) indicate that it can be applied to direct determination of niclosamide without separation of other constituents except the filtration of the suspensions. It is obvious that this method for the determination of the niclosamide is reliable, sensitive and practical in real samples.

TABLE-1
RESULTS FOR THE DETERMINATION OF NICLOSAMIDE IN LAKE WATER

| Sample No. | Found value ($10^{-6}$ mol/L) | Standard added ($10^{-6}$ mol/L) | Recovery value ($10^{-6}$ mol/L) | Recovery (n = 5, %) |
|---|---|---|---|---|
| 1 | 2.36 | 1.00 | 3.41 | 105 |
| 2 | 1.86 | 1.00 | 2.82 | 96 |
| 3 | 1.02 | 1.00 | 2.09 | 107 |

## Conclusion

In this work, a new step was made which consisted in the application of resonance light scattering technique to the studies of CTMAB interactions with niclosamide and led to a novel approach for the determination of niclosamide. The results showed that resonance light scattering method turned out to be a valuable and informative technique in the study and determination of niclosamide with CTMAB as a probe. The proposed method can be successfully applied to the determination of niclosamide in lake water samples. Therefore, we believe that the proposed assay has great potential for niclosamide assay in the field of environment measure and control science.

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