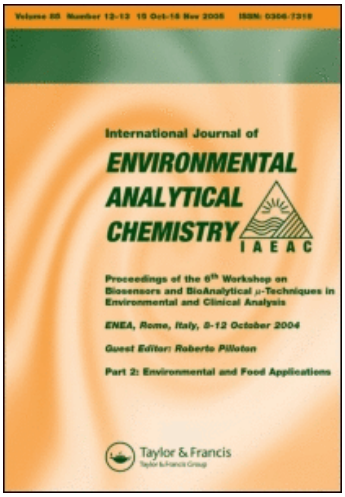

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Hou, Shicong , Cui, Yan , Du, Fengpei , Kong, Weifu , Song, Junfen , Li, Ying and Jie, Nianqin(2009) 'Determination of carbofuran by the quenching effect on resonance light scattering and its application to water and vegetable samples', International Journal of Environmental Analytical Chemistry, 89: 1, 59 — 66
To link to this Article: DOI: 10.1080/03067310802277373
URL: http://dx.doi.org/10.1080/03067310802277373

Intern. J. Environ. Anal. Chem.
Vol. 89, No. 1, 15 January 2009, 59–66

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Determination of carbofuran by the quenching effect on resonance light scattering and its application to water and vegetable samples

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(*Received 29 March 2008; final version received 16 June 2008*)

A new method for the determination of carbofuran (CF) with DNA by a resonance light scattering (RLS) technique was developed. The intensity of RLS (I_{RLS}) of DNA–HCl system was significantly quenched in presence of CF. A RLS peak at 315.6 nm was found, and the quenched intensity of RLS was proportional to the concentration of CF. The linear range of the calibration curve was $\approx$0.02–2.0 μg mL^{-1} and the detection limit (S/N = 3) 7 ng mL^{-1}. The CF in river water, cucumbers and rice samples was determined. The recovery rates were in the range of 90.0–111.1, 95.0–106, 93.0–111.0%, respectively. The mechanism of the reaction between CF and DNA is also discussed.

Keywords: carbofuran; resonance light-scattering method; DNA; quenching effect; river water; food samples

1. Introduction

The widespread use of pesticides in agriculture has undeniable repercussions in the environment and in the quality of natural waters, and it can become a serious environmental concern. It is, therefore, of interest to develop reliable analytical method to quantify pesticides at low concentration in natural water, vegetables and fruits.

Carbofuran(2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl-*N*-methyl carbamate) (CF), which is also known as Furadan (FMC) and Yaltox, is a broad-spectrum pesticide belonging to the *N*-methylcarbamate group. The major use of CF was for the control of weevils on alfalfa. Approximately, one-fourth of the total CF was applied to rice for control of water weevils. CF is registered for use in a variety of fruits, vegetables, grains and crops. It is widely used and can be found as pollutant in the water, soil and food samples. Their presence in water and food poses a potential hazard to human health. Its toxic properties include inhibitory effect on cholinesterase enzyme, violent convulsions and neuromuscular disturbance on inhalation. Due to its wide applicability and high toxicity, up to now, many techniques have been reported for the quantification of CF in vegetables, grains and environmental waters, which includes

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ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310802277373
http://www.informaworld.com

liquid chromatography [1–4], LC–MS [5,6], GC–MS [7,8], GLC [9,10], GC [11,12], flow injection [13,14], luminescence [15] and spectrophotometry [16]. These methods have good sensitivity and wide application. However, as far as we know, the use of nucleic acids (DNA) for the determination of CF by resonance light scattering (RLS) technique has not been reported.

Rayleigh scattering is a kind of scattering phenomenon. The RLS can be obtained with the simultaneous scan of the excitation/emission monochromator of a common spectrofluorometer by keeping $\Delta\lambda = 0$ nm. Huang and colleagues were the first in using this technique for trace level determination of DNA and set up a new method for molecules detection [17]. So far, the RLS technique has become very popular for the determination of biological macromolecules. Many studies on the RLS technique have been reported, however, most of the literature utilised the RLS phenomena to determine DNA [18–25]. In these methods, the enhancement of RLS by DNA was used. In this study, we firstly report DNA as a probe for the determination of CF by the quenching phenomena of the RLS. It was found that in presence of CF, RLS of DNA was remarkably quenched in acidic medium. A new method of determining CF was then developed with a common spectrofluorimeter. The recoveries of CF in natural water, cucumber and rice samples can be detected by this method with satisfactory results.

In addition, the study of interaction between the molecules of pesticides and DNA is important for us to understand the insecticidal mechanism of pesticides and their side effects, such as carcinogenesis, teratogenisis and mutagenesis. Therefore, the mechanism of the reaction between CF and DNA is also discussed in this article.

2. Experimental

2.1 *Reagents*

Stock solutions of DNA (100 mg mL^{-1}) were prepared by dissolving commercial Calf thymus DNA (ct DNA, Type 1 'Highly Polymerized', D-1501, Sigma Co., USA) with water. The solution was diluted to 10.0 μg mL^{-1} with water as working solution and fresh solutions were prepared each day. The solution of CF was made by dissolving 10.0 mg of CF (sample provided by Professor Canping Pan, China Agricultural University, $\mu = 98\%$) in 5.0 mL isopropanol, which was transferred into a flask and diluted to 10.0 μg mL^{-1} with water to make the working solution. HCl was prepared by diluting the hydrochloric acid (36–38%) (Chemical Reagent Co. of Beijing, China). Acetonitrile, acetone and NaCl were obtained from Chemical Reagent Co. of Beijing, China. River water was obtained from the Qing He River of Beijing. Cucumber and rice were purchased from grocery store of China Agricultural University.

All chemicals used were of analytical reagent grade and without further purification. The water used throughout was de-ionised and distilled.

2.2 *Apparatus*

All the light scattering spectra and intensity of light scattering were measured by a Hitachi F-4500 fluorescence spectrophotometer (Japan) with a 150W Xe lamp and quartz cell (1 cm). The pH measurements were made with a model pHS-3C pH meter

(Shanghai, China). The solid pulverizer obtained from Aglilent (Wilmington, USA) was used to crush cucumber and apple samples. EYELA rotary evaporator was used.

2.3 *Procedures*

The solutions were added to a 10 mL volumetric flask in the following order: CF, HCl, DNA. The mixture was diluted to 10 mL with water, mixed thoroughly, and allowed to stand for 15 min. The mixture was transferred to a 1 cm quartz cell for RLS measurements. The same quartz cell was used for the blank solutions. The RLS spectrum was obtained by scanning simultaneously the excitation and emission monochromators (namely, $\Delta\lambda = 0$ nm) from 200 to 600 nm. The quenching extent of RLS of the DNA–HCl system by CF was represented as $\Delta I_{RLS} = I^0_{RLS} - I_{RLS}$. The I_{RLS} and I^0_{RLS} are the intensity of the system with and without CF.

2.4 *Samples preparation*

Water sample: Water was taken from the river and allowed to stand for 24 h after which it was filtrated using filter paper. A suitable volume of the water was taken for analysis.

Cucumber sample: A 200 g cucumber was crushed by a solid pulverizer at 12,000 r min^{-1} for 1 min, and then the chopped tissue was transferred into a 500 mL beaker and stored in a freezer. A portion of 10 g frozen chopped tissue was placed in a 200 mL beaker together with 50 mL acetonitrile, allowed to churn for 3 min. The mixture was filtered under vacuum through a Büchner funnel using a piece of filter paper. The container and the filter paper were washed twice with 100 mL of acetonitrile, and the combined extracts were collected in a conical flask. A 10 g of Na_2SO_4 was added to remove water and 1 g of activated carbon was added to remove the pigment. The solution was shaken for 10 min, and then allowed to stand for 1.5 h. The activated carbon was filtered using filter paper. The acetonitrile was removed under reduced pressure on a rotary evaporator with a water bath temperature maintained at 40°C. The residue was dried under a gentle stream of nitrogen and re-dissolved in 100 mL flask. A suitable volume of the solution was taken for analysis.

Rice sample: An amount of 5 g of rice samples was weighted and placed in a conical flask. It was extracted using 20 mL of 0.25 mol L^{-1} sulphuric acid for about 12 h. After extraction, the extract was filtered and washed with water 2–3 times in order to remove excess residues in the conical flask. A suitable volume of the solution was taken for analysis.

3. Results and discussion

3.1 *Light scattering spectra*

The light scattering spectra of DNA–HCl system; DNA–CF–HCl system; DNA system; CF system and CF–HCl system are shown in Figure 1. They show that the intensity of RLS of CF or calf thymus DNA in aqueous solution is very small when they exist separately or mixed. However, when the DNA is mixed with HCl, its light scattering is strongly enhanced in the wavelength range 200–600 nm and the maximum scattering peak is located at 315.6 nm. In presence of CF, the intensity of light scattering was remarkably quenched. The wavelength of 315.6 nm was selected for further studies.

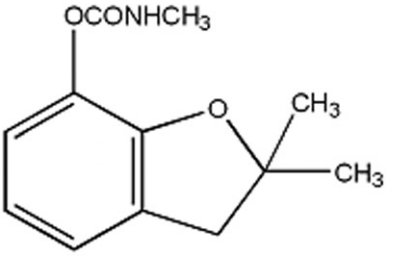

Figure 1. Structure of CF.

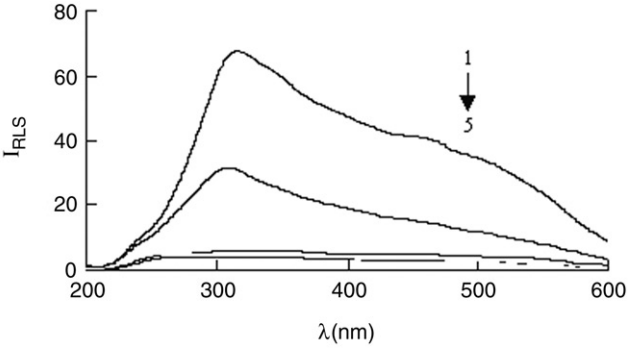

Figure 2. RLS spectra of CF–DNA–HCl system. 1. DNA–HCl; 2. DNA–CF–HCl; 3. DNA; 4. CF–HCl; 5. CF Conditions: Cf 0.6 μg mL^{-1}, DNA 1.4 μg mL^{-1}, pH = 2.22.

3.2 *Effect of addition sequence of the reagents and stability of the system*

The sequence of addition for this system was investigated. The results showed that the optimal order of addition of the reagents is CF–HCl–DNA. The effect of time on the RLS intensity was also studied under the optimum conditions. The results show that the ΔI_{RLS} reached to a maximum at 15 min after all the reagents had been added and remained stable at least 1 h. In this study, 15 min was set as the standard for all the measurements.

3.3 *Effect of pH*

The effects of pH on the RLS intensities are shown in Figure 3. The RLS intensity of the system increased firstly with increasing HCl concentration and then decreased. It can be seen that the maximum ΔI_{RLS} is obtained at a pH of about 2.22. The 0.1 mol L^{-1} HCl was used to adjust the pH value in this experiment. Further studies demonstrated that the optimum volume of 0.1 mol L^{-1} HCl was 0.6 mL.

3.4 *Effect of DNA concentration*

At the optimal pH, ΔI_{RLS} were determined with different concentration of DNA. As is shown in Figure 4, the ΔI_{RLS} of the system was increased firstly with the increasing concentration of DNA and then decreased. The concentration of DNA was chosen as 1.0 μg mL^{-1} for further study, and 1.4 mL DNA solution was added in the system.

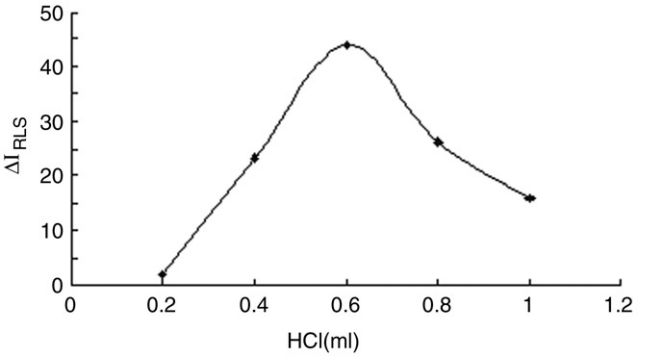

Figure 3. The effect of HCl concentration on the intensity of RLS. Concentractions: CF 1.0 μg mL^{-1}; DNA 1.0 μg mL^{-1}; HCl 0.1 mol L^{-1}.

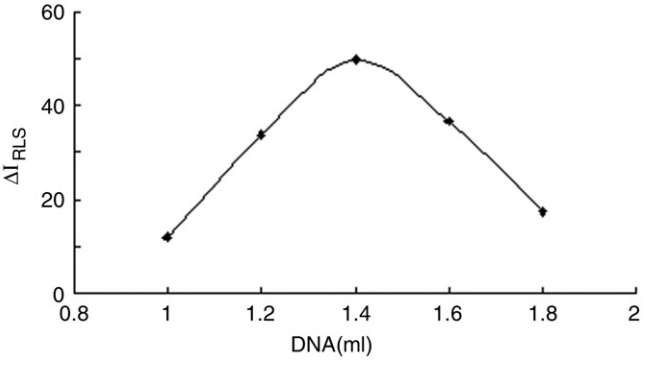

Figure 4. The effect of volume of DNA on the intensity of RLS. Concentractions: CF 1.0 μg mL^{-1}; pH 2.2; DNA 1.0 μg mL^{-1}.

3.5 *Effect of ionic strength*

The effect of the ionic strength on the light scattering intensity of DNA and CF in acidic medium was studied. In this system, NaCl is used to control the ionic strength. When the concentration of NaCl changes from 0.01 to 0.04 mol L^{-1}, there is no change of light scattering intensity. Thus, the system should avoid adding concentrated strong electrolyte.

3.6 *Calibration curve and sensitivity*

The calibration graphs for the determination of CF were drawn. Under optimal conditions, the relationship between ΔI_{RLS} and the concentration of CF was obtained. The limit of detection (LOD) was given by the equation, $LOD = KS_0/S$, where K is a numerical factor chosen according to the confidence level desired, S_0 is the standard deviation of the blank measurements ($n = 6$) and S is the slope of the calibration curve. Here a value of 3 for K was used, where 3 was the factor at the 99.7% confidence level. All the analytical parameters are presented in Table 1.

Table 1. Analytical parameters of different kinds of CF systems.[a]

	Linear range ($\mu g\,mL^{-1}$)	Linear regression eq. (C: $\mu g\,mL^{-1}$)	LOD ($ng\,mL^{-1}$)	Correlation coeff. (r)
CF	0.02–0.6	$\Delta I = 49.835C + 1.252$	0.09	0.9776
	0.6–2.0	$\Delta I = 14.016C + 22.635$	0.07	0.9914

Note: [a]Concentrations: DNA, $1.4\,\mu g\,mL^{-1}$; pH 2.22.

Table 2. Interference of foreign substances.[a]

Foreign substance	Concentration ($\mu g\,mL^{-1}$)	Relative error (%)
NH_4^+, SO_4^{2-}	0.5	3.2
K^+, Cl^-	2.5	2.2
Mg^{2+}, Cl^-	3.0	−2.6
Pb^{2+}, Ac^-	1.5	3.6
NH_4^+, Cl^-	0.8	4.1
Ca^{2+}, Cl^-	2.5	−3.6
Sodium lignin sulfonate	0.1	−4.8
Arabic gum	0.1	−5.0

Note: [a]Concentrations: CF: $0.5\,\mu g\,mL^{-1}$; DNA : $1.0\,\mu g\ mL^{-1}$; pH 2.22.

Table 3. Recovery of CF river water, cucumber and rice samples.

Sample	Added ($\mu g\,mL^{-1}$)	Found ($n = 3$, $\mu g\,mL^{-1}$)	Recovery (%)	RSD (%)
River water	1.0	1.03	103.0	3.3
		1.06	106.0	
		1.09	109.0	
	1.5	1.47	98.0	4.6
		1.35	90.0	
		1.47	98.0	
	2.0	2.02	101.0	6.1
		2.22	111.0	
		2.00	100.0	
Cucumber	1.0	1.00	100.0	6.0
		1.06	106.0	
	1.5	1.43	95.0	6.6
		1.61	107.0	
		1.59	106.0	
Rice	1.00	1.06	106.0	6.0
		1.11	111.0	
		1.03	103.0	
	1.50	1.68	112.0	9.1
		1.43	95.0	
		1.65	110.0	
	2.00	1.94	97.0	2.8
		1.90	95.0	

3.7 *Interference of foreign substances*

The influence of various substances, including common anions and cations on the RLS assay for CF was investigated. The results are listed in Table 2. It can be seen that the substances tested can be tolerated at relatively higher levels.

3.8 *Recovery test*

A known amount of CF was added to each sample at the level of 0.5–2 $\mu g\,mL^{-1}$ and the CF concentration was determined by the above procedures. The recoveries of CF in river water, cucumber and rice are summarised in Table 3. The recoveries of CF in samples were satisfactory.

3.9 *Mechanism of the interaction between CF and DNA*

As shown in Figure 2, the light scattering of CF, HCl and calf thymus DNA in aqueous solution are very small when they exist separately. The light scattering is strongly enhanced when the DNA is mixed with HCl. It was reported that HCl solution is a good probe for the determination of DNA [19]. In presence of CF, the intensity of RLS is significantly quenched.

The mechanism of the quenching phenomena of RLS was discussed as follows. The double-helical structure of native DNA results from the formation of hydrogen bonds between different base pairs. When the atoms of the bases in DNA are binding with H^+ forming cations, the hydrogen bonds of the base pairs are destroyed. The double-helical structure of native DNA is unwound and separated in HCl solution, and the single-stranded DNAs aggregate to form large particles. Therefore, the strong enhancement of light scattering intensity of DNA is observed. In this system, we consider that there is a nitrogen atom in CF which bonds with H^+ strongly, and the concentration of the H^+ decreases. Thus, the nitrogen atoms of the bases in DNA cannot bind H^+ to form cation and the single-stranded DNAs cannot be produced, and no large particles be formed. On the other hand, DNAs carry negative charge and protonised CF carry positive charge. They are attracted to each other to form an ion-association complex because of carrying different electric charges. The size of associated particles is much smaller than that of the large particles formed by the aggregation of the single-stranded DNAs. We all know that the intensity of RLS is proportional to the size of particle, therefore, the intensity of RLS of the ion-association complex is much lower than that of the aggregated single-stranded DNAs. This is probably the cause of the strong quenching of the light scattering intensity in the DNA–HCl system in the presence of CF.

4. Conclusion

In this work, a new RLS quenching method for the determination of CF has been reported. The method has satisfactorily been used for the determination of CF in actual samples. The method is simple, quick and stable. To our knowledge, DNA is scarcely used as a probe for the determination of CF. This research is practical and meaningful, especially for the study on the reaction between pesticides and DNA.

Acknowledgements

We are grateful to the financial support obtained from the National Natural Science Foundations of China (No. 20575005).

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