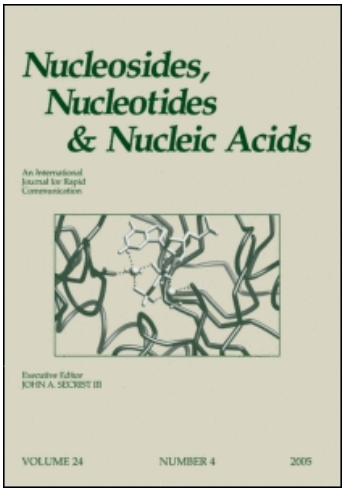

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Study on the Interaction Between Nucleic Acids and Imidacloprid and Its Application

Shicong Hou[a]; Xiaofeng Jiang[a]; Fengpei Du[a]; Huifang Cong[b]; Ying Li[a]; Nianqin Jie[a]
[a] College of Science, China Agricultural University, Beijing, P. R. China [b] Yantai Research Institute, China Agricultural University, Yantai, P. R. China

Online publication date: 24 November 2009

To cite this Article Hou, Shicong , Jiang, Xiaofeng , Du, Fengpei , Cong, Huifang , Li, Ying and Jie, Nianqin(2009) 'Study on the Interaction Between Nucleic Acids and Imidacloprid and Its Application', Nucleosides, Nucleotides and Nucleic Acids, 28: 11, 989 — 997
To link to this Article: DOI: 10.1080/15257770903362156
URL: http://dx.doi.org/10.1080/15257770903362156

Nucleosides, Nucleotides and Nucleic Acids, 28:989–997, 2009

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770903362156

Taylor & Francis
Taylor & Francis Group

STUDY ON THE INTERACTION BETWEEN NUCLEIC ACIDS AND IMIDACLOPRID AND ITS APPLICATION

Shicong Hou,[1] Xiaofeng Jiang,[1] Fengpei Du,[1] Huifang Cong,[2] Ying Li,[1] and Nianqin Jie[1]

[1]*College of Science, China Agricultural University, Beijing, P. R. China*
[2]*Yantai Research Institute, China Agricultural University, Yantai, P. R. China*

□ *The determination of imidacloprid with DNA via a resonance light scattering (RLS) technique was developed. The RLS of DNA was remarkably quenched after adding imidacloprid in aqueous medium of pH 2.10. An RLS peak at 311 nm was found, and the quenched intensity of RLS at this wavelength was proportional to the concentration of imidacloprid. The linear range of the calibration curve was approximately 0.02–2 μg/mL with the detection limit (S/N = 3) of 0.02 ng/mL. The imidacloprid in river water, cucumbers, and apple samples was determined. The recovery rates were in the range of 91.9% to 95.20%, 97.2% to 111.3%, and 94.5% to 114.8%, respectively. The mechanism of the reaction between imidacloprid and DNA is also discussed.*

Keywords Nucleic acids (DNA); imidacloprid; resonance light-scattering method; quenching effect

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 methods to quantify pesticides at low concentration in natural water, vegetables, and fruits.

Imidacloprid is a neonicotionid insecticide with an extraordinary activity in very small amounts. It acts as an agonist of acetylcholine by binding to the postsynaptic nicotinic receptors in the insect central nervous system.[1] Nowadays it is being used as a seed dressing, as a soil treatment and as a foliar

Received 2 March 2009; accepted 8 September 2009.
This work was supported by the National Natural Science Foundations of China (No.20575005).
Address correspondence to Nianqin Jie, College of Science, China Agricultural University, Beijing, 100193, P. R. China. E-mail: Nqjie826@cau.edu.cn

treatment in different crops and citrus fruits. Up to now, there are many techniques, such as high performance liquid chromatography (HPLC),[2–6] gas chromatography,[7–8] photochemical induced fluorescence,[9–10] infrared spectroscopy,[11] and RP-HPLC,[12] to determine imidacloprid in vegetables and environmental waters. These methods have good sensitivity and wide application. However, as far as we know, the use of nucleic acids (DNA) for the determination of imidacloprid by a resonance light scattering (RLS) technique has not been reported.

Rayleigh scattering is a kind of scattering phenomenon. The technique suffers from the disadvantages of low sensitivity and selectivity. RLS can be obtained with the simultaneous scan of the excitation/emission monochromator of a common spectrofluorometer by keeping $\Delta\lambda = 0$ nm. Huang et al.[13] first used this technique for a trace level of nucleic acids assay and set up a new method of molecule detection. 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 literatures[14–18] has utilized the RLS phenomena to determine nucleic acids. In these methods, the enhancement of RLS by nucleic acids was used. In this study, we first report nucleic acids as a probe for the determination of imidacloprid by the quenching phenomena of the RLS. It was found that RLS of DNA was greatly quenched after adding imidacloprid in acidic medium. A new method of determining imidacloprid was then developed with a common spectrofluorimeter. The recovery of imidacloprid in natural water, cucumber, and apple samples can be detected by this method with satisfactory results. In comparison with other methods, the method is quick, simple and stable, but the method is only suitable to the determination of imidacloprid. High performance liquid chromatography and gas chromatograph methods are able to detect many pesticides, which is an advantage over this method.

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 imidacloprid and DNA is also discussed in this article.

EXPERIMENTAL

Reagents and Apparatus

Stock solutions of nucleic acids (100 mg/mL) were prepared by dissolving commercial calf thymus DNA (ct DNA, Sigma, USA) with water. The solution was diluted to 10.0 μg/mL with water as working solution and fresh solutions were prepared each day. The solution of imidacloprid was made by dissolving 10.0 mg of imidacloprid (sample provided by Professor Canping

Pan, China Agricultural University, $w\% = 98\%$) in 5.0 mL 2-propanol, which was transferred into a flask diluted and diluted to 10.0 μg mL^{-1} with water to afford the working solution. HCl was prepared by diluting the hydrochloric acid (36–38%) (Chemical Reagent Co. of Beijing, China). Acetonitrile, acetone, NaCl were also obtained from Chemical Reagent Co. of Beijing. River water was obtained from the Qing He River in Beijing. Cucumber and apple were purchased from the vegetable mart of China Agricultural University.

All chemicals used were of analytical reagent grade and without further purification. Deionized distilled water was used throughout.

All the light scattering spectra and intensity of light scattering were measured by a Hitachi F-4500 fluorescence spectrophotometer (Japan) with a 150 W 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 (USA) was used to crush cucumber and apple samples. EYELA rotary evaporator was used.

Procedures

The solutions of DNA, imidacloprid, HCl were added successively into a 10 mL volumetric flask. The mixture was diluted to 10 mL with water, mixed thoroughly, and allowed to stand for 15 minutes. The mixture was transferred to a 1 cm quartz cell for RLS measurements, which were made against 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 imidacloprid was represented as $\Delta I_{RLS} = I^{\circ}_{RLS} - I_{RLS}$. The I_{RLS} and I°_{RLS} are the intensity of the system with and without imidacloprid.

Samples Preparation Procedure

Water sample: Water was taken from the river and allowed to stand for 24 hours, after which it was filtrated using filter paper. A known amount of imidacloprid was added successively to each sample at a level of 0.5–2 μg/mL, and the concentration of imidacloprid were determined by the above procedures.

Cucumber sample: An amount of 200 g of cucumber was crushed by solid pulverizer at 12000r min^{-1} for 1 minute, 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 minutes. The mixture was filtered under vacuum through a Büchner funnel using a 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 portion Na_2SO_4

was added to remove water in solution and 1 g of activated carbon was added to remove the pigment. The solution was shaken for 10 minutes, and then allowed to stand for 1.5 hours. The activated carbon was filtered using filter paper. The acetonitrile was removed under reduced pressure on a rotary evaporator with a water bath temperature less than 40°C. The residue was dried under a gentle stream of nitrogen and redissolved in 100 mL scale flask. A suitable volume of the solution was taken for analysis.

Apple sample: An amount of 200 g of apple was crushed by solid pulverizer at 12000r min^{-1} for 1 minute, 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 paced in a 200 mL beaker together with 25 mL acetonitrile. A 10 g Na_2SO_4 was added to remove water in solution and 1 g activated carbon was added to remove the pigment. The solution was shaken for 10 minutes, and then allowed to stand for 1.5 hours. The activated carbon was filtered using filter paper. The acetonitrile was removed under reduced pressure on a rotary evaporator with a water bath temperature less than 40°C. The residue was dried under a gentle stream of nitrogen and redissolved in 100 mL scale flask. A suitable volume of the solution was taken for analysis.

RESULTS AND DISCUSSION

Light Scattering Spectra

The light scattering spectra of DNA-HCl (1), DNA-Imidacloprid-HCl (2), DNA (3), Imidacloprid (4), and Imidacloprid-HCl (5), are shown in Figure 1. It shows that the RLS of imidacloprid or calf thymus DNA in aqueous solution is very small when they exist separately or are mixed. However, when the DNA is mixed with HCl, its light scattering is strongly enhanced in the wavelength range of 200 to 600 nm and the maximum scattering peak is located at 311 nm. After adding imidacloprid the intensity of light scattering was remarkably quenched. The wavelength of 311 nm was selected for further studies.

Effect of Addition Sequence of the Reagents and Stability of the System

The sequence of addition for this system was investigated. The results are shown in Table 1. It can be seen that the order of adding DNA-imidacloprid-HCl is optimal. The effect of time on the RLS intensity was also studied under the optimum conditions. The results showed that the ΔI_{RLS} reached a maximum at 10 minutes after all the reagents had been added and remained stable at least 1 hours. In this study, 10 minutes was set as the standard for all the measurements.

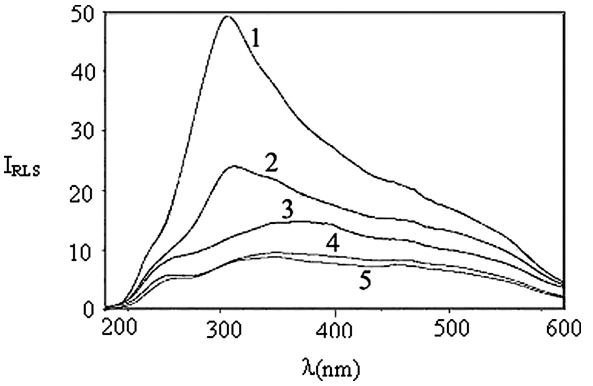

FIGURE 1 Resonance Lightlight- scattering spectra of Imidacloprid-DNA. 1) DNA-HC l; 2) DNA -Imidacloprid-HCl; 3) DNA; 4) Imidacloprid; 5) Imidacloprid-HCl. Concentrations: imidacloprid, 1.0 μg/mL; DNA, 1.0 μg/mL; PH = 2.10.

Effect of pH

The effects of pH on the RLS intensities are shown in Figure 2. The RLS intensity of the system increased initially with increasing hydrochloric acid concentration and then decreased. It can be seen that the maximum ΔI_{RLS} is obtained at a pH of about 2.1. The 0.1 mol/L hydrochloric acid was used to adjust the pH value in this experiment. Further studies demonstrated that the optimum volume of 0.1 mol/L hydrochloric acid was 0.8 mL.

Effect of DNA Concentration

At the optimal pH, ΔI_{RLS} were determined with different concentration of DNA. As is shown in Figure 3, the ΔI_{RLS} of the system was increased initially with the increasing concentration of DNA and then decreased. The concentration of DNA was chosen of 1.0 μg/mL for further study, and 2.0 mL DNA solution was added in the system.

Effect of ionic strength

The effect of ionic strength on the light scattering intensity of DNA and imidacloprid in acidic medium was studied. In this system, NaCl is used to control the ionic strength. When NaCl concentration ranges from 0.01 to

TABLE 1 The effect of order of addition

Order of addition	Change of ΔI_{RLS}	Average of ΔI_{RLS}	RSD (%)
DNA-HCl-Imidacloprid	6.59, 6.62, 4.65	5.95	19.0
DNA-Imidacloprid-HCl	22.09, 20.14, 20.11	20.78	5.5
HCl-Imidacloprid-DNA	16.44, 15.90, 17.73	16.69	5.6

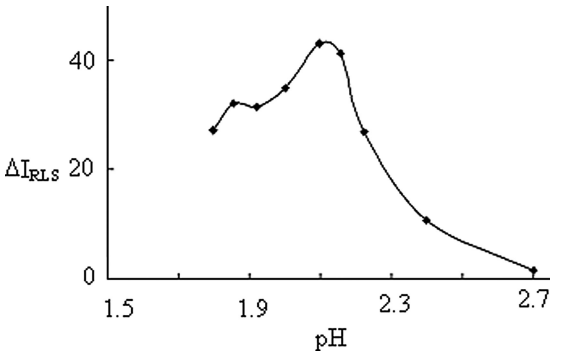

FIGURE 2 The effect of pH on the intensity of RLS concentrations: imidacloprid, 1.0 μg/mL, DNA: 1.0 μg/mL.

0.04 mol/L, there is no change of light scattering intensity. Thus, the system should avoid addition of concentrated strong electrolyte.

Interference of Foreign Substances

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

Calibration Curve and Sensitivity. At the optimal conditions, a calibration graph of a series of standard solutions of imidacloprid (0.02–2.0 μg/L) provided the following linear relationship: $\Delta I = 52.08C + 0.6006$ (C: μg/L), the limit of detection was 0.02 ng/mL (S/N = 3) and the correlation coefficient (r^2) was 0.9805.

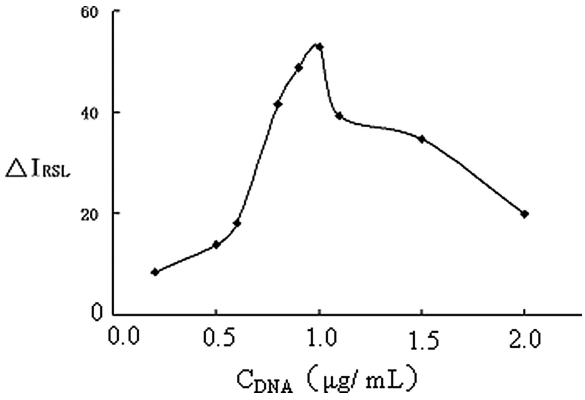

FIGURE 3 The effect of volume of DNA on the intensity of RLS concentrations: imidacloprid: 1.0 μg/mL; pH = 2.1.

TABLE 2 Interference of foreign substances[a]

Foreign substance	Concentration	Relative error (%)
NH_4^+, SO_4^{2-}	1.75×10^{-5}mol/L	−3.8
K^+,Cl^-	1.34×10^{-6}mol/L	2.8
Na^+,Cl^-	5.0×10^{-5}mol/L	−4.7
Pb^{2+}, Ac^-	7.89×10^{-5}mol/L	−4.8
NH_4^+, Cl^-	1.87×10^{-5}mol/L	3.4
Ca^{2+},Cl^-	9.1×10^{-6}mol/L	−1.9
Sodium lignin Sulfonate	2.0 μg/mL	3.5
Carbofuran	1.5 μg/mL	4.1
Cabrbaryl	1.5 μg/mL	4.8
Atrazine	2.0 μg/mL	4.5

[a]Concentrations: imidacloprid : 0.5 μg/mL; DNA:1.0 μg/mL; pH = 2.10.

Recovery Test

A known amount of imidacloprid was added successively to each sample at the level of 0.5–1.5 μg/L, and the imidacloprid concentration was determined by the above procedures. The recovery of imidacloprid in river water, cucumber and apple are summarized in Table 3. The recoveries of imidacloprid in samples were satisfactory.

Mechanism of the Interaction Between Imidacloprid and DNA

As is shown in Figure 2, the light scattering of imidacloprid, hydrochloric acid and calf thymus DNA in aqueous solution is very small when they exist separately. Its light scattering is strongly enhanced when the DNA is mixed with hydrochloric acid. Adding imidacloprid to DNA-HCl solution, the intensity scattering at 311 nm peak is remarkably quenched.

The mechanism of the quenching phenomena of RLS is discussed as follows. The double-helical structure of native DNA results from the formation hydrogen-bonds between different base pairs, and Li et al.[14]

TABLE 3 Recovery of imidacloprid in samples

Sample	Added(μg/mL)	found (n = 3, μg/mL)	Recovery (%)	RSD (%)
River water	0.5	0.476	95.2	3.5
	1.0	0.94	94.0	3.0
	1.5	1.40	93.0	4.5
Cucumbers	0.5	0.486	97.2	3.5
	1.0	1.113	111.3	3.7
	1.5	1.505	100.3	5.3
Apples	0.5	0.473	95.0	4.1
	1.0	1.14	114.0	5.5
	1.5	1.63	108.8	4.9

reported that the properties of hydrogen bonding depends on the ionic form of the bases, which in turn depends on the pH value of the solution. When the nitrogen atoms of the bases in DNA bind H^+ to form cations, the hydrogen bonds of the base pairs will be destroyed. Therefore, the double-helical structure of native DNA is unwound and separated in HCl solution, and the single stranded DNAs aggregate to form large particles, and the strong enhancement of the light scattering intensity of nucleic acids is observed. In this system, we consider that there are two nitrogen atoms in imidacloprid, which can bond with H^+ strongly, therefore the concentration of the H^+ decreases and the pH rises in solution. Thus, the nitrogen atoms of the bases in DNA cannot bind H^+ to form a cation and the single stranded DNAs cannot be produced, and no large particles are formed. This is probably the cause of the strong quenching of the light scattering intensity in the DNA-HCl system after adding imidacloprid.

REFERENCES

1. Moffat, A.S. New chemicals seek to outwit insect pests. *Science* **1993**, 261, 550–551.
2. Gilgarcia, M.D.; Galera, M.M.; Valverde, R.S.; Galanti, A.; Girotti, S. Column switching liquid chromatography and post-column photochemically flworescence detection to determination imidacloprid and 6-chloronicotinic acid in honeybees. *J. Chromatogr. A* **2007**, 1147, 17–23.
3. Fenandez-Alba, A.R.; Valvercle, A.; Contreras, M.; Chiron, S. Mariano contreras and serge chiron determination of imidacloprid in vegetables by high-performance liquid chromatography with diode-array detection. *J. Chromatogr. A* **1996**, 721, 97–105.
4. Obana, H.; Okihashi, M.; Kutsu, K.A.; Kitagawa, Y.; Hori, S. Determination of neonicotinoid pesticide residues in vegetables and fruits with solid phase extraction and liquid chromatography mass spectrometry. *J. Agric. Food Chem.* **2003**, 51, 2501–2505.
5. Ferrer, I.; Thurman, E.M.; Fernandez-Alba, A.R. Quantitation and Accurate Mass Analysis of Pesticides in Vegetables by LC/TOF-MS. *Anal. Chem.* **2005**, 77, 2818–2825.
6. Muccio, A.D.; Fidente, P.; Barbini, A.D.; Dommarco, R.; Seccia, S.; Morrica, P. Application of solid-phase extraction and liquid chromatography–mass spectrometry to the determination of neonicotinoid pesticide residues in fruit and vegetables. *J.Chromatogr. A* **2006**, 1108, 1–9.
7. Macdonald, L.M.; Meyer, T.R. Determination of Imidacloprid and Triadimefon in White Pine by Gas Chromatography/Mass Spectrometry. *J. Agric. Food Chem.* **1998**, 46, 3133–3138.
8. NaValca, A.; Gonzalez-Casado, A.; El-khatabj, R.; Vichez, J.L.; Feranandez, A.R. Determination of imidacloprid in vegetable samples by gas chromatography–mass spectrometry. *Analyst* **1997**, 122:579–581.
9. Flores, J.L.; Díaz, A.M.; Fernandoz De Córdova, M.L. Development of a photochemically induced fluorescence-based optosensor for the determination of imidacloprid in peppers and environmental waters. *Talanta* **2007**, 72, 991–997.
10. Vilchez, J.L.; Valencia, M.C.; Navalón, A.; Molinero-Morales, B.; Capitán-Vallvey, L.F. Flow injection analysis of the insecticide imidacloprid in water samples with photochemically induced fluorescence detection. *Anal. Chim. Acta* **2001**, 439, 299–305.
11. .Ma, G.X.; Wang, C.L.; Fan, D.W.; Xing, D.; Qian, L.; Wang, J.H.; Liu, S.H. Quantitative determination of imidacloprid by interared aborption spectrometry. *Spectroscopy and Spectral Analysis (Chinese)* **2006**, 126, 434–437.
12. Liu, G.S.; Li, T.T. RP-HPLC method for determination of imidacloprid. *Guangdong Chemical Industry (Chinese)* **2004**, 7, 29–30.
13. Huang, C.Z.; Li, K.A.; Tong, S.Y. Determination of nucleic acid by a resonance light-scattering technique with $\alpha,\beta,\gamma,\delta$-tetrakis [4-(trimethylammoniumy) pheny]porphine. *Anal. Chem.* **1996**, 68, 2259–2264.

14. Li, Z.P.; Li, K.A.; Tong, S.Y. Determination of nucleic acids in acidic medium by enhanced light scattering of large particles. *Talanta* **2000**, 51, 63–70.
15. Wu, X.; Wang, Y.B.; Sun, S.; Yang, J.H.; Lian, Y.X. Determination of nucleic acids at nanogram level using light scattering technique with congo red. *Spectrochim. Acta* **2005**, 61, 361–366.
16. Zhang, W.J.; Xu, H.P.; Wu, S.Q.; Chen, X.G.; Hu, Z.D. Determination of nucleic acids with crystal violet by light scattering technique. *Analyst* **2001**, 126, 513–517.
17. Chen, X.M.; Cai, C.Q.; Luo, H.A.; Zhang, C.H. Study on the resonancelight-scattering spectrum of anionic dye xylenol orange-cetyltrimethylammonium-nuleic acids and determination of nucleic acids at nanogram level. *Spectrochim. Acta* **2005**, 69, 2215–2220.
18. Liu, Z.; Qin, W.W.; Gong, G.Q. Determination of nucleic acids at nanogram level by the method of light scattering on night blue. *Anal. Lett.* **2002**, 35, 111–121.