Study on the Molecular Recognition of Herbicide Quizalofop-*p*-ethyl With *β*-cyclodextrin

Tian Shu-Li, Wu Yan-Hua, Xiong Yan-Mei, Li Xiao-Bing, Qin Zhao-Hai and Xiao Yu-Mei*
Department of Applied Chemistry, China Agricultural University, Beijing 100193, China.
xiaoyumei@cau.edu.cn*

(Received on 19th April 2013, accepted in revised form 14th October 2013)

Summary: The molecular recognition behavior of Quizalofop-p-ethyl (QpE) with *β*- cyclodextrin (*β*-CD) has been investigated quantitatively by UV-vis measurements. The stoichiometric ratios, stability constants and thermodynamics of inclusion reaction are determined. The results showed that *β*-CD could obviously enhance the water-solubility of QpE, which was favored to develop waterborne of the pesticide formulation. They could form 1:1 inclusion complexes and the formation constant reduced with the elevation of temperature, indicating that inclusion complex was unsuitable to be prepared at higher temperature. Thermodynamic parameters of inclusion reaction (ΔH、ΔS、ΔG) were all negative，suggesting that interaction process between *β*-CD and QpE was spontaneous driven by enthalpy and entropy changes. The inclusion complex of QpE with *β*-CD was prepared by coprecipitation technique and characterized using infrared spectroscopy(IR), differential scanning calorimetry(DSC) and ^{1}H-NMR spectroscopy, respectively. The ^{1}H-NMR analysis in D_2O revealed that the aromatics ring containing Cl atom entered into the cavity of *β*-CD through the wider rim.

Keywords: *β*-cyclodextrin；Quizalofop-p-ethyl；inclusion complex；UV-vis; IR; DSC

Introduction

Quizalofop-*p*-ethyl{ethyl(R)-2-[4-(6-chloroquinoxalin-2-yloxy) phenoxy] propionate} (Fig.1), is one of the most excellent aryloxyphenoxy-propionate group of herbicides introduced in the mid 1980s. It is mainly used to prevent and control annual and perennial grass weeds in vegetables and other broadleaf crops[1,2]. Based on its high efficiency and good environmental behaviors, QpE has been used widely in many countries for many years. However, because of its chemical structure characteristic, this molecule has poor solubility in water (0.61mg/L, 20□)[3]. Meanwhile, according to the report from the WHO (III) and EPA (III), QpE is toxic, which may cause the damage of human liver cell and the adverse effects on ecosystem health [4,5]. Thus improving its formulation to reduce its toxicity and increase solubility is one of the most desirable choices.

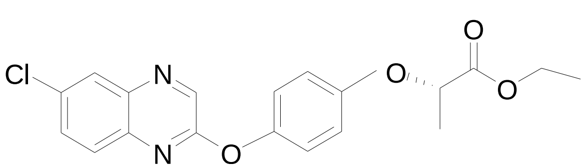

Fig. 1: Chemical structure of Quizalofop-p-ethyl (QpE).

β-CD is a kind of oligosaccharides containing seven *α*-D-glucopyranose units connected by *α*-1,4 linkages. *β*-CD has a toroidal shape with a hydrophobic internal cavity and hydrophilic external surface, which allows the guest molecule of appropriate size to be included. The non-covalent inclusion may significantly improve the physical, chemical and biological activity of guest molecule [6-10], thus CDs have a wide range of interesting applications, like in the food industry, separation science and pharmaceuticals [11-14]. In recent years, much attention has been paid to CD-based molecular recognition in pesticides. It has been reported that the solubility, wettability, unpleasant odour, chemical stability and the bioavailability of pesticide would be improved by forming host:guest complexes with CDs[15-19,]. More importantly, the utilization of organic solvent, volatility and the pollution to the environment caused by pesticide residues could be reduced by forming inclusion complex with CDs [20, 21]. Therefore, developing CD-based formulation have always been the tireless pursuit for pesticide workers.

In this article, the molecular recognition between *β*-CD and QpE was studied quantitatively by UV spectroscopy. Meanwhile, the inclusion complex was prepared and characterized by infrared spectroscopy (IR) and differential scanning calorimetry(DSC). The inclusion model was initially concluded through ^{1}H-NMR. Our results would provide good experimental and theoretical foundation on using the inclusion complex *β*-CD and QpE in the future.

*To whom all correspondence should be addressed.

Results and Discussion

Phase Solubility Studies

Phase solubility studies are widely used to evaluate the effect of CD on the water-solubility of drugs. Based on the studies, the inclusion ratio and inclusion constant K_f are also calculated. Fig. 2 showed that the solubility of QpE enhanced obviously with the increase of the concentration of β-CD, presenting that β-CD could improve the solubility of QpE greatly.

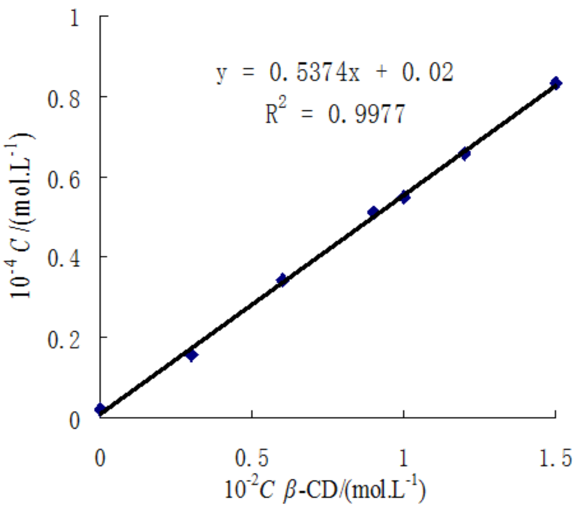

Fig. 2: Phase solubility studies of QpE in different concentration of β-CD (298K).

According to the model presented by Higuchi et al, the phase solubility diagram of QpE belongs to AL type, suggesting that the inclusion complex was formed with a molar ratio of 1:1. The forming constant K_f can be calculated through the formula of K_f =slope/S_0(1-slope), where S_0 is the solubility of QpE . The K_f value is 2251 L.mol^{-1} under 298K.

Thermodynamics Studies

Thermodynamics studies could detect complex formation process. Temperature is an important thermodynamic parameter. As can be seen from Table-1, the formation constant gradually decreases with increasing of temperature, meaning that the formation reaction between β-CD and QpE is an exothermic process. Therefore, the inclusion complex is not suitable to be prepared at higher temperature.

Table-1: The formation constants and thermodynamics parameters (pH=6.86).

T(K)	298	303	308	313	318
Ks(L.mol-1)	2251	1312	1171	582	436
ΔG(kJ.mol-1)	-18.80	-17.78	-17.20	-16.30	-15.82
ΔH(kJ.mol-1)			-61.98		
ΔS(J.mol-1.k-1)			-144.50		

The binding force of inclusion complex can be deduced from energy change of inclusion process. Fig. 3 display the relationship of In*K*s and 1/T. The enthalpy change (ΔH) , entropy change (ΔS) can be calculated from the Van`t Hoff equation (Eq. (1)). Free energy change (ΔG) at different temperature can be obtained from Eq. (2). All the results are listed in Table-1.

$$\ln Ks = -\frac{\Delta H}{R} \bullet \frac{1}{T} + \frac{\Delta S}{R} \qquad (1)$$

$$\Delta G = -RT\ln Ks \qquad (2)$$

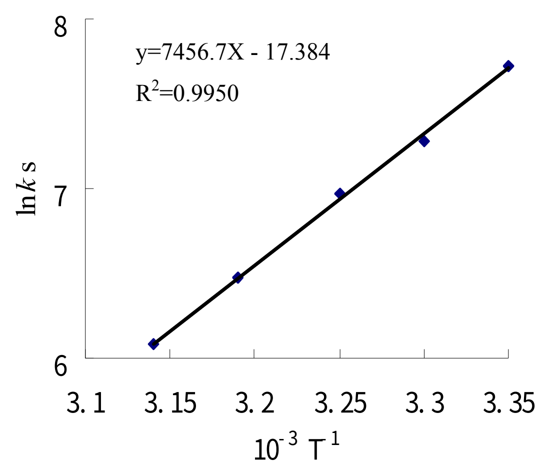

Fig. 3: Relationship between lnK_s and 1/T.

As can be seen from Table-1, ΔG is negative, which suggests that the inclusion reaction is spontaneous at the range of experimental temperature. ΔH is negative, implying that the inclusion reaction is exothermic. Relatively small negative enthalpy changes may result from some low-energy interactions between guest and host molecules, such as the dipole-dipole interaction, Van der Waals forces, formation of hydrogen bonds and so on. ΔS is also negative, which is caused by the limitation of shift and rotation degrees of OpE in β-CD cavity. The results show that the inclusion reaction between QpE and β-CD is driven by enthalpy and entropy changes.

Fourier Transform Infrared (FT-IR) Spectroscopy Studies

The inclusion complex is characterized by FT-IR spectroscopy. The spectroscopy of *β*-CD, QpE, mixture of *β*-CD and QpE and inclusion complex are shown in Fig.4. *β*-CD has four relatively strong absorption peaks at 3396, 2930, 1156 and 1033 cm^{-1}, which are O-H stretching vibration, C-H stretching vibration, C-O-C stretching vibration and O-H bending vibration and C-O and C-C stretching vibration in $-CH_2OH$ respectively (a). The IR spectra of the physical mixture QpE/*β*-CD (c) is essential combination of the spectras of *β*-CD and QpE alone (a and b), indicating that the physical mixture did not lead to inclusion. As can be seen from Fig. 4d, compared to *β*-CD, the shift and intensity of the four stong peaks in inclusion complex has no obvious change. However, compared to the IR spectra of QpE, the intensity of some QpE characistic absorption peaks in the inclusion complexes decreased significantly More importantly, there are large shift in aromatic ring vibration absorption, which are from 1446cm^{-1} to1404 cm^{-1}, 1122cm^{-1} to 1153 cm^{-1}, 828cm^{-1} to 853 cm^{-1}. The results suggest that aromatic ring of QpE enter into the cavity of *β*-CD. Meanwhile, the peaks of QpE became wider and weaker in inclusion complex, that might be caused by the overlap of *β*-CD band. The IR studies confirmed the formation of the inclusion complex. The carbonyl peak of QpE in inclusion complex has no obvious shift, indicating that carbonyl did not enter into the cavity of *β*-CD. So we conclude that the aromatic ring containing Cl atom enter into the cavity of *β*-CD.

Differential Scanning Calorimetry (DSC) Studies

DSC analysis is also used to characterize the inclusion complexes. Disappearance of the melting point peak of the guest in the inclusion complexes may be used as an evidence for the formation of complexes. As illustrate in Fig. 5, *β*-CD has one absorption peak at 98.73℃(a), which is generated by the release of high energy water molecules from the *β*-CD cavity. The absorption peak at 73.44℃ is QpE`s melting point (b). Phase transition peaks of physical mixture QpE/*β*-CD at 106.62℃ and 73.62℃ are both the melting point of *β*-CD and QpE respectively (c). However, Fig. 5d show that the DSC curve of inclusion complex is quite different from the physical mixture QpE/*β*-CD significantly, The disappearance of endothermic peak of QpE at 73.44℃ is a proof of the formation of inclusion complex. Melting point of *β*-CD increases from 106.62℃ to 115.06℃,which might be caused by the intermolecular forces between the host and guest.

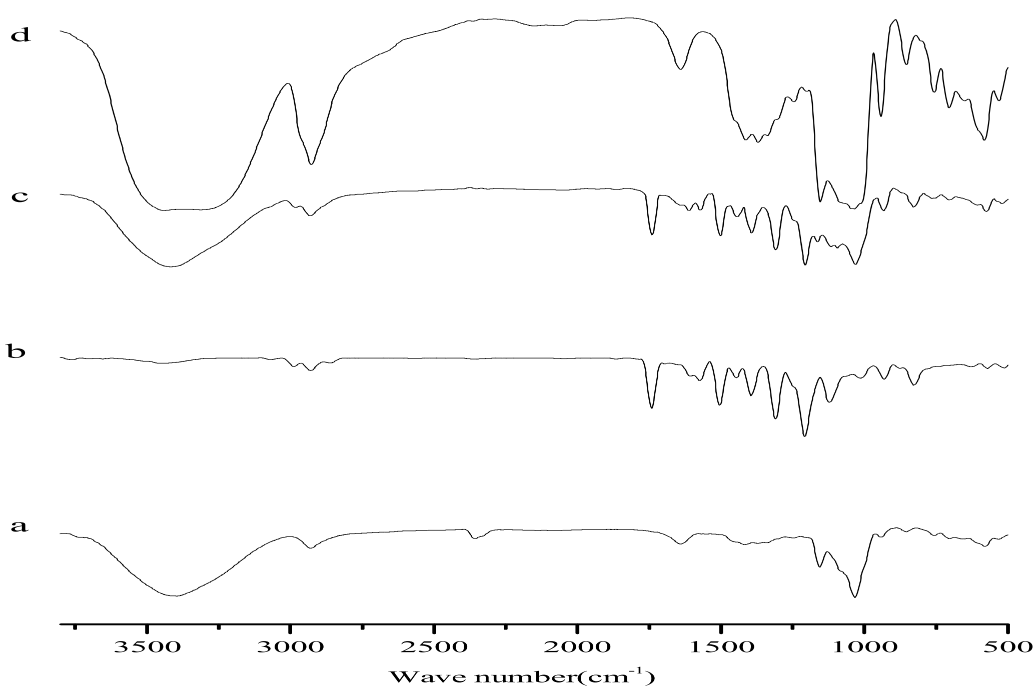

Fig. 4 : FT-IR spectra of (a) β-CD alone, (b) QpE alone, (c) mixture of *β*-CD and QpE, (d) inclusion complex.

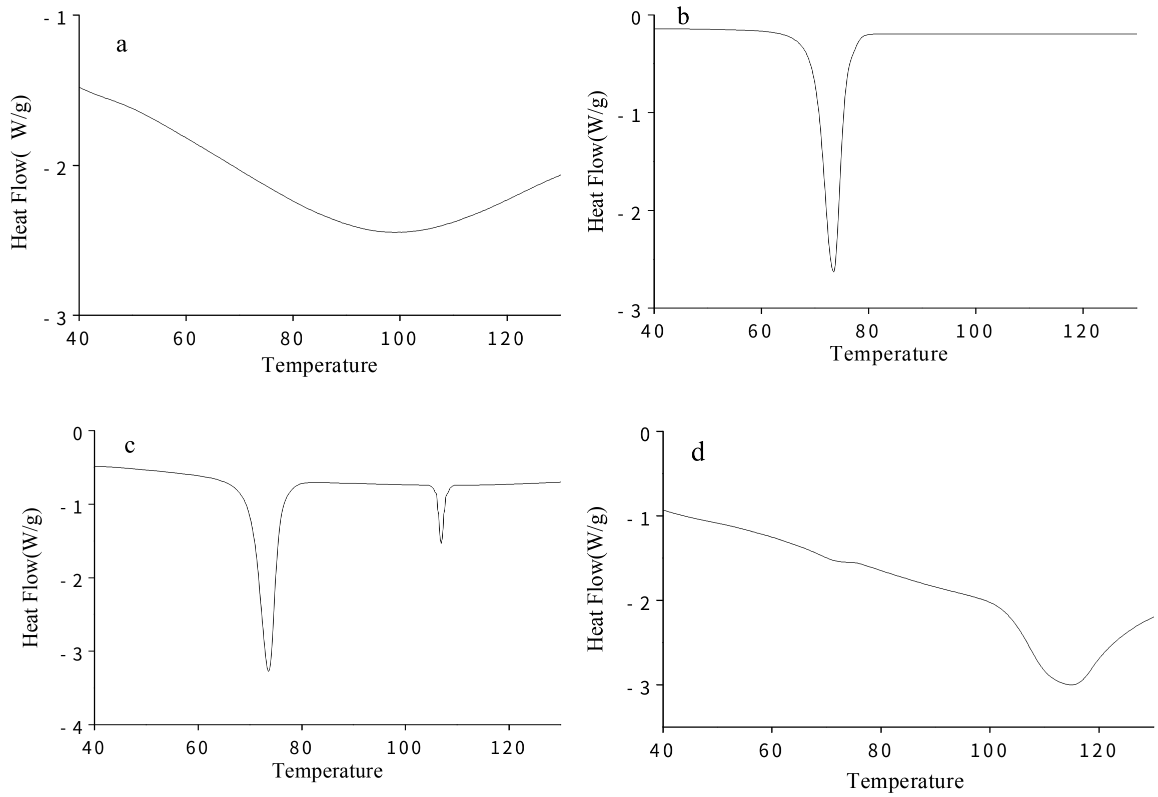

Fig. 5: DSC curve of (a) *β*-CD alone, (b) quizalofop-p-ethy alone, (c) physical mixture, (d) inclusion complex.

Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy Studies

Among all spectroscopic methods, NMR can provide valuable information about the geometry of inclusion complexes and the positions of guest in the cavity and so on. Generally, when the aromatic ring of the guest enter into the cavity, due to the anisotropic shielding of the aromatic ring, H-3 and H-5 protons compositing the inner wall of *β*-CD will shift to high field. As seen from Fig.6, the triplet of H-3 and the resonance peak of H-5 obviously shift to high field, suggesting that the aromatic ring of QpE has entered into the *β*-CD cavity. Meanwhile, shift value of H-3（Δδ=0.03）is larger than that of H-5 (Δδ=0.017) (Table-2), indicating that the aromatic ring enters into the cavity from the wide rim.

Table-2: Chemical shifts（δ）of β-CD and inclusion complex.

	H1	H2	H3	H4	H5	H6
$\delta_{\beta\text{-CD}}$	4.9379	3.5145	3.8350	3.4366	3.7202	3.7477
$\delta_{\text{inclusion complex}}$	4.9334	3.5105	3.8190	3.4368	3.7134	3.7422
Δδ	-0.0045	-0.0039	-0.02	-0.0008	-0.017	-0.0055

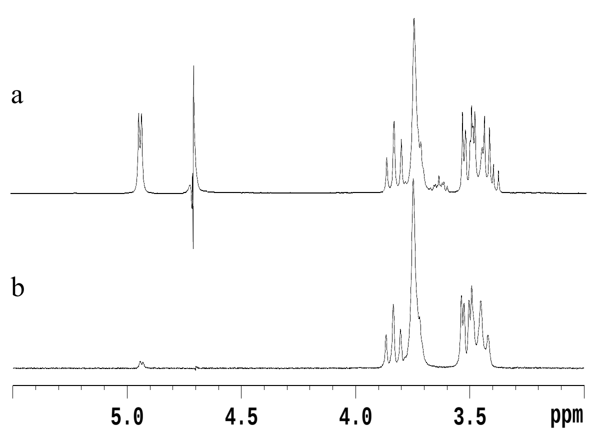

Fig.6: ^{1}H-NMR spectra of *β*-CD and inclusion complex in D_2O.

QpE is used as an ester of Quizalofop, so we also deduced that Quizalofop might has the same combining mode with*β*-CD as QpE, which is the aromatic ring containing Cl atom enter into the cavity of *β*-CD from the wide rim.

Experimental

Materials

β-CD and Quizalofop-p-ethyl were purchased from Shanghai Chemical Reagents and Anhui Huaxing Chemical Industry Co., Ltd respectively. All the other reagents were of analytical purity. *β*-CD was dissolved in water and prepared to 1.0 x 10^{-2} $mol.L^{-1}$ stock solutions. Quizalofop-p-ethyl was dissolved in methanol and prepared to 1.0 x 10^{-3} $mol.L^{-1}$ stock solutions.

Apparatus

Differential scanning calorimetry spectroscopy was measured using differential thermal gravimetric analyzer (German NET2scH, 204F1); Fourier Transform Infrared spectroscopy were recorded on Vector 22 Fourier transform infrared spectrometer; NMR spectra were performed on Bruker AV-300 spectrometer in D_2O solution.

Phase Solubility Studies

A fixed amount of pesticide, exceeding its solubility, was added to 10 mL capped tubes containing different concentration of *β*-CD (0, 0.3×10^{-2}, 0.6×10^{-2}, 0.9×10^{-2}, 1.0×10^{-2}, 1.2×10^{-2} and $1.5\times10^{-2}mol.L^{-1}$).The tubes were sealed to avoid changes due to evaporate and shaken for 72 hours in a thermostatic bath at 25.0±0.01℃. After the equilibrium was reached, the solution was centrifuged, diluted and the concentration of QpE was sectrophotomerically determined. Experiments were carried out in triplicate, and solubility data were averaged.

Preparation of Solid Inclusion Complex

Inclusion complexes were prepared with the coprecipitation method. *β*-CD（1mmol, 1.11g）was dissolved into 40ml water by heating to 70~80°C. Then added methanol solution of quizalofop-p-ethyl refluxed for 4 hours. After that, the solution was cooled to room temperature and stored at 4°C for 12 h. Finally, the precipitates were filtered across a glass filter (G4) and dired in vacuum desiccators to constant weight..

Characterization of Solid Inclusion Complexes

IR and DSC characterization was performed on mechanical mixture and the corresponding inclusion complexes of *β*-CD and QpE. A certain amount of sample was placed on the DSC disk under N_2 stream at a constant rate. Then heated the sample from 20°C to 180°C at a rate of 10°C /min and conducted DSC characterization. IR characteristic was conducted on the sample with a liquid membrane method.

Conclusion

Molecular recognition may occur between *β*-CD and herbicide QpE, the inclusion ratio is 1:1. The inclusion reaction is spontaneous exothermic process driven by enthalpy and entropy changes.High temperature is benefit to the formation of inclusion complexes. The IR spectroscopy and NMR analysis preliminarily indicate that the aromatic ring containing Cl atom enters into the cavity from the wide rim of *β*-CD. The water solubility of QpE is increased obviously by the presence of *β*-CD. Taken into account that QpE exhibits a low solubility and potential toxicity on ecosystem, the formation of inclusion complexes between *β*-CD and QpE appears a promising way to solve these problems.

Acknowledgment

The project were supported by National Science Foundation for Fostering Talents in Basic Research of China (No. J1210064) and Innovation Project for Beijing Undergraduates Students (2013).

References

1. M. Uchiyama, N. Washio, T. Ikai, H. Igarashi and K. Suzuki, *Nippon Noyaku Gakkaishi*, **11**, 459 (1986).
2. M. Zhao, *Chinese Journal Pesticide*, **42**, 32 (2003).
3. C. L. Liu, *World Pesticide Manual-Herbicide*, Chemical Industry Press (2002).
4. I. S. Elefsiniotis, G. D. Liatsos, D. Stamelakis and A. Moulakakis. *Environmental Health Perspectives*, **115**, 1479 (2007).
5. A. C. Belfroid, M. Drunen, M. A. Beek, S. M. Schrap, C. A. M. Gestel and B. Hattum, *Science of the Total Environment*, **222**, 167 (1998).
6. Y. Liu, J. Shi and D. S. Guo, The *Journal of Organic Chemistry*, **72**, 8227 (2007).
7. C. Yuan, Z. Y. Jin, X. M. Xu, H. N. Zhuang and W. Y. Shen, *Food Chemistry*, **109**,264 (2008).
8. R. Onnainty, E. M. Schenfeld, M. A. Quevedo, M. A. Fernández, M. R. Longhi and G. E. Garnero, *The Jouranl of Physical Chemistry*, **117**,

206 (2013).

9. J. H.Wang and Z. S. Cai, *Carbohydrate Polymers*, **72**, 255 (2008).
10. R. Iacovin, J. V. Caso, F. Rapuano, A. Russo, M. Isidori, M. Lavorgana, G.. Malgieri and C. Isernia, *Molecules*, **17**, 6056 (2012).
11. O. Jazkewitsch, A. Mondrzyk, R. Staffel and H. Ritter, *Macromolecules*, **44**, 1365 (2011).
12. J. E. Marcy, J. L.Koontz, S. F. OKeefe and S. E. Duncan, *Journal of Agricultural and Food Chemistry*, **57**, 1162 (2009).
13. F. V. Manakker, T. Vermonden, C. F. Van Nostrum and W. E. Hennink, *Biomacromolecules*, **10**, 3157 (2009).
14. D. W. Wang, C. B. Quyang, Q. Liu, H. L.Yuan and X. H. Liu, *Carbohydrate Polymers*, **93**, 753 (2013).
15. T. L. Neoh, T. Tanimoto, S. Ikefuji, H. Yoshii and T. Furuta, *Journal of Agricultural and Food Chemistry*, **56**, 3699 (2008).

16 S. S. Zhou, L. M. Wang, A. P. Zhang, *Journal of Agricultural and Food Chemistry*, **56**, 27089 (2008).

16. Y. M. Xiao, J. Wang, M. A Wang, J. P. Liu, H. Z. Yuan and Z. H. Qin, *Journal of The Chemical Society Of Pakistan*, **32**, 363 (2010).
17. J. Villaverde, C. Maqueda, T. Undabeytia and E. Morillo, *Chemosphere*, **69**, 575 (2007).
18. X. G. Zhang, J. X. Liu, Z. J. Fan and H. Y. Wang, *Chinese Journal of Pesticide Science*, **11**, 291 (2009).
19. C. Y. Shen, X. H. Yang, Y. Wang, J. W. Zhou and C. Chen. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, **72**, 263 (2012).
20. M. L. Alons, G. Recio, R. W. Alonso, R. M. Jiménez, J. M. Laza, J. L.Vilas and R. Fañanásal. *International Journal of Environmental Analytical Chemistry*, **92**, 963 (2012).