

Characterization and Bioactivity of the Inclusion Complex of Imazethapyr and HPCD

Jin-Yan Zhang^{1*}, Hong-Mei Bi², Bin Li³

¹College of science, Heilongjiang Bayi Agricultural University, Daqing 163319, PR China

^{2,3}Material Science and Engineering College, Northeast Forestry University Harbin 150040, PR China

Original Research Article

*Corresponding author

Jin-Yan Zhang

Article History

Received: 16.10.2018

Accepted: 27.10.2018

Published: 30.10.2018

DOI:

10.36347/sjet.2018.v06i10.004



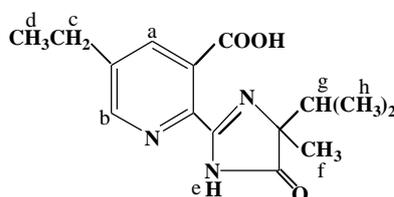
Abstract: The inclusion complex of imazethapyr with 2-hydroxypropyl-β-Cyclodextrin (HPCD), was prepared in an oscillator and its solubility was enhanced. The inclusion complexes formed were characterized by Fourier Transform Infrared Spectroscopy (FTIR), ¹H nuclear magnetic resonance (¹H NMR), X-ray diffraction (XRD), and Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TG) methods. The results indicated that the formation of a new compound, which has higher thermal stability and solubility. In the later bioactivity tests, there is still bioactivity in the new inclusion complex.

Keywords: Imazethapyr; HPCD; inclusion complex; solubilization effect.

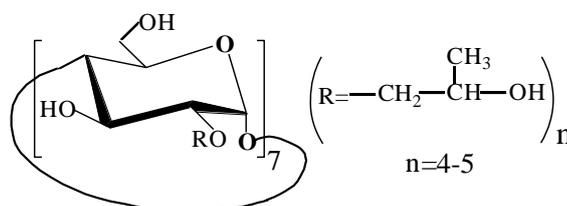
INTRODUCTION

Imazethapyr (Scheme 1) is one of three triazine herbicides with high bioactivity in China [1]. It is of high activity, and is often used to control grass and broadleaf weeds. Imazethapyr enters into China market in 1990, and it is widely employed in soybean field [2,3]. After clathration by β-cyclodextrin and its derivatives, the solubility of imazethapyr can be obviously increased. Similar studies [4, 5] have been reported. After clathration, the solubility of the inclusion complex has been obviously increased, and the bioactivity of inclusion has been kept well.

2-hydroxypropyl-β-Cyclodextrin (HPCD, Scheme 2), a macrocyclic compound of β-CD, is well known for its hydrophobic cavity. When clathrated with guest molecules, the stability and the bioavailability of the guest molecules will be enhanced greatly. There are a lot of inclusion complexes of HPCD [6-13]. The inclusion complexes of β-Cyclodextrin have been widely used to enhance water solubility and bioavailability of indissoluble herbicides [14, 15].



Scheme 1 Structure of imazethapyr



Scheme 2 Structure of 2-hydroxypropyl-β-cyclodextrin

In this paper, the inclusion complex of imazethapyr-HPCD was prepared in an oscillator, and was characterized by FTIR, ^1H NMR, XRD, DSC and TG. All of the characterization results confirmed the formation of a new compound. In addition, the bioactivity of the imazethapyr-HPCD inclusion complex was also studied.

MATERIALS AND METHODS

Chemicals and reagents

Imazethapyr (purity>99%) used in this paper was obtained from Harbin Limin Agriculture Technology Co, Ltd. HPCD (purity>99%) was purchased from Xinda Fine Chemicals Co., Ltd., Harbin. Acetone (A.R.) was purchased from the Sinopharm Chemical Reagent Co., Ltd.

Analytical Determination

FTIR spectrometer (is5) was from Thermo Fisher Nicolet. ^1H NMR spectra of the imazethapyr-HPCD complex was assessed using a Bruker AVANCE-400 NMR spectrometer (Switzerland). ^1H chemical shifts have been referred to an interior reference (tetramethylsilane). The Solvent was DMSO- d_6 .

X-ray diffraction (XRD) patterns were recorded on a D/MAX XRD-2200(Japan science Co., Ltd., Japan) using Ni-filtered, Cu K α radiation with wavelength of $k = 0.1540$ nm. Diffraction patterns were collected under ambient conditions in the 2θ range of 5–80° at a scanning rate of 4° min^{-1} , a step length of 0.02° min^{-1} . The voltage and current were 45 kV and 40 mA, respectively.

DSC data were recorded using a Diamond Instruments DSC 6300 (PerkinElmer, USA) equipped with an aluminum pan at a rate of 10°C min^{-1} between 30 and 300°C temperature range under a nitrogen flow of 40 ml min^{-1} .

TG analysis data were obtained from a TG 209 F3 Tarsus (Netzsch-Gerätebau GmbH, German). The rate was 10 °C/min over the temperature range from 30 to 600 °C, under nitrogen gas flow.

Experimental

Synthesis of imazethapyr- HPCD inclusion complex

0.001mol of imazethapyr dissolved in 30% ethanol (20ml) was mixed with 30 mL of HPCD. The mixture was heated at 40-50°C for 2h. And then the mixture was evaporated for 12h. The solution was filtered by deionized water, the white solid is placed in an oscillator for 4 hours, with the melting point 178-179.5°C.

Solubility experiments of imazethapyr-HPCD inclusion in water

Excess imazethapyr was added into different concentration of HPCD in the range from 0 to 0.025 mol/L. The solution was shaken for 48h at 37±0.5 °C in oscillator. Then the solution was filtrated by 0.22 μm microporous filtering film. 5mL of filtrate was diluted to 1000 mL, and the absorbance was measured under the maximum absorption wavelength of the solution imazethapyr.

Preparation for bioactivity tests

Germination

Superior, virus-free corn seeds (zhedan 37) were soaked in clear water for 10h. And then the seeds were germinated in climatic cabinete at 28°C for 36h.

Cultivation

Different concentrations of imazethapyr solutions were prepared. Corn seeds with sprout length of 2-4 cm were dipped into imazethapyr solutions at 28°C for 72h.

RESULTS

FTIR has been often used to characterize the formation of inclusion complex. Compared with imazethapyr, the infrared absorption position and strength of the imazethapyr-HPCD inclusion complex change obviously. 3249 cm^{-1} , 1745 cm^{-1} are attributed to the stretching vibration of C-N and C=O, the shape and strength of inclusion change compared with that of HPCD. 2937 cm^{-1} , 2877 cm^{-1} are attributed to the symmetrical stretching vibration of C-H, the shape of inclusion is the same as that of HPCD, which suggests the clathration of imazethapyr and HPCD. And the spectrum of physical mixture is totally different from that of imazethapyr and HPCD as in Fig.1. It is verified that the pyrimidine ring of imazethapyr has entered into the molecular cavity of β -CD.

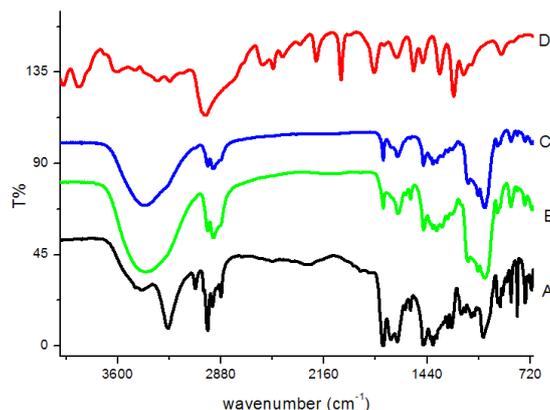


Fig-1: Spectra of imazethapyr (A), HPCD (B), imazethapyr-HPCD inclusion(C) and physical mixture (D)

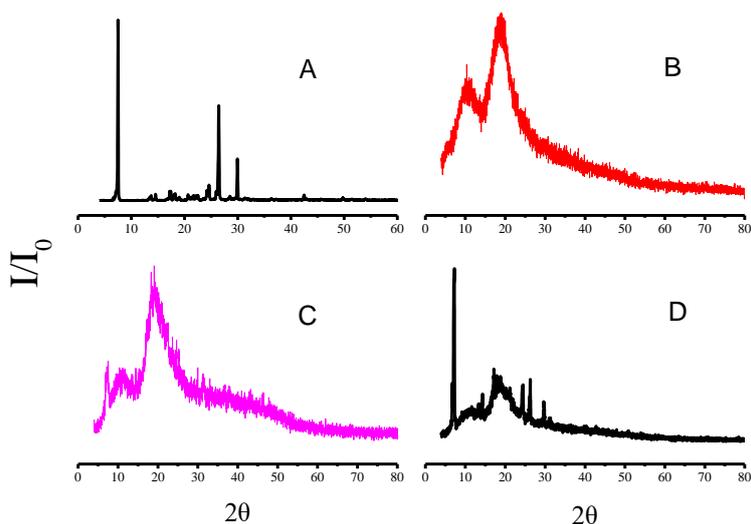


Fig-2: XRD pattern of imazethapyr (A), HPCD (B), imazethapyr-HPCD inclusion(C) and physical mixture (D)

In the XRD pattern of Fig.2, there are 2 broad peaks in the XRD pattern of HPCD, which accords with the amorphous structure of HPCD. 7.54° , 26.57° , 30.04° are attributed to the crystal characteristic peaks of imazethapyr. The XRD pattern of the physical mixture is the superposition of imazethapyr and HPCD. The XRD pattern of the inclusion complex is different

from that of imazethapyr and HPCD. While in the XRD pattern of imazethapyr-HPCD, the characteristic peaks of imazethapyr disappear, with the appearance of the diffraction of HPCD. It suggests that imazethapyr has come into the cavity of HPCD to form an inclusion complex, which means the formation of a new complex. The similar researches have been reported.

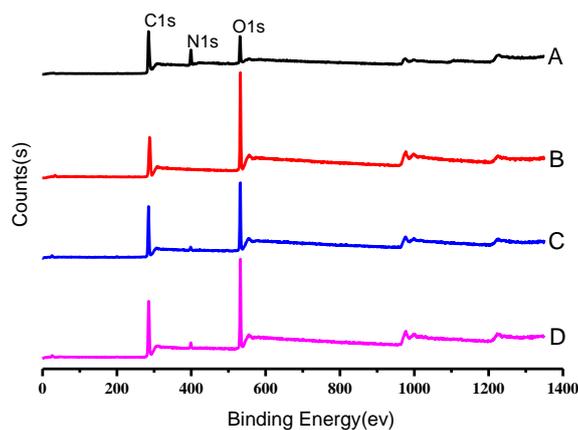


Fig-3: XPS spectra of imazethapyr(A), HPCD (B), imazethapyr-HPCD inclusion(C) and physical mixture(D)

In the XPS spectrogram of inclusion complex, the N element from imazethapyr remains. Compared with imazethapyr, the binding energy of inclusion and physical mixture all decrease 1.0eV. While the

absorption intensity of inclusion lowers 33550.6s, the absorption intensity of physical mixture lowers 22469.1s. The results of XPS show that there exists clathration between imazethapyr and HPCD.

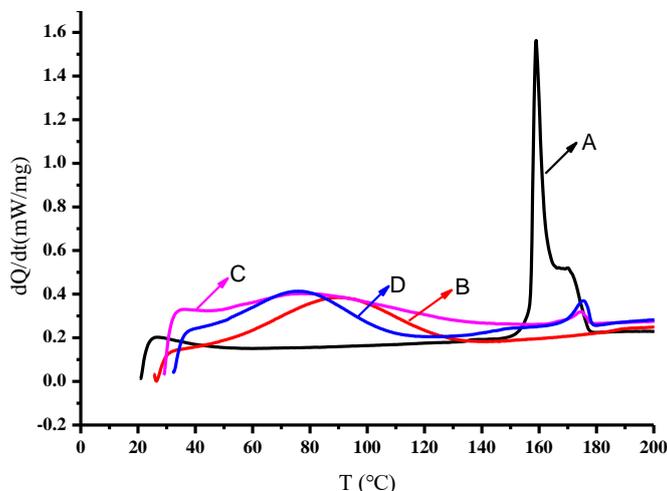


Fig-4: DSC spectra of imazethapyr (A), HPCD (B), imazethapyr-HPCD inclusion(C) and physical mixture (D)

The DSC thermal method is widely used in the characterization CDs and their derivatives. DSC of imazethapyr, HPCD, imazethapyr - HPCD inclusion complex, and their physical mixture are compared in Fig.4. Imazethapyr has a sharp endothermic peak in the range of 160-170°C. HPCD has a broader endothermic peak in the range of 70-120°C. In the DSC curve of

physical mixture, there are combination of imazethapyr and HPCD, which is different from that of inclusion complex. There is a new melting endothermic peak in the imazethapyr- HPCD inclusion complex, and the thermal stability is better than that of physical mixture. From the above information of DSC, the clathration of imazethapyr and HPCD is only a physical process.

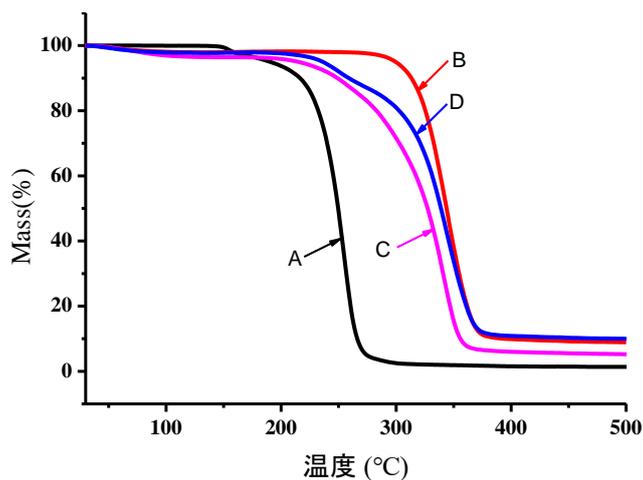


Fig-5: TG curves of imazethapyr (A), HPCD (B), imazethapyr-HPCD inclusion(C) and physical mixture (D)

There is a weightless peak of imazethapyr appeared in the range of 229.9°C to 265.9°C, and the mass fraction of weightlessness increases from 20% to 90%. That is to say that imazethapyr decomposes when temperature is higher than 230 °C; and HPCD decomposes from 325°C to 394°C. Imazethapyr- HPCD inclusion complex decomposes from 296°C to 356°C,

which has a better thermal stability compared with imazethapyr. This is consistent with the results of DSC. **Measurement of solubility of inclusion in water standard curves of imazethapyr - HPCD inclusion complex**

The solubility of the inclusion was performed through the phase solubility method [15]. S_t/S_0 is linear

with the concentration of HPCD; the mole ratio of imazethapyr and HPCD is 1:1, and can be expressed as: $S_t/S_0=1+K_{1:1}C_0$, S_t is the concentration of imazethapyr -

HPCD; S_0 is the concentration of imazethapyr aqueous solution; S_t/S_0 is the solubility ratio, $K_{1:1}$ is the solubility coefficient.

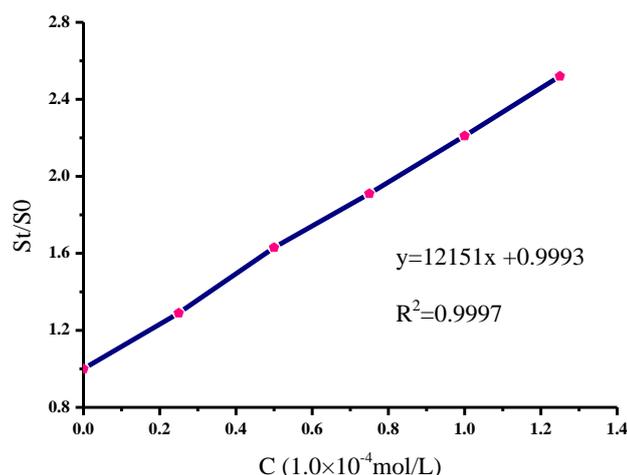


Fig-6: Solubility curve of imazethapyr in HPCD solution

Bioactivity results of imazethapyr-HPCD inclusion complex

In the following bioactivity experiments, corn is taken as indicator plant. When the concentration of imazethapyr is in the range from 0 to 200 mg/kg, the inhibition ratio of the sprout length, the taproot length, sprout fresh weight, taproot fresh weight are studied.

When the concentration reaches 50mg/kg, the inhibition ratio to corn root can reach 50%. So the EC50 of imazethapyr is 50mg/kg, and the inhibition of imazethapyr, HPCD, imazethapyr-HPCD inclusion complex is shown in tab. 1. After clathration with HPCD, the water solubility of imazethapyr-HPCD is increased, and there is still herbicidal activity.

Table-1: Inhibition of imazethapyr, cyclodextrin, inclusion and physical mixture on the growth of corn (the concentration of 50 mg/kg)

compound	inhibition ratio/%			
	the sprout length	the taproot length	sprout fresh weight	taproot fresh weight
CK	0	0	0	0
CD	-26.38	-38.28	-25.51	-32.23
HPCD	4.89	-18.97	-7.87	-19.85
imazethapyr	37.46	48.97	39.28	59.52
imazethapyr- CD inclusion	8.14	11.03	18.43	14.87
imazethapyr- HPCD inclusion	19.87	31.72	34.56	32.86

CONCLUSIONS

The imazethapyr-HPCD inclusion complex was synthesized in an oscillator. It was characterized by FTIR, ¹H NMR, XRD, DSC and TG. All the results showed that the imazethapyr had entered into the cavity of HPCD. The imazethapyr-HPCD inclusion complex had better solubility than that of imazethapyr; in the later bioactivity experiments, the imazethapyr-HPCD inclusion remained inhibition on the growth of target plant.

REFERENCES

1. Sun Ke. Market and outlook of the top ten herbicides in the world [J]. Agrochemicals. 2013, 52(5): 317-322.
2. Wu H, He X, Dong H, Zhou Q, Zhang Y. Impact of microorganisms, humidity, and temperature on the enantioselective degradation of imazethapyr in two soils. Chirality. 2017 Jul;29(7):348-57.
3. Oufqir S, El Madani M, El Belghiti MA, Zrineh A, El Azzouzi M. Adsorption of imazethapyr on six agricultural soils of Morocco: evaluation of the impact of soil properties. Arabian Journal of Chemistry. 2017 May 1; 10:S2944-9.
4. Zhang J, Li B, Bi H, Zhang P. Synthesis of atrazine-HPCD inclusion and its bioactivity. Transactions of Tianjin University. 2014 Oct 1;20(5):350-7.
5. Jinyan Zhang, Bin Li, Hongmei Bi, Ping Zhang. Clathration and Solubilization of Complex

- Synthesized by β -Cyclodextrin and Chlorimuron-ethyl [J]. *J.Pestic. Sci.*, 2015, 40(2): 33-37.
6. Wang Jia, Feng Jianguo, Ma Chao. Technology of cyclodextrin and its application in pesticide formulation processing [J]. *Chinese Journal of Pesticide Science*. 2013, 15(1): 23-31.
 7. Mao Y, Sun M, Yang X. Remediation of organochlorine esticides (OCPs) contaminated soil by successive hydroxypropylcyclodextrin and peanut oil enhanced soil washing-nutrient addition: A laboratory evaluation [J]. *Journal of Soils and Sediments*. 2013, 13(2): 403-412.
 8. Douhal A. Ultrafast guest dynamics in cyclodextrin nanocavities. *Chem. Rev.* 2004, 104(4):1955-1976
 9. Nascimento C S Jr, Anconi CP, Dos Santos HF. Theoretical Study of alpha-cyclodextrin dimer. *J. Phys. Chem. A*, 2005, 109(14): 3209–3219.
 10. Meng X, Pan Q, Liu Y. Preparation and properties of phytosterols with hydroxypropyl β -cyclodextrin inclusion complexes. *European Food Research and Technology*. 2012 Dec 1;235(6):1039-47.
 11. Garnero C, Aiassa V, Longhi M. Sulfamethoxazole: hydroxypropyl- β -cyclodextrin complex: preparation and characterization. *Journal of pharmaceutical and biomedical analysis*. 2012 Apr 7; 63:74-9.
 12. Nguyen TA, Liu B, Zhao J, Thomas DS, Hook JM. An investigation into the supramolecular structure, solubility, stability and antioxidant activity of rutin/cyclodextrin inclusion complex. *Food chemistry*. 2013 Jan 1; 136(1):186-92.
 13. Complexes – A Review. *J. Pharm. Sci. Technol.* 2010, 2: 171-183.
 14. Gidwani B, Vyas A. Synthesis, characterization and application of epichlorohydrin- β -cyclodextrin polymer. *Colloids and Surfaces B: Biointerfaces*. 2014 Feb 1; 114:130-7.
 15. Yáñez C, Cañete-Rosales P, Castillo JP, Catalán N, Undabeytia T, Morillo E. Cyclodextrin inclusion complex to improve physicochemical properties of herbicide bentazon: exploring better formulations. *PloS one*. 2012 Aug 27;7(8):e41072.
 16. Higuchi T, Connors K A. Phase-solubility techniques [J]. *Adv Anal Chem Instr.* 1965, 4(1): 117-212.