Inclusion complexes of pesticides in aqueous solutions of methylated β-cyclodextrin

Goran M. Petrović[1], Gordana S. Stojanović[1], Olga P. Jovanović[1], Aleksandra S. Đorđević[1], Ivan R. Palić[1], Sofija V. Sovilj[2]

[1]*University of Niš, Faculty of Mathematics and Natural Sciences, Niš, Serbia*
[2]*University of Belgrade, Faculty of Chemistry, Belgrade, Serbia*

Abstract

The disadvantage of some organic pesticides is their low water solubility. Among other substances, cyclodextrins and modified cyclodextrins were considered as agents for improving pesticide water solubility. The solubility of poorly soluble pesticides, dimethoate, simazine, linuron and thiram, was determined in aqueous solution of methylated β-cyclodextrin (mβCD) by ultraviolet spectrophotometry. Methylated β-cyclodextrin was obtained by the modified Hawort method and characterized by ^{1}H-NMR and HPLC data. The average degree of substitution was 13.8. Methylation was done either on C-2, C-3, and C-6 atoms of the glucopyranose unit; therefore, obtained product can be asserted as randomly methylated β-cyclodextrin. Solubility of the studied pesticides in aqueous solution of mβCD increases in relation to their solubility in water for dimethoate 506, for simazine167, for thiram 44, and for linuron 20 times. Reactions of dimethoate and simazine with mβCD were entropy-driven while the inclusion complexation of mβCD with the linuron and thiram were driven by both, enthalpy and entropy, as determined by calorimetric measurements. The observed solubility increment of the investigated pesticides in aqueous solution of mβCD, suggests that it can be efficiently used in pesticide solutions formulations and increase their bioavailability and biodegradability.

Keywords: inclusion complexes, methylated β-cyclodextrin, pesticides.

Available online at the Journal website: http://www.ache.org.rs/HI/

SCIENTIFIC PAPER

UDC 547.458.68:661.16

Hem. Ind. **67** (2) 231–237 (2013)

doi: 10.2298/HEMIND120413068P

The application of pesticides has become inevitable to protect crop plants from pests and diseases. At the same time, pesticide pollution in the environment has caused increasing concern among the public. Some hydrophobic organic pesticides have limitations for extensive application due to their low water solubility and difficulties of their removal from soil. Agents such as organic cosolvents and surfactants have been considered for improving solubility of organic pesticides [1]. Significant increase of pesticide solubility could be achieved with cosolvent volume fractions above 10%. Because of this disadvantage, cyclodextrins might become a good alternative as agents for increasing pesticide solubility [2–4] and remediation of contaminated soil and groundwater [5,6].

β-Cyclodextrin (cyclomaltoheptaose) is a cyclic oligomer of glucose containing 7 glucose residues, which are arranged in a circle with a toroidal shape. In this compound, the C-1 chain conformation of the glucose monomers imparts to the molecule a cone-like structure in which the hydroxyl groups are oriented on the exterior of the torus. This arrangement makes the cyclodextrin exterior decidedly hydrophilic. The secondary hydroxyl groups can, however, interact via hydrogen bonding to stabilize the crystalline lattice. This reduces to a large extent the solubility of cyclodextrins, especially β-cyclodextrin. In order to increase the water solubility of the cyclodextrins and their complexes, a great number of substituted derivatives have been prepared by the reaction of β-cyclodextrin with different reagents [7]. In this way, the aqueous solubility of cyclodextrin increases because of the absence of crystallinity. The solubility of the nonpolar compounds is consequently increased. The cavity of the torus has a non-polar character and in a hydrated state, it is filled with 11 water molecules. These molecules of water may be replaced with a gain of energy by molecules of a compound that is less polar than water and that can form stable inclusion complexes [8]. Since the modified β-cyclodextrin (βCD) is a water-soluble compound, the effective solubility of lipophilic substances in aqueous β-cyclodextrin solutions increases. The extent of this solubility enhancement depends on the concentration of the βCD, temperature, etc., but mainly on the chemical structure of the "guest" molecule [9]. Methylation of βCD increases its aqueous solubility, extends the hydrophobic cavity and provides a greater surface area for complexation [10]. Complexation thermodynamics is a very important factor that governs the complexa-

Correspondence: G. Petrović, Faculty of Mathematics and Natural Sciences, Višegradska 33, 18000 Niš, Serbia.
E-mail: peca@pmf.ni.ac.rs
Paper received: 13 April, 2012
Paper accepted: 19 June, 2012

tion phenomena between molecular receptor "host" and substrate "guest" molecule. The driving forces leading to the inclusion complexation of cyclodextrins include electrostatic interaction, van der Waals interaction, hydrophobic interaction, hydrogen bonding, release of conformational strain, exclusion of cavity-bound high-energy water and charge-transfer interaction [11]. Although, concerning the enthalpy-entropy compensation, the enthalpy and entropy changes of the complex formation are not good criteria to be used in judging whether a particular driving force is present, they can be useful in predicting which driving forces are important. Due to the fact that the cost of cyclodextrins is declining, the potential use of methylated β-cyclodextrin (mβCD) could be of very large interest in the pesticide solutions formulations and enhancement of the bioavailability and biological activity of the active compounds [12].

Continuing our investigation on properties and applications of cyclodextrins [13,14], herein we report the methylation of the β-cyclodextrin, characterisation of obtained mβCD, and its complexation with poorly soluble pesticides, dimethoate, simazine, linuron and thiram. Selected pesticides are typically used as water suspensions in following concentrations of active components: "dimethoate" 3–4 g/10 dm^3, "simazine" 2–3 kg/Ha, "linuron" 0.65–1.35 kg/Ha and "thiram" 15–26 g/10 dm^3. Additionally, thermodynamic parameters of complexation reactions were determined.

EXPERIMENTAL

The active components of the "dimethoate", "simazine", "linuron" and "thiram" (generic names of the pesticides) were obtained from "Župa" (Serbia) and were used without further purification. Chemical names of active ingredients are: O,O-dimethyl-S-(N-monomethyl)-carbamoyl-methyl dithiophosphate, 6-Cl-2,4-diaminoethyl-1,3,5-triazine, 3,4-dichlor-carbamoylaniline and Zn-dimethyldithiocarbamate, respectively. β-cyclodextrin was purchased from Merck (Germany) and was dried in vacuum prior to use. Dimethyl sulfate and all other chemicals were of analytical grade. Column chromatography was performed on Silica gel 60, 70–230 mesh ASTM, Merck (Germany) and the mobile phase was benzene:ethanol mixture (65:35, v/v) at a flow rate of 1 cm^3/min.

NMR spectra were obtained with deuterated dimethyl sulfoxide (DMSO-d_6) as solvent and referenced to the TMS signal using a Gemini-200 spectrometer operating at 199.98 MHz for ^{1}H. The HPLC system consisted of a LKB-Pharmacia 2248 pump, YMC-Pack Polyamine II (250 mm×4.6 mm) column, SP6040 Differential Refractometer detector and a SP 4200 Computing Integrator data module. Optical rotations were measured with model P16 polarimeter (Karl Zeiss). Ultraviolet spectra were recorded using a Perkin Elmer Lambda 15 spectrophotometer. A TRONAC model 458 isoperibol titration calorimeter was used for all of the thermodynamic measurements.

The reaction of methylation was performed by the modified Hawort method [15]. Shortly, the β-cyclodextrin (0.001 mol) was methylated with dimethyl sulfate in aqueous sodium hydroxide solution in a nitrogen atmosphere. Dimethyl sulfate (0.007 mol) was introduced drop-by-drop into the ice cooled, magnetically stirred solution. After that, the temperature was raised to 40 °C over a period of 3 h during the dropwise addition of dimethyl sulfate (0.007 mol), and the mixture was stirred at room temperature overnight. The solution was neutralized with diluted sulfuric acid, evaporated under a reduced pressure to give light-yellow powder and purified on the silica gel column. The eluent was removed under a reduced pressure to obtain white powder (yield 48%). The final product of methylation was dried in vacuum oven at 60 °C until the constant weight and characterized using the ^{1}H-NMR and HPLC spectroscopy methods.

^{1}H-NMR (DMSO-d_6, δ/ppm): 4.86–4.78 (broad doublet, anomeric protons, 1H); 4.58–4.44 (broad multiplet, OH, 1.05H, disappears on addition of D_2O); 3.74––3.47 (multiplet, cyclodextrin protons); 3.24 (singlet, methyl protons, 5.9H) (Figure 1). The average degree of substitution is 13.8, measured by comparing the integration of the seven anomeric protons of the carbohydrate with the observed methyl protons.

The results obtained from the HPLC measurements (Figure 2) show the presence of the new product (*Rt* = 2.14) of methylated β-cyclodextrin *vs*. standard of β-cyclodextrin) and confirm the high purity of the synthetized compound (area % is 99.4).

Optical rotation (20 cm path, 1% solution in water, sodium D line) α = +0.88.

Analytically calculated values for $(C_6H_{10}O_5)_7(CH_3)_{13.8}$ are: C, 50.47%; H, 7.35%. Found: C, 50.53%; H, 7.42%.

Standard aqueous solutions of the active components of pesticides were prepared in the range of concentrations up to the maximum solubility of each pesticide in water. Analytical curves of pesticides are shown in Figures 3–6, respectively. Some of the active components of pesticides had to be dissolved in small amounts of ethanol and diluted with water (15% v/v), because they are practically insoluble in water.

An aqueous solution of methyl-β-cyclodextrin (1×10^{-4} mol/dm^3) was placed in a screw-cap vial and an excess of pesticide was added. Vials were shaken at room temperature (ca. 22 °C) for at least 48 h. The contents of the vials were centrifuged, aliquots of the clear supernatant were diluted, and the concentrations of pesticides in these solutions were measured by UV spectrophotometry [16].

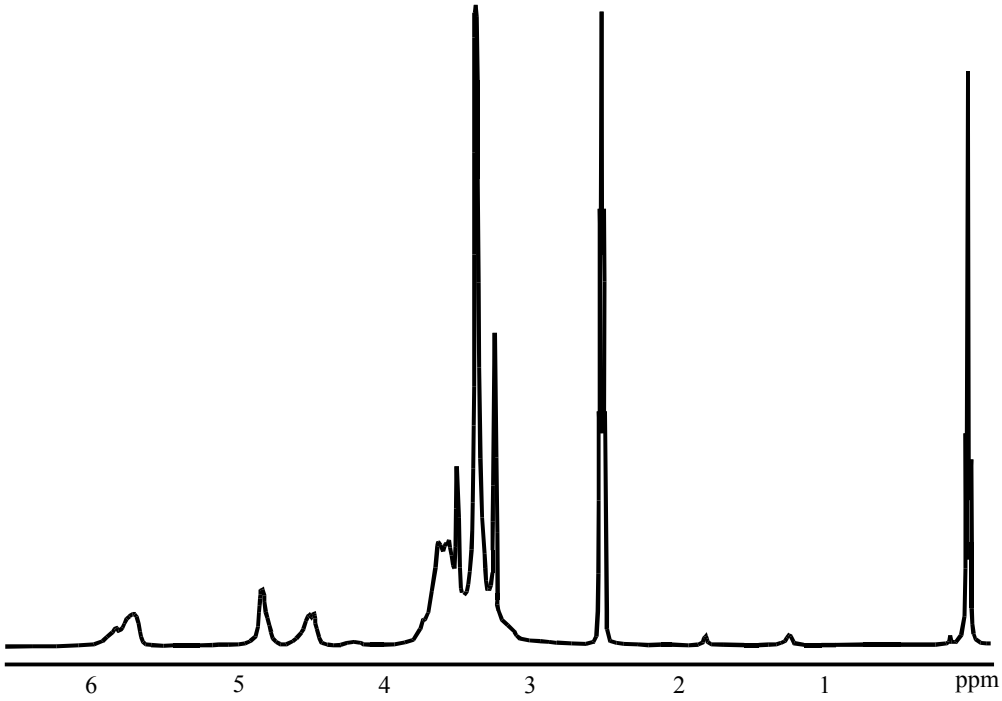

Figure 1. ^{1}H-NMR spectra of methylated-β-cyclodextrin.

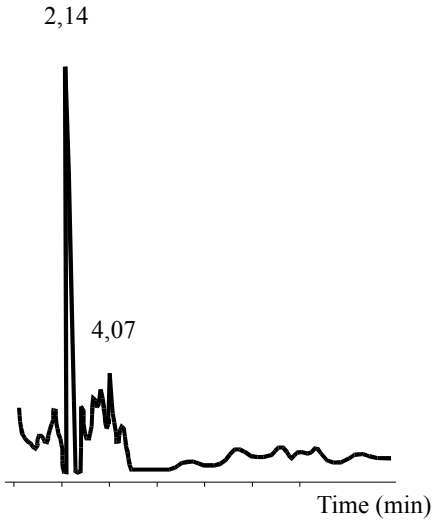

Figure 2. HPLC chromatogram of methylated β-cyclodextrin. Conditions: mobile phase, acetonitrile:H_2O = 310:190 (v/v); flow rate, 1 cm^3/min, sample concentration, 2 mg/cm^3.

Concentrations of the active components of pesticides were determined as absorbance at the wavelength at which absorbance was maximum (Table 1). There are no significant differences in the position of the absorption bands between pesticides solutions in water and in the aqueous methylated β-cyclodextrin solutions due to very weak intermolecular bonds between "host" (mβCD) and "guest" (pesticide) molecules [17].

Calorimetric measurements were performed by the calorimetric titrations in an aqueous phosphate buffer solution at pH 7.20 in a temperature-controlled water bath maintained at 22 °C. A titration curve was obtained by plotting the temperature change against the amount of the pesticide solution added, from which the complex stability constant, *K*, and the enthalpy change, Δ*H*, were calculated.

RESULTS AND DISCUSSION

Characterization of methylated β-cyclodextrin, was based on ^{1}H-NMR and HPLC data (Experimental part). The average degree of substitution was 13.8, measured by comparing the integration of the seven anomeric protons of the carbohydrate with the observed methyl protons. According to HPLC chromatogram (Figure 2), the purity of the synthesized complex was 99.4%.

The solubility of investigated pesticides in water and in aqueous mβCD solutions is presented in Table 2. The obtained results showed that the effective solubility of the investigated pesticides was substantially increased in the presence of mβCD. The solubility of some pesticides in aqueous mβCD solutions was up to three orders of magnitude higher than those in water (Table 2).

Increase the pesticide solubility with methylated β-cyclodextrin complexation reduces the average amount of pesticide applied in practice from 1.6×10^{-2} to 3×10^{-5} mol/10 dm^3 for "dimethoate", 12.3 to 7.4×10^{-2} mol/Ha for "simazine", 4 to 9.1×10^{-2} mol/Ha for "linuron" and from 8.6×10^{-2} to 4.3×10^{-3} mol/10 dm^3 for "thiram". For their complexation, assuming a 1:1 stoichiometry, the

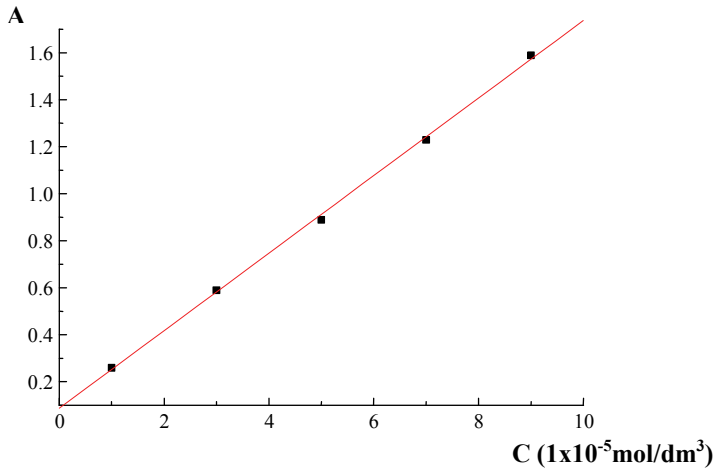

Figure 3. Analytical curve of dimethoate.

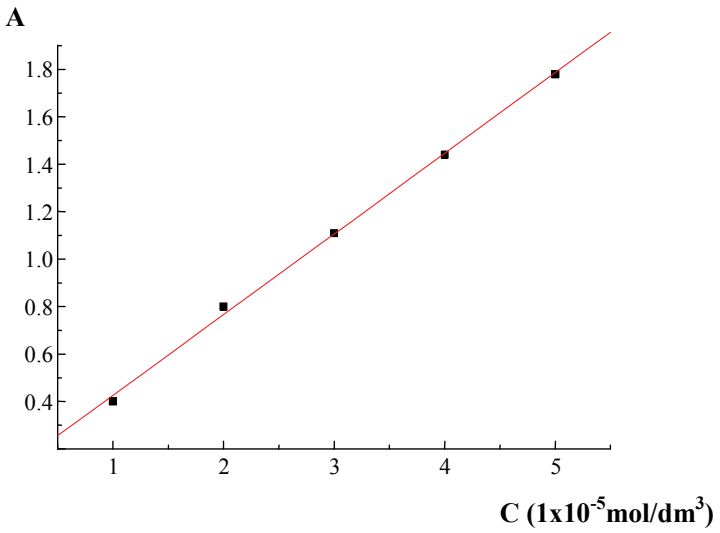

Figure 4. Analytical curve of simazine.

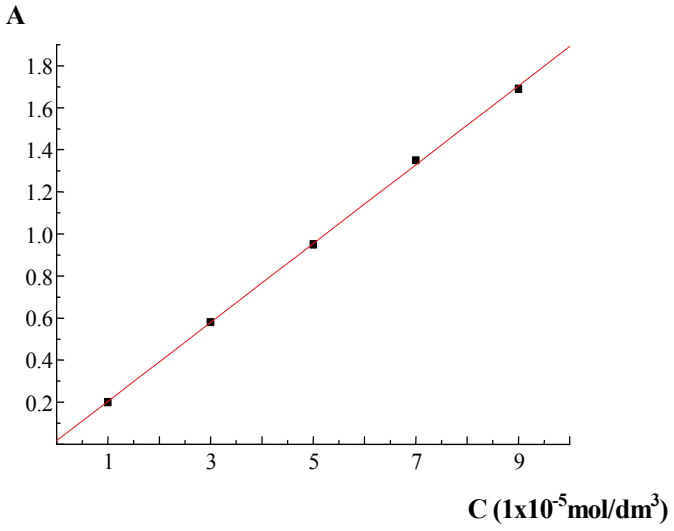

Figure 5. Analytical curve of linuron.

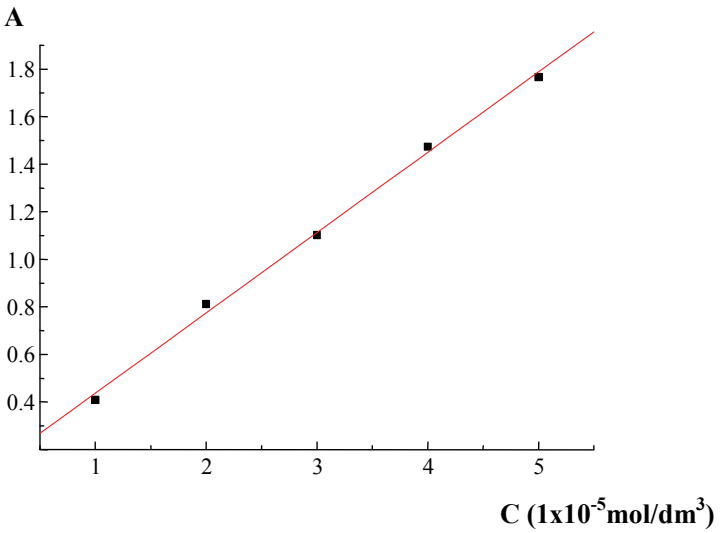

Figure 6. Analytical curve of thiram.

following masses of methylated *β*-cyclodextrin are required: for "dimethoate" 0.41 g, for "simazine" 98.28 g, for "linuron" 121.33 g and for "thiram" 5.73 g.

Table 1. λ_{max} and absorbencies of the dilute mβCD-pesticide complexes

Pesticide	λ_{max} / nm	*A*
Dimethoate	192	1.47
Simazine	221	0.87
Linuron	246	0.67
Thiram	207	0.86

The difference between the solubility enhancements is due to the molecular structure of the investigated pesticides, and binding ability and selectivity of intact molecular guest accommodated in the cyclodextrin cavity.

Assuming a 1:1 stoichiometry for the inclusion complexation of selected guest molecules (G) with methylated *β*-cyclodextrin (Eq. (1)), the complex stability constant, *K*, and the enthalpy change, ΔH^o were determined simultaneously (Eq. (2)) by using the least-squares method to minimize the error square sum (*U*):

$$G+CD \underset{}{\overset{K}{\rightleftharpoons}} G\bullet CD \quad (1)$$

$$U(K,\Delta H^o)=\sum_{t=1}^{m}(Q_t-\Delta H^o\times N_t)^2 \quad (2)$$

where Q_t refers to the net heat of complexation measured at time *t* in minutes and N_t denotes the amount in moles of the complex formed at time *t* and is directly related to the complex stability constant *K*.

The stability constant *K* and the enthalpy change ΔH° were calculated by computer simulation by continuously changing *K*, *i.e.*, N_t, to minimize the *U* value. The measurements were repeated more than three times and the *U* value obtained was minimized satisfactorily to give the optimized set of *K* and ΔH° with standard deviations. No serious deviation was found in fitting process, verifying the 1:1 stoichiometry of complexation. The complex stability constants and thermodynamic parameters obtained are listed in Table 3.

As can be recognized, the ΔH values for the inclusion complexation of methylated-*β*-cyclodextrin with the pesticide molecules are all negative and stabilizing, whereas the $T\Delta S$ values are either negative or positive. Combination of dimethoate and simazine with mβCD formed typical entropy-driven complexes. Thus, the complexes are inferred to be stabilized primarily by the entropic gain arising from the desolvation of the host and guest molecules upon complexation, as well as the release of water molecules trapped originally in the cavity. On the other hand, inclusion complexation of methyl-*β*-cyclodextrin with the linuron and thiram are enthalpy-driven reactions. In the case of thiram, negative enthalpy change indicates the dominance of van der Waals interaction but the entropy evidently assists

Table 2. The solubility of investigated pesticides in water and in aqueous mβCD

Pesticide	Solubility in water (mol/dm^3)	Solubility in aqueous mβCD (mol/dm^3)	Increase of solubility
Dimethoate	1.70×10^{-4}	8.6×10^{-2}	506
Simazine	1.44×10^{-7}	2.4×10^{-5}	167
Linuron	3.19×10^{-6}	1.4×10^{-4}	44
Thiram	1.15×10^{-6}	2.35×10^{-5}	20

process. Although it affords comparable *K* value with those of dimethoate and simazine, their thermodynamic profiles are completely different. Linuron gives the poor inclusion abilities forming the complex, which is evidently enthalpy-driven, but the enthalpic gain is exceedingly canceled out by the accompanying entropic loss. This enthalpy-entropy compensation indicates that the van der Waals interaction is the major driving force for linuron–mβCD complexation.

Table 3. Complex stability constant and their thermodynamic parameters (kcal/mol)

Pesticide	log *K*	$-\Delta G^\circ$	$-\Delta H^\circ$	$T\Delta S^\circ$
Dimethoate	3.26±0.04	4.45	1.11±0.06	3.34
Simazine	3.90±0.03	5.33	1.52±0.08	3.73
Linuron	1.92±0.06	2.69	4.42±0.04	–1.73
Thiram	3.42±0.02	4.67	4.00±0.09	0.67

CONCLUSION

Methylated β-cyclodextrin was synthesized and characterized, and its impact on solubility of commercial pesticides dimethoate, simazine, linuron, and thiram was investigated. The obtained results showed that solubility of examined pesticides was greater in the presence of mβCD than in the presence of cyclodextrin [13] or in pure water. The solubility depends of modified cyclodextrin concentration. In comparison with cosolvents and surfactants as additives in the pesticide solutions formulations, cyclodextrin derivatives have some advantages. For example, they are extremely water soluble relative to many cosolvents and surfactants, they do not form emulsions as do many surfactants and they are nontoxic and biodegradable, thus posing no hazard to the ecosystem. Because the cost of cyclodextrins is declining, water soluble methylated β-cyclodextrin may be potentially useful for improving the application of hydrophobic organic pesticides and enhancing their removal from the environment.

Acknowledgment

The research was supported by Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant no. 172047).

REFERENCES

[1] D.E. Kile, C.T. Chiou, Water solubility enhancements of DDT and trichlorobenzene by some surfactants below and above the critical micelle concentration, Environ. Sci. Technol. **23** (1989) 832–838.

[2] J. Szejtli, Cyclodextrins in pesticides, Starch **37** (1985) 382–386.

[3] O. Dailey, J. Bland, B. Trask-Morrell, Preparation and characterization of cyclodextrin complexes of the insecticides aldicarb and sulprofos, J. Agric. Food. Chem. **41** (1993) 1767–1771.

[4] M. Lezcano, W. Al-Soufi, M. Novo, E. Rodriguez-Nunez, J. Vazquez, Complexation of several benzimidazole-type fungicides with α- and β-cyclodextrins, J. Agric. Food Chem. **50** (2002) 108–112.

[5] E. Morillo, J. Perez-Martinez, J. Gines, Leaching of 2,4-D from a soil in the presence of β-cyclodextrin: laboratory columns experiments, Chemosphere **44** (2001) 1065––1069.

[6] B.B. Mamba, R.W. Krause, T.J. Malefetse, E.N. Nxumalo, Monofunctionalized cyclodextrin polymers for the removal of organic pollutants from water, Environ. Chem. Lett. **5** (2007) 79–84.

[7] Y. Liu, B.H. Han, B. Li, Y.M. Zhang, P. Zhao, Y.T. Chen, T. Wada, Y. Inoue, Molecular recognition study on supramolecular systems. Synthesis of modified cyclodextrin and their inclusion complexation thermodynamics with L-tryptophan and some naphthalene derivatives, J. Org. Chem. **63** (1998) 1444–1454.

[8] J. Szejtli, The cyclodextrins and their applications in biotechnology, Carbohyd. Polym. **12** (1990) 375–392.

[9] M.E. Brewster, J.W. Simpkins, M.S. Hora, W.C. Stern, N. Bodor, The potential use of cyclodextrins in parenteral formulations, J. Parent. Sci. and Tech. 43 (1989) 231––240.

[10] J. Szejtli, Cyclodextrins and Their Inclusion Complexes, Akademiai Kiado, Budapest, 1982.

[11] L. Liu, Q.X. Guo, The Driving forces in the inclusion complexation of cyclodextrins, J. Incl. Phenom. **42** (2002) 1–14.

[12] T. Nagai, H. Ueda, Aspects of drug formulation with cyclodextrins. In: Comprehensive Supramolecular Chemistry, Vol. 3: Cyclodextrins, Elsevier Science, Oxford, 1996, pp. 441–450.

[13] G. Petrović, G. Stojanović, R. Palić, Modified β-cyclodextrins as prospective agents for improving water solubility of organic pesticides, Environ. Chem. Lett. **9** (2011) 423–429.

[14] G. Petrović, G. Stojanović, N. Radulović, Encapsulation of cinnamon oil in β-cyclodextrin, J. Med. Plants Res. **4** (2010) 1382–1390.

[15] R.L. Whistler, M.L. Wolfram, Methods in Carbohydrate Chemistry, Vol. II, Academic Press, New York, 1963.

[16] J. Pitha, J. Milecki, H. Fales, L. Pannell, K. Uekama, Hydroxypropyl-β-cyclodextrin: preparation and characterization; effects on solubility of drugs, Int. J. Pharm. **29** (1986) 73–82.

[17] F. De Jong, D.N. Reinhoudt, Stability and Reactivity of Crown Ether Complexes, Academic Press, New York, 1981.

IZVOD

INKLUZIONI KOMPLEKSI PESTICIDA U VODENOM RASTVORU METILOVANOG β-CIKLODEKSTRINA

Goran M. Petrović[1], Gordana S. Stojanović[1], Olga P. Jovanović[1], Aleksandra S. Đorđević[1], Ivan R. Palić[1], Sofija V. Sovilj[2]

[1]*Univerzitet u Nišu, Prirodno–matematički fakultet, Niš, Srbija*
[2]*Univerzitet u Beogradu, Hemijski fakultet, Beograd, Srbija*

(Naučni rad)

Nedostatak većine organskih pesticida je njihova mala rastvorljivost u vodi. Pored ostalih supstanci za povećanje rastvorljivosti u vodi, razmatrani su ciklodekstrini i modifikovani ciklodekstrini. U ovom radu ispitivana je rastvorljivost četiri slabo rastvorna organska pesticida, dimetoata, simazina, linurona i cirama, u rastvoru metil-β-cyclodextrina (mβCD), dobijenom metilovanjem β-cyclodextrina (βCD) modifikovanom Hawort-ovom metodom. Analizom NMR spektra utvrđeno je da je prosečan stepen supstitucije 13,8 i da je metilovanje izvršeno neselektivno u položajima 2, 3 i 6 glukopiranoznih jedinica i da dobijeni proizvod spada u klasu nasumično metilovanih β-ciklodekstrina. Rastvorljivost svih ispitivanih pesticida kompleksiranih sa metil-β-ciklodekstrinom se povećava u odnosu na njihovu rastvorljivost u vodi i to za: dimetoat 506 puta, simazin 167 puta, ciram 44 puta i linuron 20 puta. Metodom kalorimetrijske titracije utvrđeno je da je građenje kompleksa dimetoata i simazina sa metil-βCD-om uslovljeno pretežno entropijskim faktorom, dok na komplekse linurona i cirama utiču i entalpijski i entropijski. Dobijeni rezultati ukazuju na moguću primenu metilovanog β-ciklodekstrina kao agensa za povećanje rastvorljivosti ispitivanih pesticida što bi omogućilo njihovu efikasniju primenu kroz smanjenje njihove aplikovane količine, povećanje biodostupnosti i biodegradibilnosti.

Ključne reči: Inkluzioni kompleksi • Metilovani β-ciklodekstrin • Pesticidi