UNIVERSITY OF PÉCS

Doctoral School of Chemistry

Thermodynamic studies on a few factors influencing the formations of some representative host–guest complexes

PhD Thesis

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1. Introduction

Crown ethers and calixarenes are two kinds of macrocyclic ligands which are able to form complex with cations, anions and neutral species due to the non-covalent interactions such as cation–dipole interaction, hydrogen bonding and π−π interaction. Their applications in chemical sensors have been numerously reported in the last two decades and numerous new derivatives have been continuously synthesized. However, factors governing their performance in the field of chemical sensor such as molecular structure, cavity size, cavity shape and solvent effect are still not completely revealed yet. For a practical purpose, investigating the nature of sensitivity and selectivity towards guests is of great importance.

In this work, I have investigated the several factors influencing the complexations between those synthesized host molecules and guest molecules, such as flexibility of the skeleton of host molecule, the solvent effect, solvation shell of guest molecule and fluorescence quenching mechanism of host molecule by transition metals ion. Besides the studying aforementioned factors, my investigation was also extended to the solvent effect on a biologically important interaction.

2. Objectives

1. Two crown ethers, namely dimethyl-pyridino-18-crown-6 ether **(1)** and dimethyl-diketo-pyridino-18-crown-6 ether **(2)** were selected as investigating models. Due to the presence of carbonyl group in **(2)**, the flexibility of the skeleton of **(2)** is smaller than that of **(1)**. Their complexations with K^+ are good platforms studying the effect of the freedom of the host' skeleton on the host–guest interaction.

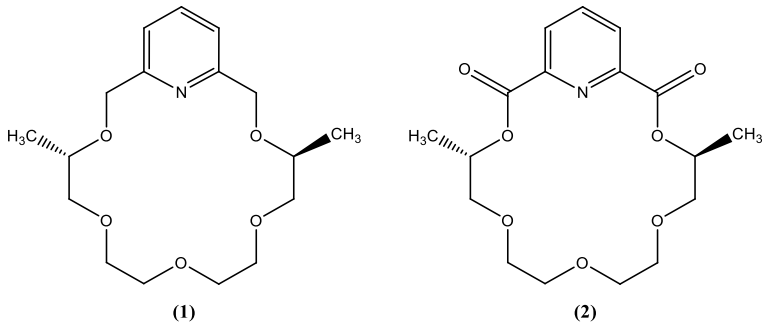
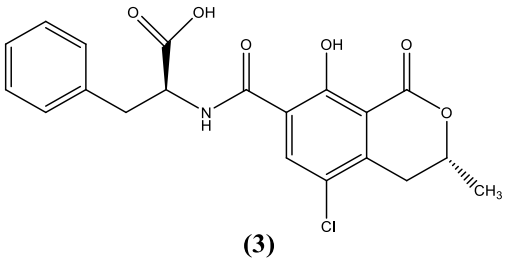


Figure 1 Structures of M_2P18C6 **(1)** and M_2K_2P18C6 **(2)**.

2. Alcohols are a kind of common solvents, however their impact on complexation has not been systemically investigated yet. Therefore, an effort on correlating the property of solvent with the thermodynamic behavior of complexation is worthwhile. In this study, I have investigated the solvent effect of primary alcohols on the complex formation of crown ether **(1)** with K^+ and Na^+ ions.

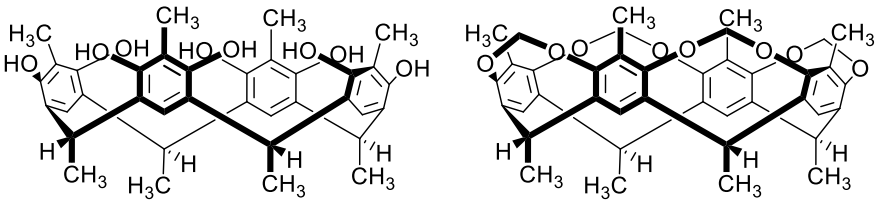
3. The role of the solvent effect has been studied in case of a biologically important interaction. Ochratoxin A (OTA) is a kind of toxic coumarin derivative, which can strongly bind to the animal's protein leading to several toxic effects such as nephrotoxicity, hepatotoxicity, neurotoxicity, teratogenicity and immunotoxicity in animals. The log *K* between OTA and human serum albumin (HSA) can reach as high as above 6. As a commonly used solvent of hydrophobic drug, ethanol effect on the protein–ligand interaction is of interest and attracts a lot of attentions. Therefore, the ethanol effect on OTA and HSA can serve as a good model to better understand the solvent effect on complexation between bioactive molecules.



(3)

Figure 2 Structure of ochratoxin A **(3)**.

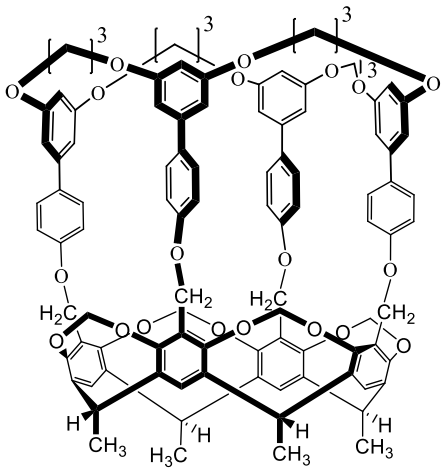
4. In liquid phase the host and guest molecules are solvated by the surrounded solvent molecules. Therefore, desolvation process should be performed prior to the physical contact occurred between the host and guest. Hence, the desolvation processes play important roles on selectivity of host molecule. In this study, I have examined how the selectivity and sensitivity of resorcinarene **(4)** and its derivative—cavitand **(5)** towards alkali metal ions are affected by the desolvation of the species interacted.



(4) **(5)**

Figure 3 Structures of resorcinarene **(4)** and cavitand **(5)**.

5. Detection of transition metal ions is of practical significance in environmental, analytical and biological chemistry. The fluorescence quenching effect of fluorescent compound by transition metal ion is a common phenomenon in these interactions. However, few work focused on the quenching mechanism by those transition metal ions. In this study, the efforts focused on the discussion of quenching mechanism of a novel 'deepened' cavitand by some transition metal ions.



(6)

Figure 4 Structure of a 'deepened' cavitand derivative **(6)**.

3. Methods

1. Calculation of binding constant

   UV–Vis and fluorescence spectrophotometric titrations were carried out to calculate the binding constant (Log *K*). Modeling of different stoichiometry and the data evaluation have been performed by a nonlinear fitting program—HyperQuad 2006.

2. Determination of thermodynamic parameters

   The thermodynamic parameters including entropy change (ΔS) and enthalpy change (ΔH) values were obtained from the intercept and the slope of the line fitted to the experimental data by applying the van't Hoff equation, respectively.

$$\log K = \frac{-\Delta H}{2.303 \cdot R} \cdot \frac{1}{T} + \frac{\Delta S}{2.303 \cdot R}$$

3. Theoretical calculation

   To obtain the background of the thermodynamic behavior at molecular level, optimized geometry and vibrational spectrum were calculated at semiempirical level using the Austin Model 1 (AM1) in the HyperChem 7.5 code.

4. The quenching model of the sphere-of-action

   It is provided that a given spherical space surrounding the excited fluorophore exists and the quenching probability is same within this region. The quenching model of the sphere-of-action is described by the following equation:

$$\frac{I_0}{I} = \exp(V \cdot \mathrm{N_A} \cdot [Q]) \cdot (1 + K_D \cdot [Q])$$

4. Results

1. The role of the skeleton's flexibility of crown ether on the complexation towards K^+ in methanol was investigated by UV spectroscopy. Results show that the entropy decreases when the 'flexible' crown ether **(1)** forms a complex with K^+, while the entropy increases during complex formation of the 'rigid' crown ether **(2)** with K^+. Quantum chemical calculations highlight that molecular vibrations of skeleton are most likely to result in the obtained entropy change. The different vibrational entropy change of the crown ether's skeleton can be identified as the molecular background of complexation phenomena at molecular level.
2. The interactions between a crown ether **(1)** and alkali metal ions including Na^+ and K^+ were studied in different alcoholic solvents including methanol, ethanol and *n*-propanol by UV spectroscopy. The results show that the stability constants of both **(1)**–Na^+ and **(1)**–K^+ complexes increase as the permittivity of the solvent decreases. A correlation between thermodynamic parameters and the permittivity of solvent was found. As the permittivity of solvent decreases, the enthalpy change and the entropy change become less favorable and more favorable, respectively. In order to understand this phenomenon, a two-step complexation model including both classic hydrophobic effect (step 1) and non-classic hydrophobic effect (step 2) was proposed. As the solvent varies from methanol to *n*-propanol, both the hydrophilicity of the solvent and the number of the released solvent molecules decrease. As a result, the classical hydrophobic effect plays more important role than the non-classical hydrophobic effect in complexations. The complexation becomes a mainly entropy-driven process in *n*-propanol.
3. The ethanol effect on OTA **(3)**–HSA interaction in PBS buffer solutions was investigated by Raman spectroscopy and steady-state fluorescence spectroscopy. The Raman spectra show that after the binding of OTA to HSA, the polarity of microenvironment of tryptophan residue on HSA decreases. The fluorescence results evidenced that the presence of ethanol induces a negative effect on the binding of OTA to HSA. Thermodynamic study suggests that as the concentration of ethanol increases, the binding process of OTA to HSA switches from being entropy-driven to enthalpy-driven. A true enthalpy–entropy compensation was also observed. This phenomenon could be attributed to the shift of the dominant role in complexation from the classical hydrophobic effect to the non-classical hydrophobic effect, arising from the more enhanced hydrogen bond network in the bulk phase as the ethanol concentration increases.
4. The interactions of the resorcinarene **(4)** and cavitand **(5)** with alkali metal ions including Li^+, Na^+, K^+ and Cs^+ in methanol were investigated by steady-state fluorescence

spectroscopy. The results showed that **(4)** is able to interact with Li^+ and Na^+, while **(5)** is able to interact with K^+ and Cs^+. Thermodynamic study shows that the complexations of **(4)** with Li^+ and Na^+ are enthalpy-favorable processes, but those of **(5)** with K^+ and Cs^+ are entropy-favorable processes. According to their thermodynamic behaviors, two different binding mechanisms, namely the binding of methanol-solvated Li^+ and Na^+ to the cavity of **(4)**, while the binding of desolvated K^+ and Cs^+ to the cavity of **(5)**, were proposed.

5. The fluorescence quenching effect of the emission intensity of a novel 'deepened' cavitand **(6)** by some transition metal ions has been investigated using steady-state fluorescence spectroscopy. The results show that amongst the used transition metal ions including Ag^+, Cd^{2+}, Cu^{2+}, Fe^{3+},Cr^{3+}, Hg^{2+}, La^{3+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and Co^{2+}, only Fe^{3+} and Cu^{2+} possess good quenching efficiency towards **(6)**. Dynamic quenching model and the simultaneous presence of dynamic and static quenching model have been used for interpretation of the quenching mechanism. The best-fit quenching mechanism was found to be the simultaneous presence of the sphere-of-action static quenching and the dynamic quenching model.

5. Thesis points

1. Flexibility of the molecular skeleton determines significantly the thermodynamics of the affinity of some crown ether derivatives towards potassium ion. The entropy change of the interaction of crown ether possessing more flexible skeletons with K^+ is more negative. Quantum chemical calculations support that molecular vibrations of skeleton is most likely reason for the obtained different entropy changes.
2. The stability of complexes formed between a crown ether derivative with Na^+ and K^+ increases in the solvent order of methanol, ethanol and *n*-propanol. The observed enthalpy–entropy compensation suggests the classical hydrophobic effect becomes more dominant than the non-classical hydrophobic effect as the hydrophilicity of solvent decreases.
3. Ethanol is able to inhibit the binding of OTA to HSA. The binding process of OTA to HSA switches from being entropy-driven to enthalpy-driven as ethanol concentration increases, which could probably arise from the more enhanced hydrogen bond network in the bulk phase when the ethanol concentration is higher.
4. Desolvation plays a dominant role in the complexations of resorcinarene and cavitand with some alkali metal ions. The complexations of resorcinarene with Li^+ and Na^+ are

enthalpy-driven processes, while those of cavitand with K^+/ Cs^+ are entropy-driven processes, which depend on the different extent of desolvation of the guest ions.

5. Cu^{2+} and Fe^{3+} are capable of quenching the 'deepened' cavitand effectively. The simultaneous presence of the sphere-of-action static quenching and the dynamic quenching model is the best model for these two quenching phenomena.

6. List of publications

I. Publications in refereed journals related to this thesis

1. **Y. Li**, Z. Czibulya, M. Poór, S. Lecomte, L. Kiss, E. Harte, T. Kőszegi, S. Kunsági-Máté: Thermodynamic study of the effects of ethanol on the interaction of ochratoxin A with human serum albumin. *Journal of Luminescence*, 148(2014): 18 – 25. **IF: 2.367** (2013)

2. **Y. Li**, Z. Csók, P. Szuroczki, L. Kollár, L. Kiss, S. Kunsági-Máté: Fluorescence quenching studies on the interaction of a novel deepened cavitand towards some transition metal ions. *Analytica Chimica Acta*, 799 (2013): 51 – 56. **IF: 4.517** (2013)

3. **Y. Li**, P. Huszthy, I. Móczár, B. Szemenyei, S. Kunsági-Máté: Solvent effect on the complex formation of a crown ether derivative with sodium and potassium ions. Thermodynamic background of selectivity. *Chemical Physics Letters*, 556 (2013): 94 – 97. **IF: 1.991** (2013)

4. **Y. Li**, P. Huszthy, S. Kunsági-Máté: Effect of molecular vibrations on the selectivity character of pyridino-18-crown-6 derivatives towards potassium ion. *Chemical Physics Letters*, 533(2012): 45 – 49. **IF: 1.991** (2013)

5. **Y. Li**, Z. Csók, L. Kollár, K. Iwata, E. Szász, S. Kunsági-Máté: The role of the solvation shell decomposition of alkali metal ions in their selective complexation by resorcinarene and its cavitand. *Supramolecular Chemistry*, 24 (2012), 374 – 378. **IF: 2.132** (2013)

II. Publications in non-refereed journals, conference abstracts, lectures and posters related to this thesis

1. **Y. Li**, S. Kunsági-Máté, Z. Csók, P. Szuroczki, L. Kollár, L. Kiss. Fluorescence quenching studies on interactions of a novel deepened cavitand towards some guests. *The Central European Conference on Photochemistry*, Bad Hofgastein, Austria, 9 – 13 February, 2014.

2. **Y. Li**, P. Huszthy, I. Móczár, B. Szemenyei, S. Kunsági-Máté: Solvent effect on the complex formation of a crown ether derivative with sodium and potassium ions. Thermodynamic background of selectivity. *The 3rd International Colloids Conference*, Xiamen, China, 21 – 24 April, 2013.

3. **Y. Li**, Zs. Czibulya, M. Poór, S. Lecomte, L. Kiss, E. Harte, T. Kőszegi, B. Desbat, S. Kunsági-Máté: Effect of molecular environment on the interaction of ochratoxin A with human serum albumin. *The 3rd International Colloids Conference*, Xiamen, China, 21 – 24 April, 2013.

4. **Y. Li**, Zs. Czibulya, M. Poór, S. Lecomte, L. Kiss, E. Harte, T. Kőszegia, S. Kunsági-Máté: Effect of ethanol on the interaction of ochratoxin A with human serum albumin. *Symposium on Weak molecular Interactions*, ISBN 978-963-642-510-4, pp66–67, Pécs, Hungary, 5 – 6 March, 2013.

5. **Y. Li**, P. Huszthy, I. Móczár, B. Szemenyei, S. Kunsági-Máté: Permittivity-dependent complexations of a crown ether derivative with Na^+ and K^+. *Symposium on Weak molecular Interactions*, ISBN 978-963-642-510-4, pp72–73, Pécs, Hungary, 5 – 6 March, 2013.

6. **Y. Li**, Zs. Csók, G. Matisz, L. Kiss, L. Kollár, S. Kunsági-Máté: A novel deepened cavitand fluorescent chemosensor for detection of Fe^{3+} and Cu^{2+}. *Symposium on Weak molecular Interactions*, ISBN 978-963-642-510-4, pp74–75, Pécs, Hungary, 5 – 6 March, 2013.

7. **Y. Li**, G. Matisz, L. Kiss, Zs. Csók, L. Kollár, S. Kunsági-Máté: Interactions of functionalized cavitand derivatives with some transition metal ions in tertrahydrofuran–water binary solutions. *The 10th Conference of Colloid Chemistry*, ISBN 978-963-9970-26-7, pp111–111, Budapest, Hungary, 29 – 31 August, 2012.

8. **Y. Li**, P. Huszthy, S. Kunsági-Máté: The solvent effect on the complex formation of a crown ether derivative with alkali metal ions. *The 10th Conference of Colloid Chemistry*, ISBN 978-963-9970-26-7, pp110–110, Budapest, Hungary, 29 – 31 August, 2012.

9. M. Poór, T. Kőszegi, **Y. Li**, Zs. Czibulya, S. Kunsági-Máté: Investigation of competitive interaction between ochratoxin A and drug molecules for serum albumin. *The 10th Conference of Colloid Chemistry*, ISBN 978-963-9970-26-7, pp109–109, Budapest, Hungary, 29 – 31 August, 2012.

10. **Y. Li**, P. Huszthy, S. Kunsági-Máté: Effect of molecular vibrations on the selectivity character of pyridino-18-crown-6 derivatives towards potassium ion. *Kémiai Szenzorok Workshop IV.*, Pécs, Hungary, 26 – 27april 2012.

11. G. Matisz, Zs. Csók, **Y. Li**, L. Kollár, S. Kunsági-Máté: Aromás szubsztituensekkel rendelkező kalix[4]arének kölcsönhatásai alkáli- és alkáliföldfém ionokkal: kvantumkémiai számítások. *Kémiai Szenzorok Workshop IV.*, Pécs, Hungary, 26 – 27 april 2012.

12. **Y. Li**, P. Huszthy, S. Kunsági-Máté: Spectrophotometric study of thermodynamics of complexation of pyridino-18-crown ether-6 with alkali cations. *The 3rd Asian Spectroscopy Conference*, pp269–269, Xiamen, China, 28 November – 01 December, 2011.

13. M. Beata Vizeli, **Y. Li**, A. Kumar, P. Sharma, S. Kunsági-Máté: Interaction of 25,26,27,28-tetrahydroxy-5,11,17,23-tetra-[4-(N-hydroxyl-3- phenylprop-2-enimidamido) phenylazo]calixarene host with ionic guests. *The 3rd Asian Spectroscopy Conference*, pp265–265, Xiamen, China, 28 November – 01 December, 2011.

14. **Y. Li**, P. Huszthy, S. Kunsági-Máté: Interaction of pyridino-18-crown-6 ligands with alkali metal ions in methanol. *Advanced Spectroscopies on Biomedical and Nanostructured Systems*, ISBN 978-973-0-11393-8, pp144–144, Cluj-Napoca, Romania, 04 – 07 September, 2011.

15. **Y. Li**, Zs. Csók, T. Kégl, L. Kollár, K. Iwata, S. Kunsági-Máté: Complex formation of functionalized cavitand derivatives with alkali metal ions in methanol solutions. *Advanced Spectroscopies on Biomedical and Nanostructured Systems*, ISBN 978-973-0-11393-8, pp110–110, Cluj-Napoca, Romania, 04 – 07 September, 2011.

III. Other publications in refereed journals

1. M. Poór, M. Kuzma, G. Matisz, **Y. Li**, P. Perjési, S. Kunsági-Máté, T. Kőszegi: Further aspects of ochratoxin A – cation interactions: complex formation with zinc ions and a novel analytical application of ochratoxin A – magnesium interaction in the HPLC-FLD system. *Toxins (BASEL)*, 6(2014): 1295 – 1307. **IF: 2.480** (2013)

2. M. Poór, **Y. Li**, G. Matisz, L. Kiss, S. Kunsági-Máté, T. Kőszegi: Quantitation of species differences in albumin–ligand interactions for bovine, human and rat serum albumins using fluorescence spectroscopy: A test case with some Sudlow's site I ligands. *Journal of Luminescence*, 145(2014): 767 – 773. **IF: 2.367** (2013)

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4. M. Poór, S. Kunsági-Máté, **Y. Li**, T. Kőszegi: A new possible mechanism of flavonoid-drug interaction: flavonoids are able to displace warfarin from human serum albumin. *Biochmica Clinica*, 37(2013): 249. **IF: 0.000** (2013)

5. M. Poór, **Y. Li**, S. Kunsági-Máté, Zs. Varga, A. Hunyadi, B. Dankó, F. Chang, Y. Wu, Protoapigenone derivatives: albumin binding properties and effects on HepG2 cells. *Journal of Photochemistry and Photobiology B: Biology*, 124(2013): 20–26. **IF: 2.803** (2013)

6. M. Poór, S. Kunsági-Máté, Zs. Czibulya, **Y. Li**, B. Peles-Lemli, J. Petrik, S. Vladimir-Knežević, T. Kőszegi: Fluorescence spectroscopic investigation of competitive interactions between ochratoxin A and 13 drug molecules for binding to human serum albumin. *Luminescence*, 28(2013): 726–733. **IF: 1.675** (2013)

7. M. Poór, **Y. Li**, S. Kunsági-Máté, J. Petrik, S. Vladimir-Knežević, T. Kőszegi: Molecular displacement of warfarin from human serum albumin by flavonoid aglycones. *Journal of Luminescence*, 142(2013): 122–127. **IF: 2.367** (2013)

8. M. Poór, S. Kunsági-Máté, G. Matisz, **Y. Li**, Zs. Czibulya, B. Peles-Lemli, T. Kőszegi: Interaction of alkali and alkaline earth ions with ochratoxin A. *Journal of Luminescence*, 135(2013): 276–280. **IF: 2.367** (2013)

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