



BIOACTIVE COMPOUNDS / CYCLODEXTRIN COMPLEXES: synthesis, characterisation, applications, and molecular modeling

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Habilitation Thesis

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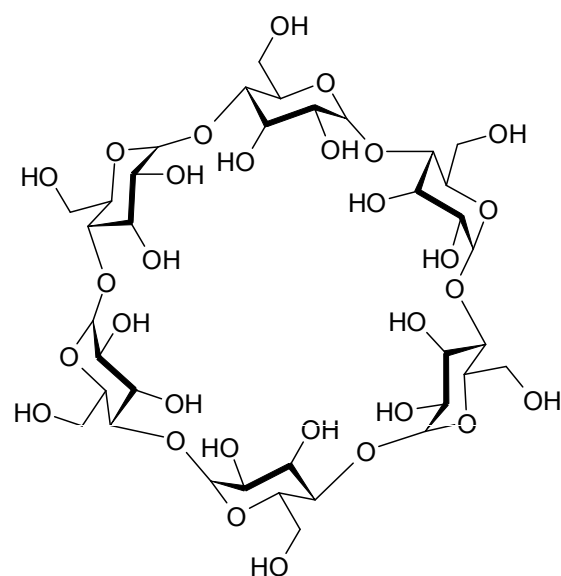
The Thesis is structured in eight Chapters:

- *Chapter one*: Introduction
- *Chapters two - seven* contain the main results in my research field from 2006 up to present, and
- *Chapter eight* on “Future trends in academic and professional directions”

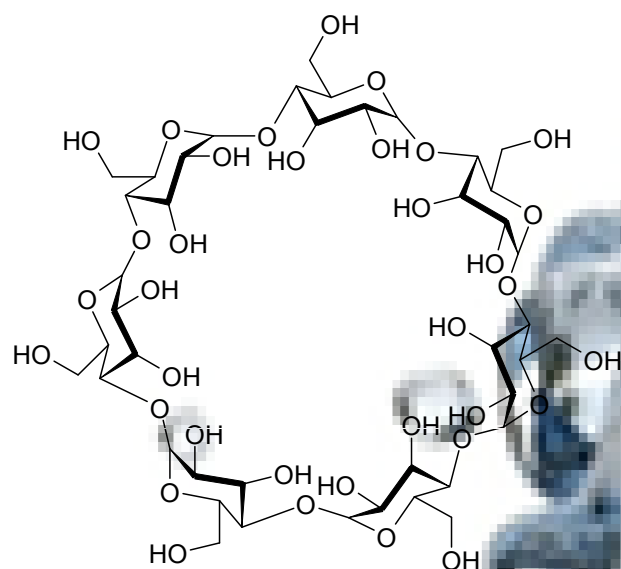
The **main objectives** of my research in the past ten years were:

1. Evaluation of the type of water molecules in cyclodextrins and their complexes
2. Synthesis, analysis, and possible applications of
 - natural antioxidants / cyclodextrin complexes
 - essential oils / cyclodextrin complexes
 - fatty acids / cyclodextrin complexes
 - alkaloids / cyclodextrin complexes
3. Molecular modeling and docking experiments on bioactive compounds / cyclodextrin complexes

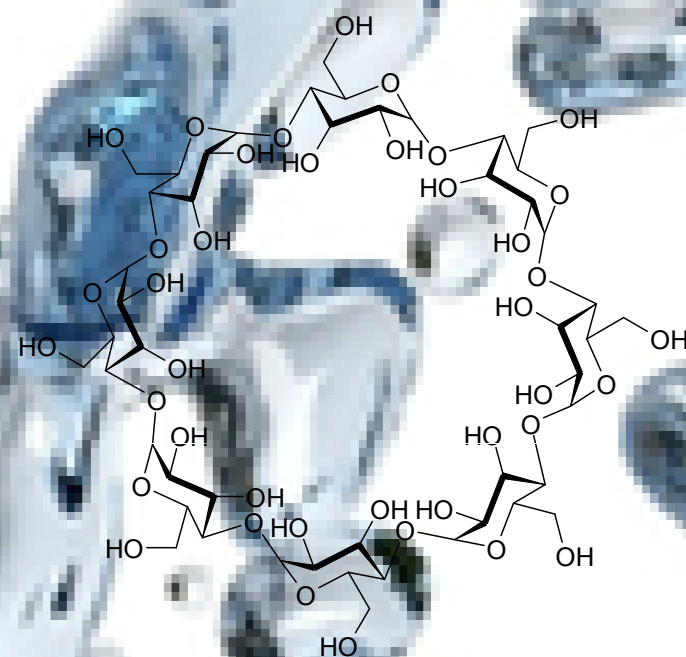
Chapter 1. INTRODUCTION



α -cyclodextrin



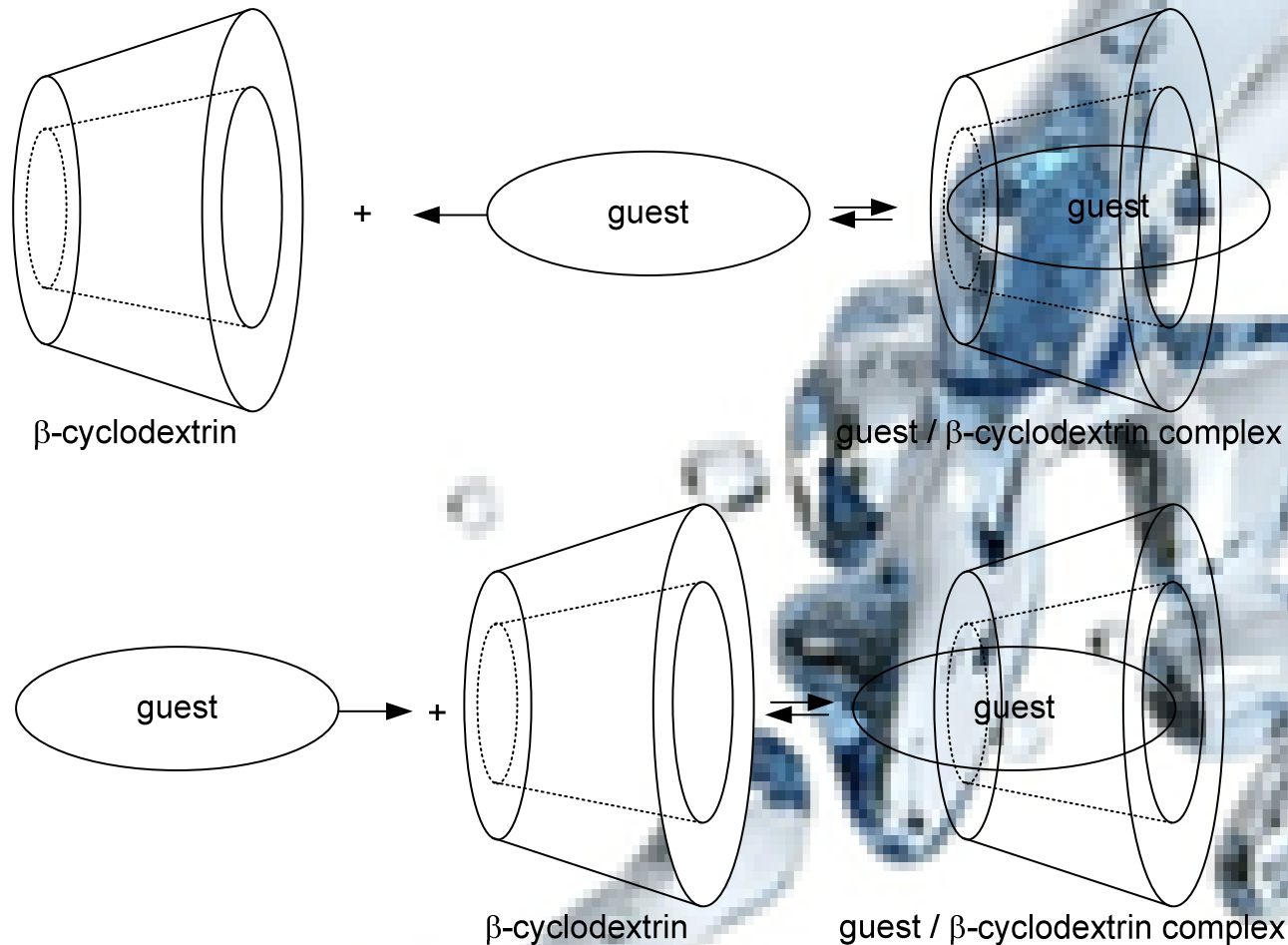
β -cyclodextrin



γ -cyclodextrin

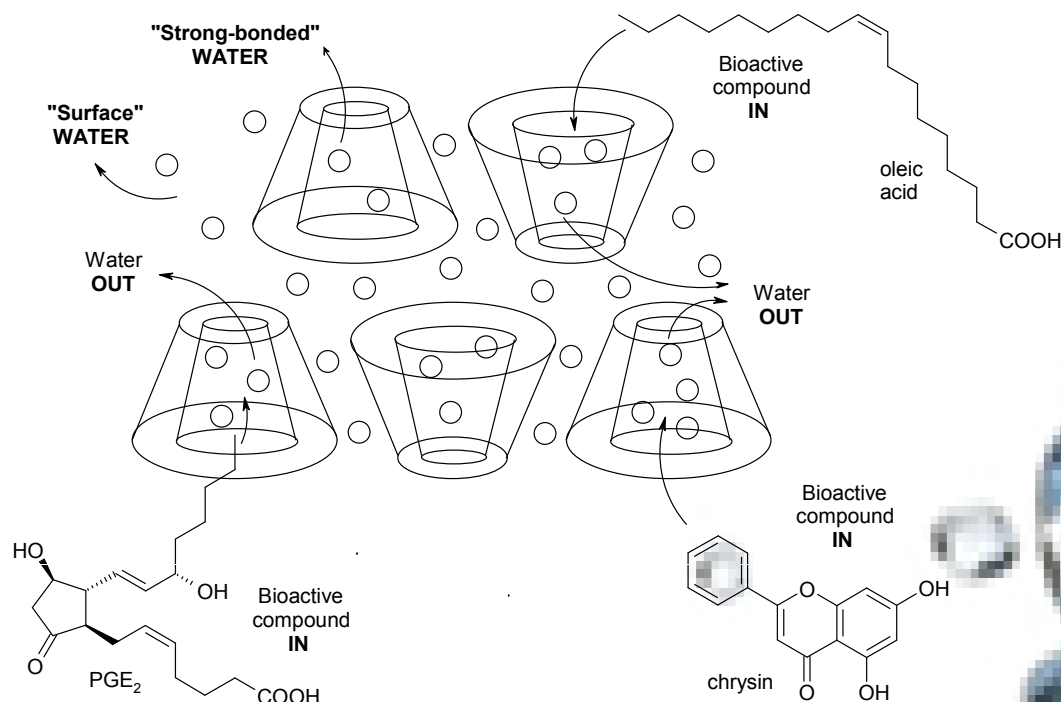
Cyclodextrins are cyclic oligosaccharides consist of six, seven, and eight glucose units corresponding to the most known natural α -, β -, and γ -cyclodextrin.

Cyclodextrins have structures like truncated cones with hydrophilic exterior and hydrophobic cavities.



Cyclodextrins can encapsulate (molecular encapsulation by guest-host interaction) geometrically compatible hydrophobic compounds. The resulting complexes (micro- or nanoparticles) protect the encapsulated compounds against degradation (oxygen, light, humidity) and allows a controlled release.

Chapter 2. "SURFACE WATER" AND "STRONG-BONDED WATER" IN CYCLODEXTRINS



The researches were focused on water implication in cyclodextrin properties:

- methods for determination of the "real" water concentration in cyclodextrins;
- evaluation of the type of water molecules in cyclodextrin structures by a new concept;
- establish the optimum conditions for determination of water content by Karl Fisher method.

In the solid state, water crystallization molecules exist both in "pure" cyclodextrins and corresponding bioactive compound complexes.

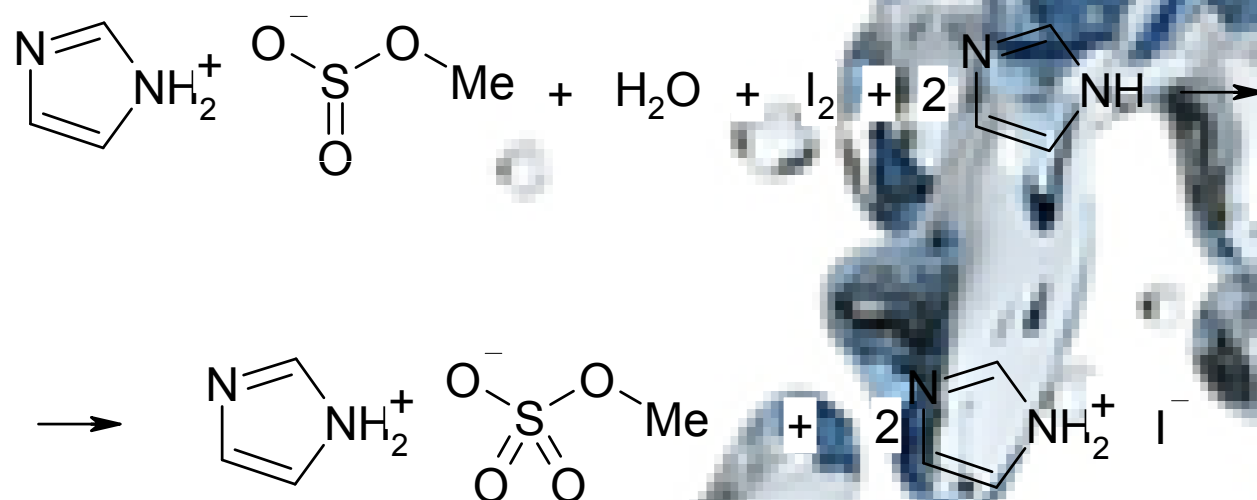
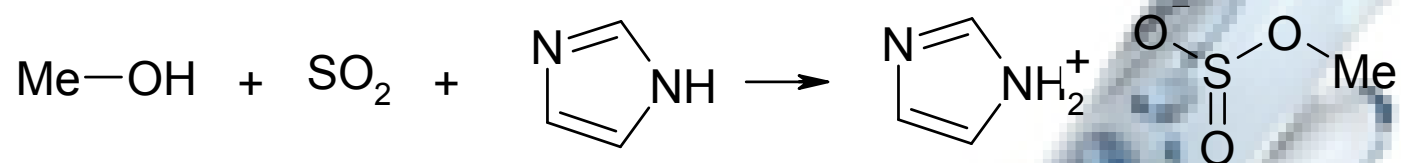
In the complexation process, hydrophobic compounds are encapsulated in the inner cavities of cyclodextrins by means of van der Waals interactions, and some of water molecules are replaced.

Cyclodextrins contain two types of water molecules:

- **"Surface" water molecules** (especially from the crystal surface) and
- **"Strong-bonded" water molecules** (especially from the cavity).

The concentration of these water molecules are relatively difficult to estimate by simple methods.

Parameter optimization of Karl Fischer water titration of cyclodextrins



The volumetric Karl Fischer titration (KFT) is a proper method to estimate the “surface water” and “strong-bonded water” molecules by means of the water reaction rate (imidazolium methylsulphite and iodine reacts with water to give imidazolium methylsulphate and iodide)

Statistically significant correlations between the water content of β - and γ -cyclodextrin, **W(%)**, and the hydrophobicity of the solvent mixture, **logP**, were obtained.

$$W(\%) = 10.81(\pm 0.10) + 0.63(\pm 0.21) \cdot \log P$$

$$n = 4; \mathbf{r = 0.951}; s = 0.17; F = 9.5$$

for β -cyclodextrin in alkane-methanol mixture

$$W(\%) = 9.60(\pm 0.12) + 0.50(\pm 0.28) \cdot \log P$$

$$n = 4; \mathbf{r = 0.784}; s = 0.23; F = 3.2$$

for γ -cyclodextrin in alkane-methanol mixture

The correlation of “strong bonded” water reaction rate (v_2) with the hydrophobicity of the solvent mixture ($\log P$) conduct to similar equations for both α - and β -cyclodextrin.

$$v_{2(aCD)} = 0.715(\pm 0.143) + 0.817(\pm 0.221) \cdot \log P_{solv.mix.}$$

$$n = 4, r = \mathbf{0.934}, s = 0.046, F = 14$$

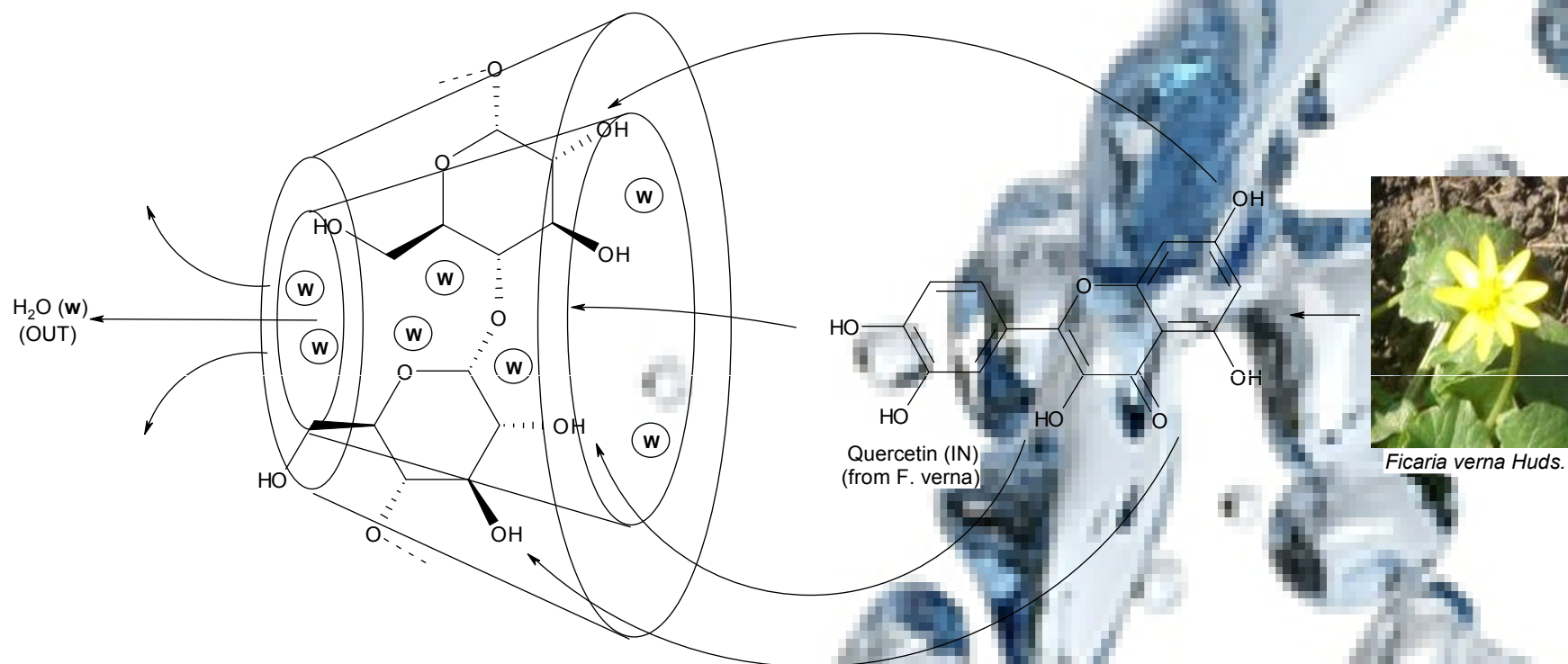
$$v_{2(bCD)} = 0.630(\pm 0.149) + 0.619(\pm 0.231) \cdot \log P_{solv.mix.}$$

$$n = 4, r = \mathbf{0.885}, s = 0.047, F = 8$$

Conclusions on KFT water titration of cyclodextrins

- (1) KFT is an appropriate method to determine the total water concentration in cyclodextrin samples;
- (2) The water reaction rate from KFT process can be a good indicator for the concentration of “**surface**” and “**strong-bonded**” water molecules from natural cyclodextrins;
- (3) The “strong-bonded” water molecules are especially those from the cyclodextrin cavity; this affirmation is sustained by the KFT behavior: the hydrophobicity of the solvent is very important because it can replace the water molecules from cyclodextrin cavity and favors the diffusion/extraction of water in KFT process.

Chapter 3. NATURAL ANTIOXIDANTS / CYCLODEXTRIN COMPLEXES



Hădărugă, D. I.; Hădărugă, N. G.; Bandur, G.; Isengard, H.-D. Water content of flavopoid/cyclodextrin nanoparticles: relationship with the structural descriptors of biologically active compounds. *Food Chemistry* **2012**, 132, 1651-1659

Hădărugă, N. G. *Ficaria verna* Huds. extracts and their β -cyclodextrin supramolecular systems. *Chemistry Central Journal* **2012**, 6, 16, doi: 10.1186/1752-153X-6-16



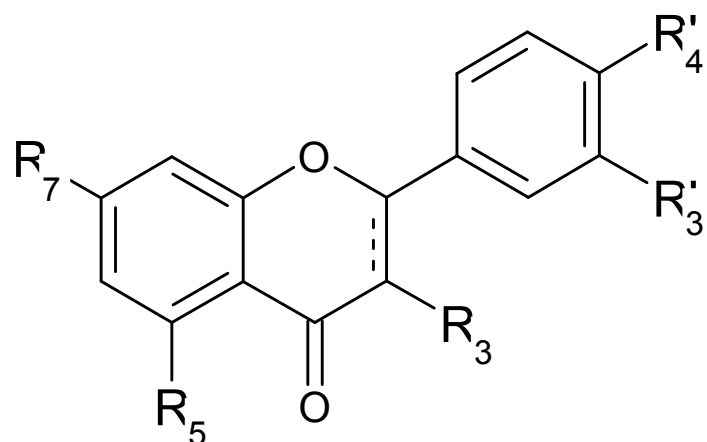
Studies on **natural antioxidants / cyclodextrin complexes** field:

(1) Synthesis and analysis of:

- flavonoids, flavonosides, flavonolignans/cyclodextrin complexes;
- fatty acid-flavonoside or -flavonolignan bioconjugate / cyclodextrin complexes;
- antioxidant compounds (*i.e. Ficaria verna* extracts) / cyclodextrin complexes;

(2) Antioxidant activity and water importance in the natural antioxidants/cyclodextrin complexes.

Flavonoid and related compounds/cyclodextrin complexes



Flavone (Flv): $R_{3,5,7} = H$; $R'_{3,4} = H$; 2,3-double

Chrysin (Chr): $R_3 = H$; $R_{5,7} = OH$; $R'_{3,4} = H$; 2,3-double

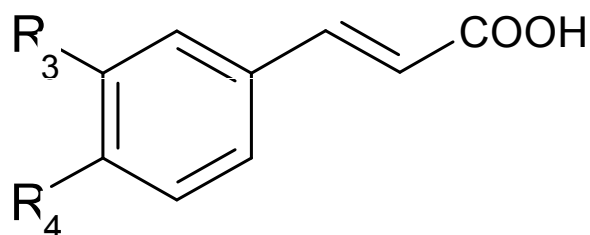
Naringenin (Nrg): $R_3 = H$; $R_{5,7} = OH$; $R'_{3,4} = H$; (2*S*)-2,3-single

Hesperetin (Hsp): $R_3 = H$; $R_{5,7} = OH$; $R'_3 = OH$; $R'_4 = OMe$; (2*S*)-2,3-single

Apigenin (Apg): $R_3 = H$; $R_{5,7} = OH$; $R'_3 = H$; $R'_4 = OH$; 2,3-double

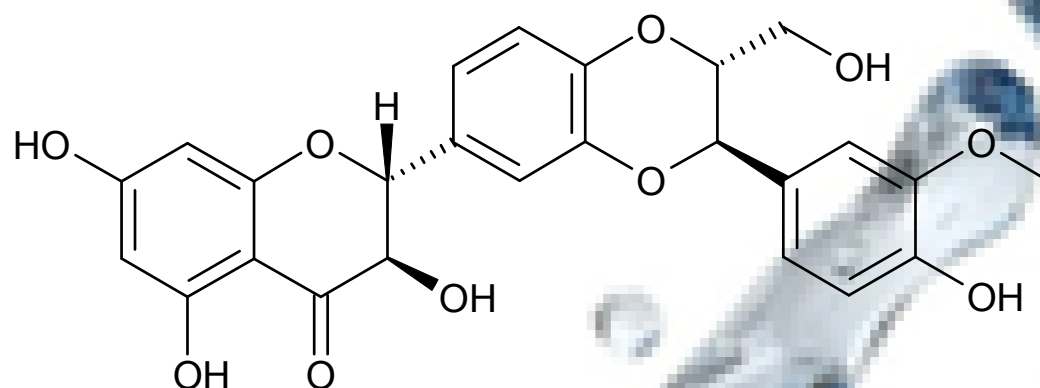
Fisetin (Fst): $R_{3,7} = OH$; $R_5 = H$; $R'_{3,4} = OH$; 2,3-double

Luteolin (Ltn): $R_3 = H$; $R_{5,7} = OH$; $R'_{3,4} = OH$; 2,3-double



Cinnamic acid (Cnm): $R_{3,4} = H$

Caffeic acid (Cff): $R_{3,4} = OH$



Silybin (Sbn)

The flavonoid, flavonoside, and flavonolignan (presented above) / cyclodextrin complexes were obtained by crystalization and by ultrasonication methods.

In the case of flavonoid (and similar compounds) / β -cyclodextrin complexes the KFT water concentration were in the range of **8.9-12.6% (crystallization method)**, and higher in the case of ultrasonication method (10.7-13.3% water).

No	Code	Description	KFT water content (%)	n (no of detn.)	TG water content (%)
1	02_Flv_bCD	Flavone/ β -cyclodextrin nanoparticles (obtained by crystallisation method)	10.59 \pm 0.10	4	10.7
2	05_Chr_bCD	Chrysin/β-cyclodextrin nanoparticles (obtained by crystallisation method)	8.85\pm0.06	5	9.3
3	08_Nrg_bCD	Naringenin/ β -cyclodextrin nanoparticles (obtained by crystallisation method)	9.95 \pm 0.40	5	9.5
4	11_Hsp_bCD	Hesperetin/ β -cyclodextrin nanoparticles (obtained by crystallisation method)	10.44 \pm 0.19	5	10.1
5	14_Apg_bCD	Apigenin/ β -cyclodextrin nanoparticles (obtained by crystallisation method)	10.07 \pm 0.47	5	9.9
6	17_Fst_bCD	Fisetin/ β -cyclodextrin nanoparticles (obtained by crystallisation method)	12.08 \pm 0.39	5	10.4
7	20_Ltn_bCD	Luteolin/ β -cyclodextrin nanoparticles (obtained by crystallisation method)	11.15 \pm 0.12	4	9.6
8	23_Sbn_bCD	Silybin/ β -cyclodextrin nanoparticles (obtained by crystallisation method)	9.43 \pm 0.22	5	8.6
9	26_Cnm_bCD	Cinnamic acid / β -cyclodextrin nanoparticles (obtained by crystallisation method)	11.52 \pm 0.42	5	10.1
10	29_Cff_bCD	Caffeic acid/ β -cyclodextrin nanoparticles (obtained by crystallisation method)	12.56\pm0.38	5	12.0

The water content (**W**, %) is good correlated with some hydrophobic descriptors of the flavonoids and similar compounds:

- **logP** – the logarithm of the octanol-water partition coefficient, and
- **N_{np}** – the number of nonpolar atoms

$$W(\%) = 13.54(\pm 0.62) - 1.14(\pm 0.24) \cdot \text{Log}P$$

$$n = 10; r = -0.871; F = 22.0; s = 0.60$$

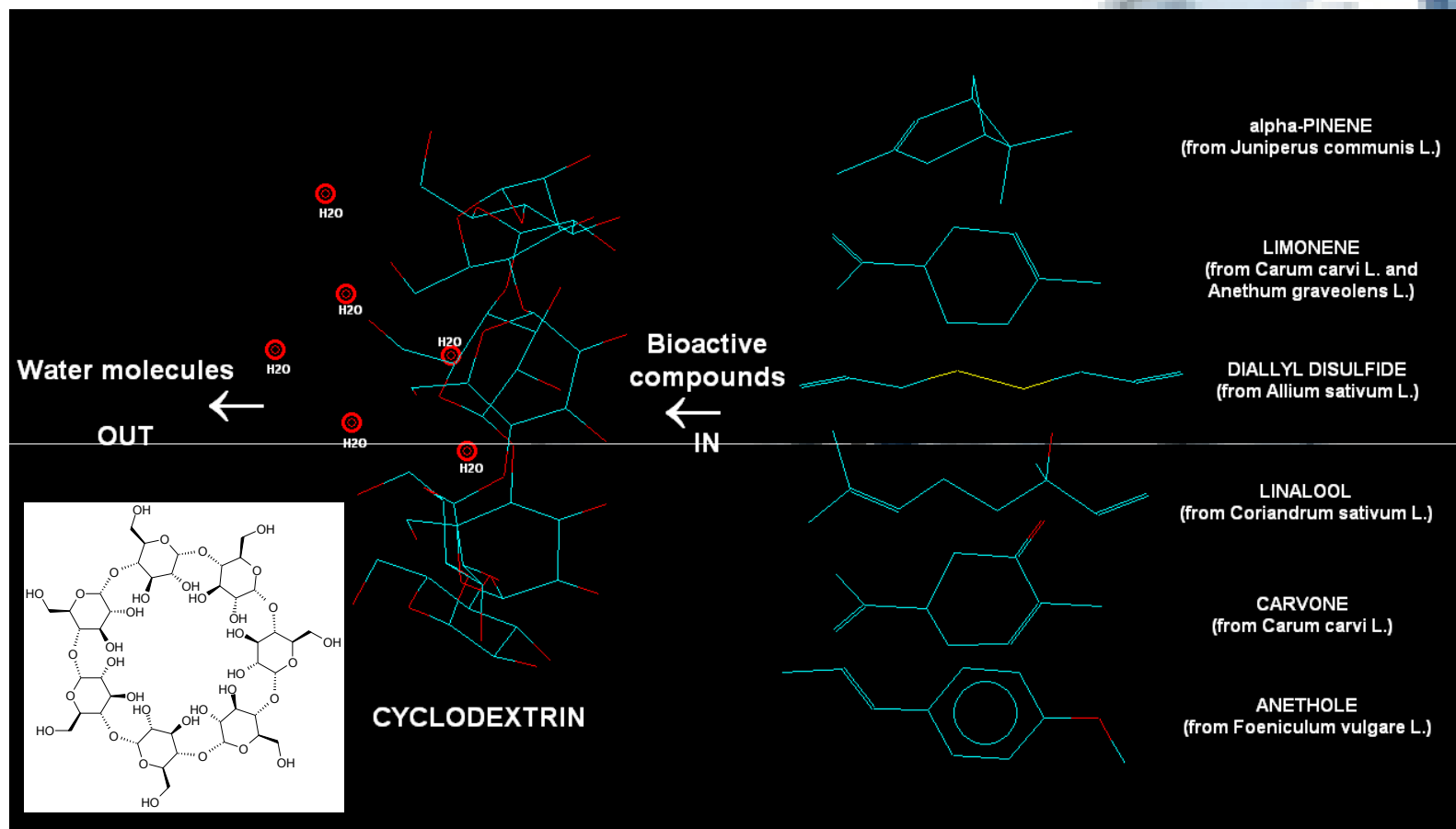
$$W(\%) = 13.77(\pm 1.00) - 0.31(\pm 0.10) \cdot N_{np}$$

$$n = 10; r = -0.752; F = 10.4; s = 0.82$$

Conclusions on natural antioxidants / cyclodextrin complexes

- (1) The study on **flavonoid / cyclodextrin complexes** indicates that the formation of the complex depends of the **hydrophobicity** of the guest compound (expressed by different descriptors), but only by **crystallization method**;
- (2) The most important descriptors are: **logP** – logarithm of octanol-water partition coefficient, **N_{np}** – total number of non-polar atoms, and the **water solubility** of the guest compound (**logS**).

Chapter 4. ESSENTIAL OILS / CYCLODEXTRIN COMPLEXES



Hădărugă, N. G.; Hădărugă, D. I.; Isengard, H.-D. Water content of natural cyclodextrins and their essential oil complexes: a comparative study between Karl Fischer titration and thermal methods. *Food Chemistry* **2012**, 132, 1741-1748

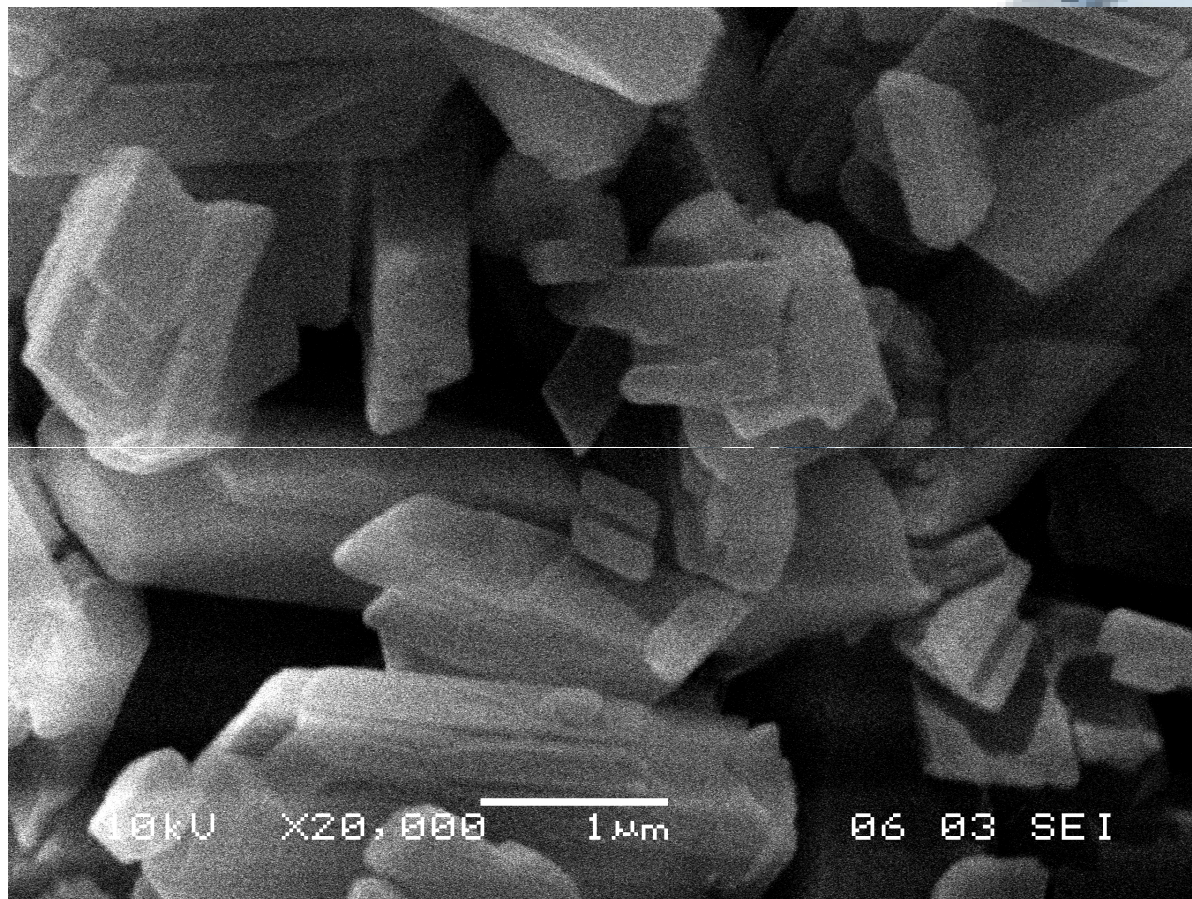
Hădărugă, N. G.; Gharibeh-Branic, A.; Hădărugă, D. I.; Gruia, G.; Pleșa, C.; Costescu, C.; Ardelean, A.; Lupea, A. X. Comparative study on *Juniperus communis* and *Juniperus virginiana* essential oils: TLC and GC analyses. *Journal of Planar Chromatography - Modern TLC* **2011**, 24, 130-135



The studies regarding the **essential oils / cyclodextrin complexes** were targeted on the following aspects:

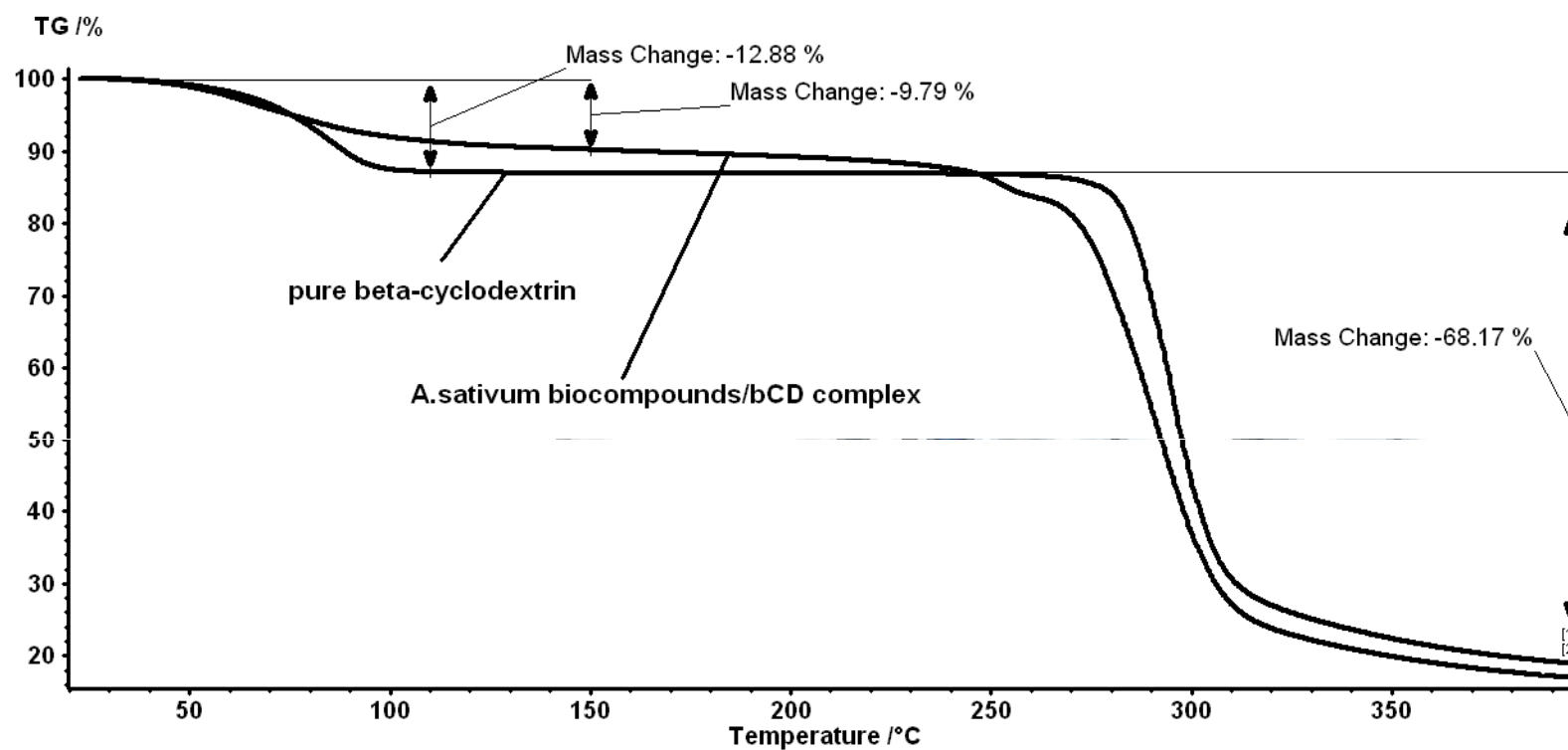
- Synthesis and characterization of essential oils (from various botanical classes such as Monocotyledonatae, Dicotyledonatae, and Pinatae) / cyclodextrin complexes;
- Evaluation of encapsulation competitiveness, protection/stability, and controlled release properties of these complexes, and
- **Establish the water importance on the encapsulation process of essential oils in cyclodextrins.**

These studies **continue** the research on essential oil/cyclodextrin complexes performed in the **PhD Thesis**. Thus, new essential oil / cyclodextrin complexes were obtained and analyzed by **SEM** ...



Scanning electron microscopy analysis for
Allium sativum L. essential oils/β-cyclodextrin complexes

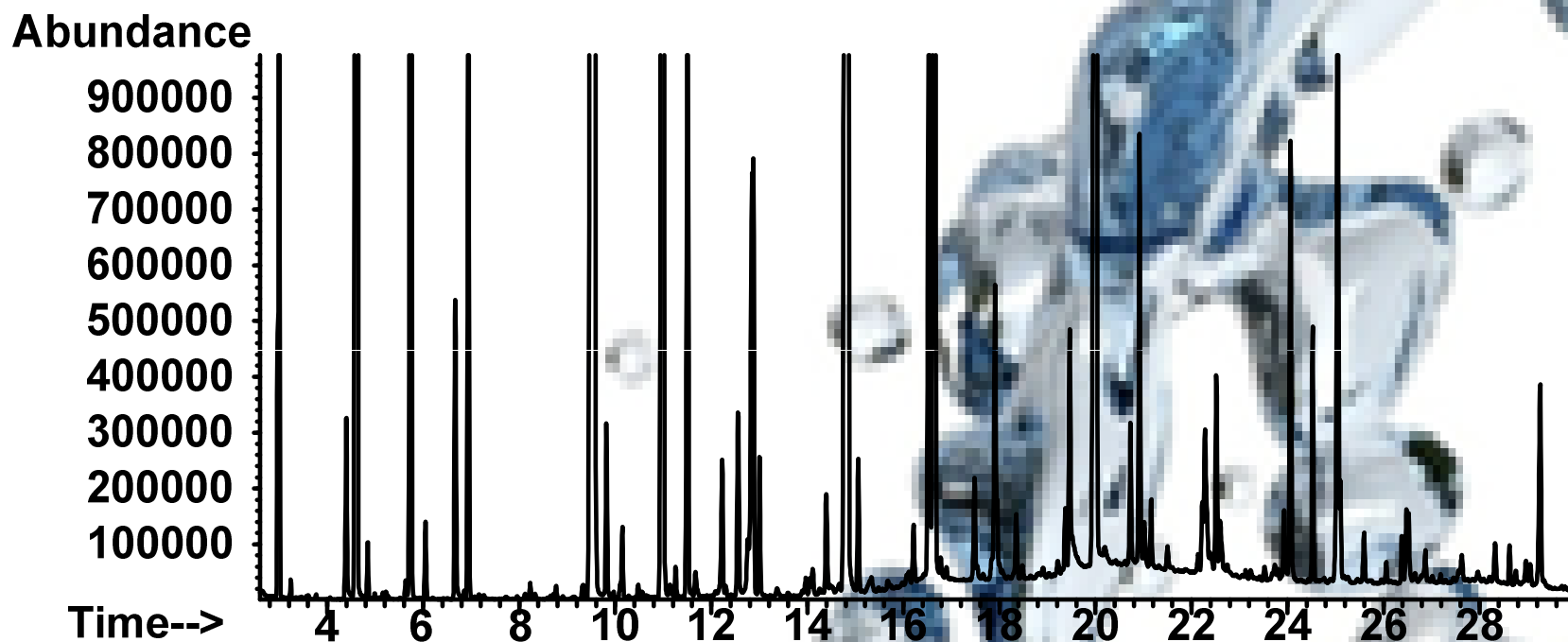
... The essential oil / cyclodextrin complexes were analyzed by DSC, TG ...



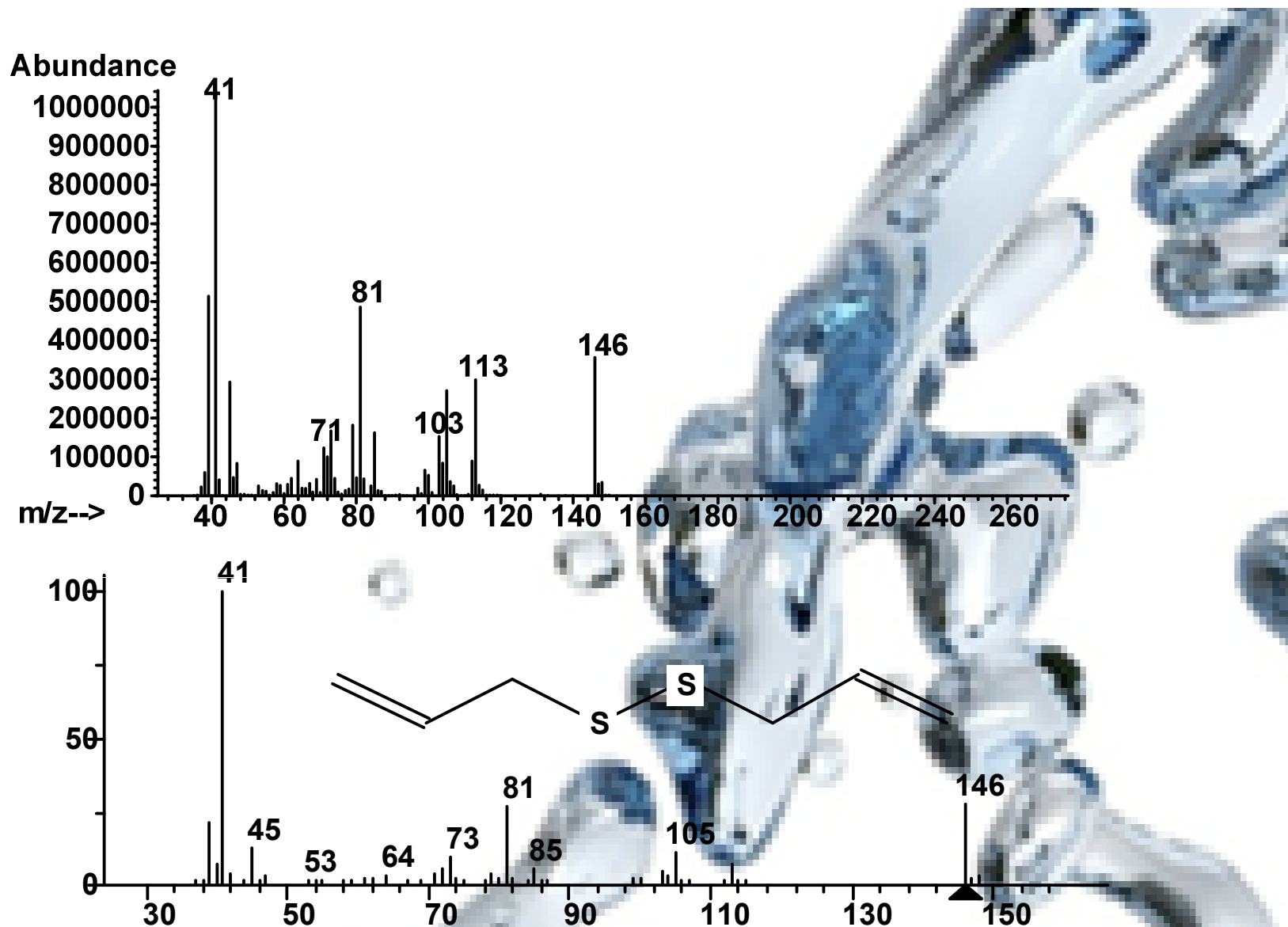
Thermograms superposition for pure β CD and *Allium sativum* L. biocompounds/ β CD nanoparticles

The raw and recovered essential oils were analyzed by **GC-MS**.

In the case of ***Allium sativum* essential oil** and its **β -cyclodextrin complex** more than one hundred compounds were identified. The most important was **diallyl-disulfide**.



The GC chromatogram from the GC-MS analysis of the essential oil of *Allium sativum* L.



Experimental and from the NIST database MS spectra for **diallyl-disulfide**

Diallyl-disulphide was better encapsulated in cyclodextrin in comparison with other similar compounds (**20%** in the **raw essential oil** and **29%** in the cyclodextrin **complex**; these are expressed as relative concentrations).

Compound name	Code	As	As/bCD
Diallyl-monosulfide	DAMS	12.3	7
1,3-Dithiane	DT	4.6	2.2
Diallyl-disulphide	DADS	19.8	29.3
Allyl-methyl-trisulfide	AM3S	8.3	8
1,3,5-Trithiane	TT	1.7	1.7
Diallyl-trisulfide	DA3T	16.2	18.4
Diallyl-tetrasulfide	DA4S	4.9	3.9

Relative concentration (%) for the non-encapsulated (As) and β -cyclodextrin encapsulated *Allium sativum* L. biocompounds (As/bCD)

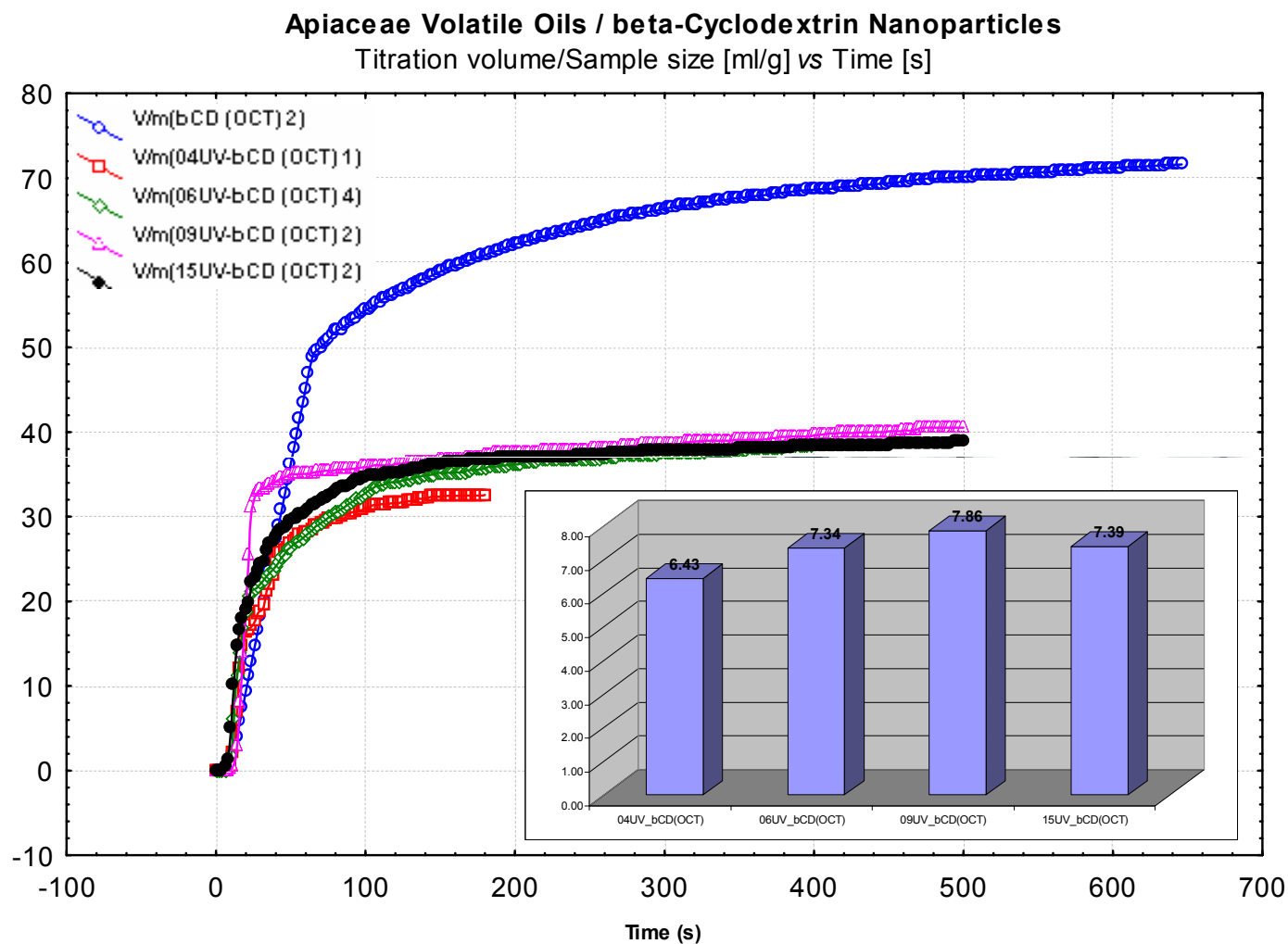
One of the most important part of the research on essential oil / cyclodextrin complexes was the **analysis of the water content by Karl Fisher titration** and thermogravimetry. Generally, the water content of essential oil/ β -cyclodextrin complexes was **higher** in the **Karl Fischer method** than from thermogravimetry.

The difference is up to **1.6%** (*Allium sativum*/ β -cyclodextrin) and is due to the presence of the strong-bonded water in these complexes.

No	Code	Water (%) by KFT ^a	TG mass loss (%) (<100°C) ^c	TG mass loss (%) (<250°C)
1	bCD	14.29 ± 0.30	13.80	14.51
2	Cc_bCD	6.43 ± 0.29	6.0	9.4
3	Cs_bCD	7.34 ± 0.13	5.4	9.5
4	Fv_bCD	7.86 ± 0.21	7.3	10.0
5	Ag_bCD	7.39 ± 0.14	5.5	9.9
6	As_bCD	6.87 ± 0.46	5.3	9.5
7	Jc_bCD	7.41 ± 0.41	6.6	7.8
8	Jc(l)_bCD	7.44 ± 0.23	6.8	9.0
9	Jc(f)_bCD	8.09 ± 0.56	6.8	9.1

Water content of β -cyclodextrin (bCD) and the corresponding *Carum carvi* L. (Cc), *Coriandrum sativum* L. (Cs), *Foeniculum vulgare* (Fv), *Anethum graveolens* L. (Ag), *Allium sativum* L. (As), and *Juniperus communis* L. (Jc) bCD complexes

The evolution of the Karl Fischer titration process can provide information about the type of water molecules from cyclodextrin complexes:



Karl Fischer water titration curves for β -cyclodextrin (upper curve) and for Apiaceae essential oil/ β -cyclodextrin complexes (lower curves)

The type of water molecules were:

- “surface water molecules” which react with higher rates: 3-8 milimolar/second
- “strong-bonded water molecules” with lower reaction rates: 0.2-1 milimolar/second

No	Code	v_1 (mM/s)	v_2 (mM/s)
1	aCD	3.99 ± 0.85	0.17 ± 0.07
2	bCD	3.23 ± 0.44	0.36 ± 0.04
3	Cc_bCD	3.58 ± 0.81	0.95 ± 0.17
4	Cs_bCD	4.12 ± 1.08	0.91 ± 0.09
5	Fv_bCD	7.76 ± 1.42	0.27 ± 0.19
6	Ag_bCD	6.05 ± 0.41	0.73 ± 0.08
7	As_bCD	5.43 ± 0.87	0.67 ± 0.09
8	Jc_bCD	4.18 ± 0.59	0.93 ± 0.19
9	Jc(l)_bCD	6.57 ± 0.73	0.85 ± 0.14
10	Jc(f)_bCD	6.26 ± 0.89	0.62 ± 0.09

Mean values for water extraction rate for three important ranges (0-50s, 50-100s, and 100-500s) for natural cyclodextrins and essential oils/ β -cyclodextrin complexes

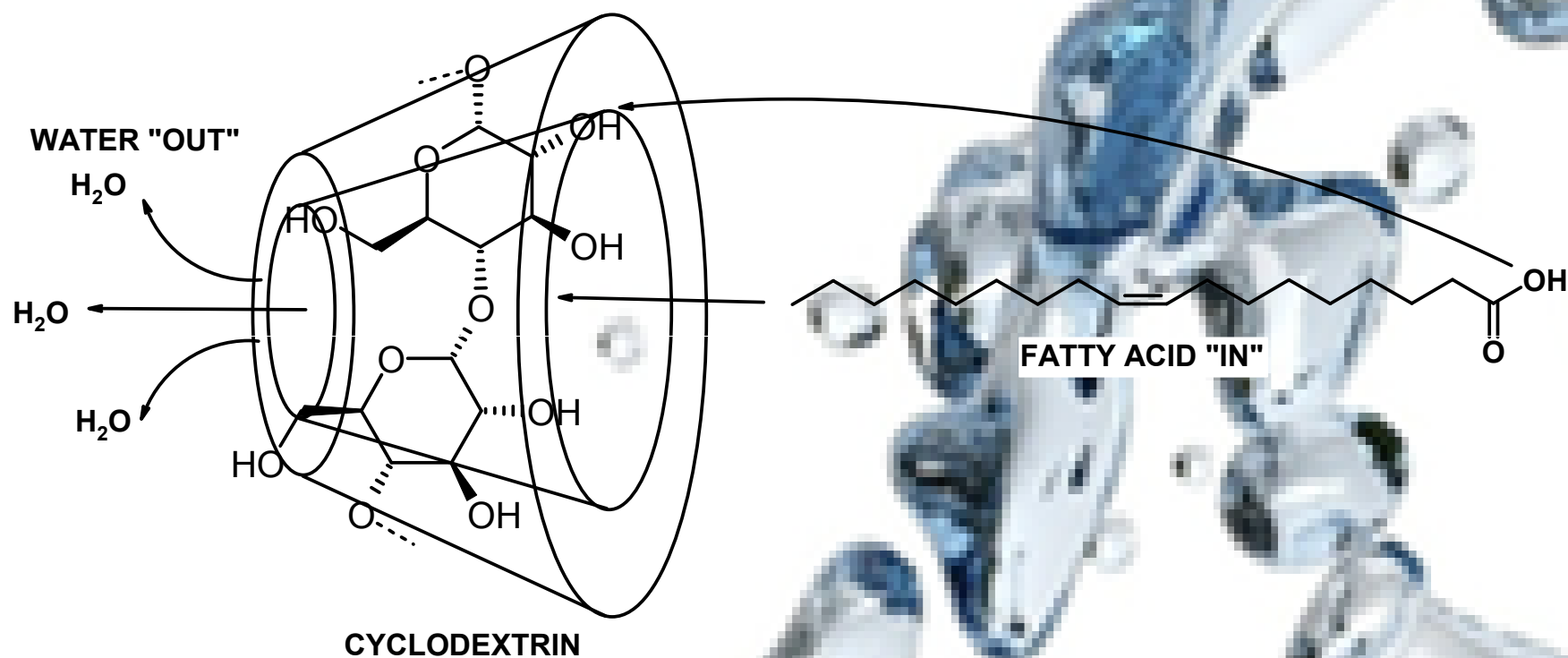
Conclusion on essential oils / cyclodextrin complexes

- (1) The hydrophobic compounds were encapsulated in higher relative concentrations comparatively with the oxygenated compounds, but the rigidity and the volume of structures are also important.

Thus, the relative concentration of the unsaturated acyclic compounds is higher than for the saturated acyclic and cyclic sulfide compounds, relative to the raw *Allium sativum* essential oil;

- (2) Classical Karl Fischer water titration is a good tool for evaluation of water concentration and type of water molecules in essential oil / cyclodextrin complexes.

Chapter 5. FATTY ACIDS / CYCLODEXTRIN COMPLEXES



Hădărugă, D. I.; **Hădărugă, N. G.**; Butnaru, G.; Tatu, C.; Gruia, A. Bioactive microparticles (10): Thermal and oxidative stability of nicotine and its complex with β -cyclodextrin. *Journal of Inclusion Phenomena and Macrocyclic Chemistry* **2010**, 68, 155-164

Hermenean, A.; Popescu, C.; Ardelean, A.; M., S.; **Hădărugă, N. G.**; Mihali, C.-V.; Costache, M.; Dinischiotu, A. Hepatoprotective Effects of *Berberis vulgaris* L. Extract/ β -Cyclodextrin on Carbon Tetrachloride-Induced Acute Toxicity in Mice. *International Journal of Molecular Sciences* **2012**, 13, 9014-9034

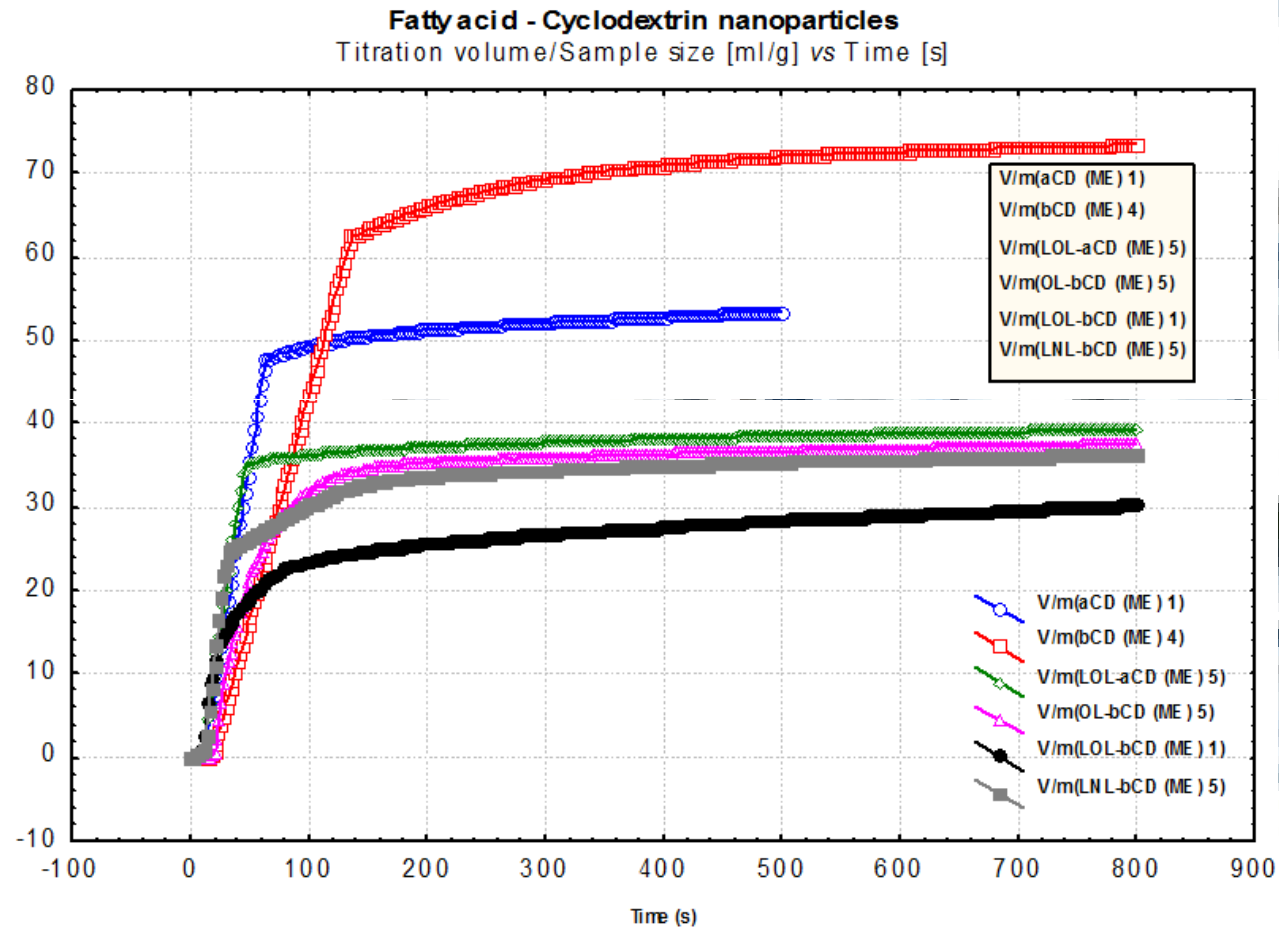


Researches on **fatty acid / cyclodextrin complexes** continue the **doctoral studies** and were focused on:

- (1) The obtaining and characterization of fatty acid / cyclodextrin complexes, establish of the protection capacity of complexes against degradation of fatty acids, and
- (2) **Evaluation the water content and types of these molecules in the cyclodextrin complexes in order to evaluate the encapsulation process.**

Water evaluation of fatty acid / cyclodextrin complexes

The most important results on the fatty acid / cyclodextrin complexes analysis was related to the Karl Fischer water titration.



Titration volume/sample size vs. Time for fatty acids/cyclodextrin complexes and for α - (blue) and β -cyclodextrin (red)

The water concentration in the fatty acid (oleic, linoleic and linolenic acids) / α - and β -cyclodextrin complexes is 4-7% lower than in the case of natural cyclodextrins. Further, the water reaction rate for “surface” and “strong-bonded” water molecules in the fatty acid/cyclodextrin complexes was determined:

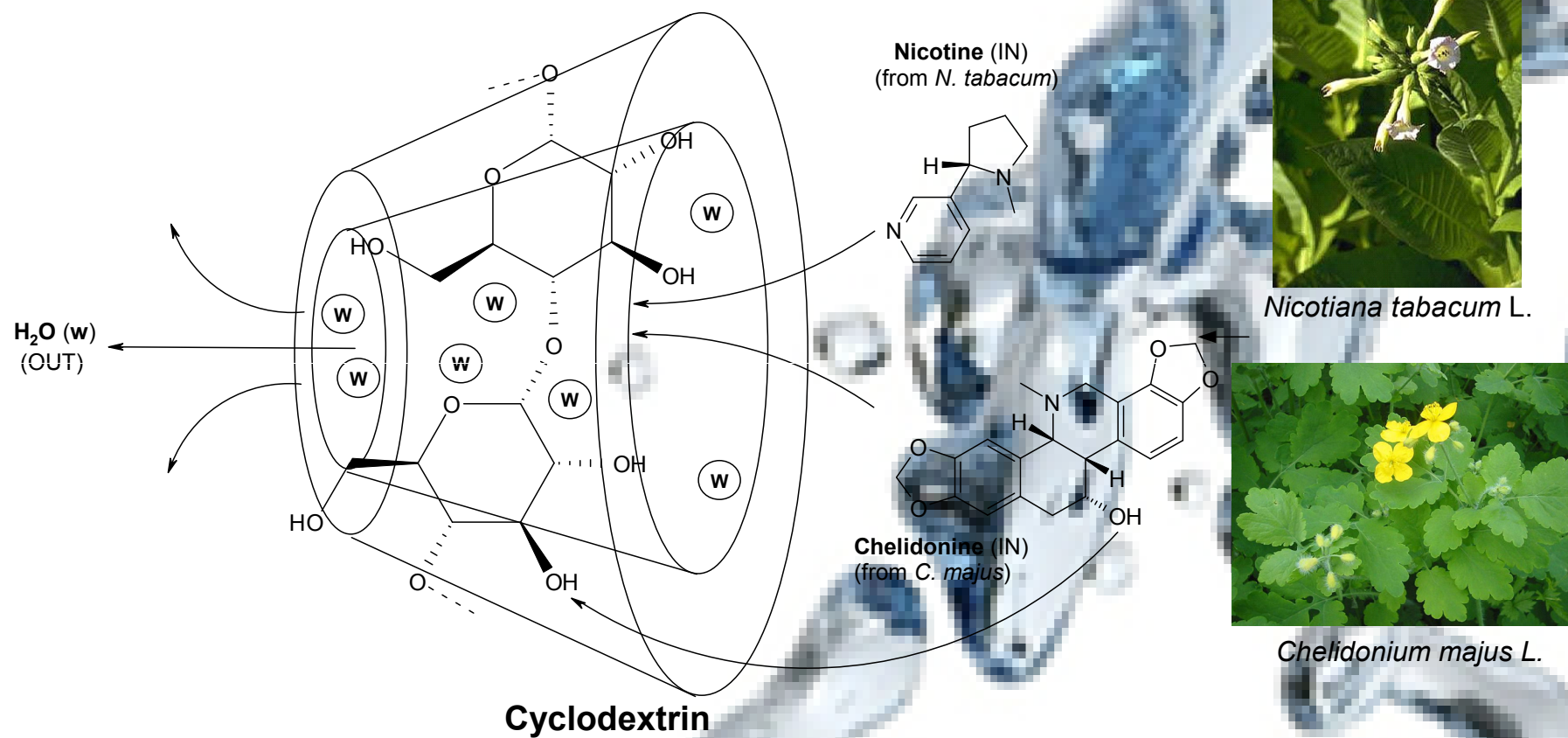
- surface water: 3.6-8.6 mM/s (depending on the unsaturation of the fatty acid for complexes); only 3.2 mM/s for β -cyclodextrin;
- strong-bonded water: 0.6-1.4 mM/s; only 0.4 mM/s for β -cyclodextrin

No	Code	Description	KFT water content (%)	v_1 (mM/s)	v_2 (mM/s)
1	LOL_aCD(ME)	Linoleic acid/ α -cyclodextrin complex (classical KFT with methanol as solvent)	7.82±0.42	6.12±0.58	2.32±0.91
2	OL_bCD(ME)	Oleic acid/ β -cyclodextrin complex (classical KFT with methanol as solvent)	7.43±0.19	3.56±0.65	0.79±0.19
3	LOL_bCD(ME)	Linoleic acid/ β -cyclodextrin complex (classical KFT with methanol as solvent)	7.28±0.11	5.42±0.91	1.44±1.25
4	LNL_bCD(ME)	Linolenic acid/ β -cyclodextrin complex (classical KFT with methanol as solvent)	7.25±0.37	8.67±1.21	0.56±0.07
5	aCD(ME)	α -Cyclodextrin (classical KFT with methanol as solvent)	10.66±0.16	3.99±0.85	0.17±0.07
6	bCD(ME)	β -Cyclodextrin (classical KFT with methanol as solvent)	14.59±0.19	3.23±0.44	0.36±0.04

Conclusion on fatty acid / cyclodextrin complexes

- (1) An important hydrophobic interaction between the fatty acid and cyclodextrin structures exists;
- (2) Good thermal stability of free fatty acid/cyclodextrin complexes was observed;
- (3) KFT water content values for these complexes are more accurate than the thermogravimetric analysis;
- (4) The water content of unsaturated fatty acid/cyclodextrin complexes is low, suggesting a better hydrophobic interaction between host and guest molecules.

Chapter 6. ALKALOIDS / CYCLODEXTRIN COMPLEXES



Hădărugă, D. I.; Hădărugă, N. G.; Butnaru, G.; Tatu, C.; Gruia, A. Bioactive microparticles (10): Thermal and oxidative stability of nicotine and its complex with β-cyclodextrin. *Journal of Inclusion Phenomena and Macrocyclic Chemistry* **2010**, 68, 155-164

Hermenean, A.; Popescu, C.; Ardelean, A.; M., S.; Hădărugă, N. G.; Mihali, C.-V.; Costache, M.; Dinischiotu, A. Hepatoprotective Effects of *Berberis vulgaris* L. Extract/β-Cyclodextrin on Carbon Tetrachloride-Induced Acute Toxicity in Mice. *International Journal of Molecular Sciences* **2012**, 13, 9014-9034

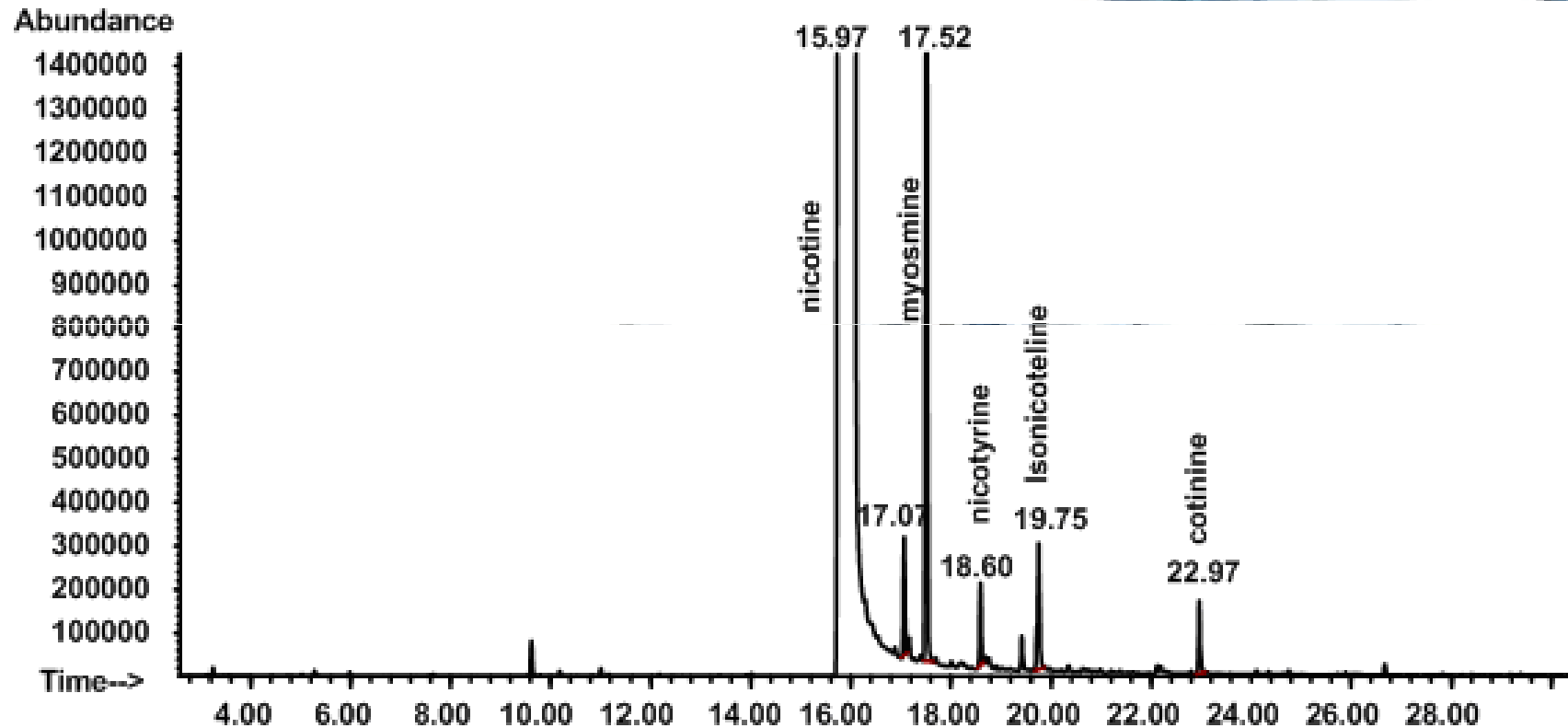


Researches on **alkaloids / cyclodextrin complexes** were focused on the:

- (1) Synthesis, analysis, and stability of nicotine / cyclodextrin complexes, and
- (2) Synthesis, analysis, and hepatoprotective effects of *Berberis vulgaris* L. / cyclodextrin complexes.

Nicotine / cyclodextrin complexes

The nicotine / cyclodextrin complex is used in smoking cessation formulas and the stability of this compound is very important.



Chromatogram from the GC-MS analysis of the raw nicotine sample

Nicotine conduct to cotinine by oxidative degradation, while the complexation in cyclodextrin reduces this degradation.

		Main alkaloid content (%)				
		Nicotine	Myosmine	Nicotyrine	Isonicoteline	Cotinine
<i>KI*</i>		1363	1437	1489	1546	1714
No	Code					
1	N**	96.0	3.1	0.2	0.5	0.2
2	N-O-t1	95.9	2.8	0.2	0.4	0.1
3	N-O-t2	94.3	3.3	0.2	0.4	0.2
4	N-O-t3	93.7	3.7	0.3	0.4	0.7
5	N-O-t4	95.7	2.3	0.1	0.3	0.6
6	N-O-t5	94.7	3.3	0.3	0.4	0.2
7	N-O-t6	92.6	4.7	0.6	0.4	0.7
8	NbCD**	98.5	0.10			-
9	NbCD-O-t4	98.4	0.08			-
10	NbCD-O-t5	98.8	0.09			-
11	NbCD-O-t6	98.0	0.05			-

Relative concentrations for the main alkaloids from commercial nicotine (N), nicotine samples thermally (30, 60, 90°C) degraded for 2 hours (N-O-t1,2,3) and for 6 hours (N-O-t4,5,6), and for the corresponding nicotine/β-cyclodextrin microparticles, non-degraded and degraded in the same conditions for 6 hours (NbCD and NbCD-O-t4,5,6)

... The nicotine/cyclodextrin complexes were analyzed by **Karl Fischer** method for the **water content**.

Good results on the complexation process were obtained in the case of **nicotine:cyclodextrin ratio** of **2:1** and a moderate temperature (**50 Celsius degree**).

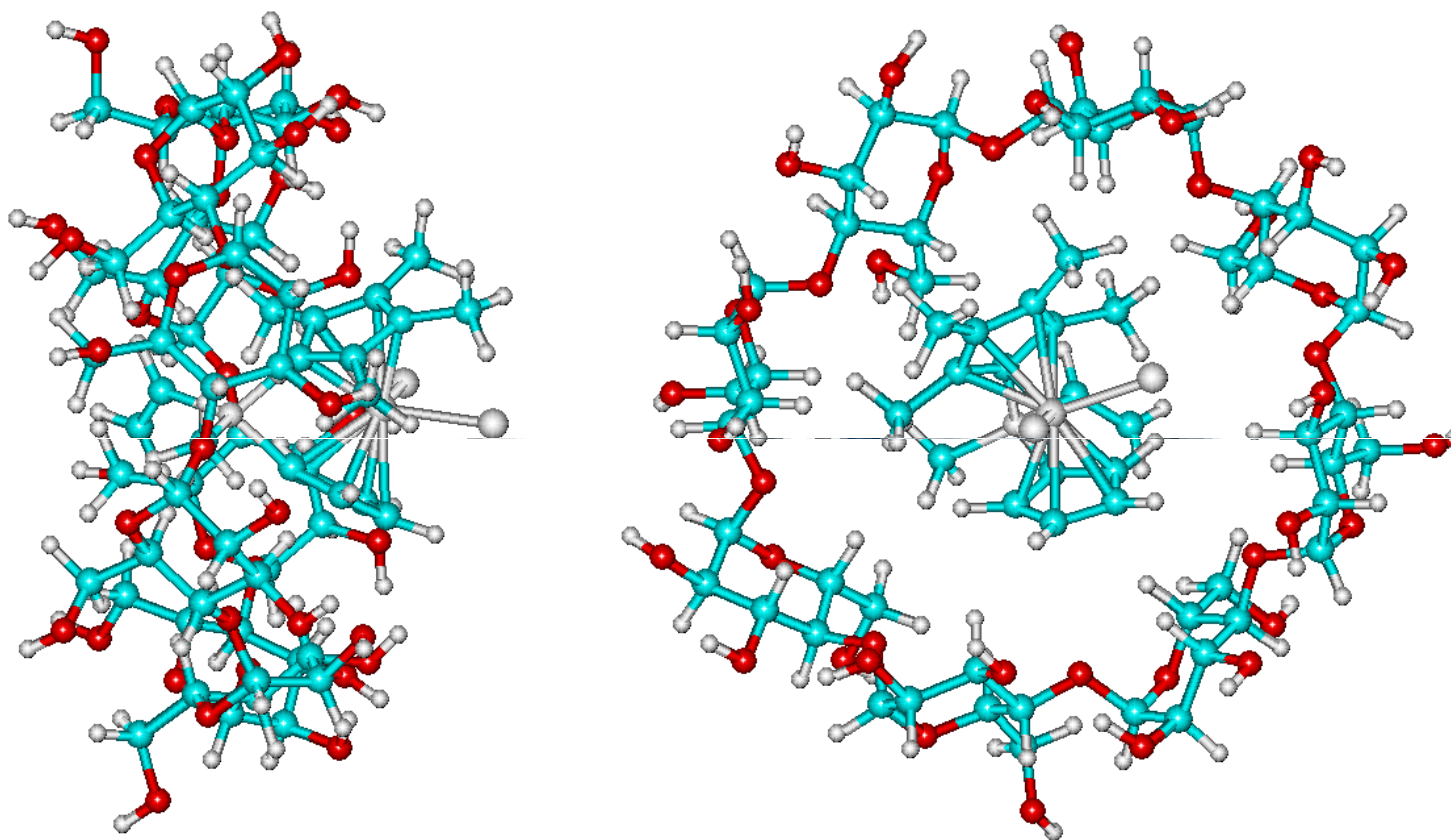
No	Code	TG mass loss (<150°C) (%)	TG mass loss (150-250°C) (%)	TG mass loss (150-200°C) (%)	Water (%) (by KFT)
1	NbCDE_r1	10.5	1.3	0.4	11.3 ± 0.7 (n = 3)
2	NbCDE_r2	9.3	2.0	0.8	11.4 ± 0.1 (n = 3)
3	NbCDE_r3	8.3	1.6	0.3	-
4	NbCDE_t1	10.5	1.8	0.8	12.0 ± 0.2 (n = 3)
5	NbCDE_t2	13.3	1.5	0.5	13.6 (n = 2)
6	NbCDM_r1	12.4	0.7	0.4	12.0 ± 0.4 (n = 3)
7	NbCDP_r1	9.8	2.2	1.1	-
8	bCD	13.0	-	-	13.9 ± 0.4 (n = 5)

Thermogravimetric analysis and classical Karl Fischer water titration (KFT) for the nicotine (N)/ β -cyclodextrin (bCD) complexes obtained in ethanol-water system at different N:bCD ratios (1:1, **2:1**, and 3:1, at **50°C**, codes NbCDE_r1,2,3), at various temperatures (50°C, 30°C, and **70°C**, codes NbCDE_r1, and NbCDE_t1,2, respectively), and in methanol- or propanol-water systems at 50°C and N:bCD ratio of 1:1 (codes NbCDM_r1 and NbCDP_r1, respectively)

Conclusion on alkaloid / cyclodextrin complexes

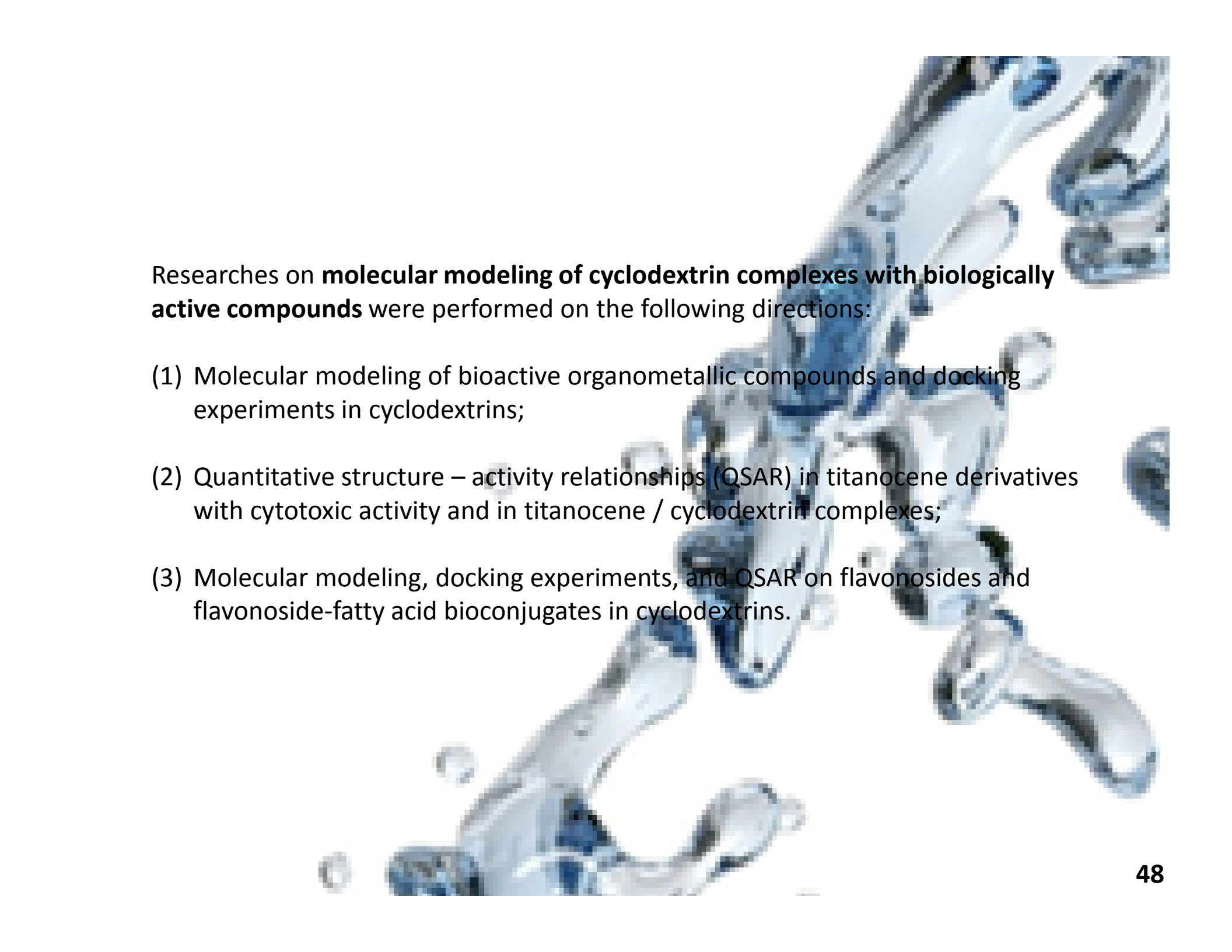
- (1) β -Cyclodextrin can act as a protecting host-molecule for nicotine-containing formulations used in smoking cessation, nicotine being stabilized in the complex;
- (2) The maximum concentration of the main alkaloid, berberine, was found in bark and also in root, and
- (3) The *Berberis vulgaris* extract/ β -cyclodextrin presents hepatoprotective effects probably due to its increased bioavailability (*not presented*)

Chapter 7. MOLECULAR MODELING OF BIOACTIVE COMPOUNDS / CYCLODEXTRIN COMPLEXES



Riviş, A.; **Hădărugă, N. G.**; Gârban, Z.; Hădărugă, D. I. Titanocene / cyclodextrin supramolecular systems: a theoretical approach. *Chemistry Central Journal* **2012**, 6, 129, doi: 10.1186/1752-153X-6-129

Pînzaru, I. A.; Hădărugă, D. I.; **Hădărugă, N. G.**; Corpaş, L.; Peter, F. Hepatoprotective flavonoid bioconjugate / β -cyclodextrin nanoparticles: DSC – molecular modeling correlation. *Digest Journal of Nanomaterials and Biostructures* **2011**, 6, 1605-1617

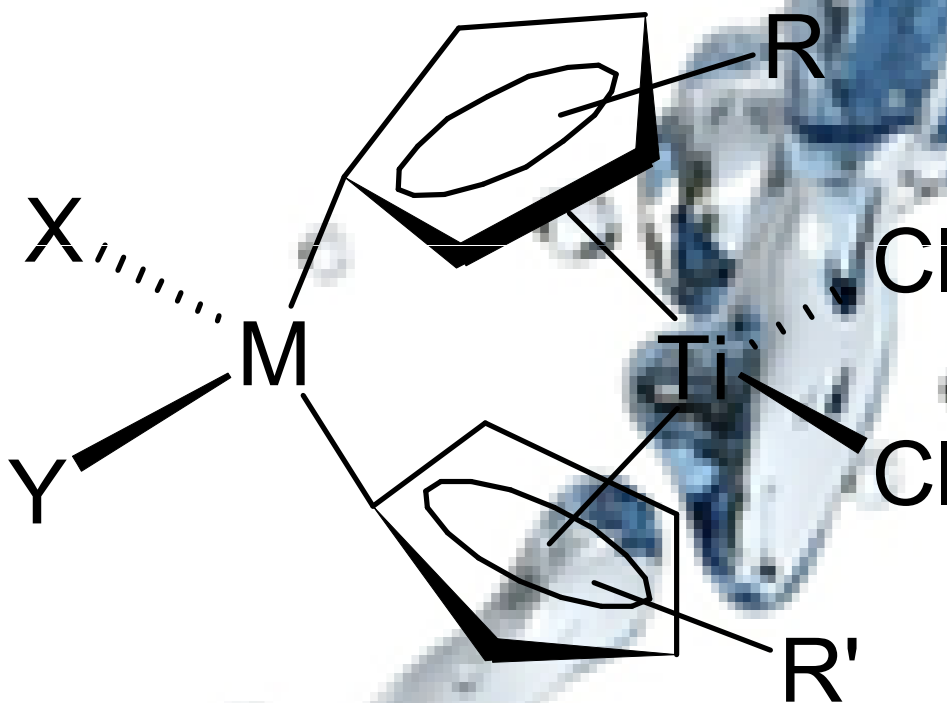


Researches on **molecular modeling of cyclodextrin complexes with biologically active compounds** were performed on the following directions:

- (1) Molecular modeling of bioactive organometallic compounds and docking experiments in cyclodextrins;
- (2) Quantitative structure – activity relationships (QSAR) in titanocene derivatives with cytotoxic activity and in titanocene / cyclodextrin complexes;
- (3) Molecular modeling, docking experiments, and QSAR on flavonosides and flavonoside-fatty acid bioconjugates in cyclodextrins.

Quantitative structure-activity relationships (QSARs) in titanocene class

The aqueous solubility of metallocenes (**titanocenes**) with **cytotoxic activity** can be enhanced by molecular encapsulation in cyclodextrins. The hydrolytic instability of these compounds can also be reduced.

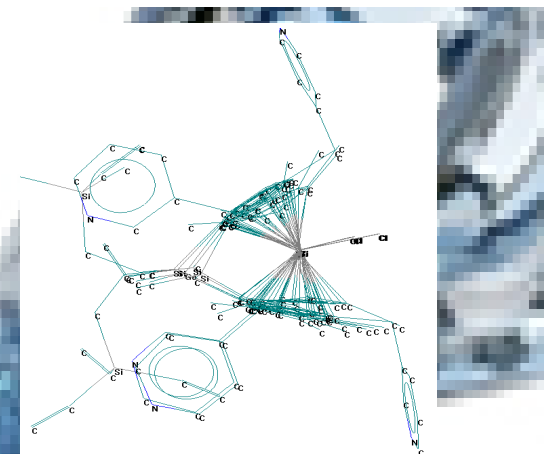


Titanocene structures with cytotoxic activities

The importance of titanocene structure to cytotoxic activity is due to the steric, electronic, and hydrophobic descriptors.

Some of the structural descriptors are intercorrelated:

- molecular surface (***S***)
- molecular volume (***V***)
- hydration energy (***E_{hydr}***)
- logarithm of the octanol/water partition coefficient (***logP***)
- refractivity (***R_f***)
- polarizability (***Pol***)



Superimposed minimum energy conformations of titanocene structures

	<i>S^{vdW}</i>	<i>V^{vdW}</i>	<i>E_{hydr}</i>	<i>logP</i>	<i>R_f</i>	<i>Pol</i>
<i>S^{vdW}</i>	1.00	0.91	0.54	-0.40	0.52	0.49
<i>V^{vdW}</i>		1.00	0.52	-0.02	0.17	0.13
<i>E_{hydr}</i>			1.00	-0.23	0.43	0.38
<i>logP</i>				1.00	-0.77	-0.80
<i>R_f</i>					1.00	1.00
<i>Pol</i>						1.00

Intercorrelational matrix for titanocene structural descriptors

The following equations were obtained, all with **cross-validation coefficient** (q^2_{cv}) greater than **0.75**:

$$pA_{1i} = 3.40(\pm 0.17) + 0.69(\pm 0.17) \cdot \log P_i$$

“HeLa” cell line $n = 11; r = 0.80; F = 16; q^2_{cv} = 0.75$

$$pA_{2i} = 3.54(\pm 0.23) + 0.69(\pm 0.30) \cdot \log P_i$$

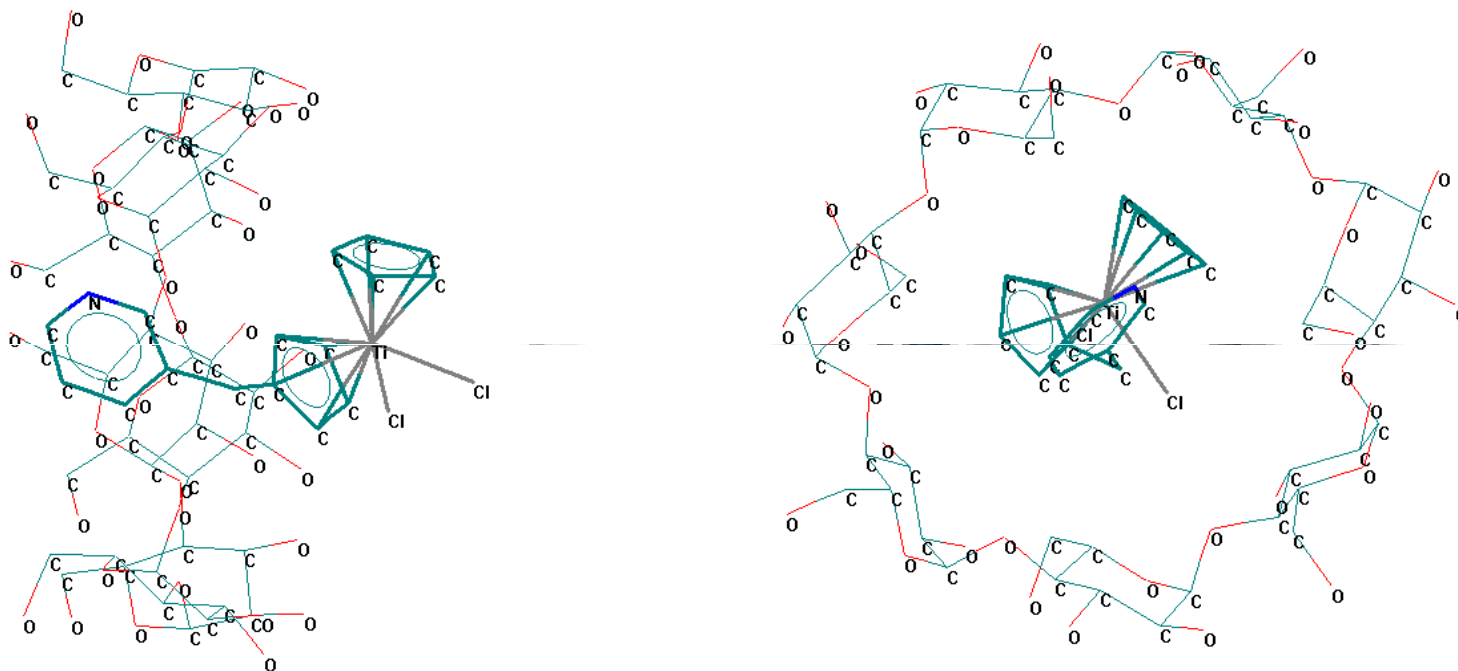
“K562” cell line $n = 8; r = 0.70; F = 5.5; q^2_{cv} = 0.88$

$$pA_{3i} = 3.63(\pm 0.12) + 0.39(\pm 0.16) \cdot \log P_i$$

“Fem-x” cell line $n = 7; r = 0.74; F = 5.9; q^2_{cv} = 0.80$

Docking of titanocenes in cyclodextrins

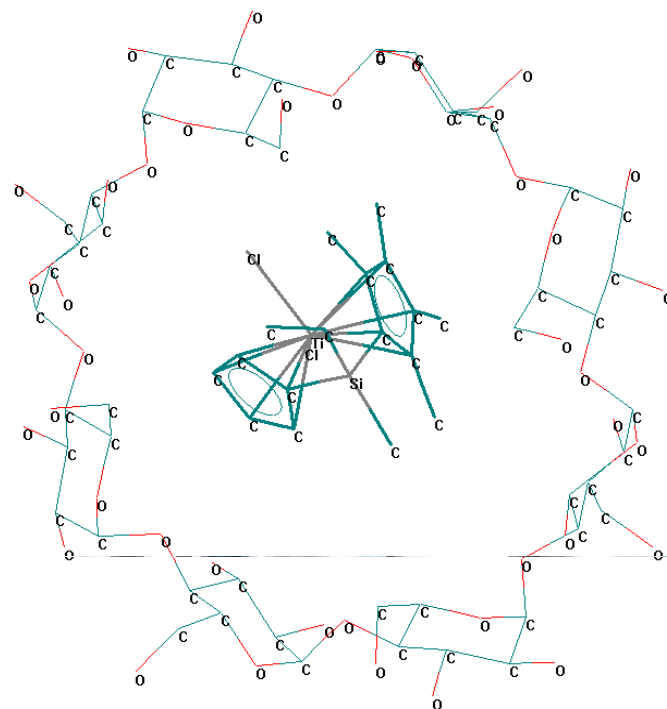
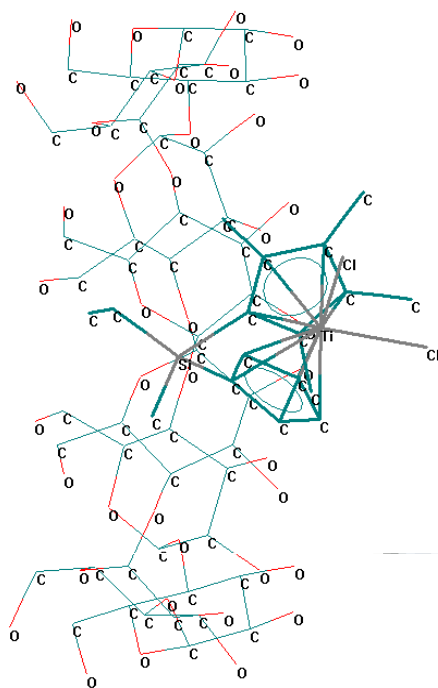
Docking of titanocenes in cyclodextrins conduct to stable complexes, with good correlation coefficients ($r \sim \mathbf{0.6-0.7}$) between the interaction energy (E_{int}) and $\log P$ of the titanocene structure.



$$E_{\text{int.}, bCD, i} = 16.24(\pm 1.64) + 4.459(\pm 1.687) \cdot \log P_{bCD, i}$$

$$n = 11; r = \mathbf{0.7}; F = 7.0$$

Titanocene / β -cyclodextrin supramolecular system
(theoretically modeled by MM+ docking experiments)



$$E_{int., gCD, i} = 21.46(\pm 1.99) + 4.347(\pm 2.047) \cdot \log P_{gCD, i}$$

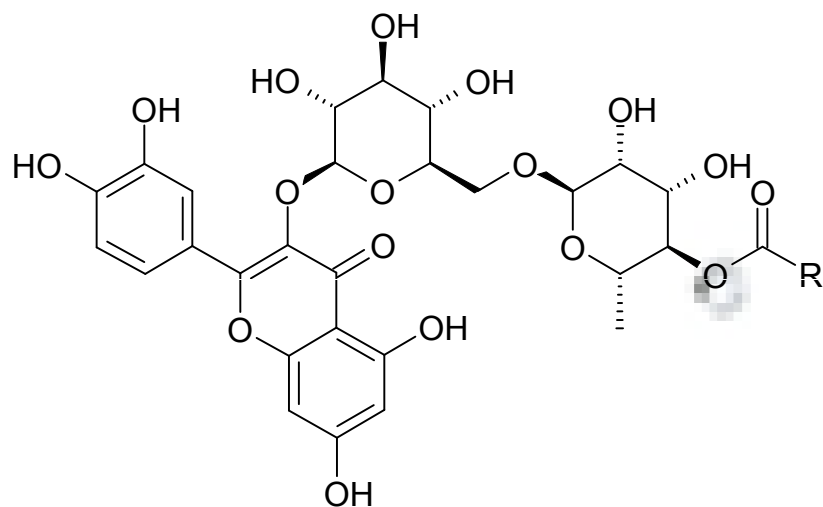
$$n = 11; r = \mathbf{0.6}; F = 4.1$$

Titanocene/ γ -cyclodextrin supramolecular system
(theoretically modeled by MM+ docking experiments)

Flavonoid-fatty acid bioconjugate / cyclodextrin complexes

In the second part, six **fatty acid-flavonoid bioconjugates** were enzymatically synthesized and complexed with β -cyclodextrin:

- **Rutin esters** (with decanoic, palmitic, and oleic acids)
- **Silybin esters** (with stearic, oleic, and linoleic acids)

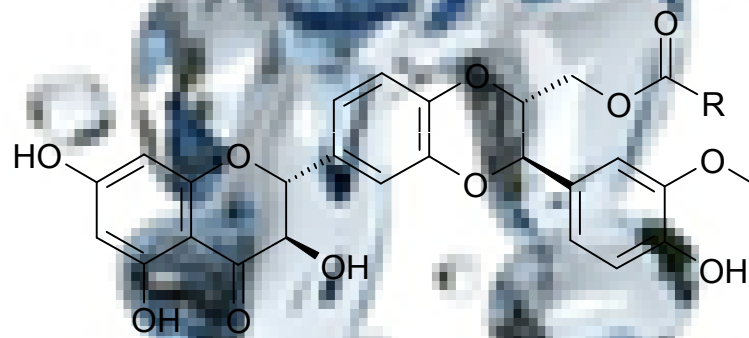


Rutin: R = H

Decanoyl derivative: R = $-(\text{CH}_2)_8-\text{CH}_3$

Palmitoyl derivative: R = $-(\text{CH}_2)_{14}-\text{CH}_3$

Oleoyl derivative: R = $-(\text{CH}_2)_7-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{CH}_3$



Silybin: R = H

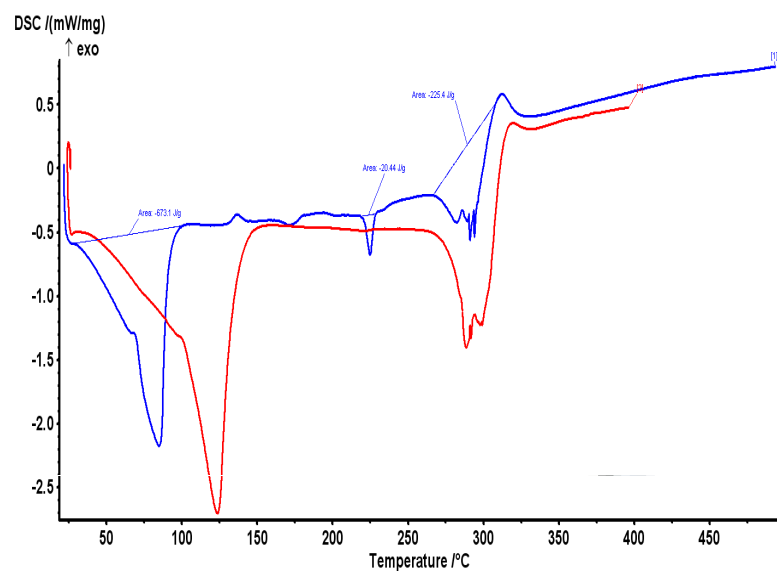
Stearoyl derivative: R = $-(\text{CH}_2)_{16}-\text{CH}_3$

Oleoyl derivative: R = $-(\text{CH}_2)_7-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{CH}_3$

Linoleoyl derivative: R = $-(\text{CH}_2)_7-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_4-\text{CH}_3$

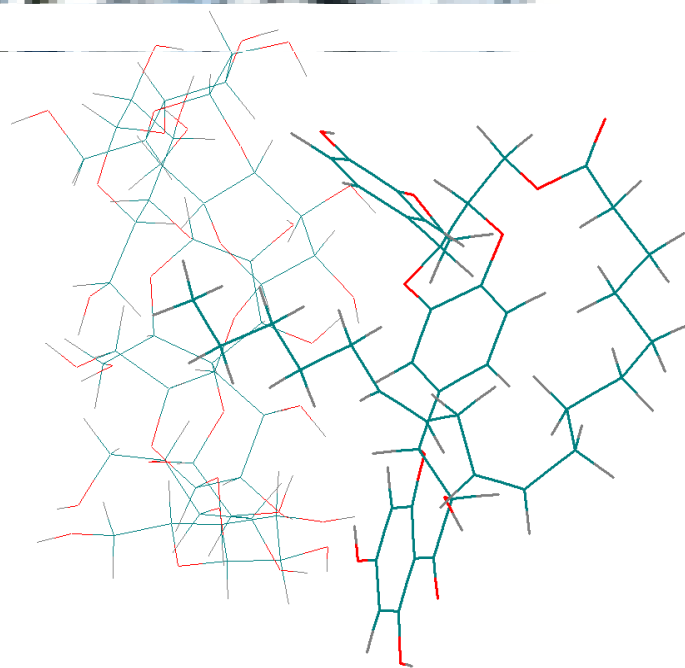
Biocompounds structures, rutin and derivatives (a), and silybin and derivatives (b), used for complexation with β -cyclodextrin

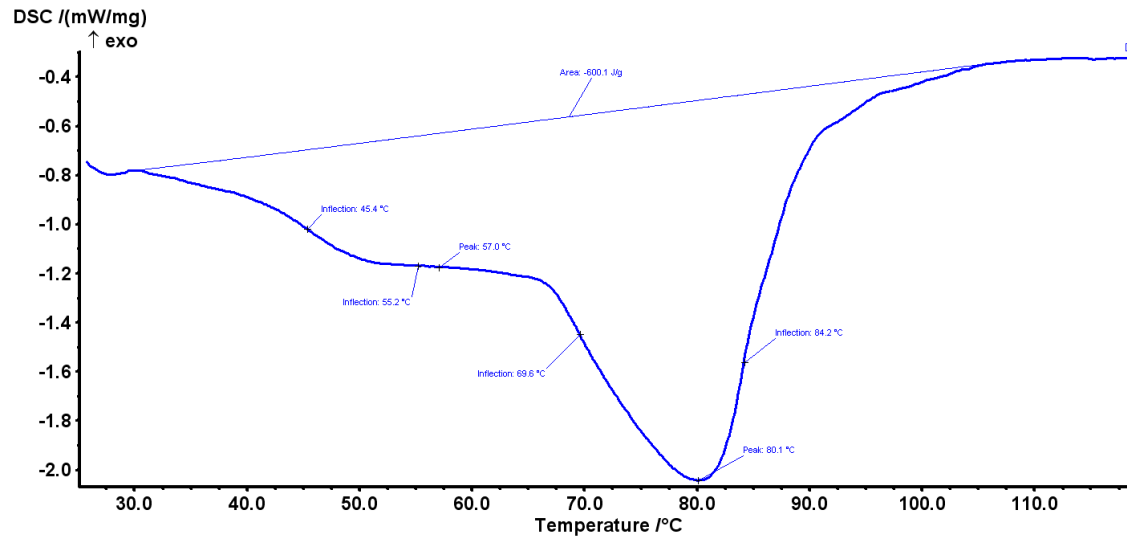
The experimental **DSC parameters** (water dissociation peak temperatures) were correlated with the theoretically determined **interaction energy**, with good correlations.



Comparative DSC analysis of rutin-oleic acid bioconjugate / β -cyclodextrin nanoparticles (1) and the starting β -cyclodextrin (2)

Silybin linoleate (S_L) bioconjugate / β -cyclodextrin supramolecular system, obtained by theoretical docking experiments





DSC analysis of
silybin-oleic acid
bioconjugate /
 β -cyclodextrin
nanoparticles

$$E_{int.,bCD,i} = 11.43(\pm 4.44) + 0.226(\pm 0.071) \cdot t_{infl.1,i}$$

$$n = 6; r = 0.847; F = 10.2$$

Conclusion on molecular modeling of bioactive compounds / cyclodextrin complexes

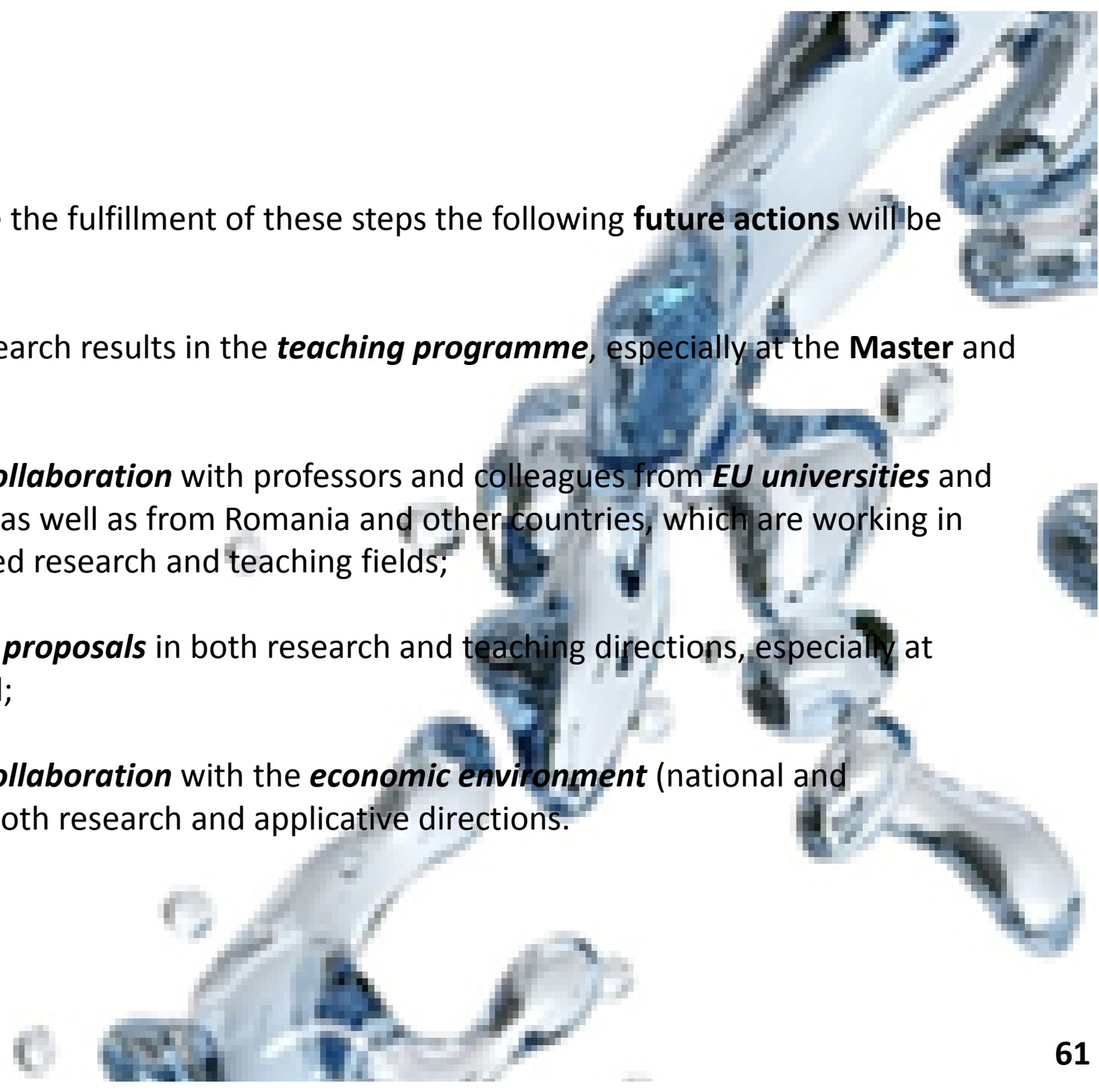
- (1) Cytotoxic activity of titanocenes increases with the overall hydrophobicity of compounds. On the other hand, increasing the hydrophobicity of titanocenes conducts to lower water solubility and reducing the transport capacity in aqueous layers; this can be resolved by molecular encapsulation in cyclodextrins, which was theoretically demonstrated by the positive interaction;
- (2) Formation of the fatty acid-flavonoid bioconjugate / β -cyclodextrin complexes can be revealed by an indirect evaluation of the remaining water from the complex hydrate; the DSC characteristics of water dissociation depends on the theoretical interaction energy.

Chapter 8. FUTURE TRENDS IN ACADEMIC AND PROFESSIONAL DIRECTIONS

8.1. Future academic development, scientific and professional career evolution and development, research and teaching directions

Researches, lectures, and practical courses development in:

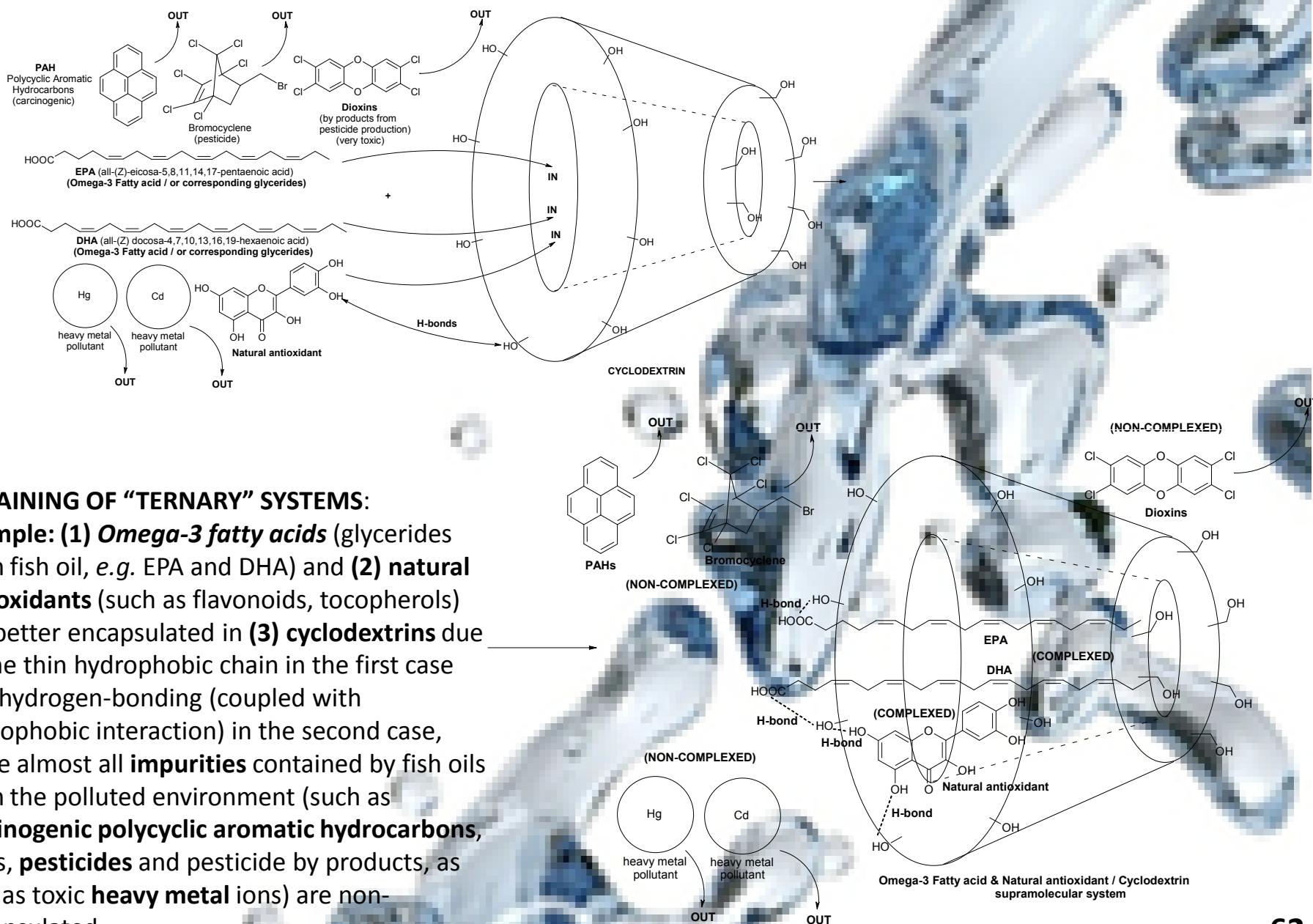
- (1) natural bioactive compounds with applications in food, medicine, and pharmacy;*
- (2) protection and controlled release systems, especially cyclodextrins and liposomes;*
- (3) combined systems containing both biologically active compounds and matrices with protection and controlled release properties;*
- (4) theoretical modeling of nanoencapsulation and controlled release processes for bioactive compound / cyclodextrin or liposome supramolecular systems;*
- (5) formulations of new food / pharmaceutical products with high social, economic, and human health impact.*



In order to ensure the fulfillment of these steps the following **future actions** will be performed:

- applying the research results in the ***teaching programme***, especially at the **Master** and **PhD** levels;
- enhancing the ***collaboration*** with professors and colleagues from ***EU universities*** and ***research centres***, as well as from Romania and other countries, which are working in the same or related research and teaching fields;
- applying ***project proposals*** in both research and teaching directions, especially at international level;
- enhancing the ***collaboration*** with the ***economic environment*** (national and international) in both research and applicative directions.

8.2. Short description of the research field (related to fatty acids)



OBTAINING OF "TERNARY" SYSTEMS:

Example: (1) Omega-3 fatty acids (glycerides from fish oil, e.g. EPA and DHA) and **(2) natural antioxidants** (such as flavonoids, tocopherols) are better encapsulated in **(3) cyclodextrins** due to the thin hydrophobic chain in the first case and hydrogen-bonding (coupled with hydrophobic interaction) in the second case, while almost all **impurities** contained by fish oils from the polluted environment (such as **carcinogenic polycyclic aromatic hydrocarbons**, PAHs, **pesticides** and pesticide by products, as well as toxic **heavy metal ions**) are non-encapsulated

8.3. Objectives of the research and teaching directions

The main **objectives** of the **research** and **teaching** directions are:

- (1) Evaluation of **omega-3 enriched** fish oil profile and the oxidative/thermal degradation products;
- (2) Evaluation of toxic/carcinogenic impurities from fish oils and omega-3 fatty acid concentrates/supplements (PAHs, organohalogenated compounds, heavy metals);
- (3) Molecular modeling of omega-3 fatty acids, natural antioxidants, and cyclodextrins, and docking experiments for evaluation of molecular encapsulation of bioactive compounds;
- (4) Obtaining and analysis of new food formulations comprise of omega-3 fatty acids/fish oil and natural antioxidants / cyclodextrins **“ternary” supramolecular systems**;
- (5) Comparatively evaluation of new omega-3 fatty acids formulations with classical omega-3 enriched products (food supplements, infant formulas, dairy and bakery food products);
- (6) Proposing **new formulations** for **omega-3 fatty acid enriched food products and supplements**.



THANK YOU!