"POLITEHNICA" UNIVERSITY OF TIMIŞOARA - ROMANIA

Faculty of Industrial Chemistry and Environmental Engineering

Water content of flavonoid | cyclodextrin nanoparticles

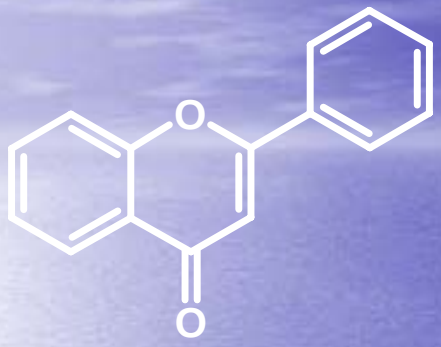
Authors:

Daniel I. Hădărugă, Nicoleta G. Hădărugă, Geza N. Bandur, Heinz-Dieter Isengard

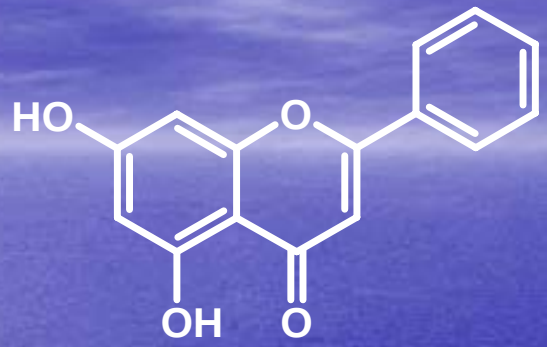
Introduction

PROPOLIS (from honey) versus FISETIN!

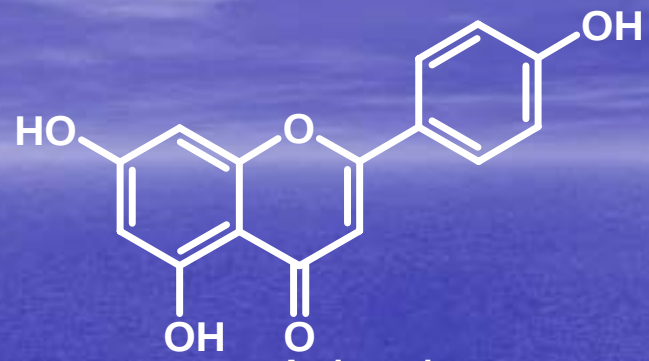
Introduction



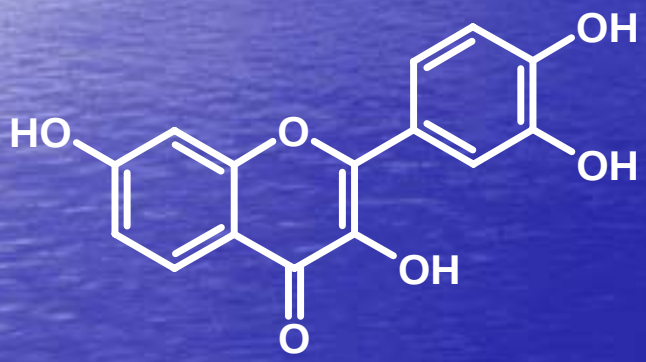
Flavone
(Flv)



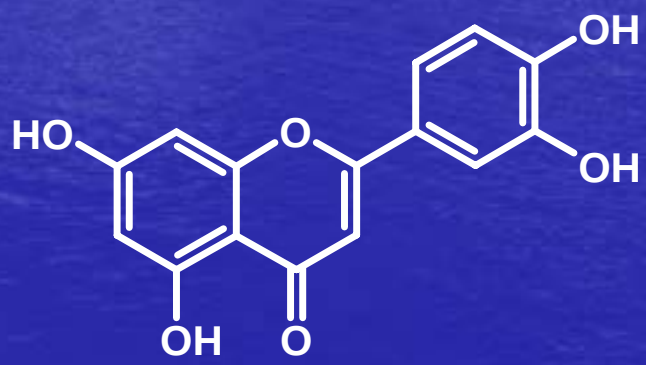
Chrysin
(Chr)



Apigenin
(Apg)

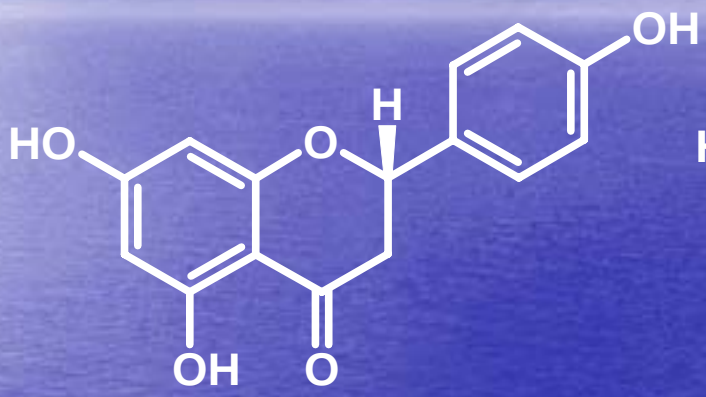


Fisetin
(Fst)

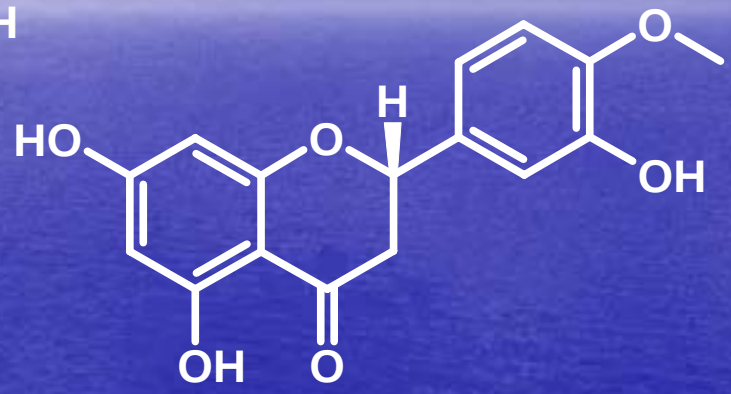


Luteolin
(Ltn)

Introduction

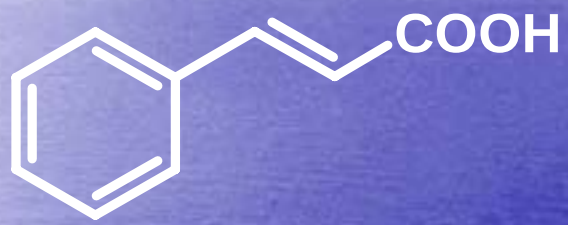


Naringenin
(Nrg)

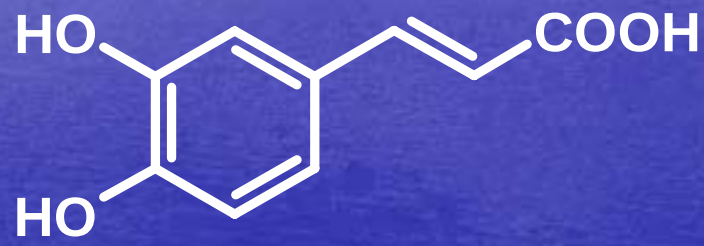


Hesperetin
(Hsp)

Introduction

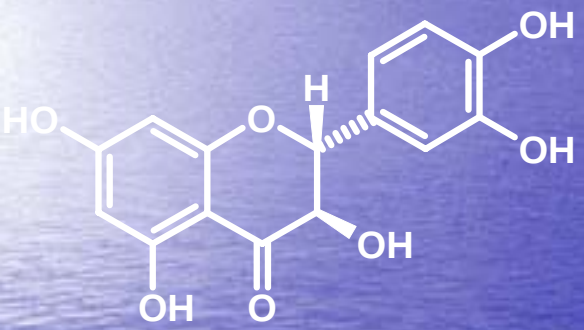


Cinnamic acid
(Cnm)

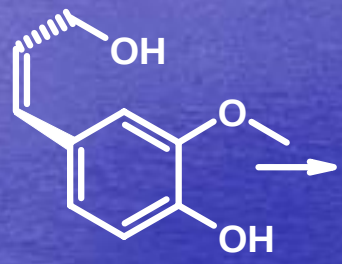


Caffeic acid
(Cff)

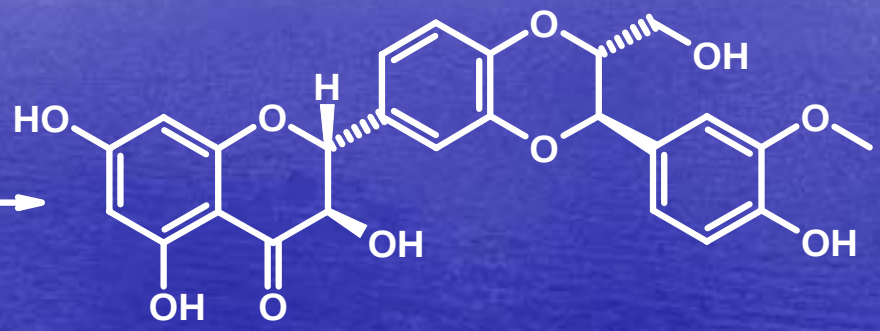
Introduction



Flavone derivative

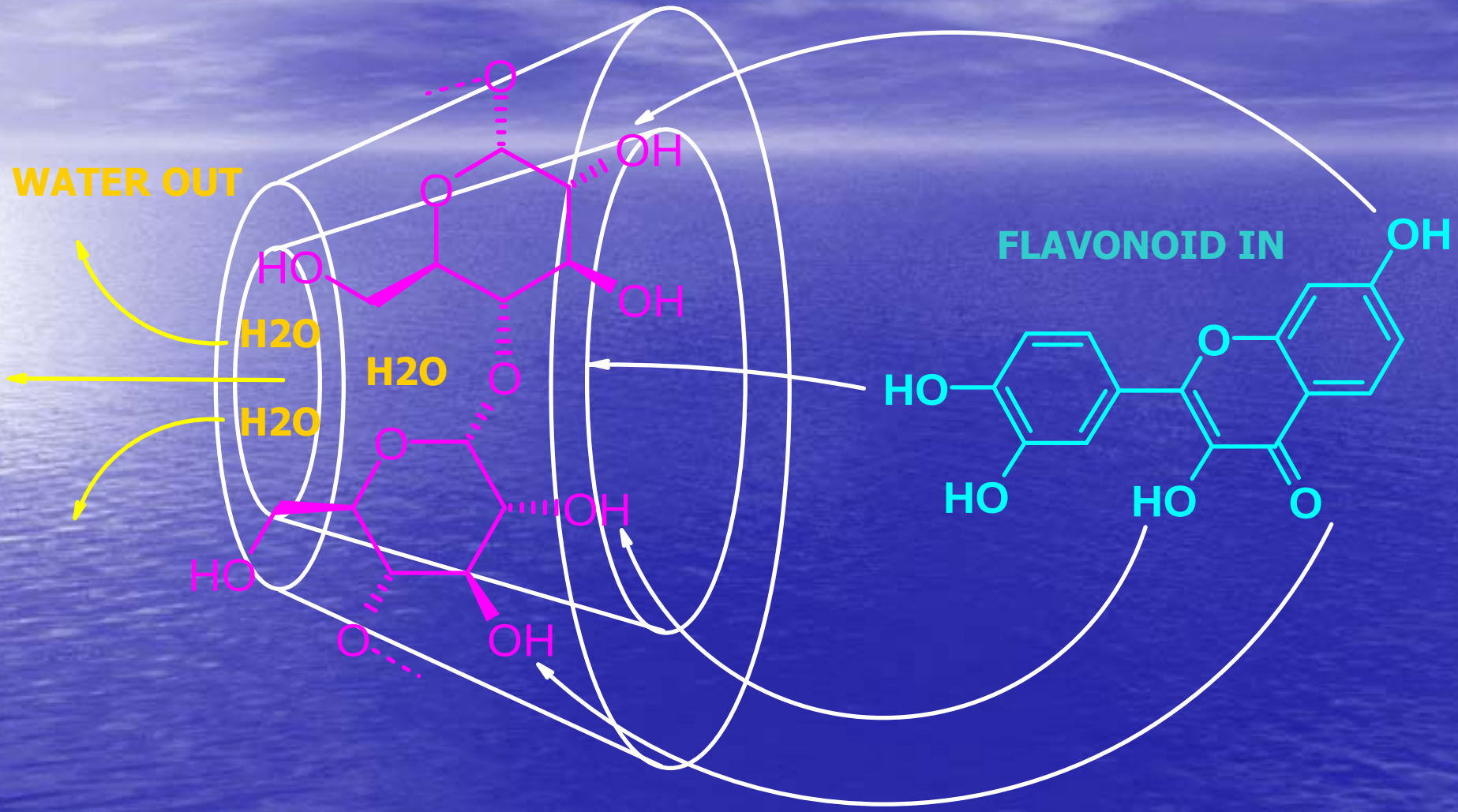


(*E*)-Ferulic acid



Silybin
(Sbn)

Introduction



Schematic representation of the nanoencapsulation process: fisetin/beta-cyclodextrin interaction

Materials and method

1. Obtaining flavonoid and related compounds/cyclodextrin complexes

a) Obtaining flavonoid/cyclodextrin complexes by crystalization from ethanol-water solution

Materials and method

1. Obtaining flavonoid and related compounds/cyclodextrin complexes

b) Obtaining the flavonoid/cyclodextrin complexes by ultrasonication

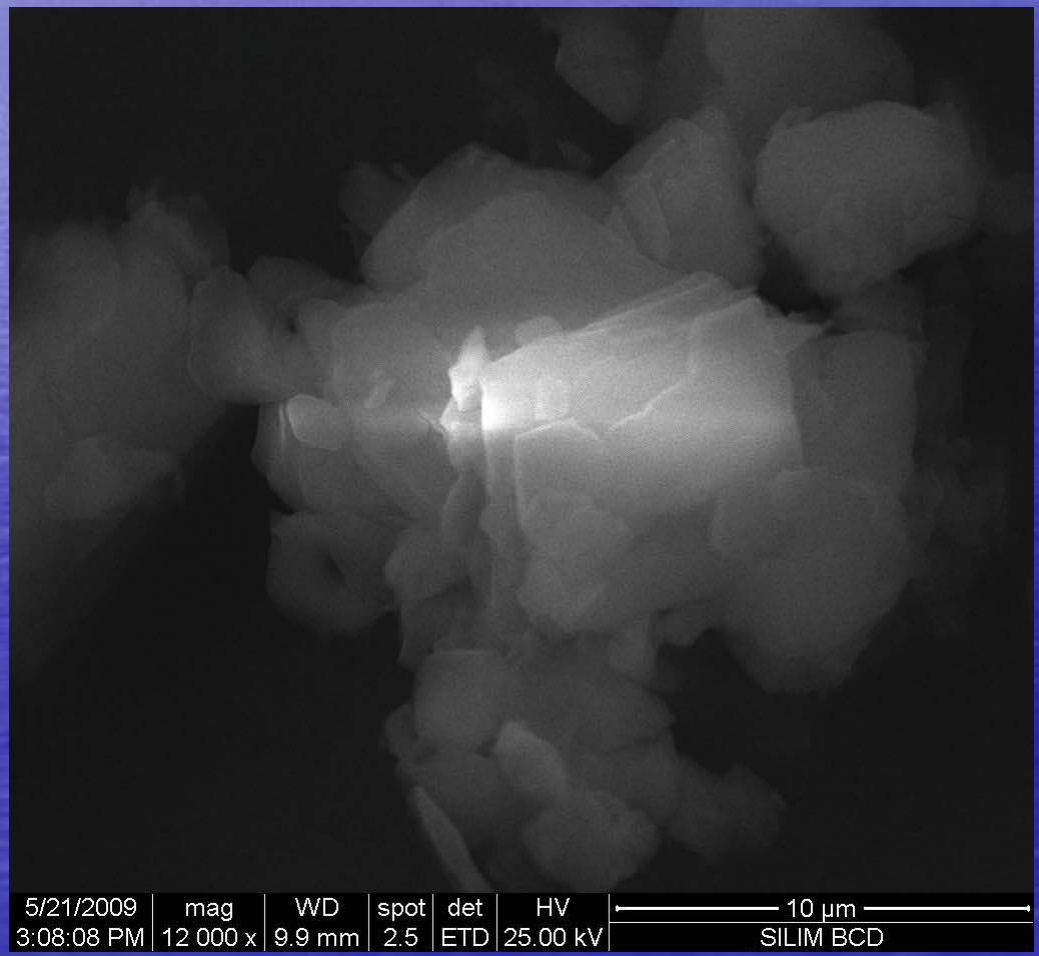
Materials and method

2. Karl Fischer water titration

Materials and method

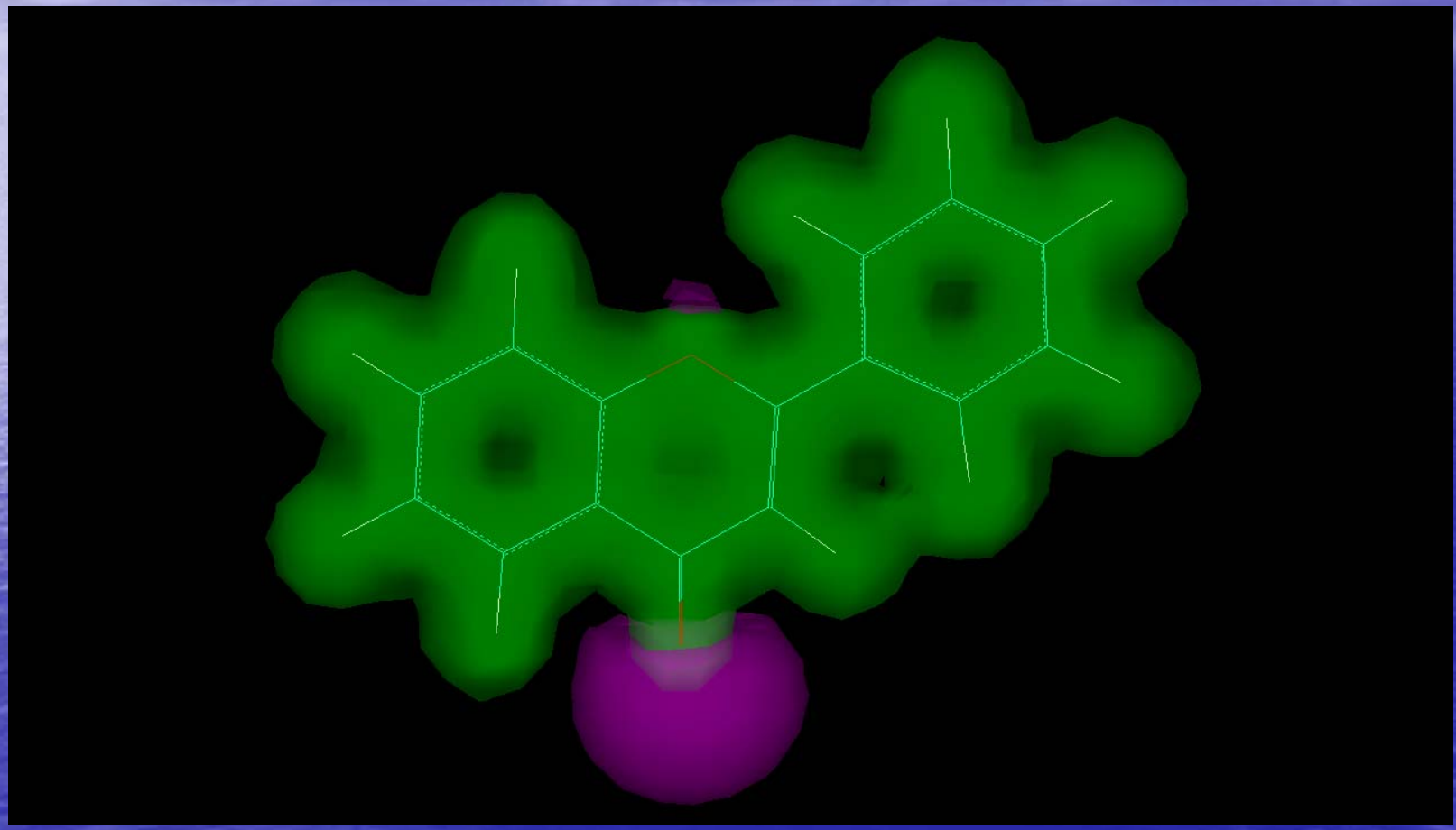
3. Thermogravimetry (TG) and Differential Scanning Calorimetry (DSC)

4. Scanning electron microscopy (SEM)



Materials and method

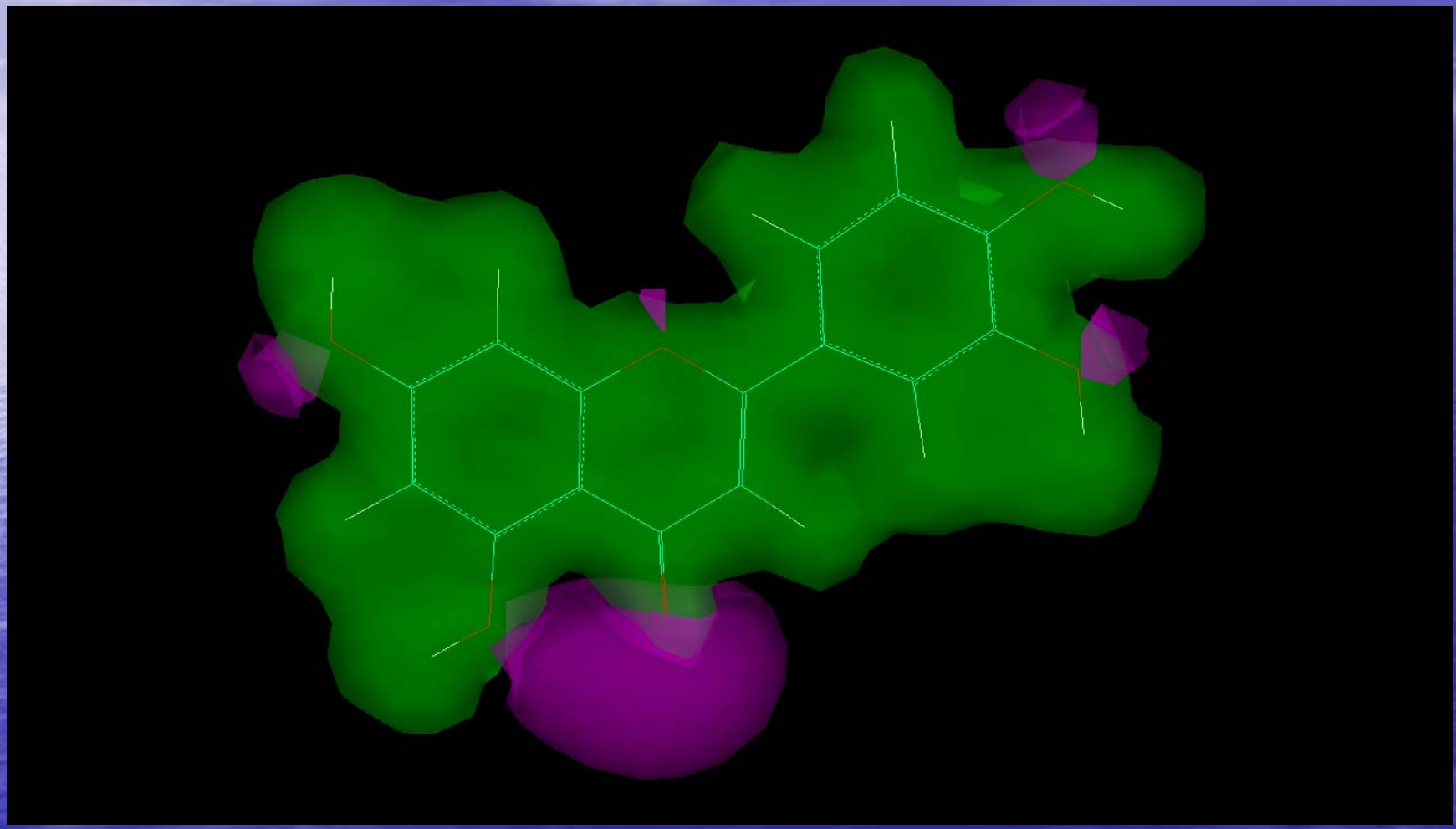
4. Structural descriptor selection



Electrostatic potential of FLAVONE
(calculated by AM1 semiempirical method from HyperChem molecular modeling package)

Materials and method

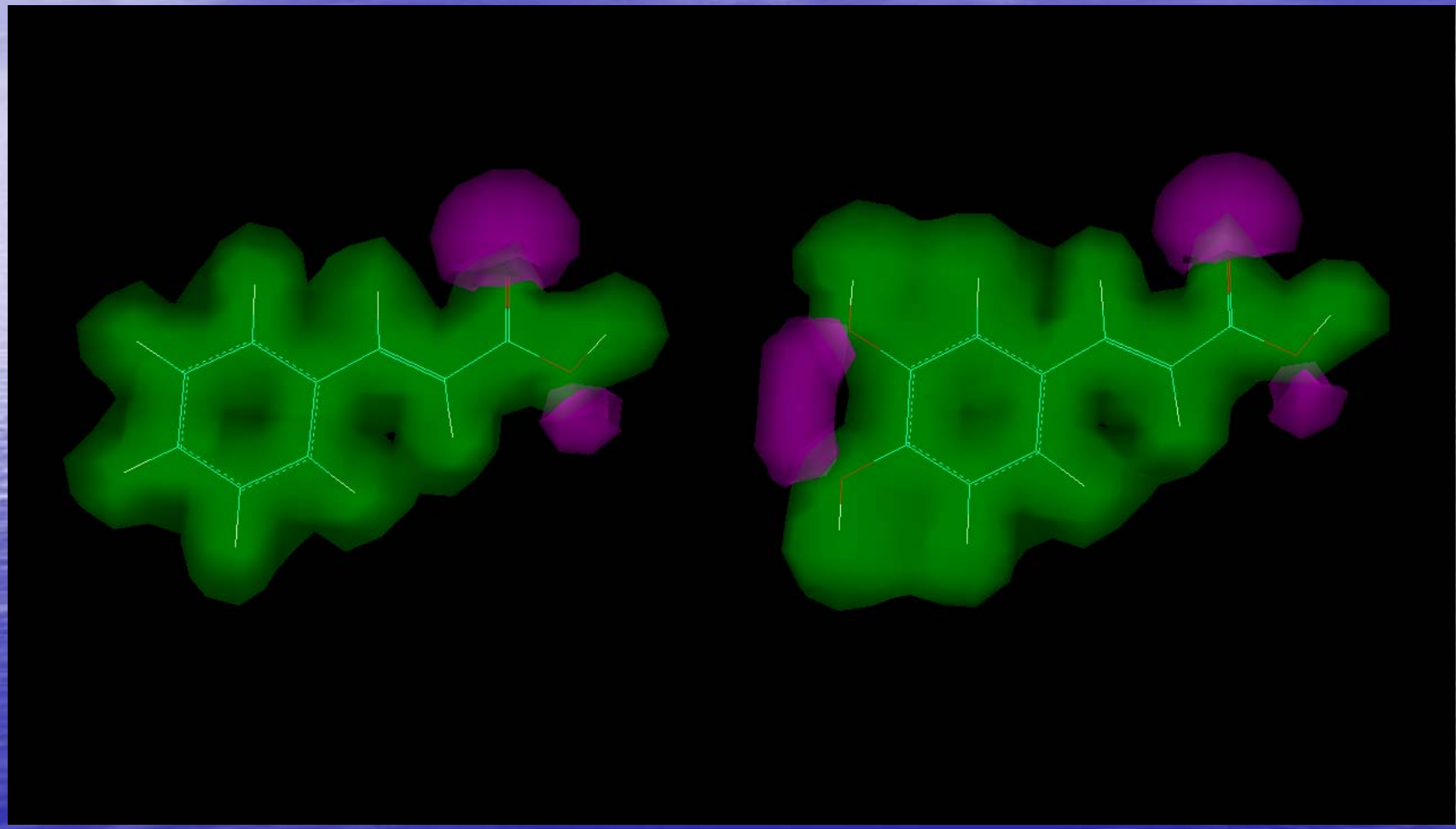
4. Structural descriptor selection



Electrostatic potential of LUTEOLIN
(calculated by AM1 semiempirical method from HyperChem molecular modeling package)

Materials and method

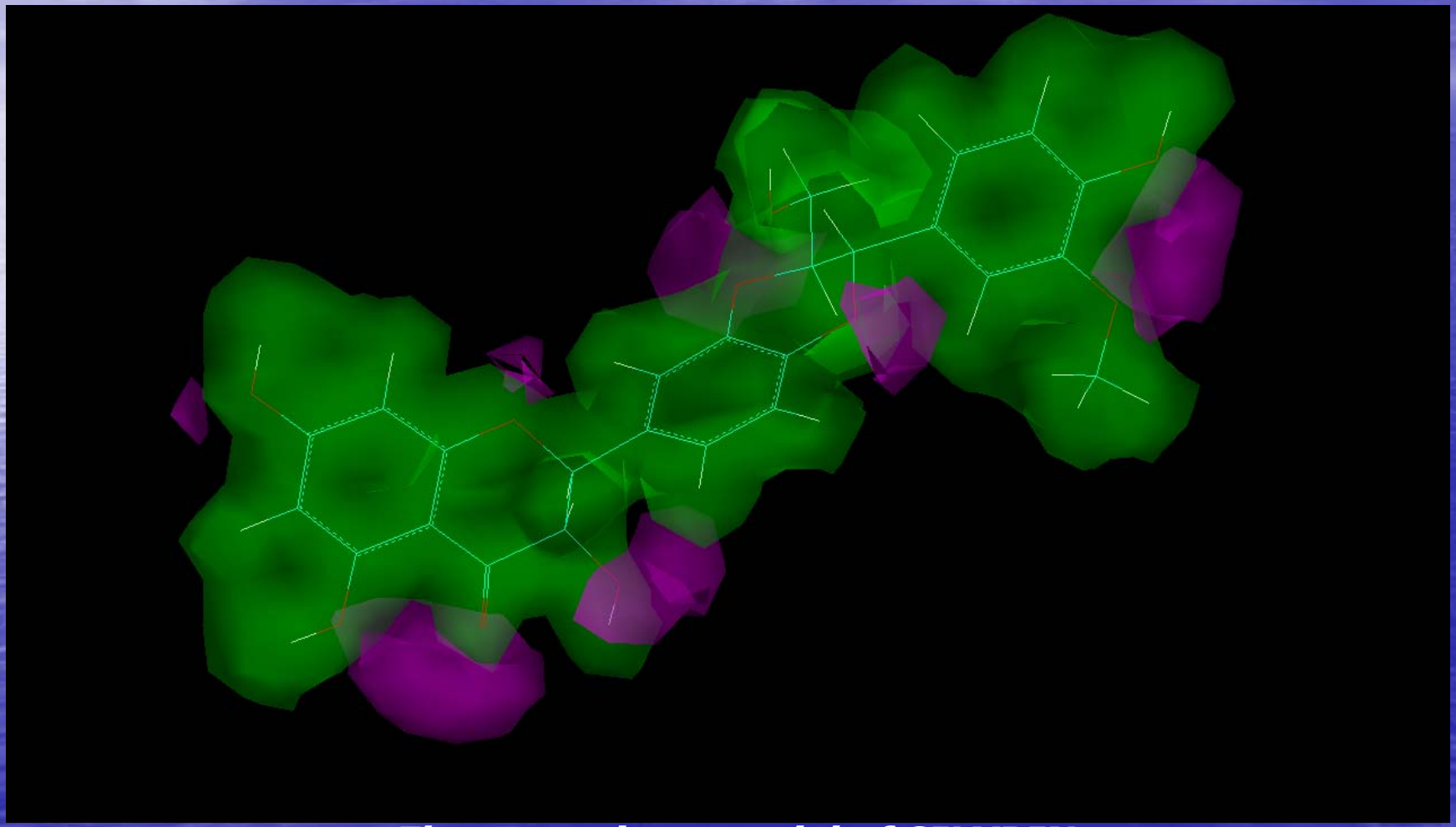
4. Structural descriptor selection



Electrostatic potential of CINNAMIC and CAFFEIC ACIDS
(calculated by AM1 semiempirical method from HyperChem molecular modeling package)

Materials and method

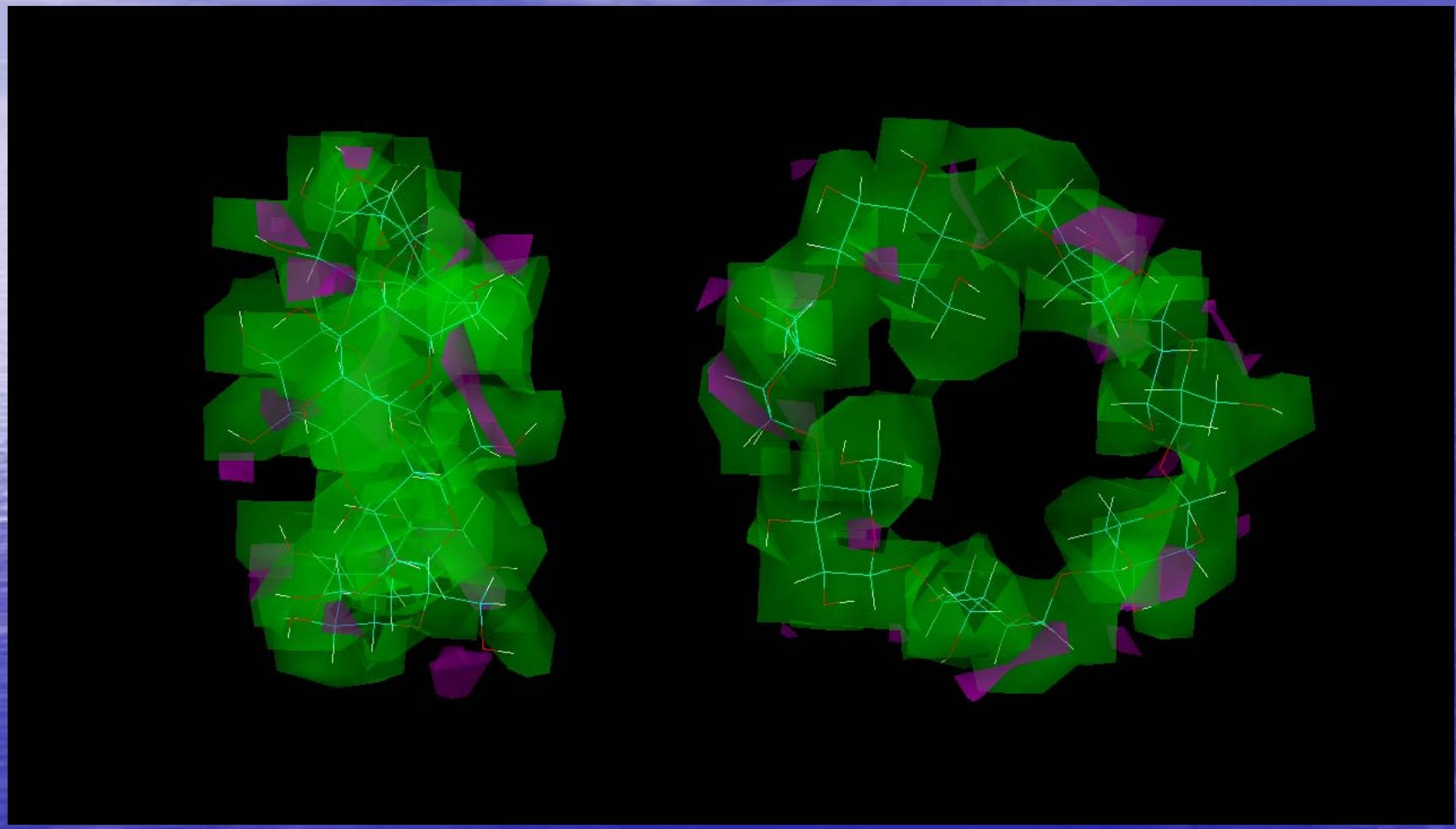
4. Structural descriptor selection



Electrostatic potential of SILYBIN
(calculated by AM1 semiempirical method from HyperChem molecular modeling package)

Materials and method

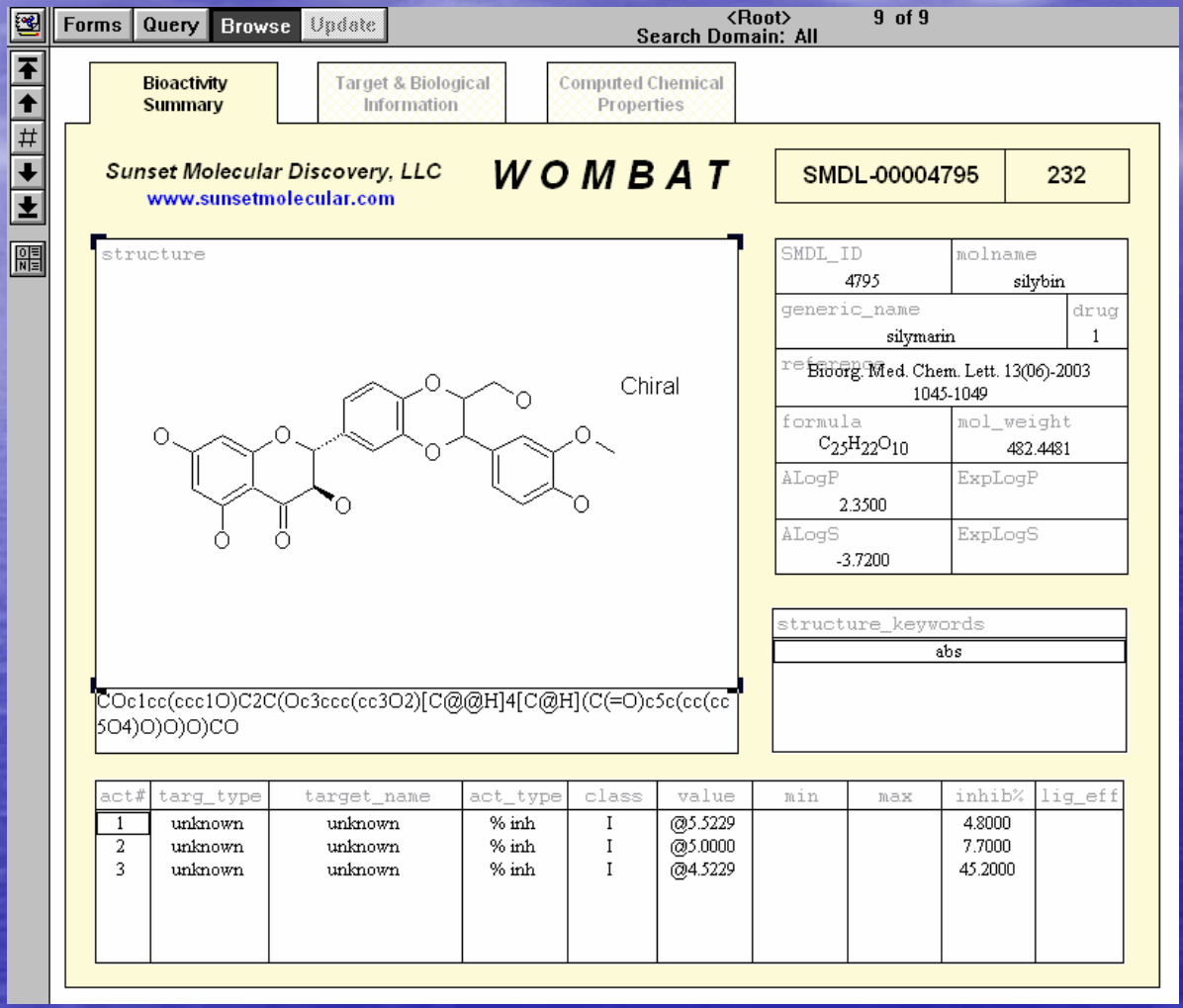
4. Structural descriptor selection



Electrostatic potential of beta-CYCLODEXTRIN
(calculated by AM1 semiempirical method from HyperChem molecular modeling package)

Materials and method

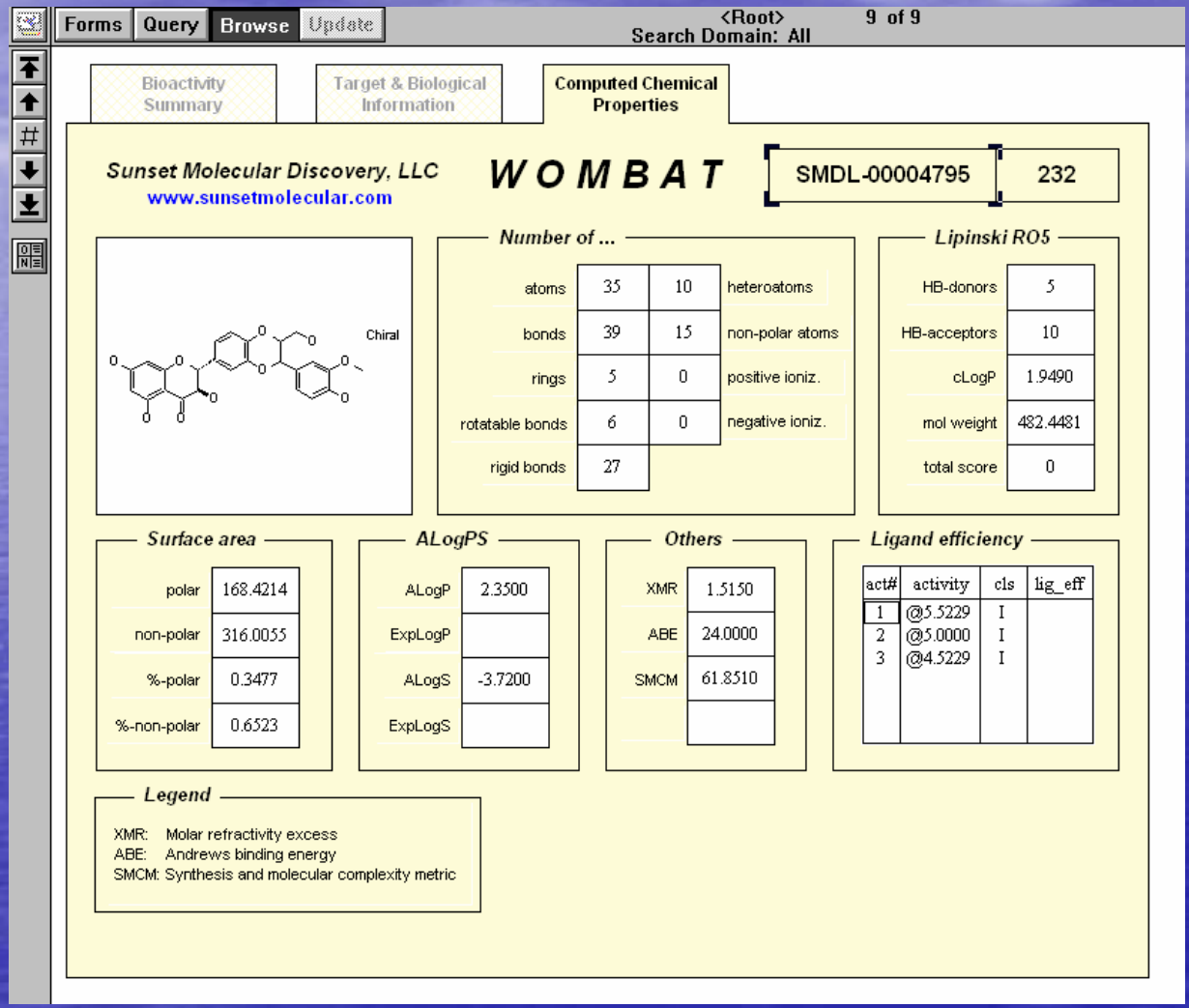
4. Structural descriptor selection



Clemons, P.A.; Olah, M.; Rad, R.; Ostopovici, L.; Bora, A.; Hădărugă, N.G.; Hădărugă, D.; Moldovan, R.; Fulias, A.; Mracec, M.; Oprea, T.I., Expanding the Genetic Code. Chemical Informatics; In: *Chemical Biology: From Small Molecules to Systems Biology and Drug Design*, Wiley-VCH, New York, 2007, pp. 723-788

Materials and method

4. Structural descriptor selection



Clemons, P.A.; Olah, M.; Rad, R.; Ostopovici, L.; Bora, A.; Hădărugă, N.G.; Hădărugă, D.; Moldovan, R.; Fulias, A.; Mracec, M.; Oprea, T.I., Expanding the Genetic Code. Chemical Informatics; In: *Chemical Biology: From Small Molecules to Systems Biology and Drug Design*, Wiley-VCH, New York, 2007, pp. 723-788

Materials and method

4. Structural descriptor selection

- *eLogK* (estimated octanol/water partition constant logarithm)
- *eLogS* (estimated water solubility logarithm)
- *N* – total number of atoms
- *Nb* – bonds
- *Nr* – rings
- *Nrtb* – rotatable bonds
- *Nrgb* – rigid bonds
- *Nh* – heteroatoms
- *Nnp* – nonpolar atoms
- *Npi* and *Nni* – positive and negative ionization atoms
- *LHd* and *LHa* – Lipinski H-donor/acceptor atoms
- *LlogP* – Lipinski CLogP
- *Sp* and *Snp* – polar and nonpolar molecular surface

Results and discussion

a) Obtaining the flavonoid and related compounds/cyclodextrin complexes

Codes, conditions, and results for the flavonoid and related compounds complexes obtained with β-cyclodextrin

No	Code	$m_{biocompound}$ (mg)	m_{CD} (mg)	Biocompound: CD molar ratio	$m_{complex}$ (mg)	Yield[(a)] (%)
1	02_Flv_bCD	111	671.3	1:1	432.6	55.30
2	05_Chr_bCD	127.5	671.6		683.9	85.58
3	08_Nrg_bCD	272.5	1342.8		1123.7	69.57
4	11_Hsp_bCD	302	1342		1344	81.75
5	14_Apg_bCD	67.6	336.7		321.8	79.59
6	17_Fst_bCD	143	672.4		680	83.39
7	20_Ltn_bCD	71.5	336.3		303.7	74.47
8	23_Sbn_bCD	241.3	671.2		668.8	73.29
9	26_Cnm_bCD	74.1	671.3		659.6	88.49
10	29_Cff_bCD	90.6	670.5		533.1	70.04

Results and discussion

a) Obtaining the flavonoid and related compounds/cyclodextrin complexes

Codes, conditions, and results for the flavonoid and related compounds complexes obtained with β-cyclodextrin by using the ultrasonication method

No	Code	$m_{biocompound}$ (mg)	m_{CD} (mg)	Biocompound: CD molar ratio	$m_{complex}$ (mg)	Yield[(a)] (%)
15	02_Flv_bCD_US	111.2	672	1:1	479.8	61.26
16	05_Chr_bCD_US	127.5	671.2		645	80.76
17	08_Nrg_bCD_US	136.7	671.1		612.9	75.87
18	11_Hsp_bCD_US	151.3	672.1		638.7	77.57
19	17_Fst_bCD_US	143.5	673		611.2	74.86
20	23_Sbn_bCD_US	241.1	670.5		745.2	81.75
21	26_Cnm_bCD_US	74.6	670.3		593.9	79.73
22	29_Cff_bCD_US	91.4	671.9		593.6	77.77

Results and discussion

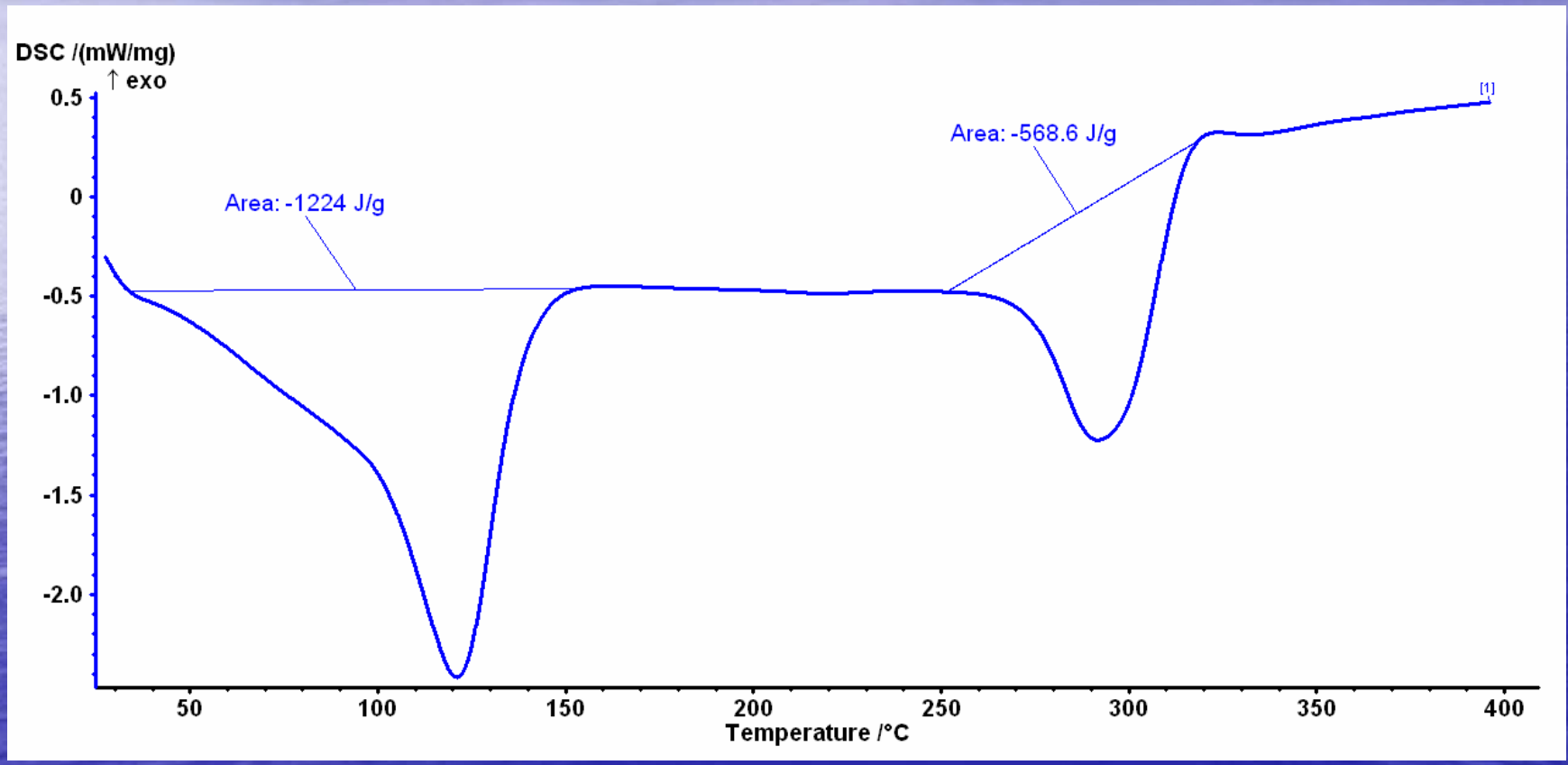
a) Obtaining the flavonoid and related compounds/cyclodextrin complexes

Codes, conditions, and results for the flavonoid and related compounds complexes obtained with α-cyclodextrin by using the crystalization method

No	Code	$m_{biocompound}$ (mg)	m_{CD} (mg)	Biocompound: CD molar ratio	$m_{complex}$ (mg)	Yield[(a)] (%)
11	01_Flv_aCD	111.3	541	1:1	620.8	95.17
12	04_Chr_aCD	127.1	540.2		579.8	86.89
13	26_Cnm_aCD	74.2	540.3		490.2	79.77
14	29_Cff_aCD	90.7	541.1		498.1	78.84

Results and discussion

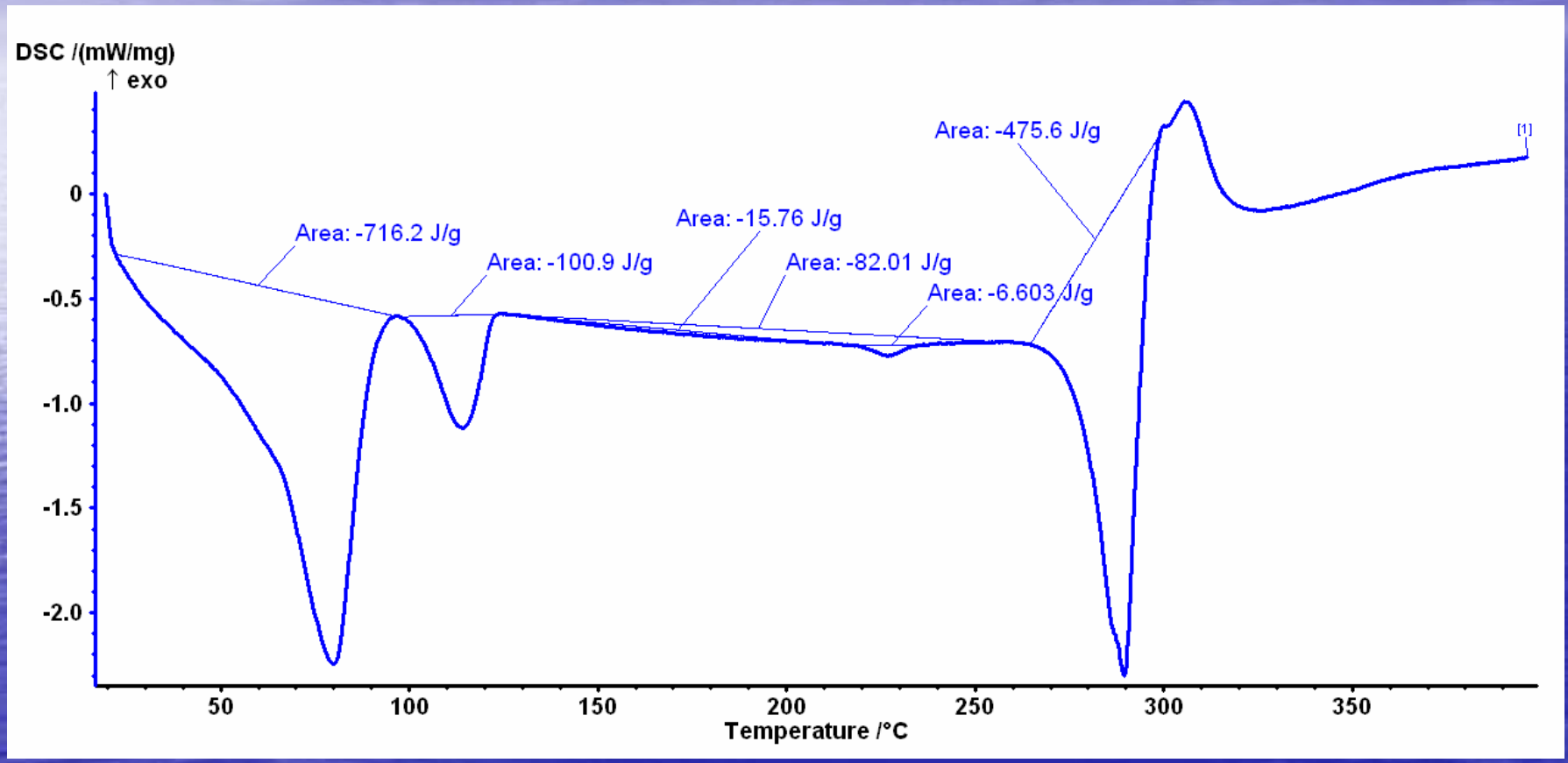
b) Differential Scanning Calorimetry (DSC) analysis of the flavonoid and related compounds/cyclodextrin complexes



DSC diagram for commercial β-cyclodextrin

Results and discussion

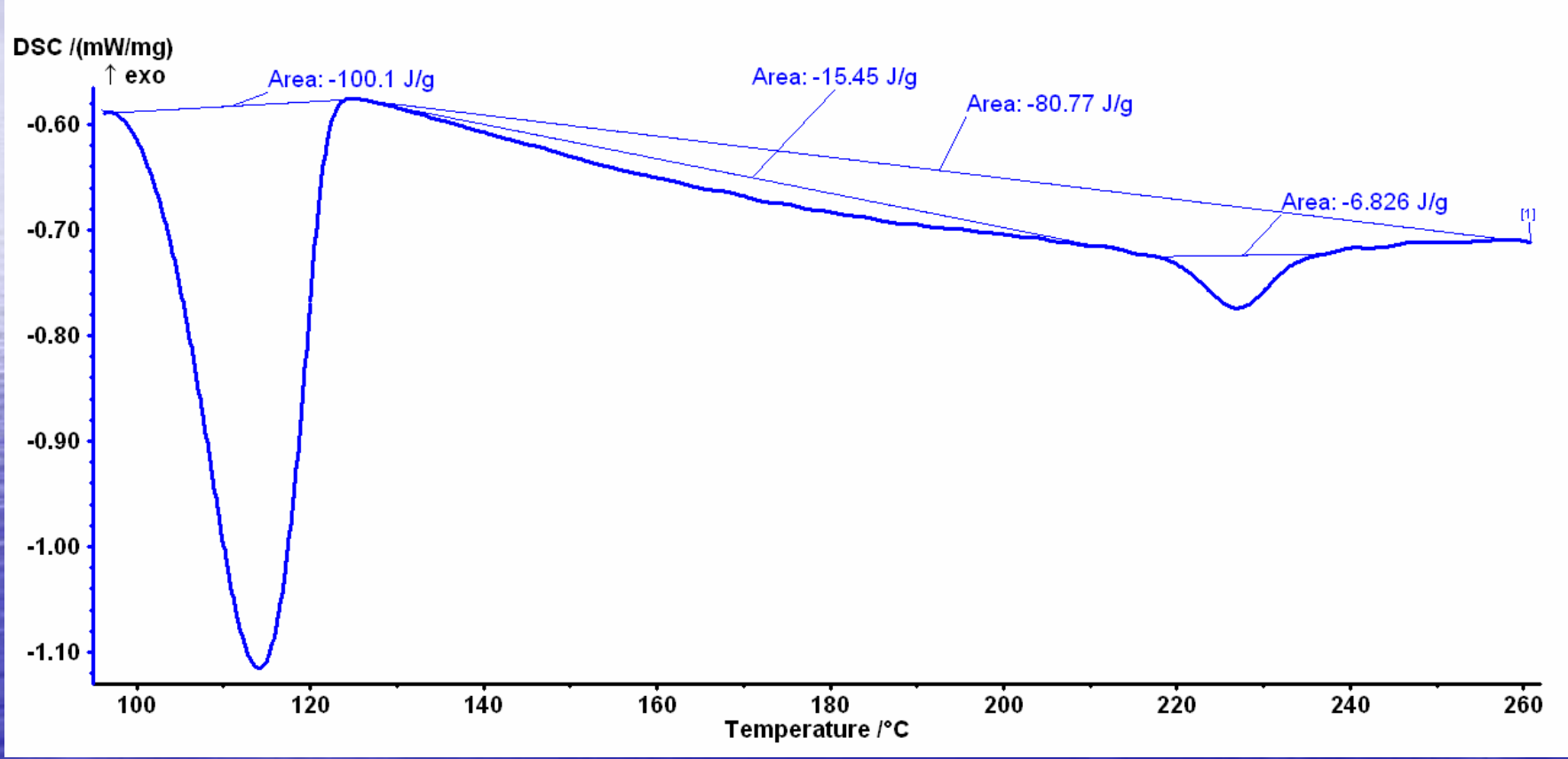
b) Differential Scanning Calorimetry (DSC) analysis of the flavonoid and related compounds/cyclodextrin complexes



DSC diagram for fisetin/β-cyclodextrin complex (all range)

Results and discussion

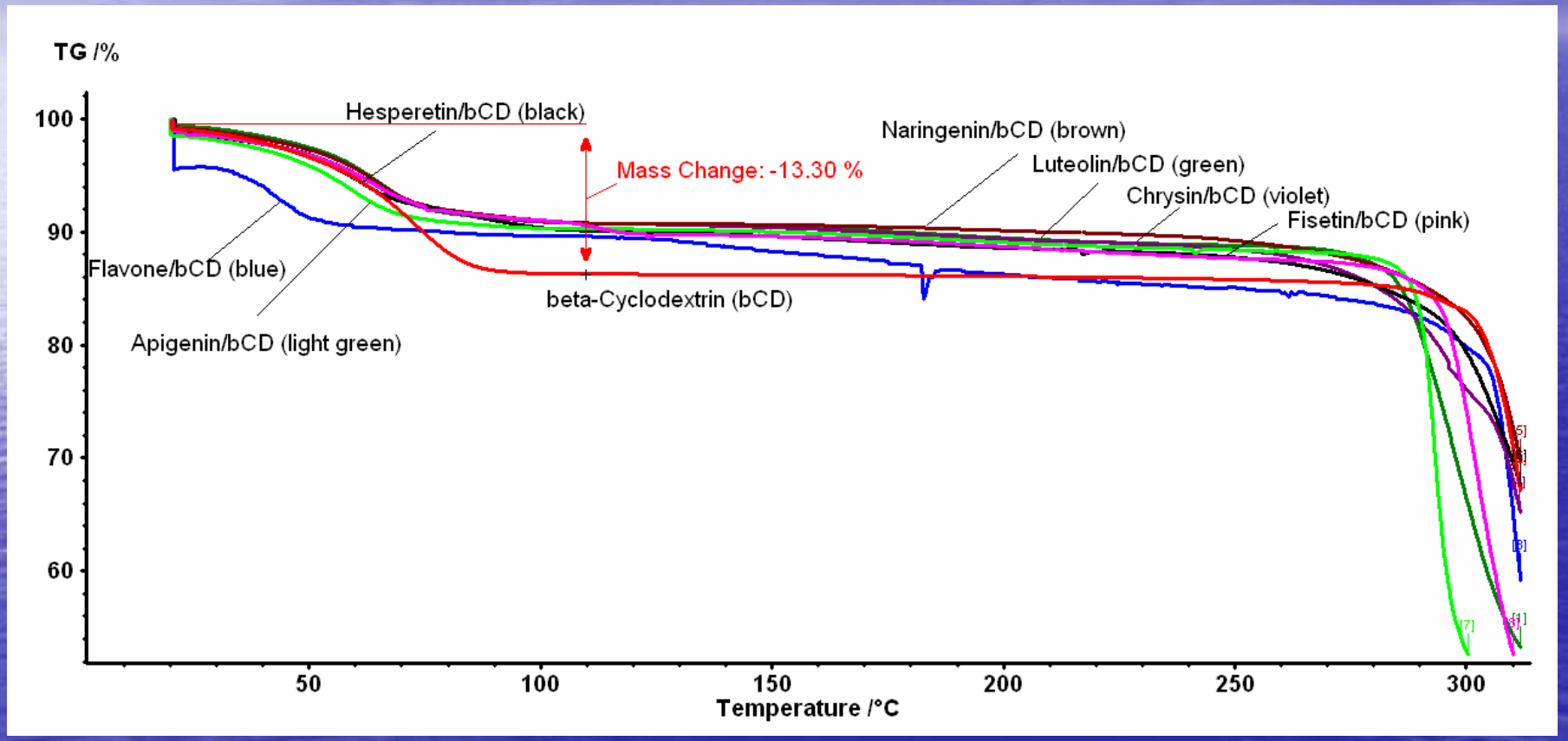
b) Differential Scanning Calorimetry (DSC) analysis of the flavonoid and related compounds/cyclodextrin complexes



DSC diagram for fisetin/β-cyclodextrin complex (dissociation range)

Results and discussion

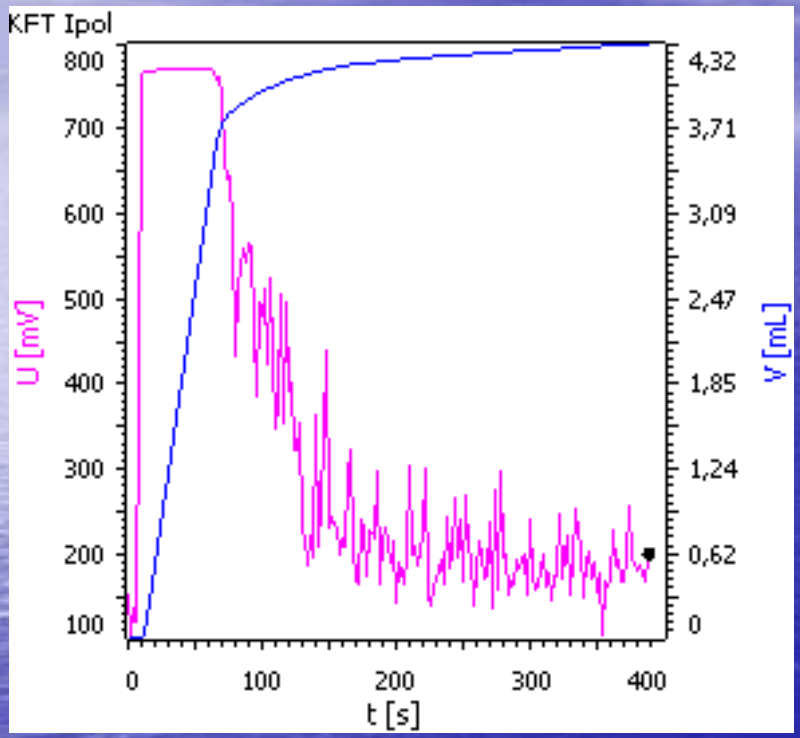
c) Thermogravimetric analysis (TG) of the flavonoid and related compounds/cyclodextrin complexes

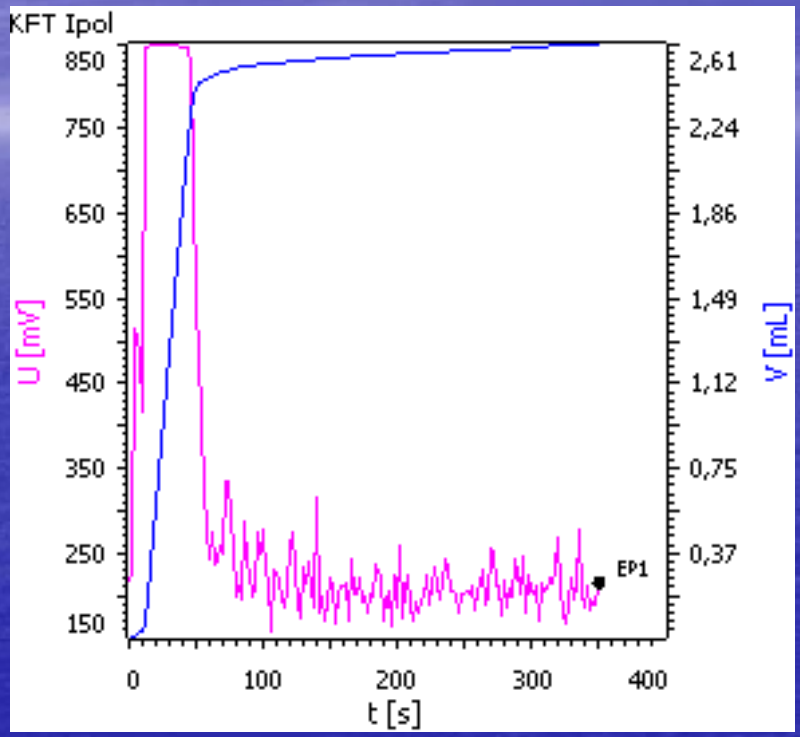


TG diagram for commercial β-cyclodextrin (red) and for flavonoid/β-cyclodextrin complexes

Results and discussion

d) Water determination in flavonoid and related compounds/cyclodextrin complexes





Ipol (U,mV/V,mL) vs. Time (s) dependence for KF water determination in Fisetin/β-cyclodextrin nanoparticles (17_Fst_bCD, left) and Silybin/β-cyclodextrin nanoparticles (23_Sbn_bCD, right)

Results and discussion

Water content (%) of flavonoid and related compounds/β-cyclodextrin complexes (obtained by ethanol-water solution method) from classical Karl Fischer titration (KF) and by thermogravimetric analysis (TG)

No	Code	Description	KF water content (%)	n (no of det.)	TG water content (%)
1	02_Flv_bCD	Flavone/β-cyclodextrin nanoparticles	10.59±0.10	4	10.7
2	05_Chr_bCD	Chrysin/β-cyclodextrin nanoparticles	8.85±0.06	5	9.3
3	08_Nrg_bCD	Naringenin/β-cyclodextrin nanoparticles	9.95±0.40	5	9.5
4	11_Hsp_bCD	Hesperetin/β-cyclodextrin nanoparticles	10.44±0.19	5	10.1
5	14_Apg_bCD	Apigenin/β-cyclodextrin nanoparticles	10.07±0.47	5	9.9
6	17_Fst_bCD	Fisetin/β-cyclodextrin nanoparticles	12.08±0.39	5	10.4
7	20_Ltn_bCD	Luteolin/β-cyclodextrin nanoparticles	11.15±0.12	4	9.6
8	23_Sbn_bCD	Silybin/β-cyclodextrin nanoparticles	9.43±0.22	5	8.6
9	26_Cnm_bCD	Cinnamic acid /β-cyclodextrin nanoparticles	11.52±0.42	5	10.1
10	29_Cff_bCD	Caffeic acid/β-cyclodextrin nanoparticles	12.56±0.38	5	12.0

Results and discussion

Water content (%) of flavonoid and related compounds/α-cyclodextrin complexes (obtained by ethanol-water solution method) from classical Karl Fischer titration (KF) and by thermogravimetric analysis (TG)

No	Code	Description	KF water content (%)	n (no of det.)	TG water content (%)
1	01_Flv_aCD	Flavone/α-cyclodextrin nanoparticles	9.78±0.20	5	10.7
2	04_Chr_aCD	Chrysin/α-cyclodextrin nanoparticles	8.24±0.49	5	9.3
3	25_Cnm_aCD	Cinnamic acid/α-cyclodextrin nanoparticles	9.13±0.21	5	9.4
4	28_Cff_aCD	Caffeic acid/α-cyclodextrin nanoparticles	9.45±0.16	5	10.0

Results and discussion

Water content (%) of flavonoid and related compounds/β-cyclodextrin complexes (obtained by ultrasonication method) from classical Karl Fischer titration (KF)

No	Code	Description	KF water content (%)	n (no of det.)
1	02_Flv_bCD_US	Flavone/β-cyclodextrin nanoparticles	12.9±0.27	5
2	05_Chr_bCD_US	Chrysin/β-cyclodextrin nanoparticles	10.7±0.24	5
3	08_Nrg_bCD_US	Naringenin/β-cyclodextrin nanoparticles	12.46±0.39	5
4	11_Hsp_bCD_US	Hesperetin/β-cyclodextrin nanoparticles	12.2±0.62	5
5	17_Fst_bCD_US	Fisetin/β-cyclodextrin nanoparticles	13.32±0.30	5
6	23_Sbn_bCD_US	Silybin/β-cyclodextrin nanoparticles	9.43±0.22	5
7	26_Cnm_bCD_US	Cinnamic acid/β-cyclodextrin nanoparticles	11.52±0.42	5
8	29_Cff_bCD_US	Caffeic acid/β-cyclodextrin nanoparticles	12.56±0.38	5

Results and discussion

e) Correlations between KF water content of cyclodextrin complexes and flavonoid and related compounds structural descriptors

Flavonoid and related compounds descriptors from Wombat database (descriptors related to the hydrophobicity/hydrophyllic properties)

Code	*eLogK*	*eLogS*	*Nh*	*Nnp*	*LHd*	*LHa*	*LlogP*	*Sp(A^2)*	*Snp (A^2)*
Flv	**3.1**	**-4.3**	2	**13**	0	2	**3.48**	27.7	208.1
Chr	**2.85**	**-3.15**	4	**11**	2	4	**3.563**	73.4	185.0
Nrg	**2.47**	**-3.14**	5	**10**	3	5	**2.445**	96.0	184.9
Hsp	**2.38**	**-3.1**	6	**11**	3	6	**2.15**	105.1	210.2
Apg	**2.47**	**-3.14**	5	**10**	3	5	**2.905**	96.3	173.5
Fst	**1.51**	**-2.5**	6	**9**	4	6	**1.244**	119.2	162.0
Ltn	**2.15**	**-2.89**	6	**9**	4	6	**2.311**	119.2	162.0
Sbn	**2.35**	**-3.72**	10	**15**	5	10	**1.949**	168.4	316.0
Cnm	**2.38**	**-2.38**	2	**7**	0	2	**2.239**	40.8	140.8

Results and discussion

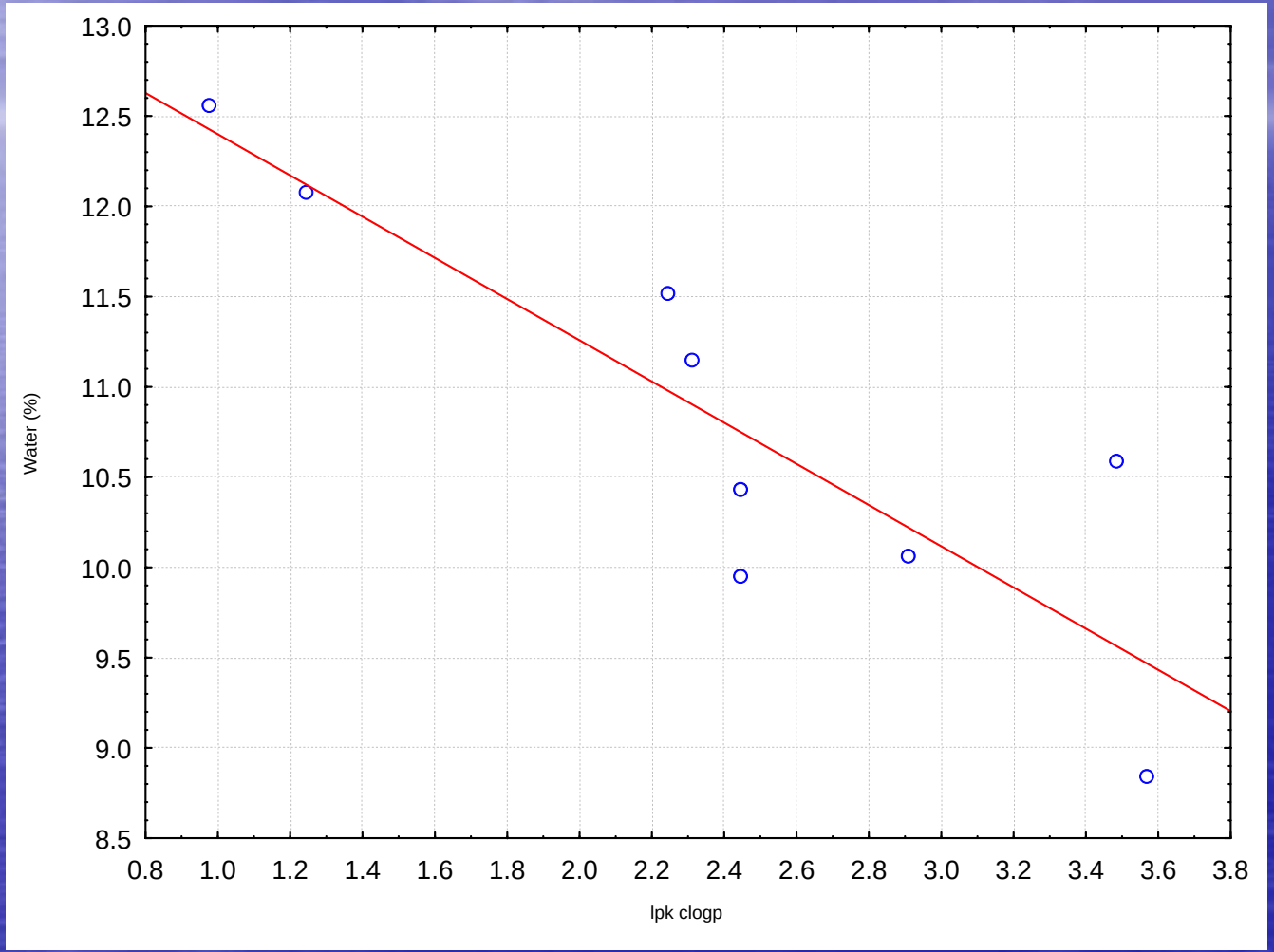
e) Correlations between KF water content of cyclodextrin complexes and flavonoid and related compounds structural descriptors

Intercorrelational matrix for the flavonoid and related compounds descriptors (significative intercorrelation coefficients are bolded)

	eLogK	*eLogS*	*Nh*	*Nnp*	*LHd*	*LHa*	*LlogP*	*Sp*	*Snp*
eLogK	1.00	**-0.70**	-0.46	0.41	-0.61	-0.46	**0.92**	-0.58	0.19
eLogS		1.00	-0.07	**-0.86**	0.11	-0.07	-0.55	0.08	-0.65
Nh			1.00	0.49	**0.93**	**1.00**	-0.54	**0.98**	**0.70**
Nnp				1.00	0.27	0.49	0.23	0.34	**0.92**
LHd					1.00	**0.93**	-0.57	**0.98**	0.43
LHa						1.00	-0.54	**0.98**	**0.70**
LlogP							1.00	-0.61	-0.05
Sp								1.00	0.55
Snp									1.00

Results and discussion

e) Correlations between KF water content of cyclodextrin complexes and flavonoid and related compounds structural descriptors



KF Water concentration vs. Lipinski CLogP dependence
for flavonoids and related compounds/β-cyclodextrin nanoparticles

Results and discussion

e) Correlations between KF water content of cyclodextrin complexes and flavonoid and related compounds structural descriptors

$$W(\%) = 13.54(\pm 0.62) - 1.14(\pm 0.24) \cdot L\log P$$

r = -0.871; *F* = 22.0; *s* = 0.60

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

r = -0.752; F = 10.4; s = 0.82

$$W(\%) = 14.28(\pm 1.46) + 1.19(\pm 0.47) \cdot eLogS$$

r = 0.667; F = 6.4; s = 0.92

Conclusion

• The formation of the complex depends of the hydrophobicity of the guest compound (expressed by different descriptors), but only in the case of obtaining of complexes by crystalization method.

The water content of cyclodextrin complexes, determined even by classical Karl Fischer titration or evaluated by thermogravimetry, depend on the hydophobic (or related) descriptors of guest compound, especially for β-cyclodextrin complexes, the best correlation being obtained for *logP* (logarithm of octanol-water partition coefficient) and *Nnp* (total number of non-polar atoms); good correlations were obtained also for the water solubility descriptor (*LogS*), which are correlated (inverse correlation) with the hydrophobicity descriptors.

These correlations, obtained only for crystallization method, can be explained by the possibility to touch the association/dissociation equilibrium between guest (flavonoid and related compounds) and host (cyclodextrin) by very slow crystallization, when some of the water molecules from cyclodextrin are replaced by the guest molecule.

If the guest molecule are more hydrophobic the van der Waals interaction with the inner cavity of cyclodextrin is better and the water content of the final complex is reduced.

Conclusion

• Obtaining of cyclodextrin complexes by ultrasonication method is inappropriate due to the facility of dissociation of guest-cyclodextrin complex in this process, as is revealed by the higher water content of these complexes

• The water content determined by classical Karl Fischer method seems to be the better method for water evaluation in cyclodextrin micro/nanoparticles, even the thermogravimetry conduct to similar results, but little bit lower than the above mentioned method, due to the analysis technique. Moreover, the KFT allow to evaluate only the water content while TG analysis indicate the mass loss by increasing temperature and do not differentiate between water and other volatile compounds (like ethanol used in synthesis)

Acknowledgements

Thanks to:

Prof. Heinz-Dieter Isengard, Georg Merkh, Erika Denzel (from Hohenheim University, Germany)

Prof. Tudor Oprea (University of New Mexico, Albuquerque &
Sunset Molecular Discovery LLC, Santa Fe, USA)

Dr. Ioan Grozescu (Electrochemistry & Condensed Matter Institute, Timisoara, Romania)

Prof. Mircea Mracec (Institute of Chemistry, Timisoara, Romania)

This work was partially supported by DAAD (Deutscher Akademischer Austausch Dienst, Germany) and Ministry of Education and Research of Romania (Grant PN2 62072/2008)

THANK YOU!