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HEPATOPROTECTIVE FLAVONOID BIOCONJUGATE / β-CYCLODEXTRIN NANOPARTICLES: DSC – MOLECULAR MODELING CORRELATION

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The paper presents the biosynthesis of some rutin- and silybin-fatty acid bioconjugates with potential enhanced hepatoprotective activity and complexation of these new compounds with β-cyclodextrin by using the crystallization from ethanol-water solution method. Bioconjugate / β-cyclodextrin nanoparticles were analyzed by differential scanning calorimetry (DSC) and the energy of dissociation of water molecules was evaluated, as well as other calorimetric parameters. On the other hand, molecular modeling and docking experiments on the molecular encapsulation of these bioconjugates in β-cyclodextrin were performed by using molecular mechanics MM+ program from the HyperChem 5.1 package. The interaction energy evaluated from docking experiments correlates with some DSC parameters (*i.e.* water dissociation energy, value or inflection temperatures of the water dissociation peaks - the dissociation peak are shifted after encapsulation). Statistically significant correlation was obtained also between water dissociation energy and log*P* (logarithm of the octanol/water partition coefficient of bioconjugates) for rutin and silybin derivative classes. All samples were better classified by using principal component analysis (PCA) with DSC and theoretical docking parameters.

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Keywords: flavonoid-fatty acid bioconjugates, β-cyclodextrin, nanoparticles, hepatoprotective agents, DSC, molecular modeling, docking experiments

1. Introduction

Flavonoids and derivatives are widely distributed in raw products as well as in many foods and have an important effect in maintaining health and preventing diseases (principally, they have anticancer, antioxidant, antimicrobial, and anti-inflammatory biological activities) [1-4]. The biochemical effects of flavonoids can be divided into four categories [5]: (1) binding affinity to biological polymers; (2) binding of heavy metal ions; (3) catalysis of electron transport; and (4) ability to scavenge free radicals.

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In order to enhance their activity, flavonoid-fatty acid bioconjugates were obtained by selective enzymatic biosynthesis, with antioxidant, antimicrobial, and antiviral activities [6-11] or antitumoral properties [12,13].

Nanoencapsulation was another method to enhance bioactivity or bioavailability of natural or biochemically modified compounds [14-23]. From the wide range of encapsulation matrices, cyclodextrins and liposomes are extensively used in medicine and food fields [14-18,24]. Naturally occurring cyclodextrins are α-, β-, and γ-cyclodextrin, which are cyclic oligosaccharides with 6, 7, and 8 glucopyranose moieties; they are obtained from starch by using various *Bacillus* species (such as *B. macerans*). The specific architecture of the cyclodextrin structure (truncated cone with exterior hydroxyl groups and hydrophobic inner cavity) determine to use them for nanoencapsulation of hydrophobic and geometrically compatible bioactive molecules (such as drugs, food additives, odorant, and flavoring compounds etc.) in order to obtain powdery formulations with higher water solubility, protecting capacity (against air, light, humidity), and controlled release properties [14,15,25-27]. Various natural antioxidants (such as flavonoids, antocyanins, and related compounds – quercetin, rutin, chlorogenic acid, *trans*-resveratrol) were encapsulated in cyclodextrins in order to enhance their stability and bioactivity [28-33].

In the present study we obtain new flavonoid-fatty acid bioconjugate / β-cyclodextrin nanoparticles, which were analyzed by differential scanning calorimetry (DSC). Bioconjugates were prior biosynthesized from rutin, silybin, and decanoic, palmitic, stearic, oleic, and linoleic acid. The same supramolecular systems were theoretically evaluated and interaction energies between flavonoid-fatty acid bioconjugates and β-cyclodextrin were determined and correlated with the experimental DSC parameters.

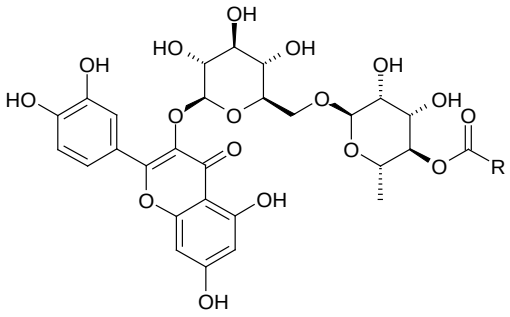
2. Materials and Method

Materials. Rutin- and silybin-fatty acid bioconjugates was biosynthesized by using Novozyme 435 (Sigma-Aldrich) in acetone media (Merck) and detailed procedure is presented elsewhere [34]. Shortly, rutin (>90%, Merck) and silybin (99%, Sigma) were enzymatically derivatized with decanoic, palmitic, stearic, oleic, or linoleic acid (>95%, Fluka) in acetone media in the presence of molecular sieves (4-8 mesh, Sigma-Aldrich), with a molar ratio for flavonoid derivative: fatty acid of 1:3, at 50ºC, for 6 days; fatty acid-flavonoid bioconjugates were separated by column chromatography (30×2 cm, silica gel 5 μm, Merck) and analyzed by thin layer chromatography (silica gel 60 F_{254}, Merck) using chloroform:methanol:water 80:20:1.5 and chloroform:ethyl acetate 60:40 as eluent mixtures for rutin- and silybin derivatives, respectively. Purified bioconjugates were characterized by FT-IR (Jasco 430), MS (Varian), and ^{1}H-NMR (Bruker 300). β-Cyclodextrin was purchased from Fluka (>99%) and ethanol 96% (v/v) was achieved from Chimopar, Bucharest. The following abbreviations were used: R and S for rutin and silybin moieties, respectively; D, P, S, O, and L for decanoic, palmitic, stearic, oleic, and linoleic acid moieties, respectively; bCD for β-cyclodextrin (*e.g.*, R_P / bCD means rutin-palmitic acid bioconjugate / β-cyclodextrin supramolecular system).

Flavonoid-fatty acid bioconjugate / β-cyclodextrin nanoparticle synthesis.

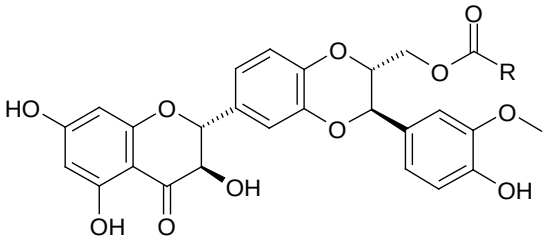
Synthesis of bioconjugate / β-cyclodextrin nanoparticles, as well as the corresponding flavonoid (rutin and silybin) / β-cyclodextrin nanoparticles was performed by using crystallization from ethanol/water solution. A quantity corresponding to 0.5 mmoles of β-cyclodextrin (0.67 g cyclodextrin hydrate, ~14% water) was suspended (cyclodextrin is partially dissolved in water) in 4 mL distilled water at 50ºC and 0.5 mmoles of bioconjugate or flavonoid (2 mL ethanolic solution; Figure 1) was slowly added (15 minutes) to the cyclodextrin solution under continuous stirring in a thermostated minireactor (equipped with reflux condenser); after another 15 minutes of complexation, the reaction mixture was slowly cooled to 20ºC in 4 hours. The crystallization of cyclodextrin nanoparticles was perfected at 4ºC over night. The resulted nanocrystals were filtered, washed with 0.5 mL ethanol and dried at maximum 40ºC until constant mass. The complexation

yield was evaluated as the ratio of the mass of bioconjugate/cyclodextrin complex and the sum of masses of bioconjugate or flavonoid compound and cyclodextrin used for complexation.



Rutin: R = H
Decanoyl derivative: R = -$(CH_2)_8$-CH_3
Palitoyl derivative: R = -$(CH_2)_{14}$-CH_3
Oleoyl derivative: R = -$(CH_2)_7$-CH=CH-$(CH_2)_7$-CH_3

(a)



Silybin: R = H
Stearoyl derivative: R = -$(CH_2)_{16}$-CH_3
Oleoyl derivative: R = -$(CH_2)_7$-CH=CH-$(CH_2)_7$-CH_3
Linoleoyl derivative: R = -$(CH_2)_7$-CH=CH-CH_2-CH=CH-$(CH_2)_4$-CH_3

(b)

Fig. 1. Biocompounds structures, rutin and derivatives (a), silybin and derivatives (b), used for complexation with β-cyclodextrin

Differential scanning calorimetry (DSC)

Calorimetric analysis of cyclodextrin complexes was performed by using a Netzsch 204 scanning electron microscopy apparatus. Aluminum oxide dishes were used for weighting and analysis of the samples (10±2 mg of sample). The DSC conditions were: temperature program 20-500ºC, with a heating rate of 4ºC/min; cooling of the sample was achieved with liquid nitrogen. Data acquisition was performed by using the DSC Netzsch 204-Acquisition soft/2000 and data handling was realized with the Netzsch Proteus-Thermal Analysis ver. 4.0 / 2000 program. The following DSC parameters were evaluated: water dissociation energy ($E_{dis.w}$, J/g), maximum/minimum peak temperature (temperatures) for water dissociation ($t_{Peak1\ or\ 2}$, ºC), temperatures corresponding to DSC inflections for different intervals ($t_{Infl.1,2,3}$, ºC, for intervals corresponding to water dissociation peaks; see below).

Molecular modeling of flavonoids, bioconjugates, and β-cyclodextrin

Molecular modeling of bioconjugate molecules, flavonoids, and β-cyclodextrin was performed by using the molecular mechanics MM+ program from the HyperChem 5.1; a RMS of 0.005 kcal/mole and a Polak-Ribiere algorithm were used in the molecular modeling process.

Conformational analysis of flavonoids, bioconjugates, and β-cyclodextrin

The most stable conformations for bioconjugates, flavonoids or β-cyclodextrin were obtained after conformational analysis using the *Conformational Search* program from HyperChem package. All flexible rings from bioconjugates, flavonoids, and β-cyclodextrin, as well as all flexible rings (pyranone ring from aglycone moiety and the two pyranose rings from disaccharide moiety for rutin and also dioxane ring for silybin; the glucopyranose rings and the corresponding macrocyclic ring for β-cyclodextrin) were considered for conformational analysis. The following conditions were set up for conformational search: variation of the flexible torsion angles ±60º ÷ ±180º, energy criterion for acceptance of the conformation 4 kcal/mole above minimum, all conformations with atomic distances lower than 0.5 Å and differences between

torsion angles lower than 15º were not considered as well as conformations with energy differences lower than 0.05 kcal/mole (duplicates); the maximum number of optimization and iterative calculations was 1000 and maximum 100 conformations were retained. The hydrogen atoms were neglected.

Docking of bioconjugates and flavonoids in β-cyclodextrin

The docking of the more stable conformations of studied bioconjugates in β-cyclodextrin was realized by using the molecular mechanics interactions of the host-guest molecules in vacuum. The bioconjugate or flavonoid and β-cyclodextrin structure in minimal energy conformations were set up at distances of ~8Å between the gravity centres of the host-guest molecules, and the biologically active compound structure was oriented with fatty acid moiety in front of the primary (A) or secondary (B) face of cyclodextrin (the principal axis corresponding to the biocompound was perpendicular to the A or B plan of cyclodextrin). The complex was modeled in absence of water molecules by using the same MM+ program and the interaction was stopped when the RMS gradient was lower than 0.005 kcal/mole. The biocompound-cyclodextrin interaction energy was evaluated as the difference between the the overall energies of these two molecules and the energy of the complex. The log*P*, logarithm of octanol/water partition coefficient, calculated with the *QSAR Properties* from the HyperChem package, was also evaluated for bioactive compounds and used in the experimental – theoretical correlations.

Principal Component Analysis (PCA)

The multivariate statistical analysis of the theoretical and experimental data for bioconjugate / bCD supramolecular systems was achieved using the PCA analysis (*in house* program) in order to find similarities-disimilarities of the samples. Principal component analysis is the basis of the multivariate analysis of the data [35-37]. PCA presumes an approximation of the *X* matrix (data) as a product of two reduced matrices, *T* and *P*, which retain only the useful information from *X*. The graphical representation of *T* columns conduct to the "object shape" images of *X*, and the graphical representation of *P* rows conduct to the "variable shape". Thus, the first direction (first principal component, PC_1) in the properties space, for which the data have maximum variance, conduct to the monodimensional representation of the data as projections on this PC_1; the second direction (named PC_2) has the same particularities, but it is perpendicular to PC_1. Other directions can be obtained in the same way, but only some of them will be PCs. The *X* matrix can be described as a sum of a useful matrix (**X*), which is a product of score matrix (**T*) and loadings matrix (**P*), and an error matrix (*E*). Representation of the *t* vectors (one to another) can conduct to information about similarities and possible grouping of the studied objects; the same representation of the *p* vectors can furnish the similarities between properties and the importance of these properties for the model.

3. Results and discussion

Bioconjugate / β-cyclodextrin nanoparticle synthesis and analysis

All β-cyclodextrin complexes of flavonoid (rutin and silybin) and their bioconjugates with saturated (decanoic, palmitic, and stearic acids) or unsaturated fatty acids (oleic and linoleic acids) were obtained with yields over than 70% (calculated as percent of recovered nanocrystals in comparison with the starting compounds amount). The calorimetric analysis (among other analyses, presented elsewere [31,33]) revealed that the biologically active compounds (flavonoids or bioconjugates) / β-cyclodextrin inclusion complexes are obtained due to the main DSC characteristics: lowering and shifting (mainly the "surface water" is presented) of the water dissociation energy. The DSC analysis of the starting β-cyclodextrin indicates a water dissociation energy of 1194 J/g, with a temperature of the endothermal process of 124 ºC (Figure 2), higher in

comparison with the water boiling temperature (in normal conditions), most probably due to the "strong bonded" water molecules from the cyclodextrin hydrate; two peaks (partially superimposed) can be observed: a smaller one with a maximum (shoulder like) for endothermal effect at ~95ºC and an inflection at 90.5ºC, most probably corresponding to the "surface water" molecules, and a bigger one, with the maximum at 124ºC and inflections at 117.3ºC and 127.2ºC, corresponding to dissociation of "strong bonded" water molecules. No calorimetric effect appear until 270ºC, when the decomposition of cyclodextrin take place.

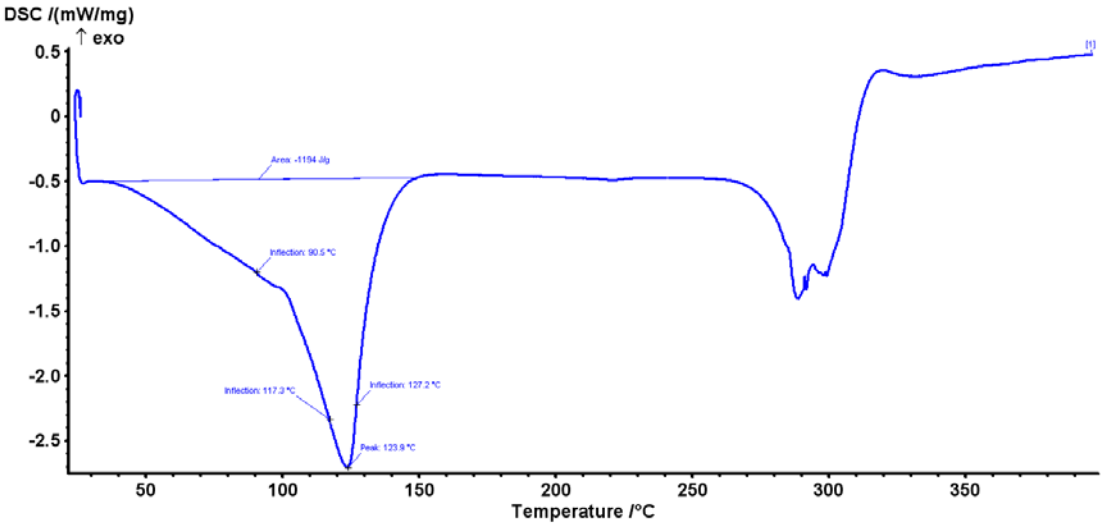


Fig. 2. DSC analysis of commercial β-cyclodextrin

In comparison, the DSC analysis of rutin- and silybin-bioconjugate / β-cyclodextrin nanoparticles revealed two aspects which suggest the formation of the inclusion complex: the endothermal effect corresponding to the dissociation of water molecules is smaller that for the starting β-cyclodextrin (this seems that the water concentration is lowered, but the endothermal effect corresponding to the cyclodextrin decomposition is approximately the same) and the peak temperature is shifted to a low temperature (with a difference of 30-40ºC, most probably due to the replacing of "strong-bonded" water molecules from the cyclodextrin cavity with the hydrophobic biologically active compound) (Figure 3).

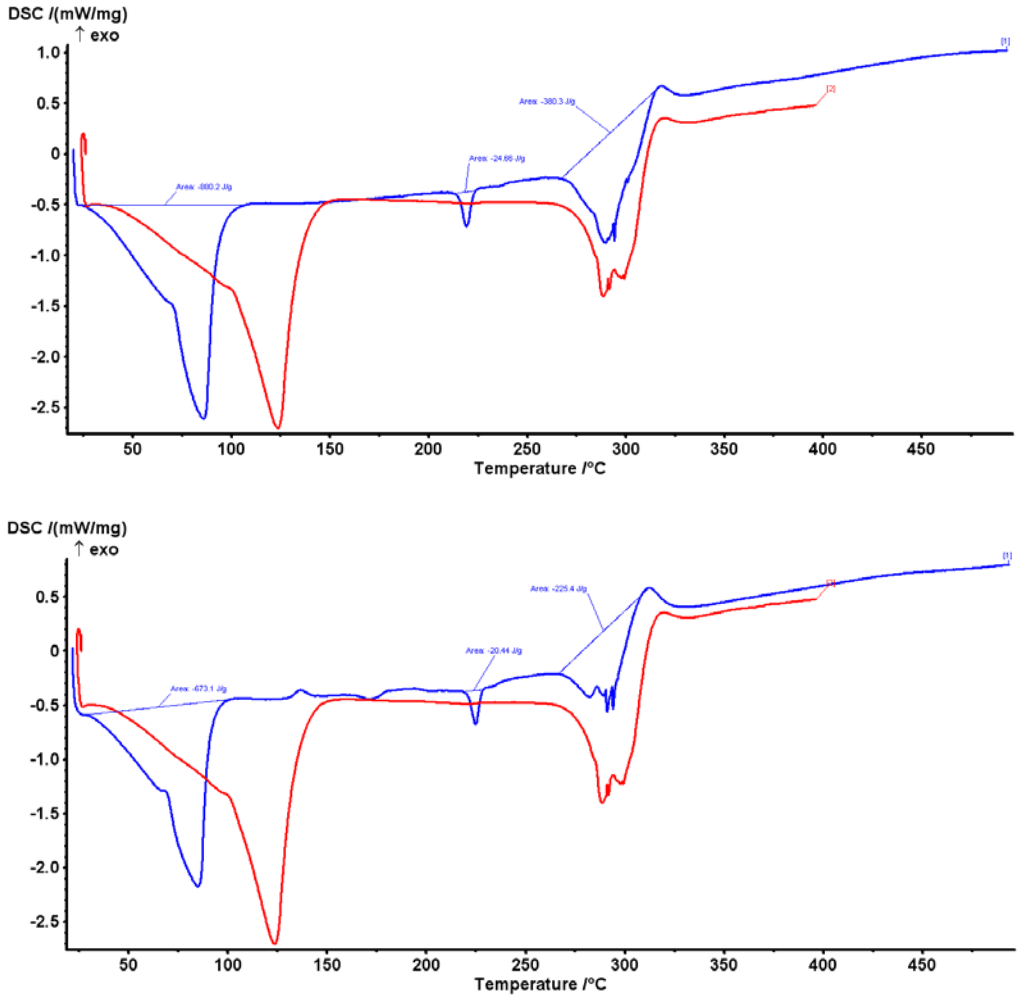


Fig. 3. Comparative DSC analysis of rutin-decanoic acid (up) and rutin-oleic acid (down) bioconjugate / β-cyclodextrin nanoparticles (1) and the starting β-cyclodextrin (2)

Thus, the R_D / bCD complex has water dissociation energy of 850 J/g with a temperature peak (t_{peak2}) of 86.2ºC (Figure 4), close to that corresponding to R_P / bCD complex (86.6ºC). In the case of unsaturated fatty acid bioconjugate complex R_O / bCD this parameter is little bit lower (85.1ºC) (Figure 5). All rutin bioconjugates having more hydrophobic fatty acid moieties (palmitic and oleic acid derivatives) conduct to complexes with lower water dissociation energy (601 J/ g and 666 J/g, respectively) most probably due to a better interaction with cyclodextrin cavity, in comparison with the case of decanoic acid bioconjugate; as a result, water molecules are replaced in a higher level for palmitic and oleic acid bioconjugates, which is revealed also by the inflection temperature for the first interval considered (47.5ºC for decanoic acid derivative in comparison with ~60ºC for the other two cases), even the inflection temperatures on second and third intervals are close (Table 1).

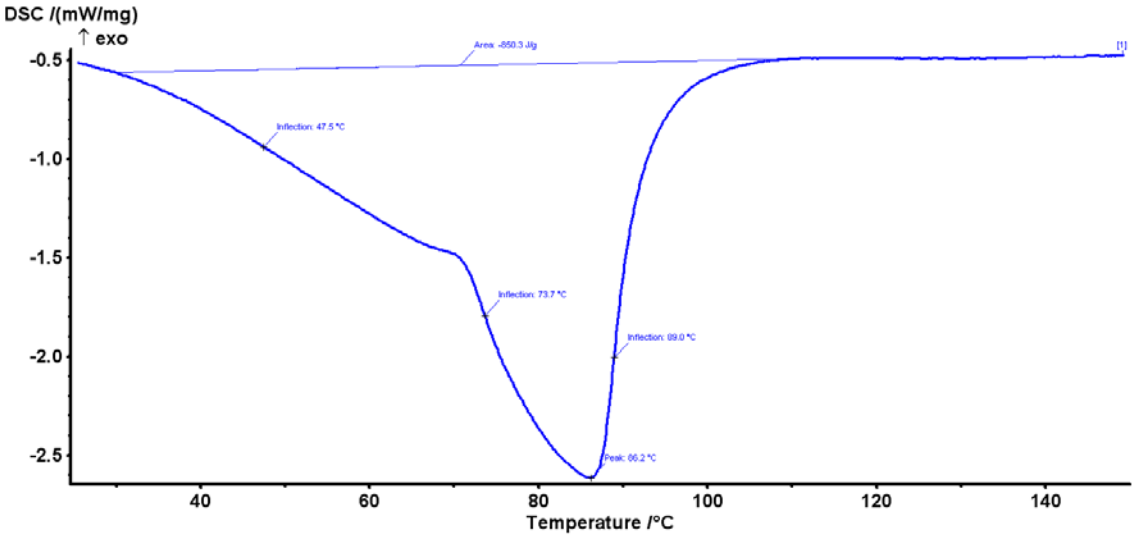


Fig. 4. DSC analysis of rutin-decanoic acid bioconjugate / β-cyclodextrin nanoparticles

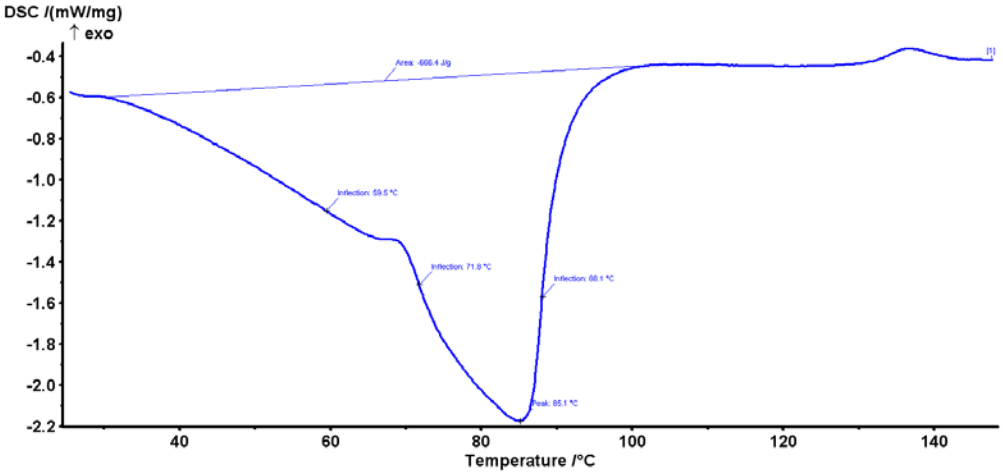


Fig. 5. DSC analysis of rutin-oleic acid bioconjugate / β-cyclodextrin nanoparticles

In the case of silybin bioconjugate / β-cyclodextrin nanoparticles the DSC analysis conduct to similar behavior, excepting the case of saturated fatty acid derivative, where traces of uncomplexed bioconjugate can be observed (at 68.2ºC); the general behavior of the S_S / bCD complex is very close to the corresponding rutin-saturated fatty acid bioconjugate / bCD nanoparticles. Generally, the two peaks corresponding to water dissociation appear, especially in the case of unsaturated fatty acid derivatives (oleic and linoleic acid bioconjugate / bCD nanoparticles). The peaks for the "surface" and "strong-bonded" water dissociation for S_O / bCD and S_L / bCD complexes appear at 57 / 85ºC and 80.1 / 107.5ºC, respectively (Figure 6 and Table 1).

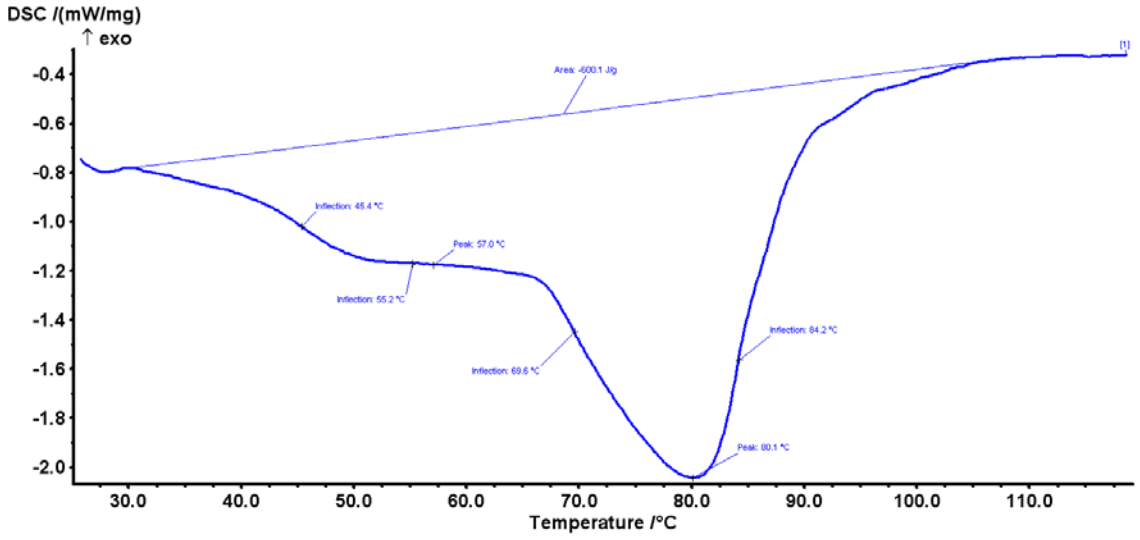


Fig. 6. DSC analysis of silybin-oleic acid bioconjugate / β-cyclodextrin nanoparticles

Table 1. DSC and molecular modeling / docking parameters for bioconjugate / β-cyclodextrin complexes ($E_{int.}$ – theoretical interaction energy between flavonoids or bioconjugates and bCD, evaluated from molecular modeling and docking experiments, kcal/mole; $E_{dis.w}$ – water dissociation energy, evaluated from DSC analysis, J/g; $t_{peak1,2}$ – temperature of the peaks corresponding to the water dissociation, from DSC analysis, ºC; $t_{infl.1,2,3}$ – the main inflection temperatures from DSC analysis, corresponding to water dissociation, ºC; logP – logarithm of the octanol/water partition coefficient for flavonoids and bioconjugates, calculated with QSAR Properties program).

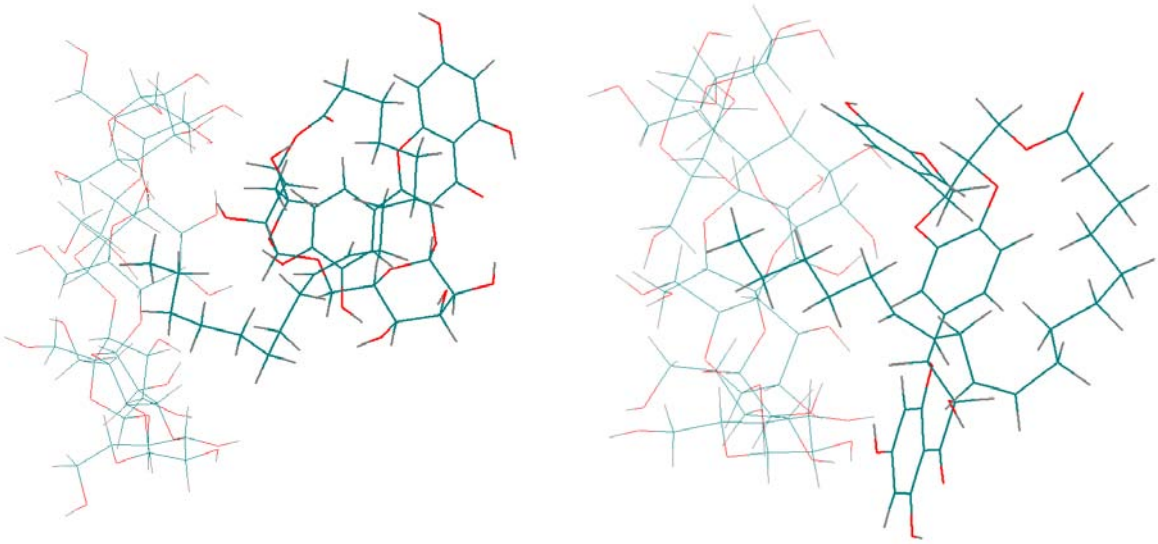
No	Code	$E_{int.}$ (kcal/mole)	$E_{dis.w}$ (J/g)	t_{Peak1w} (ºC)	t_{Peak2w} (ºC)	$t_{infl.1}$ (ºC)	$t_{infl.2}$ (ºC)	$t_{infl.3}$ (ºC)	log*P*
1	R/bCD	22.64	-	-	-	-	-	-	-1.61
2	R_D/bCD	23.46	850.3	-	86.2	47.5	73.7	89	1.92
3	R_P/bCD	27.33	601	63	86.6	62	75.1	91	4.29
4	R_O/bCD	23.43	666.4	-	85.1	59.5	71.8	88.1	4.83
5	S/bCD	16.34	646.6	-	80.1	-	78.2	82.8	2.1
6	S_S/bCD	27.34	901.1	83.2	-	69.7	-	85.2	8.8
7	S_O/bCD	21.99	600.1	57	80.1	55.1	69.6	84.2	8.54
8	S_L/bCD	28.88	627	85	107.5	76.9	99.1	111.1	8.28

Molecular modeling and docking experiments for flavonoid and bioconjugate / β-cyclodextrin supramolecular systems

All flavonoids, bioconjugates, and β-cyclodextrin molecules were molecular modeled by using molecular mechanics MM+ program from the HyperChem package, and further conformationally analyzed (*Conformational Search* program) in order to obtain the most stable conformations which were used in docking experiments. Some of these theoretically experiments were already published (rutin-saturated fatty acid bioconjugate / β-cyclodextrin supramolecular systems [38]) and are completed with similar experiments for other rutin-unsaturated fatty acid bioconjugate and silybin-fatty acid bioconjugate / β-cyclodextrin supramolecular systems. Thus, all bioconjugates have a spiral-like conformation for fatty acid moiety (which interact with benzopyranone moieties from rutin and silybin parts by hydrophobic bonds). The β-cyclodextrin conformations were very close in the range up to 0.5 kcal/mole above best, almost all

hydroxymethyl moieties being oriented close to the main axis of molecule. The structure is stabilized by the hydrogen bonds formed between hydroxyl groups.

The maximum energy interaction between flavonoids or bioconjugates and cyclodextrin was obtained by using the starting position with bioconjugate structure oriented to the secondary face of bCD along the cyclodextrin symmetry axis, at a distance of ~8 Å between the gravity centre of molecules; the hydrophobic fatty acid moiety of bioconjugate was oriented to the hydrophobic cavity of cyclodextrin. The bioconjugate/cyclodextrin supramolecular system was optimized by using the same MM+ program (in vacuum) (Figure 7) and the interaction energy was evaluated as the difference between the sum of energies for the singular bioactive compounds implied in complexation and the energy of the complex. The higher interaction energy was observed in the case of R_P / bCD for the rutin derivative series (27 kcal/mole), and for S_S / bCD and S_L / bCD for the silybin derivative series (27-29 kcal/mole); for the starting flavonoid derivatives (rutin and silybin) the interaction energy was lower (with 5 kcal/mole and 11 kcal/mole in comparison with the above mentioned bioconjugates); this is in good agreement with the log*P* parameter for the guest bioactive compound (flavonoids and derivatives) (Table 1).



(a) (b)

Fig. 7. R_O (a) and S_L (b) bioconjugate / β-cyclodextrin supramolecular system, obtained by theoretical docking experiments

DSC – molecular modeling / docking correlations and PCA analysis

Correlations between theoretical and experimental parameters corresponding to flavonoid-fatty acid bioconjugate / β-cyclodextrin supramolecular systems have been tried. Theoretical bioconjugate / cyclodextrin interaction energy ($E_{int.}$) correlate with experimental DSC parameters (peak and inflection temperatures); however, all DSC parameters (temperature parameters) considered are intercorrelated (t_{peak2} correlate with $t_{infl.1,2,3}$ with correlation coefficients >0.85). No correlation with theoretical parameter log*P* can be obtained with experimental parameters.

Statistically significant correlation was obtained in the case of DSC experimental parameter $t_{infl.1}$ with the theoretical interaction energy ($E_{int.}$) for all bioconjugate / cyclodextrin supramolecular systems, the correlation coefficient being r = 0.85 (Figure 8). Other correlations with higher coefficients were obtained but only for separated biocompound classes (rutin or silybin derivatives). Thus, in the rutin derivative class log*P* correlate with $E_{dis.w}$ (r = -0.91) and also $t_{infl.3}$ correlate with $E_{int.}$ (r = 0.96); due to the intercorrelation of DSC parameters, these also correlate with the theoretical interaction energy. Energy of dissociation of water molecules seems to correlate with interaction energy but only with a correlation coefficient of r = -0.70, which is in agreement with the log*P* – $E_{dis.w}$ correlation.

In the case of silybin derivative / β-cyclodextrin complexes the experimental – theoretical correlations were the same. First, interaction energy is correlated with the hydrophobicity, log*P* (*r* = 0.85), but the lower log*P* of silybin itself seems to be important for this correlation. Second, the DSC experimental parameters are good correlated with the theoretical MM calculations for bioconjugate / bCD supramolecular systems: a correlation coefficient of 0.99 was obtained in the case of $t_{infl.1}$, r = 0.99 in the case of t_{peak1}, and 0.89 in the case of t_{peak2}. However, the statistical analysis for both sets of flavonoid derivatives is poor due to the low case numbers, which is only 3 or 4.

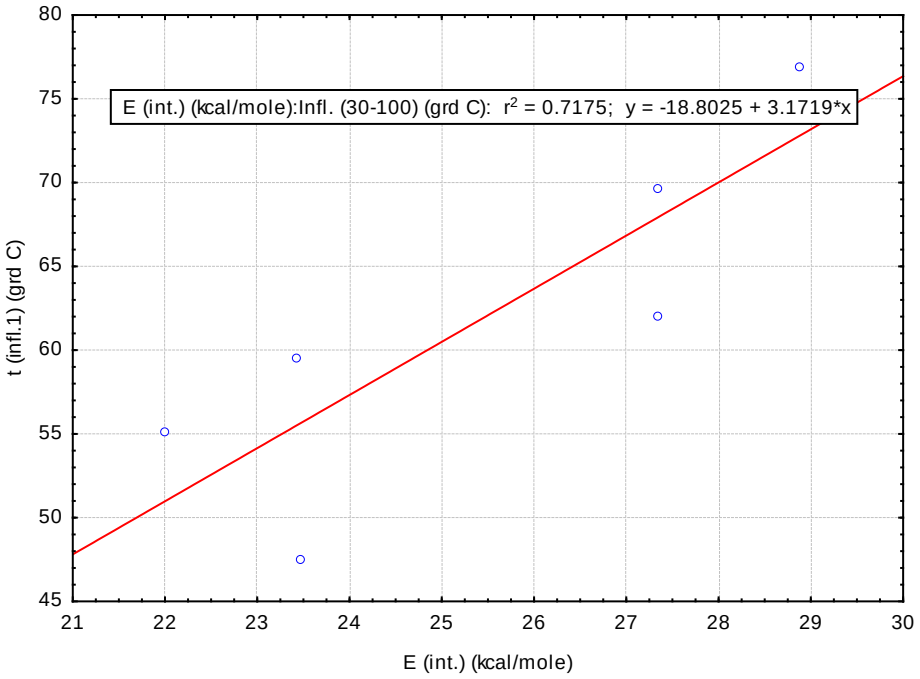


Fig. 8. Experimental (DSC) – theoretical (MM) correlation for bioconjugate / β-cyclodextrin supramolecular systems ($t_{infl.1}$ vs. $E_{int.}$)

Statistical multivariate analysis (PCA) of the theoretical molecular modeling / docking data (hydrophobicity, bioconjugate-cyclodextrin interaction energy) as well as of the experimental DSC results (water dissociation calorimetric effect, peaks and inflections temperatures corresponding to water dissociation) conduct to a good classification of the samples. Thus, the PCA analysis indicate a grouping of rutin bioconjugate complexes in the lower side of the "score" plot, while the silybin bioconjugate complexes as well as the starting silybin / bCD complex are distributed in the upper side of this plot; only R / bCD and bCD alone are outside of these groups (Figure 9). The variance is explained by 99% for the first two principal components (97% for PC_1 and 2% for PC_2) and the most important variables for this classification are the water dissociation energy for PC_1 and the corresponding DSC temperatures (peaks and inflections) as well as the energy of complex for PC_2 (Figure 9). Less important seem to be the theoretical interaction energy for this classification. Similar results (two groups corresponding to rutin and silybin derivatives) were obtained when it used only the bioconjugate / bCD complexes in PCA analysis.

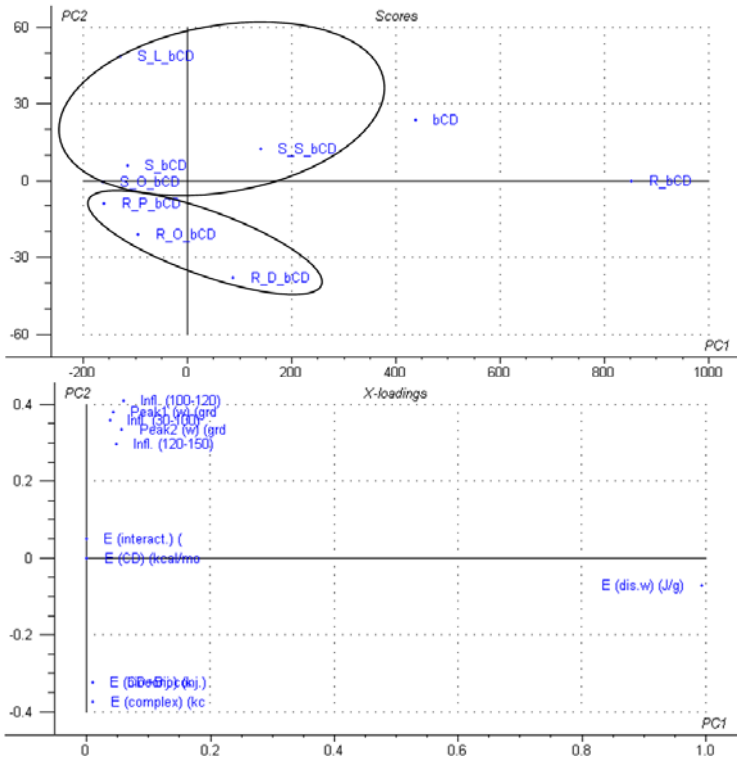


Fig. 9. Scores (left) and loadings (right) plots from the PCA analysis of the theoretical MM and experimental DSC data for flavonoid derivatives / β-cyclodextrin supramolecular systems

4. Conclusion

The following conclusion can be draw among the synthesis and analysis of flavonoid-fatty acid bioconjugate / β-cyclodextrin supramolecular systems: (1) formation of rutin- and silybin-fatty acid bioconjugate / β-cyclodextrin complexes could be revealed by an indirect evaluation of the remaining water from the complex hydrate: the endothermal calorimetric effect corresponding to the dissociation of water molecules is reduced in the bioconjugate / β-cyclodextrin complex in comparison with the non-complexed β-cyclodextrin; further, the dissociation of water appears at a lower temperature (with ~40ºC) than in the case of β-cyclodextrin, which could be due to the dissociation of "surface" water molecules, the water molecules from the inner cavity of cyclodextrin being replaced by the hydrophobic moiety of biologically active compound (molecular encapsulation); (2) experimental calorimetric analysis is in good agreement with theoretical calculations for flavonoid derivative-fatty acid / β-cyclodextrin interactions, especially in the case of separated rutin and silybin derivative classes. Interaction energy, evaluated by theoretical molecular modeling and docking calculations, correlates with DSC parameters corresponding to dissociation of water molecules. The above presented conclusions (reducing and shifting of the water dissociation calorimetric effect) demonstrates that the water molecules from the inner cavity of cyclodextrins, which are "strong-bonded" water molecules, are replaced by the more hydrophobic biologically active molecules or moieties; these molecules interact by van der Waals bonds (hydrophobic interaction) with the hydrophobic cavity of cyclodextrin.

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