JAP&T
TPA-Timişoara

Available on-line at www.japt.tpa.usab-tm.ro

Journal of Agroalimentary Processes and Technologies 2009, *15*(3), 441-448

Journal of Agroalimentary Processes and Technologies

Quercetin and rutin/2-hydroxypropyl-β-cyclodextrin nanoparticles: obtaining, characterization and antioxidant activity

Georgeta Coneac[a], Eliza Gafiţanu[b], Nicoleta G. Hădărugă[c], Daniel I. Hădărugă[d*], Adrian Riviş[c], Dorel Pârvu[c]

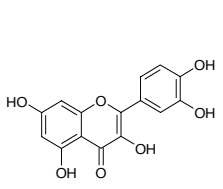
[a] *"Victor Babeş" University of Medicine and Pharmacy Timişoara, Faculty of Pharmacy, 300041-Timişoara, P-ţa Eftimie Murgu 2, Romania*
[b] *University of Medicine and Pharmacy "Gr. T. Popa" Iaşi, Faculty of Pharmacy, 700115-Iaşi, Universităţii Street 16, Romania*
[c] *Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Processing Technology, Food Quality Department, 300645-Timişoara, C. Aradului 119, Romania*
[d] *"Politehnica" University of Timişoara, Faculty of Industrial Chemistry and Environmental Engineering, Applied Chemistry and Organic-Natural Compounds Engineering, 300006-Timişoara, P-ţa Victoriei 2, Romania*

Abstract
This paper presents the obtaining, characterization and antioxidant activity evaluation of quercetin/2-hydroxypropyl-β-cyclodextrin and rutin/2-hydroxypropyl-β-cyclodextrin complexes. For synthesis the crystallization from ethanol-water solution method was used, but the yields were relatively low (up to 23%). The morphology and dimensional characterization was realized by using scanning electron microscopy. The formation of complexes was revealed by differential scanning calorimetry and the antioxidant activity of nanoparticles comparatively with the nonencapsulated quercetin and rutin clearly indicate the controlled release capacity of flavonoids from complexes.

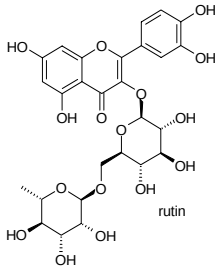
Keywords: quercetin, rutin, propolis, 2-hydroxypropyl-β-cyclodextrin, nanoparticles, antioxidant activity, DPPH

1. Introduction

Quercetin and rutin (see the structures below) are two of the most important flavonoidic compounds with antioxidant capacity from propolis, a brownish, waxy product collected from the buds of certain trees by bees and used by them to cement or caulk their hives [1-5]. Propolis has been used for centuries and it was always mentioned as an immunomodulatory, antitumor, antimicrobial, anti-inflammatory, antioxidant agent, among others. It contains hundreds of compounds, the main being resins (benzoic and cinnamic acid derivatives and flavonoids), waxes and fatty acids, essential oils, pollen, and minerals [3,5]. Flavonoids are principally responsible for the antibacterial activity of propolis, but also for the anticancerigene and immunomodulating activity. Among the flavonoids used in this study, other flavonoids which can be found in propolis are: chrysin, apigenin, acacetin, kaempferol, pinocembrine etc [2,3,5].



quercetin



rutin

Quercetin and rutin can also be found in fruits and vegetables, as well as olive oil, red wine, and tea; they possess many biological effects including antioxidation by scavenging free radicals, anticancer, antiviral, prevention of atherosclerosis, and chronic inflammation activities [3]. In order to protect them against environmental

* Corresponding author: *e-mail address*: daniel.hadaruga@chim.upt.ro

oxidation and to obtain formulations with controlled release properties molecular encapsulation can be used. Some of the better matrices for nanoencapsulation are cyclodextrins, which are naturally occurring cyclooligosaccharides containing 6-8 glucopyranose moieties (α-, β-, and γ-cyclodextrin), and having a hydrophobic inner cavity with a capacity to enclose (partial or total) small organic molecules [7-9]. The presence of primary and secondary hydroxyl groups to the exterior increase the water solubility of these compounds and the corresponding host-guest complexes. The water solubility of cyclodextrins can be increased by semi-synthetic modifications, as in the case of 2-hydroxypropyl-β-cyclodextrin, which has 50-fold higher water solubility and lower toxicity [10]. Some studies on the complexation and properties of quercetin and rutin in cyclodextrins were done [11-16].

In this study we continue the research on the enhancing the bioavailability of propolis extracts and biocompounds by nanoencapsulation [17-18]. A comparative study between nonencapsulated quercetin and rutin and the corresponding 2-hydroxypropyl-β-cyclodextrin complexes on the overall antioxidant activity was performed.

2. Materials and Method

Materials. Quercetin (99%) and rutin, >90% (as important biocompounds from propolis with antioxidative capacity) used for cyclodextrin nanoencapsulation were purchased from Fluka Chemie AG; 2-hydroxypropyl-β-cyclodextrin (2HPbCD) used as host molecule was obtained from CycloLab R&D Ltd (>99%, DS ~4.6). Ethanol 96% (v/v) used as solvent in the complexation process and also for antioxidant activity evaluation was purchased from Chimopar, Bucureşti, and DPPH (2,2-diphenyl-1-pycrylhydrazyl) used for antioxidant activity determination was purchased from Sigma (analytical grade, >99%).

Obtaining the quercetin and rutin/2HPbCD nanoparticles. Approximately 0.79 g (0.5 mmoles) 2HPbCD and 0.5 ml water were introduced in a minireactor equipped with a magnetic stirrer, thermostatable mantle, reflux condenser and dropping funnel. The cyclodextrin solution was heated to 50ºC and after that 0.5 mmoles quercetin (0.151 g) or rutin (0.332 g) ethanolic solution (2 ml) was slowly added to the cyclodextrin solution under continuous stirring in about 15 minutes. The complexation was perfected for another 15 minutes and after slowly cooling at room temperature (in about 4 hours), the complex solution/suspension was put in a refrigerator over night. The obtained crystals were filtered, washed with little ethanol and dried at room temperature. The complexation yield was calculated as the ratio of the dried complex mass and the sum of the flavonoid and cyclodextrin mass (as percent). The quercetin or rutin/2HPbCD crystals were subjected to the scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and spectrophotometric analyses. The last analysis was used in order to evaluate the antioxidant activity of the complexes in comparation with the case of non-encapsulated flavonoids, by using the DPPH method.

Scanning electron microscopy (SEM). In order to evaluate the morphological and dimensional characteristics of the quercetin or rutin/2HPbCD particles scanning electron microscopy was used. An INSPECT S SEM apparatus, with a voltage of 25 kV, magnitude level between 3000 and 12000×, and focussing of 10-14.1 mm was used.

Differential scanning calorimetry (DSC). DSC analysis of the quercetin or rutin/2HPbCD complexes was realized by using a Netzsch 204 DSC apparatus. Approximately 7 mg complex was used for analysis in Al_2O_3 dishes. The following DSC conditions were used: temperature program 20-400ºC, with a heating rate of 4ºC/min, liquid nitrogen was used for cooling, the acquisition and handling of the data were realized with the Netzsch

Proteus-Thermal Analysis ver. 4.0/2000 program.

Antioxidant activity evaluation. The antioxidant activity of commercial quercetin or rutin, and quercetin, rutin/2HPbCD complexes was spectrophotometrically evaluated, by using the DPPH method. A CamSpec M501 spectrophotometer was used and the acquisition and data handling were realized with the UV-Vis Analyst ver. 4.67, Camspec Ltd. soft. For spectrophotometric time scan 0.5 ml quercetin or rutin ethanolic solution (1%) or 0.5 ml complex (10%), 0.5 ml DPPH 1mM ethanolic solution and 2 ml ethanol were added to the cuvette and the recording was realized at 517 nm for 1800s. The antioxidant activity was determined even for the undiluted samples or for diluted ones (using a progressive 1:10 dilution factor). The antioxidant activity was calculated as residual percentage absorbance (*A%*), as a percentage ratio of the momentan and initial absorbance of the mixture. The mean DPPH reaction rate could be also calculated by using the momentan absorbance and the DPPH calibration curve (obtained for ethanolic solutions at 517 nm: *Absorbance (DPPH) = 0.025 + 0.011·Concentration (DPPH, mM)*.

3. Results and Discussion

The complexation yields in the case of some biocompounds (which can occur in propolis) were relatively lower, probably due to the high solubility of 2HPbCD and also the corresponding complex in ethanol-water system. Thus, the quercetin/2HPbCD complex was obtained with a yield of 12% and rutin/2HPbCD complex with a yield of 23%. Probably, higher yields of encapsulation in the case of cyclodextrins with higher water solubility can be obtained by using other methods which cannot allow the loose of cyclodextrin and/or corresponding complex by solubilization (like kneading or spray-drying methods).

The SEM analysis indicates a significative difference between the morphology of the quercetin/2HPbCD and rutin/2HPbCD crystals. Thus, in the case of quercetin/2HPbCD complex, the crystals have acicular shapes, with higher uniformity and dimensions from micrometers (in length) to hundreds on nanometers (in width), while in the case of rutin/2HPbCD complex the crystals have irregular prismatic shapes, with higher dimensions (Figures 1 and 2).

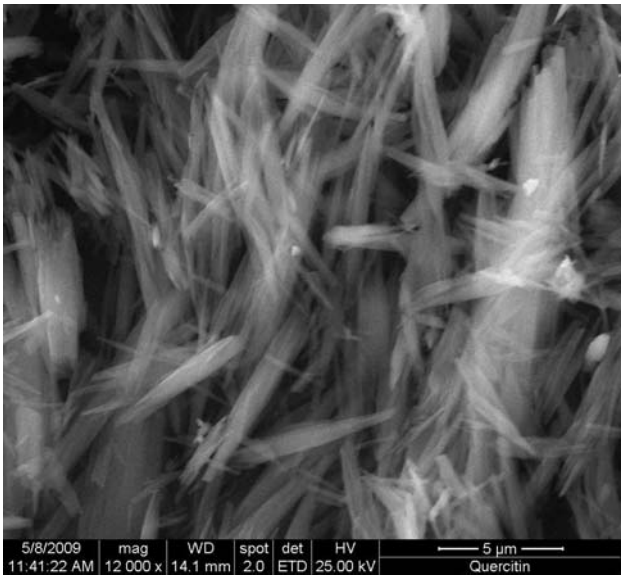


Figure 1. SEM micrograph for the quercetin/2HPbCD complex (12000× magnitude)

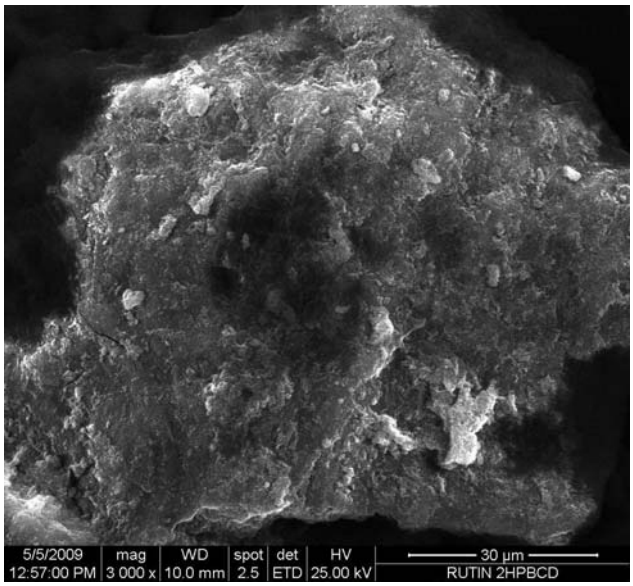


Figure 2. SEM micrograph for the rutin/2HPbCD complex (3000× magnitude)

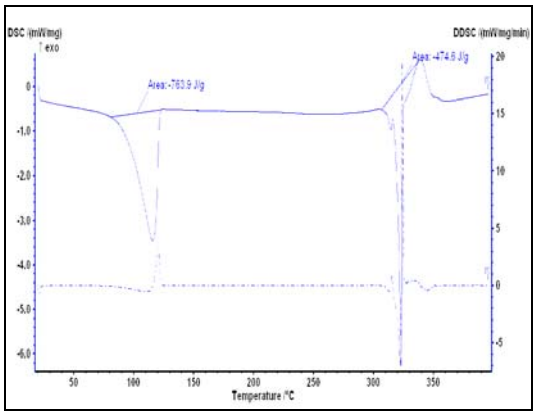


Figure 3. DSC analysis for quercetin

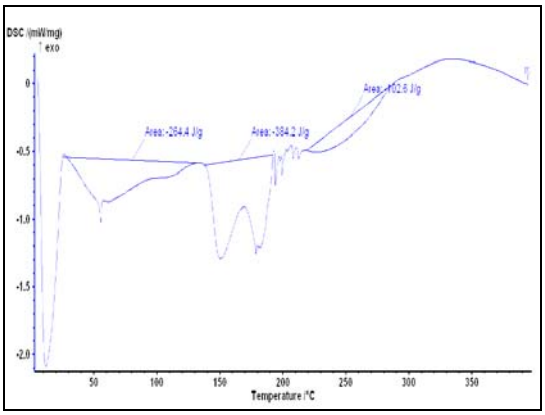


Figure 4. DSC analysis for rutin

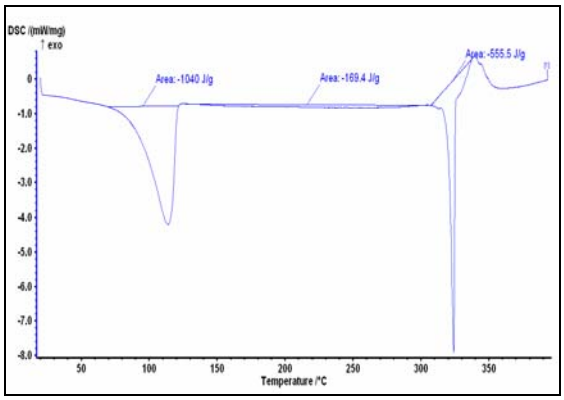


Figure 5. DSC analysis for quercetin/2HPbCD complex

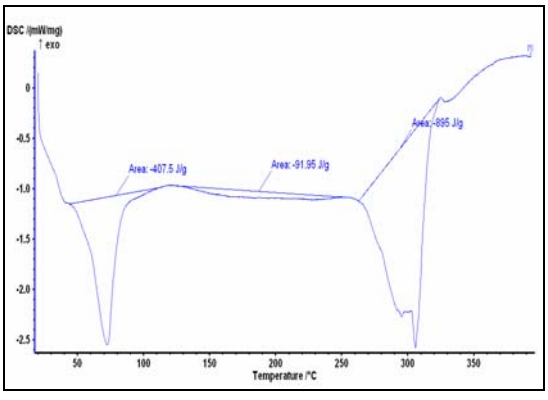


Figure 6. DSC analysis for rutin/2HPbCD complex

The DSC analysis of the non-encapsulated and encapsulated quercetin and rutin revealed that the complexation process appear due to the endothermic peak for the range interval of 120-300ºC, which correspond to the complex dissociation (especially for the rutin/2HPbCD complex). For the quercetin/2HPbCD complex the same endothermal peak appear in the range of dissociation, but smaller.

Thus, the water dissociation in the case of 2HPbCD and also in the case of corresponding complex appear at ~100ºC. Quercetin starts to dehydrate at ~90ºC and at ~320ºC decompose. Rutin loose the water molecules up to 120ºC and at ~190ºC become plastic; at the temperature of 220ºC the decomposition of rutin appear. This modification is not relevant in the case of complex due to the protecting capacity of 2HPbCD (Figures 3-6).

The complex formation is revealed also by the spectrophotometric analysis for non-encapsulated and encapsulated quercetin and rutin in 2HPbCD. A decrease of the absorbance of the nonencapsulated or encapsulated quercetin and rutin-DPPH mixture at 517 nm was observed in all cases, even for 100th fold dilution, but in the case of nonencapsulated flavonoids no decrease of the absorbance above 500s can be observed, while for the 2HPbCD complexes this decrease appear even after 30 minutes; this means that the quercetin and rutin are slowly released from the complex and the antioxidant activity exists even at 30 minutes (Figures 7 and 8).

The highest antioxidant activity was observed for nonencapsulated quercetin (residual percent absorbance of 10%, Figure 9) and also for rutin (20%, Figure 10). For the corresponding complexes the overall antioxidant activity was lower, but it exists even after 1800s, in comparation with the nonencapsulated flavonoids, which have no activity after 500s. Thus, the residual percent absorbance was 87.5% for quercetin/2HPbCD complex and 60% for rutin/2HPbCD complex (Figures 11 and 12).

The profile of the DPPH reaction can be revealed by using the mean DPPH reaction rate for different reaction time range. In order to evaluate this rate the DPPH calibration curve was used.

The DPPH reaction rate for a specific time interval was calculated as the negative ratio of the DPPH concentration variation (mM, obtained from the DPPH calibration curve by using the corresponding spectrophotometric absorbance at 517 nm) and the reaction time for this interval.

For the nonencapsulated quercetin and rutin this rate was similar for the initial time interval (0-50s), where the dependence was approximately linear; the rate values were 0.0011 mM/s for quercetin and 0.0009 mM/s for the rutin (Figure 13). Generally, the DPPH reaction rate decrease drastically after 500s for nonencapsulated flavonoids, being negligible.

For the corresponding complexes the DPPH reaction rate was lower, but this is non-null even after a reaction time of 1800s (Figure 14). For these complexes three reaction time ranges can be established: *i1*, 0-50s (less significative), *i2*, 50-200s, and *i3*, 200-1800s. In all cases the reaction rate for *i1* interval is about 50-500 times higher than for *i3* interval.

Thus, the mean reaction rate for *i1* interval, *v(i1)*, decrease from $8.2 \cdot 10^{-5}$ mM/s to $2.6 \cdot 10^{-5}$ mM/s for *i2*, and to $1.7 \cdot 10^{-6}$ mM/s for *i3* in the case of quercetin/2HPbCD complex and from $8 \cdot 10^{-4}$ mM/s for *i1* to $1.7 \cdot 10^{-6}$ mM/s for *i3* in the case of rutin/2HPbCD complex. The controlled release properties of the flavonoid/2HPbCD complexes is clearly revealed by these DPPH reaction rates.

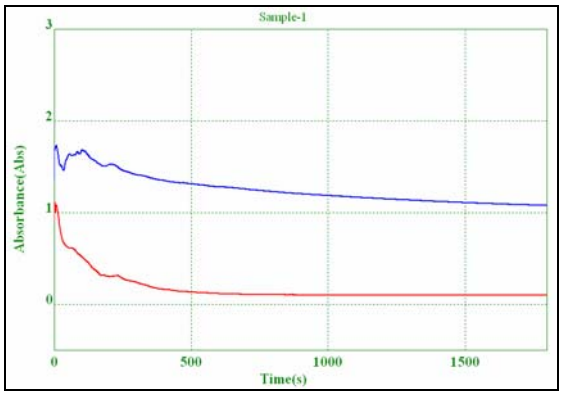


Figure 7. Superposition of the *Absorbance vs. Time* curves for quercetin (standard solution, 1:10 dilution – red, and 1:100 dilution – blue)

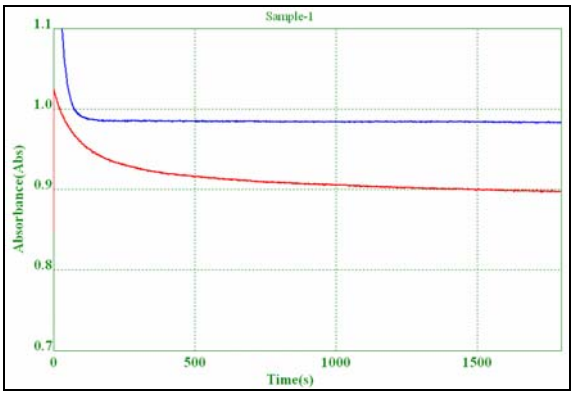


Figure 8. Superposition of the *Absorbance vs. Time* curves for quercetin/2HPbCD complex (standard solution, 1:10 dilution – red, and 1:100 dilution – blue)

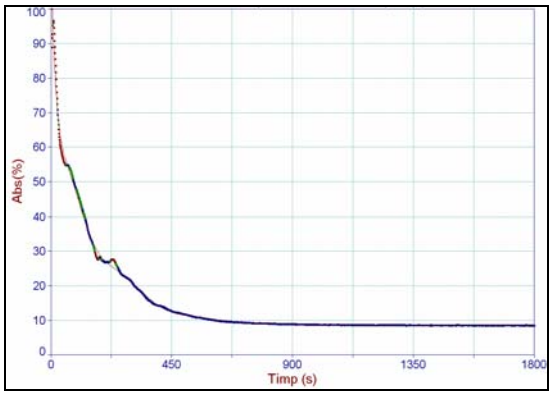


Figure 9. Antioxidant activity for quercetin (0.5 mg/ml)

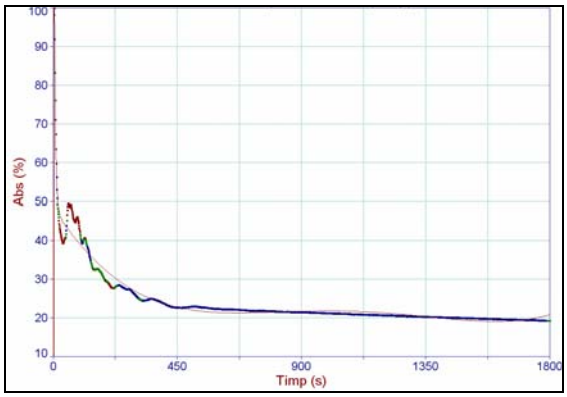


Figure 10. Antioxidant activity for rutin (0.5 mg/ml)

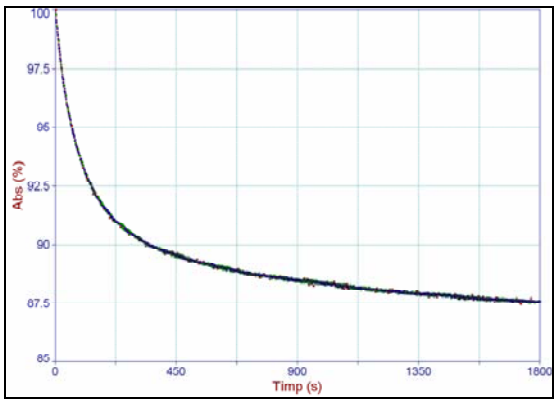


Figure 11. Antioxidant activity for quercetin/2HPbCD complex (0.5 mg/ml)

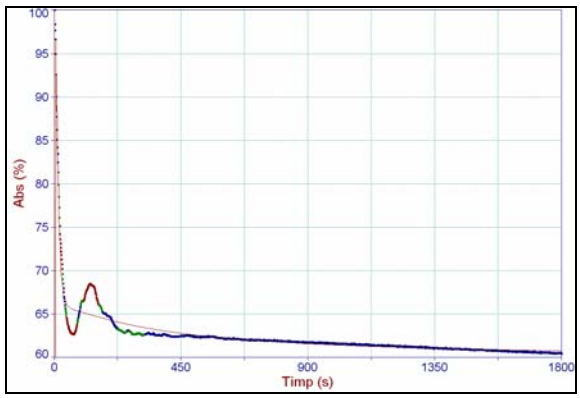


Figura 12. Antioxidant activity for rutin/2HPbCD complex (0.5 mg/ml)

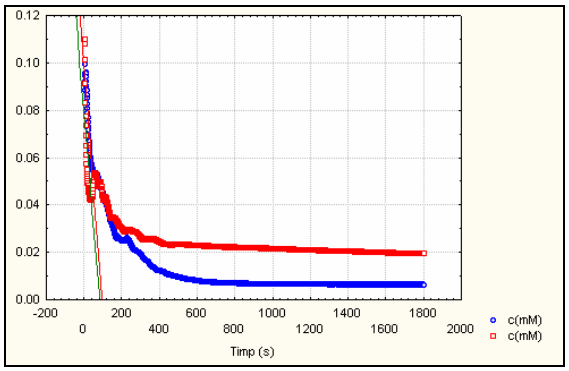


Figure 13. *Concentration (mM) vs. Time (s)* dependence for nonencapsulated quercetin (blue) and rutin (red)

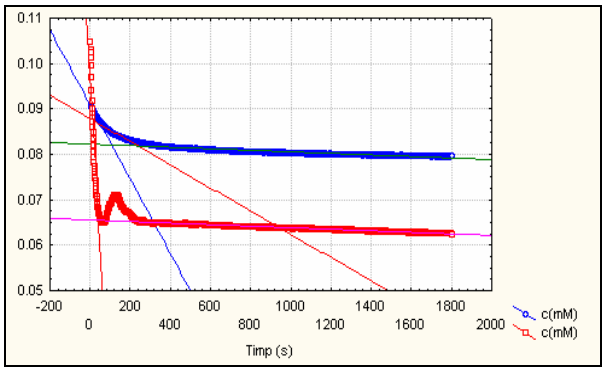


Figure 14. *Concentration (mM) vs. Time (s)* dependence for quercetin/2HPbCD (blue) and rutin/2HPbCD (red) complexes

4. Conclusion

The following conclusions can be drawn among the complexation of quercetin and rutin in 2-hydroxypropyl-β-cyclodextrin and antioxidant activity evaluation: (1) the complexation of flavonoids like quercetin and rutin (which occur in propolis and confer the antioxidant property) with the cyclic semi-synthetic oligosaccharides, like 2-hydroxypropyl-β-cyclodextrin, a compound with a high water solubility, is realized with lower yields if the crystalization from ethanol-water solution method is used; therefore, another method like kneading or spray-drying ones are proper; (2) the presence of the quercetin, rutin/2-hydroxypropyl-β-cyclodextrin interaction is revealed by the differential scanning calorimetry (DSC) analysis and also by the spectrophotometry of the complex solution in the presence of a free radical (like 2,2-diphenyl-1-pycrylhydrazyl, DPPH); (3) the antioxidant activity of quercetin and rutin is important even for nonencapsulated forms or for cyclodextrin encapsulated ones, but the antioxidant activity exists for a long time only for flavonoid/cyclodextrin nanoparticles (revealed by the DPPH/flavonoid reaction rate); this observation demonstrate the controlled release properties of the 2-hydroxypropyl-β-cyclodextrin in the case of the cyclodextrin complexes of some main flavonoids from propolis.

Acknowledgements

The authors want to thanks to Dr. Ioan Grozescu (INCDEMC Timişoara, Romania) for the permission to evaluate the particle dimensions by scanning electron microscopy and to Dr. Geza Bandur ("Politehnica" University of Timişoara, Romania) for the help in differential scanning calorimetry analysis

References

1. Nafady, A.M.; El-Shanawany, M.A.; Mohamed, M.H.; Hassanean, H.A.-H.; Nohara, T.; Yoshimitsu, H.; Ono, M.; Sugimoto, H.; Doi, S.; Sasaki, K.; Kurod, H., Cyclodextrin-Enclosed Substances of Brazilian Propolis, *Chemical Pharmaceutical Bulletin* **2003**, *51*(8), 984-985
2. Nagai, T.; Inoue, R.; Inoue, H.; Suzuki, N., Preparation and Antioxidant Properties of Water Extract of Propolis, *Food Chemistry* **2003**, *80*, 29-33
3. Sforcin, J.M., Propolis and the immune system: a review, *Journal of Ethnopharmacology* **2007**, *113*, 1–14
4. Weston, R.J., The contribution of catalase and other natural products to the antibacterial activity of honey: a review, *Food Chemistry* **2000**, *71*, 235-239
5. Burdock, G.A., Review of the biological properties and toxicity of bee propolis (propolis), *Food and Chemical Toxicology* **1998**, *36*, 347-363
6. Jullian, C.; Moyano, l.; Yanez, C.; Olea-Azar, C., Complexation of quercetin with three kinds of cyclodextrins: An antioxidant study, *Spectrochimica Acta Part A* **2007**, *67*, 230-234

7. Brewster, M.E.; Loftsson, T., Cyclodextrins as pharmaceutical solubilizers, *Advanced Drug Delivery Reviews* **2007**, *59*, 645–666
8. Szejtli, J.; Szente, L., Elimination of bitter, disgusting tastes of drugs and foods by cyclodextrins, *European Journal of Pharmaceutics and Biopharmaceutics* **2005**, *61*, 115–125
9. Stella , V.J.; Rao, V.M.; Zannou, E.A.; Zia, V., Mechanisms of drug release from cyclodextrin complexes, *Advanced Drug Delivery Reviews* **1999**, *36*, 3–16
10. Wang, Z.; Zhang, X.; Deng, Y.; Wang, T., Complexation of hydrophobic drugs with hydroxypropyl-β-cyclodextrin by lyophilization using a tertiary butyl alcohol system, *Journal of Inclusion Phenomena and Macrocyclic Chemistry* **2007**, *57*, 349-354
11. Yan, C.; Li, X.; Xiu, Z.; Hao, C., A quantum-mechanical study on the complexation of beta-cyclodextrin with quercetin, *Journal of Molecular Structure: THEOCHEM* **2006**, *764*, 95-100
12. Calabro, M.L.; Tommasini, S.; Donato, P.; Raneri, D.; Stancanelli, R.; Ficarra, P.; Ficarra, R.; Costa, C.; Catania, S.; Rustichelli, C.; Gamberini, G., Effects of alpha- and beta-cyclodextrin complexation on the physico-chemical properties and antioxidant activity of some 3-hydroxyflavones, *Journal of Pharmaceutical and Biomedical Analysis* **2004**, *35*, 365-377
13. Pralhad, T.; Rajendrakumar, K., Study of freeze-dried quercetin–cyclodextrin binary systems by DSC, FT-IR, X-ray diffraction and SEM analysis, *Journal of Pharmaceutical and Biomedical Analysis* **2004**, *34*, 333-339
14. Calabro, M.L.; Tommasini, S.; Donato, P.; Stancanelli, R.; Raneri, D.; Catania, S.; Costa, C.; Villari, V.; Ficarra, P.; Ficarra, R., The rutin/beta-cyclodextrin interactions in fully aqueous solution: spectroscopic studies and biological assays, *Journal of Pharmaceutical and Biomedical Analysis* **2005**, *36*, 1019-1027
15. Haiyun, D.; Jianbin, C.; Guomei, Z.; Shaomin, S.; Jinhao, P., Preparation and spectral investigation on inclusion complex of beta-cyclodextrin with rutin, *Spectrochimica Acta Part A* **2003**, *59*, 3421-3429
16. Lederer, M.; Leipzig-Pagani, E., A simple alternative determination of the formation constant for the inclusion complex between rutin and beta-cyclodextrin, *Analytica Chimica Acta* **1996**, *329*, 311-314
17. Coneac, G.; Gafiţanu, E.; Hădărugă, D.I.; Hădărugă, N.G.; Riviş, A.; Bandur, G.N.; Pînzaru, I.A..; Rusu, G.; Urşica, L.; Păunescu, V.; Gruia, A.; Sebeşan, M.; Grozescu, I.; Lazău, C.; Sfîrloagă, P., Propolis Extract/β-Cyclodextrin Nanoparticles: Synthesis, Physico-Chemical, and Multivariate Analyses, *Journal of Agroalimentary Processes and Technologies* **2008**, *14*(1), 58-70
18. Coneac, G.; Gafiţanu, E.; Hădărugă, D.I.; Hădărugă, N.G.; Pînzaru, I.A.; Bandur, G.; Urşica, L.; Păunescu, V.; Gruia, A., Flavonoid Contents of Propolis from the West Side of Romania and Correlation with the Antioxidant Activity, *Chem. Bull. "Politehnica" Univ. (Timişoara)* **2008**, *53*(1-2), 56-60