Scientific paper

Property Studies of Coenzyme Q_{10}–Cyclodextrins complexes

Maja Milivojević Fir, Luka Milivojević, Mirko Prošek and Andrej Šmidovnik*

National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

** Corresponding author: E-mail: andrej.smidovnik@ki.si; Phon ++386 1 47 60265, Fax: ++386 1 47 60300*

Received: 11-02-2009

Abstract

Complexes of coenzyme Q_{10} with β- and γ-cyclodextrin were obtained by using co-precipitation method. Phase solubility profiles with both cyclodextrins employed were classified as A_L type, indicating the formation of 1:1 stoichiometric complexes. Water-solubility, thermo- and photo-stability, and antioxidant activity of coenzyme Q_{10} were significantly increased by complexation with cyclodextrins. Water-solubility of complexes was examined under various conditions (temperature and pH), stability studies in the solid state were performed under stress conditions (T = 80 °C, λ = 254 nm), and coenzyme Q_{10} concentration was determined by HPLC/MS and HPLC/UV, respectively. The DPPH radical-scavenging method was used for measuring antioxidant activity.

Keywords: Coenzyme Q_{10}, cyclodextrin, complex, photostability, thermostability, antioxidant activity.

1. Introduction

Coenzyme Q_{10} (CoQ_{10}) – also known as ubiquinone 50 – belongs to a family of compounds known as quinones. CoQ_{10} is an essential component in the production of cellular energy in the form of adenosine triphosphate, and acts as an antioxidant that blocks oxidative injuries to DNA, lipids, proteins and other essential molecules.[1,2] Positive effects of CoQ_{10} use need to be investigated further in future and determined with some clinical trials, however, CoQ_{10} has been proposed for the prevention and treatment of cardiovascular diseases, neurodegenerative diseases, such as Parkinson's and Huntington's disease, angina pectoris, hypertension, diabetes and cancer.[3–5]

Although CoQ_{10} is classified as lipophilic compound, its degree of solubility in lipids is extremely limited, while it is practically insoluble in aqueous solutions. Due to its high molecular weight and poor aqueous solubility, it is poorly and slowly absorbed from gastrointestinal tract.[6] The importance of CoQ_{10} formulation was recognized during the development of different CoQ_{10} preparations. For these reasons, CoQ_{10} provokes chemists to develop a formulation for oral administration with better water-solubility and therefore better bioavailability.[7–13]

Since CoQ_{10} is only soluble in lipids and fats, it is practically impossible to add it to water-based formulations. It is precisely because of this reason that we wanted to prepare a water-soluble form of CoQ_{10}, which would enable the production of water-based preparations in food, pharmaceutical and cosmetic industry.

Cyclodextrins are well known inclusion-complexing agents for small and large molecules.[14] The most common cyclodextrins (CD) are α-CD, β-CD and γ-CD, ring molecules consisting of six, seven and eight glucopyranose units, respectively. Having a hydrophilic outer surface and hydrophobic inner cavity give them a unique ability to form inclusion complexes with lipophilic compounds and increase their water-solubility, stability and/or bioavailability.[15–17]

The aim of this work was to obtain the complexes of CoQ_{10} with β-CD and γ-CD by co-precipitation method in aqueous solution in order to improve CoQ_{10} water-solubility, stability and antioxidant activity. Simultaneously, the stoichiometry and the stability constant were determined. For these purpose, HPLC technique combined with different solvent washing procedures, and radical scavenging method for antioxidant activity were used. Physicochemical characteristics of the resulting complexes, such as solubility in relation to temperature and pH, and the influence of temperature and ultra-violet (UV) light on its stability were also examined.

2. Materials and Methods

2. 1. Materials

CoQ_{10} (863 g/mol). – Bulk Medicines & Pharmaceuticals GmbH (Hamburg, Germany).

β-cyclodextrin (1135 g/mol), γ-cyclodextrin (1297 g/mol) and potasium bromide.–Sigma-Aldrich (Steinheim, Germany).

2,2-diphenyl-1-picrylhydrazyl radical. –Sigma-Aldrich (St. Louis, MO, USA).

Methanol, ethanol, 1,4-dioxane, hexane, diethyl ether, acetic acid, and hydrochloride acid.–HPLC grade from Merck (Darmstadt, Germany).

2. 2. Phase Solubility Studies

Increasing amounts of β-CD or γ-CD solutions (3 mL, 2–14 mM) were added to seven 10 mL vessels containing 32 mg (37 mmol) of CoQ_{10} and magnetic stirrer. Vessels were closed airtightly and the suspensions were vigorously stirred at room temperature for 48 hours. The suspensions were filtered (Milipore Millex-HV PVDF membrane filter, 0.45 μm) and the concentration of CoQ_{10} was determined by HPLC/MS.

The stability constants of complexes, K_s, were calculated from the linear section of the solubility diagrams as:

$$K_s = \frac{k}{n(1-k)} \quad (1)$$

where k is the slope and n the intercept of the linear function.[18]

HPLC/MS determination of CoQ_{10} was performed on a Surveyor LC system (Thermo Finnigan, San Jose, CA, USA). HPLC analyses were performed on a Gemini C18 reversed phase column, 150 mm · 4.6 mm, 5 μ (Phenomenex, Torrance, CA, USA). Mobile phase consisted of ethanol, 1,4-dioxan and acetic acid (92 : 8 : 0.1, v/v/v/), respectively. A flow rate of 1.0 mL/min gave adequate resolution, and separation was performed at ambient temperature. The retention time of CoQ_{10} was 4.3 ± 0.1 min. The injection volume was 10 μL and the LC eluent was directed into the Finnigan LCQ mass spectrometer (Finnigan MAT, San Jose, CA, USA). MS identification and quantification were done in positive APCI ionization mode. Ionization discharge voltage was 6.0 kV, discharge current 5.0 μA, and source temperature 450 °C. Capillary voltage was 3.0 V, tube lens offset was –5.0 V, capillary temperature was 250 °C, sheath gas pressure was 0.2 MPa, and auxiliary gas flow was 1.7 L/min. The chromatograms were obtained in SIM mode. Molecular mass $(M + H)^+$ m/z 863.4 ± 1 was used for quantitative determination of CoQ_{10}. Data processing was done with Xcalibur 1.3 software (Thermo Finnigan Corporation, San Jose, CA, USA).

2. 3. Sample Preparation

Physical mixtures: Physical mixtures of CoQ_{10} with β-CD (fmβ-CDQ_{10}) or γ-CD (fmγ-CDQ_{10}) in 1:1 molar ratio were prepared by 1 hour gentle dry mixing of exactly weighed amounts of CDs (1.317 g and 1.50 g, respectively) with CoQ_{10} (1.00 g) using a mortar and pestle.

Complexes: β-CD (1.500 g, 1.32 mmol) or γ-CD (7.50 g, 5.78 mmol) was dissolved in 15 mL of distilled water at 80 °C. CoQ_{10} (1.122 g, 1.30 mmol and 4.983 g, 5.77 mmol, respectively) was added to dissolved CD. Reaction mixture was stirred at 80 °C until the formation of yellow homogeneous paste. The paste was washed with n-hexane at 4 °C to remove CoQ_{10} adsorbed on the outside surface of the CD. The redundant CD was washed with distilled water at 4 °C. The precipitate was 70% water solution of complex of CoQ_{10} with β-CD (β-CDQ_{10}) or γ-CD (γ-CDQ_{10}), and it was used for solubility and stability tests, while the lyophylizated precipitate of complex was characterized by infrared spectroscopy, thermal property analysis and X-ray diffraction.

2. 4. Effects of Solvents on Complexes and Physical Mixtures

Samples of CoQ_{10} complexes with β-CD or γ-CD and corresponding physical mixtures were suspended in appropriate solvents (methanol, ethanol, hexane, 1,4-dioxane and diethyl ether). 58 mg of 70% precipitate of β-CDQ_{10}, 63 mg of 70% precipitate of γ-CDQ_{10}, 40 mg of fmβ-CDQ_{10} or 43 mg fmγ-CDQ_{10} were suspended in 3 mL of solvent, vortexed for 10 seconds and centrifuged for 10 min at 4 °C. Supernatant was filtered through hydrophylic membrane filter, 0.45 μm. These CoQ_{10} removing steps were repeated four times. CoQ_{10} content in solvents was measured by HPLC/UV.

Chromatography conditions: HPLC analyses of CoQ_{10} samples and standard solutions of CoQ_{10} (5, 10, 25, 50 in 100 mg/L) were performed on a LUNA C18(2) reversed phase column, 100 mm · 4.6 mm, 3 μm. Mobile phase constituted of ethanol and 1,4-dioxane (93 : 7, v/v), respectively. A flow rate of 1.0 mL/min gave an adequate resolution, and separation was performed at ambient temperature. A UV detector was operated at 280 nm. The retention time of CoQ_{10} was 3.9 ± 0.1 min.

2. 5. Solubility Studies

Temperature dependence: Into 20 mL of water (pH = 6.5, adjusted with 0.0010 M HCl) 15 mg of fmβ-CDQ_{10}, 25 mg of fmγ-CDQ_{10}, 20 mg of 70% water suspension of β-CDQ_{10} or 35 mg of 70% water suspension of γ-CDQ_{10} was weighted. Suspensions were stirred for 120 minutes, divided to 2 mL per sample, and thermostated for 5 minutes at 25, 30, 40, 50, 60, 70, 80, 90 and 95 °C. The suspensions were filtered through membrane filter (Milipore

Millex-HV PVDF, 0.45 μm) and the concentration of CoQ_{10} in the filtrates was determined by HPLC/MS, as described earlier.

pH dependence: 20 mL of water with different pH (adjusted with 0.010 M HCl) was placed in 50 mL reaction vessel and thermostated to 37 °C, then 15 mg of physical mixture or 20 mg of 70% water suspension of complex was added. The samples were stirred using magnetic stirrer at 37 °C for 30 min. The suspensions were filtered through membrane filter (Milipore Millex-HV PVDF, 0.45 μm) and the concentration of CoQ_{10} in the filtrates was determined by HPLC/MS, as described earlier.

2. 6. Stability Studies

For the photo- and thermo-stability studies, 180 mg of CoQ_{10} was dissolved in 6 mL of n-hexane, 500 mg of 70% water suspension of β-CDQ_{10} in 5 mL of water and 535 mg of 70% water suspension of γ-CDQ_{10} in 5 mL of water. 300 μL of sample was dispersed over a quartz-glass plate (25 mm · 30 mm) and evaporated at room temperature.

Thermo-stability of the samples was determined in the dark at 25 and 80 °C.

Photo-stability of the samples was determined under UV irradiation (254 nm), using a UV lamp, at 25 and 80 °C. The distance between the sample and light source was 15 cm. Samples were withdrawn at fixed time intervals and assayed for CoQ_{10} and its major photolytic decomposition products using the European Pharmacopoeia 4.[19]

2. 7. Antioxidant Activity

CDQ_{10}: 100 μL of complex solution (0.8 mmol/L, Section 2.7.) was diluted with 900 μL of distilled water (0.08 mmol/L). The sample was divided into two parallels of 500 μL, and 1.5 mL of 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) in ethanol (0.01 mg/mL) was added.

CoQ_{10}: solution CoQ_{10} in ethanol was prepared in molar concentration 0.08 mmol/L. 500 μL of solution was diluted with 1.5 mL of $DPPH^{\cdot}$ in solvent mixture ethanol : water (2 : 1, v/v, 0.01 mg/mL).

Blind sample: 500 μL of distilled water was diluted with 1.5 mL of $DPPH^{\cdot}$ in ethanol (0.01 mg/mL).

CDQ_{10} and CoQ_{10} reacted with $DPPH^{\cdot}$ for 60 minutes at room temperature, and the absorbance changes were measured at 514 nm.

Antioxidant activity was calculated from the equation (2) and expressed in percentages:

$$AU = 1 - \left(\frac{A_v}{A_s} \right) \quad (2)$$

AU is the antioxidant activity, A_v represents the absorbance of the sample, and A_s represents the absorbance of the blind sample.

3. Results and Discussion

3. 1. Phase Solubility Studies

Solubility test was designed to determine the apparent total molar solubility of the CoQ_{10} as a function of total molar concentration of β-CD and γ-CD. The solubility of pure CoQ_{10} at pH 6.5 was $9.3 \cdot 10^{-8}$ mol L^{-1}, and increased in relation to CD concentration in water. After adding of CoQ_{10} to the aqueous solution of β-CD or γ-CD, the CoQ_{10} solubility was 75- and 160-times higher ($6.83 \cdot 10^{-6}$ and $1.46 \cdot 10^{-5}$ mol/L), respectively.

The slopes in the phase-solubility diagrams were linear ($R^2 \geq 0.98$) as an A_L diagram type according to Higuchi and Connors, what confirmed the formation of soluble complexes with CoQ_{10}-CD stoichiometric ratio of 1:1.[18] The differences in slope showed the relative affinity of ligands to the different types of CD. The apparent stability constants, calculated from the slope of solubility diagrams and intercept were 432.1 and 2207.9 dm^3 mol^{-1} for complexes of CoQ_{10} with β-CD and γ-CD, respectively.

3. 2. Effects of Solvents on Complexes and Physical Mixtures

Washing the samples of physical mixtures and complexes of CoQ_{10} with β-CD, or γ-CD with various solvents confirmed that CoQ_{10} insertion into the cavity of CD is affected by the cavity size and hydrophobicity of CD. The washing process can provide some information about the type of association in complex, as reported by several researchers.[20–22] Park and co-workers used methanol, ethanol and diethyl ether to remove both adsorbed and included linoleic acid from CD cavity.[20]

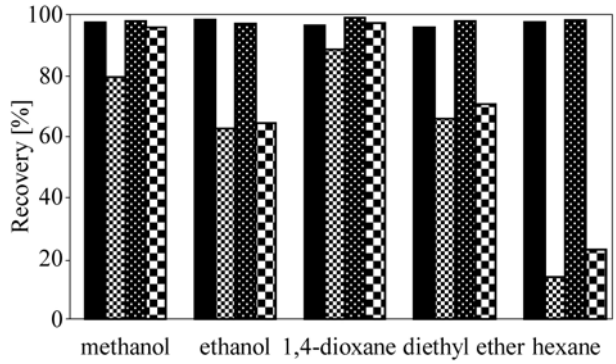

Figure 1:Effects of solvents (methanol, ethanol, 1,4-dioxane, diethyl ether, hexane) on the removal of CoQ_{10} from physical mixtures fmβ-CDQ_{10} (■) and fmγ-CDQ_{10} (▩), and corresponding complexes β-CDQ_{10} (▩) and γ-CDQ_{10} (▩).

All solvents used in washing procedure can almost completely extract CoQ_{10} from physical mixtures (Figure 1). Solvents such as methanol, 1,4-dioxane, ethanol and diethyl ether can remove both adsorbed and included CoQ_{10}. The obtained portions of extracted CoQ_{10} from

β-CDQ_{10} and γ-CDQ_{10} were more than 62% when methanol, 1,4-dioxane, ethanol or diethyl ether were used as washing solvents. As seen in Figure 2 and Figure 3, hexane removed 11.9 and 19.4% of CoQ_{10} from the β-CDQ_{10} and γ-CDQ_{10} in the first washing, respectively. No further CoQ_{10} was removed by increasing the number of washing times. Subsequently, hexane was used to remove adsorbed CoQ_{10} from CDQ_{10} complexes for the preparation of β-CDQ_{10} and γ-CDQ_{10} inclusion complexes.

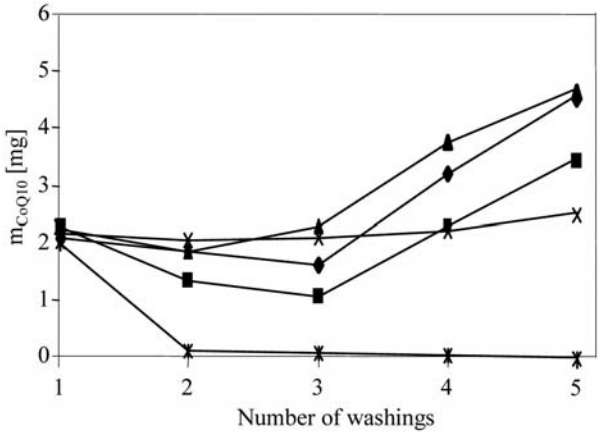

Figure 2: : Portions of extracted CoQ_{10} (17 mg) ffrom samples of β-CDQ_{10} complex (methanol ◆, ethanol ■, 1,4-dioxane ▲, diethyl ether ✕, hexane ✱)

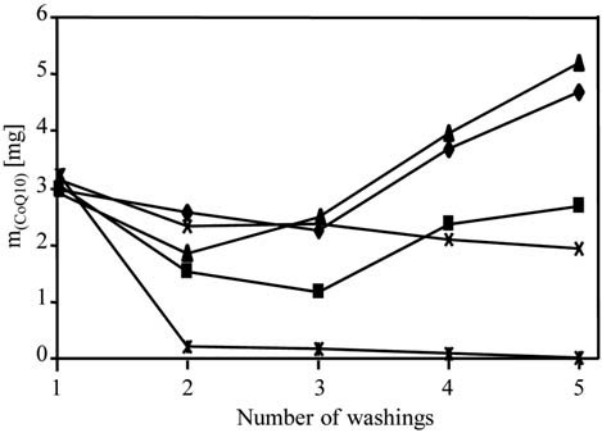

Figure 3: Portions of extracted CoQ_{10} (17 mg) from samples of β-CDQ_{10} complex (methanol ◆, ethanol ■, 1,4-dioxane ▲, diethyl ether ✕, hexane ✱).

The results of the washing processes show significant differences between physical mixtures and complexes. Negligible interactions between CoQ_{10} and CD are present in physical mixtures, while stronger associations between guest and host can be found in complexes.

3. 3. Solubility Studies

About 6.0 mg of CoQ_{10} in a form of inclusion complex or physical mixture was dissolved in 20 mL of distilled water. The aliquots of 2 mL were thermostated at 25, 30, 40, 50, 60, 70, 80, 90 and 95 °C. Filtrates were analysed for CoQ_{10} concentration using the HPLC/MS method.

The results presented in Figure 4 and Figure 5 show that the solubility of CoQ_{10} in the form of complex or physical mixture increases with temperature. The solubility of CoQ_{10} in the form of complexes is linearly dependent on temperature, while the CoQ_{10} in the form of physical mixtures shows exponential increasing of solubility versus temperature.

The increase of CoQ_{10} solubility depends on the type of CD. The solubility of CoQ_{10} at room temperature is 13.88 mg L^{-1}, and 19.26 mg L^{-1} in the form of β-CDQ_{10} and γ-CDQ_{10}, respectively. The difference in water-solubility is even higher at 95 °C. The solubility of CoQ_{10} in the form of γ-CDQ_{10} is almost two times higher (109.36 mg L^{-1}) than in the form of β-CDQ_{10} (57.03 mg L^{-1}). As temperature exceeds the boiling point of solvent, air bubbles replace CoQ_{10} in hydrophobic CD cavity, and the solubility of CoQ_{10} in all forms decreases drastically.

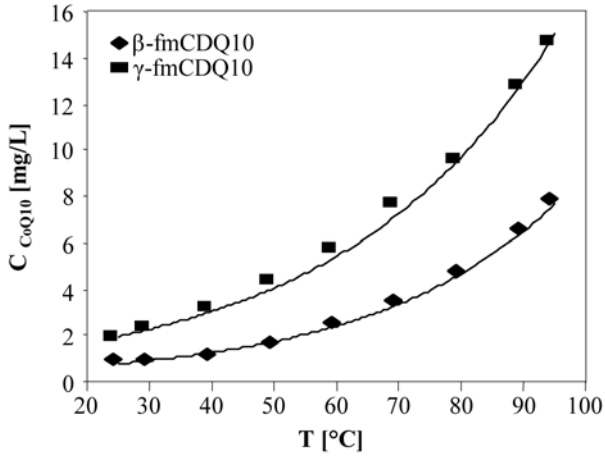

Figure 4: Effect of temperature on the CoQ_{10} solubility in the form of physical mixture.

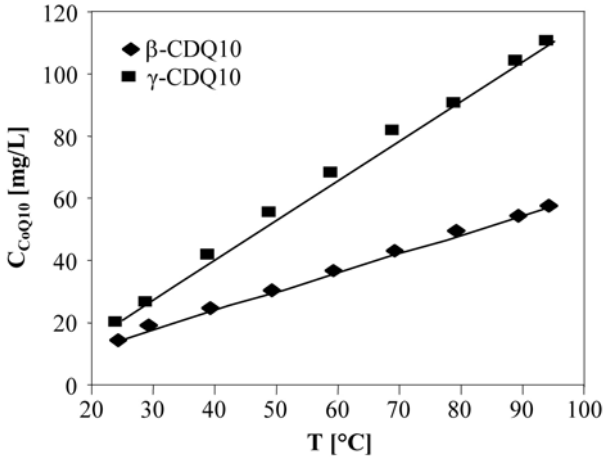

Figure 5: Effect of temperature on the CoQ_{10} solubility in the form of complex.

The solubility of CoQ_{10} in the form of physical mixture shows no significant differences. It is not certain why the solubility is about 7-fold higher at 95 °C than at 25 °C, but it might be that this is partly due to the incorporation of CoQ_{10} into the CD cavity, and adsorption of CoQ_{10} onto the outside surface of CD.

The solubility of complexes is also pH-dependent. The glygosidic bond of CDs is hydrolytically cleaved by lowering pH. In normal conditions, the ring-opening rate increases by increasing the number of glucosidic units, α-CD is more stable than β-CD, and β-CD is more stable than γ-CD, but included guest molecules in new conditions can markedly decelerate acid-catalysed ring opening.[23, 24]

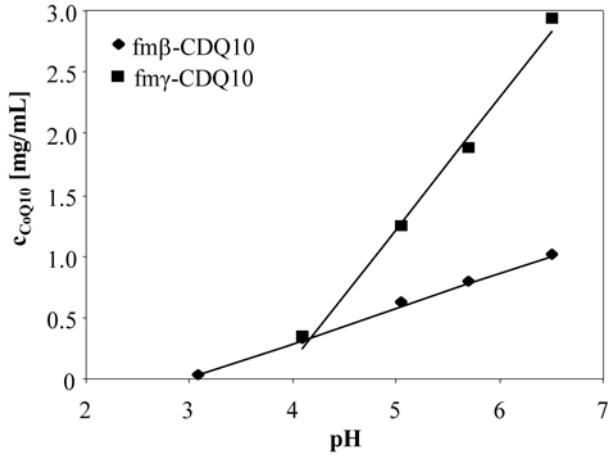

Figure 6: Effect of pH on the CoQ_{10} solubility in the form of physical mixture.

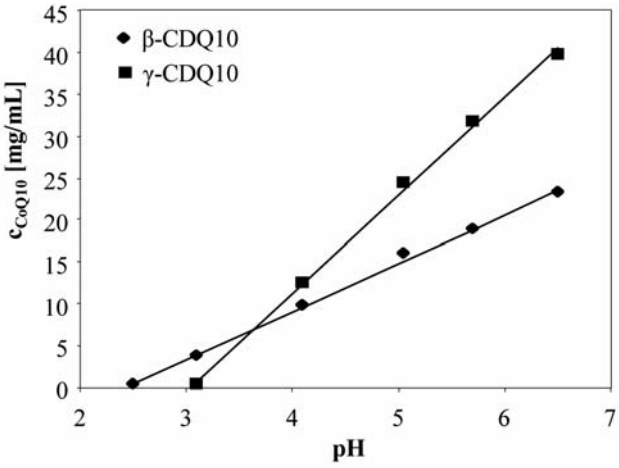

Figure 7: Effect of pH on the CoQ_{10} solubility in the form of complex.

The results presented in Figure 6 and Figure 7 show a strong correlation between the solubility and stability of complexes at lower pH. The apparent stability constant determined at room conditions and pH 6.5 is 4-fold higher for γ-CDQ_{10} than the apparent stability constant for β-CDQ_{10}. A resembling trend was also expected at a lower pH. The stability constants for complexes were not calculated at a lower pH; lower or higher stability can be predicted only by relying upon the solubility determined at various pH values. The results show relatively high solubility of CoQ_{10} in water at pH 6.5 and 37 °C in the form of β-CDQ_{10} (23.05 mg of CoQ_{10} per L), and low solubility at pH 2.5 (0.29 mg of CoQ_{10} per L). The solubility of β-CDQ_{10} shows linear dependence of pH value and small influence of CoQ_{10} upon β-CD stability. CoQ_{10} has even less influence on stability of γ-CD. At pH 6.5, 39.56 mg of CoQ_{10} per L was determined, while at pH 3.09, only 0.30 mg CoQ_{10}/L stayed in the form of complex.

3. 4. Stability Studies

CoQ_{10} is a light-sensitive compound and CDs are well known hosts with the ability to improve photo-stability.[25, 15, 16] Therefore, effect of UV irradiation and temperature on CoQ_{10} degradation was investigated as described in section *2.6*.

Overheating of CoQ_{10} at high temperatures in the dark does not affect the stability of the investigated substance significantly, as after 92-hour monitoring of the CoQ_{10} concentration in the sample of pure CoQ_{10}, we observed that 96.3% of CoQ_{10} remained. Even lesser impact of heat was observed in the case of inclusion complexes, where after 92 hours, we determined less than 1% of degradation products of CoQ_{10}. At lower temperatures, the heat effect is even smaller, or practically negligible, as after preserving the samples for 92 hours at room temperature in the dark, we determined 99.9 ± 0.1% of CoQ_{10}, which is the value within the measurement error.

Greater influence of temperature on the stability of CoQ_{10} in various forms would most likely be seen across a longer period of time. Kommuru and his associates were monitoring the stability of CoQ_{10} for 16 months at various temperatures; 37, 45 and 55 °C. They observed degradation to a large extent at 45 and 55 °C, while CoQ_{10} was relatively stable at 37 °C. Using the Arrhenius plot of log*K* versus T^{-1}, they predicted shelf life at room temperature (time to 90% potency) to be about 6.3 years.[26] The above mentioned results suggest that the use of CDs as protective agents would prolong the shelf life of CoQ_{10} even more than what was determined by Kommuru.

CoQ_{10} is yellow to orange crystalline powder, and upon exposure to light, CoQ_{10} gradually decomposes, and the colour changes to dark yellow.[27, 28]

Protection from UV light-induced decomposition by CD complexation has been demonstrated for many substances.[29, 30] When examining the photostability of CoQ_{10} with low melting temperature, it is of value to know how the photostability of complexes will be affected by UV light at room and elevated temperature. On the basis of the results shown in Figure 8, it can be deduced that UV light

has much greater effect on the pure CoQ_{10} than CoQ_{10} complexes with CDs. After 120 minutes at room temperature, β-CD and γ-CD almost entirely protect CoQ_{10}, while 27.7% of the pure CoQ_{10} was decomposed.

The photolytic degradation of CoQ_{10} is also believed to be affected by heat and the type of CD. It was evident that almost no degradation occurred in the presence of β-CD (2.8%) or γ-CD (7.0%) at room temperature. However, at 80 °C, the difference is greater, about 72.3% of pure CoQ_{10} was degraded, while γ-CD offered 3-fold higher photo-protection against UV irradiation (64.9% of CoQ_{10} remained unchanged). The best protection of CoQ_{10} towards the combination of UV light and high temperature seems to be β-CD, where 79.2% of CoQ_{10} remained unchanged.

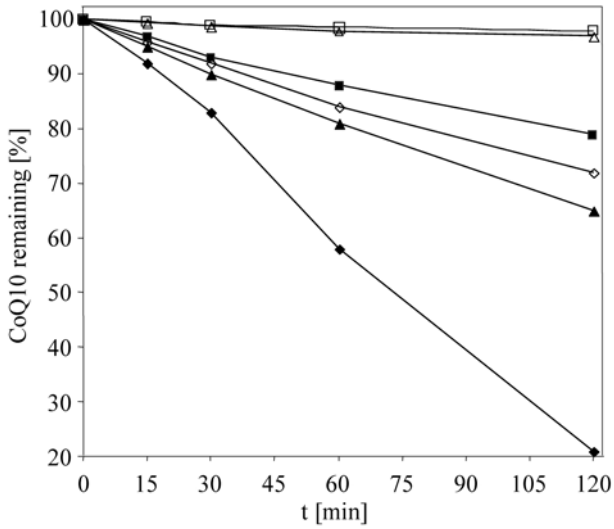

Figure 8: Effect of CDs on the photostability of CoQ_{10} (λ = 254 nm) at 25 (CoQ_{10} ◇, β-CDQ_{10} □, γ-CDQ_{10} △) and 80 °C (CoQ_{10} ◆, β-CDQ_{10} ■, γ-CDQ_{10} ▲).

3. 5. Antoxidant Activity

The antioxidant activity of CoQ_{10} and its complexes with CD was estimated using a slightly modified spectrophotometric method published by Blois.[31] The equimolar solutions of CoQ_{10} and CDQ_{10} were prepared, and antioxidant activity was tested with free radical $DPPH^{\cdot}$, which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of plant extracts.[32, 33]

The radical-scavenging activity against $DPPH^{\cdot}$ radical for CoQ_{10} was determined to be 1.8%. The CDQ_{10} complexes show higher antioxidant activity than pure CoQ_{10}. Complexation of CoQ_{10} with β-CD and γ-CD resulted in a 26% and 21% increase of antioxidant activity, respectively. The radical-scavenging activity against $DPPH^{\cdot}$ radical for CoQ_{10} in the presence of β-CD and γ-CD was 2.3 and 2.2%, respectively, meaning that the kind of cyclodextrin has small effect on antioxidant activity. We believe that increased water-solubility of CoQ_{10} in the form of CDQ_{10} complexes has improved antioxidant activity.

4. Conclusion

Summing up the results of the described investigation, it can be concluded that complexation of CoQ_{10} with cyclodextrins increases aqueous solubility, thermostability, photostability and antioxidant activity of CoQ_{10}, while the increase depends on the kind of CD. γ-CD increases the solubility of CoQ_{10} to the largest extent, while β-CD offers the best protection towards high temperature, UV light, and the combination of them. In the case of antioxidant activity no major differences can be found when using β-CD or γ-CD. There are some indicators that inclusion complexes are formed between CoQ_{10} and CD, however, further investigation has to be carried out in order to prove this theory.

5. References

1. E. G. Bliznakov, D. J. Wilkins, *Adv. Ther.* **1998**, *5*, 218–228.
2. F. L. Crane, Y. Hatefi, R. L. Lester, C. Widmer, *Biochem. Biophys. Acta* **1957**, *25*, 220–221.
3. I. Eggens, P. G. Elmberger, P. Löw, *Br. J. Exp. Pathol.* **1989**, *70*, 83–92.
4. A. Kalen, E. L. Appelkvist, G. Dallner, *Lipids* **1989**, *24*, 579–584.
5. G. P. Littarru, M. Battino, K. Folkers, in: E. Cadenas (Ed.) and L. Packer (Ed.): Handbook of antioxidants, Marcel Decker, New York, **1996**, pp. 203–239.
6. S. Greenberg, W. H. Frishman, *J. Clin. Pharmacol.* **1990**, *30*, 590–608.
7. C. H. Hsu, Z. Cui, R. J. Mumper, M. Jay, *AAPS PharmSciTech* **2003**, *4*, Article 32.
8. H. Takeuchi, H. Sasaki, T. Niwa, T. Hino, Y. Kawashima, K. Uesugi, H. Ozawa, *Int. J. Pharm.* **1992**, *86*, 25–33.
9. S. Nazzal, N. Guven, I. K. Reddy, M. A., Khan, *Drug. Dev. Ind. Pharm.* **2002**, *28*, 49–57.
10. S. Nazzal, I. I. Smalyukh, O. D. Lavrentovich, M. A. Khan, *Int. J. Pharm.* **2002**, *235*, 247–265.
11. R. K. Chopra, R. Goldman, S. T. Sinatra, H. N. Bhagavan, *Internat. J. Vit. Nutr. Res.* **1998**, *68*, 109–113.
12. T. R. Kommuru, B. Gurley, M. A. Khan, I. K. Reddy, *Int. J. Pharm.* **2001**, *212*, 233–246.
13. U. Ullmann, J. Metzner, C. Schulz, J. Perkins, B. Leuenberger, *J. Med. Food* **2005**, *8* (3), 397–399.
14. J. Szejtli, in: J. Szejtli (Ed.) and T. Osa (Ed.): Comprehensive Supramolecular Chemistry, Pergamon, Oxford, **1996,** *3*, pp. 189–203.
15. J. Szejtli, *Kontakte* **1988,** *1*, 31–36.
16. H. Van Doorne, *Eur. J. Pharm. Biopharm.* **1993,** *39*, 133–139.

17. M. Prosek, J. Butinar, B. Lukanc, M. Milivojevic Fir, L. Milivojevic, M. Krizman, A. Smidovnik, *J. Pharm. Biomed. Anal.* **2008,** *47*, 918–922.
18. T. Higuchi, K. A. Connors, *Adv. Anal. Instr.* **1965**, *4*, 117–212
19. European Pharmacopoeia 4, Council of Europe, Strasbourg, **2001,** pp. 2099–2100.
20. C. W. Park, S. J. Kim, S. J. Park, J. H. Kim, J. K., Kim, G. B. Park, J. O. Kim, Y. L. Ha, *J. Agric. Food Chem.* **2002**, *50*, 2977–2983.
21. N. E. Polyakov, T. V. Leshina, T. A. Konovalova, E. O. Hand, L. D. Kispert, *Free Radic. Biol. Med.* **2004**, *36*, 872–880.
22. J. Szejtli, E. Bánky-Elöd, *Die Stärke* **1975**, *11*, 368–376.
23. K. Uekama, T. Irie, in: J. Szejtli (Ed.) and T. Osa (Ed.): Comprehensive Supramolecular Chemistry, Pergamon, Oxford, **1996,** *3*, pp. 451–482.
24. F. Hirayama, M. Kurihara, T. Utsuki, K. Uekama, *J. Chem. Soc., Chem. Commun.* **1993**, 1578–1580.
25. Y. Matsuda, R. Masahara, *J.Pharm.Sci.* **1983,** *72*, 1198–1203.
26. T. R. Kommuru, M. Ashraf, M. A. Khan, I. K. Reddy, *Chem. Pharm. Bull.* **1999,** *47*, 1024–1028.
27. Y. Matsuda, R. Masahara, *J. Pharm. Sci*. **1983,** *72*, 1198–1203.
28. H. Takeuchi, H. Sasaki, T. Niwa, T. Hino, Y. Kawashima, K. Uesugi, H. Ozawa, *Int. J. Pharm.* **1992,** *86*, 25–33.
29. K. Uekama, T. Irie, F. Hirayama, *Chem. Lett.* **1978,** 1109–1112.
30. K. Uekama, T. Irie, M. Sunada, M. Otagiri, K. Iwasaki, Y. Okano, T. Miyata, Y. Kase, *J. Pharm. Pharmacol.* **1981,** *33*, 707–710.
31. M. S. Blois, *Nature* **1958,** *181*, 1199–1200.
32. J. Bao, Y. Cai, M. Sun, G. Wang, H. Corke, *J. Agric. Food Chem.* **2005,** *53*, 2327–2332.
33. I. A. Castro, M. M. Rogero, R. M. Junqueira, M. M. Carrapeiro, *Int. J. Food Sci. Nutr.* **2006,** *57*, 75–82.

Povzetek

Kompleksa koencima Q10 z β- in γ-cyclodextrinom sta bila pridobljena s koprecipitacijsko metodo. Fazna diagrama topnosti za oba kompleksa sta bila opredeljena kot diagrama tipa A_L, kar nakazuje nastanek kompleksov v stehiometrijskem razmerju 1 : 1. S kompleksacijo koencima Q_{10} s ciklodekstrini so bile dosežene bistveno večja topnost v vodi, termo- in foto-stabilnost ter antioksidativna učinkovitost. Topnost v vodi je bila določena pri različnih pogojih (temperatura in pH). Študije stabilnosti v trdnem stanju so bile izvedene pod stresnimi pogoji (T = 80 °C, γ = 254 nm). Koncentracije koencima Q_{10} so bile določene s tekočinsko kromatografijo z mason ali UV detekcijo. Antioksidativna učinkovitost koencima Q_{10} je bila določena s spektrofotometrično metodo. Kot stabilen prosti radikal je bil uporabljen $DPPH^{\cdot}$.