Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Pharmaceutics 309 (2006) 115–122
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Solubilization and quantification of lycopene in aqueous media in the form of cyclodextrin binary systems ☆

Maria Vertzoni [a], Theodora Kartezini [a], Christos Reppas [a], Helen Archontaki [b], Georgia Valsami [a,*]

[a] *Laboratory of Biopharmaceutics and Pharmacokinetics, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, 15771 Athens, Greece*

[b] *Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, 15771 Athens, Greece*

Received 22 July 2005; received in revised form 10 November 2005; accepted 15 November 2005
Available online 10 January 2006

Abstract

An optimized kneading method for the preparation of lycopene–cyclodextrin binary systems was developed leading to solubilization of lycopene in water and 5% (w/v) dextrose solution. Lycopene quantification in the prepared binary systems was performed by a developed spectrometric method that followed a successful single-step extraction with dichloromethane. Storage stability characteristics of the binary systems were studied at 4 °C in solution and at −20 °C in the lyophilized products. Lycopene content was monitored at λ_{max} = 482 nm, the limit of detection was 0.41 μg/ml and relative standard deviation was less than 3.1%. The results obtained with the spectrometric method were confirmed by a HPLC method. In the presence of cyclodextrins, lycopene concentration in water was 8.0 ± 1.0, 27.1 ± 3.2 and 16.0 ± 2.2 μg/ml for β-CD, HP-β-CD and Me-β-CD, respectively. In 5% (w/v) aqueous dextrose solutions the corresponding values were 16.0 ± 1.8, 48.0 ± 5.1 and 4.0 ± 0.5 μg/ml, respectively. At 4 °C, storage stability of lycopene–cyclodextrin binary systems in water or 5% (w/v) aqueous dextrose solutions, was limited ($t_{1/2}$ = 1–4 days). Addition of the antioxidant sodium metabisulfite increased the stability of lycopene–HP-β-CD binary system in water. At −20 °C, the lyophilized lycopene–cyclodextrin binary systems were stable for at least 2 weeks.

Keywords: Lycopene solubilization; Lycopene quantification; Spectrometric method; Kneading technique; Lycopene–cyclodextrin binary systems; Lyophilization

1. Introduction

Lycopene is the main carotenoid in tomatoes and tomato products (Clinton, 1998) and occurs also naturally in some other fruits and vegetables, guava, watermelon and pink grapefruit (Nguyen and Schwartz, 1999). Among the common dietary carotenoids, lycopene has the highest singlet oxygen capacity in vitro (Paiva and Russell, 1999; Di Mascio et al., 1989). Numerous clinical studies have concluded that use of carotenoids, such as β-carotene and lycopene in the diet has been inversely correlated with prostate cancer incidence, digestive tract cancers, pancreatic cancer and cancers in other tissues (Franceschi et al., 1994; Giovannucci et al., 1995; Clinton et al., 1996; Rao and Agarwal, 1998; Giovannucci, 1999; Gann et al., 1999; Bowen et al., 2002).

However, the extremely high lipophilicity (clog *P* = 17.64, www.syrres.com/default.htm, accessed on 20-10-2005) of lycopene resulting in its extremely low aqueous solubility and its sensitivity to air and light are significant barriers to its oral formulation and bioavailability.

To date, only one approach has been reported in the literature as a possible way to improve solubility of lycopene, i.e molecular encapsulation into the cavity of α- and β-cyclodextrin (Mele et al., 2002). Indeed, the ability of cyclodextrins (CDs) to form inclusion-complexes with various molecules (Szejtli, 1982) is used in pharmaceutical industry in order to increase aqueous solubility of lipophilic drugs and enhance the stability of various substances sensitive to degradation caused by external factors, such as light and oxygen (Duchêne, 1987;

☆ Part of this work has been presented at EUFEPS 2004. New safe medicines: towards mechanistic prediction, October 17–20, 2004, Brussels, Belgium.

* Corresponding author. Tel.: +30 210 727 4022; fax: +30 210 727 4027.
E-mail address: valsami@pharm.uoa.gr (G. Valsami).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.11.021

Loftsson and Brewster, 1996). Therefore, a drug may show improved bioavailability when administered as a complex with CDs (Rajewski and Stella, 1996; Stella and Rajewski, 1997). Furthermore, it was recently reported that drug/cyclodextrin complexes could self-associate to form water soluble aggregates that can further solubilize the drug through non-inclusion complexation (Loftsson et al., 2002, 2004; Mele et al., 1998).

However, quantitative information on the extent that CDs can improve lycopene solubility and even more on stability characteristics of lycopene–cyclodextrin (Lyc–CD) binary systems is lacking.

In this investigation, an optimized kneading method was developed for the preparation of Lyc–CD binary systems, by which considerable increase of solubilization of lycopene in water and 5% (w/v) dextrose solution was achieved. The main novelty of this study was the quantification of lycopene in the above aqueous media. Quantification was accomplished using an effective extraction procedure followed by a spectrometric method which was developed, validated and compared to an existing HPLC method (Vertzoni et al., 2005). Moreover, interaction of lycopene with 2-hydroxypropyl-β-cyclodextrin (which has been approved for intravenous administration) and methyl-β-cyclodextrin (a lipophilic derivative of β-cyclodextrin) was studied for the first time.

The ultimate goal of relevant research in our laboratory is the assessment of the absolute bioavailability of various dosage forms of lycopene and therefore, the development of a lycopene formulation that could be administered intravenously.

2. Materials and methods

2.1. Apparatus

A Perkin-Elmer Lambda 6 double-beam UV/vis spectrometer (Illinois, USA) was used for spectrophotometric measurements. The operational conditions were: scan speed 100 nm/min, scan range 190–700 nm.

The chromatographic system, consisted of a Spectra System P1000 pump, a Spectra System UV 2000 absorbance detector extended to the visible region and an autosampler AS 3000. The above system was controlled by a Spectra System Controller SN 4000 and a software package Chromquest (Thermoquest Inc., San Jose, USA).

Differential scanning calorimetry (DSC) was performed on a DSC-4 differential scanning calorimeter with System 4 Microprocessor Controller and Thermal Analysis 3700 Data Station, Perkin-Elmer.

2.2. Reagents and materials

All chemicals were of analytical purity grade. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) of molar substitution (MS) 0.6 ($\bar{M}_r = 1383$) and methyl-β-cyclodextrin (Me-β-CD) of MS 1.6–2.0 ($M_r = 1310$) were purchased from Sigma–Aldrich (St. Louis, USA). β-Cyclodextrin (β-CD) ($\bar{M}_r = 1134.9$) was purchased from Serva Electrophoresis GmbH (Heidelberg, Germany). Lycopene powder from tomatoes was kindly donated by the Laboratory of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens. Lycopene was extracted from commercial tomato and its purity (93.5%) was tested spectrometrically at 482 nm, against a standard solution prepared from pure all-*trans*-lycopene powder (purchased from Sigma–Aldrich). The identity of the isolated all *trans*-lycopene was deduced by its ^{1}H and ^{13}C NMR data and comparison with literature data (Hengartner et al., 1992). Lycopene powder was stored at −70 °C. Dichloromethane, acetonitrile and methanol (HPLC grade) were obtained from Labscan Ltd. (Dublin, Ireland). Absolute ethanol (analytical grade) was obtained from Panreac Quimica SA (Barcelona, Spain). Dextrose 5% (w/v) solution for intravenous injection was purchased from Demo A.E. (Greece). Water purified with Labconco water pro ps system (Kansas City, MI, USA) was used in all procedures. Sodium metabisulfite was donated from Uni-Pharma S.A (Athens, Greece). Nylon syringe filters Titan® of 5 μm pore size and regenerated cellulose syringe filters Titan® of 0.45 μm pore size, were purchased from Scientific Resources Inc. (Eatown, NJ, USA). Whatman® paper filters No. 44 of 3 μm pore size were purchased from Whatman International Ltd. (Maidestone, England).

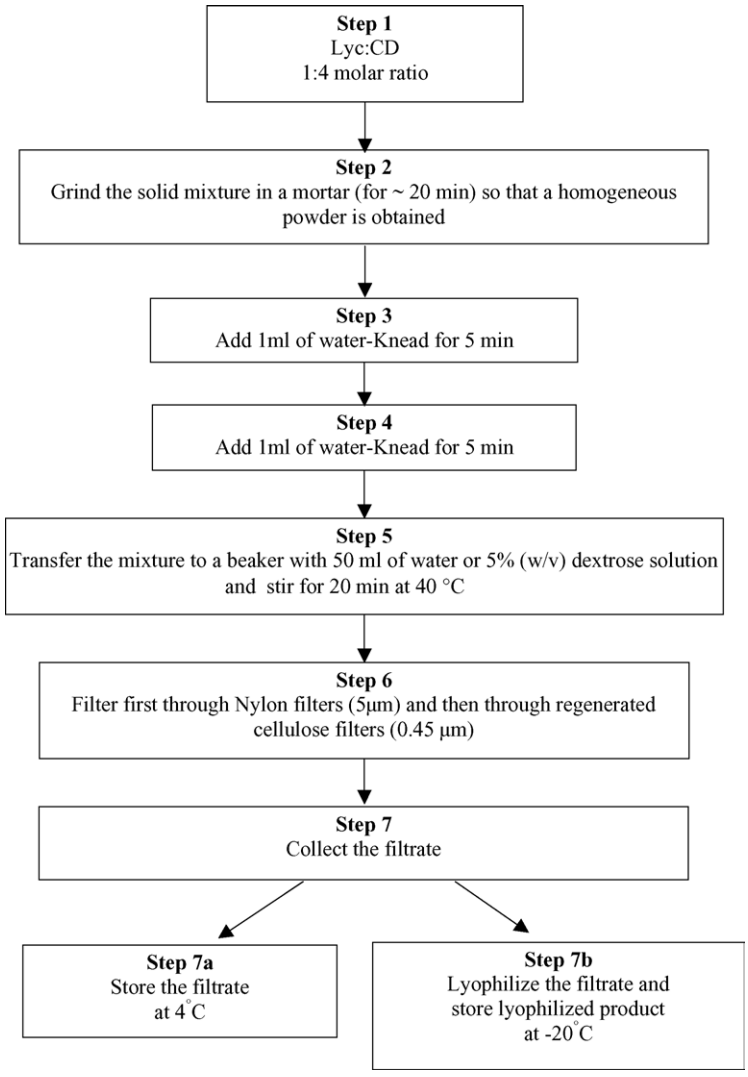
2.3. Procedures

2.3.1. Preparation of Lyc–CD binary systems

The procedure used for the preparation of Lyc–CD binary systems is shown schematically in Flow chart 1. A quantity of 0.0215 g of lycopene was mixed in a mortar with the appropriate amount of cyclodextrin (1:4 molar ratio). The above mixture was ground for 20 min until a homogeneous powder was obtained. One milliliter of water was added and kneaded for 5 min. Then, 1 ml of water was added again and kneading was continued for another 5 min in order to obtain a homogeneous red paste. The paste was transferred into a beaker with 50 ml of either water or 5% (w/v) isotonic solution of dextrose at 40 °C. The suspension was stirred for 20 min at 40 °C. Then, it was filtered, first through nylon membrane syringe filters (pore size 5.0 μm) and then through regenerated cellulose syringe filters (pore size 0.45 μm). An appropriate amount of the red filtrate (Flow chart 1, step 7) was used for quantification of lycopene and for the study of storage stability characteristics of the formed lycopene–CD binary system in aqueous solution (Flow chart 1, step 7a). The remaining amount of the filtrate was lyophilized and used for the study of storage stability characteristics at −20 °C (Flow chart 1, step 7b).

2.3.2. Spectrometric quantification of lycopene in its cyclodextrin binary systems

Filtrate (Flow chart 1, step 7a): One milliliter of this solution was transferred to a glass tube (after appropriate dilution with water) together with 1 ml of absolute ethanol. After vortexing for 1 min, 2 ml of CH_2Cl_2 were added. After additional vortexing for 1 min, the solutions were centrifuged (1828 *g*, 10 min, 10 °C).



Flow chart 1. Preparation of Lyc–CD binary systems.

Using a pasteur pipette, the lower layer was transferred into a quartz cuvette (10 mm path length × 5 mm) and its lycopene content was monitored at 482 nm.

Lyophilized product (Flow chart 1, step 7b): An accurately weighed amount of lyophilized product was transferred into a 10 ml volumetric flask, dissolved with a small amount of water and diluted to volume with water. One milliliter of this solution was transferred into a glass tube and the procedure, described above for the quantification in filtrate (Flow chart 1, step 7a), was followed.

Calibration curve: For construction of lycopene calibration curves, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 ml of lycopene stock solution in CH_2Cl_2 (50 μg/ml) were diluted with absolute ethanol up to 10 ml so that a concentration range of 1–10 μg/ml was obtained (working standard solutions). One milliliter of each working solution was mixed with 1 ml of water and after vortexing for 1 min, 2 ml of CH_2Cl_2 were added. After vortexing for 1 min, these solutions were centrifuged (1828 *g*, 10 min, 10 °C), as above. Similarly, using a pasteur pipette, the lower layer was transferred into a 1 cm path length quartz cuvette (10 mm × 5 mm) and its lycopene content was monitored at 482 nm.

2.3.3. Chromatographic quantification of lycopene

A Hypersil BDS RP-C18 column (150 mm × 4.6 mm), 5 μm particle size, was equilibrated with a mobile phase composed of acetonitrile and methanol (50:50, v/v). The flow rate was 1.5 ml/min and detection was performed at 472 nm. Sample treatment and calibration curve construction was performed as previously described (Vertzoni et al., 2005).

2.3.4. Storage stability characteristics of Lyc–CD binary systems

Storage stability characteristics of Lyc–CD binary systems in aqueous solutions (Flow chart 1, step 7) and in lyophilized products were studied for 2 weeks at 4 and −20 °C, respectively. Samples were obtained at 0, 1, 2, 4, 6, 8, 10 and 14 days. For HP-β-CD, the storage stability characteristics in solution were

also studied in the presence of the antioxidant compound sodium metabisulfite (0.01%, w/v).

2.3.5. DSC analysis of Lyc–CD lyophilized products

Samples of 5–12 mg of lycopene, CDs, Lyc–CD physical mixtures and Lyc–CD lyophilized products, were sealed into aluminum pans. Samples were heated from 10 to 260 °C with a heating rate of 10 °C/min under nitrogen atmosphere. The power and temperature scales were calibrated against the enthalpy of fusion and melting temperature of indium.

3. Results and discussion

3.1. Development of a modified kneading method

Lycopene concentration in Lyc–β-CD binary systems in water, prepared with a previously described method (Mele et al., 1998), was found to be less than 0.1 μg/ml. Therefore, development of an optimized kneading method was attempted to increase the amount of lycopene present in such a binary system. The following modifications were applied:

a. Overnight standing of Lyc–CD paste under nitrogen atmosphere was omitted, to avoid possible lycopene degradation during the drying process. Instead, suspension of the paste in water or 5% (w/v) isotonic dextrose solution was performed immediately after kneading (Flow chart 1, steps 5 and 6) and lyophilization was followed immediately after filtration (Flow chart 1, step 7b).
b. The volume of water used to suspend the Lyc–CD paste in the mortar was reduced to 50 ml instead of 200 ml (Flow chart 1, step 5). This quantity was found to significantly improve the efficiency of the method.
c. Titan® nylon syringe filters of 5 μm pore size in combination with Titan® regenerated cellulose syringe filters of 0.45 μm pore size were used in the filtration procedure after optimization of the type of filters to be used in the filtration step (Flow chart 1, step 6). The filters used previously (Mele et al., 1998) (Whatman® No. 44 paper filters 3 μm pore size), proved to be inadequate as shown from the high background signal in Fig. 1(c). The background was reduced when filtration through Whatman® No. 44 paper filters of 3 μm pore size was followed by filtration through Titan® regenerated cellulose syringe filters of 0.45 μm pore size. However, this reduction was accompanied by significant reduction of the absorbance signal (Fig. 1(e)). Filtration through Titan® nylon syringe filters of 5 μm pore size gave the highest absorbance value but a low background signal still existed (Fig. 1(a)). Filtration through Titan® regenerated cellulose syringe filters of 0.45 μm pore size resulted in significant lower absorbance value without background (Fig. 1(d)), while the combination of Titan® nylon syringe filters (5 μm) with Titan® regenerated cellulose syringe filters (0.45 μm) gave the optimum results (Fig. 1(b)).

In the present study the above optimized kneading method was applied to the preparation of lycopene binary systems with

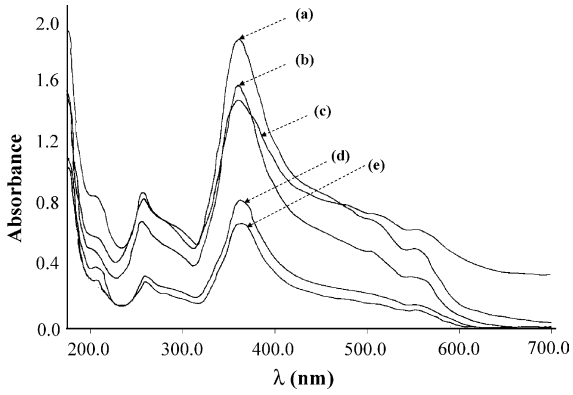


Fig. 1. Absorption spectra of lycopene binary system with β-CD in water, showing the effect of different type of filters (Flow chart 1, step 6): (a) Titan® nylon syringe filters pore size 5 μm, (b) Titan® nylon syringe filters pore size 5 μm followed by Titan® regenerated cellulose syringe filters pore size 0.45 μm, (c) Whatman® No. 44 paper filter pore size 3 μm, (d) Titan® regenerated cellulose syringe filters pore size 0.45 μm and (e) Whatman® No. 44 paper filter pore size 3 μm followed by Titan® regenerated cellulose syringe filters pore size 0.45 μm.

β-CD, HP-β-CD and Me-β-CD in water and in media appropriate for intravenous administration, such as normal saline and 5% (w/v) dextrose solution. Formation of Lyc–CD binary systems was not possible in normal saline, presumably because NaCl acted as a competing guest molecule for the CD cavity (Szejtli, 1982).

3.1.1. DSC results of Lyc–CD binary systems

Supporting evidence of the interaction between lycopene and β-cyclodextrins was attained with differential scanning calorimetric results (Fig. 2). β-CD shows an endotherm at 135 °C

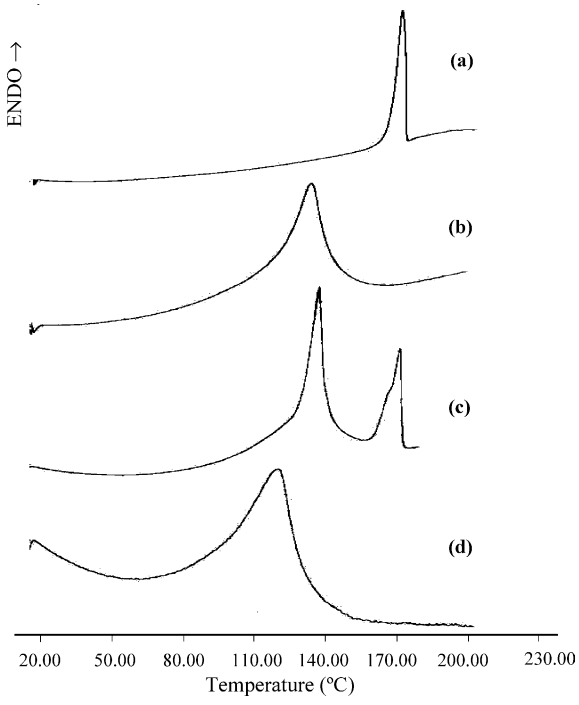


Fig. 2. DSC thermograms of (a) lycopene, (b) β-CD, (c) Lyc–β-CD physical mixture and (d) lyophilized product of Lyc–β-CD binary system.

($\Delta H = 60$ cal/g) corresponding to its dehydration. Lycopene shows an endotherm at 172 °C ($\Delta H = 18.47$ cal/g) corresponding to its melting point. The physical mixture of lycopene and β-CD shows the two characteristic endotherms of lycopene and β-CD, slightly shifted. However, on the Lyc–β-CD lyophilized product the peak of β-CD dehydration shifted to 123 °C ($\Delta H = 52$ cal/g) and that of lycopene completely disappeared. The shift of β-CD peak to lower temperature, its broadening and reduction in ΔH was definitely a strong evidence of a disorder happened in the water molecules inside cyclodextrin cavity, that was probably caused by the presence of lycopene.

Similar DSC results were also observed in the case of HP-β-CD and Me-β-CD (not shown).

3.1.2. Spectrometric determination of lycopene in its binary systems with cyclodextrins

Absorption spectra of lycopene solutions with β-CD, HP-β-CD and Me-β-CD in water and 5% (w/v) aqueous dextrose solutions are shown in Fig. 3(A and B), respectively. These spectra could not be used for the determination of lycopene in the presence of CDs, because standard aqueous solutions of lycopene for the construction of standard curves could not be prepared. For this reason, optimization of lycopene extraction from aqueous CD solutions was attempted with various organic solvents prior to spectrometric analysis. Quantitative extraction, assumed by complete decolorization of the aqueous phase, was accomplished by dichloromethane and the developed procedure, as described in the experimental part, was followed. However, it should be noted that the key point for the successful lycopene extraction to dichloromethane was the addition of ethanol in the aqueous sample prior to the extraction step.

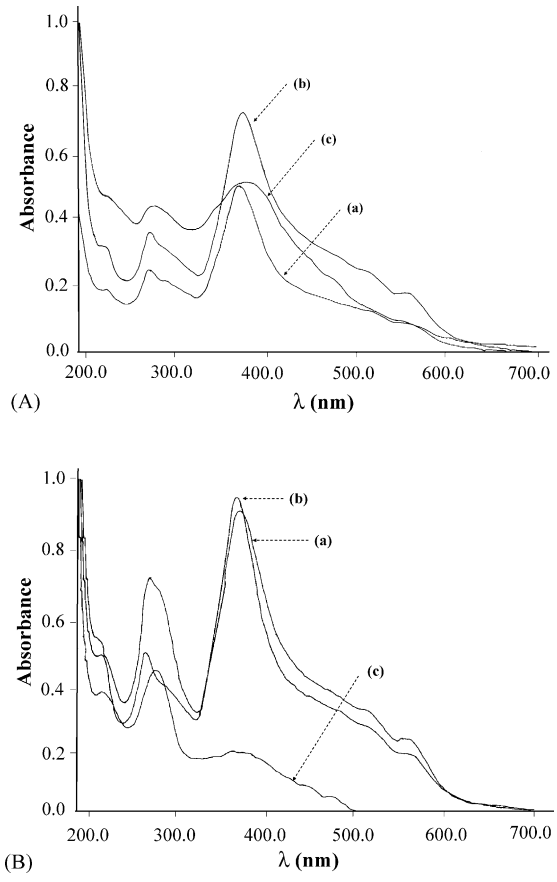


Fig. 3. Absorption spectra of lycopene binary systems with (a) β-CD, (b) HP-β-CD and (c) Me-β-CD in water (A) and in 5% (w/v) dextrose solution (B).

Table 1
Analytical parameters of calibration curves of lycopene constructed with the spectrometric method (concentration range covered 1–10 μg/ml)

Day	Slope $b \pm s$ ($\times 10^3$)	Intercept $a \pm s$ ($\times 10^2$)	r^2 ($n = 6$)
1	86.1 ± 2.8	−5.4 ± 1.8	0.9991
2	87.4 ± 1.7	−3.1 ± 1.1	0.999
3	85.3 ± 1.4	−2.3 ± 1.6	0.9993

Analytical parameters of lycopene standard curves (concentration range 1–10 μg/ml) are shown in Table 1. The limits of detection and quantification were 0.41 and 1.2 μg/ml, respectively, and between days relative standard deviation was less than 3.1%. An HPLC–vis method (Vertzoni et al., 2005) was used for comparison and in the case that lycopene concentration was below the LOQ of the spectrometric method. The results of this comparison showed no statistical difference between the two methods (*t*-test, $P \leq 0.05$) in the concentration range used for the spectrometric assay.

Absorption spectra of lycopene in dichloromethane and dichloromethane extracts of its aqueous β-CD, HP-β-CD and Me-β-CD solutions (Flow chart 1, step 7), after appropriate dilution, are shown in Fig. 4. Similar spectra of lycopene were also obtained after respective extraction from 5% (w/v) aqueous dextrose solutions (not shown). The fact that all these spectra presented the same spectral features revealed that the measured absorbance corresponded only to lycopene extracted from each CD aqueous solution (Flow chart 1, step 7). Thus, based on these spectra, quantification of lycopene in the formed binary systems with CDs became possible by monitoring the absorbance at λ_{max} 482 nm. Intensity of absorbance was proportional to lycopene solubility in every cyclodextrin aqueous solution. The results are presented in Table 2 and reflect the solubility

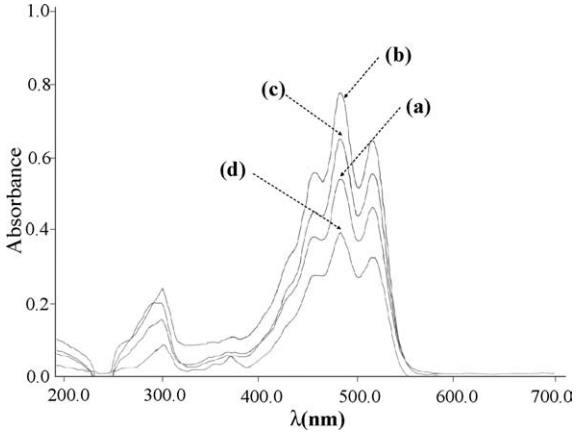


Fig. 4. Absorption spectra of lycopene in dichloromethane extracts from its binary systems with (a) β-CD, (b) HP-β-CD and (c) Me-β-CD prepared in water. For comparative purposes the spectrum of a 5 μg/ml solution of lycopene in dichloromethane is also included (d).

Table 2
Lycopene concentration (μg/ml) in Lyc–CD binary systems in water and 5% (w/v) dextrose solution (Flow chart 1, step 7a) measured spectrometrically ($n = 3$)

Complexation agent	Lycopene concentration (μg/ml)[a]	
	Water	Dextrose solution 5% (w/v)
β-CD	8.0 ± 1.0	16.0 ± 2.0
HP-β-CD	27.0 ± 3.0	48.0 ± 5.0
Me-β-CD	16.0 ± 2.0	4.0 ± 1.0

[a] The solubility of lycopene in water and dextrose solution 5% (w/v) was <0.002 μg/ml (measured by the HPLC method of Vertzoni et al., 2005).

enhancement of lycopene by the presence of CDs in aqueous media.

Solubilization ability of CDs increased in the order β-CD < Me-β-CD < HP-β-CD (Table 2), when water was used to collect the paste after the kneading process (Flow chart 1, step 5). Solubilization affinity of CDs increased in the order Me-β-CD < β-CD < HP-β-CD in presence of dextrose. The presence of dextrose favored the formation of lycopene binary systems with HP-β-CD and β-CD, as it was assured by the increased concentration of lycopene in these solutions, and revealed a dramatic decrease of lycopene concentration in solutions with Me-β-CD (Table 2).

It was reported recently, that drug/cyclodextrin complexes can self-associate to form water-soluble aggregates or micelles, which can further contribute to solubilize drugs through non-inclusion complexation (Loftsson et al., 2002). On the other hand, Mele et al. (1998) have demonstrated the non-covalent associations of cyclodextrins with carotenoids in water and formation of large aggregates. Therefore, the rank order of solubility increase could be attributed to a combination of inclusion- and non-inclusion-complexation. The greater hydrogen-bonding interaction occurring between Lyc–HP-β-CD and Lyc–β-CD binary systems and dextrose may lead to a greater non-inclusion-complexation (and hence a greater solubility) than that occurring in the presence of Me-β-CD. In contrast, the rank order observed in water may be considered in parallel with the solubility order of the cyclodextrins employed [i.e. solubility of β-CD is much lower than that of HP-β-CD or Me-β-CD; Szejtli (1982)].

3.1.3. Storage stability characteristics of lycopene–CD binary systems

Stability of Lyc–CD binary systems was studied either in aqueous solution at 4 °C (Flow chart 1, step 7a) or as a lyophilized product at −20 °C (Flow chart 1, step 7b) for a period of 2 weeks.

Concentration of lycopene in solution decreased exponentially with time. Fitting the results on SigmaPlot® 4.0 for Windows®95 (SPSS Inc., IL USA), first-order kinetics was observed (Fig. 5) and relevant degradation rate constants

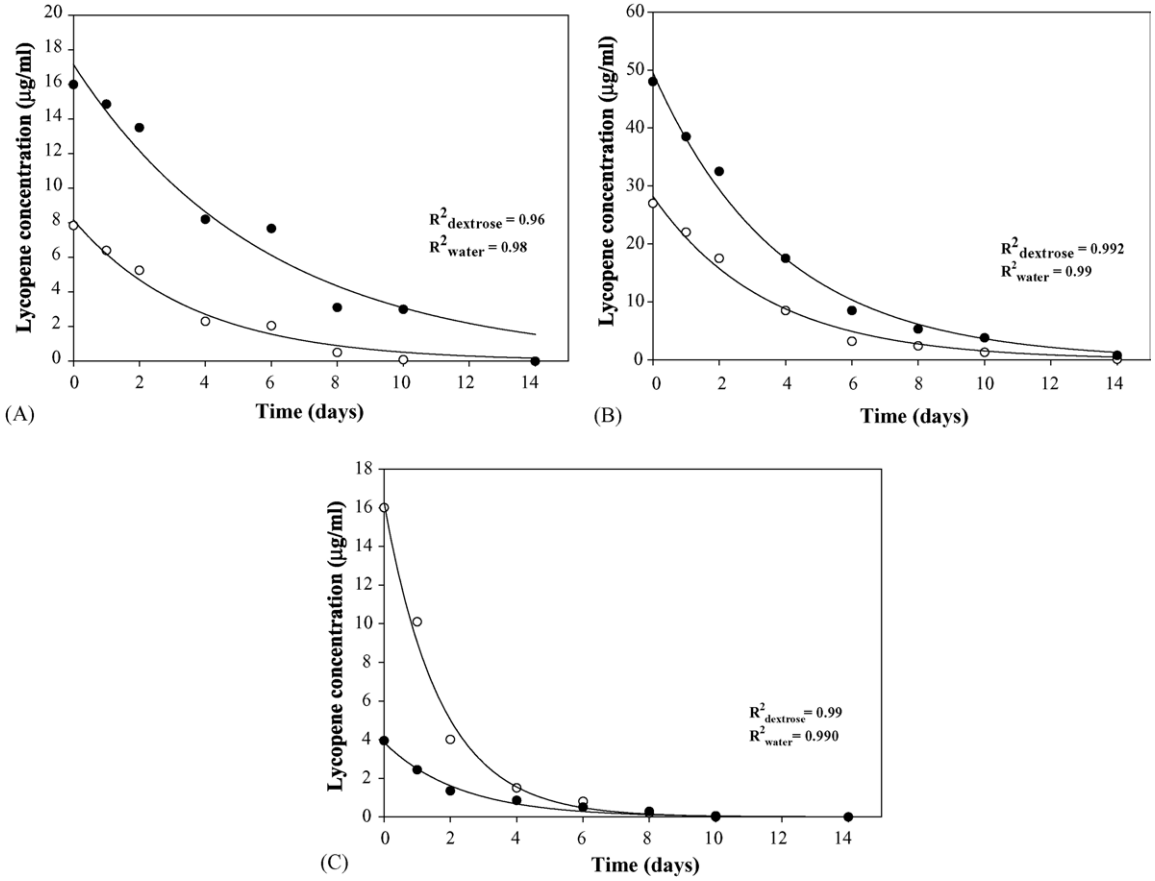


Fig. 5. Two-week stability study of lycopene binary systems with (A) β-CD, (B) HP-β-CD and (C) Me-β-CD prepared in water (◯) and in 5% (w/v) dextrose solution (●).

Table 3
Estimated values of rate constants, k_d, after a 2-week stability study on Lyc–CD binary systems (Flow chart 1, step 7a) in water and in 5% (w/v) dextrose solutions, at 4 °C

Complexation agent	Water		Dextrose solution 5% (w/v)	
	k_d (days^{-1})	$t_{1/2}$ (days)	k_d (days^{-1})	$t_{1/2}$ (days)
β-CD	0.270 ± 0.025	2.57	0.171 ± 0.021	4.05
HP-β-CD	0.290 ± 0.022	2.39	0.261 ± 0.021	2.66
Me-β-CD	0.580 ± 0.050	1.20	0.435 ± 0.040	1.59

were estimated along with $t_{1/2}$ values, which are tabulated in Table 3. The $t_{1/2}$ values were derived from the relationship $t_{1/2} = 0.693/k_{d(obs)}$, which holds for first and pseudo-first-order kinetics.

Presence of dextrose improved the stability of lycopene in aqueous solution of β-cyclodextrin (Flow chart 1, filtrates of step 7) [$t_{1/2\,(dextrose)} \approx 4$ days, $t_{1/2\,(water)} \approx 2.5$ days] while it did not practically affect the stability of lycopene in HP-β-CD ($t_{1/2} \approx 2.5$ days) and Me-β-CD ($t_{1/2} \approx 1.5$ days) aqueous solutions (Table 3).

Addition of an antioxidant reagent, sodium metabisulfite (0.01%, w/v) in the lycopene–HP-β-CD aqueous solution of 5% (w/v) dextrose solution (Flow chart 1, step 7) resulted in significant improvement of its stability at 4 °C ($t_{1/2} \approx 10$ days). This was desired considering that ultimately lycopene in HP-β-CD solution would be given for intravenous administration to dogs and sodium metabisulfite is a commonly used compound for stability enhancement in such occasions.

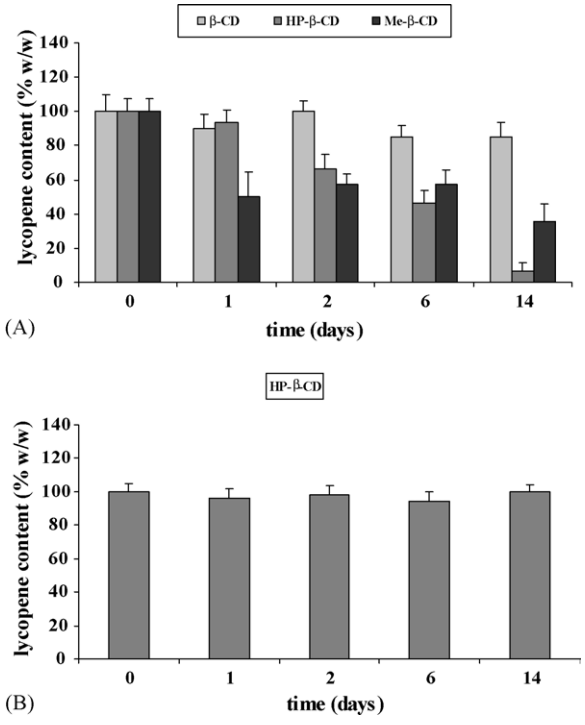


Fig. 6. Two-week storage stability data of lyophilized Lyc–CD binary systems, at −20 °C (Flow chart 1, step 7b), prepared in: (A) water and (B) 5% (w/v) dextrose solution.

Stability of lycopene–cyclodextrin lyophilized products (Flow chart 1, step 7b) is shown in Fig. 6. When water was used to collect the paste after the kneading process (Flow chart 1, step 5), the lyophilized product between lycopene and β-CD was stable at −20 °C for about 2 weeks (Fig. 6(A)). However, products of lycopene with HP-β-CD and Me-β-CD decomposed substantially within 6 days after preparation. Presence of dextrose was found to stabilize the solid lycopene–HP-β-CD for at least 14 days under the same storage conditions (Fig. 6(B)). Due to the extremely low aqueous solubility of lycopene, it was not possible to perform phase solubility experiments in order to determine stability constants of the formed binary systems. As a consequence, the mechanism of complexation between lycopene and CDs could not be investigated.

4. Conclusions

The modified kneading procedure, the appropriate single-step extraction with dichloromethane and the fast and simple spectrometric method developed in this study, enabled the considerable enhancement in solubility and the reliable quantification of the extremely lipophilic substance, lycopene, in aqueous media in the presence of β-cyclodextrin derivatives. These findings could be used for improving the oral bioavailability and/or for administering lycopene intravenously.

Acknowledgments

The authors would like to thank Prof. L. Skaltsounis and his research group (Laboratory of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens) for providing all-*trans*-lycopene powder and Prof. K. Viras (Laboratory of Physical Chemistry, Department of Chemistry, N&K University of Athens) for valuable suggestions on DSC analysis.

References

Bowen, P., Chen, L., Stacewicz-Sapuntzakis, M., Duncan, C., Sharufi, R., Ghosh, L., Kim, H.-S., Christov-Tzelkov, K., van Breemen, R., 2002. Tomato sauce supplementation and prostate cancer: lycopene accumulation and modulation of biomarkers of carcinogenesis. Exp. Biol. Med. 227, 886–893.

Clinton, S.K., Emenhiser, C., Schwartz, S.J., Bostwick, D.G., Williams, A.W., Moore, B.J., Erdman, J.W., 1996. Cis-trans lycopene isomers, carotenoids and retinol in the human prostate. Cancer Epidemiol. Biomark. Prev. 5, 823–833.

Clinton, S.K., 1998. Lycopene: chemistry, biology and implications for human health and disease. Nutr. Rev. 56, 35–51.

Di Mascio, P., Kaiser, S., Sies, H., 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. Biophys. 274, 532–538.

Duchêne D. (Ed.) 1987. Cyclodextrins and Their Industrial Uses. Editions de Santé, Paris, France.

Franceschi, S., Bidoli, E., La Vecchia, C., Talamani, R., D'vanzo, B., Negri, E., 1994. Tomatoes and risk of digestive-track cancers. Int. J. Cancer 59, 181–184.

Gann, P.H., Ma, J., Giovannucci, E., Willett, W., Sacks, F.M., Hennekens, C.H., Stampfer, M.J., 1999. Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. Cancer Res. 59, 1225–1230.

Giovannucci, E., Ascherio, A., Rimm, E.B., Stumpfer, M.I., Colditz, G.A., Willett, W.C., 1995. Intake of carotenoids and retinol in relation to risk of prostate cancer. J. National Cancer Inst. 87, 1767–1776.

Giovannucci, E., 1999. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J. National Cancer Inst. 91, 317–331.

Hengartner, K., Benhardt, K., Meyer, K., Englert, G., Glinz, E., 1992. Synthesis, isolation and NMR spectroscopic characterization of fourteen (Z) isomers of lycopene and of some acetylenic didehydro- and tetrahydrolycopenes. Helv. Chim. Acta 75, 1848–1865.

Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.

Loftsson, T., Magnusdottir, A., Masson, M., Sigurjonsdottir, J., 2002. Self-association and cyclodextrin solubilization of drugs. J. Pharm. Sci. 91, 2307–2316.

Loftsson, T., Masson, M., Brewster, M., 2004. Self-association of cyclodextrins and cyclodextrin complexes: minireview. J. Pharm. Sci. 93, 1091–1099.

Mele, A., Mendichi, R., Selva, A., 1998. Non-covalent associations of cyclomaltooligosaccharides (cyclodextrins) with trans-β-carotene in water: evidence for large aggregates by light scattering and NMR spectroscopy. Carbohydr. Res. 310, 261–267.

Mele, A., Mendichi, R., Selva, A., Molnar, P., Toth, G., 2002. Non-covalent associations of cyclomaltooligosaccharides (cyclodextrins) with carotenoids in water. A study on the α- and β-cyclodextrin/ψ, ψ-carotene (lycopene) systems by light scattering, ionspray ionization and tandem mass spectrometry. Carbohydr. Res. 337, 1129–1136.

Nguyen, M.L., Schwartz, S.J., 1999. Lycopene: chemical and biological properties. Food Technol. 53, 38–45.

Paiva, S.A., Russell, R.M., 1999. Beta-carotene and other carotenoids as antioxidants. J. Am. Coll. Nutr. 18, 426–466.

Rajewski, R.A., Stella, V.J., 1996. Pharmaceutical applications of cyclodextrins 2. In vivo drug delivery. J. Pharm. Sci. 85, 1142–1169.

Rao, A.V., Agarwal, S., 1998. Bioavailability and in vivo antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. Nutr. Cancer 31, 199–203.

Stella, V.J., Rajewski, R.A., 1997. Cyclodextrins: their future in drug formulation and delivery. Pharm. Res. 14, 556–567.

Szejtli J., 1982. Cyclodextrins and their Inclusion Complexes. Akadémiai Kiadó, Budapest.

Vertzoni, M., Reppas, C., Archontaki, H., 2005. Optimized determination of lycopene in canine plasma using reversed-phase high-performance liquid chromatography. J. Chromatogr. B 819, 149–154.