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Stabilization and Solubilization of Lipophilic Natural Colorants with Cyclodextrins

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Abstract. The paper provides data on the practical utilization of the benefits of the molecular encapsulation of natural colorants by cyclodextrins. Experimental results on the stability of cyclodextrin complexed curcumin, curcuma oleoresin, β-carotene and carotenoid oleoresins against light-, heat- and oxygen prove the benefits of molecular encapsulation of colorants. The parent β-cyclodextrin stabilized most effectively the curcumines, while the stability of carotenoids was most effectively achieved by α-cyclodextrin complexation. Methylated β-cyclodextrin was found to be the most potent solubilizing agent for both carotenoids and curcuminoids.

Key words: carotenoids, curcuminoids, cyclodextrins, stabilization, solubilization.

1. Introduction

The application of cyclodextrins for the formulation of natural colors has been a subject of over 190 publications and patent applications as CYCLOLAB's database indicates. Most of the publications deal with the stabilizing effect of the molecular encapsulation of naturally occurring dyes. The intensity of the R&D activity in the field of application of cyclodextrins in colorants can be illustrated by the figures of a literature survey on the number of relevant papers and patent applications (Table I).

As seen from the above data, the most intensively studied field is the complexation of synthetic dyes, mainly azo-dyes, with cyclodextrins. As far as the cyclodextrin/natural color interaction is concerned, the most studied field is the carotene-cyclodextrin interaction.

The aim of the present study was to select the most appropriate types of cyclodextrins for stabilization and solubilization of lipophilic natural colorants. Particular attention was paid to the stability and solubility of the carotene/cyclodextrin inclusion complexes.

Table I. Number of papers and patent applications on cyclodextrin/color complexation found in CYCLOLAB's database in August 1997.

Key words	No. of papers	No. of patents	Total No.
Color	100	96	196
Dye	188	68	256
Carotene	14	17	31
Anthocyanine	9	2	11
Curcumin	4	2	6
Betalains	1	0	1
Chlorophyll	1	4	5
Xanthophyll	1	0	1

2. Materials and Methods

2.1. MATERIALS

The parent α-cyclodextrin and the methylated-β-cyclodextrin of DS =1.8 (RAMEB) were produced by Wacker Chemie, Munich, Germany and used without further treatment.

β-cyclodextrin (βCD), 2-hydroxypropylated-β-cyclodextrin of DS = 2.7–5.0 (HPBCD), the water soluble-β-cyclodextrin polymer and the per-O-acetylated, (heptakis 2,3,6-tri-O-acetyl)-β-cyclodextrin were prepared by Cyclolab Ltd., Budapest, Hungary

The branched (glucosylated, maltosylate) β-cyclodextrin (G_2BCD) was the product of Ensuiko Sugar Refining Co., Yokohama, Japan. Crystalline β-carotene of over 95% purity was purchased from Sigma Co. (St. Louis, USA). Curcumin (over 98% purity) was the product of Carl Roth (Germany).

Lipophilic curcuma and capsicum oleoresins were prepared by Soxhlet-extraction using *n*-hexane solvent from dry Curcuma longa (turmeric) and Capsicum annum (Hungarian paprika) plants. All other reagents used were of analytical purity.

2.2. PREPARATION OF CYCLODEXTRIN COLORANT COMPLEXES

The complexation of natural colorants was carried out by using suspension and co-crystallization techniques [1, 2].

2.3. ANALYSIS OF THE COMPLEXES PREPARED

The total colorant load of the solid inclusion complexes was determined by UV-VIS spectrophotometry on a Hewlett Packard 89532Q type diode-array spectropho-

tometer according to the published method [3]. The curcumin calibration and the curcumin content of complexes were determined at an absorption maximum of 422 nm in 50% (v/v) aqueous ethanolic solution.

The spectrophotometric determination of the β-carotene in the cyclodextrin complexes was done at the absorption maximum of 465 nm. The calibration curves for β-carotene determination were recorded in chloroform solution. A linear absorption concentration relationship was obtained in the concentration range of 1–15 μg/mL. For spectrophotometric analysis the solid carotene/cyclodextrin complexes were dissolved in dimethyl-formamide then diluted with chloroform.

Capillary gas-chromatography was used for measurement of the curcuma oleoresin content in solid samples according to Harangi et al. [4].

2.4. THERMOANALYTICAL INVESTIGATIONS

Evolved Gas Analysis of the free-, adsorbed- and complexed colorants was carried out on a DuPont 630 thermoanalytical system using a FID detector to detect only the loss of volatile organics – and not water – upon heating in a nitrogen stream according to a previously published method. The Evolved Gas Analysis provides reliable quantitative data on the extent of release of entrapped volatile organics from solid formulations (mechanical mixtures, inclusion complexes). In the temperature range at which the guest substances show a complete thermal loss the corresponding true inclusion complexes will release only a small fraction of their guest content. Mechanical mixtures of the host and guest have thermal release profiles similar to that of the original plain guest [5].

3. Results and Discussion

3.1. STABILIZATION OF CURCUMINOID COLORANTS BY CYCLODEXTRINS

The use of **e**volved **g**as **a**nalysis (EGA) was found to be an adequate method for the quantitative description of the thermal release of volatiles from solid Curcuma oleoresin/βCD complexes upon heating. The amount of fractions of volatile organics released form the solid samples are listed in Table II. These data can be considered as a measure of the heat resistance of molecularly entrapped curcuminoid type natural colorants over the non-complexed species.

The stabilizing effect of the molecular entrapment of curcuminoids by cyclodextrins is obvious by the loss of organic volatiles in the range of 65–130 °C. The non-complexed form lost more than 80% of its original organic volatile content upon heating. It contained only a small amount of its curcuma oleoresin content in the temperature range of 140–200 °C.

From the β-cyclodextrin complexed curcuma oleoresin only about 20% of the curcuma content escaped when heated up to 130 °C. The complexed curcuma volatiles were heat resistant in the temperature range of 65–90 °C. The thermal decomposition of the solid complex started only above 140 °C. The lactose-adsorbed

Table II. Quantitative description of the thermal stability of Curcuma oleoresin formulations by Evolved Gas Analysis (EGA).

Sample	Released organic volatiles in percentage of the curcuma content of samples upon heating in different temperature ranges			
	65–90 °C	100–130 °C	140–160 °C	170–200 °C
Curcuma oleoresin alone	62	21	6	2
Curcuma oleoresin on lactose	71	18	4	Not detectable
Curcuma/βCD complex	8	12	48	26

Table III. Loss of curcumin content in the lactose-adsorbed and βCD complexed pure curcumin samples upon a 12-hour irradiation with UV_{365nm} light at 25 °C in the solid state spread in 1 mm layers onto a glass surface.

Sample	Remnant curcumin content (%) of samples determined by UV spectroscopy at λ = 422 nm			
	Time zero	2 hours	6 hours	12 hours
Curcumin-lactose mixture	11	8.8	7.2	6.0
Curcumin/βCD complex	10.9	10.5	10.6	9.8

curcuma showed a thermal release profile similar to that of the curcuma oleoresin itself.

4. Light Stability of Pure Curcumin/βCD and Curcuma Oleoresin/βCD Complexes

Both the curcuma oleoresin and the crystalline curcumin substance have been known to exhibit sensitivity toward light irradiation especially when exposed to ultraviolet light. The comparative stress light stability tests were done by exposing the free, adsorbed and complexed samples of curcumin to λ = 365 nm ultraviolet light-irradiation and following the loss of the intact curcumin content by a UV-spectrophotometric method. Samples were taken at different time intervals, dissolved and assayed for curcumin content by spectrophotometry. The results of the light stability test are listed in Table III.

5. Long-Term Storage Stability Data

Since the first curcumin- and curcuma oleoresin/β-cyclodextrin inclusion complexes were prepared in May 1981 there has been a possibility to see how the curcumin content of these samples changes during storage in closed, colorless

Table IV. Results of the long term (14 years) storage test of β-cyclodextrin complexed curcumin and curcuma oleoresin in closed glass vials under normal conditions.

Samples	Remnant curcumin content (%) of complexes determined by UV spectroscopy at λ = 422 nm						
	In 1981	In 1982	In 1984	In 1986	In 1988	In 1992	In 1996
Curcumin βCD complex	10.9	9.7	9.0	8.8	8.6	8.9	8.4
Curcuma oleoresin/βCD complex	8.9	7.3	6.7	6.3	5.9	4.5	4.6

glass vials under normal storage conditions. Samples have been taken periodically during the past 14 years and the intact curcumin content of the samples was determined using UV-VIS spectrophotometry. The results of the long term storage test are summarised in Table IV.

As can be seen from the above data the β-cyclodextrin complexation provided a remarkable shelf life improvement for curcumin and a less pronounced effect on the curcuma oleoresin, when compared to the analytical standard curcumin and curcuma oleoresin sample stored in a freezer under nitrogen, in sealed ampoules. This latter effect might be due to the interaction of sequiterpenoid constituents of the extract with the cyclodextrin cavity, thus replacing less lipophilic curcumin (diferuloyl-methane derivative) in the molecular container by the more ideal terpenoid-type guests present in the extract.

5.1. STABILIZATION OF CAROTENOIDS BY CYCLODEXTRINS

Most of the literature data – altogether 38 papers dedicated to carotene/CD interactions – on the complexation of carotenoids refer to the fact that carotenes, as such, are good complex forming guests in terms of their chemical structure, polarity and molecular dimensions.

The good complex forming property of the carotenoids, however, does not always manifest in the improved chemical stability of the complexed colorant. The following stress stability data supports this statement and helps to select the most appropriate type of cyclodextrin for carotenoid stabilization.

6. Thermal Stability of Carotene Cyclodextrin Complexes

It has been observed that the complexation of pure crystalline β-carotene with β-cyclodextrin results in a solid formulation that exhibits a rather poor stability against heat. However, when the carotene was formulated with natural plant oils, like a mixture of β-carotene with peanut oil, the complexes formed with oily β-carotene showed much better stability against heat and light. The list and com-

Table V. Composition of cyclodextrin complexes prepared from pure, crystalline β-carotene.

Sample	Carotene content (%)	Water content (%)
β-carotene/αCD	8.9	5.5
β-carotene/βCD	10.1	6.8
β-carotene/γCD	7.5	4.9
β-carotene/ RAMEB	6.0	3.3
β-carotene/ HPBCD	6.6	4.6
β-carotene/ per-O-acetyl-βCD	7.0	3.9

Table VI. Accelerated heat stability of adsorbed and cyclodextrin complexed β-carotene in the solid state at 60 °C.

Sample	Remnant β-carotene content of solid formulations (%)					
	Time zero	8 hrs	24 hrs	48 hrs	72 hrs	144 hrs
Zeolite-carotene mixture	10.2	5.5	1.9	0.7	0.5	0.3
αCD complex	8.9	8.4	8.5	8.2	7.7	6.9
βCD complex	10.1	6.8	3.2	0.9	0.6	0.6
γCD complex	7.5	7.0	7.0	6.5	5.7	3.4
RAMEB complex	6.0	6.2	5.7	5.4	4.2	4.3
HPBCD complex	6.6	4.3	3.0	2.5	2.7	2.2
Per-O-Ac-βCD complex	7.0	7.1	6.7	6.8	6.4	6.3

position of β-carotene/cyclodextrin complexes prepared and studied are shown in Table V.

Among the above complexes the improved heat stability in the solid state was observed only with αCD, γCD, DIMEB and per-O-acetyl-βCD. It has been found that β-cyclodextrin does not provide the required stabilizing effect, it does not appear to be more stable, than corresponding adsorbed β-carotene on a zeolite carrier. The results of stress heat stability data at 60 °C in the solid state are listed in Table VI.

As can be seen from the above data, among parent cyclodextrins the most feasible stabilizing agent of carotene is α-CD. This result is in a good agreement with earlier published observations [6–8].

However, it was surprisingly found that in the case of commercially available vegetable oil diluted β-carotene mixtures the stabilizing effect of β-cyclodextrin was improved. This might be due to the formation of some ternary complexes between β-cyclodextrin, β-carotene and a suitable fatty acid component of the vegetable oil diluent, resulting in better or deeper fit of the carotene molecule into

Table VII. Accelerated heat stability test of adsorbed and β-cyclodextrin complexed β-carotene diluted in peanut oil.

Sample	Remnant β-carotene content of the formulations (%)					
	At 40 °C			At 60 °C		
	Time zero	8 hrs	24 hrs	Time zero	8 hrs	24 hrs
β-carotene on zeolite	4.5	2.5	1.3	4.5	0.8	0.06
β-carotene /β-CD complex	4.4	4.2	3.8	4.4	4.0	3.6

Table VIII. Change of β-carotene content (%) in complexes and mechanical mixtures prepared from the oily β-carotene upon exposure to UV_{365nm} irradiation in the presence of oxygen at 25 °C.

Samples	Remnant β-carotene content in samples (%)					
	Time zero	2 hrs	4 hrs	8 hrs	12 hrs	24 hrs
Lactose-β-carotene mixt.	4.5	3.1	2.3	1.8	1.2	0.7
Zeolite-β-carotene mixt.	3.7	1.8	1.1	0.7	0.4	0.06
αCD complex	4.5	4.5	4.2	4.3	4.0	4.1
βCD complex	4.4	4.0	3.7	3.7	2.9	2.7

the β-cyclodextrin cavity. If the 30% β-carotene containing peanut oil/β-carotene suspension was used for complexation – instead of pure, crystalline β-carotene – the resulting carotene/βCD complex showed improved stability in the accelerated stability test both at 40 and at 60 °C as detailed in Table VII.

7. Light Stability of β-Carotene-Cyclodextrin Complexes

The resistance of carotenoids to light, in particular in the presence of air, is rather low, most of these polyenes decompose rapidly with the simultaneous loss of their color and biological activity. The effect of UV-irradiation of solid cyclodextrin complexes of β-carotene has been studied both in the case of the complexes of pure synthetic β-carotene and complexes made from peanut oil-β-carotene suspension. Again the tendency observed was that the light stabilizing potency of the studied cyclodextrins for the pure crystalline β-carotene followed the rank order of α-cyclodextrin $>$ methyl-β-cyclodextrin $>$ acetyl-β-cyclodextrin $>$ γ-cyclodextrin $>$ β-cyclodextrin. For demonstration of the stabilizing effect of cyclodextrins the decoloration kinetics of cyclodextrin complexed β-carotene/peanut oil suspension and of the zeolite and lactose based mixtures are shown in Table VIII, when exposed to UV_{365nm}-irradiation under aerated conditions at 25 °C.

Table IX. The solubilizing potency of chemically modified cyclodextrins on curcuma oleoresin in deionised water at 25 °C after a 48 hour equilibration.

CD	Dissolved curcuma oleoresin (mg/mL) in the presence of increasing concentration of CDs				
	Without CDs	5% CD	10% CD	30% CD	40% CD
RAMEB	About 0.01	1.20	7.75	18.20	31.30
HPBCD	About 0.01	0.88	2.21	5.58	8.90
G_2BCD	About 0.01	0.75	2.15	6.00	8.55

As can be seen the exposure of the mechanical mixtures of β-carotene with zeolite and lactose lose their color within five to ten hours, while both the α- and β-cyclodextrin complexes appear to be more resistant toward UV-irradiation.

8. Solubilization of Natural Colors with Cyclodextrins

The complexation of lipophilic natural colors with highly soluble cyclodextrin derivatives leads to significant improvement of their solubility in water under normal conditions. The technological advantages of this type of solubilization method are obvious, since the use of surfactants or organic solvents in certain cases needs to be avoided.

For the solubilization of natural colors only highly water soluble cyclodextrin derivatives, like methylated-, hydroxypropylated- and branched-β-cyclodextrins can be taken into consideration. All these cyclodextrin derivatives are technically feasible, are produced industrially, have reasonable price and possess toxicological data.

The solubilization potency of the above highly water soluble cyclodextrins have been found to be rather different to the natural colors.

In general it can be stated that the solubilizing power of these CD derivatives follows the rank order given below:

methylated-β-cyclodextrin $\gg$ hydroxypropylated-β-cyclodextrin

= branched-β-cyclodextrin

The use of these cyclodextrin derivatives in the concentration range of 10–40% results in practically useful solubility enhancements, below 10% the solubilizing effect is practically less feasible, as shown in detail in Table IX.

A similar tendency was found in the solubilization of natural capsicum oleoresin, the solvent extract of *Capsicum annuum* L., which contained among a number of plant lipids capsanthin as a major colorant, and this extract has a very high color value. The technologically feasible improvement of the aqueous solubility of

Table X. Solubilization of capsicum oleoresin with highly soluble cyclodextrin derivatives in deionised water at 25 °C after a 48-hour equilibrium time.

CD	Dissolved capsicum oleoresin (mg/mL)				
	Without CDs	5% CD	10% CD	20% CD	40% CD
RAMEB	0.03	0.81	2.2	5.8	11.8
HPBCD	0.03	0.06	0.9	1.2	2.2
G2BCD	0.03	0.08	1.1	1.7	2.9
βCD polymer	0.03	0.34	0.7	1.6	3.0

capsicum oleoresin was attained only with randomly methylated-β-cyclodextrin at its higher concentrations (Table X).

The 1000-fold enhancements of the aqueous solubility of multicomponent natural lipophilic colorants attained with 40% methylated-β-cyclodextrin solutions can be of real practical significance.

In a similar manner applying 40% aqueous methyl-β-cyclodextrin solution for solubilization of β-carotene, 10.5 mg/mL dissolved β-carotene concentration can be achieved at room temperature. This aqueous β-carotene solution when filtered sterile, into sterile glass vials remains stable for 6 months at room temperature, exposed to normal daylight. The aqueous β-carotene solution can serve as a water-soluble substrate for tissue cultures and for special microbiological culture media, as a bioavailable polyene source for living cells, without the untoward side effects of the commonly applied organic co-solvents or detergents [9].

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