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Fatty Acid-Cyclodextrin Complexes: Properties and Applications

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Abstract. The complexation of fatty acids (both saturated and unsaturated) with various cyclodextrins and cyclodextrin derivatives greatly modifies their properties. Inclusion complex formation – depending upon the type of host cyclodextrin – may result in protection against the environment, in improved water solubility and bioavailability. Thus lipid complexation enables the preparation of more reliable diagnostic reagents, better chromatographic separations and higher yields in biotechnological processes. The relevant literature is reviewed with particular emphasis on the practical utility of the molecular encapsulation of fatty acids with cyclodextrins.

Key words: Fatty acids, cyclodextrins, stabilization, solubilization, tissue culture, diagnostics.

1. Introduction

Fatty acids, their methyl-, ethyl and saccharose esters, monoglycerides, and similar long apolar alkane-chain containing molecules, steroids, detergents, etc. are generally good complex-forming 'guest' molecules for the cyclodextrin (CD) 'host'. The stability of the complexes formed generally decreases in the following order: fatty acids > mono- > di- > triglycerides.

The solubility of the lipid/CD complexes is generally very low, less than 0.1 mg/mL: therefore, by forming such complexes, lipids can easily be isolated (e.g. from biological systems), or can be protected (e.g. against oxidation). The solubility of the lipid/methylated-CD complexes is, however, quite surprising: e.g. 20–25 mg/mL clear aqueous steroid solutions can be prepared from practically insoluble steroids. Therefore lipid/CD and lipid/methylated-CD complexes should be treated separately.

The aim of the present paper is to highlight the practical significance of fatty acid/CD complex formation partly through relevant experimental observations and partly by summarizing the available literature dealing with the complexation of fatty acids by cyclodextrins.

The first systematic studies on fatty acid/CD complexes were published by Schlenk and Sand in 1961 [1]. They studied the composition of fatty acid/CD

complexes (their molar ratio) and the length of the channel in the complex as a function of the number of carbon atoms in the acid.

The physical properties (e.g. the solubility in water) of apolar homologues of organic compounds are usually a logarithmic function of the chain length. In the presence of CD this relationship does not hold, because complex formation brings about differences in the stretching of the various fatty acids. The available channel length is more or less identical with the actual length of the fatty acid molecule.

On average, five CH_2 groups are coordinated to one CD molecule, but deviations can be found. In the case of αCD, the C_8, C_{10} and C_{11} acids require channels longer then the actual length of the acid. In the case of βCD the opposite tendency has been observed: the C_{13}, C_{14} and C_{15} acids occupy the same space as the C_{12} acid. A similar leveling off has been observed with C_{17} and longer acids at a 3 : 1 CD/acid molar ratio. Constant values have been found at about 1.5, 2 and 3 CD/acid molar ratios; these may probably be regarded as the theoretical values for the acids.

The solubility of members of the homologous series of aliphatic fatty acids in an aqueous CD solution increases from caproic acid onwards; for smaller fatty acids it is slightly lower than the solubility in water. The increase in solubility is 1.2–30-fold [1].

From the solubility data it has been calculated that a C_{18} acid and a C_{12} acid are associated with about 2.8 and 1.9 αCD molecules. Miyajima *et al.* [2] reported that an αCD accommodates 6.3 CH_2-units of the monophosphathidylcholine. From similar studies [3, 4] it is concluded that about 6 CH_2-units of a fatty acid need one CD capsule.

Szejtli and Bánky [5] investigated the relationship between the parameters of fatty acid complex formation and the composition of the product. The increase of the fatty acid concentration was the only parameter which could appreciably increase the fatty acid content of the complex; with the amylose complex the maximum fatty acid content was only 4.1%, but 10% could be incorporated by βCD.

Szejtli *et al.* [6] have found that on reacting with a mixture of saturated and unsaturated fatty acids βCD prefers the unsaturated species over the saturated ones. According to Pauli and Lach [7] the derivatives of saturated fatty acids form βCD complexes more readily than the corresponding unsaturated fatty acid derivatives.

The exact structures and stoichiometries of α- and βCD complexes of linoleic and arachidonic acids in solution were characterized by ^1H spin-lattice relaxation time measurements and one-dimensional difference nuclear Overhauser enhancement (1D NOE) studies. These studies of Jyothirmayi *et al.* [4] concluded that the carboxylic functions of both linoleic and arachidonic acids are located inside the CD cavity and hydrogen bonded to a primary C_6-OH group. The $C_9{=}C_{10}$ double bond of linoleic acid is at least partly buried in the CD cavity, therefore it is protected against oxidation. The remaining segments of the fatty acid (in D_2O, under conditions of NMR spectrometry) are located outside of the cavity. Because of the geometry of the *cis* double bonds at positions 5, 8, 11 and 14 in arachidonic acid,

both arms of the molecule, one with the carboxyl and the other with the methyl end lie close to one another in this 'horseshoe' configuration. The carboxyl arm penetrates deeply in the cavity, while the methyl-end arm lies parallel to the carboxyl arm, but outside the cavity.

The molecular encapsulation of linoleic and arachidonic acid by CDs resulted in the inhibition of lipoxygenase oxidation of the sensitive substrates. This effect was due to the inclusion complex formation by which the oxidizable free, monomeric forms of substrates were reduced. The activity of lipoxygenase enzyme was found not to be affected by CDs [8].

The pronounced and selective affinity of α-, β- and γCDs to lipid-hydroperoxides was studied by following the changes of peroxide values of auto-oxidized substrates (linoleic, and oleic acids) upon addition of aqueous CD solutions [9].

2. Experimental

The parent α- and β-cyclodextrins of over 99% purity, and RAMEB (randomly methylated β-cyclodextrin with an average degree of substitution DS = 1.8) were products of Wacker Chemie GmbH, Munich, Germany. The HPBCD (2-hydroxypropylated βCD) of DS = 2.8 and the crystalline DIMEB (= heptakis (2,6-di-*O*-methyl)-βCD) of DS = 2.0 were the products of CYCLOLAB, Ltd., Budapest.

The studied fatty acids were purchased from Sigma Co. (St. Louis, USA) of analytical purity and used without further purification. The vegetable oils – common cold-pressed sunflower oil and linseed oil – were purchased from a local food store and used as obtained.

The solid α- and βCD complexes were prepared by the suspension technique at room temperature as described earlier [5].

The water soluble fatty acid complexes are produced and marketed by CYCLOLAB Ltd. [10].

The interaction of fatty acids with the chemically modified CDs in solution was characterized by registering the phase solubility isotherms in distilled water at room temperature after 48 h equilibration with stirring. The dissolved fatty acid concentration in the clear filtrates was determined by HPLC. The chromatographic analyses were performed on a Hewlett-Packard HP 1050 Series chromatograph, equipped with an autosampler and a multiple wavelength detector operating at 200 nm (bandwidth 6, the reference wavelength 350 nm, bandwidth 80). The separation was achieved using a reversed-phase 10 cm × 4 mm I.D. column (Nucleosil 120-3 C18, Macherey-Nagel, Düren, Germany). The samples were eluted with acetonitrile-water-phosphoric acid (75 : 25 : 0.15) at a flow rate of 1.5 mL/min. Integration and calculations were carried out using a Hewlett-Packard HPLC Chem-Station.

The solid state of the complexes was characterized by X-ray diffractometry on a Philips PW 8010 type diffractometer. The thermal behaviour of the free and

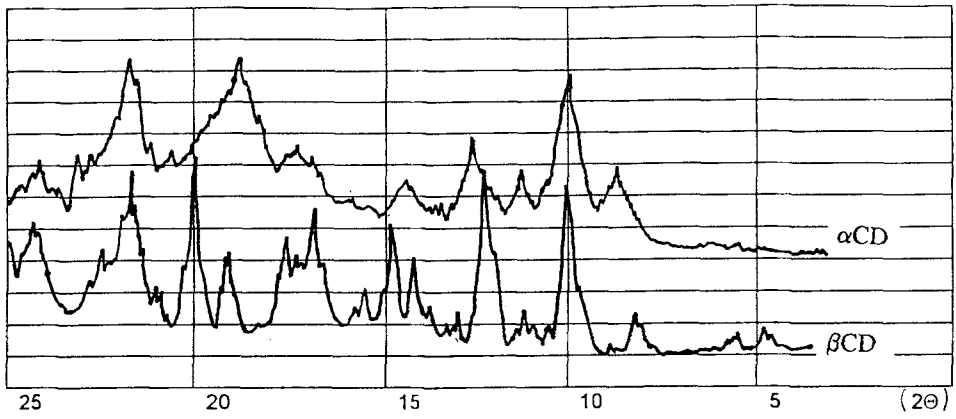

Fig. 1. X-ray powder diffractograms of sunflower oil α- and β-CD complexes prepared under identical conditions.

complexed fatty acids in the solid state was characterized by thermal analysis (TEA and DSC) on a Du Pont 630 thermoanalytical system. The resistance of the complexed unsaturated fatty acids to pure oxygen was demonstrated by determining the oxygen uptake at 37°C in a Warburg apparatus [11].

3. Results and Discussion

3.1. Poorly Soluble Fatty Acid/CD Complexes

The aqueous solubility of fatty acid/CD complexes is generally less than 0.1 mg/mL, i.e. expressed as fatty acid less than 0.01 mg/mL. This value is higher than without CD and depends on the chain length. This seemingly very low, but improved solubility seems to have a significance e.g. in tissue cultures, microbiological nutrient media, etc. A hundred- to thousand-fold better solubility enhancement can be attained by the chemically modified CDs.

Considering the diameter of the CD cavities it is not surprising that for the C_{10} to C_{18} long slim fatty acid molecules (both as free acids or glycerides) αCD is the best complexing host.

The crystallinity and disorder parameters are different in the complexes of α-, β- and γCDs prepared under identical conditions. The X-ray diffractograms of sunflower oil/ α- and β-cyclodextrin complexes show that in the solid state quite different crystallinity is established in the different hosts (Figure 1).

The stabilizing effect against oxygen as measured by the Warburg-technique interestingly does not prove the superiority of αCD over βCD. As Figures 2 and 3 illustrate the oxygen consumption of the complexed linoleic acid and linseed oil is almost negligible when compared to that of the free, noncomplexed species.

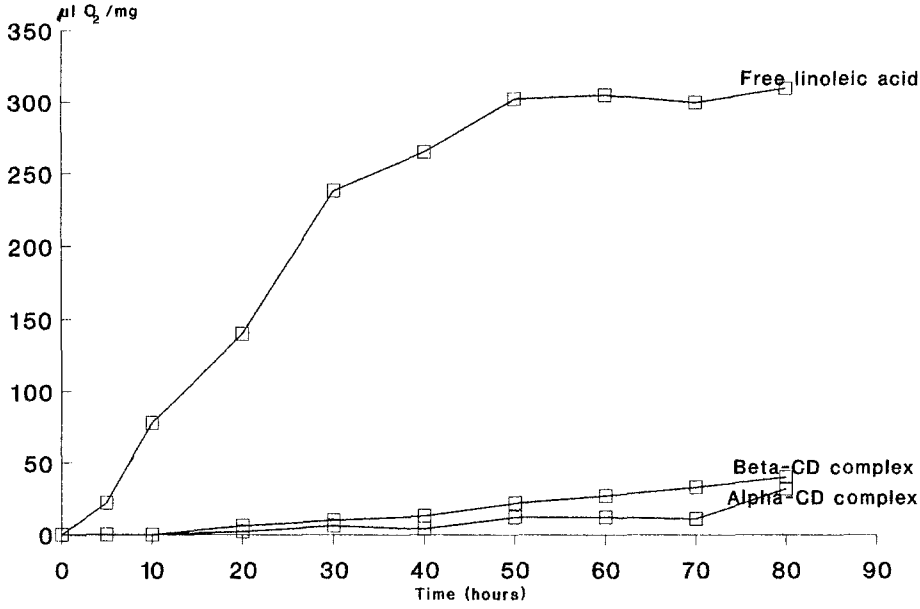

Fig. 2. Oxygen uptake of free- and cyclodextrin-complexed linoleic acid measured by the Warburg method at 37°C in a pure oxygen atmosphere.

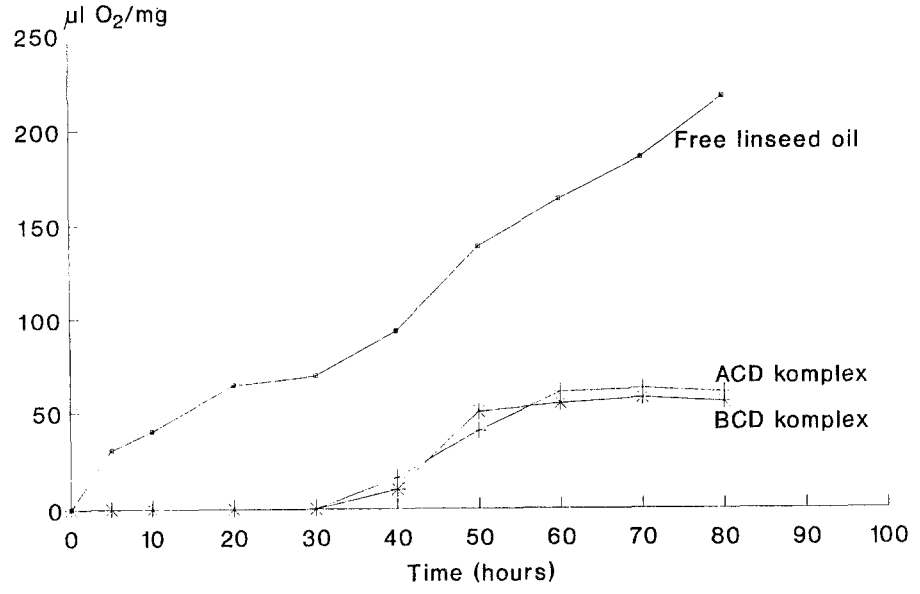

Fig. 3. Oxygen consumption of free- and cyclodextrin-complexed linseed oil in a pure oxygen atmosphere at 37°C measured by the Warburg method.

Interestingly no significant differences are observed between the stabilizing effect of α- and βCDs. Generally it can be stated that CD-complexed unsaturated fatty acids are fairly well protected against atmospheric oxygen. These findings are in good agreement with the results of a previous paper dealing with the stabilization of eicosapentaenoic and docosahexaenoic acids by CDs [12].

The actual load of a proper inclusion complex of an average lipid molecule with βCD varies between 8–10% by weight due to the molecular stoichiometry of fatty acid to CD of approx. 1 : 2 or 1 : 3.

During the last few years the number of papers on the advantages of using methylated CDs in biotechnological processes has been rapidly increasing. In these cases directly or indirectly the CD-complexation of some lipid plays the key role.

3.2. Water Soluble Inclusion Complexes of Fatty Acids

Among the several hundred published chemically modified CDs only two are produced industrially, and readily available: the methylated and the hydroxypropylated βCDs.

Three different types of methylated CDs can be considered [11].

TRIMEB = heptakis(-2,3,6-tri-*O*-methyl)-β-CD = permethylated βCD. This CD derivative is used e.g. in gas chromatography, but produced only on a laboratory scale. Its aqueous solubility is lower than that of the partially methylated CDs, moreover the permethylation of βCD is not a simple task. Considering its properties and price its broad utilization in lipid chemistry is not expected.

DIMEB = heptakis(2,6-di-*O*-methyl)-βCD = dimethyl-βCD. Theoretically this compound contains 14 methoxy groups, seven substitute the primary hydroxyl groups on the narrower edge of the cyclodextrin cylinder, and seven substitute all C(2) hydroxyls while all C(3)-OH remain unaltered. This substance is crystalline and possesses the highest known solubilizing property for lipids, but because of its complicated preparation and price it is not produced on an industrial scale.

RAMEB = randomly methylated βCD. This partially methylated βCD contains 1.7 to 2.0 methoxy groups/glucopyranose units. Being a rather heterogeneous, amorphous, non-crystallizable product, in most cases it is somewhat inferior in its properties as compared to DIMEB, but as its production is simpler, much cheaper, and produced industrially, it is easily available.

The other feasible highly water soluble CD derivative is the 2-hydroxypropylated-β-cyclodextrin (HPBCD). This substance is a multicomponent, heterogeneous, amorphous hygroscopic mixture, with an average degree of substitution of DS = 2.7 to 3 or 5 to 7 hydroxypropyl groups/CD ring. This derivative can be considered as a potential future parenteral drug carrier [11].

Relevant data on lipid/modified-CD interactions have been published on studies using DIMEB, RAMEB and HPBCD.

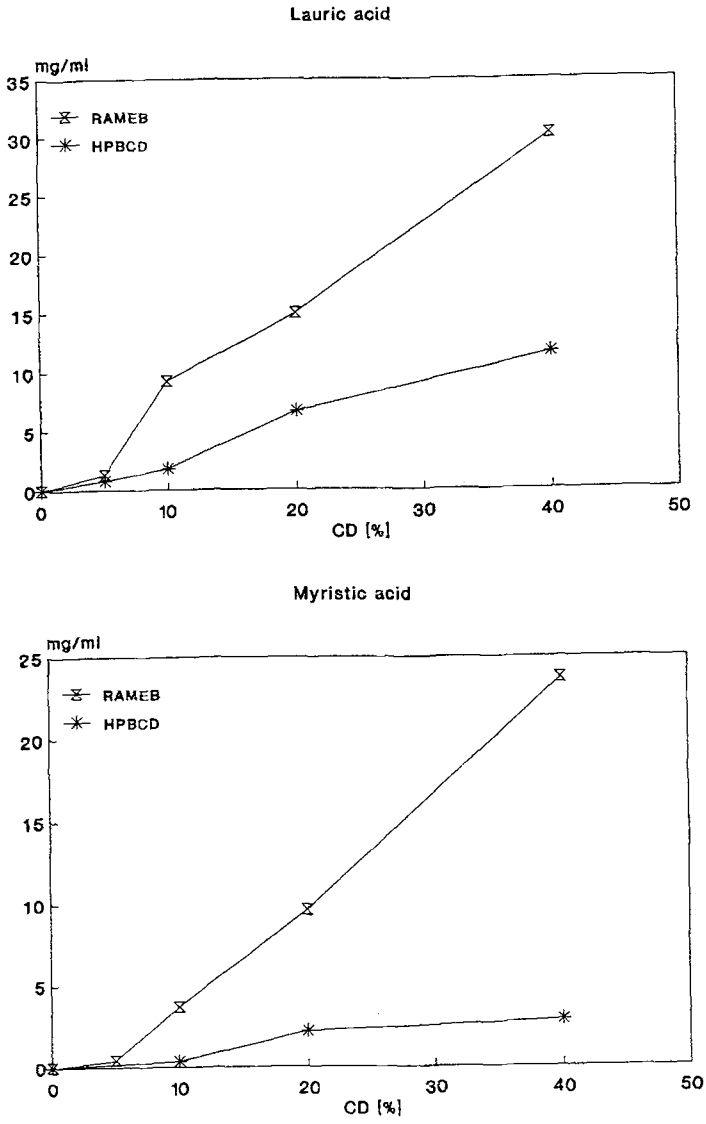

Fig. 4. Solubility isotherms of lauric and myristic acids in aqueous RAMEB and HPBCD solutions at room temperature.

The solubility of fatty acids in water was found to be significantly improved by highly water soluble CDs. In general it was found that the methylated βCDs were the most potent solubilizing agents for all the studied substrates regardless of the chemical structure and geometry of the fatty acids. Figures 4 and 5 show the phase solubility diagrams of different model fatty acids, in aqueous solutions of methylated- and 2-hydroxypropylated βCDs.

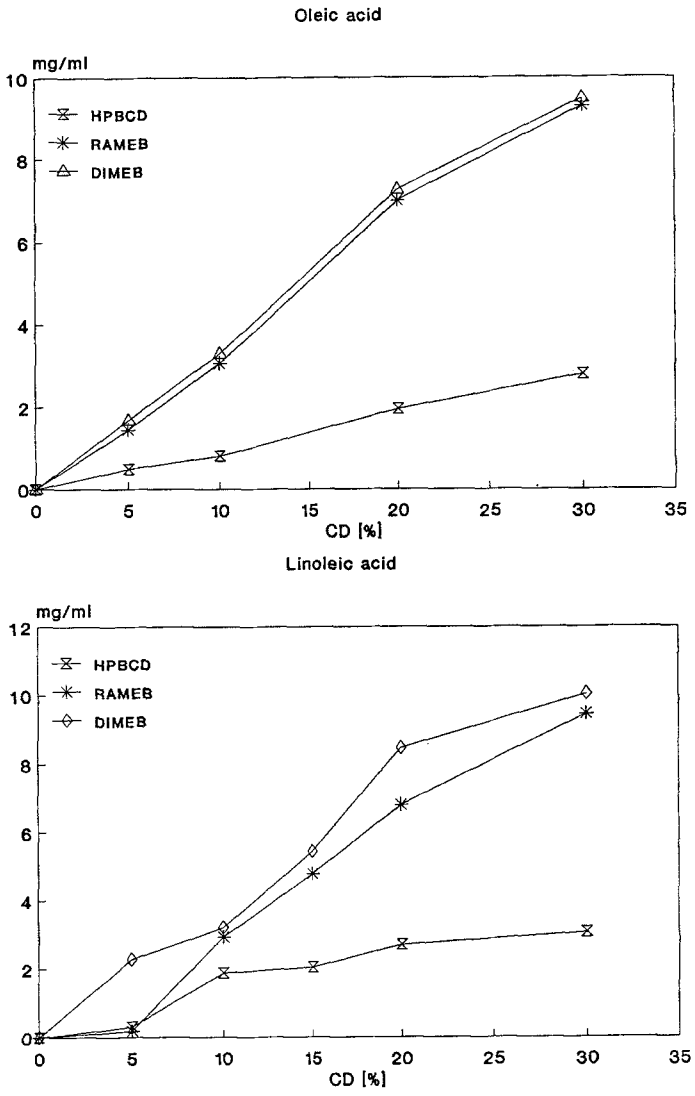

Fig. 5. Solubility isotherms of oleic and linoleic acids in aqueous RAMEB, DIMEB and HPBCD solutions at room temperature.

As the solubility profiles illustrate the solubilizing power of methylated βCDs greatly surpasses that of the hydroxypropyl-βCD. The solubility enhancements obtained with randomly methylated-βCD were not much different from those achieved by the crystalline heptakis-2,6-di-*O*-methyl-βCD.

The solubility enhancement data of oleic, linoleic, linolenic, eicosapentaenoic and docosahexaenoic acids in aqueous solutions of methylated and hydroxypropylated βCDs shows that the increasing number of double bonds within a fatty acid

TABLE I. Fatty acid content (% w/w) of water soluble lipid complexes prepared by freeze-drying the aqueous solutions of RAMEB or DIMEB solubilized fatty acids.

Substrate	RAMEB	DIMEB
myristic acid	2.8	3.0
lauric acid	3.3	3.8
stearic acid	1.7	2.0
palmitic acid	1.8	1.6
Na palmitate	2.0	1.9
ascorbic palmitate	4.0	4.8
cholesteryl palmitate	1.1	1.2
palmitoleic acid	2.0	2.2
ethyl palmitoleate	3.1	3.3
oleic acid	4.0	3.3
linoleic acid	3.6	3.1
linelenic acid	2.5	2.3
eicosapentaenoic acid		
docosahexaenoic acid	2.0	1.8
1 : 1 mixt.		

molecule results in the formation of a more stable inclusion complex. This might be due to the non-linear structure and more compact geometry of the fatty acids which provides a better fitting into the cavities of CDs than the fitting of a more linear long chain 'thread'. The entrapped fatty acid contents of the water soluble solid complexes were found to vary in a range of 1 to 5% by weight (see Table I).

The normal and high resolution DSC curves of palmitic acid and RAMEB formulations (free fatty acid, mechanical mixture and the inclusion complex) indicated that the characteristic heat flow due to the melting process of the palmitic acid at 63°C disappeared proving that practically no free fatty acid was present in the complex sample (Figure 6). The stabilizing potency of the inclusion complex formation for the volatile lipids against evaporation was demonstrated by the example of water soluble eicosapentaenoic acid (EPA) with RAMEB and DIMEB. Figure 7 presents the results of TEA (Thermal Evolution Analysis) on the free, adsorbed and complexed EPA in a nitrogen atmosphere upon heating. As clearly seen the free and adsorbed EPA show a well defined thermal release in the temperature range of 100 and 190°C with a maximum EPA vapour concentration at 155–162°C.

However, the really complexed species exhibits an almost negligible thermal escape of any volatile organic throughout the heating process up to the temperature of 270°C, where the thermal decomposition of the carbohydrate matrix (cyclodextrin) takes place. The solid, water soluble lipid complexes were also found to possess good storage properties under normal conditions in paper and polyethy-

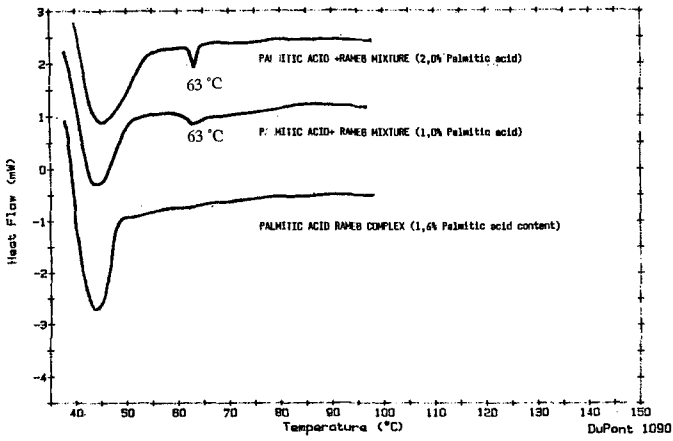

Fig. 6. Heat flow curves (DSC) of free-, adsorbed- and RAMEB-complexed palmitic acid.

TABLE II. Remnant active ingredient content of solid water soluble lipid complexes upon storage at 60°C in open glass vials, under normal humidity. Results are expressed in % (w/w).

Sample	Time zero	2 Months	6 Months	8 Months
Eicosapentaenoic-RAMEB	2.3	2.6	no data	1.7
Ascorbic-Palmitate-RAMEB	4.0	3.7	4.2	3.9
Ascorbic-Palmitate-DIMEB	4.8	4.8	4.0	4.6
Cholesteryl-Palmitate-RAMEB	1.1	1.3	1.0	0.8
Cholesteryl-Palmitate-DIMEB	1.2	no data	1.0	1.2
Oleic acid-RAMEB	4.0	4.1	3.7	3.7
Linoleic acid-RAMEB	3.6	3.6	3.2	3.4
Linolenic acid-RAMEB	4.1	3.9	3.3	3.0
Lauric acid-RAMEB	3.6	3.2	3.2	3.3
Myristic acid-RAMEB	4.2	4.3	4.0	4.2
Cetylalcohol-RAMEB	4.0	4.0	no data	no data
Myristylalcohol-RAMEB	4.6	4.8	4.2	no data

lene bags, as well as in glass vials. The results of the accelerated storage stability test in open glass vials at 60°C are summarized in Table II.

The sensitivity of the fatty acid DIMEB complexes against humidity was found to be acceptable, even under 92% R.H.; a six-day storage did not cause significant increase in the clumping tendency of solid complexes. However, far less humidity-resistant are the water soluble lipid RAMEB complexes in the solid state. These formulations cannot be stored without the risk of considerable moisture sorption in open bottles at higher than 60% R.H. for days.

The redissolution properties of the water soluble solid formulations are generally acceptable, however, in certain cases the 5 to 15 fold dilution of the concentrated

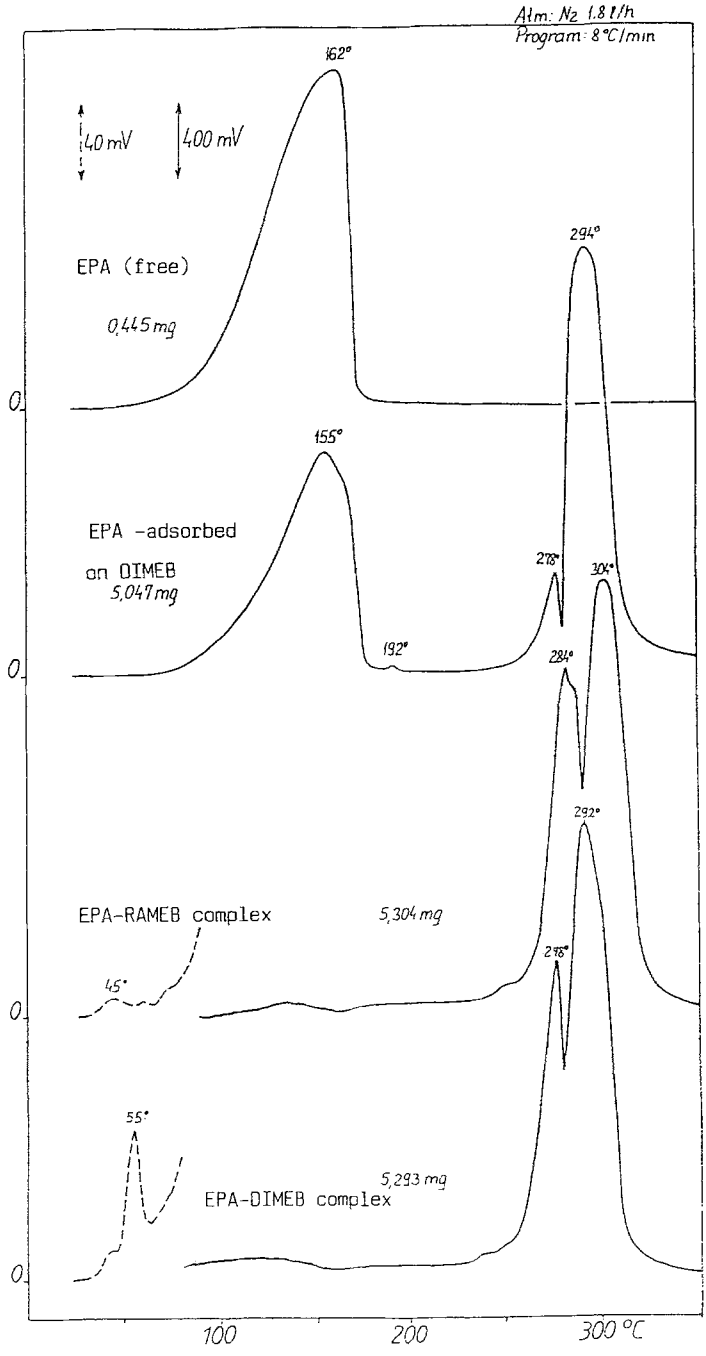

Fig. 7. Thermal Evolution Analysis (TEA) of free- and complexed eicosapentaenoic acid (EPA) with RAMEB and DIMEB.

stock solutions with water results in more or less intense turbidity, depending on the association constant of the complex formed. This can be practically avoided by using buffered aqueous cyclodextrin solutions as diluents instead of water.

The water soluble fatty acid complexes with RAMEB can be autoclaved, without any crystallization in contrast to the lipid complexes with DIMEB (heptakis-2,6-di-*O*-methylated-βCD) and its inclusion complexes have been known to crystallize in aqueous solutions upon heating.

This practical inconvenience can easily be avoided using RAMEB-based water soluble complexes for such purposes where heat sterilization is necessary (e.g. in microbiological studies, in tissue cultures, in enzymology and biotechnology).

The reconstituted, sterile filtered solutions from the water soluble lipid complexes were stored in sealed glass ampoules for six months and neither precipitate formation nor opalescence were observed, the solutions remained clear.

3.3. Practical Utilization of Fatty Acid-CD Interactions

Even in the early 1960s aqueous-ethanolic αCD was recommended for the detection of lipids on thin layer chromatographic plates, based on a publication of French [13] of 1949.

The determination of the calcium level in the blood of hemodialysis patients is a frequent task. Non-esterified fatty acids present in heparinized samples from non-fasting patients cause artificially reduced calcium values in some colorimetric methods. A 10 min preincubation of the serum sample with αCD can fully eliminate this interference [14].

The treatment of serum with non-modified CDs produces an immediate and selective flocculation of lipoproteins. The almost quantitative formation of water insoluble inclusion complexes enables the effective and selective removal of lipids from serum samples with the avoidance of harmful organic solvents upon sample preparation and purification in clinical diagnosis [15].

The complex forming potency of αCD with chylomicrons, very-low, low- and high-density lipoproteins was used for clarifying lipemic serum. In contrast to the use of organic solvents or ultracentrifugation, this method provides a simple, mild, non-hazardous and effective means for removal of interfering lipid particles from biological specimens.

The ability of non-modified CDs to form inclusion complexes with lipoproteins was found to be in the order of βCD $>$ αCD $>$ γCD. Among lipoproteins the order of reactivity with a given CD was: low density lipoproteins $>$ high density lipoproteins $>$ very low density lipoproteins [16].

The CD complexes of unsaturated fatty acids can be utilized as serum substitutes in mammalian cell cultures. Both oleic acid-βCD and linoleic acid-βCD complexes exhibited a growth-enhancing effect of human lymphoblast cells, up to 100 mg/L of medium. At higher concentration, the fatty acid-βCD complex proved to be toxic, but this can probably be attributed to the fatty acids: no toxic effects resulted from

the 100 mg fatty acid-βCD complex and 1000 mg free βCD, but this exhibited a stable and reproducible growth-promoting effect.

In human diploid fibroblast cultures, growth similar to that in a medium supplemented with bovine albumin was observed after bringing the fatty acid-βCD complex solution to a final concentration of 10 to 20 mg/mL. Bovine serum albumin can be partially or completely substituted by fatty acid-βCD complexes in mammalian cell cultures [17], e.g. in human interferon production [18].

Mouse mammary tumour cells can be cultured under serum-free conditions when the bovine albumin is substituted by an αCD complex of oleic acid [19, 20].

During the last years there has been a rapid increase in the number of papers on the advantages of using methylated CDs in biotechnological processes. In these cases directly or indirectly the CD complexation of some lipid plays the key role.

The first recognition that the formation of inclusion complexes between CDs and fatty acids results in biological consequences was done by studying the interaction of bacterial Palmitoyl-CoA and methylated CDs [21].

Fatty acid synthesis by *Mycobacterium phlei* is stimulated by CDs, most markedly by α- and the least by γCD. Methylated (dimethyl-αCD and dimethyl-βCD) CDs are much more efficient stimulants, but also the heptakis(2-*O*-propyl)-, pentakis(6-*O*-methyl)-, heptakis(3-*O*-methyl)- and permethylated βCDs showed such fatty acid synthesis stimulating effect [22].

Because the methylated CDs are not noxious for living microbial cells, they do not denaturate their enzymes, their application particularly in lipid chemistry and biochemistry opens quite new possibilities. Using appropriate CDs, reactions can be performed in homogeneous lipid/water systems which until now seemed impossible. It has to be stressed that CD complexation is not a 'micellization' that can be achieved by detergents, but a true 'molecular dispersion', i.e. a true solution of the lipid.

Water-soluble palmitic acid and sodium palmitate in complexed form with methylated βCD have been found to act as a bioavailable energy source for cellular ATP formation in *Mycobacterium leprae* strains under laboratory conditions [23, 24].

In culture media containing a water soluble complex of dimethyl-βCD (DIMEB)-Na palmitate, facultative psychrophilic mycobacteria were cultivable from *M. Leprae* infected tissues. The medium contained in 1 L water 0.3 g Na palmitate/DIMEB complex as a water soluble carbon source for *de novo* ATP synthesis. In another experiment the RAMEB complexes of palmitic acid, Na palmitate, myristic acid, cetyl alcohol or myristyl alcohol at a concentration of 0.3 g/L were used.

The water soluble complexed C_{14} myristic acid or myristyl alcohol in the media resulted in a considerably higher bacterial count and shorter latency growth period as compared to the C_{16} palmitic acid, palmitate or C_{16} cetyl alcohol in media inoculated with host grown *M. Leprae* cells and incubated at +10°C or 30°C [25].

The production of monensin – a polyether antibiotic – was almost doubled on a methyl oleate containing medium in the presence of 2 mg/mL dimethyl- βCD [26].

TABLE III. Removal efficacy (in %) of washing liquids on contaminated surfaces, using model lipophilic contaminants as propolis, palmitic acid and deposited proteins as real contaminants from textile, glass surface, worn contact lens, and tracheal canula.

Sample	Distilled water	Detergent	HPBCD	RAMEB
canula/protein (metal)	12%	38%	24%	80%
contact lens/protein (plastic)	8%	22%	18%	78%
cotton/propolis (textile)	6%	30%	32%	66%
glass/palmitic acid (glass)	3%	45%	38%	85%

Pertussis toxin (leukocytosis promoting factor, LPF-heamagglutinin) is one of the main protective antigens against whooping cough infection, and is also one of the components of a pertussis vaccine. It is produced by *Bordatella pertussis*, which is very susceptible to a number of inhibitors, e.g. fatty acids (palmitic or oleic acid), and already at a concentration of 10 μmol/L cell propagation stops [27]. However, by adding 0.5 mg/mL of dimethyl βCD (or trimethyl-βCD), an increase in cell growth was observed.

The enhancement of the production of filamentous heamagglutinin was even higher: several hundred times more was produced in the presence of dimethyl-βCD, than without the solubilizer [28].

The diagnosis of whooping cough is not an easy task in clinical practice, because *Bordatella pertussis* phase I is a finicky, fastidious, slow-growing bacterium that is difficult to isolate on laboratory media. Now a medium containing dimethyl-βCD with a long shelf life has been reported, which overcomes the former problems [20]. Similarly, CDs stimulated the *in vitro* growth of *Mycobacterium lepraemurium* [29].

RAMEB can be used for the effective removal of water insoluble lipid-protein complex and other type of lipophilic contaminations, deposits from sensitive, valuable surfaces (e.g. contact lenses, fiber optics and other medicinal accesories) where the application of common organic solvents or detergents are to be avoided [30]. The efficacy of washing liquids consisting of 10%(w/w) hydroxypropylated or 10% of randomly methylated βCDs and the same amount of a common detergent was tested by following the dissolved contaminant concentration in washing liquids. The results of such cleansing experiments are listed in Table III.

The hydrolysis of triglycerides by lipases in aqueous systems is a very slow process. Either a lipid-dissolving, water-miscible organic solvent has to be added to the system (which is tolerated only up to a relatively low concentration because

of enzyme/protein denaturation), or an appropriate detergent, for example natural bile, has to be present. It has been found that the hydrolysis of olive oil by lipase in the presence of hog bile, or dimethyl-βCD as a bile substitute takes place as in the presence of bile [31].

The observation that phosphatides (lignoceric acid, cerebroside, ceramide) can be solubilized with cyclodextrins will certainly be exploited in lipid enzymology [32].

The RAMEB solubilized sterile, aqueous solutions of cerebroside, sphingomyeline and D-sphingosine with a dissolved 10 mg/mL lipid concentration are commercially available [10].

Studying the lignoceryl-CoA ligase activity in a microsomal fraction prepared from rat brain, the lignoceric acid, solubilized by αCD was really utilized, but the one solubilized by Triton WR 1339 was not utilized [33].

Prostaglandins and their derivatives prostacyclins, leukotrienes, thromboxanes etc., being unsaturated hydroxy-fatty acid derivatives are of limited water solubility and are rather unstable. The majority of these compounds are low melting crystals, or viscous oils and are difficult to prepare in homogeneous dilution without considerable degradation. For molecular encapsulation by cyclodextrin complexation prostanoids are ideal guest substances.

The complex structures in solution are different, as are the effects attainable with α-, β- and γCDs. ^{13}C-NMR and magnetic relaxation time investigations show that in an αCD complex ($K = 250\,M^{-1}$) only the alkyl side chain of $PGF_{2\alpha}$ is included in the cavity. In contrast, in the wider βCD cavity the cyclopentane ring and its immediate neighbours are accommodated, resulting in a more stable complex ($K = 1240\,M^{-1}$). In both cases the terminal carboxylic group is assumed to interact with the outside of the cavity through hydrogen bonding. $PGF_{2\alpha}$ forms a 1 : 1 complex with γCD. PGE_1, however, forms a 1 : 2 complex [34, 35].

The decomposition of the prostacyclin methylester (PGI_2-Me) is decelerated by βCD in aqueous solution. The stabilizing effect of βCD is better at low pH values, the ratio of $t_{1/2}$ values for the complexed and the free PGI_2Me is 1.64 at pH 7.6 while it is 3.66 at pH 2.6. Solid PGI_2Me-βCD complex can be stored without decomposition for 20 months at +4°C. Humidity accelerates the decomposition of PGI_2Me in complexed form [36].

The first CD containing drug on the market was the PROSTAMON E tablet, it contains 0.5 mg PGE_2+6 mg βCD (for induction of labour), the second one was the PGE_1/αCD complex (PROSTAVASIN). This formulation comprises 20 μg PGE_1+646.7 μg αCD and is administered as an intra-arterial infusion for treatment of stenosis in extremities or thrombophlebitis.

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