Protection and controlled release of fatty acids and essential oils by nanoencapsulation in cyclodextrins (a review)

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Abstract

The paper presents a review on the obtaining, analysis, and controlled release for the fatty acids (oleic, linoleic, linolenic acids) and essential oils (Dicotyledonatae and Pinatae family plants) / cyclodextrin nanoparticles.

Keywords: nanoencapsulation, controlled release, protection, thermal stability, bioactive compounds, fatty acids, essential oils, cyclodextrins, thermal analysis, chromatography, molecular modeling, multivariate analysis, PCA

1. Introduction

The protection of the bioactive compounds against the environmental, alimentary, or biological degradative factors (air/oxygen, light/UV radiation, humidity etc.) is a major problem due to the degradation products (especially those oxygenated) which have a negative impact to the human health. In the case of the unsaturated fatty acids, the epoxydes and hydroperoxydes, which can be formed in the presence of the oxygen, can lead to free radicals and interact with nucleic acids or proteins from the organism [1]. The linear epoxydes (like those resulted by epoxydation of the oleic, linoleic, and linolenic acids) can easily penetrate the cell membrane and interact especially with the DNA. It is well known that the limonene-dioxide, a degradation compound that appears in the citrus essential oils or other biosystems by oxidation, has neoplastic activity.

One of the best matrices used to protect and controlled release of the bioactive molecules are cyclodextrins (CDs), which are natural cyclic oligosaccharides, containing six (*α*CD), seven (*β*CD), eight (*γ*CD), or more glucopyranose moieties. Cyclodextrins have structures like truncated cones, with hydrophobic inner cavities, which can accommodate geometric compatible bioactive molecules by van der Waals interactions, providing stable complexes (molecular inclusion compounds, supramolecular systems) with very good protecting properties [1-3].

Cyclodextrins were used especially for drug and food additive complexation in order to improve the solubility, the stability, to mask disagreeable odor and taste, and to provide systems with controlled release of the encapsulated biomolecules. In the pharmaceutical field, some studies were performed on the solubilization of the trimethoprim, sufadiazine, and sulfamethoxazole by molecular

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encapsulation in CDs and modified CDs, the encapsulation of the alkylparabens, carbamazepine, and oxyphenbutazone in natural CDs, or the complexation of the barbiturates or steroids with modified CDs. In the case of barbiturates, molecular dynamic studies were performed in order to evaluate the encapsulation process. A very interesting review was recently published on the possibility of application of the cyclodextrins in food industry, authors reviewing over than 14 years of determinations on the flavoring compounds/βCD complexes [1,3].

The goal of the present review is the study of the nanoencapsulation and the protection capacity of the bioactive compounds or systems (unsaturated fatty acids and essential oils) by α- and β-cyclodextrins [3-24].

2. Fatty acid/CD nanocapsules

The nanoencapsulation of the fatty acids in α- and βCD was achieved in good yields of 60-91%, the higher ones being obtained in the case of αCD. Due to the high quantity of data, only the case of oleic acid is presented here.

The DSC analysis (Figure 1) clearly indicates the formation of the complex. For the pure CDs the DSC analysis indicate the dehydration in the range of 80-130°C for αCD and 70-115°C for βCD. The DSC analysis indicate no physical or chemical modification. The DSC analysis shows that the oleic acid/αCD complex do not contain any quantity of water; some transitions appear at 116°C and 147°C. A small quantity of water (and/or ethanol) is present in the oleic acid/βCD complex.
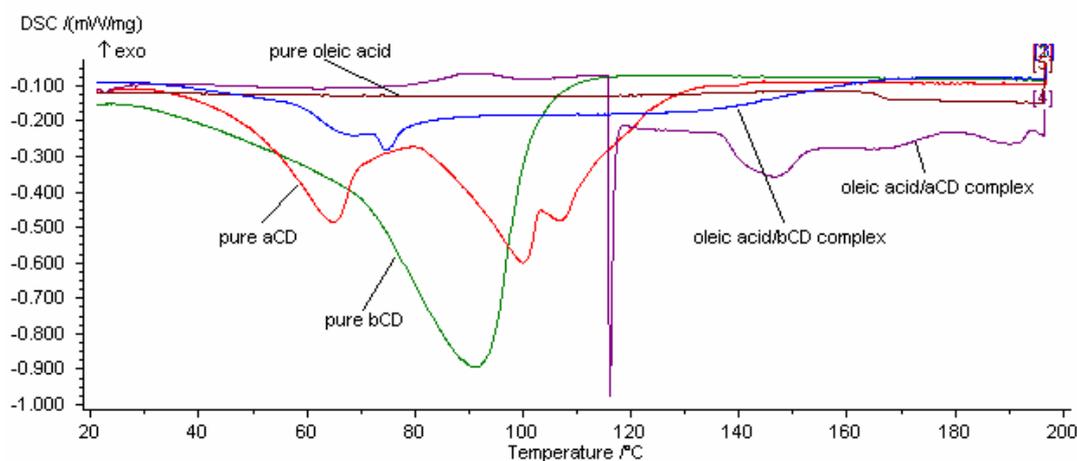

Figure 1. DSC analysis for pure oleic acid, pure CDs, and for the oleic acid/α- and βCD complexes

In order to establish the free and degraded oleic acid sample composition, the GC-MS analysis was performed for the free oleic acid sample not degraded and degraded at 50-150°C temperature range, and for the samples separated from the complexes not degraded or degraded at 50-150°C temperature range. The concentration of pure oleic acid was 98.8% (as methyl ester), the trace impurities being caproic, enanthic, caprilic, pelargonic, caprinic, myristic, and palmitic acid, and some dicarboxylic acids.

A small quantity of ethyl oleate was identified in the samples extracted from the complexes, probably formed in the complexation process.

After the thermal degradation of the free oleic acid, in air at normal pressure, a series of degradation products could be identified, especially for the samples degraded at temperatures above 100°C. These compounds belong to the aldehyde class (by oxydation processes), epoxy derivatives and dihydroxy-acids resulting by

epoxydation-hydratation processes. The concentration of oleic acid in the sample degraded at 50°C was approximately 99%, the number and the quantity of degradation products being very small. The concentration of oleic acid in the samples degraded at 100°C and 150°C was 95.1% and 81.7%, respectively. A small quantity of elaidic acid (*E* isomer of oleic acid) of 8% could be identified in the sample degraded at 150°C, probably resulted from an addition-eliminating reaction with one water molecule.

In the first half of the GC chromatogram aldehydes were the main degradation products of the oleic acid (identified as corresponding dimethyl-acetals). The higher concentration was determined for octanal (in concentration of 0.03, 0.16, and 0.18% for the samples degraded at 50, 100, and 150°C, respectively). The same variation was observed for nonanal and decanal. For the sample degraded at 100°C the 9,10-epoxi and 9,10-dihydroxy derivative of oleic acid were identified.

The excellent protective capacity of CDs can be observed from the GC-MS analyses of the oleic acid/CDs samples degraded in the same conditions. For the oleic acid/αCD sample degraded at 50°C, no important degradation products were identified, the concentration of oleic acid (as methyl ester) being 99.8%. For the sample degraded at 100°C, the oleic acid (as methyl ester) concentration was 97.4%. The main degradation product was malon-aldehyde (identified as acetal, 0.9%). The *E* isomer of oleic acid (elaidic acid) was not identified neither in the samples degraded at 50-100°C, nor in those degraded at 150°C (Figure 2).

For the oleic acid/*β*CD complex degraded at 50-150°C, small quantities of C_6-C_9 aldehydes were identified, but the concentration of oleic acid was relatively higher (96.5% in both samples degraded at 50-100°C, and 92.8% in the sample degraded at 150°C; 5.8% of elaidic acid was identified in this case).
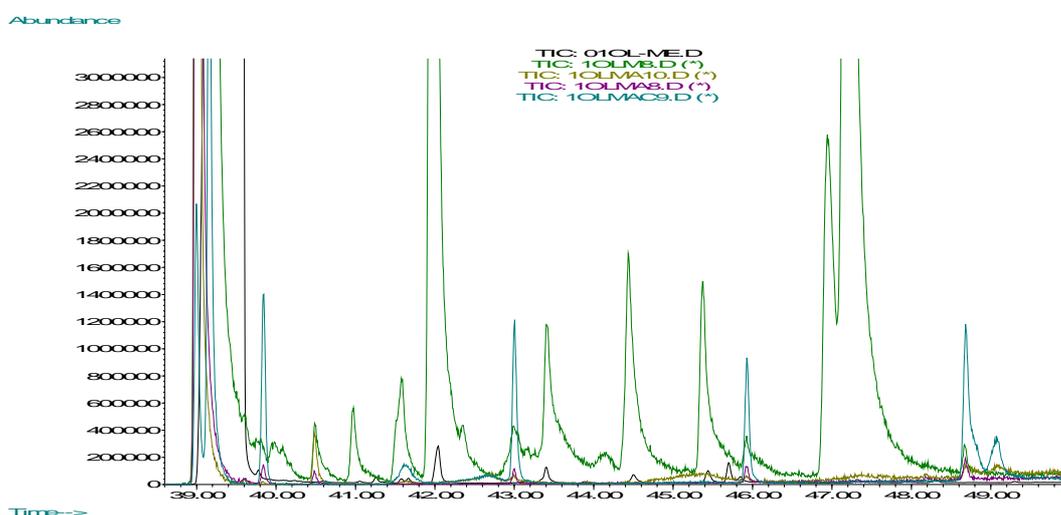

Figure 2. The superposition of the GC chromatograms for free oleic acid not degraded (black) and degraded at 150°C (green), and for the samples corresponding to the oleic acid/αCD complex degraded at 50°C (violet), 100°C (yellow), and 150°C (cyan), respectively (the KI range of 2100-2600)

For the theoretical evaluation of the interaction energy, the most stable fatty acid conformation was orientated along to the *Z* axis, with the hydrophobic moiety of the fatty acid pointed to CD. The best

docking results were obtained in the case of orientation of the fatty acid conformation to the secondary OH-groups of the CDs. The optimized conformations of the complexes indicate the formation of the van der Waals bonds between the inner cavity of the CD

and the hydrophobic moiety of the fatty acid structure and the formation of the *H*-bonds between the secondary OH-groups of

the CD and carbonyl or hydroxyl groups from the fatty acid carboxyl (Figure 3).
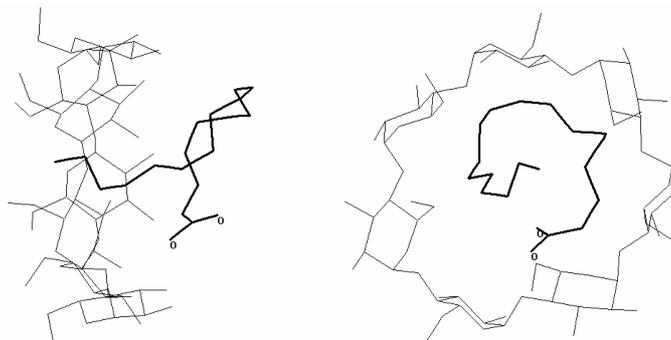

Figure 3. Molecular modeling (MM+) of the interaction of the oleic (bolded) with βCD; two representations (along to the OX-left and OZ-right axes) of the optimized conformations of complexes are presented

The attempt to correlate the interaction energy obtained by DSC analysis and by molecular mechanics calculations conducts to a very good correlational equation, with a correlation coefficient of 0.92.

$$E_{int(MM+)} = 17.368(\pm0.933) + 1.457(\pm0.321) \cdot E_{int(DSC)}$$

$n = 6; r = 0.92; s = 1.62; F = 21$

3. Essential oil/*β*CD nanocapsules

Eighteen essential oil/*β*CD nanocapsules (caraway, coriander, fennel, dill-Apiaceae family, basil, thyme, lavender, marjoram, mint, spearmint, sage-Lamiaceae family, bergamot, lemon, orange-Rutaceae family, clove, eucalyptus-Myrtaceae family, cinnamomum-Lauraceae family, and rose-Rosaceae family from Dicotyledonatae

botanical class), one essential oil (garlic-Liliaceae family, Monocotyledoneae class)/*β*CD complex, and six bionanocapsules (fir, spruce, turpentine-Pinaceae family, juniperus plant, leaf, and fruit-Cupressaceae family) were obtained with good yields of 55-84%, but only the Apiaceae family (especially the case of caraway essential oil) are presented here.

The thermogravimetric analysis indicates that the concentration of the Apiaceae family essential oils encapsulated was in the range of 7.6% (for the coriander oil/*β*CD complex) to 9% (for the fennel oil/*β*CD complex) (Figure 4), comparatively with the pure *β*CD analysis, that revealed a mass loss of 14.1% (up to 200°C), corresponding to 12 water molecules (the pure *β*CD are crystallized with water molecules).
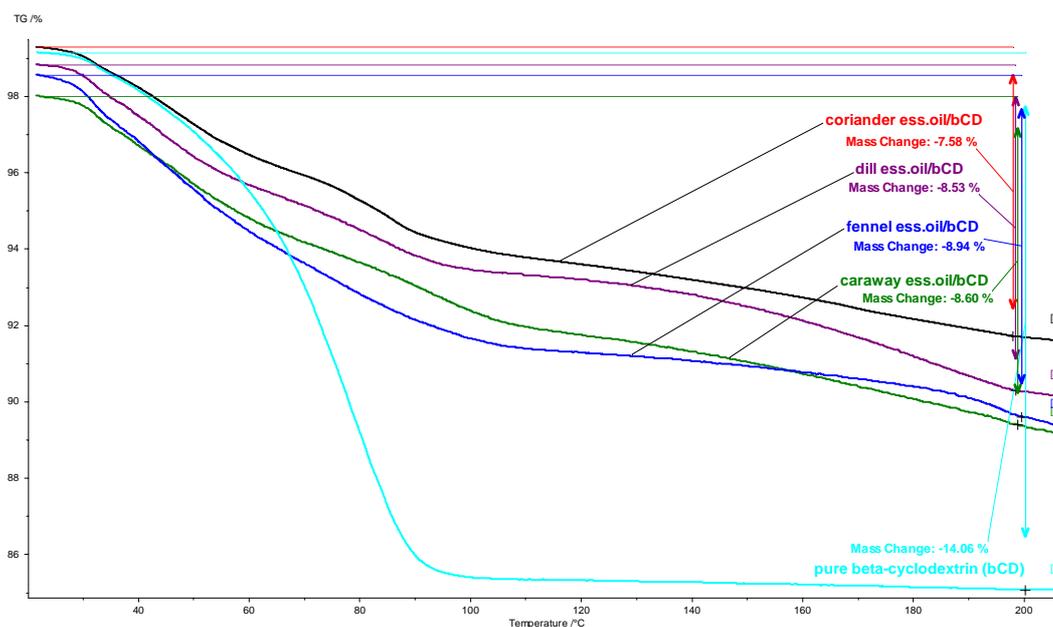
Figure 4. TG analysis of the essential oils from Apiaceae family plants/βCD complexes

The main components identified in the essential oils from the Apiaceae family plants were the oxygenated compounds like (*S*)-(+)-carvone, linalool and anethol. More than 170 compounds were separated by GC analysis of the caraway essential oil (Figure 5), the main compound being carvone (47.3%) and limonene (28.4%) (Figure 6). The minor components were *α*- and *β*-pinene, carveole and some sesquiterpenes (*β*-elemene, *β*-cubebene, *β*-bourbonene, *γ*-cadinene).In order to evaluate de composition of the encapsulated oil, this was recovered in hexane by multiple extraction (no more than four extractions because the fifth one indicate only traces of essential oil compounds by GC-MS analysis). The recovered essential oil was analyzed in the same way and the composition was compared with that of the raw essential oil (by means of the ratio between the concentrations of compounds in the recovered and raw essential oils). Limonene (a hydrophobic monoterpene) was encapsulated in higher relative concentration (the concentration in the recovered essential oil) than in the raw essential oil, the ratio between these concentrations being 1.9. In the case of carvone, this ratio was < 1, probably due to the presence of the carbonyl group (which decrease the hydrophobicity of the molecule and the hydrophobic interaction with the inner cavity of the cyclodextrin).

Figure 5. The superimposed GC chromatograms of the raw (black) and recovered caraway essential oils

The multivariate analysis (PCA-principal component analysis) of the GC data (concentration of the compounds), both for the raw and recovered essential oils from the Dicotyledonatae and Pinatae botanical classes, indicates a good classification of these samples. The Dicotyledonatae class was very good classified in the along to the PC1 and PC3 components and the Pinaceae class was grouped in the upper region, along to the PC2 component (Figure 6).

Figure 6. PC2 vs PC1 loadings plot for the GC data of the essential oils from the Dicotyledonatae and Pinatae botanical classes

These three principal components explain 95% from the variance of the data (73% PC1, 14% PC2 and 8% PC3). Only dual grouping of the raw and recovered samples was obtained in the attempt to classify these raw and complexed essential oils (not shown). The most important compounds for the classification of the samples are cinnamaldehyde, linalyl anthranilate, camphene, anethol for the first principal component (PC1), carvone and carvacrol for PC2.

4. Conclusion

- The unsaturated fatty acid/CD complexes were obtained with good yields and the DSC analysis suggests the formation of the complex. The DSC analysis also indicates that a part of the encapsulated molecules can be ethanol and water molecules. The peak corresponding to the dissociation of the complex (with energy assumed to be equal with the interaction energy) has an elongated profile and the interaction energy is difficult to evaluate. The experimental interaction energy of the oleic acid/CD decrease to a half for the linoleic acid/CD and forward for the linolenic acid/CD. The same variation can be observed in the case of the theoretical interaction energy evaluated by molecular mechanics calculations. A linear correlation can be observed between the experimental and theoretical interaction energies that confirm the formation of the fatty acid/CD complexes;

- Higher relative concentration of degradation products can be identified in the case of thermally degraded fatty acid samples, the main compounds being aldehydes (malondialdehyde, hexanal, heptanal, octanal, nonanal, decanal) resulted by oxidation processes, especially at higher temperature. A relatively high concentrations of the fatty acids were transformed (probably by hydration-dehydration processes) to the more

stable isomers, most of them in the case of samples degraded at 100 and 150°C;

- Very good thermal stability was observed for fatty acid/α- and βCD complexes, the relative concentration of the fatty acid recovered from the complex being >90% in the most case (especially for the oleic and linoleic acids), comparatively with the non-encapsulated samples degraded at the same temperatures, where these concentration were lower;

- The nanoencapsulation of the essential oils from plants belong to the Dicotyledonatae, Monocotyledoneae, and Pinatae botanical classes in β-cyclodextrin was performed with good yields of 55-84%. The total encapsulated sample determined by thermogravimetry was in the range of 6.5-11.5%. Probably, a part of the mass loss is due to the remanent water molecules from the cyclodextrin crystal. A competitive binding can occur between the hydrophobic flavoring compound from the essential oils and ethanol (used in the encapsulation process);

- The main compounds in the essential oils from the Dicotyledonatae botanical class were oxygenated compounds (exception for the Rutaceae family essential oils), which are encapsulated in relative concentrations in the recovered essential oils close to those from the raw ones. A large number of compounds (especially mono- and sesquiterpenes) were observed in the case of essential oils from the Pinatae botanical class, the main compounds being α-pinene and limonene. All concentrated monoterpenic hydrocarbons were encapsulated in higher relative concentrations comparatively with the corresponding concentration in the raw essential oils;

- The complexation of the essential oil of garlic (from Monocotyledoneae class) in βCD provides a product with no "garlic" odor. The relative concentration of the unsaturated acyclic

sulfur compounds in the recovered essential oil is higher than for the saturated acyclic and cyclic sulfur compounds;

- From the multivariate analysis of the GC data, the botanical classes were clearly classified by cinnamaldehyde, linalyl anthranilate, camphene, anethol, carvone and carvacrol, but the attempt of grouping the raw and recovered essential oils provides only poor classifications. Very good results were obtained in the case of Cupressaceae family, which are clearly grouped in two classes (raw and recovered essential oils) by α-pinene.

- These good results obtained for the nanoencapsulation of the bioactive compounds, recommends the CDs (GRAS accepted natural food additives) for the protection of biomolecules in the industrial processes (some attempts were made for the application of the Rutaceae essential oil/CDs in the pastry industry) and the multivariate PCA methods for HACCP system implementation.

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