Chapter 13
Solubility of Fullerenes in Fatty Acids Esters: A New Way to Deliver *In Vivo* Fullerenes. Theoretical Calculations and Experimental Results

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Abstract The biological effects of fullerenes and, in particular, of C_{60} have been recognized since long time. One of the problems which hindered the application of fullerenes in medicinal chemistry regards their insolubility in water and water-based fluids. In the present chapter it is reported that C_{60} and C_{70} fullerenes are soluble in vegetable oils, in general, in esters of fatty acids and in free fatty acids. These results pave the way in the utilization of vegetable oils as vehicles in the delivery of fullerenes for both topical applications and internal use (e.g., intramuscular injection).

It is shown that the solubility of fullerenes in vegetable oils can be predicted and justified on the basis of the solubility parameters of C_{60} and C_{70} and of the glycerol esters of fatty acids. A detailed procedure for the calculation of the solubility parameters of fullerenes and vegetable oils by group increment is reported.

The solubility of C_{60} and C_{70} in a series of vegetable oils, namely: olive, sunflower, peanut, soybean, linseed and castor oil, has been determined quantitatively spectrophotometrically. Additionally, the solubility of C_{60} and C_{70} has been determined quantitatively in the methyl esters of brassica oilseed and only qualitatively in molten cow butter, molten stearic acid and molten behenamide. The experimental results show that the solubility of fullerenes appears to be dependent on the unsaturation level of the fatty acids composing the vegetable oils being lower in oils with higher unsaturation level. The solubility has been found dependent also on the polarizability parameter of the vegetable oils.

The stability of C_{60} and C_{70} solutions in vegetable oils has been studied in air and under inert atmosphere, after thermal processing and under the action of UV radiation. In all cases it has been found that C_{60} and C_{70} are prone to form adducts with the fatty acid chains of the vegetable oils. The adducts are formed both by radical and Diels-Alder mechanisms. The pharmaceutical valency and potential of such adducts has also been discussed.

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F. Cataldo, T. Da Ros (eds.) *Medicinal Chemistry and Pharmacological Potential of Fullerenes and Carbon Nanotubes,*

Keywords Adducts, C_{60}, C_{70}, esters of fatty acid, excipients, fatty acids, fullerenes, glycerol esters of fatty acids, grafting, group increment method, solubility, solubility parameter, vehicles for drug delivery, vegetable oils, triglycerides

13.1 Introduction

One of the most promising applications of fullerenes is in biochemistry and medicinal chemistry (Wilson, 2000; Tabata et al., 1997; Dugan et al., 2000; see also other chapters of the present book). For such application the biocompatible solubility of C_{60} is mandatory. Therefore, a lot of efforts have been made in preparing fullerene derivatives with functional groups able to permit the C_{60} dissolution in water and similarly, also the encapsulation of C_{60} into host–guest complexes, for instance, with cyclodextrin (Braun et al., 1994; Cataldo, 2002). In this specific context it came quite unexpected the discovery of C_{60} solubility in various vegetable oils (Braun et al., 2007; Cataldo and Braun, 2007). This implies the possible utilization of fatty acids esters of glycerol for the delivery of fullerene or its derivatives into living organisms or its topical use on skin. Furthermore, it has been observed (Sayes et al., 2005) that C_{60} fullerene exerts a cytotoxic activity just on the cell membranes through a lipid peroxidation mechanism. This fact offers a possibility for the realization of the idea of the "magic bullet", i.e., a chemical, which selectively can destroy only the undesired cells leaving untouched the "normal cells" (Guanti et al., 2005).

The solubility of C_{60} fullerene in numerous solvents was studied since when these molecules become available in bulk quantities (Ruoff et al., 1993; Beck and Mandi, 1997). Similarly, attention was paid to the solubility of C_{70} (Sivaraman et al., 1994). Numerous papers appeared in the literature on this specific subject and among the most recent and complete surveys it should be mentioned the works of Korobov and Smith (2000), Marcus et al. (2001) and Makitra et al. (2003). In general, it was reported that fullerenes are soluble in solvents with low dielectric constant, large refractive indices and large molecular volume (Ruoff et al., 1993). A correlation with solvent polarizability and fullerene solubility has also been proposed (Sivaraman et al., 1994). Murthy and Geckeler (2001) have correlated the fullerene solubility with the solvent to fullerene molar volume ratio. Particularly surprising are the works of Heymann (1996a, b), where it was shown an increasing solubility of fullerenes in a series of straight chain alcohols: the solubility of both C_{60} and C_{70} grows dramatically from methanol to 1-octanol, hence by increasing the size of the solvent molecules and by reducing their hydrophilic character. Liu et al. (2005) have reported that C_{60} dissolves in ionic liquids.

Despite the numerous modelling works on the subject (e.g. Sivaraman et al., 2001; Huang, 2005) and the great interest on the solubility of fullerenes in hydrophilic solvents, in water and in biologically active solvents for the potential application of fullerenes as drugs, none was able to predict that C_{60} and C_{70} fullerenes

are soluble in fatty acid esters and primarily in fatty acids triglycerides such as olive oil (Braun et al., 2007) and in a series of other vegetable oils such as linseed, sunflower and soybean oil (Cataldo and Braun, 2007). The solubility of C_{60} was discovered not only in methyl esters of fatty acids such as the methyl ester of brassica oilseed (Cataldo and Braun, 2007), but also in other long-chain molecules such as fatty amides as we will show in this chapter.

This chapter shows that the solubility of C_{60} and C_{70} fullerenes can be predicted from the solubility parameter of these two molecules and the calculated solubility parameters of fatty acids and their esters. Furthermore, the solubility of C_{60} and C_{70} fullerenes in a series of vegetable oils and fatty acid esters will be presented and discussed.

13.2 Experimental

13.2.1 Materials and Equipment

Fullerenes were high purity grades (99 + %) from Southern Chemicals LLC. Vegetable oils were commercially available oils from olive, linseed, soybean, sunflower, peanut and castor. A methyl ester of brassica oilseed also was employed.

The solubility was determined spectrophotometrically on a Shimadzu UV160A using the reference quartz cuvette filled with pure vegetable oil under testing and the sample cuvette filled with the solution of C_{60} or C_{70} in a given vegetable oil.

13.2.2 Solubility Determination

In a typical procedure, about 40 mg of fullerene (C_{60} or C_{70}) was stirred with 15–17 g of oil at room temperature. After 3 days of stirring at room temperature the solution was left overnight before filtering it through a paper filter "Rapida A". The filtered solution was then employed for the spectrophotometric determination of solubility. In the case of C_{60}-saturated solutions, there was no necessity to dilute the solution before the spectrophotometric measurement. Thus, the measurement was made by reading the absorbance at 530 nm using an averaged molar extinction coefficient of 724 M^{-1} cm^{-1} (Catalan et al., 1995). Instead, the spectrophotometric determination of C_{70} solubility always required a dilution with fresh oil before measurement. In general, 5 ml of filtered saturated solution was diluted to 50 ml prior to measurement. A molar extinction coefficient of ε = 28,444 M^{-1} cm^{-1} was used at 379 nm or ε = 15,166 M^{-1} cm^{-1} at 469 nm (Sivaraman et al., 1994).

13.3 Results and Discussion

13.3.1 The Solubility Parameter

The solubility parameter has been defined by Hildebrand and Scott (1950) as:

$$\delta = [(\Delta H_{vap} - RT) / V_m]^{0.5} \tag{13.1}$$

The evaporation enthalpy ΔH_{vap} was taken as the parameter of the cohesion energy between molecules minus the thermal energy needed to separate them (RT) divided by the molar volume V_m. So, equation (13.1) can be re-written as:

$$\delta = [(E_{coh}) / V_m]^{0.5} \tag{13.2}$$

The cohesive energy E_{coh} of a substance in a condensed state is defined as the increase in internal energy ΔU per mole of substance if all the intermolecular forces are eliminated.

Hansen (2007) has shown that the solubility parameter proposed by Hildebrand and Scott does not take into account the contribution of polar forces and hydrogen bonding, therefore, a more complex solubility parameter has been proposed:

$$\delta^2 = \delta^2_d + \delta^2_p + \delta^2_h \tag{13.3}$$

derived from the contribution of three components of the cohesive energy:

$$E_{coh} = E_d + E_p + E_h \tag{13.4}$$

respectively due to the contribution of dispersion and polar forces plus an hydrogen bonding contribution.

It is possible to calculate the solubility parameter and the solubility parameter components of almost all molecules and polymers by a group contribution method (Van Krevelen, 1990; Bicerano, 1996). For this purpose, as explained by Van Krevelen (1990) it is useful to introduce the molar attraction constant simply defined as:

$$\varphi = (E_{coh} V_m)^{0.5} \tag{13.5}$$

A set of equations has been proposed by Van Krevelen (1990) for the calculation of the solubility parameter components using the molar attraction by a group contribution methodology:

$$\delta_d = \left(\sum \varphi_d\right) / V_m \tag{13.6}$$

$$\delta_p = \left(\sum \varphi^2{}_p\right)^{0.5} / V_m \tag{13.7}$$

$$\delta_h = \left[\left(\sum E_h \right) / V_m \right]^{0.5} \tag{13.8}$$

The total solubility parameter can be calculated as follows:

$$\delta_t = \left[\delta^2{}_d + \delta^2{}_p + \delta^2{}_h \right]^{0.5} \tag{13.9}$$

It can be observed from equation (13.8) that the hydrogen bond parameter δ_h cannot be calculated from the molar attraction, but directly from the hydrogen bonding energy E_h (Van Krevelen, 1990).

13.3.2 The Solubility Parameter of C_{60} and C_{70} Fullerenes

Fullerenes are highly symmetrical molecules free from any polar groups. For such types of molecules equations (13.7) and (13.8) give $\delta_p = \delta_h = 0$. Therefore, the solubility parameter of fullerenes calculated according to Van Krevelen (1990) can be calculated from equation (13.6).

The density of C_{60} and C_{70} fullerenes is known from literature (Beckhaus et al., 1994) being 1.76 and 1.69 g/ml, respectively. Thus, the molar volume of C_{60} and C_{70} is as follows:

V_{C60} = 409.5 ml/mol
V_{C70} = 497.5 ml/mol

It is interesting to note that such molar volumes can be calculated also by group increments using the van der Waals molar volume for a carbon atom, which is 6.95 ml/mol (Van Krevelen, 1990).

Thus, the calculated molar volumes are:

V_{C60} = 417.0 ml/mol
V_{C70} = 486.5 ml/mol

Both calculated values are in fair agreement with the experimental value. Therefore, with this approach it is possible to calculate also the molar volumes of higher fullerenes, whose density is not known. Table 13.1 reports the calculated molar volumes for a series of fullerenes.

Knowing the fullerene molar volume, either the experimental or the calculated value, it is quite straightforward the calculation of the solubility parameter by substituting the tabulated φ_d values for group increments in equation (13.6).

The calculation should consider the number of hexagonal rings present in the fullerene molecule. It is known that each stable fullerene cage is characterized for having 12 pentagons and a number of hexagons, which depends on the total number of carbon atoms present in the cage according to the Euler's relation:

$$N^* + 2(10 + \xi) \tag{13.10}$$

Table 13.1 Solubility parameters of fullerenes

Fullerene	Molar volume (ml/mol)	Calc. molar volume (ml/mol)	Hexagons (number of)	Solub. param $(MPa)^{1/2}$
C_{60}	409.5	417.0	20	19.5
C_{70}	497.5	486.5	25	19.4
C_{76}		528.2	28	20.1
C_{78}		542.1	29	20.2
C_{82}		569.9	31	20.4
C_{84}		583.8	32	20.5
C_{90}		625.5	35	20.7

where N^* is the number of carbon atoms in the fullerene cage and ξ is the number of hexagons in the fullerene structure.

Thus, for example, for C_{60} fullerene that has 20 hexagons, the calculation of the solubility parameter is:

$$\delta_d(C_{60})=(\Sigma\varphi_d)/V_m=[(70\times60)+(190\times20)/409.5]=19.5\ MPa^{1/2}\ (or\ J^{1/2}\ cm^{-3/2}) \quad (13.11)$$

In fact, the tabulated φ_d value for each sp^2 hybridized carbon atom is $70\,J^{1/2}\ cm^{3/2}\ mol^{-1}$, while the φ_d value for each hexagonal ring is $190\,J^{1/2}\ cm^{3/2}\ mol^{-1}$. Thus, for C_{70}, which has 25 hexagonal rings the calculation is:

$$\delta_d(C_{70})=(\Sigma\varphi_d)/V_m=[(70\times70)+(190\times25)/497.5]=19.4\ MPa^{1/2}\ (or\ J^{1/2}\ cm^{-3/2}) \quad (13.12)$$

In the fullerene case, $\delta_d = \delta_t$.

The calculated values found are in fair agreement with the solubility parameter found experimentally by Sivaraman et al. (1994) who reported $\delta_t\ (C_{60}) \approx \delta_t\ (C_{70}) \approx 18\,MPa^{1/2}$. Furthermore, Hansen and Smith (2004) have reported an experimental solubility parameter $\delta_d\ (C_{60}) = 19.7\,MPa^{1/2}$ in excellent agreement with the calculated value reported in equation (13.11). Strangely enough, Hansen and Smith (2004) have reported also the $\delta_p\ (C_{60}) = 2.9\,MPa^{1/2}$ and a $\delta_h\ (C_{60}) = 2.7\,MPa^{1/2}$ so that the total solubility parameter $\delta_t\ (C_{60}) = 20.1\,MPa^{1/2}$ calculated according to equation (13.9). However, both for structural and for symmetry considerations Van Krevelen (1990) has suggested that $\delta_p = \delta_h = 0$ in the case of high symmetry molecules like C_{60}, which are free from polar substituents.

Table 13.1 reports the solubility parameters of a series of fullerenes. Higher fullerene homologues up to C_{90} are predicted to have a solubility parameter $\delta_t \approx \delta_d \approx 20\,MPa^{1/2}$, thus, not far from the value calculated for the most common fullerenes C_{60} and C_{70}.

13.3.3 The Solubility Parameter of Fatty Acids and Fatty Acids Esters of Glycerol

Being a mixture of different fatty acids esters of glycerol, the calculation with group increment of the solubility parameter of vegetable oils presents some

difficulties. Furthermore, no data are available on the solubility parameters of such oils even in a comprehensive book on solubility parameter such that of Hansen (2007).

The approach employed for the calculation of the solubility parameters of fatty acids and fatty acid esters with the group increment technique according to the Van Krevelen (1990) method has involved the following steps. First of all the density ρ of fatty acids or of a given vegetable oil such as olive oil was taken from literature (Martinenghi, 1963). In the case of vegetable oils, the average molecular weight ω of their fatty acids mixture was considered in the calculation of the molar volume. The value of ω was taken from literature (Martinenghi, 1963).

The general equation for the calculation of the three components of the vegetable oils are:

$$\delta_d=(\Sigma_i x_i \varphi_{di})/(\omega\rho^{-1}) \tag{13.13}$$

$$\delta_p=(\Sigma_i x_i \varphi^2_{pi})^{0.5}/(\omega\rho^{-1}) \tag{13.14}$$

$$\delta_h=[(\Sigma_i x_i E_{hi})/(\omega\rho^{-1})]^{0.5} \tag{13.15}$$

where x_i is the weight fraction of a given component of a vegetable oil (considered always as triglyceride).

Let us consider, for example, the approximate composition of olive oil: we have considered 0.2% of glyceryl trimyristate, 13% of glyceryl tripalmitate, 0.8% of glyceryl tripalmitoleate, 2.5% of glyceryl tristearate, 73.3% of glyceryl trioleate, 9% of glyceryl trilinoleate, 0.3% of glyceryl triarachidate, 0.7% of glyceryl trilinolenate and 0.2% of glyceryl eicosenoicate. For each triglyceride the relative values $\Sigma\varphi_d$, $(\Sigma\varphi^2_p)^{0.5}$ and ΣE_h have been calculated. Then, these values were multiplied by the weight fraction of each triglyceride present in the oil as shown in equations 13–15 and divided by the molar volume of the oil. The results of such calculation are shown in Table 13.2. Table 13.3 reports the average composition of the vegetable oils studied in the present work. From the data in Table 13.3 the solubility parameters of the vegetable oils reported in Table 13.2 have been calculated.

The data in Table 13.2 show that, as expected the polar and hydrogen-bonding component is more pronounced in the case of free fatty acids rather than in vegetable oils, where the fatty acids are all locked as glyceryl esters. An exception is offered by castor oil, which is characterized by a high content of ricinoleic acid, a fatty acid with one hydroxyl group attached to the chain. Thus, its hydrogen-bonding component is rather high than any other vegetable oil considered.

Table 13.2 Solubility parameters of fatty acid and vegetable oils

	δ_d (MPa)$^{1/2}$	δ_p (MPa)$^{1/2}$	δ_h (MPa)$^{1/2}$	δ_t (MPa)$^{1/2}$	$\Delta\delta_d$ [a] using calc. δ_d (C_{60})	$\Delta\delta_t$ [b] using calc. δ_t (C_{60})	$\Delta\delta$t [c] using Hansen δ data
Myristic acid	16.1	5.6	9.0	19.2	11.1	**0.3**	7.7
Palmitic acid	15.5	4.8	8.3	18.3	10.4	**1.2**	7.3
Stearic acid	15.6	4.3	7.9	18.0	9.8	**1.5**	6.8
Oleic acid	16.3	4.7	8.2	18.8	10.0	**0.7**	6.7
Linoleic acid	16.1	4.8	8.2	18.7	10.1	**0.8**	6.8
Linolenic acid	16.0	4.8	8.3	18.7	10.2	**0.8**	7.0
Olive oil	16.2	1.5	4.7	16.9	5.9	**2.6**	4.3
Soybean oil	15.4	1.5	4.6	16.2	6.3	**3.3**	4.9
Sunflower oil	16.0	1.5	4.7	16.7	6.0	**2.8**	4.4
Peanut oil	15.3	1.5	4.6	16.1	6.4	**3.4**	5.0
Lineseed oil	15.7	1.5	4.7	16.4	6.2	**3.1**	4.7
Castor oil	15.8	1.2	9.1	18.3	9.9	**1.2**	7.7
Brassica oil	16.2	1.5	4.4	16.9	5.7	**2.6**	4.1
Glyceryl trioleate	16.1	1.5	4.7	16.9	6.0	**2.6**	4.3
Glyceryl tristearate	17.0	1.6	4.7	17.7	5.6	**1.8**	3.6

[a]Calculated according to equation (13.17).
[b]Calculated according to equation (13.18).
[c]Calculated according to equation (13.16).

13.3.4 Solubility Criteria from Solubility Parameter Comparison of Fullerenes in Fatty Acids and Fatty Acid Esters of Glycerol

There are many different solubility criteria available in order to establish the solubility of a certain molecule in a given solvent. Hansen (2007) and Hansen and Smith (2004) have made extensive review of such criteria. For simplicity of the mathematical treatment we prefer to follow the criteria proposed by Van Krevelen (1990) for the solubility of a polymer in a solvent. The first point is that we assume that C_{60} and C_{70} are polymeric forms of elemental carbon and treat them on these terms. To predict the solubility according to Van Krevelen (1990), the difference in the solubility parameters between solute and solvent should conform to the following equation:

$$\Delta\delta = [(\delta_{d,\,\text{fullerene}} - \delta_{d,\,\text{solv}})^2 + (\delta_{p,\,\text{fullerene}} - \delta_{p,\,\text{solv}})^2 + (\delta_{h,\,\text{fullerene}} - \delta_{h,\,\text{solv}})^2]^{1/2} < 5\text{MPa}^{1/2} \quad (13.16)$$

Since according to our calculations $\delta_{p,\,\text{fullerene}} = \delta_{h,\,\text{fullerene}} = 0$, the above equation simplifies as follows:

$$\Delta\delta = [(\delta_{d,\,\text{fullerene}} - \delta_{d,\,\text{solv}})^2 + (\delta_{p,\,\text{solv}})^2 + (\delta_{h,\,\text{solv}})^2]^{1/2} \quad (13.17)$$

The results of such calculations are reported in Table 13.2 (third column from right) and suggest that C_{60} (and C_{70}) are not soluble or only slightly soluble in molten free

Table 13.3 Composition of vegetable oils studied in the present work[a]

Fatty acid	Chain length	Unsaturations	Brassica oilseed (%)	Sunflower (%)	Soybean (%)	Linseed (%)	Olive (%)	Peanut (%)	Castor (%)
Myristic	C_{14}	NONE	0.5		0.3	0.9	0.2		
Palmitic	C_{16}	NONE	2.8	3.6	9.8	4.5	13.3	8.3	
Palmitoleic	C_{16}	1 at C_9					0.8		0.5
Stearic	C_{18}	NONE	1.2	2.9	2.4	6.3	2.5	3.1	1.4
Arachidic	C_{20}	NONE	0.9	1.6	0.9	0.3	0.3	2.4	
Beenic	C_{22}	NONE	0.6	0.4				3.1	
Lignoceric	C_{24}	NONE	0.7						
Hexadecenoic	C_{16}	1 at C_8	2.5						
Oleic	C_{18}	1 at C_9	15.5	34.0	28.9	26.2	73.0	56.0	7.4
Ricinoleic	C_{18}	1 at C_9; 1 OH at C_{12}							87.0
Linoleic	C_{18}	1 at C_9; C_{12}	11.0	57.5	50.7	14.7	9.0	26.0	3.1
Linolenic	C_{18}	1 at C_9; C_{12}; C_{15}	7.6		6.9	47.0	0.7		
Eicosenoic	C_{20}	1 at C_{11}	3.5						
Erucic	C_{22}	1 at C_{13}	52.2						
Others			1.0		0.1	0.1	0.2	1.1	0.6

[a]From Martinenghi (1963).

fatty acids, but are expected to be soluble in glyceryl esters of fatty acids and their mixtures since the $\Delta\delta$ value is close to $5\,MPa^{1/2}$ with the exception of the case of castor oil, where the fullerenes are not expected to be soluble.

In Table 13.2 also another calculation has been considered comparing the total solubility parameters of the solvents and the fullerenes. In the case of fullerenes $\delta_{d,\,fullerene} = \delta_{t,\,fullerene}$ and

$$\Delta\delta_t = [(\delta_{t,\,fullerene} - \delta_{t,\,solv})^2]^{1/2} \tag{13.18}$$

with equation (13.18) it is expected that C_{60} and C_{70} fullerenes are easily soluble both in a series of fatty acids and in a series of glyceryl esters of fatty acids. The prediction made in such a way is confirmed by the experimental evidence, which is shown in Table 13.4. In fact both C_{60} and C_{70} are soluble in a series of different glyceryl esters of fatty acids, but also in molten stearic acid, a free fatty acid (in the latter case it was not possible to determine the solubility).

The last column in the right of Table 13.2 shows $\Delta\delta$ values obtained using equation (13.16) and the published solubility parameters of C_{60} proposed by Hansen and Smith (2004), i.e. δ_d (C_{60}) = $19.7\,MPa^{1/2}$, δ_p (C_{60}) = $2.9\,MPa^{1/2}$ and a δ_h (C_{60}) = $2.7\,MPa^{1/2}$. By substituting these values in equation (13.16) together with the calculated δ_d, δ_p and δ_h values for the glyceryl esters of fatty acids as well as the values for the free fatty acids one obtains $\Delta\delta$ values, which suggest solubility of fullerenes in glyceryl esters of fatty acids since $\Delta\delta < 5$ with the exclusion of castor oil. Additionally, last column in the right of Table 13.2 suggests that fullerenes are not soluble or slightly soluble in free fatty acids. Instead, it has been found experimentally that fullerenes are soluble also in free fatty acids and even in fatty acids amides like behenamide as reported in Table 13.4.

Based on these considerations, it appears that the best calculation approach for the prediction of the solubility of fullerenes in free fatty acids and in fatty acids derivatives is granted by equation (13.18), but simply because we are reasoning a posteriori. It is evident from our calculations that the surprising solubility of fullerenes in free fatty acids and glyceryl esters of fatty acids, or which is the same in vegetable oils, was not easily predictable and expected on the basis of theoretical considerations. Only the intuition of Braun et al. (2007) (see also Cataldo and Braun, 2007) has led to this important discovery, which may have implications and applications in medicine, cosmetics and possibly also in other fields.

13.3.5 Experimental Solubility of C_{60} and C_{70} Fullerenes

The solubility of C_{60} in vegetable oils was initially found very low. For instance, in olive oil a value of 6 mg/l has been reported (Braun et al., 2007). Further work has reported that the solubility of C_{60} in a series of vegetable oils is instead at the level of 100–200 mg/l (Cataldo and Braun, 2007). The differences may be, at least in part, ascribed to the different analytical procedures employed, but certainly they

Table 13.4 Solubility of C_{60} and C_{70} in natural glyceryl esters of fatty acids and derivatives

Oil or solvent	C_{60} solubility (mg/l)	C_{70} solubility (mg/l)	C_{60} solubility (mol fract. × 10^4)	C_{70} solubility (mol fract. × 10^4)	C_{60} solubility (molarity × 10^3)	C_{70} solubility (molarity × 10^3)	δ_t $(MPa)^{1/2}$
Olive	909	1035	12.00	11.71	1.261	1.231	16.9
Castor	392	636	5.36	7.45	0.544	0.756	18.3
Peanut	751	852	10.25	9.97	1.042	1.013	16.1
Brassica[a]	859	1034	12.65	13.05	1.192	1.230	16.9
Sunseed	522	867	6.95	9.89	0.724	1.031	16.7
Soybean	495	753	6.77	8.82	0.687	0.896	16.2
Linseed	365	727	4.87	8.31	0.506	0.865	16.4
Molten cow butter	Soluble	Soluble					
Molten stearic acid	Soluble	Soluble					
Molten behenamide	Soluble	Soluble					
Toluene[b]			4.71	1.8	4.44	1.70	18.2
1,3,5-trimethylbenzene[b]			3.28	2.43	2.36	1.75	18.0
1,2 dichlorobenzene[b]			38.0	40.2	34.2	35.7	20.5
1,3 dichlorobenzene[b]			23.1	25.4	19.2	22.3	20.5
Tetralin[b]			30.1	20.1	22.2	14.6	19.9
N-hexane[b]			0.084	0.02	0.064	0.015	14.9
Dodecane[b]			0.325	0.27	0.143	0.12	16.1
Carbon tetrachloride[b]			0.43	0.14	0.44	0.14	17.8

[a]Methyl ester.

[b]From Korobov and Smith (2000).

are due to the different conditions employed in the preparation of the solutions. For instance, Braun et al. (2007) stirred the fullerene/oil mixture (about 15 mg in 5 ml of oil) for 72 hours at room temperature and then determined the solubility by liquid chromatography. On the other hand, Cataldo and Braun (2007) stirred the fullerene/oil mixture (30 mg in 50 ml of oil) for a shorter time (2 hours) but at a much higher temperature (75°C). The solubility determination was made at room temperature on the cooled solution and found two orders of magnitude higher than that originally reported by Braun (2007). It turned out, however, that heating a vegetable oil with C_{60} fullerene causes a reaction between the solute and the solvent. Spectrophotometric evidences have already been published about the reactivity of fatty acid esters with C_{60} (Cataldo and Braun, 2007). Therefore, in the present work, the C_{60} fullerene solubility has been redetermined in a more rigorous procedure and also the C_{70} solubility has been determined for the first time: the data are reported in Table 13.4. In each determination about 40 mg of C_{60} or C_{70} fullerenes was mechanically stirred for 3 days at room temperature with about 15 g of oil. After settling and filtration, the solubility was determined spectrophotometrically and found to be about 4.5 times the values reported previously for C_{60} (Cataldo and Braun, 2007). As shown in Table 13.4, if the solubility of C_{60} and C_{70} is expressed in terms of mol fraction ($\times 10^4$) or in terms of molarity ($\times 10^3$), then one discovers that the solubility level of these molecules in the triglycerides of fatty acids is comparable or even better to the solubility in some good solvents like toluene and trimethylbenzene, but still considerably lower than the fullerenes solubility in certain chlorobenzene derivatives. In any case such result comes as a complete surprise. One thing is to predict the solubility level with the solubility parameter; another matter is to have found unexpectedly high solubility levels for fullerenes in fatty acids esters.

Previously (Cataldo and Braun, 2007), it has been noted the interesting correlation between C_{60} fullerene solubility and the unsaturation level of the fatty acids present in the vegetable oils and measured by the "iodine number", an analytical test, which determines the number of double bonds present in the oil by the addition of iodine (Martinenghi, 1963). This correlation is confirmed for C_{60} also with the new measurements and is extendable also to C_{70}. The correlation is shown in Fig. 13.1 and suggests that the solubility S expressed in milligrams per litre (mg/l) is linked to the iodine number of the oil (NI) according to the following equations:

$$S_{C60} = -5.46(\mathrm{NI}) + 1307 \tag{13.19}$$

and

$$S_{C70} = -2.85(\mathrm{NI}) + 1221 \tag{13.20}$$

These equations suggest that C_{60} is more sensitive to the degree of unsaturation of the oil employed as solvent than C_{70}. Additionally, a limiting solubility value for the fully saturated triglycerides (those with NI = 0) can be predicted from equations

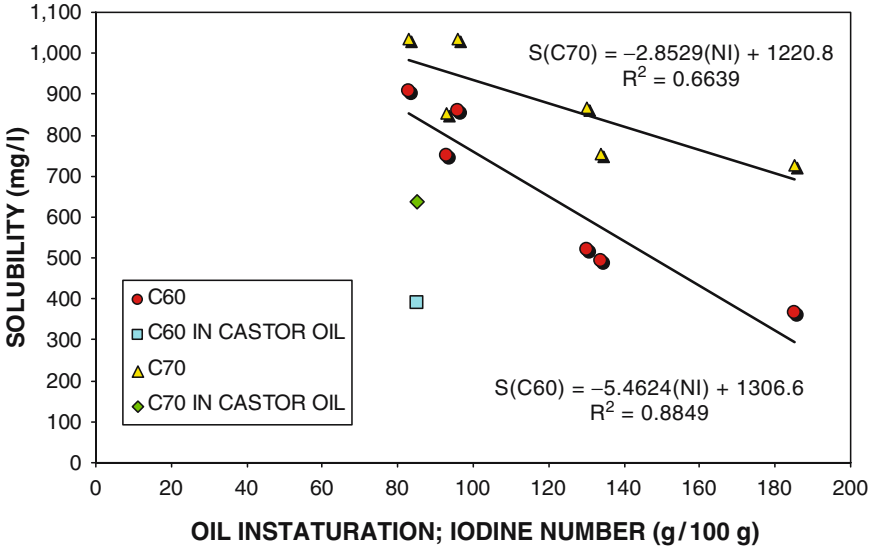

Fig. 13.1 The solubility of C_{60} and C_{70} fullerene is maximum in vegetable oils with lower level of unsaturation (i.e. number of double bonds) and decreases as the insaturation level grows. Having a peculiar chemical structure, castor oil cannot be included in the correlation rule (*See Color Plates*)

(3.19) and (13.20) to be ≈ 1,300 mg/l for both fullerenes. In fact, as shown in Table 13.4, both C_{60} and C_{70} fullerenes have been found to be easily soluble also in molten cow butter, a largely saturated mixtures of triglycerides with iodine number between 25 and 35 g/100 g. Furthermore, C_{60} and C_{70} are easily soluble also in molten stearic acid, a C_{16} free fatty acid (not a triglyceride) having NI = 0. Similarly, an easy solubility of C_{60} and C_{70} was observed in molten behenamide, a fully saturated fatty acid with 22 carbon atoms in the chain whose terminal carboxyl group has been converted into an amide group.

At first glance the relationship between fullerene solubility and fatty acids unsaturation may appear as a surprise. However, it should be noticed that the unsaturation level of vegetable oils correlates also with their refractive index (Martinenghi, 1963). Thus, following the approach of Sivaraman et al. (1994), it is possible to show the change in solubility as function of the polarizability parameter of the solvent defined as:

$$P_{\mathrm{P}} = [(n^2 - 1)/(n^2 + 2)] \tag{13.21}$$

Thus, by plotting the log of the fullerene molar fraction × 10^7 (Log χ) against the polarizability parameter of the various oils one obtains a reasonable linear correlation, which shows the same trend of the unsaturation level. Such a graph is shown in Fig. 13.2 and suggests that the fullerene molar fraction decreases by increasing the polarizability parameter.

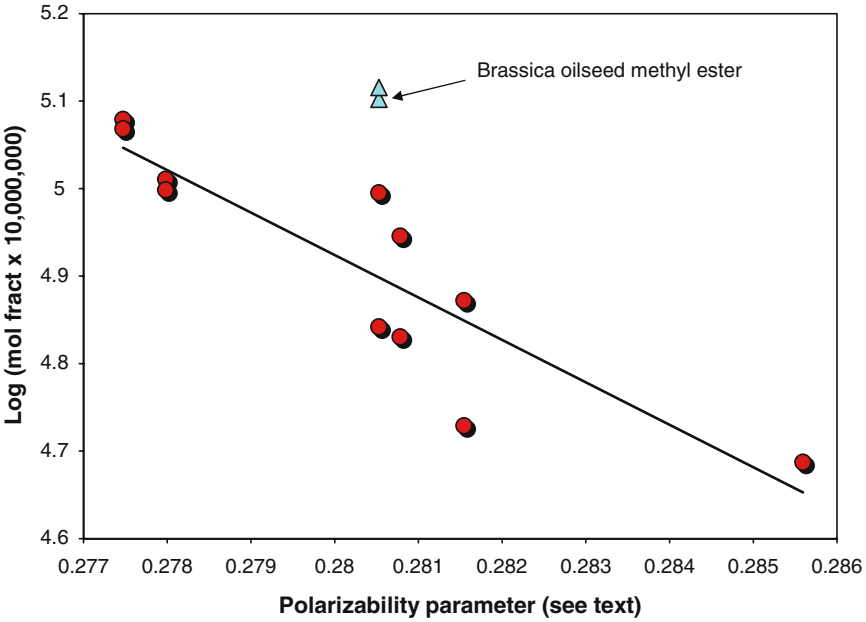

Fig. 13.2 Variation of Log (Mole Fraction × 10^7) with the polarizability parameter $P_p = [(n^2 - 1)/(n^2 + 2)]$ for both C_{60} and C_{70} in various triglycerides of fatty acids. The triangles refer to C_{60} and C_{70} dissolved in methyl ester of brassica oilseed (*See Color Plates*)

13.3.6 Stability of the C_{60} and C_{70} Fullerenes Solutions in Vegetable Oils

When C_{60} fullerene is dissolved in vegetable oils, its electronic absorption spectra show a new absorption band, which is not present in the spectrum of pristine fullerene in other solvents (see Fig. 13.3). The new band appears at 435 nm almost in all fatty acid esters tested as solvents (Cataldo and Braun, 2007). Curiously, the intensity of this band at 435 nm depends just on the unsaturation level of the fatty acids present in the ester, being more intense in oils with higher unsaturation and hence higher iodine number (in decreasing order: linseed > sunflower ~ soybean) and is much less intense with oils having lower iodine number such as olive oil and biodiesel. The explanation of such behaviour is based on the fact that the addition is favoured by fatty acids with at least two double bonds or more in their chains. In other words, olive oil and brassica oilseed have only 10% content of linoleic acid (see Table 13.3) while the other oils, which instead display a stronger band at 435 nm have all a high levels of linoleic acid and linseed oil has also a high level of linolenic acid with three double bonds. The addition may require the presence of at least two double bonds since it is assumed to be a Diels-Alder type addition reaction. Although the double bonds of linoleic and linolenic acids are not conjugated, moderate thermal processing in the presence of catalysts or the action of light can

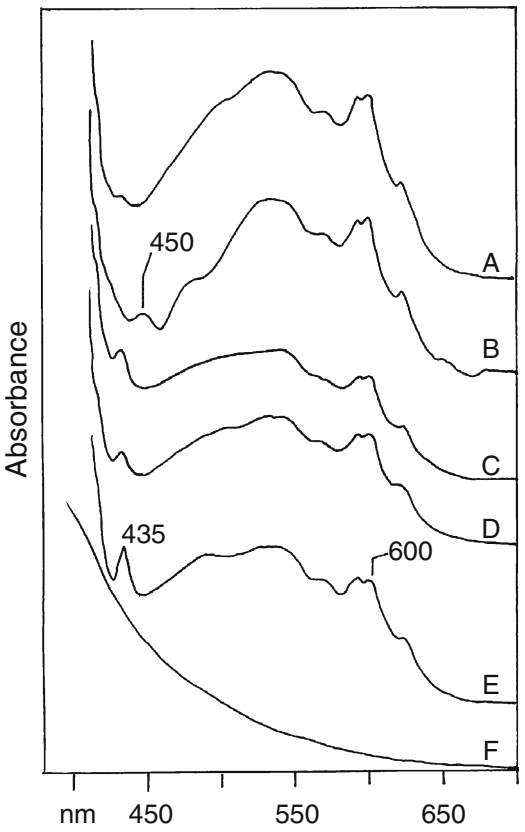

Fig. 13.3 Electronic absorption spectra of C_{60} fullerene dissolved in: (A) brassica oilseeds methyl ester (biodiesel); (B) olive oil; (C) sunflower oil; (D) soybean oil; (E) linseed oil; (F) linseed oil and C_{60} fullerene after heating at 150°C for 15 minutes

cause the slippage of the double bonds to a conjugated position. Fullerenes are highly prone to undergo the Diels-Alder addition reactions where they act essentially as dienophyle rather than as dienes (Taylor, 1999).

Additionally, it should be observed that the thermal oxidability and oxidative polymerization of the unsaturated fatty acids follows the trend linolenic > linoleic > oleic > > palmitoleic (Martinenghi, 1963). The oxidation involves, as first step, the abstraction of a hydrogen atom in allylic position to the double bonds. Certainly, this process is favoured in the case of fatty acids with two or more unconjugated double bonds where the formation of a free radical by allylic hydrogen abstraction leads quite necessarily to double bonds slippage with formation of conjugated double bonds:

$$\text{R–CH=CH–CH}_2\text{–CH=CH–(CH}_2)_n\text{–COOH} \rightarrow \text{R–CH=CH–CH–CH=CH– (CH}_2)_n\text{–COOH} \rightarrow$$
$$\rightarrow \text{R–CH–CH=CH–CH=CH–(CH}_2)_n\text{–COOH}$$

Thus, the addition of fullerenes to the fatty chains cannot be exclusively through a Diels-Alder mechanism, but involves also a radical mechanism.

The new absorption band at 435 nm in the C_{60} spectrum has been attributed to the 1,2 addition to the fullerene cage to the fatty acid chains either across to the double bonds by a Diels-Alder addition or, more simply, by radical addition (Cataldo and Braun, 2007). Thus, fatty acid esters are able to not only dissolve C_{60}, but also react with this molecule causing the addition of the fatty chain to the fullerene cage. In fact, the bands at 435 nm shown in Fig. 13.3 appear only when C_{60} is stirred at 75°C for a couple of hours in the esters of fatty acids. Only for olive oil the new band appears much weaker than in the other cases and displaced at 450 nm (Fig. 13.3B). Since this oil contains chlorophyll, the displacement may be probably due also to a charge–transfer interaction between C_{60} and chlorophyll or with other impurities.

On standing in air, at room temperature the C_{60} fullerene solutions in vegetable oils are not stable, but change their colour from violet to reddish. The electronic absorption spectra show a gradual increase in the absorption band in the visible initially in the range between 450 and 550 nm. Similar results are obtained both by heating the solutions in air or under nitrogen. In the latter case prolonged heating is needed to achieve the same results. Heating C_{60} solutions in linseed or other oils for 15 minutes at 150°C (in air) causes the entire spectrum of C_{60} in the visible to disappear completely as shown in Fig. 13.3F.

Contrary to C_{60}, C_{70} solutions in vegetable oils prepared at room temperature do not display any new absorption band in their visible spectra and show also a better stability than C_{60} solutions. In fact, after 1 week in air a diluted solution of C_{70} in methyl ester of brassica oilseed shows only slight spectral changes: essentially a reduction in the intensity of the band originally located at 469 nm, which after "ageing" appears at about 467 nm and a less-pronounced reduction in the intensity of the band at 379 nm. In any case, these changes should be interpreted in terms of slower reaction of C_{70} with the solvent oil. Heating C_{70} solutions in sunseed or linseed oil at 160°C for 1–2 hours under argon flow causes profound changes in the visible part of the electronic absorption spectra: the band originally located at 469 nm disappears completely in linseed oil solution or is reduced dramatically in intensity and shifted to shorter wavelengths in the case of sunseed oil solution. A brown colour develops in both cases from the original reddish colour. It is evident that also C_{70} reacts with the fatty acid chains of the solvent as in the case of C_{60}, although the reaction appears slower and seems to require longer times or slightly more drastic conditions.

The stability of C_{60} and C_{70} solutions in vegetable oils has been examined also towards the action of UV light. A C_{60} solution in linseed oil has been irradiated in a quartz reactor with UV light from a 12 W low-pressure Hg lamp having its main emission at 254 nm under N_2. In less than 1 hour irradiation, all the visible part of the electronic spectrum of C_{60} with bands at about 530 and 600 nm have been bleached. Simultaneously, a growth in absorption intensity as function of the irradiation time has been observed at about 410 nm.

Irradiation of C_{70} solution in peanut oil under the same conditions just described for C_{60} fullerene in linseed oil after 70 minutes of irradiation causes the partial reduction of the intensity of the band at 464 nm and of a shoulder at 398 nm.

The spectral changes undergone by C_{60} and C_{70} solutions in vegetable oils appear very similar to those observed by thermal processing. Therefore, the interpretation is the same: grafting of fullerene molecule onto fatty acid chains.

13.3.7 Potential Application of Vegetable Oils as Vehicle for Drug Delivery of Fullerenes and Fullerene Derivatives

The solubility of C_{60} and C_{70} fullerenes in vegetable oils will permit to employ these molecules for topical use in creams, lotions and ointments, which are adsorbed by skin. Vegetable oils, especially olive oil, are considered excellent excipients for injectable preparation where the active principle is soluble in fats. Their absorption in the subcutaneous tissues is slow and limited and ensures a gradual release of the active principle (Adami, 1960).

The C_{60} and C_{70} reactivity with the vegetable oils at first glance could appear as an obstacle in the use of fullerene solutions in vegetable oils. Apart from the fact that one could use fully saturated fatty acids derivatives as vehicle for fullerenes delivery, which are not reactive with them, the formation of adducts between the unsaturated fatty acids and fullerenes could be exploited not only in the stabilization of the systems fullerenes–vegetable oils, but also in the alteration and, may be in the attenuation of the fullerene reactivity in *in vivo* and in a very gradual release of the fullerenes–fatty acids derivatives in living systems.

13.4 Conclusions

We have confirmed from the earlier observations (Braun et al., 2007; Cataldo and Braun, 2007) that C_{60} fullerene is soluble in vegetable oils, which are glycerol esters of fatty acids. The solubility levels in a series of vegetable oils have been determined and revised in comparison with earlier determinations. For the first time the solubility of C_{70} fullerene have been determined in vegetable oils and found comparable to those found for C_{60}. According to the most recent determinations the solubility of C_{60} and C_{70} in fatty acid esters expressed in molarity or in mole fractions are even higher than the fullerene solubility in common solvent like toluene (Table 13.4). It has been confirmed that the solubility of both C_{60} and C_{70} fullerene is affected by the unsaturation level of the fatty acids composing the vegetable oils. Higher solubility being observed in most saturated oils (Fig. 13.1). The fullerenes solubility appears also related to the polarizability parameters of the vegetable oils: lower values of the polarizability parameters imply higher solubility values (Fig. 13.2).

A theoretical justification of the surprising solubility of C_{60} and C_{70} fullerenes in vegetable oils has been proposed in terms of solubility parameter of fullerenes and vegetable oils. It has been shown that the solubility parameter of C_{60} and C_{70} fullerenes can be calculated by a group increment approach following Van Krevelen

(1990). The calculated solubility parameter derived from the Van Krevelen approach has been compared with the experimental solubility parameters of C_{60} and C_{70} reported in the literature and derived experimentally. An excellent agreement has been found between the calculated and the experimental δ_d values. A comparison with the calculated solubility parameter of the vegetable oils, under certain conditions, permits to show that a good solubility of fullerenes in glycerol esters of fatty acids can be expected. Fullerene solubility in molten free fatty acids can be predicted on the basis of solubility parameters comparison and it has been verified by dissolving C_{60} and C_{70} in molten fatty acids.

It has also been found a certain level of reactivity between C_{60} and C_{70} with the unsaturated fatty acids. The electronic absorption spectra show evidences that C_{60} or C_{70} are grafted onto the fatty chains when the fullerene solutions in vegetable oils are left for days in air, by heating the solutions under N_2, Ar and air, or by UV irradiation of the solutions in inert atmosphere.

In any case the discovery of the solubility of C_{60} and C_{70} in vegetable oils paves the way in the utilization of these molecules in biology, biochemistry and medicinal chemistry, since the vegetable oils can be used as vehicles of excipients of fullerenes. The formation of adducts between fullerenes and vegetable oils appears as a further opportunity to be exploited in the application of such derivatives in medicinal chemistry.

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