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The prolongation of the lifespan of rats by repeated oral administration of [60]fullerene
Tarek Baati, *et al.*

Abstract
Countless studies showed that [60]fullerene (C(60)) and derivatives could have many potential biomedical applications. However, while several independent research groups showed that C(60) has no acute or sub-acute toxicity in various experimental models, more than 25 years after its discovery the in vivo fate and the chronic effects of this fullerene remain unknown. If the potential of C(60) and derivatives in the biomedical field have to be fulfilled these issues must be addressed. Here we show that oral administration of C(60) dissolved in olive oil (0.8 mg/ml) at reiterated doses (1.7 mg/kg of body weight) to rats not only does not entail chronic toxicity but it almost doubles their lifespan. The effects of C(60)-olive oil solutions in an experimental model of CCl(4) intoxication in rat strongly suggest that the effect on lifespan is mainly due to the attenuation of age-associated increases in oxidative stress. Pharmacokinetic studies show that dissolved C(60) is absorbed by the gastro-intestinal tract and eliminated in a few tens of hours. These results of importance in the fields of medicine and toxicology should open the way for the many possible -and waited for- biomedical applications of C(60) including cancer therapy, neurodegenerative disorders, and ageing.

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https://carbon60oliveoil.com/about/our-process/

Research Grade C60 Olive Oil Solution

Our Process
We are the only company known to strictly follow the original Baati / Paris method of Carbon 60 olive oil preparation. We start with 99.9+ % purified, research grade, solvent-free Carbon 60. We use 5 grams of our Carbon 60 per liter of olive oil. This is more than 6 times the amount of Carbon 60 that can possibly saturate the solution. This is very important, as not all of the Carbon 60 being mixed with olive oil will dissolve in a two week mixing time.

Many of our competitors cut corners by using an industrial grade Carbon 60 (containing dangerous solvents), and/or simply putting in 0.8 or 0.9 grams of Carbon 60 per liter of olive oil, which always results in an inferior product. Our own in-house experiments have shown that you cannot achieve a fully saturated 0.8 grams per liter (0.8 mg per ml) solution if you do not start with the entire 5 grams per liter mix ratio described in the Baati process. Some companies are even boiling their olive oil to try to dissolve the carbon faster. This destroys the polyphenols in the olive oil, and results in a damaged, black oil.

After extensively researching olive oil quality we found that small batch extra virgin olive oil is notoriously inconsistent in quality. It is our determination that the best, most consistent quality olive oil available is Kirkland brand Organic Extra Virgin Olive Oil, which is what we have been using for several years.

We mix our 5 grams of Carbon 60 per liter of olive oil for 2 weeks using specialized equipment that ensures full suspension of Carbon 60 particles during the entire mixing operation. The mixing occurs in complete darkness, in temperature controlled conditions, to avoid Carbon 60 degradation from light or heat.

Our fully saturated Carbon 60 solution is then centrifuged at 5,000 g for one hour to remove the undissolved Carbon 60 .

This clarified, fully saturated Carbon 60 solution is then sterile filtered through a 0.22 micron filter.

We then bottle our solution in amber glass bottles, and package for delivery to you. Our packaging is designed to be extremely robust to prevent breakage, and conforms to international standards for shipping liquids in glass containers. We have not experienced a single broken bottle delivery since adopting our current shipping system in 2013.

We are the only company proven to consistently provide a fully saturated, 0.8 mg per ml Carbon 60 olive oil solution. Don’t settle for the slightly less expensive, vastly inferior products available from our competitors. Insist on fully saturated Carbon 60 olive oil solution from the only company that has been doing it right from the beginning!

US2014140985
FULLERENE AND ITS USE TO MAINTAIN GOOD HEALTH AND TO PROLONG THE EXPECTED LIFESPAN OF MAMMALS

[PDF]

Inventor: Fathi MOUSSA, et al.

[0001] This application claims benefit of Tunisian Provisional Application No. TN 2011/327 filed Jun. 30, 2011 the contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention concerns [60]fullerene and stable biocompatible compositions comprising [60]fullerene dissolved in a carrier. The present invention also relates to a method for increasing the expected lifespan (longevity) of a mammal, which comprises a step of administering

[60]fullerene or a composition comprising a therapeutically effective amount of [60]fullerene. The present invention further concerns a method for preserving a mammal to damages caused by free radicals using said [60]fullerene or said composition.

[0004] 2. Description of Related Art

[0005] Free radicals, such as oxygen radicals and other reactive oxygen/nitrogen/chlorine species (hydroxyl, nitric oxide radicals), are constantly formed in vivo. Some of these molecules are physiologically useful, but they can also result in pathological oxidative stress to cells and tissues. Endogenous defences include both antioxidants and repairing systems. However, excess production of free radicals, their production in inappropriate relative amounts or deficiencies in endogenous defences can have deleterious effects. Free radicals can cause oxidative damage to lipids, DNA, bio molecules, rises in the concentration of intracellular calcium, as well as activation of proteases, nucleases and protein kinases. Considerable evidence supports the view that oxidative damage involving free radicals occurs in most, if not all, human diseases. Oxidative stress is now recognized as an important contributor to the development of many human diseases including liver fibrosis, ischemia-reperfusion, atherosclerosis, neurodegenerative disease and age-related cancer as well as to process of ageing. Thus antioxidants and systems that can protect against oxidative stress are needed to maintain health. A large body of scientific evidence supports that oxidative stress is directly responsible for aging (Aging Cell. 2009, 8(3):258-69) and an array of neuropathology conditions (Nutrition 2010, 26:595-603. Neurochem Res. 2007, 32:757-73). The free radical theory of aging proposes that the organism is unable to repair all of them and that, with time, unrepaired damages accumulate and put the organism at risk: in other words, free radicals provoke aging and death (FEBS Letters 2009, 498: 183-186. J. Neurochem. 2009, 108:1251-65). Antioxidants are the substances able to react with free radicals and to protect the body from the damage caused by these molecules (Ital J Biochem. 2006, 55:263-282). In particular, consumption in excess of some foods which are rich sources of antioxidants is considered to promote good health and longevity. It is now believed that the maintenance of redox balance within the body can forestall aging and promote good health and longevity.

[0006] Due to its 30 carbon double bonds, [60]fullerene (Buckminsterfullerene, Ceo) is a powerful free radical scavenger which characterize it as a radical sponge (Science 1991, 254, 1 83-1185). Biological applications of fullerenes and derivatives, in particular as antioxidants, have been extensively reviewed (Bioorg. Med. Chem. 1996, 4: 767-779. Eur. J. Med. Chem. 2003, 38: 913-923. Biomedicine & Pharmacotherapy, 2005, 59: 351-358). Ceo is only soluble in a limited number of organic solvents, such as toluene, benzene, chloronaphtalene and dichlorobenzene. Availability of biocompatible aqueous solutions of Ceo and its derivatives that are insoluble in water have been major obstacles to toxicity and in vivo studies of this new family of compounds. Biological properties of water-insoluble fullerenes are still misunderstood and to our knowledge there are no certified toxicology data about them. Most of the fullerenes studied until now are water-soluble derivatives, since study of water-insoluble fullerenes, such as pristine Ceo, in biological medium proves difficult. It is a common practice to derivatize the fullerene core with substituants such as OH, COOH, NH2 to increase hydrophilicity (Bioorg. Med. Chem. 1996, 4: 767-779. Eur. J. Med. Chem. 2003, 38: 913-923. Biomedicine & Pharmacotherapy, 2005, 59: 351-358). Water-soluble Ceo derivatives have been found to retain in vitro the free radical scavenger properties of their parent fullerene molecule, allowing these properties to be exploited in biological systems. Many patents already exist for a broad range of biomedical applications and other commercial applications of water-soluble fullerenes, including anticancer and anti-HIV therapies, drugs for neurodegenerative diseases, drug delivery systems, and preparations that retard aging. In particular, a group of hydrophilic Ceo derivatives, carboxyfullerenes, were proposed to increase metazoan's lifespan (U.S. Patent Application 2003/0162837). However, water-soluble fullerenes are difficult to synthesize and to purify. Besides, in contrast to pristine Ceo, which is non-toxic, some C6o-derivatives can be highly toxic (Adv Exp Med Biol, 2007, 620, 168-80).

[0007] Pristine C6o has been shown to be more effective as an antioxidant than certain carboxyfullerenes in Wang, I. et al., J. Med. Chem. 1999, 42, 4614-4620. However, Ceo has not been employed as an active ingredient to develop an in vivo treating method in this publication. Aqueous suspensions of Ceo are well known in the art. They are stable for long periods and can be delivered to cells. A study of 14C-labeled C-60 reported that it is possible to form suspensions of C6o in water that are stable for long periods (J. Am. Chem. Soc. 1994, 116, 4517-4518). However, the authors failed to detect the fullerene inside the cells and these suspensions containing very low concentrations of fullerene (typically 0.1 mg per ml) were inadequate to perform in vivo studies, especially toxicity studies. and metabolic fate investigations ((J. Am. Chem. Soc. 1994, 116, 4517-4518). Other vectorisation methods include the formation of inclusion complexes with cyclodextrins, calixarenes, tween-20, micelles, liposomes, and vesicles; however the Ceo concentrations reached by such methods are still very low (1 mg/mL at most) and inadequate to perform in vivo toxicity studies. Further, these methods present another drawback because they generally necessitate a preliminary dissolution step of the fullerene in an organic solvent. Other studies proposed the use of Ceo nanoparticles suspended in aqueous media to form a colloidal solution so-called nC60, however such solutions prpved to be highly toxic because they contain impurities linked to the oxidation byproducts of the solvents used during their preparation (Adv Exp Med Biol, 2007, 620, 168-80. Journal of Nanoscience Letters 2011, 1: 62-63). Another method, disclosed in J. Med. Chem. 2000, 43, 3186-3188 uses polyvinyl-pyrrolidone to solubilize C6o; however this vehicle can react with fullerene and the formed complex may cause harmful effects on mice embryos.

[0008] Moussa et al. described in Fullerene Science & Technology 1995, 3, 333-342 that partially micronized Ceo particles can be incorporated into living human phagocyte cells. C6o was directly suspended in the culture media and did not exhibit acute toxicity. Moussa et al. also described in Fullerene Science & Technology 1996, 4, 21-29 that micronized particles of water-insoluble fullerenes may be administered to mice on the form of a biocompatible aqueous suspension comprising a surfactant (tween 80) and a suspending agent (carboxym ethyl cellulose) which stabilizes the suspension. The authors disclosed that C6o is non-toxic, can cross cellular membranes and accumulates in liver and spleen (Fullerene Science & Technology 1996, 4:21-29). The same group headed by F. Moussa have already used Micronized Ceo suspensions as free radical scavenger in vivo (Nano Letters 2005, 5: 2578-2585). However, the effective doses were very high (i.e. >1 g/kg of body-weight) and intra peritoneal (i. p.) administration was the only route of administration for such suspensions. The authors also disclosed that Ceo can solubilize in vivo inside lipid droplets (Nano Letters 2005, 5: 2578-2585). This result has been confirmed in vitro by other authors whom studied Oßo solubility in vegetable oils (Fullerenes, Nanotubes, and Carbon Nanostructures, 2007, 15: 311-314. Fullerenes, Nanotubes, and Carbon Nanostructures, 2007, 15: 331-339). Stable biocompatible compositions comprising water insoluble fullerenes dispersed and/or dissolved in a carrier selected from the group consisting of fats and oils in an amount ranging from 0.2 to 10% by weight relative to the total weight of the composition, preferably from 0.1 to 2% by weight, were already proposed by N Gharbi and F Moussa for preventing damages caused by free radicals (2005/International Application No, PCT/EP2005/004963). However, in such compositions the water-insoluble fullerene is not fully dissolved and their oral absorption was unknown. Further, large aggregates of the administered fullerene can be filtered by liver and spleen and confined in their reticulo-endothelial system (RES) thus altering the diffusion and biodistribution of Ceo in the whole body. Thus, the in vivo use of water-insoluble fullerenes as free radical scavengers through delivery thanks to a non-aqueous carrier is still not satisfactory.

[0009] The inventors of the instant invention have now discovered a surprising use of [60]fullerene as agent that promotes an increases in the overall length of the expected lifespan of mammals.

[0010] Compositions comprising [60]fullerene and their use for preventing damages caused to metazons by free radicals are disclosed in TN Patent No.TN 2011/327 issued Jun. 30, 2011 to Moussa et al. which is incorporated herein by reference in its entirety.

SUMMARY OF THE INVENTION

[0011] It is in view of the above problems that the present invention was developed. The main objective of the invention is to provide a process or method for extending the longevity of a mammal, which comprises a step of administering to said mammal a composition comprising an effective amount of [60]fullerene, which avoids the drawbacks of the prior art processes, and in particular: 1—avoids the use of charge transfer complexes, 2

—avoids the use of organic solvents, and 3—avoids in situ aggregation of the administered fullerene. It has now been discovered by the inventors that the compositions comprising [a]fullerene dissolved in a suitable carrier selected from the group consisting of oils and fats proved suitable to achieve the aforementioned objectives. In particular, not only they allow [60]fullerene to be administered orally or intramuscularly or intra peritoneally to prolong the longevity of mammals but they are at least several times more active than previous compositions. Thus, a first embodiment of the instant invention comprises a stable biocompatible composition comprising (a) a carrier selected from the group consisting of fats and oils; and (b) [60]fullerene, wherein [60]fullerene is almost dissolved in said carrier. The embodiment is further drawn to compositions, in which [60]fullerene is dissolved in the carrier. Another embodiment of the instant invention is a method to prolong the longevity of mammals, which comprises a step of administering to said mammal a stable biocompatible composition comprising an effective amount of [60]fullerene dissolved in a carrier selected from the group consisting of fats and oils. In a preferred embodiment, the invention is drawn to a method of prolonging the longevity of mammals, which comprises a step of adding to food or any nutritional composition a stable composition comprising an effective amount of [60]fullerene dispersed in a carrier selected from the group consisting of fats and oils.

[0012] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The foregoing and other objects, features and advantages of the present invention will become readily apparent to those skilled in the art from a reading of the detailed description hereafter when considered in conjunction with the accompanying drawings wherein:

[0014] FIG. 1 is a representation of the growth rate of rats as a function of time, which were treated or not with a composition according to the present invention.

[0015] FIG. 2 shows whole blood Ceo concentrations-time plot (mean ±S.E . . .) following single dose oral administration (4 mg/kg, n=3) or single dose intra-peritoneal (ip) bolus injection of the same dose (n=3) of Ceo dissolved in olive oil (0.8 mg/ml) (n=3),

[0016] FIGS. 3 and 4 show the results of some biochemical tests for Ceo pre-treated and control rats before CCl4 administration, and

[0017] FIG. 5 represents the survival percentage of rats (n=6 per group) orally treated with Ceo at the age of 10 months (1 ml/kg of body-weight, weekly until the end of the second month then every two weeks until the end of the 7th month, with water, olive oil or Ceo dissolved in olive oil (0.8 mg/ml)). Table 1 summarizes the mean pharmacokinetic parameters obtained in rats after oral (n=3) or intra-peritoneal (n=3) administration of Ceo dissolved in olive oil and table 2 summarizes Ceo concentrations in whole blood (WB), liver, spleen and brain of rats daily treated with a single dose of Ceo dissolved in olive oil (4 mg/kg body weight) by oral (n=3) or ip route (n=3).

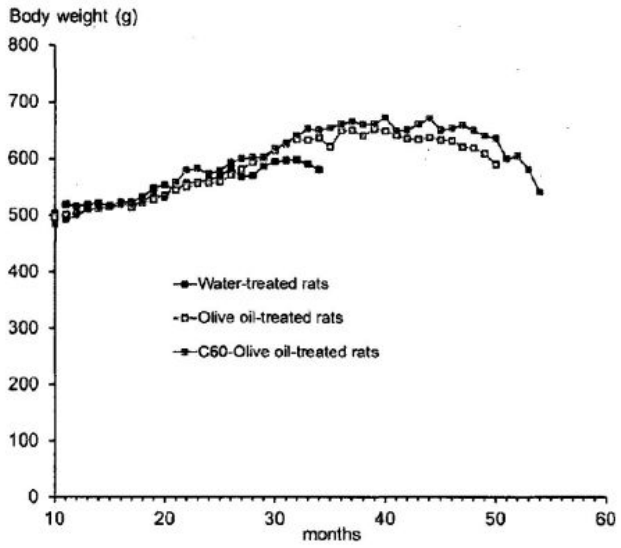
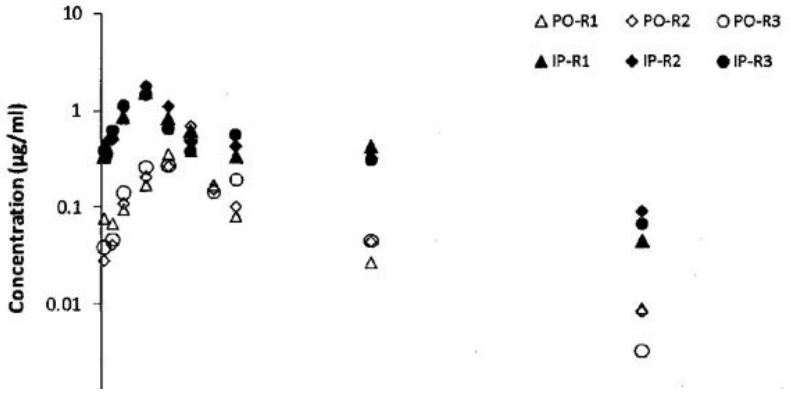


Figure 1. Growth of surviving rats (n = 6 per group) treated at the age of ten months with gavages (daily during one week, then weekly until the end of the second month then every two weeks until the end of the 7th month) of 1 ml of water or olive oil or C_{60} dissolved in olive oil (0.8 mg/ml) (BW = body-weight).



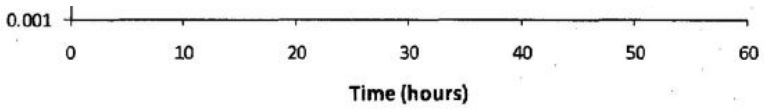


Figure 2. Individual rat plasma C_{60} concentrations *versus* time plot following single dose (4 mg/kg) of C_{60} dissolved in olive oil (0.8 mg/ml), administered by intra-peritoneal route (IP) or oral route (PO) (R1, R2, R3 = rat 1, rat 2 and rat 3).

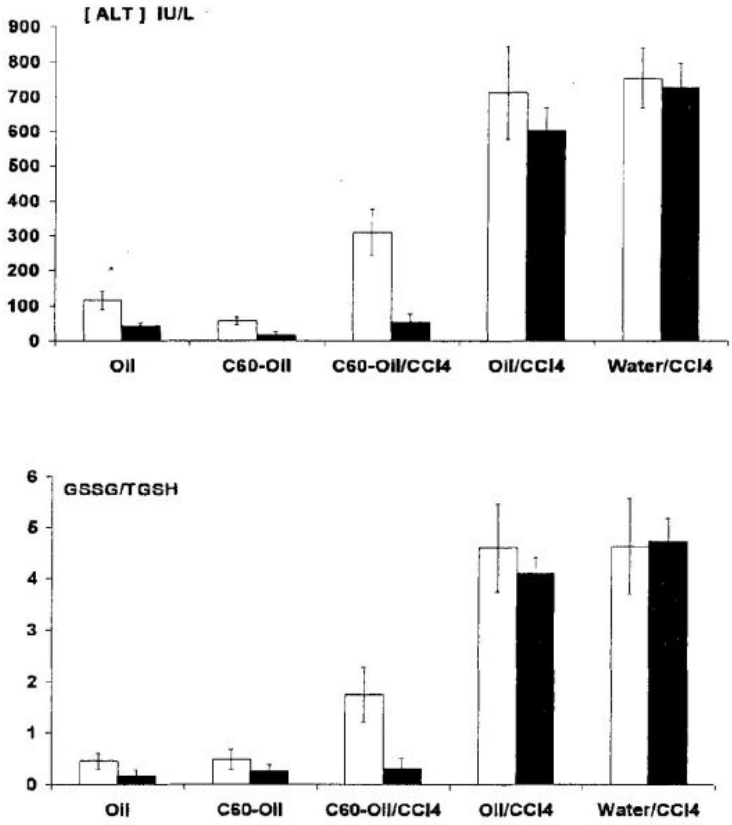


Figure 3. Effect of C_{60} pretreatment (4 mg/kg bw, 7 successive days) on (top) serum alanine aminotransferase (ALT) activity, used as biochemical marker of liver injury, and (down) circulating levels of oxidized glutathione/total glutathione ratio, used as gauge of oxidative stress, of rats intoxicated by CCl_4 administration at day 8. (GSSG) oxidized and (GSH) reduced glutathione, TGSH: total glutathione (GSSG + GSH). (White bars) orally pretreated rats and (black bars) i.p. pretreated rats. Data are the mean ± SD for six rats.

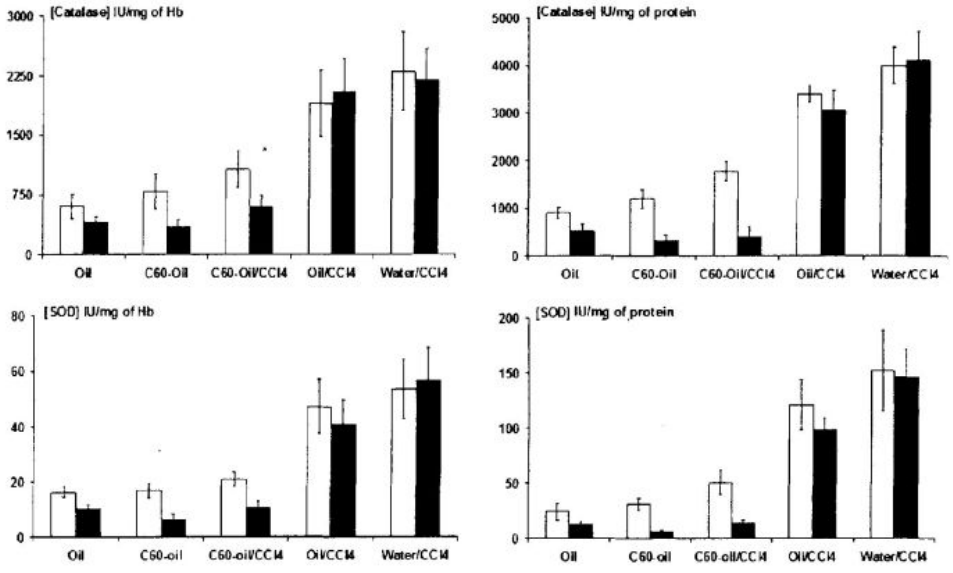


Figure 4. Effect of C_{60} pretreatment (4 mg/kg bw, 7 days) on (top) catalase and (down) superoxide dismutase (SOD) activities, used as biochemical markers of oxidative stress, inside (left) erythrocytes and (right) livers of orally (white bars) and i.p. (black bars) pretreated rats of rats intoxicated by CCl_4 administration at day 8. Data are the mean ± SD for six rat.

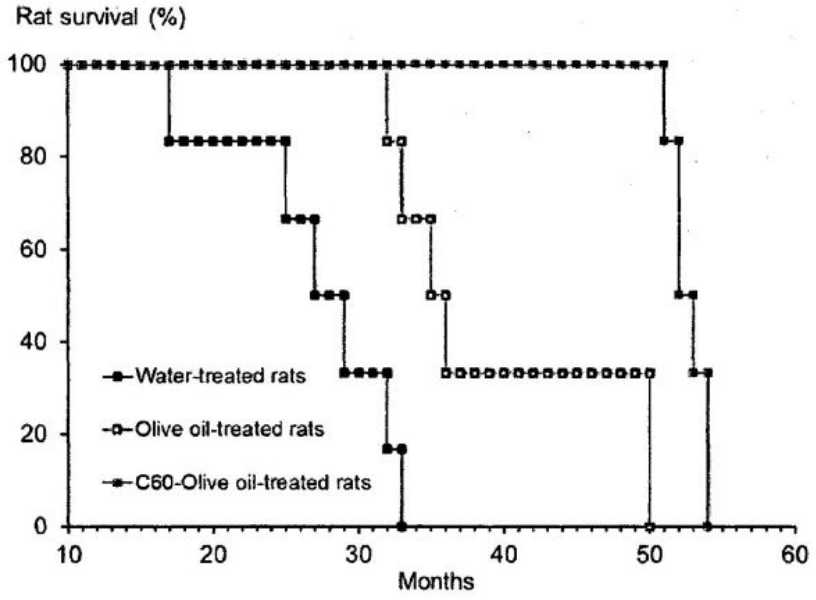


Figure 5. Survival percentage (Kaplan-Meier plot) of rats (n = 6 per group) treated at the age of ten months daily for one week, then weekly until the end of the second month and then every two weeks until the end of the 7th month, by gavages with 1 ml of water or olive oil or C_{60} dissolved in olive oil (0.8 mg/ml), respectively.

Table 1: Mean pharmacokinetic parameters obtained in rats after oral (n = 3) or intra-peritoneal (n = 3) administration of C_{60} dissolved in olive oil

	Oral Value ± SD (CV %)	IP Value ± SD (CV %)
$t_{½}$ (h)	9.3 ± 2.7 (28.6 %)^{NS}	13.9 ± 2.9 (20.8 %)
Cmax (µg/ml)	0.52 ± 0.16 (30.7 %)***	1.47 ± 0.15 (10.2 %)
Tmax (h)	8.0 ± 0.1	4.0 ± 0.1
AUC_0^{∞} (µg.h.ml^{-1})	4.37 ± 0.60 (0.14 %)***	21.21 ± 1.50 (7.1 %)
Cl/F (ml.h^{-1})	185.5 ± 27.5 (14.8 %)***	37.8 ± 2.6 (6.9 %)
Vd/F (L)	2.56 ± 1.09 (42.6 %)*	0.75 ± 0.12 (15.5 %)
MRT (h)	12.6 ± 0.9 (7.1 %)*	18.0 ± 1.9 (10.6 %)

Statistical differences were evaluated by T-test. *p<0.05, **p<0.01, ***p<0.001

Table 2. C_{60} concentrations at day 1 (D_1) and day 8 (D_8) in whole blood (WB), liver, spleen and brain of rats daily treated with a single dose of C_{60} dissolved in olive oil (4 mg/kg bw) by oral gavages or i.p. route (IP, Mean ± SD, n = 3) (Lw = liver weight; Sw = spleen weight; Bw = brain weight; TAD = total administered dose).

	Oral (D_1)	Oral (D_8)	IP (D_1)	IP (D_8)
WB (C_{60}, µg/ml)	0.03 ± 0.01	0.18 ± 0.06	0.36 ± 0.06	0.56 ± 0.17
Liver (C_{60}, µg/g)	0.21 ± 0.04	2.92 ± 0.82	4.91 ± 1.52	31 ± 12
Lw (g)	5.2 ± 0.6	7.5 ± 0.8	7.7 ± 0.8	10.0 ± 1.1
C_{60} (%/TAD)	0.14	0.39	4.73	5.54
Spleen (C_{60}, µg/g)	2.99 ± 1.37	51 ± 14	23 ± 6	191 ± 40
Sw (g)	0.48 ± 0.10	0.56 ± 0.13	0.54 ± 0.17	0.70 ± 1.3
C_{60} (%/TAD)	0.18	0.51	1.55	2.39
Brain (C_{60}, µg/g)	0.013 ± 0.003	0.20 ± 0.08	0.54 ± 0.17	3.78 ± 1.25
Bw (g)	1.81 ± 0.03	1.83 ± 0.03	1.85 ± 0.05	1.82 ± 0.04

C_{60} (%/TAD)	0.003	0.007	0.125	0.123

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

[0018] Free radicals are constantly formed in vivo. Some of these molecules are physiologically useful, but they can also result in pathological oxidative stress to cells and tissues. Endogenous defences include both antioxidants and repairing systems. However, excess production of free radicals, their production in inappropriate relative amounts or deficiencies in endogenous defences can have deleterious effects. Free radicals can cause oxidative damage to lipids, DNA, bio molecules, rises in the concentration of intracellular calcium, as well as activation of proteases, nucleases and protein kinases. Considerable evidence supports the view that oxidative damage involving free radicals occurs in most, if not all, human diseases. Oxidative stress is now recognized as an important contributor to the development of many human diseases including liver fibrosis, ischemia-reperfusion, atherosclerosis, several neurological disorders and age-related cancer as well as to process of ageing. Thus antioxidants and systems that can protect against oxidative stress are needed to maintain health and prolong the expected lifespan in metazoans. This has led to attempts to develop additional antioxidants to supplement the antioxidant defences of cells as potential therapeutic agents. Diet-derived antioxidants and a number of small molecules that can scavenge free radicals as well as super oxide dismutase-mimetics and chelators of transition metal ions were proposed as potential therapeutic agents against oxidative stress. Compositions according to the invention comprising [60]fullerene have been found to exhibit highly efficient antioxidant properties in vivo. The fullerene core, i.e. the fullerene skeleton without lateral substituants, used in the practice of this invention comprise clustered carbon structures generally spherical in shape and having a carbon content of 60 carbon atoms. Typically, [60]fullerene according to the invention is present in an amount ranging from 0.01 to 0.0.08% by weight relative to the total weight of the composition, preferably 0.08% by weight. [60]fullerene is preferably dissolved in the carrier, i.e. the composition can be filtered through a 0.2 pm filter. The stable, biocompatible compositions according to the invention comprise a carrier selected from the group consisting of fats and oils; and [60]fullerene, wherein said fullerene is mostly dissolved in said carrier. The carrier used in the present invention is a pharmaceutically acceptable and biocompatible carrier, selected from the group consisting of fats and oils. The fat or oil may be any natural or synthetic fat or oil suitable for administration to a mammal. They are not particularly restricted inasmuch as they are components which can be used in pharmaceutical preparations or in foods. Oils and fats can be hydrogenated or partially hydrogenated. They are used at a solid, a semisolid, or a liquid state. Vegetable and animal fats and oils are preferred, vegetable fats and oils are most preferred. Oils and fats include, without limitation fatty acid esters, fatty acids, fatty alcohols and fatty alcohol esters. Synthetic lipids can also be used. Fatty acids, as defined herein, are intended to mean aliphatic monocarboxylic acids having a chain of 4 to 40 carbon atoms, which may be branched or unbranched, saturated or unsaturated, cyclic or acyclic. Fatty acids may be natural or synthetic, polyunsaturated, mono-unsaturated or saturated. Natural fatty acids, which are usually unbranched and C4-C28 even-numbered, are preferred. Examples of fatty acids include, but are not limited to, linoleic acid, arachidonic acid, linolenic acid, gamma-linolenic acid, caprylic acid, stearic acid, myristic acid, a palmitic acid, behenic acid, undecylenic acid, oleic acid, an decosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), isostearic acid, 12-hydroxy-stearic acid. Salts thereof [e.g. alkali metal salts (sodium salts, potassium salts, etc.), alkaline earth metal salts (calcium, magnesium salts etc.)] can also be employed. Fatty acid esters are preferably esters of fatty acid as defined hereinabove with C-1-C40 aliphatic or aromatic alcohols, preferably aliphatic, saturated or unsaturated, straight-chain or branched-chain, cyclic or acyclic. Alcohols can be polyols, having preferably up to five hydroxyl groups. Examples of fatty acid esters include, but are not limited to, triglycerides i.e. tri-esters of glycerol with fatty acids cited above, sterids i.e. esters of sterols with fatty acids cited above, the group consisting of the lower alkyl esters thereof (preferably methyl, propyl, butyl, isopropyl and hexyl), 1,2- or 1,3-diglycerides, 1- or 2-monoglycerides, polyglycolysed glycerides such as sucrose fatty acid esters, polyglyceryl fatty acid esters, propylene glycol fatty acid esters. Specific examples of fatty acid esters are octyldodecyl behenate; isocetyl behenate; isocetyl lactate; isostearyl lactate; linoleyl lactate; oleyl lactate; isostearyl octanoate, isocetyl octanoate, decyl oleate, isocetyl isotearate, isocetyl laurate; isocetyl stearate; isodecyl octanoate; isodecyl oleate; isononyl isononanoate; isostearyl palmitate; myristyl isostearate; octyl isononanoate; 2-ethylhexyl isononanoate; octyl isostearate; octyldodecyl erucate; isopropyl palmitates, 2-ethylhexyl palmitate, 2-octyldecyl palmitate, branched alkyl myristates such as isopropyl myristate, t-butyl myristate, 2-octyldodecyl myristate, hexyl isostearate, butyl isostearate, isobutyl stearate, hexyl laurate, 2-hexyldecyl laurate, propylene glycol monostearate and distearate. Examples of giycerides (fatty acid esters) include, without limitation, triolein, trilinolein, tripalmitin, tristearin, trimyristin, and triarachidonin. Examples of sterids (fatty acid esters) include, without limitation, cholesteryl oleate, cholesteryl linoleate, cholesteryl myristate, cholesteryl palmitate, cholesteryl arachidate. Examples of fatty alcohols include, without limitation, cetyl alcohol, stearyl alcohol, lauryl alcohol, myristyl alcohol, palmityl alcohol, behenyl alcohol, hexadecyl alcohol, oleic alcohol, isostearyl alcohol, cetostearyl alcohol. They can be used as esters with C4-C40 dicarboxylic, tricarboxylic or tetracarboxylic acids. Oils may be natural oils such as vegetable oils and animal oils (composed predominantly of triglycerides), or mineral oils such as silicon oils, fluorinated oils. Liquid paraffin can also be used. Examples of natural oil include, but are not limited to, oils from plant sources, such as corn oil, wheat germ oil, soybean oil, rice bran oil, rapeseed oil, canola oil, sesame oil, palm (kernel) oil, olive oil, camellia oil, peanut oil, coconut oil, sunflower oil, peanut oil, orange oil, evening primrose oil, borage oil, blackcurrant seed oil, cottonseed oil, beaver oil, pineapple oil, safflower oil, copra oil, oils found in coffee, and animal oils such as turtle oil, fish oil, cod-liver oil. Fats may be mineral fats or natural fats such as vegetable fats and animal fats. Petrolatum, paraffin can also be used. Examples of natural fat include, but are not limited to, butter, cocoa butter, theobroma, peanut butter, lard, beef fat, chicken fat, horse fat, lanolin and lanolin derivatives. Oils and fats can be polyunsaturated such as corn, soybean, safflower oils, or saturated, such as palm, coconut oils and butter, or mono-unsaturated, such as olive oil and canola oil. Other suitable carriers according to the invention are diisopropyl sebacate; diisopropyl adipate; diisostearyl adipate; octyldodecyl stearoyl stearate; pentaerythrityl tetra-isononanoate; pentaerythrityl tetraisostearate; triisopropyl citrate; triisostearyl citrate; and trioctyldodecyl citrate. Preferred carriers according to the invention are butter, cocoa butter, peanut butter, olive oil, soybean oil, cod-liver oil and liquid paraffin. As defined above, carriers may be used each alone or in a combination of two or more species. [60]fullerene is dissolved in the carrier, depending on the nature of the carrier. Some carriers are able to dissolve substantial amounts of water-insoluble fullerenes (several mg/g of carrier). In one embodiment, at least 0.8 mg of fullerene is dissolved per ml of the carrier (the carrier being a liquid). As an example, it is possible to dissolve a total weight up to 1 mg of C60 per g of olive or soybean oil in less than one week. The compositions according to the invention may be pharmaceutical compositions comprising the fullerene in a therapeutically effective amount. Preferably, said fullerene can protect against biologically reactive radical species, which means chemicals that are free radicals or contribute to the generation of free radicals. Generally, the biologically reactive radical species are generated from O2 or H2O2. Thus, the invention also concerns a method to prolong the longevity of a mammal, which comprises a step of administering to said mammal a stable biocompatible composition as defined hereinabove. [60]fullerene, when dissolved in the carriers of the present invention, can be administered to mammals and this compound is well absorbed by said mammals. Generally, the said fullerene is administered in an amount of at least 0.1 mg/kg of body weight per day. According to the method of the invention, the inventive compositions may be administered orally, intramuscularly, subcutaneously, intra dermally or intra peritoneally, rectally by suppositories or sublingually. For oral ingestion by a mammal to be treated, the carrier is preferably an edible carrier. In at least one embodiment, said composition is administered in a pure form. In another embodiment, it is administered in the form of an emulsion in water. In another embodiment the fullerene is administered as a labile C6o-derivative derivative that can deliver Ceo after administration. The compositions of the instant invention can be in any liquid or solid conventional pharmaceutical formulation. The carrier enables the fullerene to be formulated as tablets, pills, dragees, capsules, liposome, pomade, ointment, cream , lotion, emulsions, gels, syrups, slurries and the like. The compositions of the present invention are preferably presented for oral administration to mammals in unit dosage forms, such as tablets, capsules, and oral solutions, containing suitable quantities of [60]fullerene. The compositions may be sterilized and/or may contain some adjuvants such as preservatives, stabilizers, acidity regulators, natural or synthetic flavour, anti-foaming agents, viscosity-control agents, emulsifiers, salts for varying the osmotic pressure and/or other buffers. In addition, compositions may contain other pharmaceutically active agents. The level of free radicals and reactive oxygen species in mammal cells decreases following treatment as compared to the level of reactive oxygen species in a cell that has not been contacted with a composition according to the invention. Indeed, [60]fullerene according to the invention can act as antioxidant and supplement the antioxidant defences of cells. That means they inhibit oxidation or inhibit reactions promoted by reactive oxygen species.

Physiologically relevant reactive oxygen species, which contribute to the generation of free radicals, include hydrogen peroxide, super oxide anion, and the like. The protective method of the invention reduces cell damage and death, and thus generally maintains the health of treated mammals. Further, the inventors discovered that [60]fullerene administered as biocompatible composition as described herein—1) can be absorbed after oral administration (FIG. 2);—2) they can react inside the liver with vitamin A (retinol) and esters thereof following a Diels-Alder-like reaction without any toxic effect (New J Chem, 1998, 989-992);—3) they are eliminated through the bile ducts (Nano Letters 2005, 5 (12): 2578-2585); and—4) despite the large amounts administered, no acute or sub-acute toxicity could be observed in mice and rats. No behaviour or growth disorder could be observed in treated animals either, which can be seen on FIG. 1. The latter shows growth rate of three groups (n=6) of rats which received weekly per os 1 ml of olive oil containing 0.8 mg of Ceo or 1 ml of olive oil only or 1 ml of water only. The in vivo new properties of [60]fullerene are due to fullerenes themselves and/or to the fullerene-retinol and fullerene-retinyl ester adducts formed after administration inside the liver. Also disclosed herein is a method for preparing a composition according to the present invention, comprising a carrier and particles of [60]fullerene. Said method comprises the steps of:—(a) Charging a milling vessel with the fullerene, the carrier and balls, said milling vessel and balls being made out of any biocompatible metal or polymer;—(b) Agitating the mixture resulting from step (a) until a homogeneous dissolution is obtained; and—(c) Sterilizing the composition resulting from step (b) by filtration. Direct mechanical milling in the carrier presents the advantages to accelerate the dissolution. Said method comprises the steps of:—(a) Charging a milling vessel with the fullerene, the fat or oil and balls, said milling vessel and balls being made out of any biocompatible metal or polymer;—(b) Agitating the mixture resulting from step (a) until complete homogenization of the solution;—(c) Agitating the composition resulting from step (b) until complete dissolution of the fullerene; and—(d) Sterilizing by filtration the composition resulting from step (c). Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present disclosure. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements. The invention is further illustrated by the examples described below. These examples are meant to illustrate the i nvention and are not to be interpreted as limiting the scope of the invention.

EXAMPLES

General Considerations

[0019] C60 (Purity: 99.98%) was purchased from Term USA (Fort Bragg, Calif., USA). Its characterization and its purity were assessed by HPLC, UV, C-NMR, and Mass Spectrometry. No impurity could be observed. It was used without further purification as well as after sublimation. All the other reagents were analytical grade and were purchased from Sigma (St Louis, Mo.). Animals received human care and the study protocols complied with general guidelines for the care and use of laboratory animals. Male Wistar rats (Charles River, France) were housed by groups of 3 in polypropylene cages at constant temperature (22° C.) and humidity (60%) and with a 12 h light/dark cycle, and fed a standard diet ad libitum. All rats were allowed to acclimate to this facility for at least one week before being used in the experiments. At the end of the experiment, body weights were determined and the animals were sacrificed under the same conditions by bleeding through the thoracic aorta after sodium pentobarbital (1.0 mL/kg of body weight) anaesthesia.

[0020] Biochemical tests, Cso determinations and statistics were processed as previously described in Nano Letters 2005, 5 (12): 2578-2585.

Example 1

Direct Dissolution of [60]Fullerene in a Vegetable Oil

[0021] In the stainless steel milling vessels of a Pulverisette 7 (Fritsch, Idar-Oberstein, Germany) or a similar device, add 8 mg of [60]fullerene and 10 mL of olive oil or 10 g of butter and 6 stainless steel balls (8 mm of diameter) (the milling vessels and the balls can be made out of any biocompatible metal or polymer such as stainless steel, tempered chrome steel, silicon nitride, corundum, tungsten carbide, agate, oxide of zirconium etc). Agitate the mixture during several hours (at 600 rpm for instance) until complete dissolution. The resulting homogenous solution or paste is then ready for use for oral administration or by any route of administration after appropriate sterilization. Sterilization may be achieved by filtration under vacuum (pore size: 0.2 μ?L). The sterilized composition is stable for at least 1 month. It is also possible to dissolve water-insoluble fullerenes in natural or mineral oils without stirring however the dissolution may be time consuming (up to several days at room temperature). Therefore, the former protocol is preferred. The fullerene concentration in compositions according to the invention can be determined by HPLC after adequate dilution in mobile phase as described previously (J. Chromatogr. B 1997, 696: 153-159).

Example 2

Pharmacokinetics and Biodistribution of an Oily Solution after Oral and Intra-Peritoneal Administration in Rats

[0022] Pharmacokinetic studies were carried out with male Wistar rats (weighing 200-220 g). Rats were housed in individual cages and maintained in an air-conditioned room (22-25° C.) on a 12 h light/dark cycle with water and food available. The rats were acclimated for 7 days and they were fasted overnight but with access to water, before treatment.

[0023] Under general anesthesia, a catheter was introduced into the rat right jugular vein, positioned subcutaneously with the tip in the inter-scapular region. The prepared rats were then allowed to recover for 24 h, and the blood catheters were flushed with 0.9% NaCl solution containing 20 I U/ml of heparin to avoid possible clot obstruction. Before Ceo administration, the rats were fasted overnight but with access to water. The same single dose of Ceo (4 mg/kg) was delivered orally, by a gavages needle, or intra-peritoneally to two groups of three rats. Blood (0.20 ml) was withdrawn via the canular prior to dosing (f=0) and at 15, 30, 60 min and then at 2, 4, 8, 10, 12, 24 and 48 h post-dosing. Antithrombin heparin (20 IU/m1) was added in each blood sample. After each blood collection 0.20 ml of sterile 0.9% NaCl solution were injected to the animal, to avoid hypovolemia. The rats were sacrificed 48 h after eo administration for organ collection (livers, spleens, and brains).

[0024] It was now discovered by the inventors that:—1) Soluble Ceo is absorbed and eliminated after either intraperitoneal or oral administrations. Table 1 represents the main pharmacokinetic parameters; 2—the maximal concentrations (Cmax) are reached 4 and 8 hours after i.p. and oral administrations, respectively (FIG. 2); 3—the maximal concentration after i.p. administration (1.47±0.15 pg/ml) is higher than that after oral administration (0.52±0.16 Mg/ml). Consequently, the area under the curve (AUC) is about 5 times larger when Ceo is administered by i.p. route as compared to oral route. Although these results do not allow determining the bioavailability of Ceo, they clearly show that a non-negligible % of the orally administered dose is absorbed as compared to the i.p. administered one (FIGS. 2); and—3) the fullerene is well distributed in the whole body, in particular it can cross the brain barrier (table 2).

Example 3

Ceo-Induced Protection of the Liver against Acute Toxicity of Carbon Tetrachloride (CCU) in Rats

[0025] Carbon tetrachloride is a classical hepatotoxicant that causes rapid liver damage progressing from steatosis to centrilobular necrosis. CCU intoxication in rodents is an important model for elucidation of the mechanism of action of hepatotoxic effects such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity. These effects are consistent with the known induced metabolic activation of CCl4 to reactive intermediates, including CCl3? and CCl02' free radicals, and mobilization of intracellular calcium. Kupffer cells (liver resident macrophages) participate in the mechanism of toxicity of CCl4 in vivo by release of chemoattractants for neutrophils and a series of chemical mediators

(cytokines). Both expression and synthesis of these cytokines are mainly modulated through redox-sensitive reactions. Further, involvement of reactive oxygen species and lipid peroxydation products can be demonstrated in other fundamental events of hepatic fibrogenosis, like activation of hepatic stellate cells (HSC: liver resident nonparenchymal cells also referred to as fat-storing or perisinusoidal cells, lipocytes and Ito cells). In a previous work, the effects of C60-pretreatments on acute carbon tetrachloride intoxication in rats, a classical model for studying free-radical-mediated liver injury was reported. The results obtained by the authors leaded by F Moussa (Nano Letters 2005, 5 (12), 2578-2585) showed that aqueous C6o suspensions not only have no acute or subacute toxicity in rodents but they also protect their livers in a dose-dependent manner against free-radical damage. The most effective dose of C6o reported in the latter paper was about 2.5 g/kg of body-weight and was administered intra-peritoneally and the better protection was obtained at day 14 after administration. It was now discovered by the inventors that the fullerene is about 100 times more active it is administered in solution than in suspension, and the effect is more rapid (24 hours after administration) as compared to the results published previously in the same experimental model (Nano Letters 2005, 5: 2578-2585).

Example 4

Ceo Prolongs the Longevity in Rats without Chronic Toxicity

[0026] The rats were housed one per cage and acclimated for 14 days, before dosing. Three groups of 10 rats (10 months old, weighing 495±31 g) were administered daily for one week, then weekly until the end of the second month and then every two weeks until the end of the 7th month, by gavages with 1 ml of water or olive oil or Ceo dissolved in olive oil (0.8 mg/ml), respectively. The rats were weighed before each dosing. Routine observations following official recommendations (EC Commission Directive 2004/73/EC of 29 Apr. 2004 Adapting to Technical Progress for the Twenty-Ninth Time Council Directive 67/548/EEC on the Approximation of the Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labeling of Dangerous Substances. O.J. No. L1522004) were made on all animals inside and outside the cage once a day throughout the study for signs of departure from normal activity, morbidity and mortality.

[0027] This experiment was initiated after observing that Ceo is absorbed through oral administration. To study the chronic toxicity of Ceo we designed a protocol according to the general guidelines of US food and drug administration (Chronic Toxicity Studies with Rodents in Toxicological Principles for the Safety Assessment of Food Ingredients. Redbook 2000, revised July 2007, Chapter IV.C.5.a. IV.C.5.a.) with some modifications. It was now discovered by the inventors that oral administration of Ceo increases significantly the longevity of rats (FIG. 5). At 25 months after the beginning of the treatment, the % of survival is equal to 25%, 67% and 100% for the rats treated with water, olive oil or C6o-dissolved in olive oil, respectively (FIG. 5). At 37 months, after the beginning of the treatment, this percentage is always equal to 100% for the rats treated with C6o-dissolved in olive oil, 17% for the rats treated with olive oil, and 0% for the rats treated with water (FIG. 5). The increase of the expected lifespan reported herein has never been reported for any other substance, to our knowledge.

TN2009000493
Oily Solutions of C60 Fullerene and their use for preventing damages caused to meatazoans by free radicals
Moussa FATHI, et al.
[PDF]

TN2011000327
Fullerene and Its Use to Maintain Good Health and Prolong the Expected Lifespan of Mammals
Tarek BAATI, et al.
[PDF]

Related Patents:

CN105596368
Fullerene olive oil composition and application thereof in treatment of Parkinson's disease
WANG, et al.
[PDF]

Abstract
The present invention discloses a fullerene olive oil composition and its application in the treatment of Parkinson's disease. The fullerene olive oil composition is an olive oil dispersion of fullerenes and / or metal fullerenes, and the fullerene and / or the metal fullerene have a concentration of 0.01-0.8 mg / mL. In the present invention, fullerene has strong ability of scavenging free radicals by in vitro ESR test, and then it is proved by cell-level experiments that it has no damage to the cells and is able to repair cell damage caused by free radicals. Finally, by constructing The Parkinson's mouse model, treated with the fullerene olive oil composition of the present invention, was found to actually improve the motor ability of mice, demonstrating the efficacy of the fullerene olive oil compositions of the present invention in the treatment of Parkinson's disease.
A fullerene olive oil composition and its use in the treatment of Parkinson's disease...

In a specific embodiment provided by the present invention, the concentration of C 60 in the C 60 - olive oil composition is 0.8 mg / mL, and the practically used fullerene olive oil is recommended to be administered in vivo at a dose of 0.1 to 10 mg / kg, The best dose is 4mg / kg. In another specific embodiment, the concentration of GdC82 in the GdC82-olive oil composition is 0.8 mg / mL and its dosage in vivo is 10-1000 μg / kg. The organism is a mammal, such as a human...

Example 1 Preparation of Fullerene Olive Oil Compositions

(1)GdC 82 - Preparation of olive oil composition
Weigh 20mL olive oil, weighed 20mgGdC 82 (particle size of 0.7 ~ 1nm), mixed and stirred evenly, and then the mixture was placed in a ball mill ball mill, 10h, the mixture was removed after the ball mill, cool and dry dark protected, static After 1 h of centrifugation, filtration was performed using a 220 nm filter to obtain a fullerene-olive oil solution, GdC 82 - olive oil composition, in which the content of GdC 82 was 0.4 mg / mL

(2)C 60 - Olive oil composition
Weigh 20mL olive oil, weighed 20mgC 60 (particle size of 0.7 ~ 1nm), mixed and stirred evenly, and then the mixture was placed in a ball mill ball mill, 10h, the mixture was removed after the ball mill, cool and dry dark protected, static After 1 h, centrifuged and filtered through a 220 nm filter to give a fullerene-olive oil solution, C 60 - olive oil composition with a C 60 content of 0.8 mg / mL...

The result of FIG. 6 proves that C60 can eliminate hydroxyl radicals generated by H 2 O 2 and reduce cell damage caused by free radicals...

RU2283273
FULLERENE SOLUTION PREPARATION METHOD

SUBSTANCE: invention can be used in manufacture of cosmetics, therapeutical agents, and other biologically active preparations. Fullerene is mixed with organic solvent to achieved homogenous mass. Resonance frequency of ultrasonic emission providing appearance of resonance state in system ultrasonic emitter-above prepared mixture is found. The mixture is then affected by ultrasonic emission at thus found frequency for at least 15 min at 40-70°C. Ultrasonic emissions with sinusoidal, rectangular, sawtooth pulse forms are suitable. Organic solvent is selected from those containing unsaturated carboxylic acid, e.g. oleic, linoleic, linolenic, arachidonic acid, or mixture thereof; sea-buckthorn oil, cedar oil, linseed oil, olive oil, or mixture thereof; cod-liver oil, animal fat, or mixture thereof; citric, orange, cypress essential oils, turpentine oil, camphor oil, or mixture thereof. ^ EFFECT: simplified fullerene dissolution procedure and extended range of nontoxic effective solvents compatible with biological structures...

A method is known for the preparation of fullerene solutions in solvents, including those tolerant with respect to biological structures (see RF patent No. 2198136 IPC 01/01 B 31/02, published October 16, 2001), which coincides with the claimed decision on the largest number of essential features and adopted for prototype.

A known method for the preparation of fullerene solutions comprises mixing the starting components by introducing fullerenes into a volume of a solvent, which is selected from an unsaturated fatty acid or a mixture of such acids, natural oil or natural fat, mineral oil, and a mixture of these components is prepared to obtain a homogeneous mass, and dissolution on the composition is effected by ultrasound and electromagnetic microwave radiation, while the parameters of ultrasonic radiation with a frequency of 2 5-40 kHz and a rectangular waveform with a maximum input power density of 50-100 mW / cm3, and electromagnetic microwave radiation parameters are selected with a wavelength of 9.4 mm, a maximum input power density of up to 1 W / cm3 at a pressure in the reaction volume of 50- 74 bar and a temperature in this volume of 80-150 ° C and an exposure time of 10 to 30 minutes. As an unsaturated fatty acid take oleic acid, and the mixture of acids is prepared from oleic and arachidonic acids, as an oil, sea buckthorn is used, and the mixture is prepared by adding cedar oil to it.

As fat, fish oil is taken, and the mixture is prepared by adding animal fat to it. Mineral oil is mixed with synthetic oil before introducing fullerenes into this mixture.

The known prototype method allows the preparation of fullerene solutions in non-toxic biocompatible solvents that can be used as ingredients in the synthesis of pharmaceuticals and cosmetic compositions.

However, the known method for producing fullerene solutions is rather complicated in the implementation, since it requires the simultaneous application of ultrasound and electromagnetic microwave radiation at high temperature (80-150 ° C).

The object of the present invention was to develop a method for dissolving fullerenes that would simplify the process of dissolution of fullerene, and also expand the range of nontoxic effective solvents compatible with biological structures for use in medicines and therapeutic cosmetic products.

This object is achieved by a method in accordance with the invention, comprising mixing the fullerene to obtain a homogeneous mass with an organic solvent selected from the group: an unsaturated carboxylic acid or a mixture of such acids, an oil containing an unsaturated carboxylic acid, or a mixture of such oils, natural fat containing an unsaturated carboxylic acid or a mixture of such fats, vegetable essential oil or a mixture of such oils, finding the resonant frequency of ultrasonic radiation that provides the appearance of a resonance state in Istemi: an ultrasonic emitter - the volume of said mixture, and then subjecting said mixture to ultrasonic radiation frequency found for at least 15 minutes at a temperature of 40-70 ° C.

You can use ultrasonic radiation with a sinusoidal pulse shape, with a rectangular pulse shape, with a sawtooth pulse shape.

As the unsaturated carboxylic acid, an acid selected from the group of oleic acid, linoleic acid, linolenic acid, arachidonic acid can be introduced.

As an oil containing an unsaturated carboxylic acid, a mineral oil or a synthetic oil can be used, as well as a natural vegetable oil.

As a natural vegetable oil containing unsaturated carboxylic acid, it is possible to introduce an oil selected from the group: sea buckthorn oil, cedar oil, linseed oil, olive oil.
As natural fat containing unsaturated carboxylic acid, it is possible to use fish or animal fat, for example badger fat.

Unsaturated carboxylic acids, which are fullerenes dissolving agents, are included in all known vegetable oils, in fish oil and in fat of mammals.

As a vegetable essential oil, it is possible to introduce an oil selected from the group: orange essential oil, lemon essential oil, cypress essential oil, eucalyptus essential oil, camphor oil, turpentine turpentine.

Vegetable essential oils have the same property necessary for the formation of a suspension - they are hydrophobic substances and when dispersed in water they form micelles. At the same time, there is no chemical interaction between them and water, which causes a change in their chemical structure.

The research conducted by the authors showed that the effect of resonant frequency on the fullerene and solvent mixture by ultrasonic radiation provides a significant increase in the efficiency of the fullerene dissolution process, which makes it possible to avoid additional processing of the mixture by electromagnetic microwave radiation, and also to lower the temperature at which ultrasonic resonance frequency 80-150 ° C to 40-70 ° C.

The use of natural vegetable essential oil as a solvent also allowed to expand the spectrum of nontoxic effective solvents, since natural vegetable essential oils are nontoxic and less flammable in comparison with conventionally used solvents.

The effect on the mixture of fullerene and solvent by ultrasonic radiation of a resonance frequency of less than 15 minutes at a temperature of less than 40 ° C leads to a significant decrease in the efficiency of the fullerene dissolution process. When the extraction process is carried out at a temperature above 70 ° C, there is a risk of ignition of the solvent.

The obtained solutions contain fullerenes in an amount up to 65 mg / cm3, do not give a precipitate, retain high biological activity with prolonged storage (for at least a year) and can be used as components of medicines and cosmetics (creams, lotions, shampoos, ointments etc.).

The claimed method for extracting fullerenes from fullerene-containing soot is explained in the drawings, where

FIG. 1 shows absorption spectra in the ultraviolet (UV) region of the fullerene solution in turpentine turpentine (1) and camphor oil (2);

FIG. 2 shows the absorption spectrum in the ultraviolet (UV) region of the C60 fullerene solution in linoleic acid;

FIG. 3 shows the spectrum of the disturbed total internal reflection in the infrared region of the spectrum of the C60 solution in linseed oil (1) and the differential spectrum (2) obtained by subtracting the spectrum of pure linseed oil from the spectrum of the fullerene C60 solution in linseed oil;

4 shows the spectrum of the disturbed total internal reflection in the infrared region of the spectrum of the C60 solution in cypress etheric oil;

FIG. 5 shows the differential spectrum of the disturbed total internal reflection in the infrared region of the spectrum of the C60 solution in lemon essential oil obtained by subtracting the spectrum of pure lemon essential oil from the spectrum of the fullerene C60 solution in lemon essential oil;

Table 1 shows the solubility of fullerenes in some solvents.

The claimed method is carried out as follows.

Based on the required concentration of fullerene in solution, the appropriate amount of fullerene and organic solvent is measured. Next, the fullerene is mixed with the organic solvent until a homogeneous mass is obtained. Depending on the purpose of the fullerene solution, the organic solvent is selected from the group: an unsaturated carboxylic acid or a mixture of such acids, an oil containing an unsaturated carboxylic acid, or a mixture of such oils, natural fat containing an unsaturated carboxylic acid, or a mixture of such fats, vegetable essential oil or a mixture such oils. Further, a resonant frequency of ultrasonic radiation is obtained, which ensures the appearance of a resonance state in the system: the ultrasonic radiator is the volume of the prepared mixture. Then, the mixture of fullerene and organic solvent is exposed to ultrasonic radiation of the resonant frequency found for at least 15 minutes at a temperature of 40-70 ° C.

The resulting solutions contain fullerene in an amount of up to 54 mg / ml and perfectly mix in cosmetic and drug compositions with the rest of the ingredients, without precipitating and maintaining a high level of biological activity. It was found that the dispersion and dissolution are not accompanied by the destruction of fullerene molecules.
Below are some results of the study of the solubility of fullerenes in organic solvents.

Example 1. The solubility of fullerene C60 in linoleic acid was investigated. Linoleic acid (grade OCT) in volume of 20 ml was placed in a transparent quartz tube. After that, fullerene C60 was added to linoleic acid with a purity of 99.5% in discrete portions of 5 mg and thoroughly mixed. After each addition of fullerene was added, the mixture was ultrasounded using a submerged cylindrical radiator, the frequency of the ultrasound generator being tuned such that a resonance was observed in the system being treated, the ultrasound emitter. The processing time by ultrasound after each addition to the solution of each new portion was 5 minutes, the resonance frequency was 28.5 kHz, the temperature was 50 ° C. This procedure was repeated until after the introduction of the next batch of fullerene and ultrasonic action at the bottom of the tube did not appear the precipitate of the non-dissolved fullerene. The solution was then centrifuged at 20,000 g (where g is gravity acceleration) and then filtered through a membrane filter with a pore diameter of 0.2 μm from Sartorius (Germany).
After filtration, a sample was taken for spectrophotometric measurements and a 10 ml sample to determine the specific solubility.

FIG. 2 shows the absorption spectrum of C60 fullerene in the ultraviolet region, in which characteristic absorption bands with maxima near 280 nm and 330 nm are present. The spectrum of the C60 solution in linoleic acid and in ethanol was measured with respect to linoleic acid in ethanol at a dilution of 1: 100 on a spectrophotometer Spectroscan II from LKB (Sweden). A 10 ml sample was placed in a container of known weight measured on an analytical balance of MC210S from Sartorius (Germany) of the first accuracy class with a sampling rate of 0.01 mg and then evaporated at 235 ° C to a constant weight measured on the same weights, after which the specific maximum concentration of the dissolved fullerene was determined. For linoleic acid, this value was 34.2 mg / ml.

Example 2. A procedure similar to that described in Example 1 was followed, but as a solvent, flax seed oil was used, and the exposure parameters used were: resonant frequency of the ultrasonic generator 34.6 kHz, temperature 55 ° C. Centrifugation and filtration were carried out as in Example 1. A sample was then taken for spectrophotometry and a sample of 10 ml for evaporation at a temperature of 250 ° C. The transmission spectrum of the C60 solution in linseed oil was removed in the infrared region by the method of violated total internal reflection (FTIR) on a FTIM 1202 Fourier spectrophotometer.

FIG. 3 shows the IRR spectrum of the C60 solution in flax oil and the differential spectrum obtained by subtracting the spectrum of pure linseed oil from the spectrum of the fullerene C60 solution in linseed oil. 3, all 4 active absorption bands characteristic of the C60 molecule are present in this spectrum, with maxima near 1430-1449 cm-1, 1170-1180 cm-1, 580 cm-1 and 530 cm-1. Measurements of the specific solubility of fullerene C60 in linseed oil yielded a value of 53.1 mg / cm3.

Example 3. A procedure similar to that described in Example 1 was followed, but oleic acid was used as the solvent, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic oscillator was 24.2 kHz, the temperature was 40 ° C. Centrifugation and filtration were carried out as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 250 ° C. Measurements of the specific solubility of fullerene C60 in oleic acid gave a value of 22.4 mg / cm3.

Example 4. A procedure similar to that described in Example 1 was followed, but linolenic acid was used as the solvent, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 25.1 kHz, the temperature was 52 ° C. Centrifugation and filtration were carried out as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 250 ° C. Measurements of the specific solubility of the fullerene of the solubility of C60 in linolenic acid gave a value of 35.5 mg / cm3.

Example 5 A procedure similar to that described in Example 1 was followed, but sea-buckthorn oil was used as the solvent, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 31.4 kHz, the temperature was 55 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 250 ° C. Measurements of the specific solubility of C60 fullerene in sea-buckthorn oil gave a value of 43.2 mg / cm3.

Example 6. A procedure similar to that described in Example 1 was followed, but the cedar oil was used as the solvent, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 34.3 kHz, the temperature was 70 ° C. Centrifugation and filtration were carried out as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 250 ° C. Measurements of the specific solubility of fullerene C60 in cedar oil gave a value of 51.8 mg / cm3.

Example 7. A procedure similar to that described in Example 1 was followed, but a mixture of cedar and sea-buckthorn oil was used as a solvent, and the following parameters were used: the resonant frequency of the ultrasonic generator 32.8 kHz, the temperature of 60 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A 10 ml sample was taken for evaporation at a temperature of 250 ° C. Measurements of the specific solubility of fullerene C60 in a mixture of cedar oil and sea buckthorn oil yielded a value of 47.5 mg / cm3.

Example 8. A procedure similar to that described in Example 1 was followed, but olive oil was used as a solvent, and the exposure parameters used

were as follows: the resonant frequency of the ultrasonic generator was 25.6 kHz, the temperature was 50 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 250 ° C. Measurements of the specific solubility of fullerene C60 in olive oil gave a value of 23.6 mg / cm3.

Example 9. A procedure similar to that described in Example 1 was followed, but fish oil was used as the solvent, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 28.4 kHz, the temperature was 65 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 250 ° C. Measurements of the specific solubility of C60 fullerene in fish oil gave a value of 53.0 mg / cm3.

Example 10. A procedure similar to that described in Example 1 was followed, but the cypress essential oil (purity 99.9%) was used as the solvent, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 23.1 kHz, the temperature was 50 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. After filtration, a sample was taken for spectrophotometric measurements and a 10 ml sample to determine the specific solubility.

4 shows the infrared spectrum of the disturbed total internal reflection of the C60 fullerene solution in cypress ether oil in which all four C60 bands active in the infrared region are present with maxima near 1430-1449 cm -1, 1170-1180 cm -1, 580 cm-1 and 530 cm-1. A 10 ml sample was placed in a container of known weight measured on an analytical balance MC210S from Sartorius (Germany) of the first accuracy class with a sampling rate of 0.01 mg and then evaporated at a temperature of 160 ° C to a constant weight measured on the same weights, after which the specific maximum concentration of the dissolved fullerene was determined. For cypress essential oil, this value was 20.6 mg / ml.

Example 11. A procedure similar to that described in Example 1 was followed, but the lemon essential oil (99.9% purity) was used as the solvent, and the exposure parameters used were the following: the resonant frequency of the ultrasonic generator was 25.7 kHz, the temperature was 50 ° C. Centrifugation and filtration were carried out in the same manner as in Example 10. After filtration, a sample was taken for spectrophotometric measurements and a 10 ml sample to determine the specific solubility.

FIG. 5 shows the differential infrared spectrum of the disturbed total internal reflection of the C60 fullerene solution in lemon essential oil, which was obtained by subtracting the spectrum of pure lemon essential oil from the spectrum of the C60 solution in lemon essential oil. As can be seen from FIG. 5, in the spectrum there are all four C60 bands active in the IR region with maxima near 1430-1449 cm-1, 1170-1180 cm-1, 580 cm-1 and 530 cm-1. A 10 ml sample was placed in a container of known weight measured on an analytical balance MC210S from Sartorius (Germany) of the first accuracy class with a sampling rate of 0.01 mg and then evaporated at a temperature of 160 ° C to a constant weight measured on the same weights, after which the specific maximum concentration of the dissolved fullerene was determined. For lemon essential oil, this value was 18.8 mg / ml.

Example 12. A procedure similar to that described in Example 1 was followed, but orange orange oil was used as the solvent, and the exposure parameters used were the following: the resonant frequency of the ultrasonic generator was 25.6 kHz, the temperature was 56 ° C. Centrifugation and filtration were carried out in the same manner as in Example 10. A 10 ml sample was then taken for evaporation at a temperature of 160 ° C. Measurements of the specific solubility of fullerene C60 in orange essential oil yielded a value of 21.3 mg / cm3.

Example 13. A procedure similar to that described in Example 1 was followed, but the eucalyptus essential oil was used as the solvent, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 23.8 kHz, the temperature was 55 ° C. Centrifugation and filtration were carried out in the same manner as in Example 10. A 10 ml sample was then taken for evaporation at a temperature of 160 ° C. Measurements of the specific solubility of fullerene C60 in eucalyptus essential oil gave a value of 23.4 mg / cm3.

Example 14. A procedure similar to that described in Example 1 was performed, but as a solvent, turpentine was used as a turpentine, and the exposure parameters used were the following: the resonant frequency of the ultrasonic generator was 27.3 kHz, the temperature was 65 ° C. Centrifugation and filtration were carried out in the same manner as in Example 10. A 10 ml sample was then taken for evaporation at a temperature of 160 ° C. Measurements of the specific solubility of fullerene C60 in the live turpentine yielded a value of 25.6 mg / cm3.

Example 15 A procedure similar to that described in Example 1 was followed, but a mixture of fullerenes obtained by extraction from fullerene black was used as a solvent, and turpentine was used as a solvent, and the mixture was processed in an ultrasonic bath. The exposure parameters were as follows: the resonant frequency of the ultrasonic generator was 26.8 kHz, the temperature was 60 ° C. Centrifugation and filtration were carried out in the same manner as in Example 10. A 10 ml sample was then taken for evaporation at a temperature of 160 ° C. Measurements of the specific solubility of fullerenes in living turpentine yielded a value of 24.7 mg / cm3.

The resulting solution was analyzed on a Specord M 40 UV spectrometer from Karl Zeis (Germany). The removed absorption spectrum in the UV region is shown as a curve (1) in FIG. As can be seen from FIG. 1, the spectrum is a typical spectrum of a fullerene mixture.

Example 16 A procedure similar to that described in Example 1 was followed, but a mixture of fullerenes obtained by extraction from fullerene black was used, camphor oil was used as the solvent. The exposure parameters were as follows: the resonant frequency of the ultrasonic oscillator was 26.6 kHz, the temperature was 55 ° C.
Centrifugation and filtration were carried out as in Example 10.
A 10 ml sample was then taken for evaporation at a temperature of 160 ° C. Measurements of the specific solubility of fullerenes in camphor oil gave a value of 23.1 mg / cm3. The resulting solution was analyzed in the same manner as in Example 15. The absorption spectrum in the UV region is shown in the form of a curve (2) in FIG. As can be seen from FIG. 1, the spectrum is a typical spectrum of a fullerene mixture.

Example 17 A procedure similar to that described in Example 1 was followed, but arachidonic acid was used as the solvent, and the following parameters were used: the resonant frequency of the ultrasonic generator was 27.8 kHz, the temperature was 50 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 250 ° C. Measurements of the specific solubility of fullerene C60 in arachidonic acid yielded a value of 49.2 mg / cm3.

Example 18. A procedure similar to that described in Example 1 was followed, but the domestic mineral motor oil M88 was used as the solvent, and the exposure parameters used were the following: the resonant frequency of the ultrasonic generator was 25.7 kHz, the temperature was 55 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A sample of 10 ml was then taken for evaporation at a temperature of 260 ° C. Measurements of the specific solubility of fullerene C60 in oil gave a value of 18.2 mg / cm3.

Example 19. A procedure similar to that described in Example 1 was followed, but synthetic engine oil VISCO 5000 5W40 (COMMA, UK) was used as the solvent, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 28.4 kHz, the temperature was 60 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 270 ° C. Measurements of the specific solubility of fullerene C60 in synthetic motor oil gave a value of 14.7 mg / cm3.

Example 20. A procedure similar to that described in Example 1 was followed, but as a solvent, a mixture of oleic and arachidonic acids taken in a ratio of 1: 1 was used, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 25.6 kHz, and the temperature was 50 ° C. Centrifugation and filtration were carried out as in
Example 1. A 10 ml sample was then taken for evaporation at a temperature of 250 ° C. Measurements of the specific solubility of fullerene C60 in a mixture of oleic and arachidonic acids gave a value of 38.2 mg / cm3.

Example 21. A procedure similar to that described in Example 1 was followed, but the badger fat was used as a solvent, and the exposure parameters used were: resonant frequency of the ultrasonic generator 29.6 kHz, temperature 70 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 270 ° C. Measurements of the specific solubility of fullerene C60 in badger fat gave a value of 48.2 mg / cm3.

Example 22. A procedure similar to that described in Example 1 was followed, but the dog fat was used as a solvent, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 28.7 kHz, the temperature was 70 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 270 ° C. Measurements of the specific solubility of fullerene C60 in badger fat gave a value of 47.1 mg / cm3.

Example 23. A procedure similar to that described in Example 1 was followed, but a mixture of canine fat and badger oil taken in a 1: 1 ratio was used as the solvent, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 29.1 kHz, the temperature was 70 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A 10 ml sample was then taken for evaporation at a temperature of 270 ° C. Measurements of the specific solubility of fullerene C60 in a mixture of badger and canine fats gave a value of 47.9 mg / cm3.

Example 24. A procedure similar to that described in Example 1 was carried out, but as a solvent, a mixture of domestic mineral motor oil M88 and synthetic engine oil VISCO 5000 5W40, taken in a ratio of 1: 1, was used, and the exposure parameters used were as follows: the resonant frequency of the ultrasonic generator was 27.6 kHz, temperature 60 ° C. Centrifugation and filtration were carried out in the same manner as in Example 1. A sample of 10 ml was then taken for evaporation at a temperature of 260 ° C. Measurements of the specific solubility of fullerene C60 in a mixture of oils gave a value of 16.1 mg / cm3.

The claimed method of obtaining a fullerene solution allows obtaining stable, transparent, non-precipitating solutions in non-toxic solvents while maintaining a high level of their biological activity (most natural essential oils themselves have medicinal properties, and are also widely used in the manufacture of cosmetics, including medicinal products) .