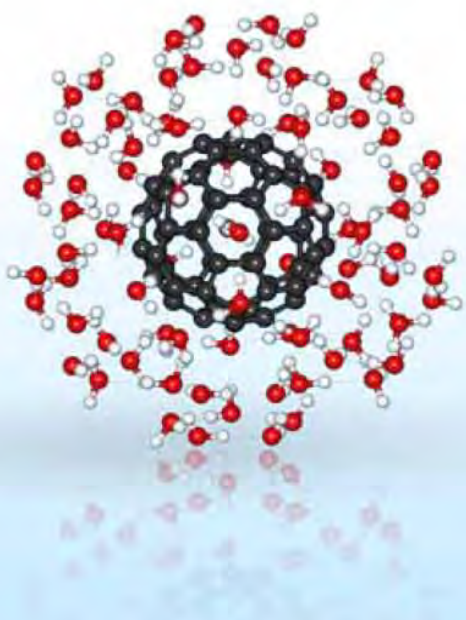
HYDRATED C_{60} FULLERENE

ГИДРАТИРОВАННЫЙ ФУЛЛЕРЕН C_{60}



HYDRATED C_{60} FULLERENE : SOME MOST IMPORTANT PHYSICAL-CHEMICAL AND BIOLOGICAL PROPERTIES

Andrievsky G.V.

Institute of Physiological Active Compounds, LLC, Kharkov, Ukraine

E-mail: yard@kharkov.ua and science@ipacom.com

ГИДРАТИРОВАННЫЙ ФУЛЛЕРЕН C_{60}: НЕКОТОРЫЕ НАИБОЛЕЕ ВАЖНЫЕ ФИЗИКО-ХИМИЧЕСКИЕ И БИОЛОГИЧЕСКИЕ СВОЙСТВА

Андриевский Г.В.

Институт Физиологически Активных Соединений, Харьков, Украина

A N D

WATER SPECIFICALLY STRUCTURED WITH HYDRATED C_{60} FULLERENES AS AN UNIVERSAL ANTIOXIDANTS AND REGULATOR OF FREE RADICAL PROCESSES IN BIOLOGICAL SYSTEMS.

Grigoriy Andrievsky

Institute of Physiological Active Compounds, LLC, Kharkov, Ukraine.

ОСОБЫЕ СТРУКТУРЫ ВОДЫ (ОСВ), КАК УНИВЕРСАЛЬНЫЕ БИОАНТИОКСИДАНТЫ, РЕГУЛЯТОРЫ СВОБОДНО-РАДИКАЛЬНЫХ ПРОЦЕССОВ В БИОЛОГИЧЕСКИХ СИСТЕМАХ: ЧТО ОБ ЭТОМ ПОВЕДАЛИ ГИДРАТИРОВАННЫЕ ФУЛЛЕРЕНЫ.

Андриевский Г.В.

Институт Физиологически Активных Соединений, Харьков, Украина

MAIN TERMS:

- C_{60}HyFn

HYDRATED C_{60} FULLERENES

- FWS

WATER SOLUTIONS of C_{60}HyFn

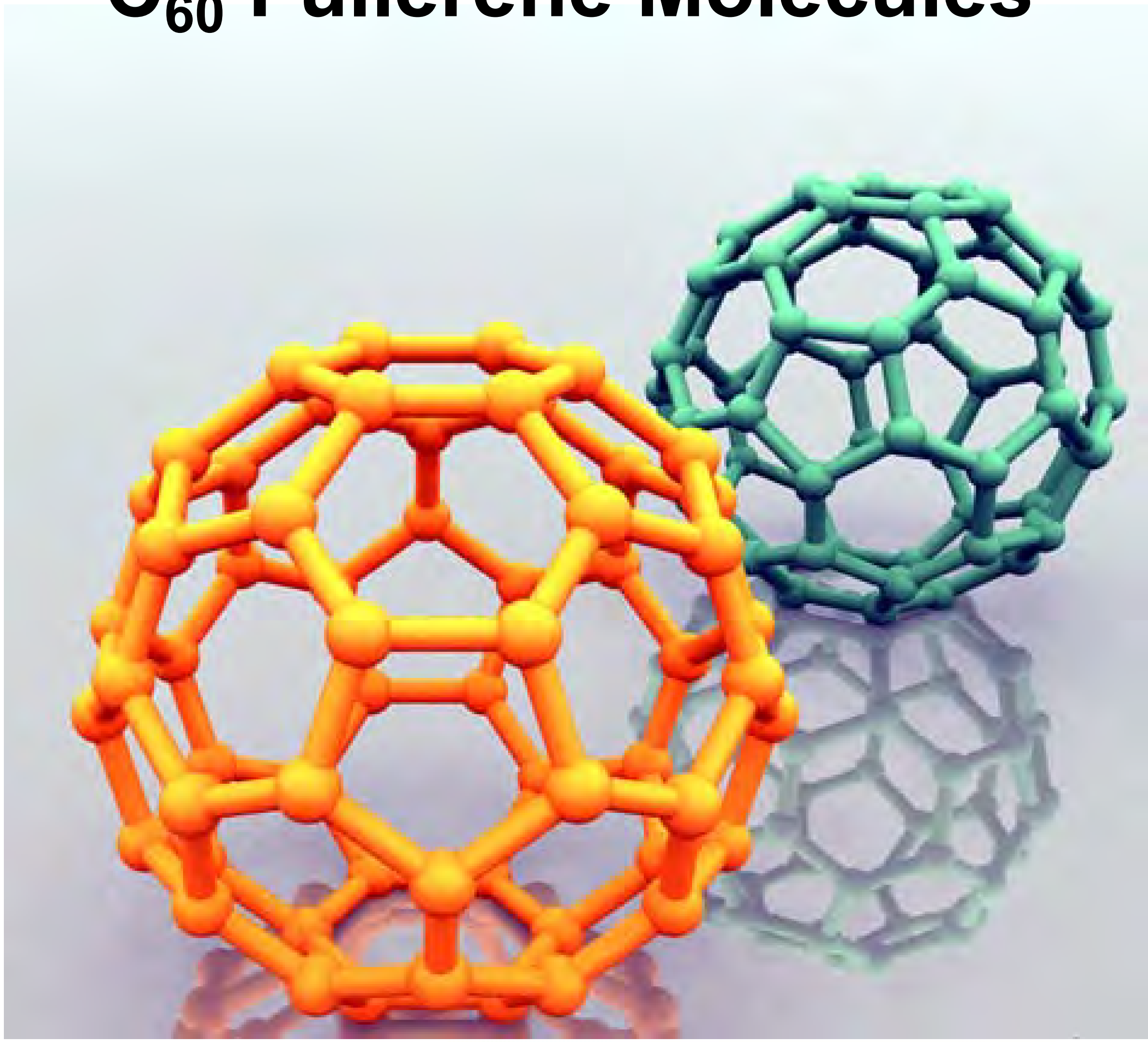
(Fullerene-Water-Solutions)

- CLOSE BOUNDED WATER

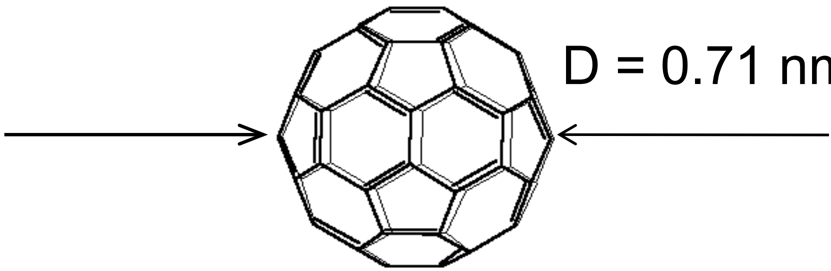
- ORDERED WATER

- WATER CLUSTERS

C_{60} Fullerene Molecules



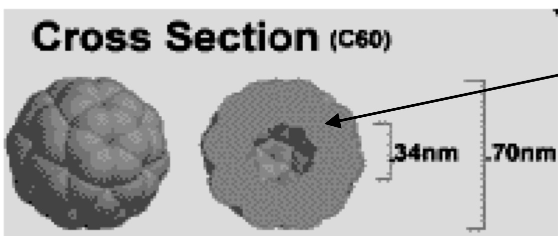
FULLERENES – VACUUM BUBBLES WITH ABSOLUTE PHYSICAL VACUUM INSIDE



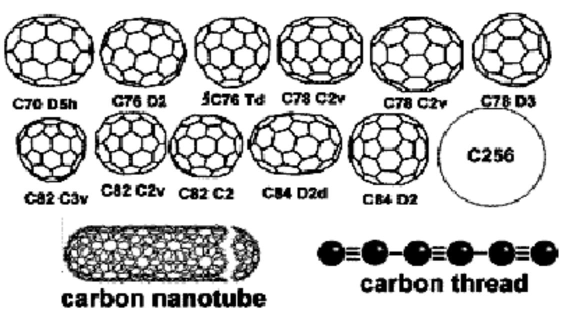
D = 0.71 nm

FULLERENE C60

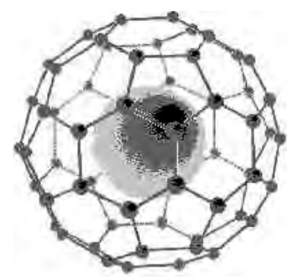
The distance "centre to centre" between two opposite C atoms is 0.71 nm.
If to it to add the values of two covalent radiuses of C atom (2 x 0.077 = 0.154 nm) we will receive real diameter of isolated C_{60} molecule in vacuum - 0.854 nm.
It is strange, but that the diameter of C_{60} molecule is equal to 1.0 nm is generally accepted currently. Why!?



Cavity
~ 0.3 – 0.4 nm



In this cavity it is possible to place the various atoms (A), for example, such as Li, K, N, Kr, La, Dy, Er and H_2O molecule (!).
In this case so-called endohedral C_{60} fullerenes ($A@C_{60}$) are formed.



$A@C_{60}$

MONUMENT FOR C_{60} FULLEREN IN KHARKOV, UKRAINE

PHYSICAL-CHEMICAL PROPERTIES OF HYDRATED C_{60} FULLERENE AND IT WATER SOLUTIONS (C_{60}FWS).

Evolution of Our Knowledge (1994-2004): From an Ultramicroheterogeneous Colloidal Dispersions to the Aqueous Molecular-Colloidal Solutions of the Single Hydrated C_{60} Fullerenes.

Additional Information see on our site:

1. http://www.ipacom.com/index.php/en/publications-about-c60hyfn/71 ;
2. http://www.ipacom.com/index.php/en/researches/90 .
3. http://www.ipacom.com/images/Articles/c60hyfn_phys_chem_bio_prop_51.pdf

The General Method of the Production of an Aqueous Molecular-Colloidal Solution of Fullerenes Based on Transfer of Fullerenes from Non-Polar Solvents to Polar One Under Ultrasonic Treatment was Described in:

G.V.Andrievsky, M.V.Kosevich, O.M.Vovk, V.S. Shelkovsky, L.A.Vashchenko. **ARE FULLERENES SOLUBLE IN WATER?** // Recent Advances in The Chemistry and Physics of Fullerenes and Related Materials /Edit R.S.Ruoff and K.M.Kadish, Proc.187th Meeting of The Electrochemical Society, Reno, 16-21 May 1995, The Electrochem. Soc. Inc., 1995, p. 1591-1602.

G.V. Andrievsky, M.V. Kosevich, O.M. Vovk, V.S. Shelkovsky, L.A.Vashchenko**. ON THE PRODUCTION OF AN AQUEOUS COLLOIDAL SOLUTION OF FULLERENES**. J.Chem.Soc., Chem. Commun., 1995, 12, p.1281-1282.

In accordance with:

1). **"MODE OF THE PRODUCTION OF MOLECULAR-COLLOIDAL SOLUTION OF FULLERENES IN WATER"**, *UA patent N29540 of 11/15/2000*.
2). **"AQUEOUS MOLECULAR-COLLOIDAL SOLUTION AT LEAST OF ONE HYDRATED FULLERENE",** *RU patent N 2213692 of 07/16/2002*.
3). **"BIOLOGICALLY ACTIVE ADDITIVES" (**BASED ON THE NATIVE FULLERENES), *UA patent N27669 of 09/15/2000*.

and

after modification and optimization of many technological parameters

now we are capable to receive

C_{60}FWS with C_{60} concentration up to ~4 mg/ml (5.5 mmol/L)

C_{60}FWS – WATER SOLUTION of HYDRATED C_{60} FULLERENE (C_{60}HyFn)

http://www.ipacom.com/index.php/en/production

C_{60}FWS samples.
<u>Left:</u> mother solution containing 0.36 mM (0.26 mg/ml) of C_{60}.
<u>Right</u>: diluted ten times with water.
The sample was presented to professor Eiji Osawa on June 30, 2003, who was performed dilution on July 11, 2003, and photo was made on October 4, 2003.

As of history of Colvin's methods of nano-C_{60} (THF/C_{60}) preparation and it applications:

C_{60} Fullerene In Water.

1- Dispersion receivd by V. Colvin method with C_{60} concentration of $70*10^{-6}$ mol/l (~ 50 mg/l = 36 ppm);

2- FWS with C_{60}HyFn concentration $70*10^{-6}$ mol/l (50 mg/l);

3- FWS with C_{60}HyFn concentration $265*10^{-6}$ mol/l (190 mg/l).

The sample '2' was prepared by means of dilution with water of the sample '3'.

1. J. D. Fortner, D. Y. Lyon, C. M. Sayes, A. M. Boyd, J. C. Falkner, E. M. Hotze, L. B. Alemany, Y. J. Tao, W. Guo, K. D. Ausman, V. L. Colvin, and J. B. Hughes. C_{60} IN WATER: NANOCRYSTAL FORMATION AND MICROBIAL RESPONSE. Environ. Sci. Technol., 39(11) (2005) 4307 - 4316.

2. C.M. Sayes, J.D. Fortner, W. Guo, D. Lyon, A.M. Boyd, K.D. Ausman, Y.J. Tao, B. Sitharaman, L.J. Wilson, J.B. Hughes, J.L. West, and V.L. Colvin. THE DIFFERENTIAL CYTOTOXICITY OF WATER-SOLUBLE FULLERENES. Nano Lett., 4(10) (2004) 1881 – 1887.

3. Oberdörster, E. MANUFACTURED NANOMATERIALS (FULLERENES, C_{60}) INDUCE OXIDATIVE STRESS IN BRAIN OF JUVENILE LARGEMOUTH BASS. Environmental Health Perspectives, 112 (2004) 1058–1062.

4. S. Deguchi, R. Alargova and K. Tsujii, K. STABLE DISPERSIONS OF FULLERENES, C_{60} AND C_{70}, IN WATER PREPARATION AND CHARACTERIZATION. Langmuir, 17 (2001) 6013–6017.

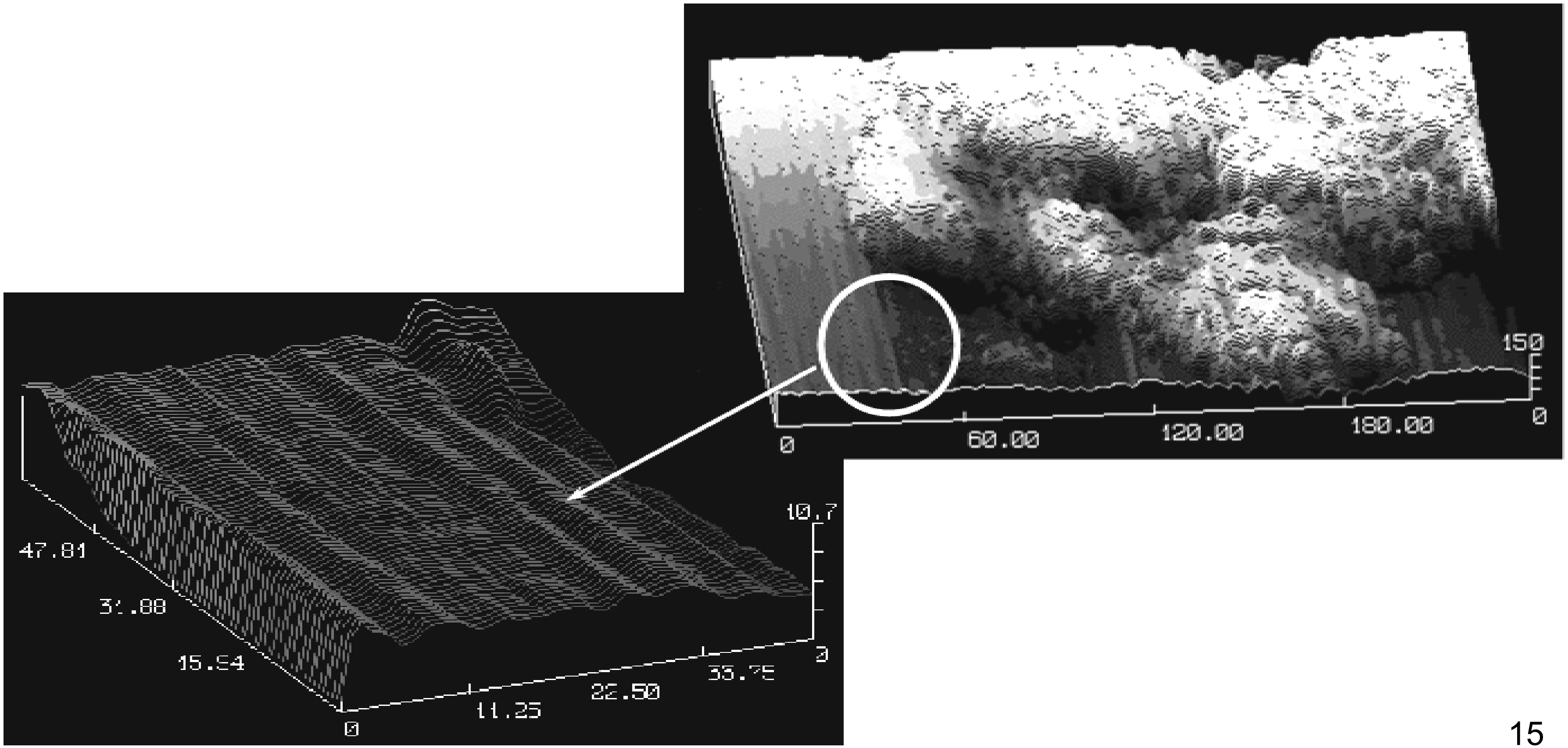
5. G.V. Andrievsky, V.K. Klochkov, L.I. Derevyanchenko. IS C_{60} FULLERENE MOLECULE TOXIC?! Fullerenes, Nanotubes and Carbon Nanostructures, 13 (4), (2005) 363-376 (*before publication this article it was rejected by Nano Lett., J. Med. Chem., Science News and Nannotechweb*).

6. T.B. Henry, E.J. Petersen, R.N. Compton. AQUEOUS FULLERENE AGGREGATES (nC_{60}) GENERATE MINIMAL REACTIVE OXYGEN SPECIES AND ARE OF LOW TOXICITY IN FISH: A REVISION OF PREVIOUS REPORTS. Current Opinion in Biotechnology, 2011 (doi: 10.1016/j.copbio.2011.05.511).

List of Basic Methods Used for Investigation of Chemical-Physical Properties of Molecular-Colloidal Solutions of Hydrated Fullerenes (HyFn) in Water == FWS ("Fullerene-Water-Solution").

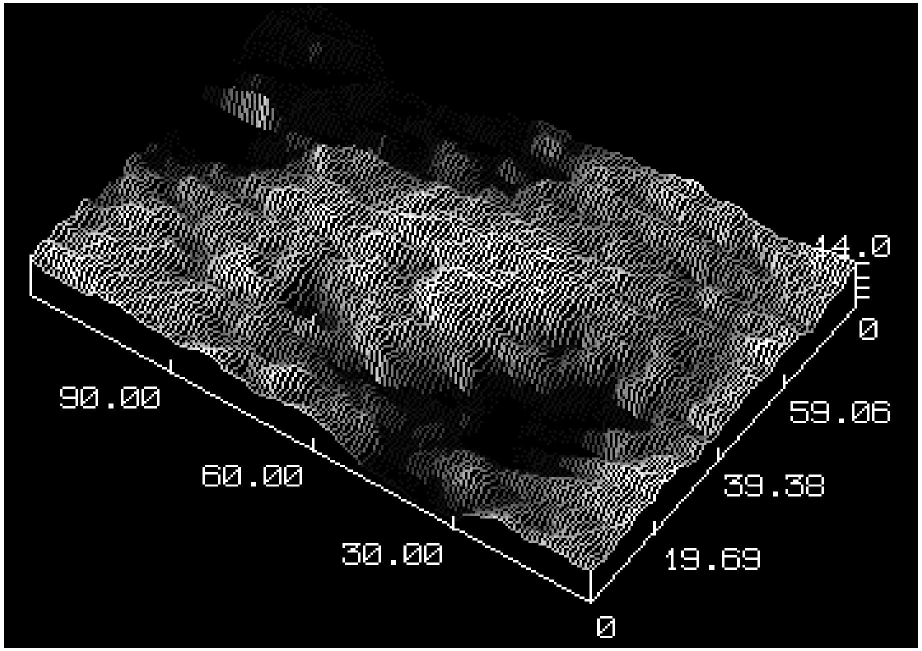
1. **Transmittance Electron Microscopy** (*TEM*)
2. **Scanning Tunneling Microscopy** (*STM*)
3. **$^{252-}$Cf-Plasma Desorption Mass Spectrometry** (*PD MS*)
4. **^{13}C Nuclear Magnetic Resonance** (*^{13}C NMR*)
5. **Spectroscopy in *UV-Vis* and *Near IR* Region** (*UV-Vis*)
6. **IR Spectroscopy** (*including FTIR and SEIRA in Reflectance Mode*)
7. **Luminescence Spectroscopy** (*including at Helium Temperatures*)
8. **Electron Paramagnetic Resonance** (*EPR*)
9. **Small-Angle Neutron Scattering** (*SANS*)
10. **Dynamic, Rayleigh, Raman and Brillouin Light Scattering** (*DLS, etc.*)
11. **LT Differential Scanning Calorimetry** (*DSC*)
12. **Piezogravimetry**
13. **Macro- and Micro-Electrophoresis**
14. **Ultracentrifugation and Ultrafiltration**
15. **Atomic Absorption Spectrometry**
16. **TL-, GL- and Ion Exchange Chromatography**
17. **Coagulation and another Techniques of Colloidal and Organic Chemistry.**
18. **Molecular Dynamics Simulation.**

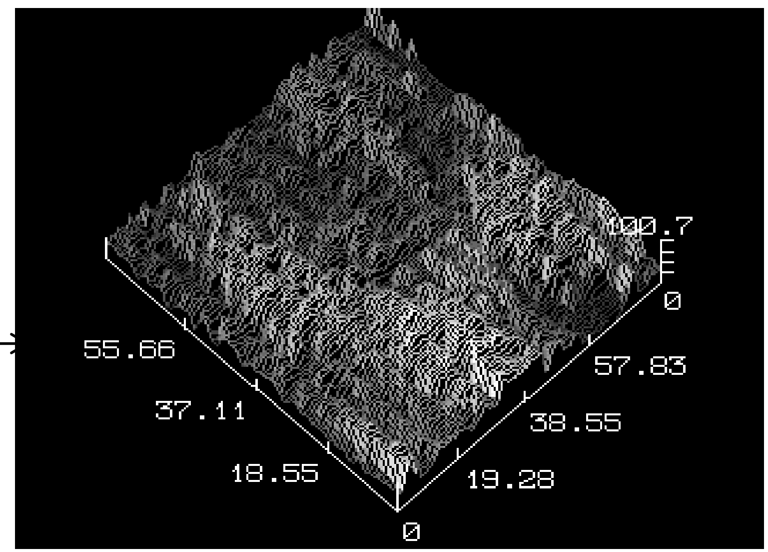
The Images of C_{60} Aggregates Adsorbed on the Monocrystalline Graphite Surface from C_{60}FWS and Observed by Scanning Tunneling Microscopy (STM) (in nm scale)



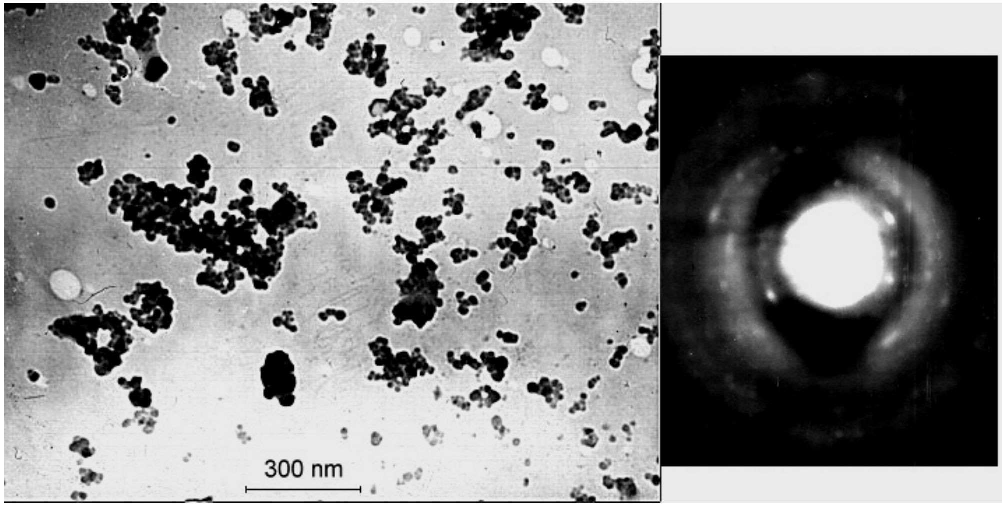
The Images of C_{60} Aggregates Adsorbed on the Monocrystalline Graphite Surface from C_{60}FWS and Observed by Scanning Tunneling Microscopy (STM)

(in nm scale)



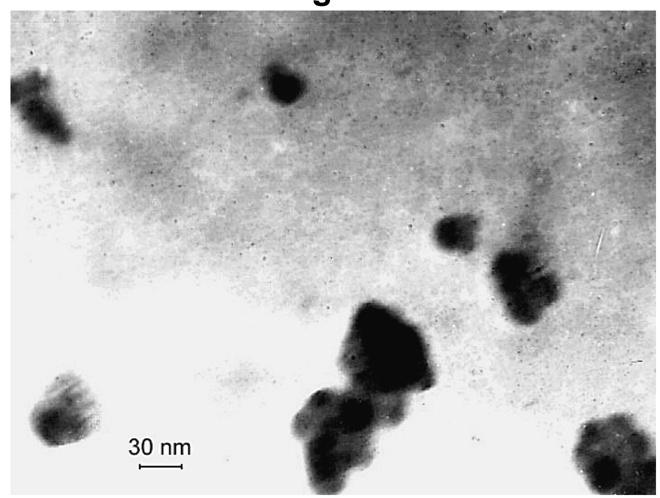


The secondary associates (~7.1 nm) consisting from first spherical clusters (~3.4 nm) that contain 13 individual C_{60}HyFn



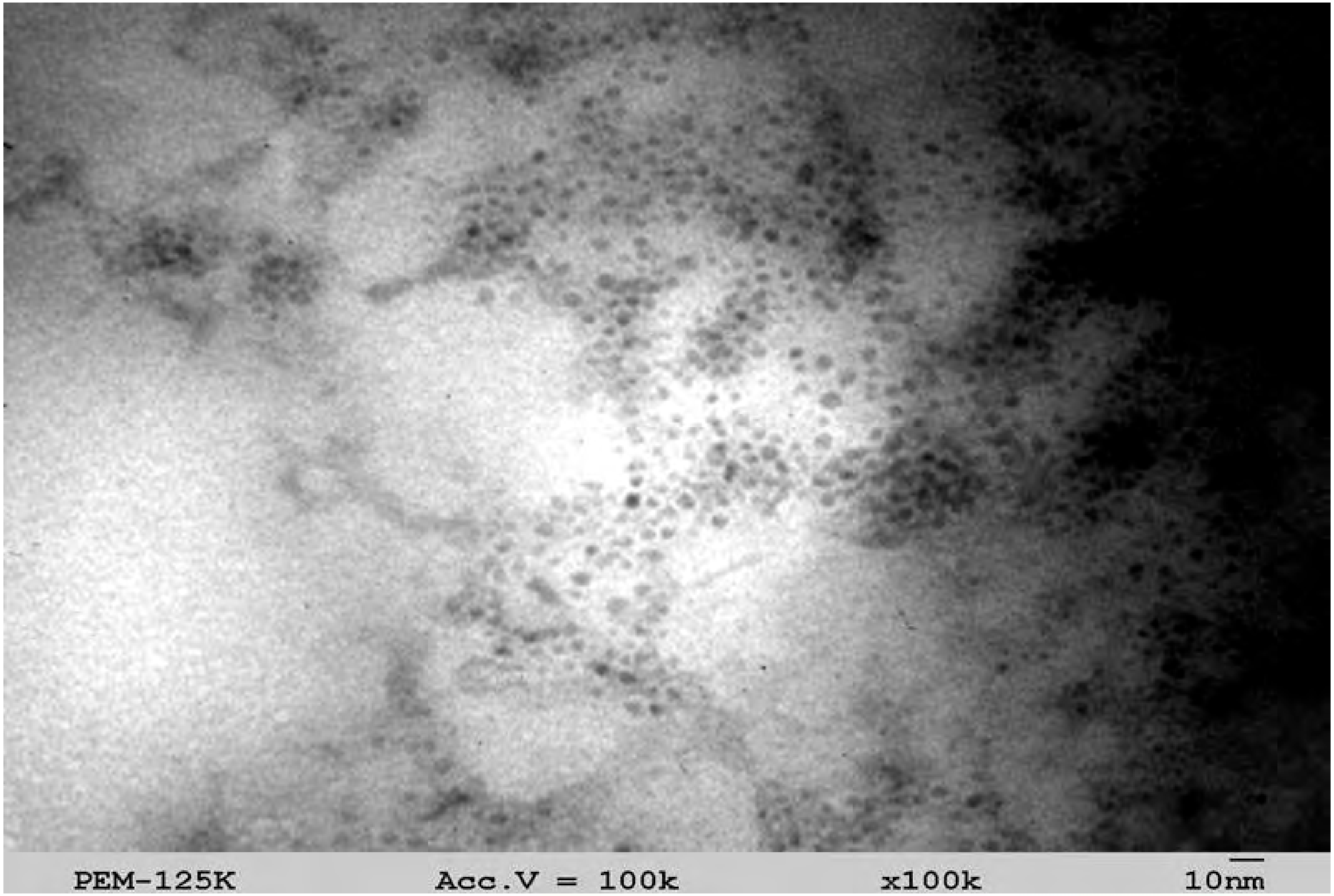
Transmission Electronic Micrographs (TEM)
of C_{60} ColloidaParticles Precipitated from C_{60}FWS

The Electron Micrograph with 'Vague' Zones

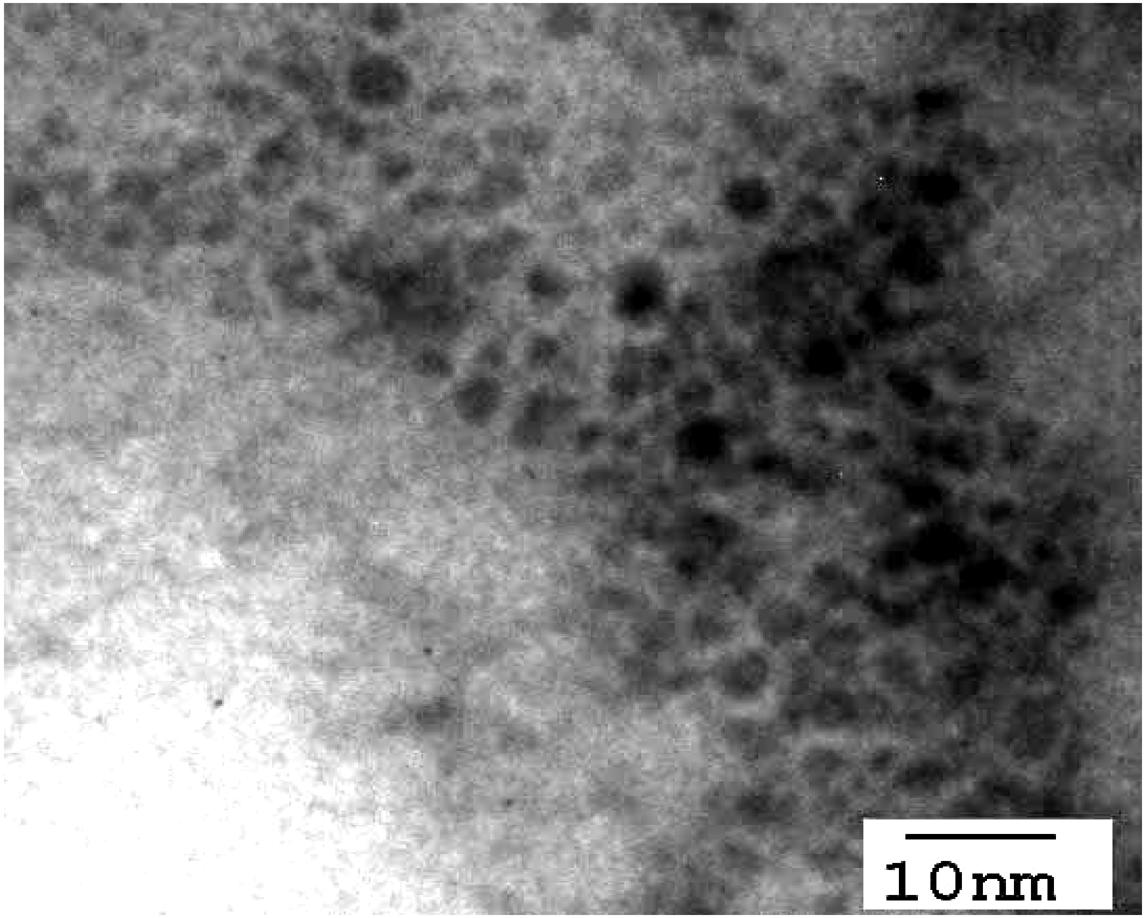


G.V. Andrievsky, V.K. Klochkov, E.L. Karyakina, N.O. Mchedlov-Petrossyan. STUDIES OF AQUEOUS COLLOIDAL SOLUTIONS OF FULLERENE C_{60} BY ELECTRON MICROSCOPY.. Chem.Phys.Lett., 300 (1999) 392-396.

Transmission Electronic Micrographs (TEM) C_{60} particles sorbed from C_{60}FWS on graphite thin film



Transmission Electronic Micrographs (TEM) C_{60} particles sorbed from C_{60}FWS on graphite thin film



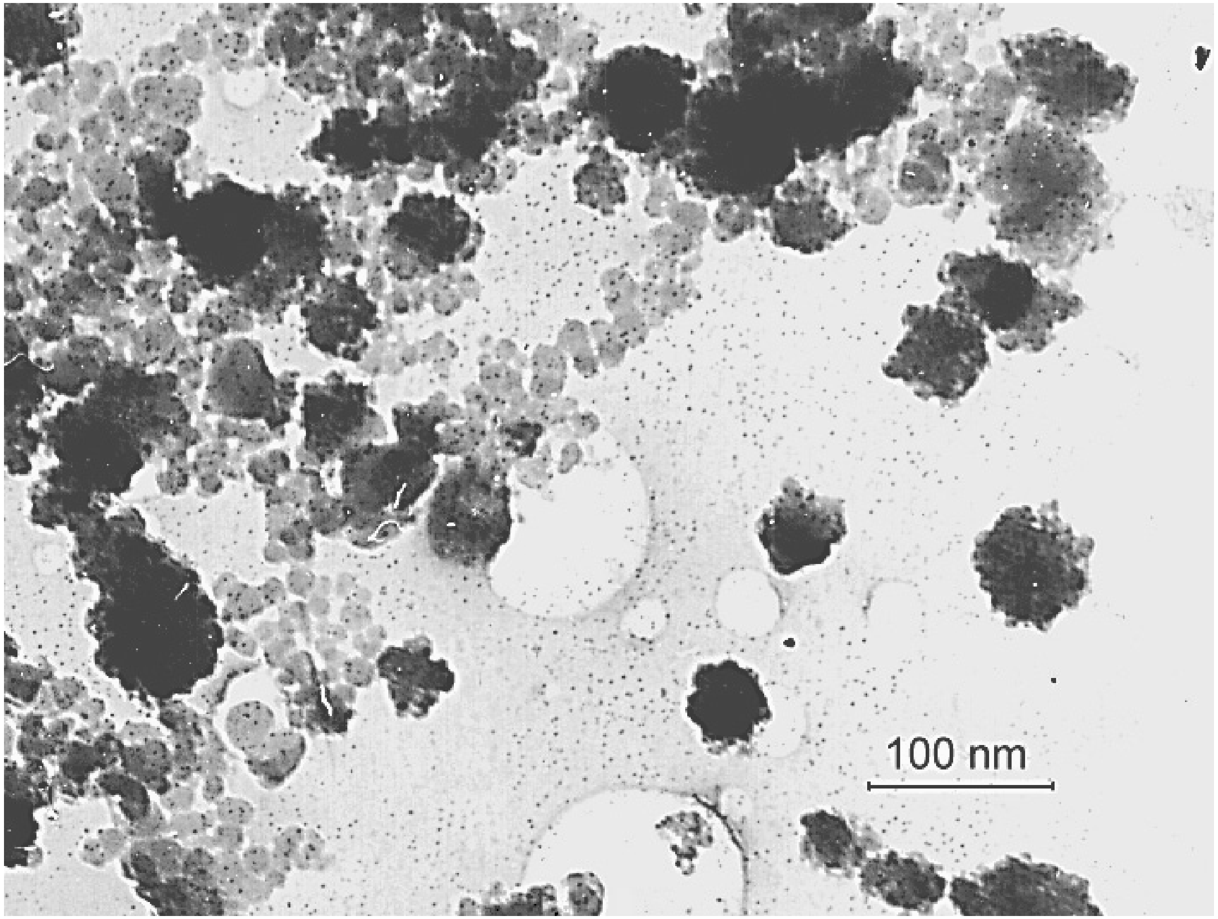
In:

G.V. Andrievsky, V.I. Bruskov, A.A. Tykhomyrov, S.V. Gudkov.

PECULIARITIES OF THE ANTIOXIDANT AND RADIOPROTECTIVE EFFECTS OF HYDRATED C_{60} FULLERENE NANOSTUCTURES *IN VITRO* AND *IN VIVO*.

Free Radical Biology & Medicine, 47 (2009) 786–793

The electron micrograph of C_{60} fullerene aggregates after coagulation C_{60}FWS with NaCl

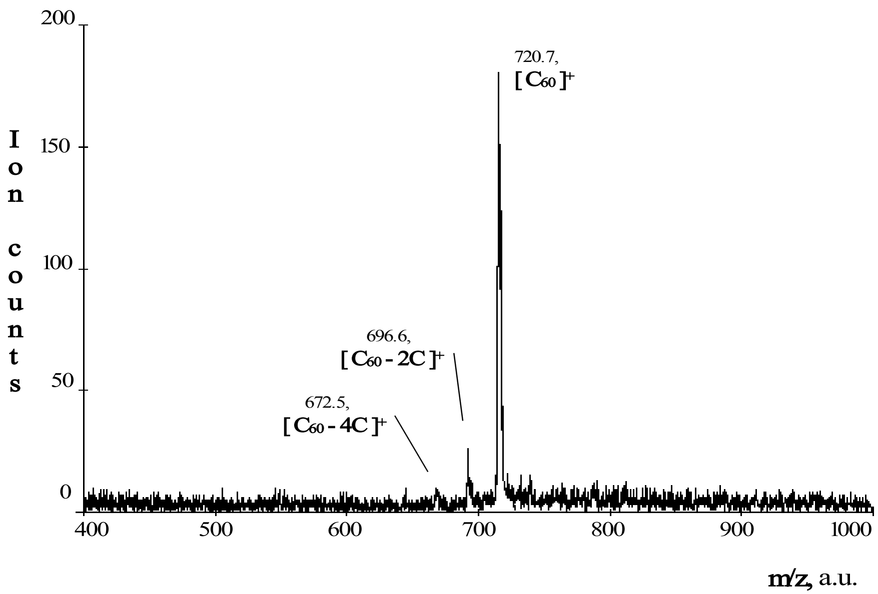


"...The C_{60}FWS are shown to be the ultramicroheterogeneous and polydisperse systems containing spherical structures (from ~1.5 to 72 nm). By analyzing the sizes of these structures, it was revealed that their diameters regularly rise within the range from 3.4 to 36.0 nm and are equal to 3.4, 7.1, 10.9, 14.5, 18.1, 21.8, 25.4, 28.8, 32.4, 36.0 nm….."

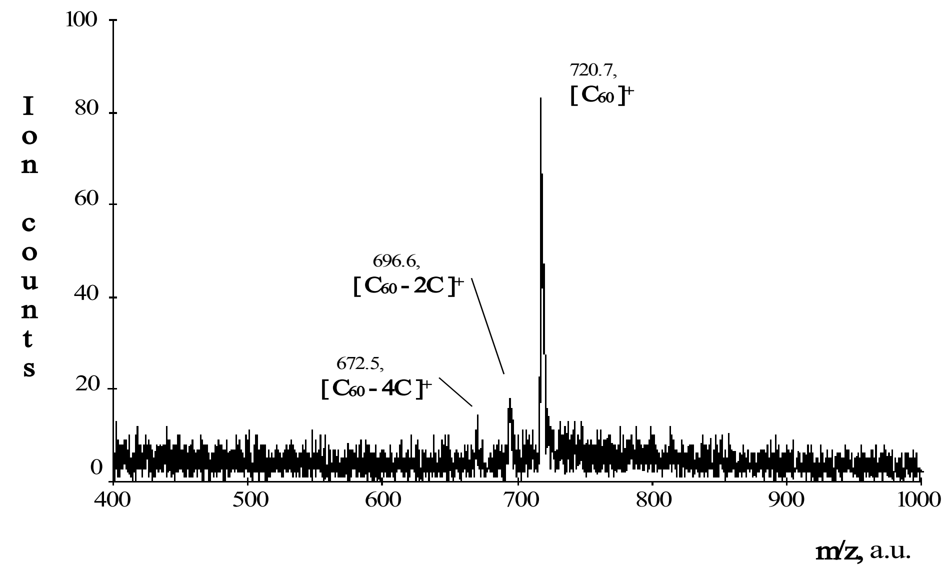
Diameters of C_{60}HyFn Associates Rise Regularly Within the Range from 1.6 to 36.0 nm and are Equal to

1.6, 3.4, 7.1, 10.9, 14.5, 18.1, 21.8, 25.4, 28.8, 32.4, 36.0 nm

[252]Cf-Plasma Desorption Mass Spectra of C_{60} Samples Precipitated from Its Toluene and Aqueous Solutions.

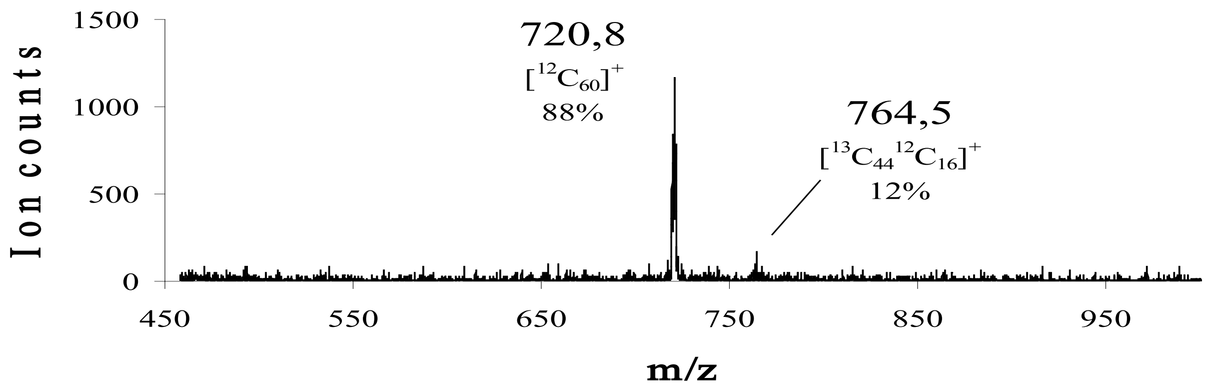


C_{60} from toluene solution

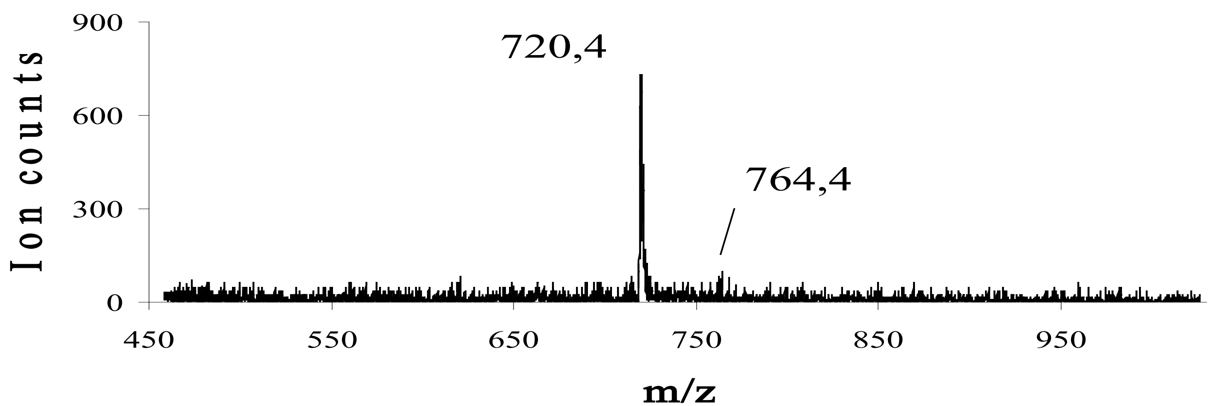


C_{60} from C_{60}FWS

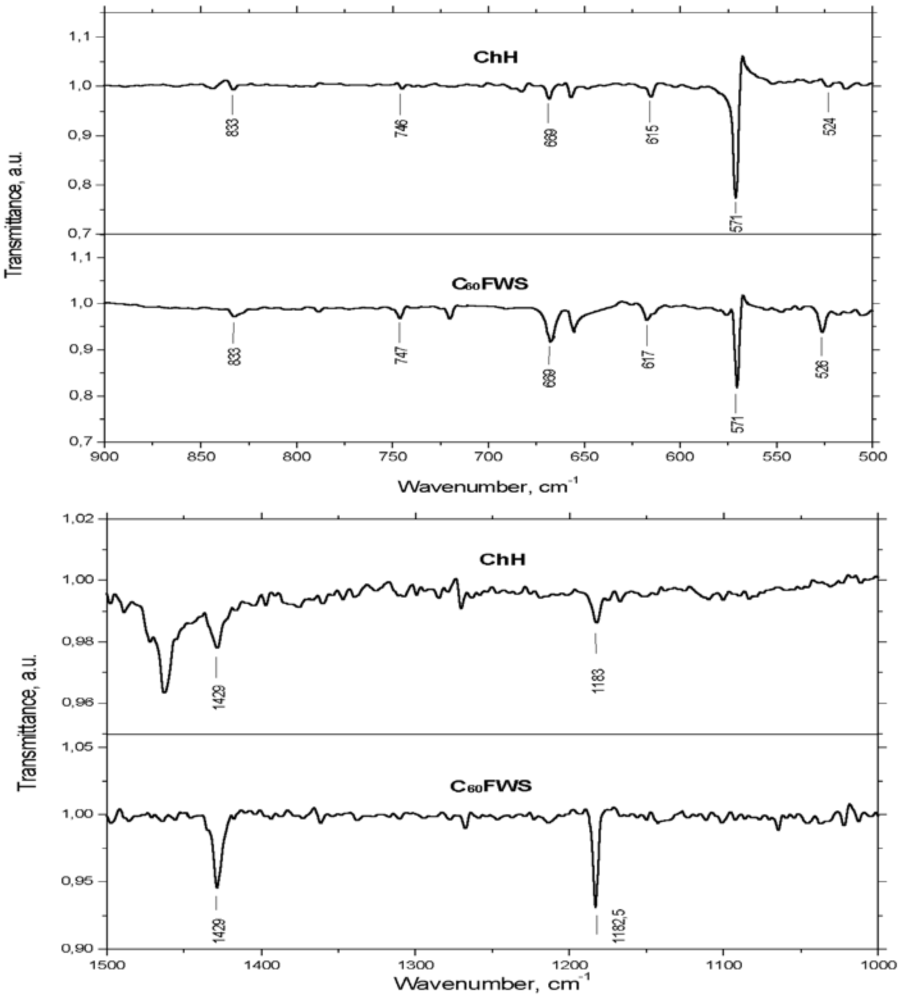
^{252}Cf PD mass spectrum of ^{13}C enriched C_{60} from benzene solution



^{252}Cf PD mass spectrum of ^{13}C enriched C_{60} from water solution (= C_{60}FWS)



FT-IR Spectra (*in the Reflectance Mode*) of Equal Amounts of C_{60} Precipitated from C_{60}FWS and ChH

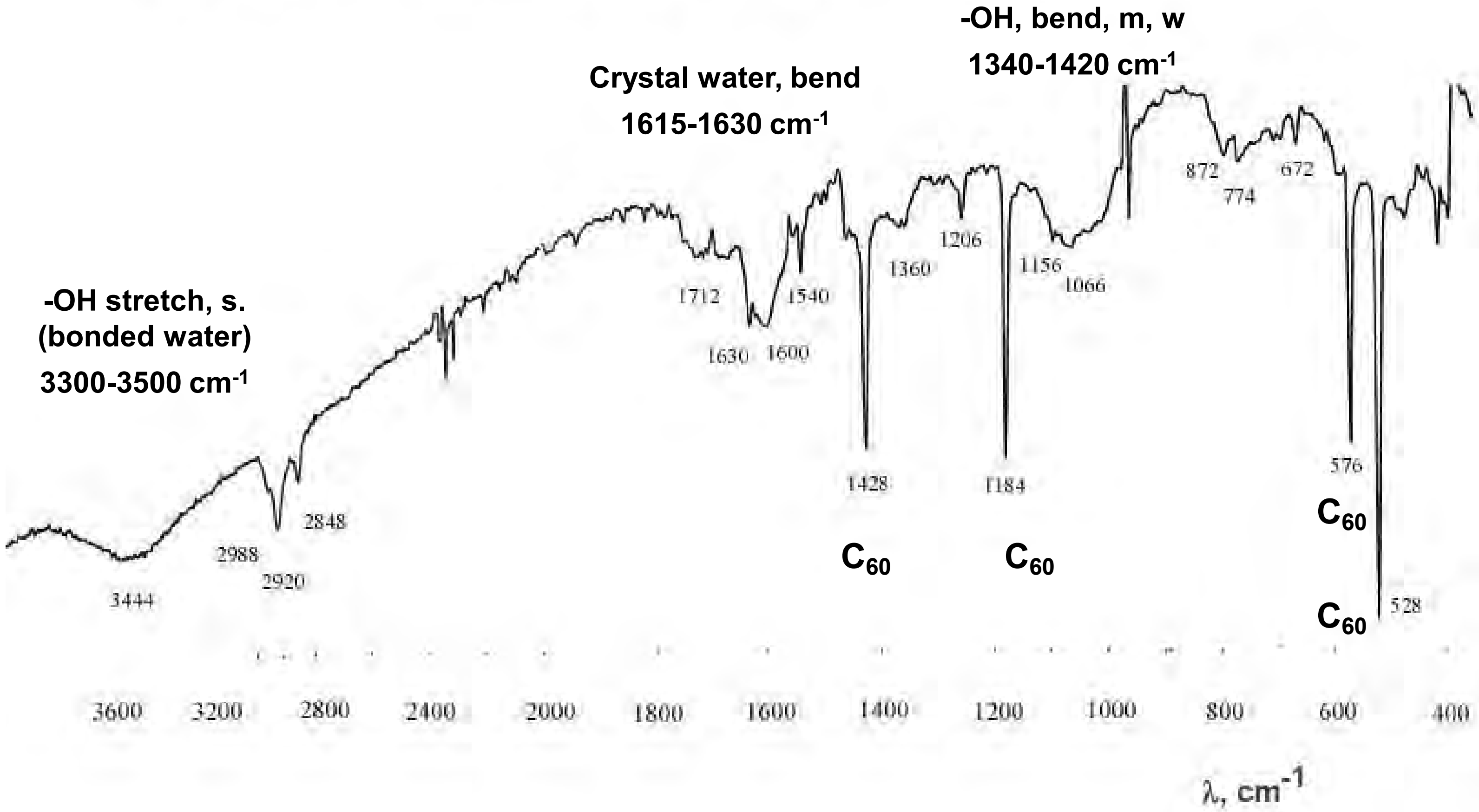


G.V. Andrievsky, V.K. Klochkov, A. Bordyuh, G.I. Dovbeshko.

COMPARATIVE ANALYSIS OF TWO AQUEOUS-COLLOIDAL SOLUTIONS OF C_{60} FULLERENE WITH HELP OF FT-IR REFLECTANCE AND UV-VIS SPECTROSCOPY.

Chem. Phys. Letters, 364 (2002) 8-17

IR-spectrum of C60HyFn precipitated from C60FWS (in KBr)

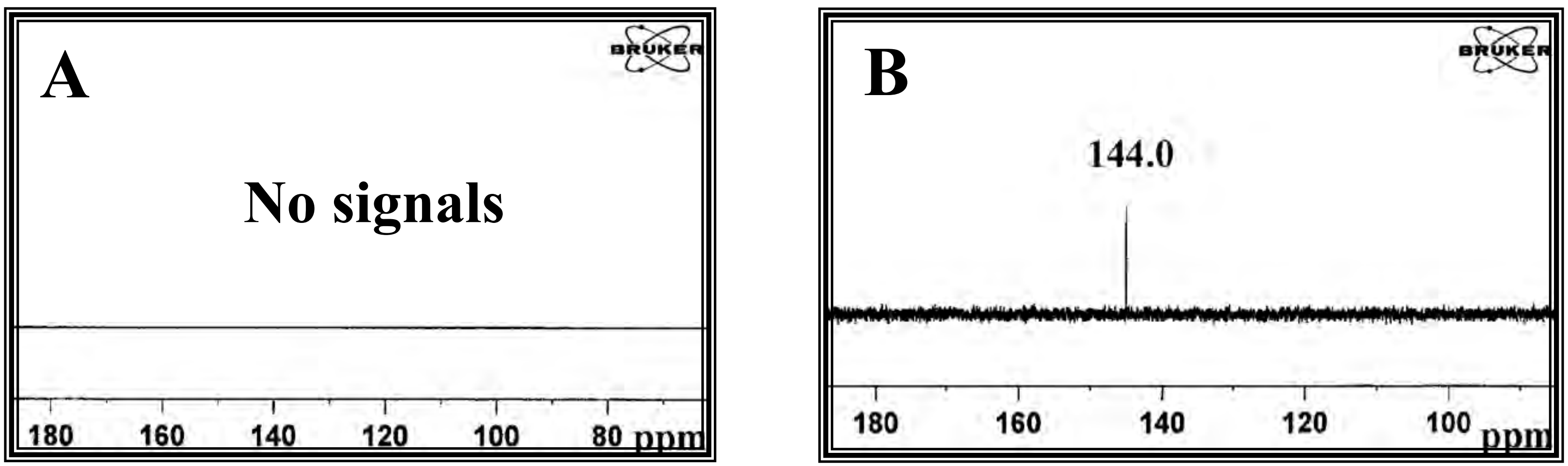


Proff. V. Senyavin and Proff. M. Korobov. Chemistry Dept., Moscow State University, Russia, December, 2001

^{13}C NMR Spectra of ^{13}C Enriched C_{60} for:

A - FWS = Aqueous Solution of Hydrated C_{60} Fullerene
$[C_{60}]$ = 0.2 mg/ml

B - Solution of C_{60} Obtained after FWS Drying and further Redissolving of Precipitate in Organic Solvent ($CDCl_3/CS_2$, 1:1 v/v)**;**
$[C_{60}]$ = 0.2 mg/ml



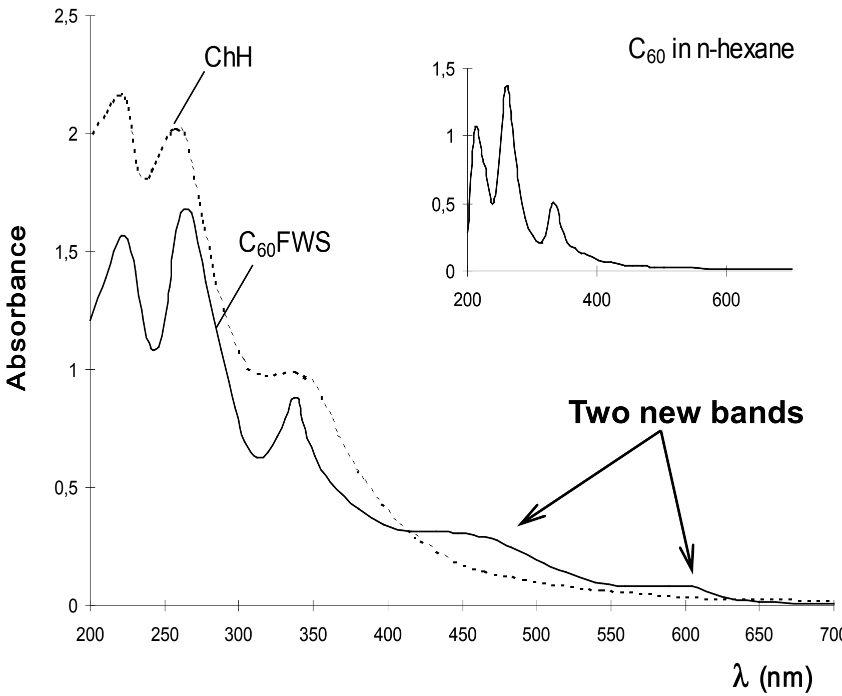
S.R. Wilson, K.S. Reinhard. New York University, Chemistry Department, July, 2004.

UV-VIS Adsorption Spectra of C_{60} in:

C_{60}FWS - polydisperse aqueous solution of hydrated C_{60} fullerene;

ChH - typical hydrophobic and monodisperse (*~10 nm*) C_{60} colloid;

And C_{60} in n-hexane solution.



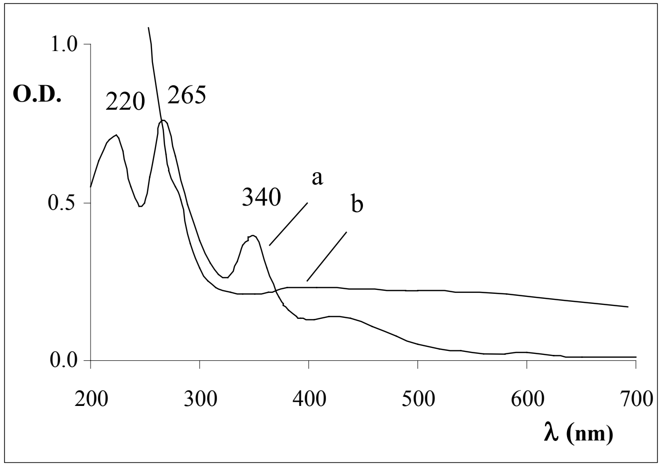
For comparison, the same C_{60} absorption bands have:

1. **In n-hexane solution - λ_{max} = 329 nm and ε = 51 000 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$;**
2. **In ChH - λ_{max} = 340 nm and ε = 14 000 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$;**
3. **In C_{60}FWS λ_{max} = 343 nm and ε = 68 000 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$**

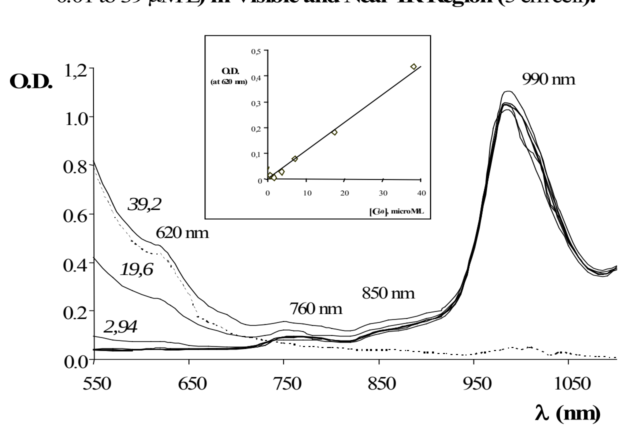
For C_{60}FWS the linear dependence of the optical absorbance from the C_{60} concentration (3-4 000 μM and more) is strictly valid (*the Beer-Lambert law*)!!!

G.V. Andrievsky, V.K. Klochkov, A. Bordyuh, G.I. Dovbeshko. COMPARATIVE ANALYSIS OF TWO AQUEOUS-COLLOIDAL SOLUTIONS OF C_{60} FULLERENE WITH HELP OF FT-IR REFLECTANCE AND UV-VIS SPECTROSCOPY. Chem. Phys. Letters, 364 (2002) 8-17

UV-VIS ABSORPTION SPECTRA OF C_{60} SOLUTIONS IN WATER:



(a) C_{60}FWS = Molecular-Colloidal Solution of Hydrated C_{60} Fullerene (received at 3.5<pH<12.5);
(b) Typical Hydrophobic C_{60} Colloids (received at pH< 3,5).



Absorption spectra of FWS with various C_{60} concentrations (from 0.01 to 39 μM/L) in Visible and Near-IR Region (5 cm cell).

C_{60}FWS is not typical hydrophobic, light-scattering colloid.

In C_{60}FWS absorption spectra in Vis and Near-IR region it is not revealed the component connected with light scattering on nanostructures both singl C_{60}HyFn, and small (3.4 - 36 nm) C_{60}HyFn associates.

BNC Experimental Report	Experiment title: Structure and Stabilization Mechanism of Unmodified Fullerene Water Solutions by SANS	Proposal No. SANS Local contact A. Len
Principal proposer: A. Khokhryakov, Joint Institute for Nuclear Research, Dubna, Russia Experimental team: M.V.Avdeev, V.L.Aksenov, L.Rosta		Date of Experiment Oct 2005 Date of Report May 2006

Objectives

Previous studies of the coagulation dynamics with inorganic salts in unmodified fullerene water solutions (FWS) [1] showed that these systems exhibit properties of typical hydrophobic colloidal systems. Our first experiments on the UV-Vis light absorption confirmed this fact and revealed that coagulation depends strongly on temperature and concentration of the coagulative ion, as well as on the age and origin (preparation procedure) of the sample. In the beginning of the coagulation process light absorption curves show an increase in the intensity which we connect with the scattering from growing colloidal particles. The aim of SANS experiments was to detect changes in the shape and size of colloidal particles in FWS following the coagulation. Also, the ageing effect and the influence of the preparation procedure on the coagulation were of current interest.

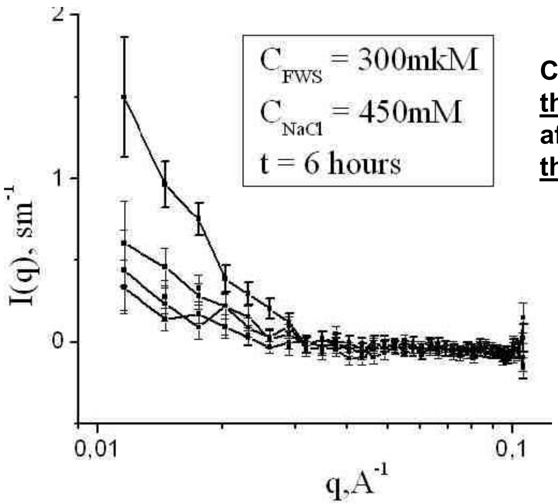


Fig. 1

Change in the SANS curves <u>during the coagulation of the FWS system</u> after NaCl is added. <u>No growth of the scattering intensity is observed</u>.

Results

In contrast to UV-Vis light absorption the small-angle neutron scattering <u>(SANS)</u> experiments on coagulating systems (e.g. in Fig.1) do not show any particle size growth, which testifies the formation of quite loose structures of particles during their coalescence. One can assume the presence of quite large water layers around initial particles which, then, are included in the aggregate structure. In this connection, the most probable stabilization mechanism of FWS is the formation of weak donor-acceptor complexes C_{60}-$(H_2O)_n$.

During the long period of time (up to 1 year) the effect of sample ageing is observed for FWS with respect to the coagulation dynamics. Colloidal properties of the system are significantly modified. The system approaches the more rigid state and coagulates much slower. Along with it, the SANS method, again, does not detect any difference in the structure of colloidal particles. Experimental curves. completely repeat the data of earlier investigations [2]. The possible explanation is a weak particle aggregation in time, which is, however, cannot be resolved by SANS because of large size of secondary aggregates.

Finally, several samples prepared by other techniques [3] were investigated. In addition to the main steps of the solvent substitution method [1] long-range sonication and ozonation were used. The initial solvent was toluene. The maximal concentration of fullerene dispersed in water in this case is about an order lower (20-40 mkM) than in solutions prepared by the basic procedure [1]. <u>Small-angle neutron scattering does not shows any principal difference in the structure of particles on the nanoscale in all solutions</u> after normalization on concentration.

Note that sensitivity of SANS method is less than 2 nm.

"mkM = microM"

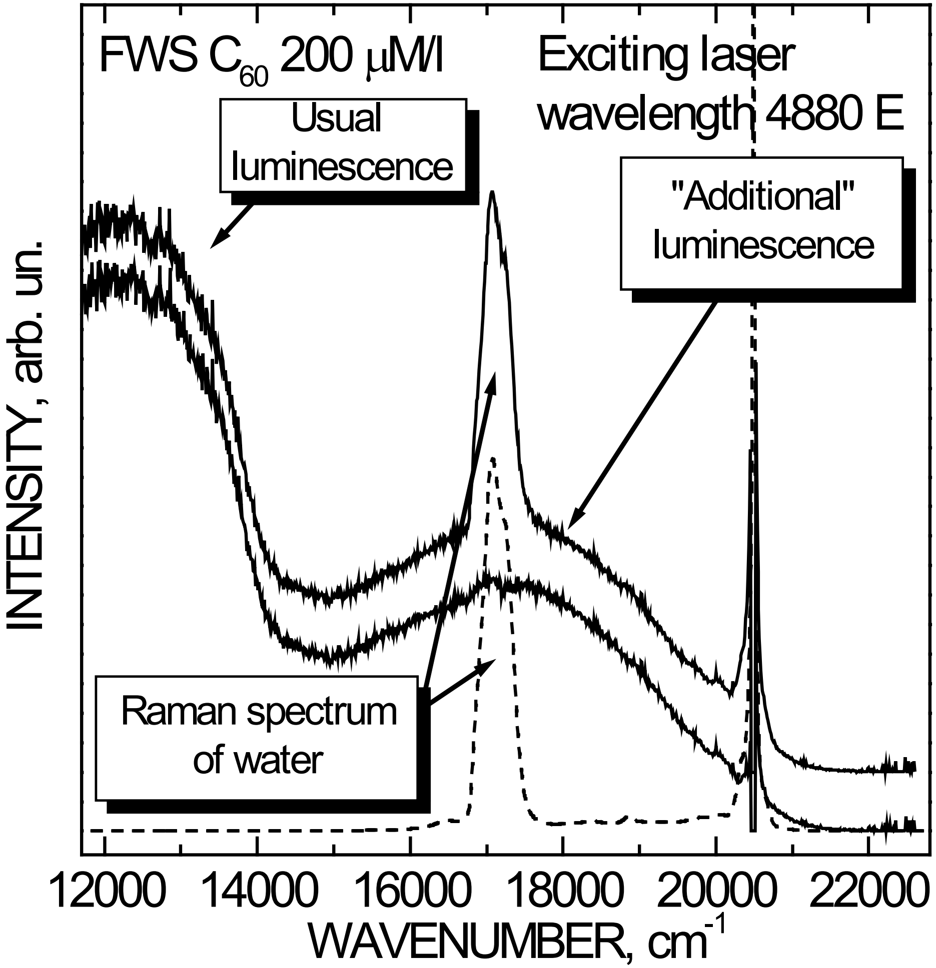
Future prospects

<u>The formation of unexpectedly large solvation shell which retains its structure during coagulation is of further interest</u>. Spectroscopic experiments with the purpose of evaluation of binding energy of the water molecules with the C_{60} molecule are discussed.

References

1. G. Andrievsky, M. Kosevich, O. Vivk, et al., J. Chem. Soc. Chem. Comm. 1995, 1281.
2. M.V.Avdeev, A.A.Khokhryakov, T.V.Tropin et al., Langmuir 20, 2004, 4363
3. P. Scharff, C Sigmund, K. Risch et al., Fullerenes Nanotubes Carb. Nanostruct., 13, 2005, 497.

Photoluminescence spectra C_{60}FWS contain two components: (1) – 'red' band of Usual luminescence of C_{60} and (2) - a wide 'blue-green' band of an Unusual ('Additional') luminescence, which is absent in spectra of crystal samples of C_{60}!!!

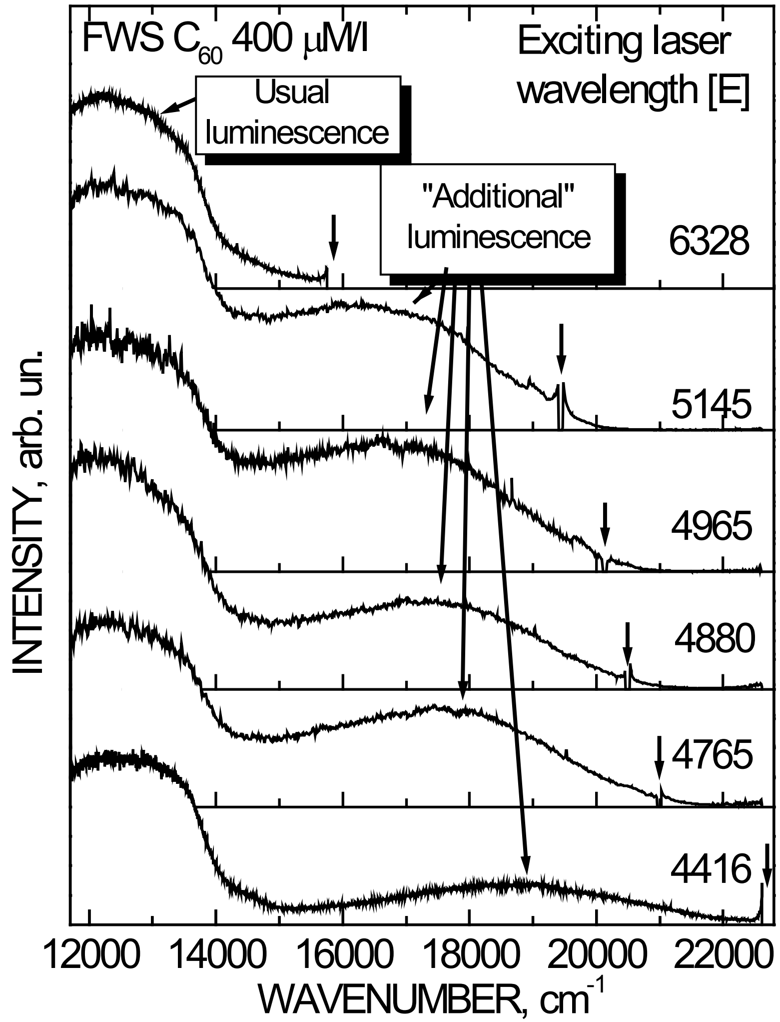


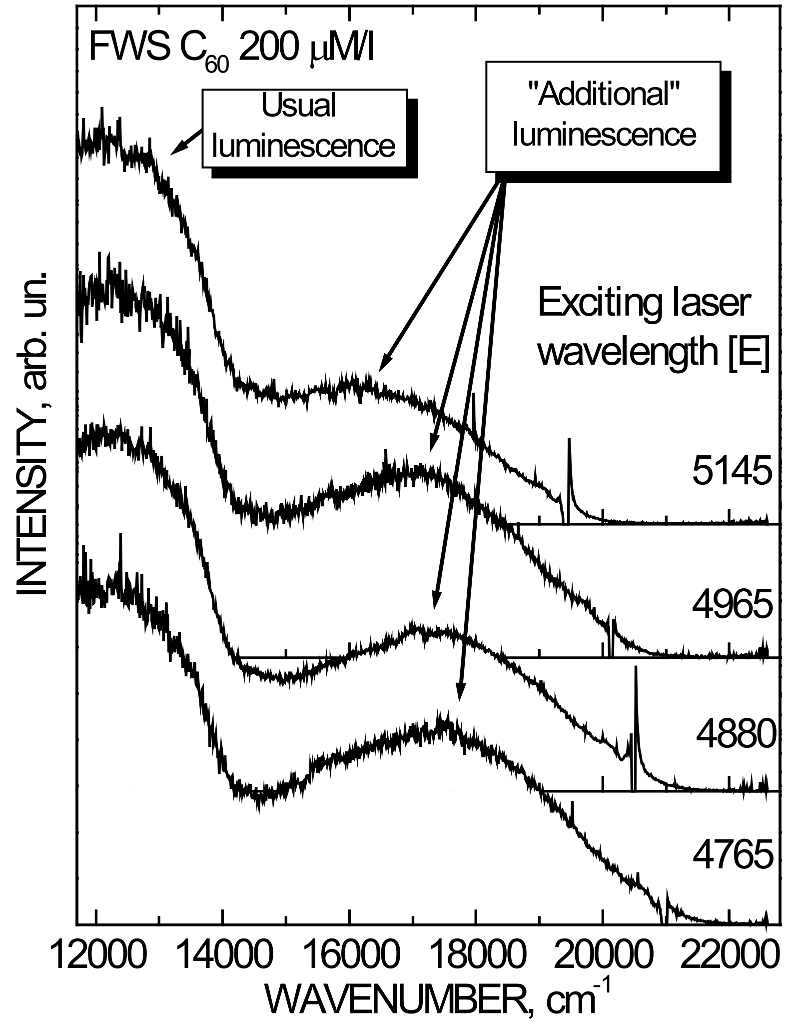
G.V. Andrievsky, A.A. Avdeenko, L.I. Derevyanchenko, V.I. Fomin, V.K. Klochkov, V.S. Kurnosov, A.V. Peschanskii.

PECULIARITIES FOR LUMINESCENCE IN SYSTEMS WITH FULLERENE C_{60}-WATER INTERFACE.

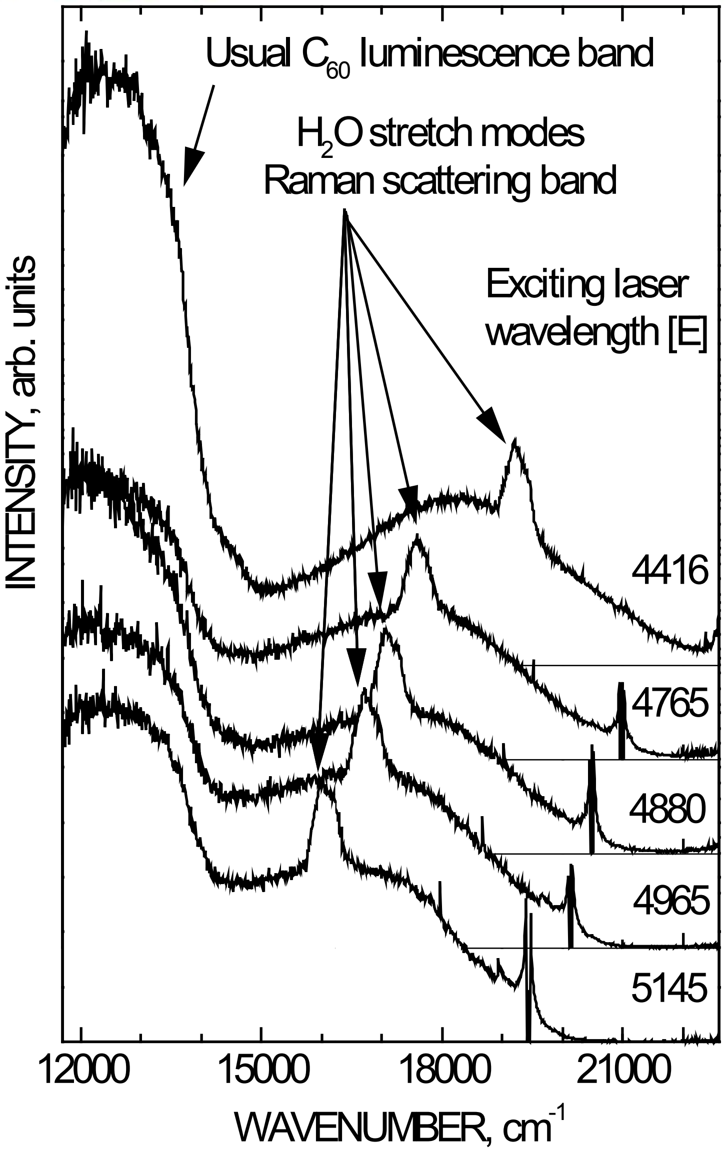
In "Spectroscopy of Emerging Materials" eds. E.C. Faulques et al.,. NATO Science Series II: Mathematics, Physics and Chemistry, Kluwer Academic Publishers in 2004 and Springer Netherlands, 165 (2005) 151-160; doi: 10.1007/1-4020-2396-0_12 (http://www.springerlink.com/content/r12368n382x37627/)

Here is an example of a spectrum correction by means of exclusion of the contribution of water Raman spectrum.





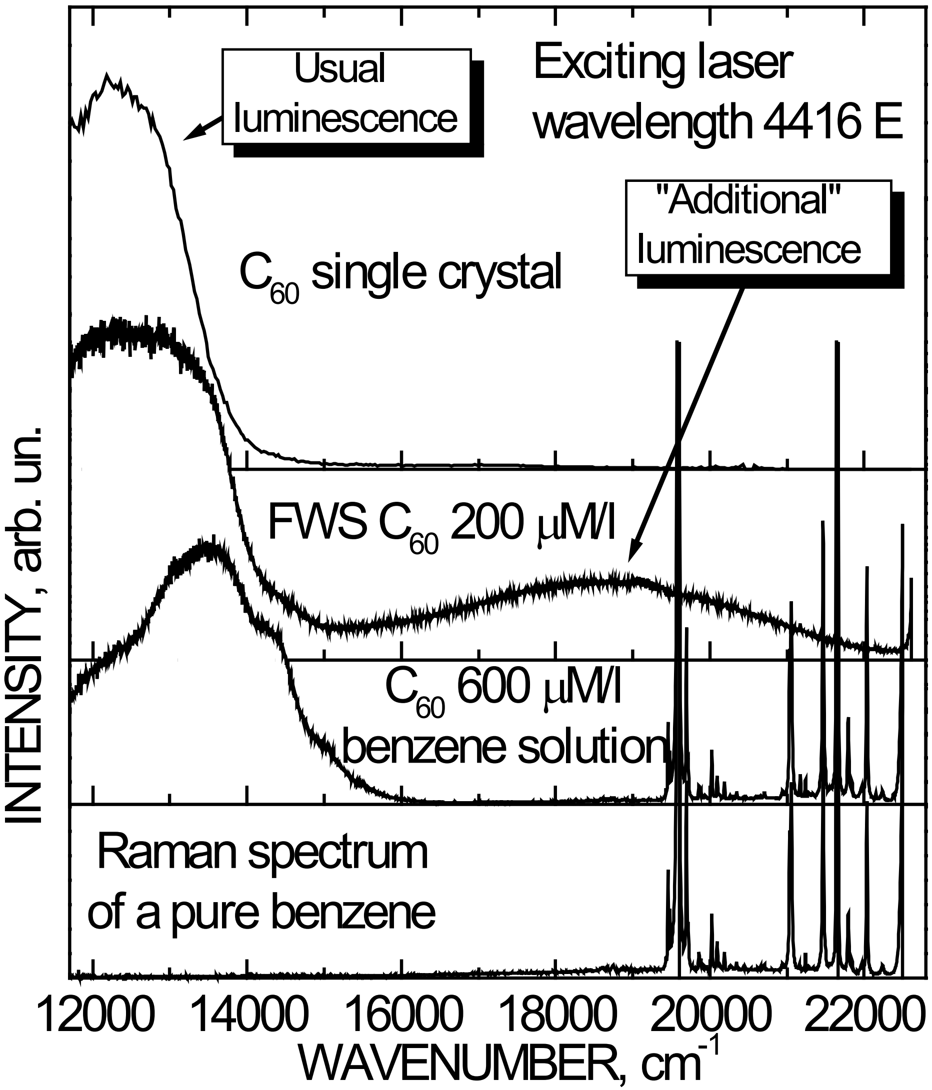
Spectral position of the "unusual" luminescence band maximum depends on the exciting laser wavelength. This phenomena seems to be similar to Raman process and not usual for a luminescence.



But it isn't true entirely. Full experimental spectra without subtraction of the water Raman spectrum clearly show that the shift of the observed "unusual" luminescence band doesn't exactly follow the exciting light frequency, as it must take place for Raman process. Position of Raman band of water stretch modes clearly shows this discrepancy.

And what is more, it was checked that for exciting light wavelengths shorter than 430 nm the shift of the "unusual" luminescence band comes to a halt.

So, the "unusual" luminescence is unusual twice.



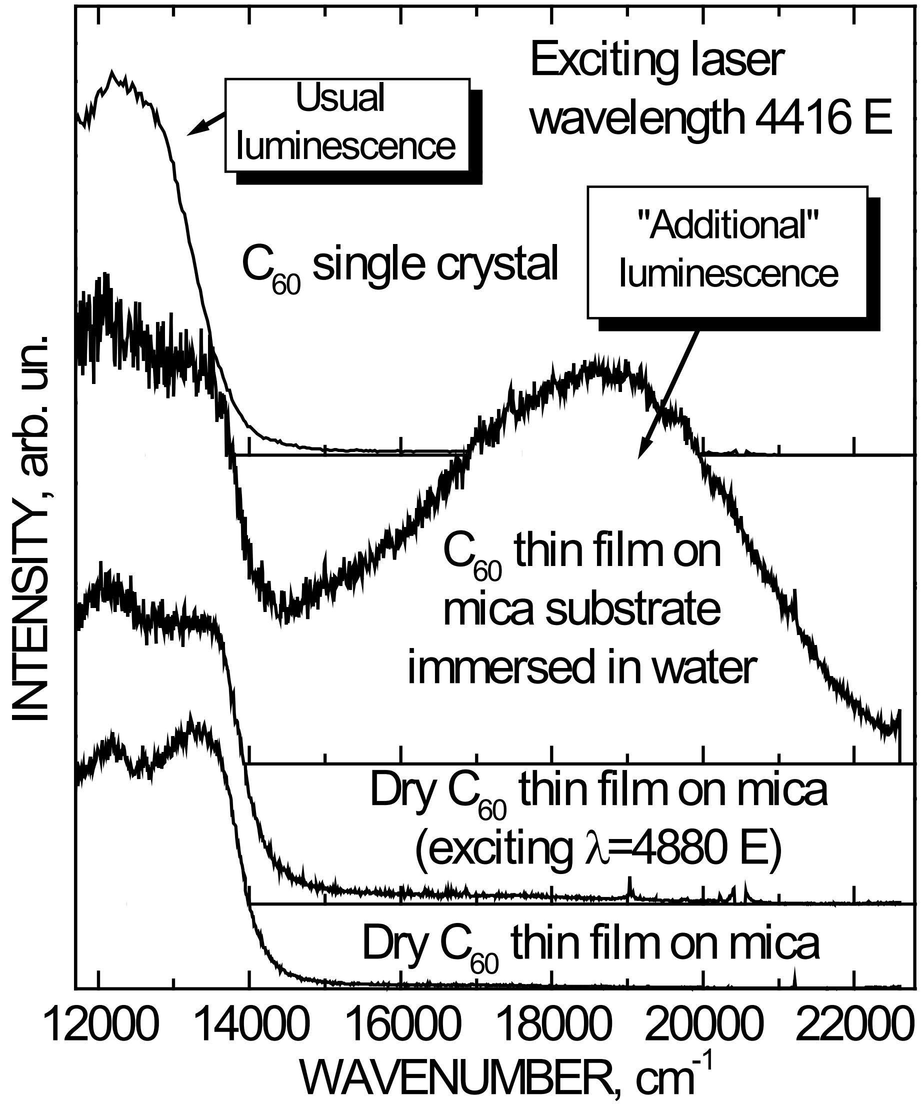
Comparison of luminescence spectra of

- a C_{60} single crystal,
- C_{60} FWS,
- and C_{60} benzene solution

had shown presence of an “additional” or “unusual” very wide luminescent band in the middle of the range of visible light only in water contained system.

It was a surprise!

Note: Here the spectrum of C_{60} FWS is shown after the subtraction of the Raman spectrum of water.

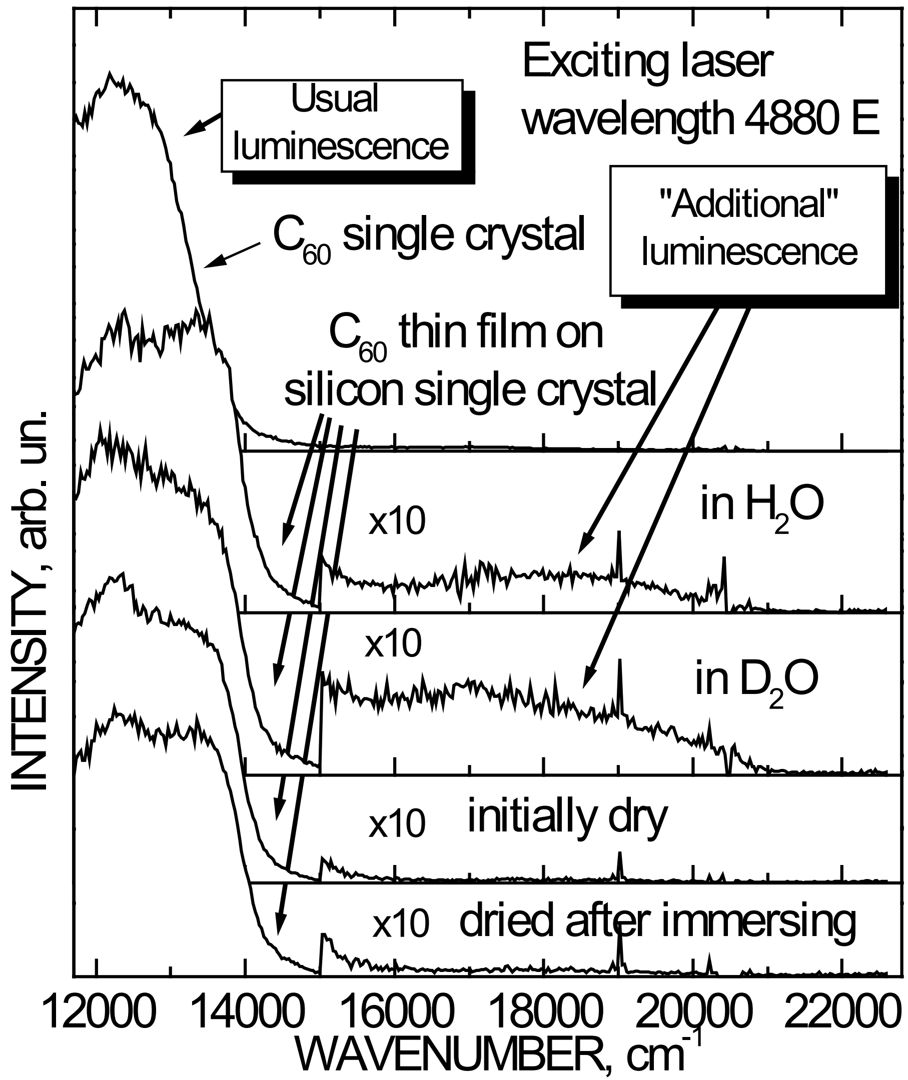


Comparison of photoluminescence spectra of C_{60} single crystal and C_{60} thin solid film deposited in vacuum on mica substrate in the conditions of initially dry and immersed in water.

Unfortunately the experiments with C_{60} films on mica was irreversible. After the film was immersed in water and then it was subsequently dried, even at vacuum and heating up to 170 °C during several hours, its initial state was not restored.

The dried film showed presence of “unusual” luminescent band like as in moisturized state!!!!!

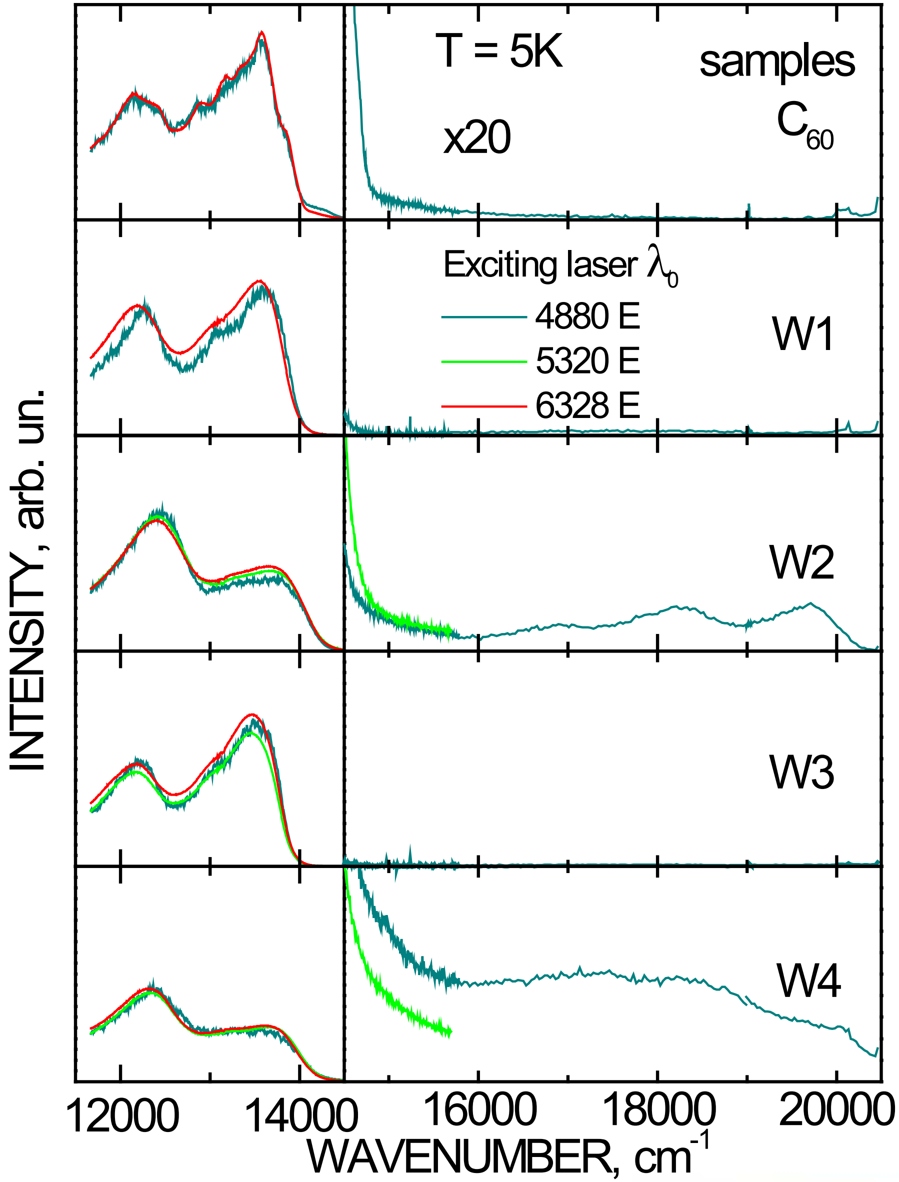
The film is enough thin and maybe enough porous.



Another solid state fullerene samples were prepared by deposition in vacuum on silicon single crystal substrate. The films were thicker, than in the case with mica, and had optically perfect surface. Their immersing in water and subsequent drying had shown reproducible character of the spectra.

Also, it is important that contact angle for water droplets on these C_{60} crystal films is equal to 90-95°!!!

(Proff. M.V. Korobov. Chemistry Dept., Moscow State University, Russia, May 2003)

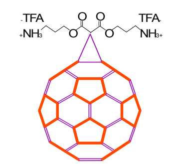


Series of experiments were carried out at low temperature of **5 K**. They showed dependence of usual luminescence band from frequency of exciting light.

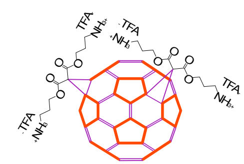
Derivatives, which are associated with two organic molecules (W2 and W4), reveal luminescent bands in the “orange-green” range of visible light also, while ones (W1 and W3) with one associated molecule don’t.

But in this experiments the more important is that **for pure C_{60} samples the above mentioned unusual luminescence band does not arise absolutely!!!**

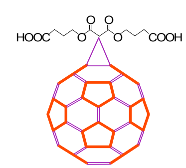
(Technical report by Grant UC2-2440-KH-02 of the U.S. Civilian Research & Development Foundation for the Independent States of the Former Soviet Union (CRDF), 2003-2004.)



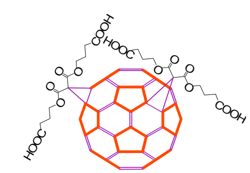
W1 C_{60} mono-amine



W2 C_{60} bis-amine e-isomer



W3 C_{60} mono-acid



W4 C_{60} bis-acid e-isomer

The Main Conclusions Derived from Spectroscopic Experiments:

- The Unusual ('Additional') luminescence arises at luminescence excitation in the region of 430-550 nm. This region in absorption spectra of C_{60}FWS corresponds to the New band, the occurrence of which is the consequence of C_{60} molecule hydration.

- Individual C_{60} molecules and its pure crystal forms are not capable to give the Unusual luminescence in the region of 450-650 nm!!!

- Fullerene C_{60} easily interacts with water in conformity with the mechanism of hydrophobic hydration (*about donor-acceptor complexes of C_{60} and H_2O look in the subsequent slides*).

- In spite of the fact that crystal forms of C_{60} are not dissolved spontaneously in water, the individual molecule of C_{60} fullerene is not typically hydrophobic or typically hydrophilic. It can show simultaneously both properties!!!!

STANDARD INSTRUMENTS FOR DLS ANALYSIS OF PARTICLE SIZES IN C_{60} AQUEOUS DISPERSIONS (nano-C_{60}, nC_{60}, THF/C_{60}, FAS and suchlike) THAT HAVE BEEN SYNTHESISED USING "BOTTOM-UP" TECHNOLOGY FAIL TO YIELD OBJECTIVE RESULTS

(**i.e. they actually yield results that are 3 to 10 times higher, compared to data obtained when such dispersions are analysed using TEM, STM and AFM!!!! [1-7,** see below**]).**

The root causes of this are as follows:

- It is incorrect to use standard DLS equipment (e.g. Malvern Zetasizer NanoZS and many similar instruments), which employs laser radiation sources with a wavelength of less than 700 nm [8];

- For similar DLS analysis equipment, the lower limit for detecting particle sizes in C60 aqueous dispersions that have been synthesised using “bottom-up” technology is within 30 nm (usually 60 nm and more). These instruments fail to “sense” the smaller “light scattering” nanostructures in such dispersions.

- Crystal solvate (crystal hydrate) nanostructures of particles of similar dispersions (less than 30 nm) are “transparent” (i.e. they do not reflect and scatter) in terms of incident light from laser radiation sources that are typically used.

- Small nanostructures (less than 30 nm) of similar dispersions are highly hydrophilic and strongly associated with the hydrophilic medium. Hence, similar to the behaviour of substances in true solutions, their behaviour cannot be characterised from the viewpoint of the Brownian motion of typical colloid particles. Therefore, attempts to detect their hydrodynamic radius by means similar DLS analyzers are meaningless.

References

1. Our personal observations: **(i)** G.V. Andrievsky, et al. STUDIES OF AQUEOUS COLLOIDAL SOLUTIONS OF FULLERENE C_{60} BY ELECTRON MICROSCOPY. Chem.Phys.Lett., 300 (1999) 392-396; **(ii)** M.V. Korobov, et al. DSC OF C_{60} WATER SYSTEM: UNEXPECTED PEAKS. In: “The exciting world of Nanocages and Nanotubes”, P.Kamat, D.Guldi, K.Kadish, Fullerenes V.12, Proc. Series of 201st Meeting of The Electrochem. Soc., Pennington, NJ, PV 2002-12 (2002) 799-804; **(iii)** G.V. Andrievsky, et al. PECULIARITIES OF THE ANTIOXIDANT AND RADIOPROTECTIVE EFFECTS OF HYDRATED C_{60} FULLERENE NANOSTUCTURES IN VITRO AND IN VIVO. Free Radical Biology & Medicine, 47 (2009) 786–793, doi: 10.1016/j.freeradbiomed.2009.06.016.

2. V.P. Belousov, et al. AQUEOUS MICELLAR SOLUTION OF C_{60}: PREPARATION, PROPERTIES, AND CAPABILITY FOR GENERATION OF SINGLET OXYGEN. Russian Journal of General Chemistry, 76 (2) (2006) 251-257. doi: 10.1134/S1070363206020150.

3. N. Levi, et al. C_{60}-FULLERENES: DETECTION OF INTRACELLULAR PHOTOLUMINESCENCE AND LACK OF CYTOTOXIC EFFECTS. Journal of Nanobiotechnology 2006, 4:14; doi:10.1186/1477-3155-4-14.

4. D.Y. Lyon, et al. ANTIBACTERIAL ACTIVITY OF FULLERENE WATER SUSPENSIONS: EFFECTS OF PREPARATION METHOD AND PARTICLE SIZE. Environ. Sci. Technol., 40 (2006) 4360-4366. Compare with:

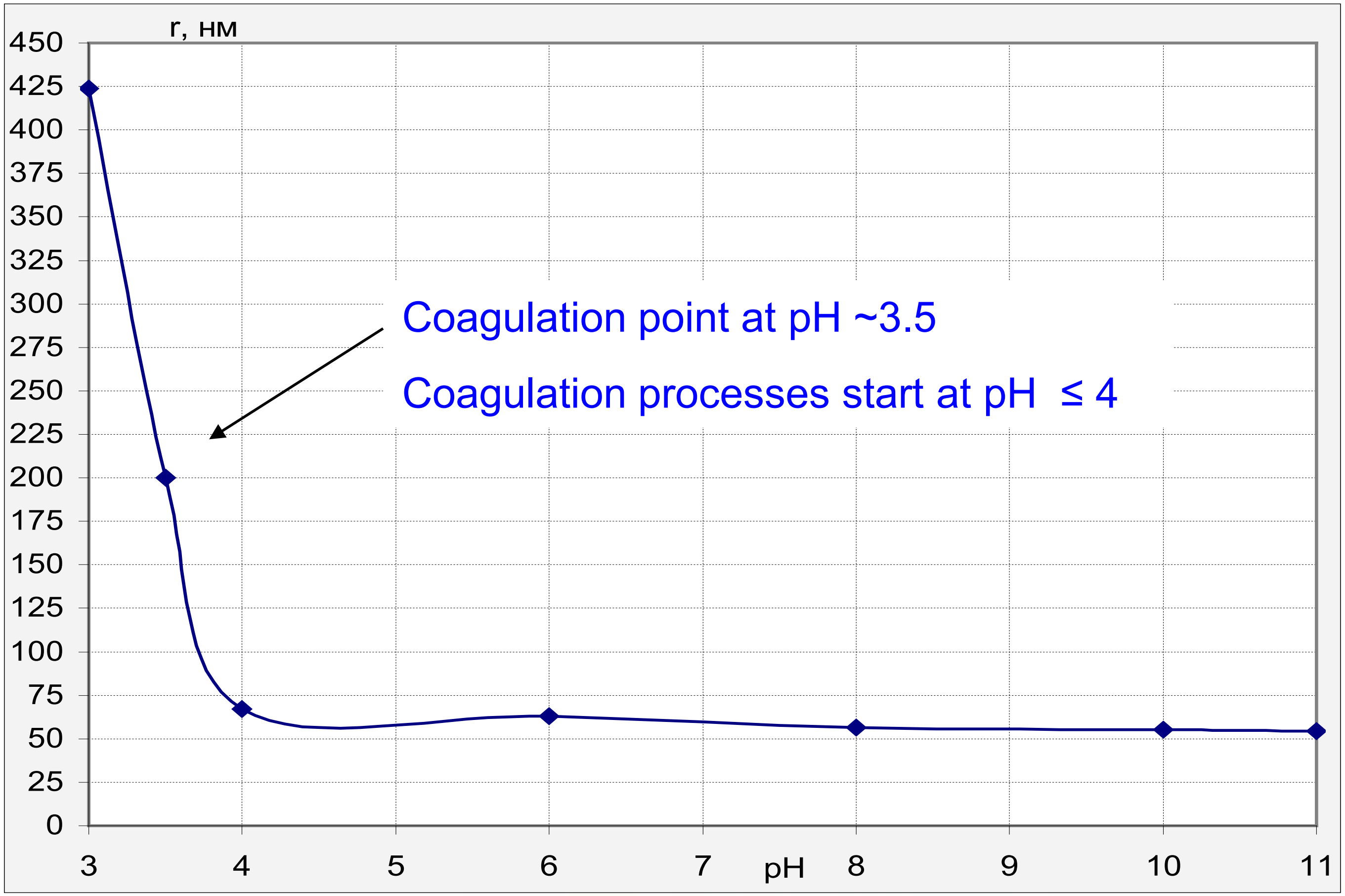
5. T.A. Spurlin, A.A. Gewiirth. EFFECT OF C60 ON SUPPORTED LIPID BILAYER. Nano Letters, 7(2) (2007) 531-535.

6. P. Spohn, et al. C_{60} FULLERENE: A POWERFUL ANTIOXIDANT OR A DAMAGING AGENT? THE IMPORTANCE OF AN IN-DEPTH MATERIAL CHARACTERIZATION PRIOR TO TOXICITY ASSAYS. Environ. Pollut., 157(4) (2009) 1134-1139; doi:10.1016/j.envpol.2008.08.013.

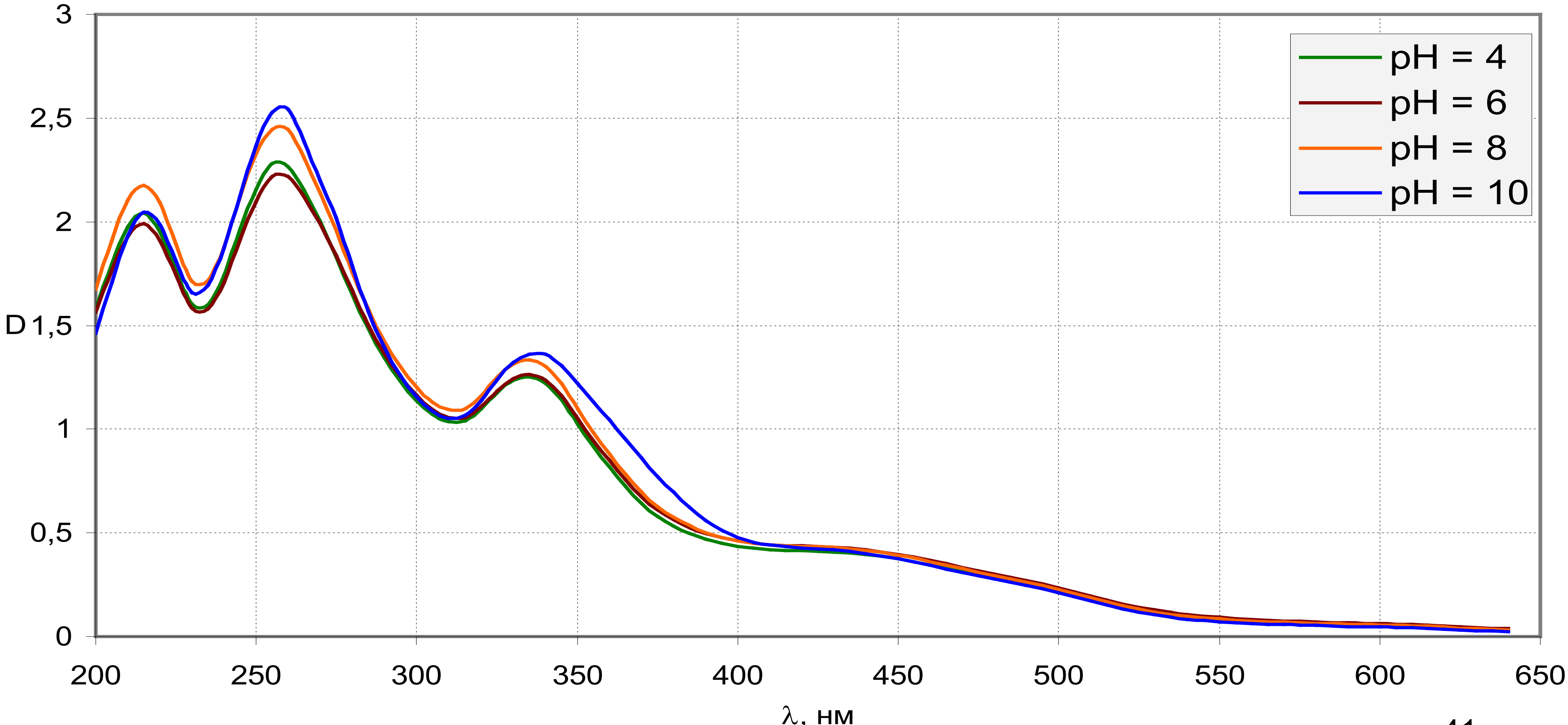
7. L.K. Duncan, et al. C_{60} COLLOID FORMATION IN AQUEOUS SYSTEMS: EFFECTS OF PREPARATION METHOD ON SIZE, STRUCTURE, AND SURFACE CHARGE. Environ. Sci. Technol. 42 (2008) 173–178.

8. Q.Ying, J. Marecek, B. Chu. J. SOLUTION BEHAVIOR OF BUCKMINSTERFULLERENE (C_{60}) IN BENZENE. Chem. Phys., 101 (1994) 2665-2672.

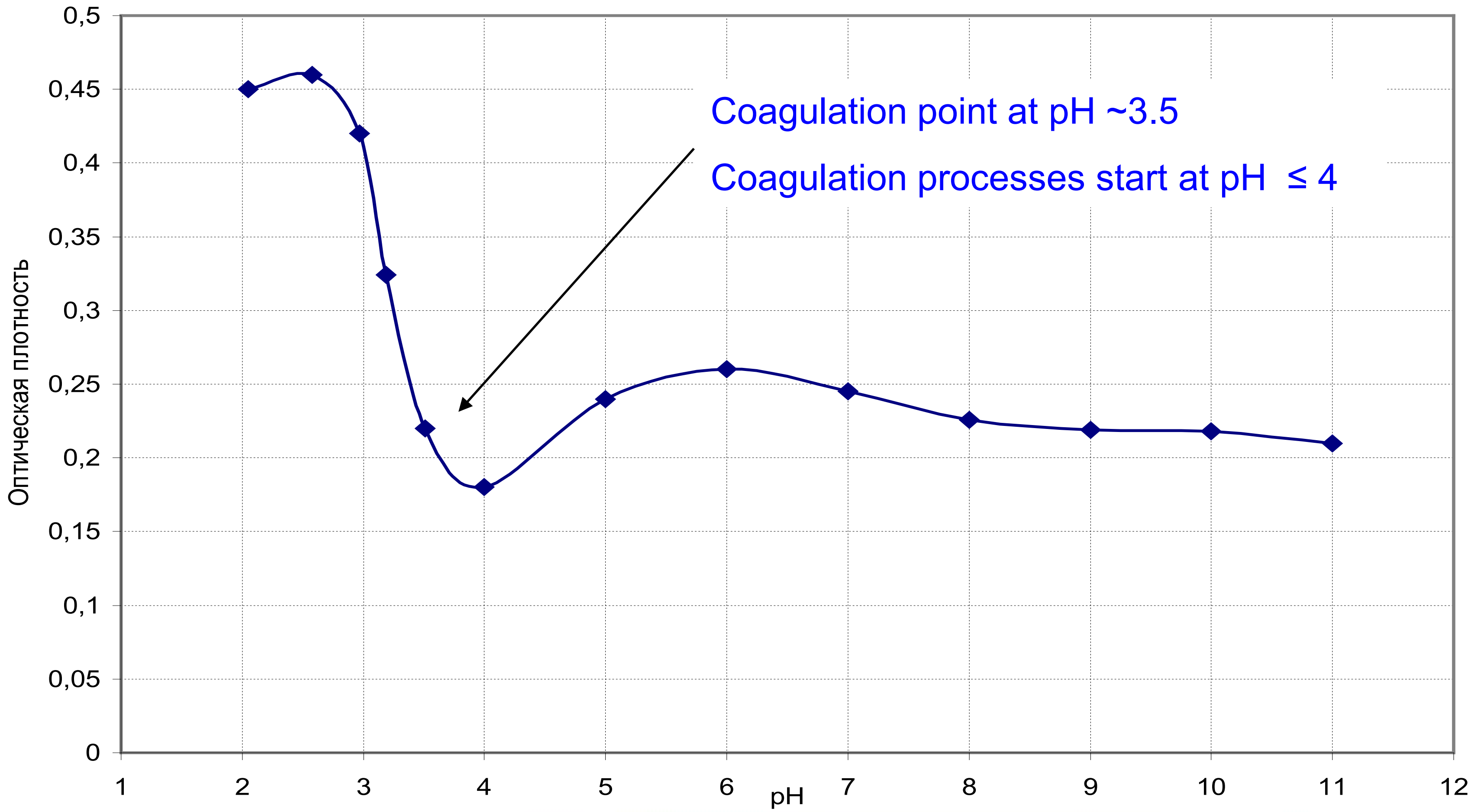
Dependence of the Sizes of Light Scattering Structures in Water Solutions of Hydrated C_{60} Fullerene from pH Value in Solution (from DLS data)



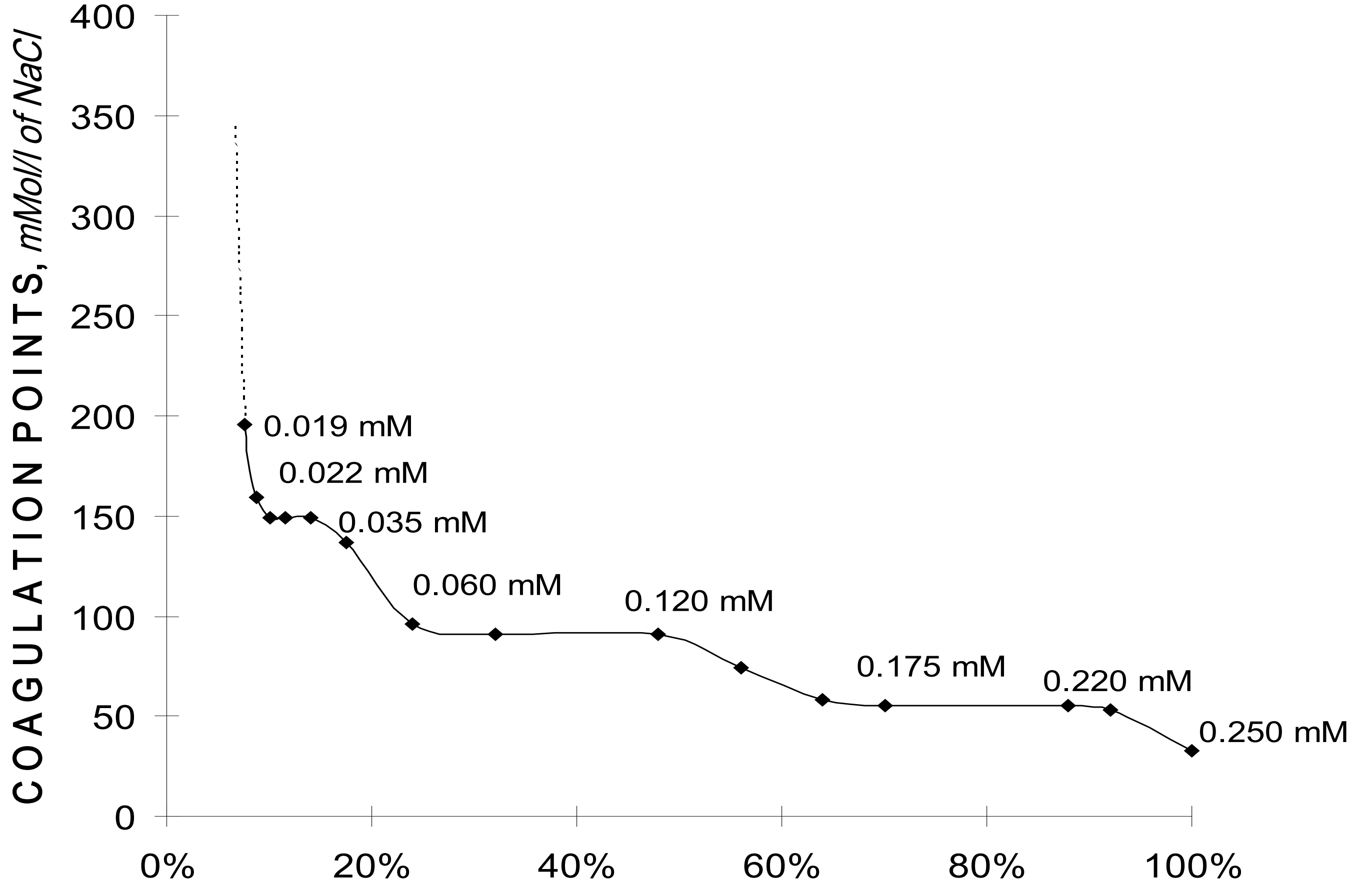
Absorption spectra of aqueous solutions of C_{60}HyFn in UV-Vis regions at various pH values.



Dependence of optical density of C_{60}HyFn aqueous solution (C_{60}FWS) on the pH values in its.



Dependence of coagulation points (C_{NaCl}, *mM/L*) from the C_{60} percentage (*%*) in solutions obtained by the water dilution of C_{60}FWS with the initial concentration of C_{60} = 0.25 *mM/L*



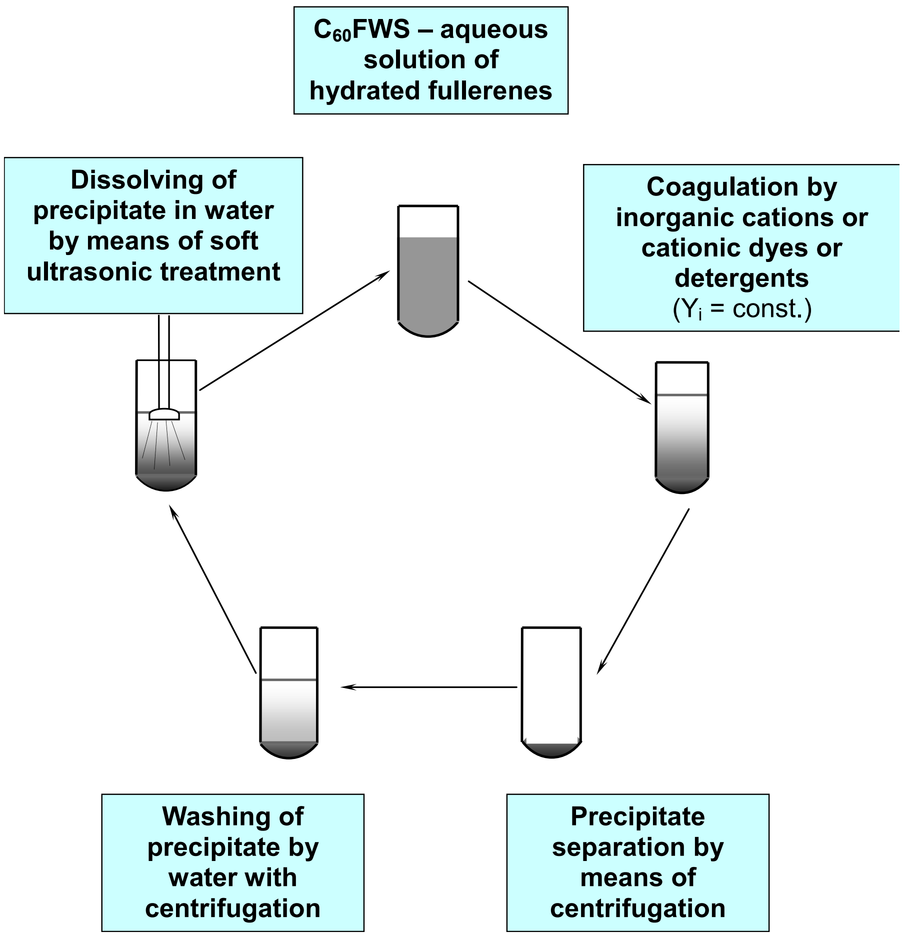
EXTRACTION BY ORGANIC SOLVENTS

The simple and important property of C_{60}FWS is that C_{60} cannot to be extracted neither by toluene nor by benzene (“good” solvents for C_{60}) from such aqueous solutions. However, using UV–Vis spectroscopy, we detected that the minimal extraction of C_{60} is occurred from water-colloidal solutions, and the extent of C_{60} extraction coincides with solubility of the water in the abovementioned organic solvents.

Also, C_{60} from C_{60}FWS cannot be extracted by toluene in any essential extent in the case when C_{60}HyFn were precipitated by coagulation of inorganic cations (salts). It denotes that the coagulation cannot destroy strong hydrated shell surrounding C_{60} molecule.

G.V. Andrievsky, V.K. Klochkov, A. Bordyuh, G.I. Dovbeshko. COMPARATIVE ANALYSIS OF TWO AQUEOUS-COLLOIDAL SOLUTIONS OF C_{60} FULLERENE WITH HELP OF FT-IR REFLECTANCE AND UV-VIS SPECTROSCOPY. Chem. Phys. Letters, 364 (2002) 8-17

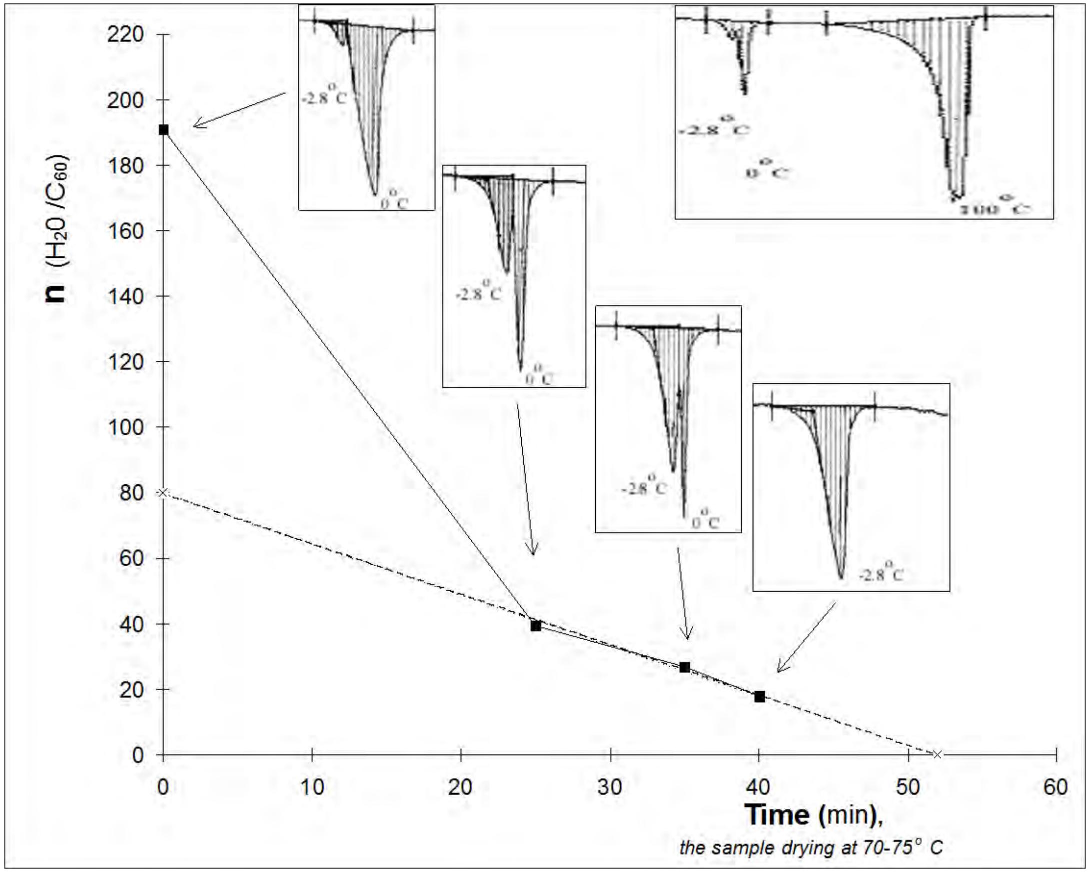
CYCLIC PROCEDURE OF HYDRATED C_{60} FULLERENES "PRECIPITATION – DISSOLVING'



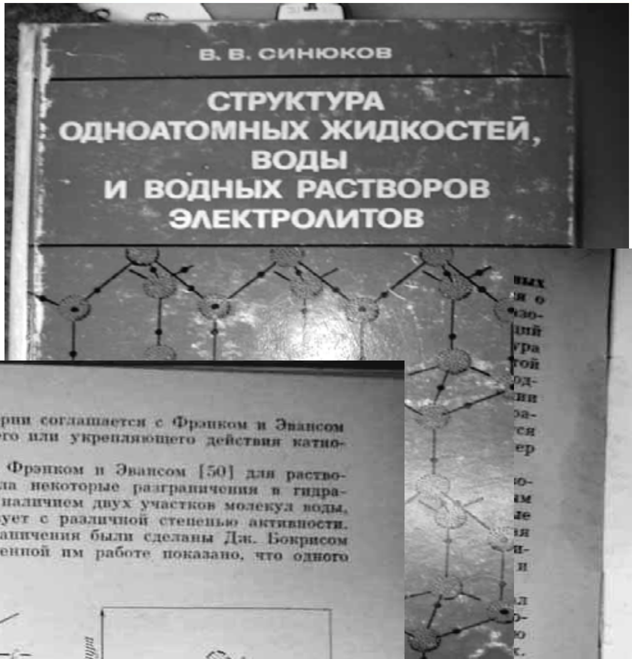
Differential Scanning Calorimetry of Hydrated Fullerenes (HyFn).

Dependence of "H_2O/C_{60}" Composition on a Drying Time (at 70-75° C) for Wet Precipitates of HyFn with Proper DSC Traces of Water Melting.

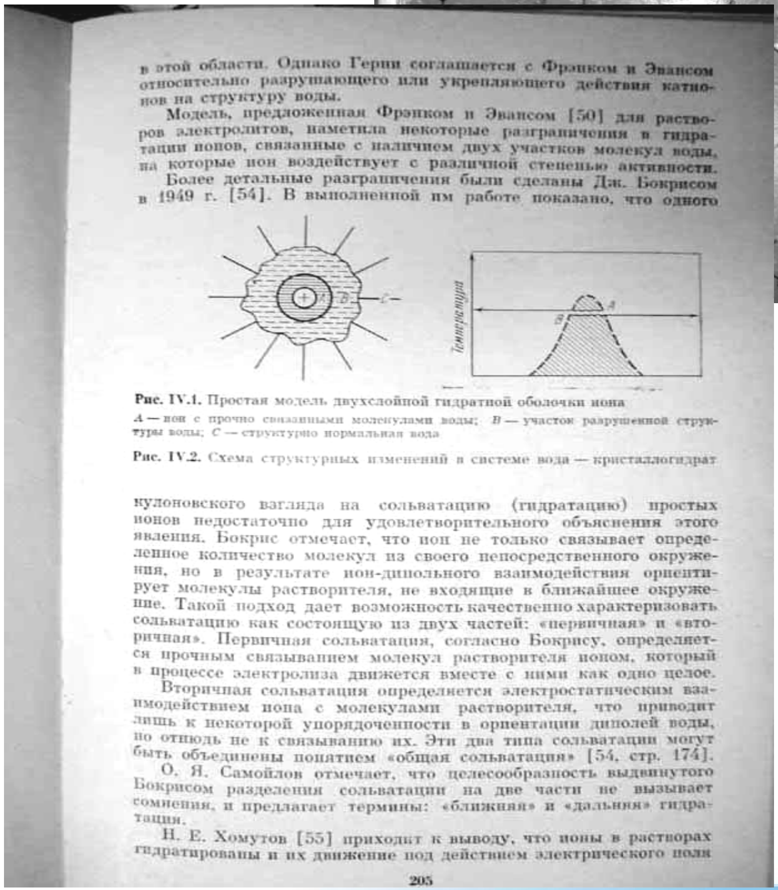
The Dashed Line - it is the Prognosis of Number of "Close Bounded " Water Molecules on C_{60} surface in FWS (= Aqueous Solution of HyFn).



Proff. M.V. Korobov. Chemistry Dept., Moscow State University, Russia, May 2003


В. В. СИНЮКОВ

СТРУКТУРА ОДНОАТОМНЫХ ЖИДКОСТЕЙ, ВОДЫ И ВОДНЫХ РАСТВОРОВ ЭЛЕКТРОЛИТОВ


в этой области. Однако Герни соглашается с Фрэнком и Эвансом относительно разрушающего или укрепляющего действия катионов на структуру воды.

Модель, предложенная Фрэнком и Эвансом [50] для растворов электролитов, наметила некоторые разграничения в гидратации ионов, связанные с наличием двух участков молекул воды, на которые ион воздействует с различной степенью активности.

Более детальные разграничения были сделаны Дж. Бокрисом в 1949 г. [54]. В выполненной им работе показано, что одного

Рис. IV.1. Простая модель двухслойной гидратной оболочки иона

A — ион с прочно связанными молекулами воды; *B* — участок разрушенной структуры воды; *C* — структурно нормальная вода

Рис. IV.2. Схема структурных изменений в системе вода — кристаллогидрат

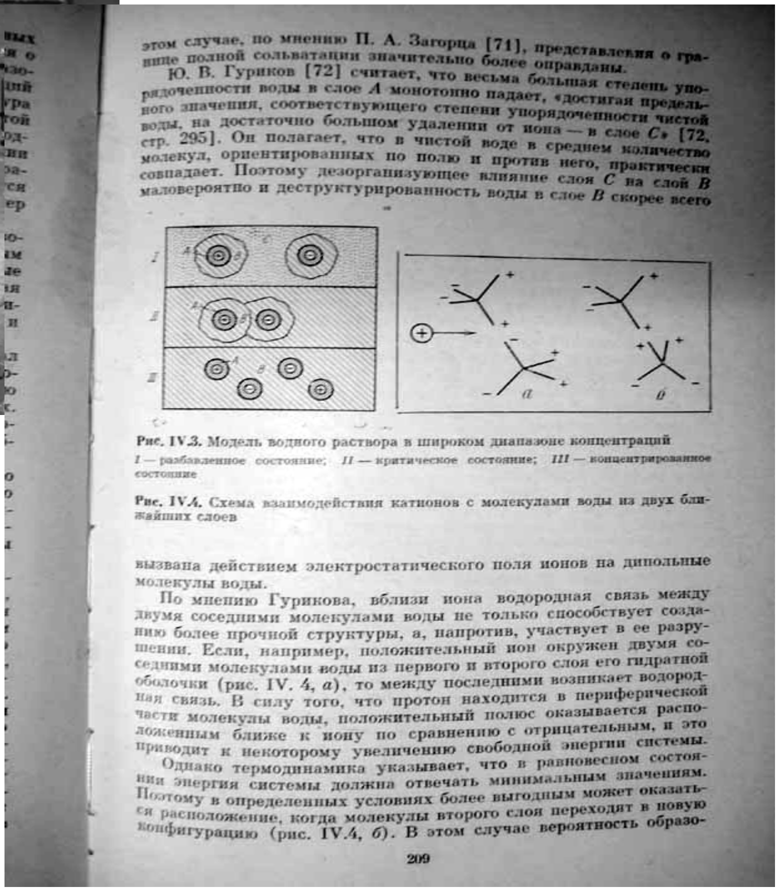
кулоновского взгляда на сольватацию (гидратацию) простых ионов недостаточно для удовлетворительного объяснения этого явления. Бокрис отмечает, что ион не только связывает определенное количество молекул из своего непосредственного окружения, но в результате ион-дипольного взаимодействия ориентирует молекулы растворителя, не входящие в ближайшее окружение. Такой подход дает возможность качественно характеризовать сольватацию как состоящую из двух частей: «первичная» и «вторичная». Первичная сольватация, согласно Бокрису, определяется прочным связыванием молекул растворителя ионом, который в процессе электролиза движется вместе с ними как одно целое.

Вторичная сольватация определяется электростатическим взаимодействием иона с молекулами растворителя, что приводит лишь к некоторой упорядоченности в ориентации диполей воды, но отнюдь не к связыванию их. Эти два типа сольватации могут быть объединены понятием «общая сольватация» [54, стр. 174].

О. Я. Самойлов отмечает, что целесообразность выдвинутого Бокрисом разделения сольватации на две части не вызывает сомнения, и предлагает термины: «ближняя» и «дальняя» гидратация.

Н. Е. Хомутов [55] приходит к выводу, что ионы в растворах гидратированы и их движение под действием электрического поля

205


этом случае, по мнению П. А. Загорца [71], представления о границе полной сольватации значительно более оправданы.

Ю. В. Гуриков [72] считает, что весьма большая степень упорядоченности воды в слое *A* монотонно падает, «достигая предельного значения, соответствующего степени упорядоченности чистой воды, на достаточно большом удалении от иона — в слое *C*» [72, стр. 295]. Он полагает, что в чистой воде в среднем количество молекул, ориентированных по полю и против него, практически совпадает. Поэтому дезорганизующее влияние слоя *C* на слой *B* маловероятно и деструктурированность воды в слое *B* скорее всего

Рис. IV.3. Модель водного раствора в широком диапазоне концентраций

I — разбавленное состояние; *II* — критическое состояние; *III* — концентрированное состояние

Рис. IV.4. Схема взаимодействия катионов с молекулами воды из двух ближайших слоев

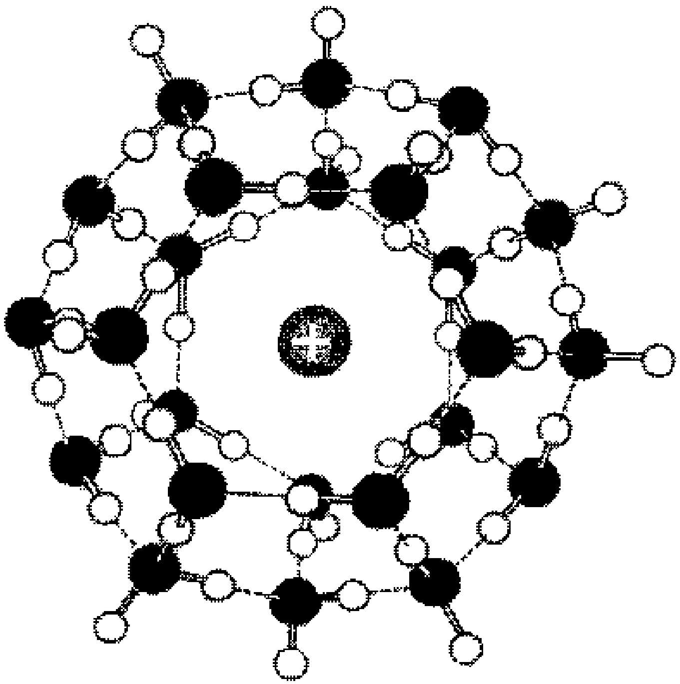
вызвана действием электростатического поля ионов на дипольные молекулы воды.

По мнению Гурикова, вблизи иона водородная связь между двумя соседними молекулами воды не только способствует созданию более прочной структуры, а, напротив, участвует в ее разрушении. Если, например, положительный ион окружен двумя соседними молекулами воды из первого и второго слоя его гидратной оболочки (рис. IV. 4, *a*), то между последними возникает водородная связь. В силу того, что протон находится в периферической части молекулы воды, положительный полюс оказывается расположенным ближе к иону по сравнению с отрицательным, и это приводит к некоторому увеличению свободной энергии системы.

Однако термодинамика указывает, что в равновесном состоянии энергия системы должна отвечать минимальным значениям. Поэтому в определенных условиях более выгодным может оказаться расположение, когда молекулы второго слоя переходят в новую конфигурацию (рис. IV.4, *б*). В этом случае вероятность образо-

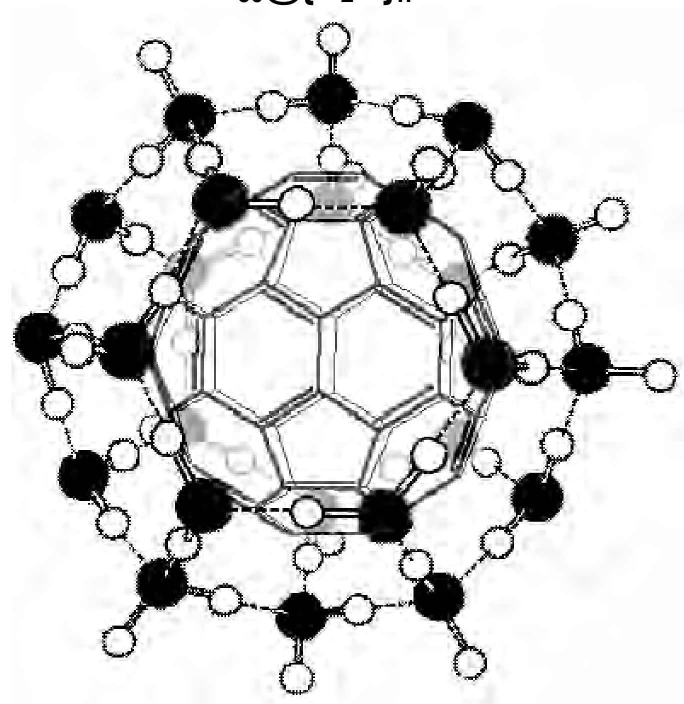
209

Water Clusters with H_3O^+ or Metal Ions (+) Encaged Inside the Clathrate of $(H_2O)_{24}$



[S. Wei, A.W. Castleman Jr., *(Using Reflectron Time-of-Flight Mass Spectrometer Techniques to Investigate Cluster Dynamics and Bonding)* Int. J. Mass Spectrom. Ion Proc. 131 (1994) 233.]

Hypothetical Model of Hydrated Fullerene - Supramolecular, Donor-Acceptor Complex of H-bonded Water Molecules with C_{60} - $C_{60}@\{H_2O\}_n$



$$C_{60}@\{xH_2O\cdot yOH^-\}^{y-}\cdot yH^+,$$

in which the part of counter-ions (H^+) is substituted by metal ions (Me^{x+}).

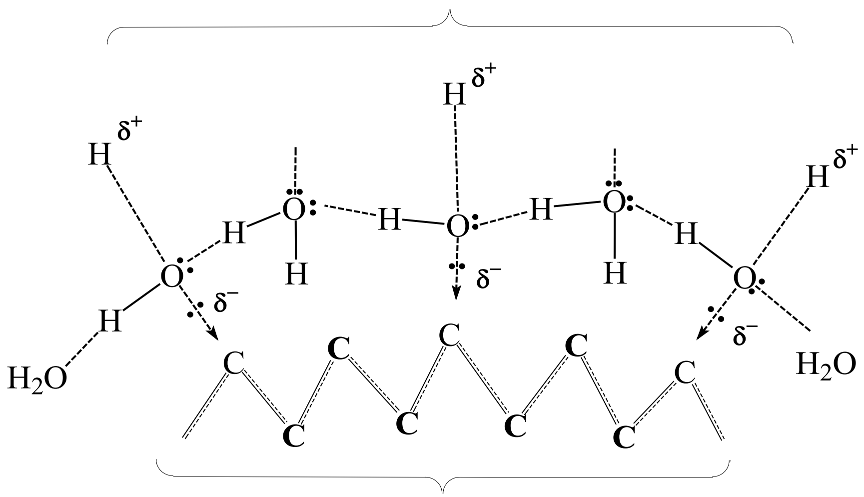
Hypothetical Model of Hydrated Fullerene - $C_{60}@\{H_2O\}_n$

The Fragment of a Structure, $C_{60}@\{yOH^{-}\ xH_2O\}^{y-}\ yH^{+}$,

in which the Part of Counter-Ions (H^{+})

can be Substituted by Metal Ions (Me^{z+}).

Close bounded water molecules on C_{60} fullerene surface



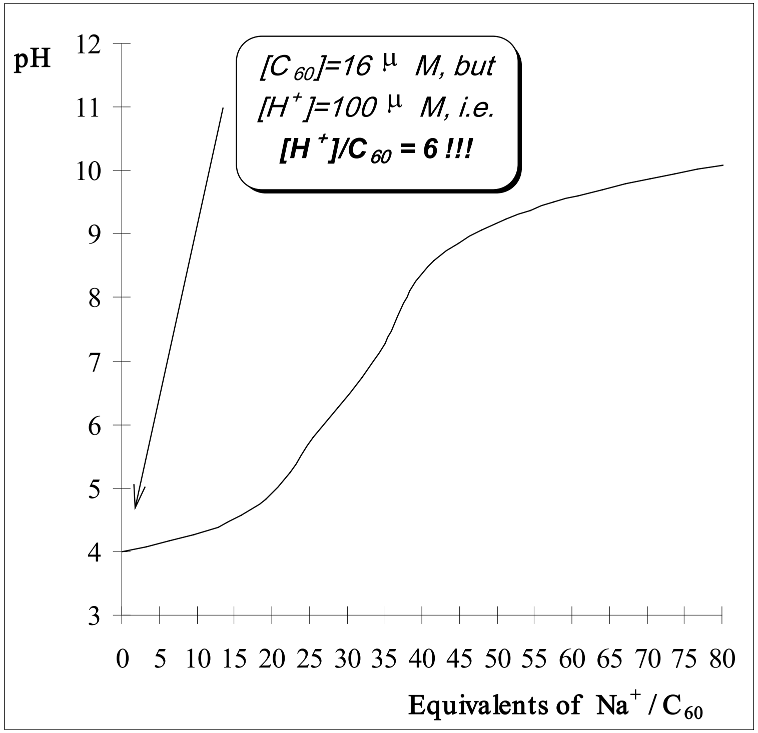
Carbon atoms of electron-acceptor surface of C_{60} fullerene

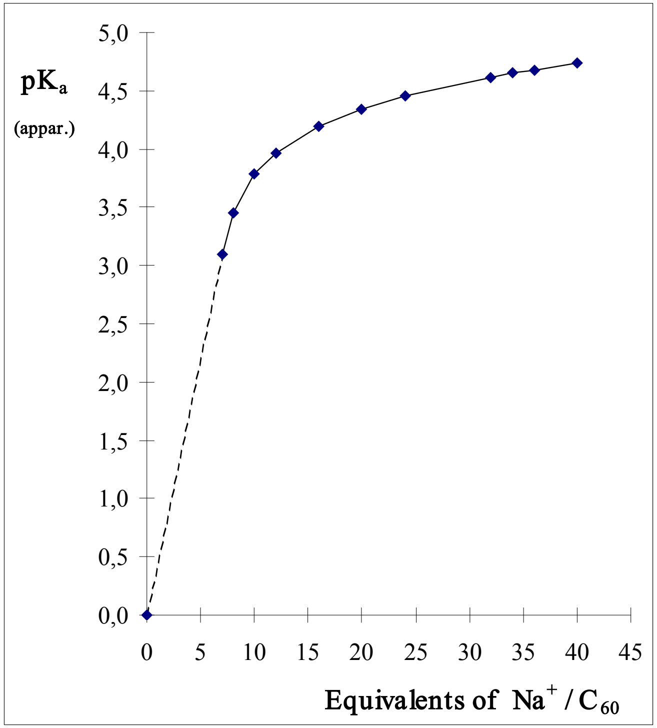
G.V. Andrievsky, et.al. COMPARATIVE ANALYSIS OF TWO AQUEOUS-COLLOIDAL SOLUTIONS OF C_{60} FULLERENE WITH HELP OF FT-IR REFLECTANCE AND UV-VIS SPECTROSCOPY. **Chem. Phys. Letters**, **364, (2002) 8-17.**

The changes of acid-basic properties of C_{60}HyFn aqueous solution (C_{60}FWS) at the titration of it by NaOH

(after deduction of titration of CO_2 that normally presents in deionized water at ambient conditions)

Before titration: [C_{60}] = 16 μM and C_{60}HyFn in H^+- form (see below)



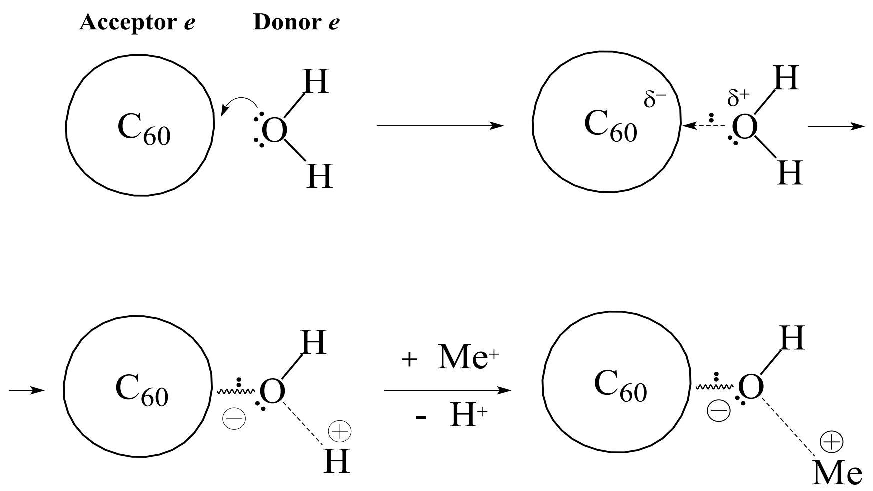


G.V. Andrievsky, V.K. Klochkov, A. Bordyuh, G.I. Dovbeshko. COMPARATIVE ANALYSIS OF TWO AQUEOUS-COLLOIDAL SOLUTIONS OF C_{60} FULLERENE WITH HELP OF FT-IR REFLECTANCE AND UV-VIS SPECTROSCOPY. Chem. Phys. Letters, 364 (2002) 8-17

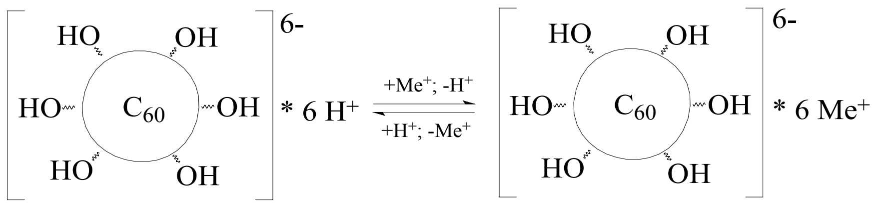
Hydrated C_{60} Fullerene:

Localized Hydrolysis of Water Molecules on C_{60} Surface and

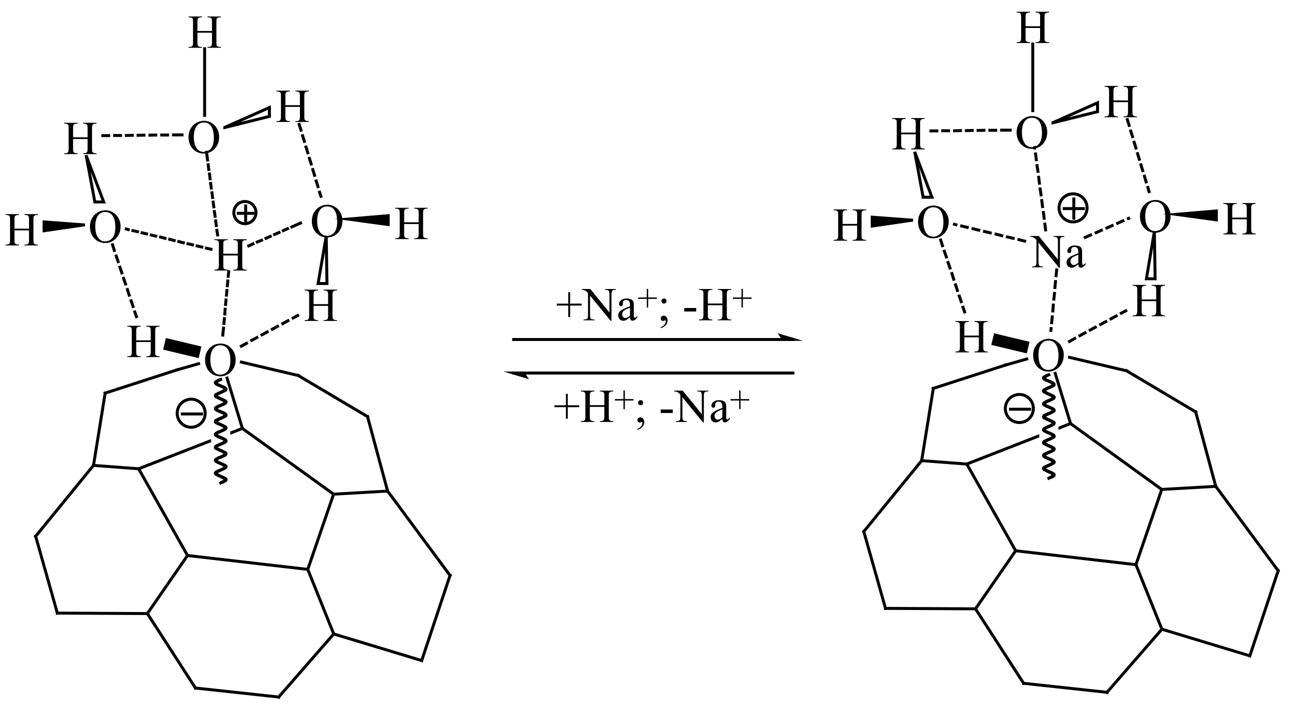
Cation Exchange of *H^+ / Me^+* in the First Nearest Aqueous Shell



at pH < 4

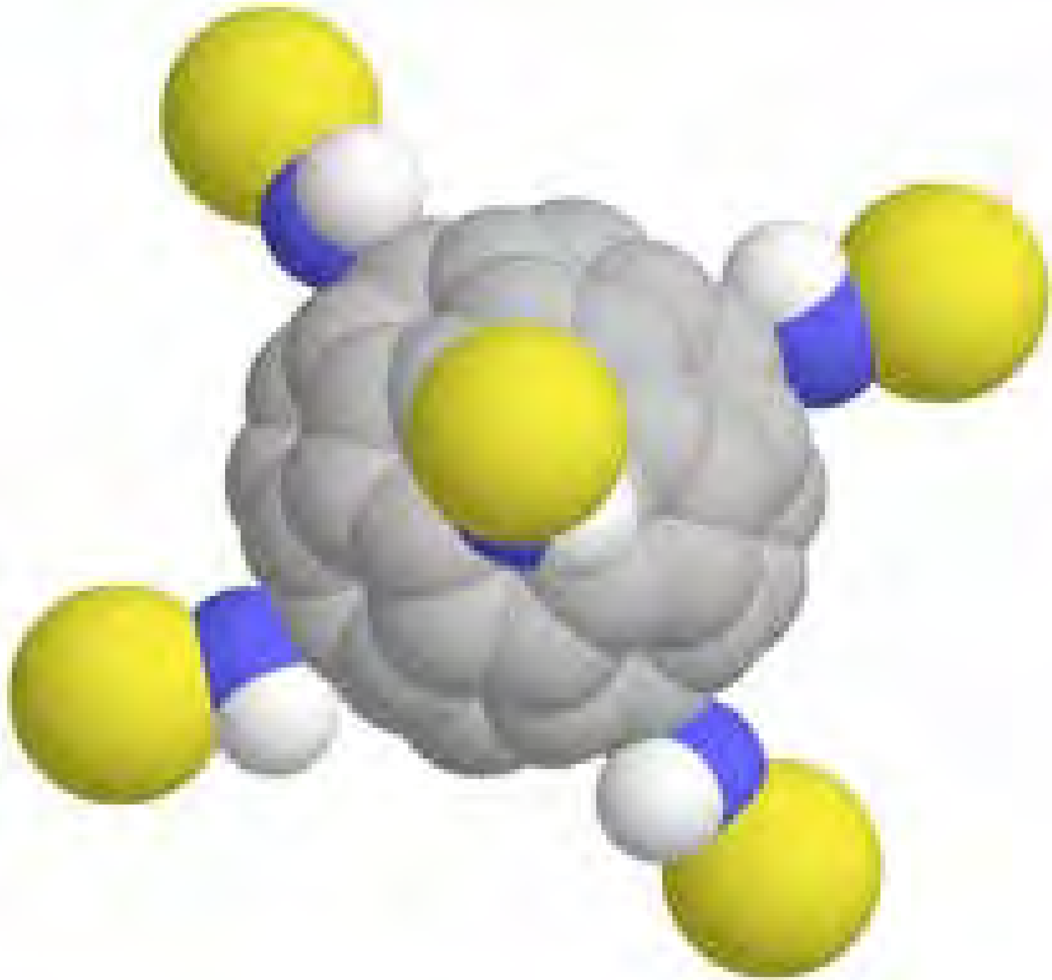


If Me^{+} = Na^{+}, then:



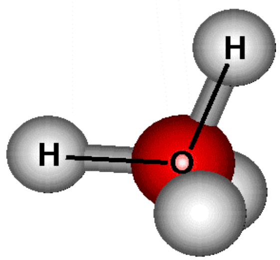
C_{60}HyFn in H^{+}- form

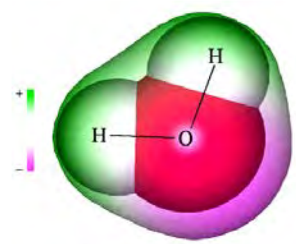
C_{60}HyFn in Na^{+}- form



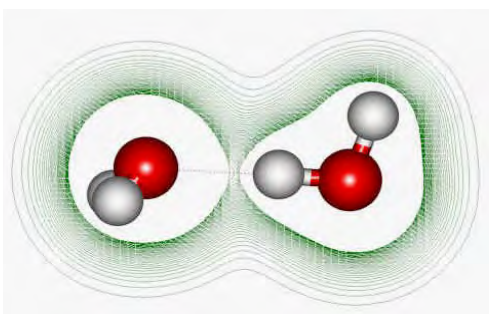
Water Block and to the Questions About Model of C_{60} Hydration

WATER MOLECULE - H_2O

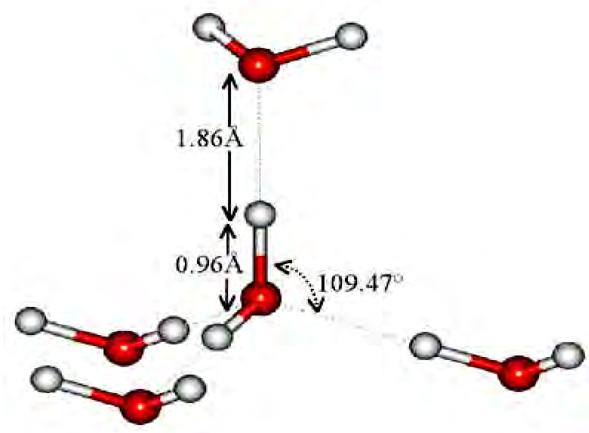




Hydrogen Bonded Two Water Molecules



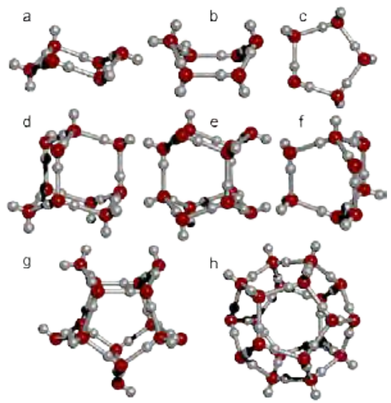
Hydrogen Bonded Water Pentamer

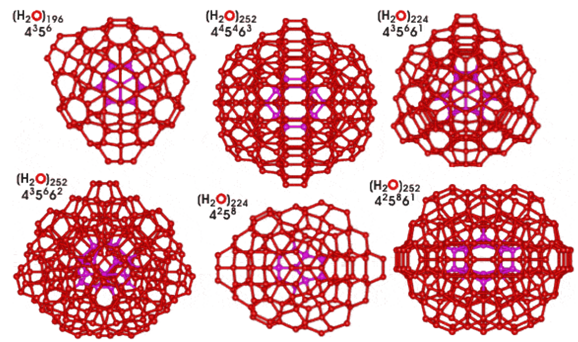


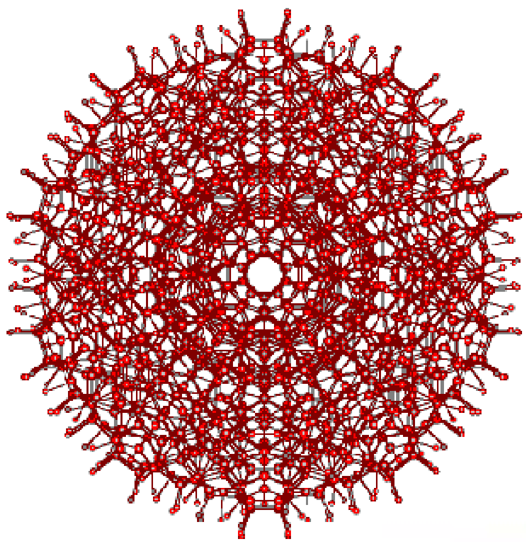
In accordance with:
M. Chaplin, *Water Structure and Science*, South Bank University, London, UK (http://www.lsbu.ac.uk/water)

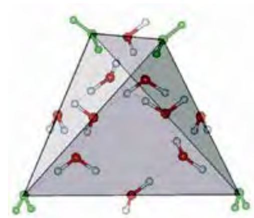
Various Water Clusters and C_{60} Fullerene

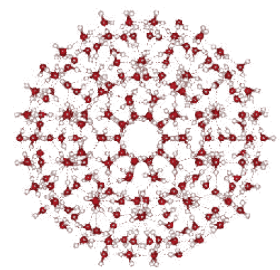
(by M. Chaplin, http://www1.lsbu.ac.uk/water/)

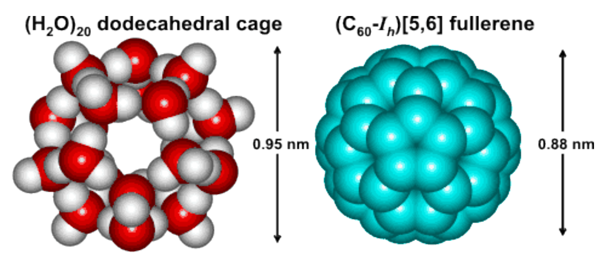






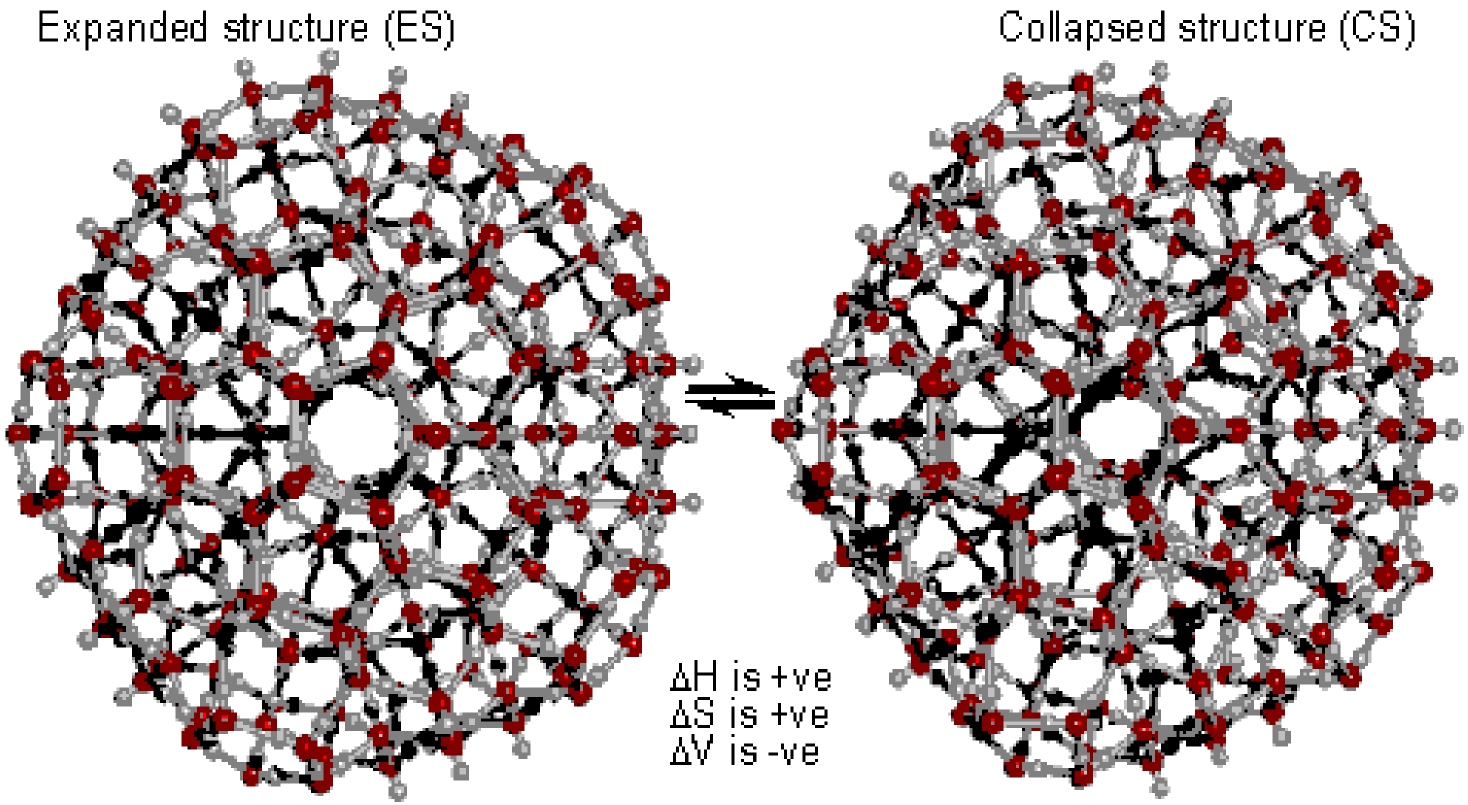






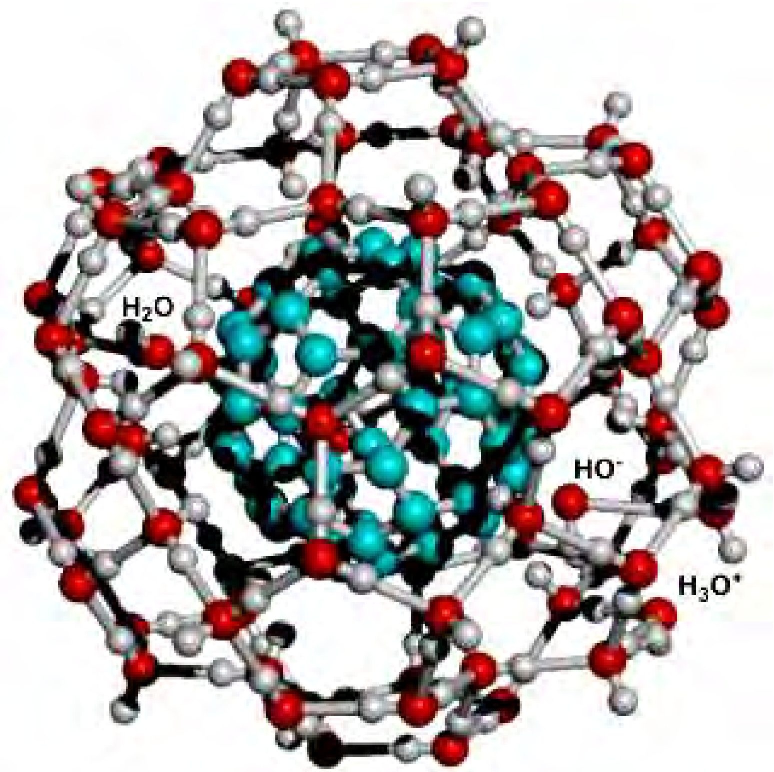
Icosahedral Water Clusters

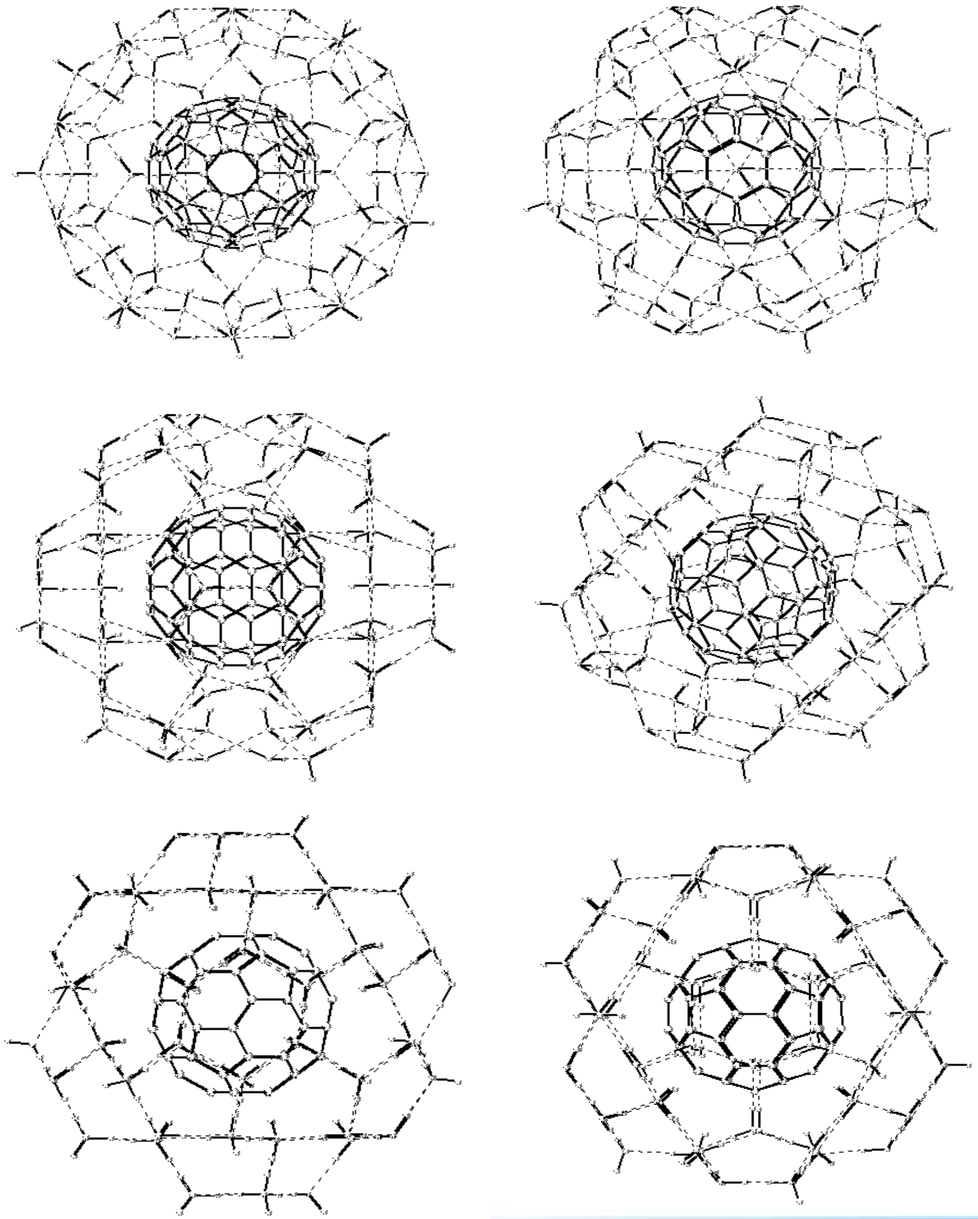
by Professor Martin Chaplin (December 20, 2001,
School of Applied Science South Bank University
London SE1 0AA (http://www.lsbu.ac.uk/water/icosahedral.html),



Fullerene C_{60} Encaged Inside the Icosahedral Water Cluster of $(H_2O)_{80}$ = = Hydrated C_{60} Fullerene (HyFn).

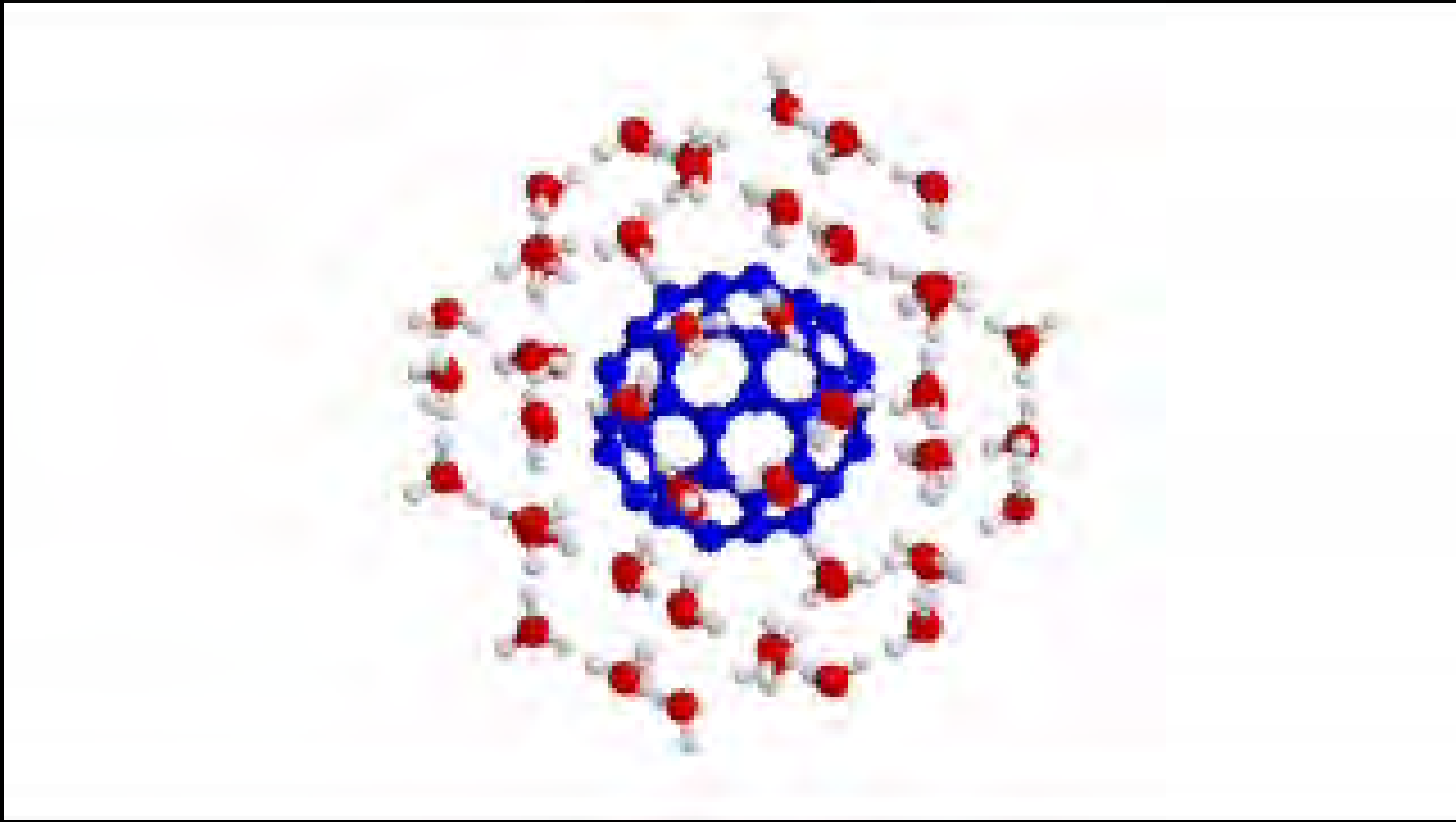
Designed by Professor Martin Chaplin in December 20, 2001,
School of Applied Science
South Bank University
London SE1 0AA
(http://www.lsbu.ac.uk/water/buckmin.html),
in accordance with recommendations of Andrievsky G.V.
and Chem.Phys.Lett., 300 (1999) 392-396.

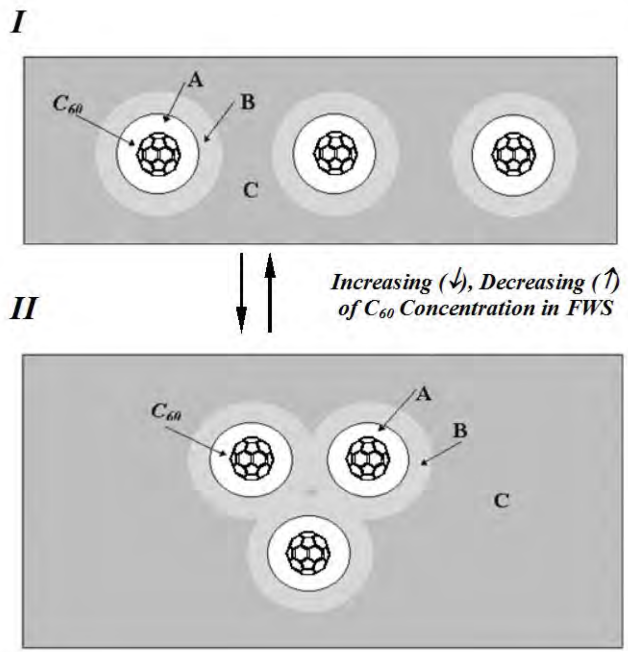




Various Projections of Fullerene C_{60} Encaged Inside the Icosahedral Water Cluster of $(H_2O)_{80}$

Designed by Martin Chaplin in November 14, 2001 (http://www.lsbu.ac.uk/water/buckmin.htm) in accordance with Andrievsky G.V., et.al.,. Chem.Phys.Lett., 300 (1999) 392-396





I - **Model of Aqueous Solution of Hydrated C_{60} Fullerene (HyFn).**

II - **Model of Clusters Formation of Hydrated C_{60} Fullerene in Water Solution (FWS).**

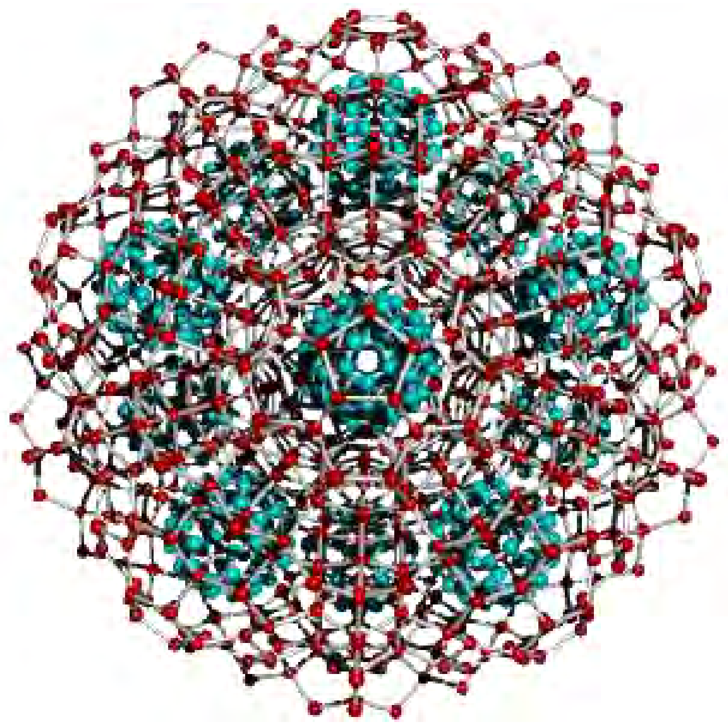
Were:

A - **First Water Layer of Close Bonded Water Molecules (~20-24 H_2O);**

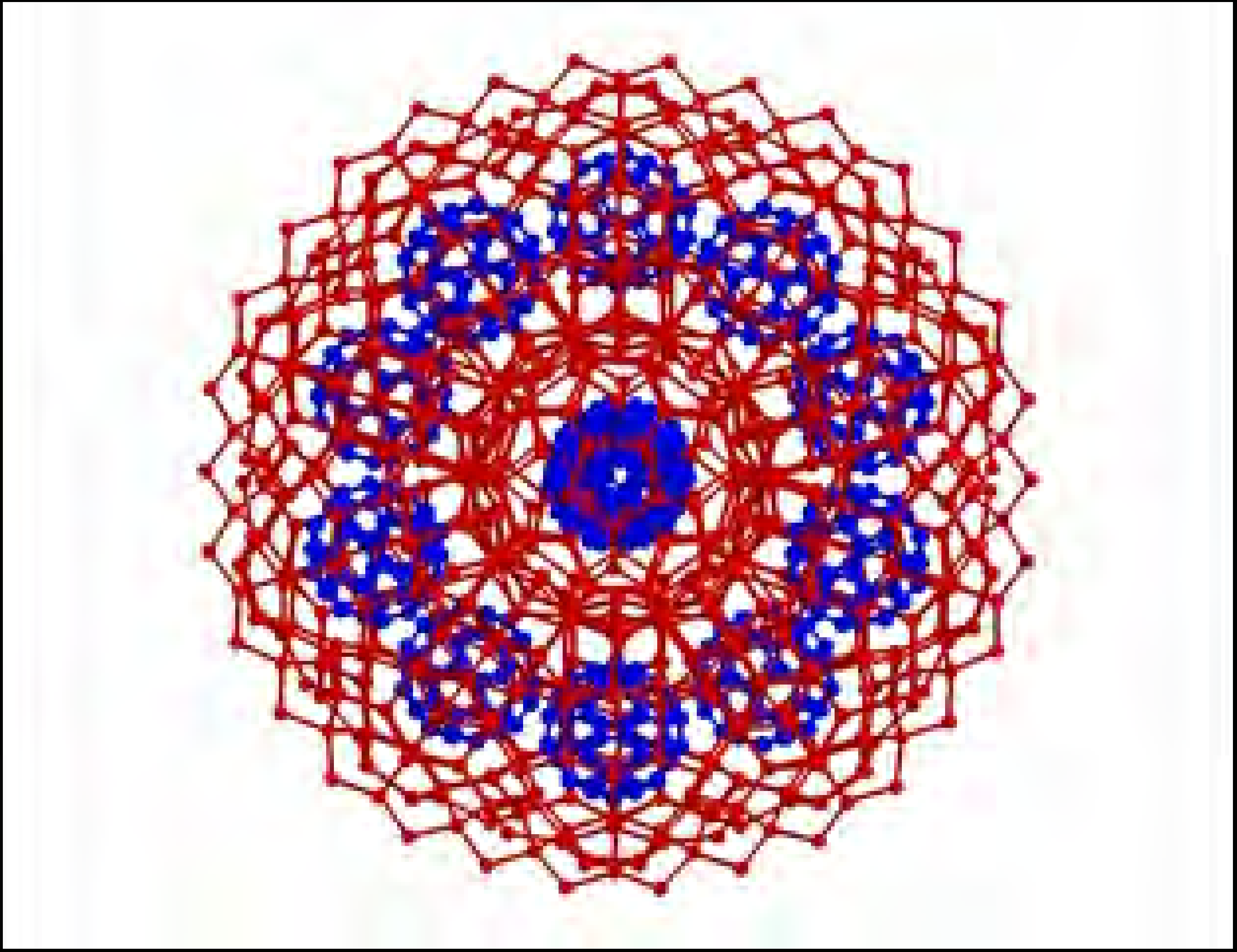
B - **Transitional Layers of Water Molecules;**

C - **Bulk («Free») Water.**

Designed by Professor Martin Chaplin in December 20, 2001, School of Applied Science
South Bank University, London SE1 0AA
(http://www.lsbu.ac.uk/water/buckmin.html),
in accordance with recommendations of Andrievsky G.V.
and Chem.Phys.Lett., 300 (1999) 392-396.

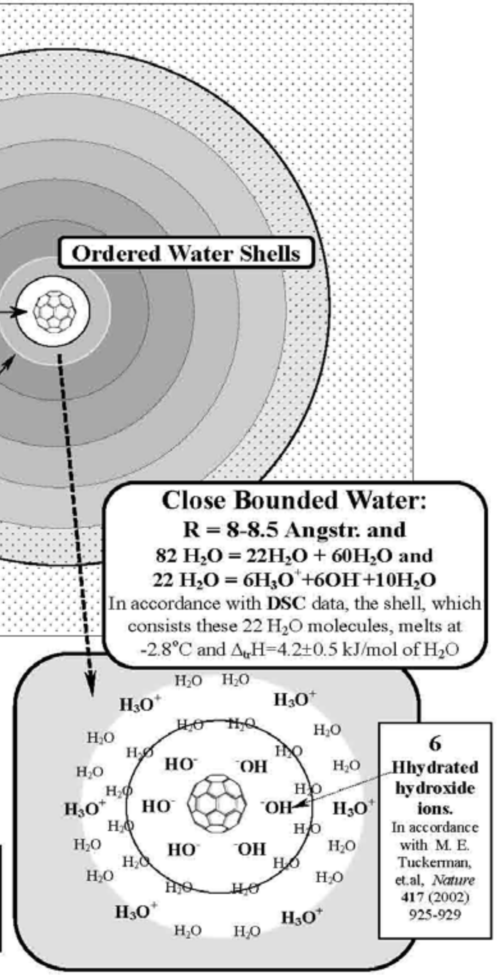


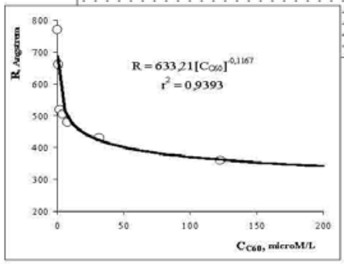
The predicted model of the elementary spherical (icosahedral) cluster with the diameter of 3.4 nm which consists of 13 hydrated C_{60} fullerenes.



THE HYDRATED C_{60} FULLERENE (HyFn = DONOR-ACCEPTOR COMPLEX OF $C_{60}@\{H_2O\}_n$ **) SURROUNDED BY ORDERED WATER SHELLS.**

The Probable Model of HyFn Which Based On Data of Dynamic Light Scattering (DLS), Small-Angle Neutron Scattering (SANS), Low Temperature Differential Scanning Calorimetry (DSC) and Molecular Simulation of professor M. Chaplin .

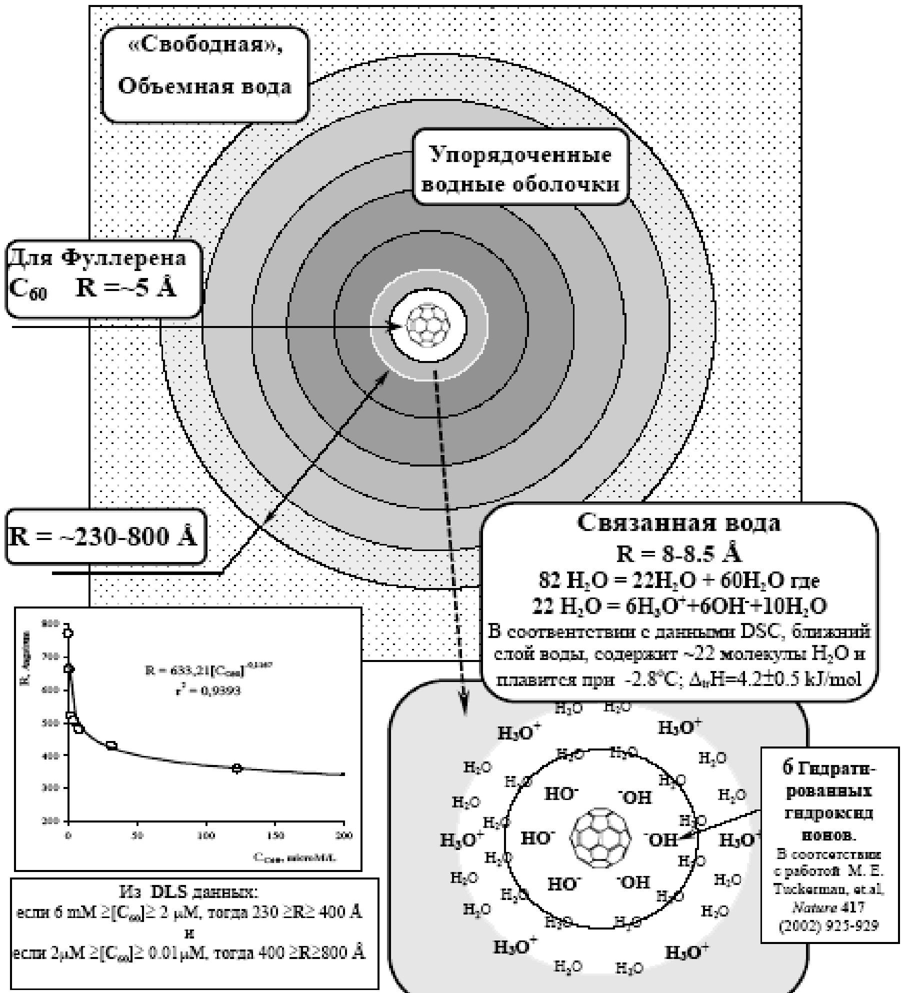




From **DLS** data:
if 6 mM ≥**[C_{60}]**≥ 2 µM, then 230 ≥**R**≥ 400 Å
and
if 2µM ≥**[C_{60}]**≥ 0.01µM, then 400 ≥**R**≥800 Å

Рис.2. ГИДРАТИРОВАННЫЙ C_{60} ФУЛЛЕРЕН (HyFn = донорно-акцепторный комплекс вида $C_{60}@\{H_2O\}_n$), **ОКРУЖЕННЫЙ УПОРЯДОЧЕННЫМИ ВОДНЫМИ ОБОЛОЧКАМИ.**

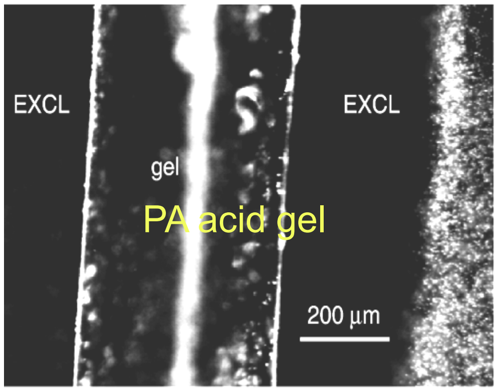
Вероятная модель, основанная на данных динамического светорассеяния (DLS), малоуглового нейтронного рассеяния (SANS) [18] и низкотемпературной дифференциальной сканирующей калориметрии (DSC) [17] для HyFn, выделенных из C_{60}FWS [9], а также учитывающая результаты молекулярного моделирования профессора M. Chaplin [19,40].

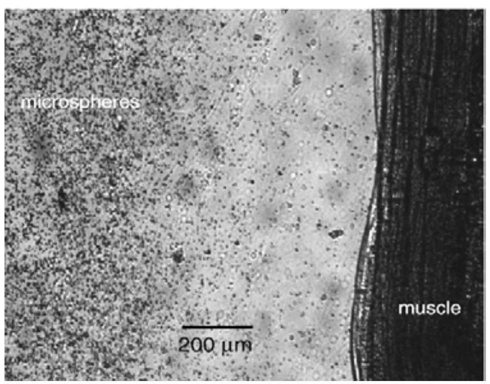


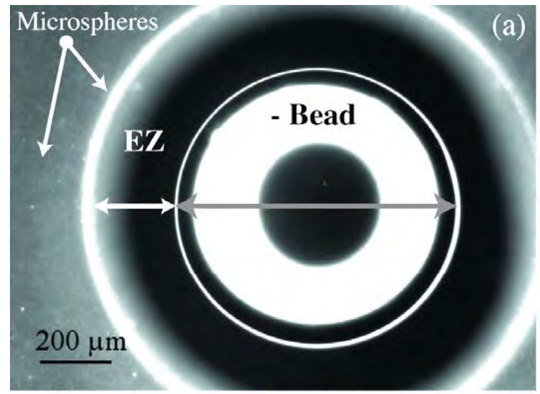
From experimental data:
--- there are ~24 H_2O molecules in the "first" shell of close bounded water - $C_{60}@\{H_2O\}_{24}$ – hydrated C_{60} fullerene (C_{60}HyFn).

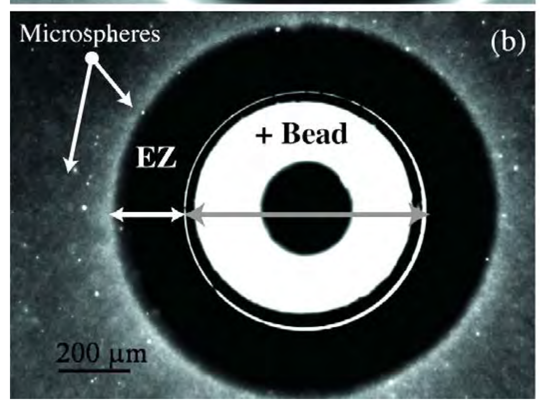
For C_{60}HyFn:
--- phase transformation (melting) of this close bounded water occur at **-** 2.8 ^oC;
--- enthalpy of C_{60} hydration - ΔHf_{ull} = 79±10 kJ/mol = ~ 0.87 eV;
--- enthalpy of H_2O molecule hydration - ΔH_{water} = 4.2±0.5 kJ/mol = ~ 0.04 eV (70% of the enthalpy of melting of the bulk water!!!);
--- among such 24 H_2O there are 6 molecules that symmetric placed around C_{60} and formed with it 6 donor-acceptor (DA) bonds due to charge transfer (CT) from oxygen atom of H_2O to C_{60} molecule. Energy of each DA-bond is equal to ~0.093 eV;
--- due to DA-bond formation, in such water molecules O-H bonds lengths are non equivalent and due to localized hydrolysis of water on C_{60} surface the C_{60}HyFn in aqueous solutions is negatively charged structure that behaves oneself like hexa-anion of weak polyacids;
--- taking into account energies all DA- and H-bonds, stabilization energy of highly stable supramolecular complex $C_{60}@\{H_2O\}_{24}$ is equal to ~15.5 eV.
--- due to the existence of ordered outer water shells melting at 0 ^oC, $C_{60}@\{H_2O\}_{24}$ structure is fully stable in its aqueous solutions (C_{60}FWS) at heating at least up to 125 ^oC.

EXCLUSION ZONE WATER (EXCL or EZ) by G.Pollack









J. Zheng, W.-C. Chin, E. Khijniak, E. Khijniak Jr., G. H. Pollack. Surfaces and interfacial water: Evidence that hydrophilic surfaces have long-range impact. *Advances in Colloid and Interface Science* 127 (2006) 19–27.
J.-M. Zheng, A. Wexler, G.H. Pollack, Effect of buffers on aqueous solute-exclusion zones around ion-exchange resins, *Journal of Colloid and Interface Science* (2009), doi: 10.1016/j.jcis.2009.01.010

O_2 and CO_2 Solubility in Water (at 18-20 ^oC):

O_2 ~ **1** mM/L
CO_2 ~ **45** mM/L

but only **0.2%** of CO_2 dissolved converts to HCO_3^- !!!

$CO_2 + H_2O \leftrightarrow HCO_3^-$
and $[HCO_3^-]$ = **0.09** mM/L

What does it amount to? It means that CO_2 is dissolved in water in a kind of CO_2 Nanobubbles :

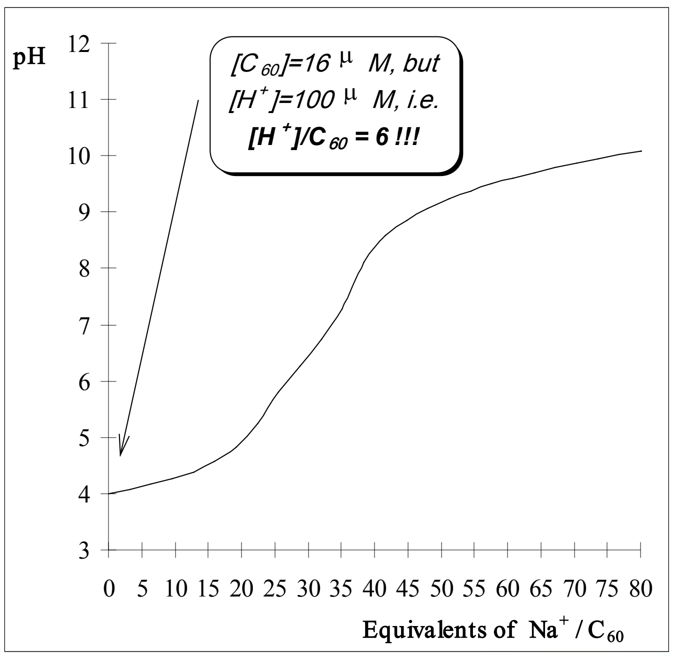
$$(\mathbf{CO_2})_n@(\mathbf{HCO_3^-})_m * \mathbf{m\ H^+}$$

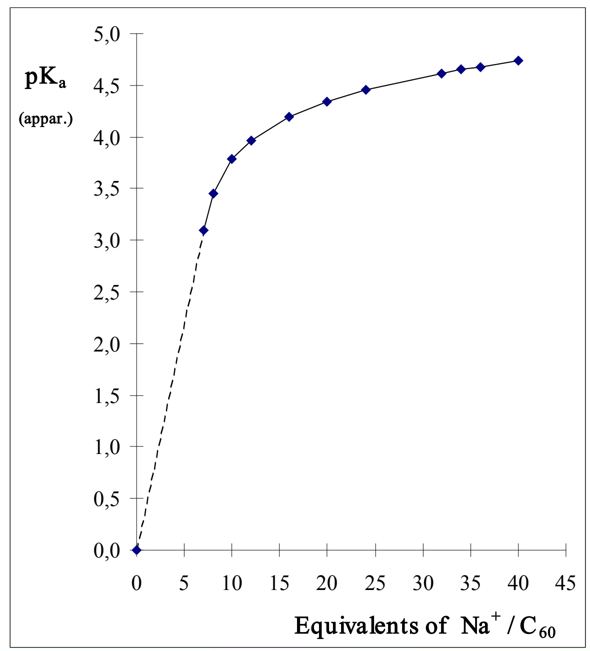
(with size app. 100 nm)

F. Jin, J. Ye, L. Hong, H. Lam, C. Wu. Slow Relaxation Mode in Mixtures of Water and Organic Molecules: Supramolecular Structures or Nanobubbles? *J. Phys. Chem. B*, 111 (9) (2007) 2255–2261

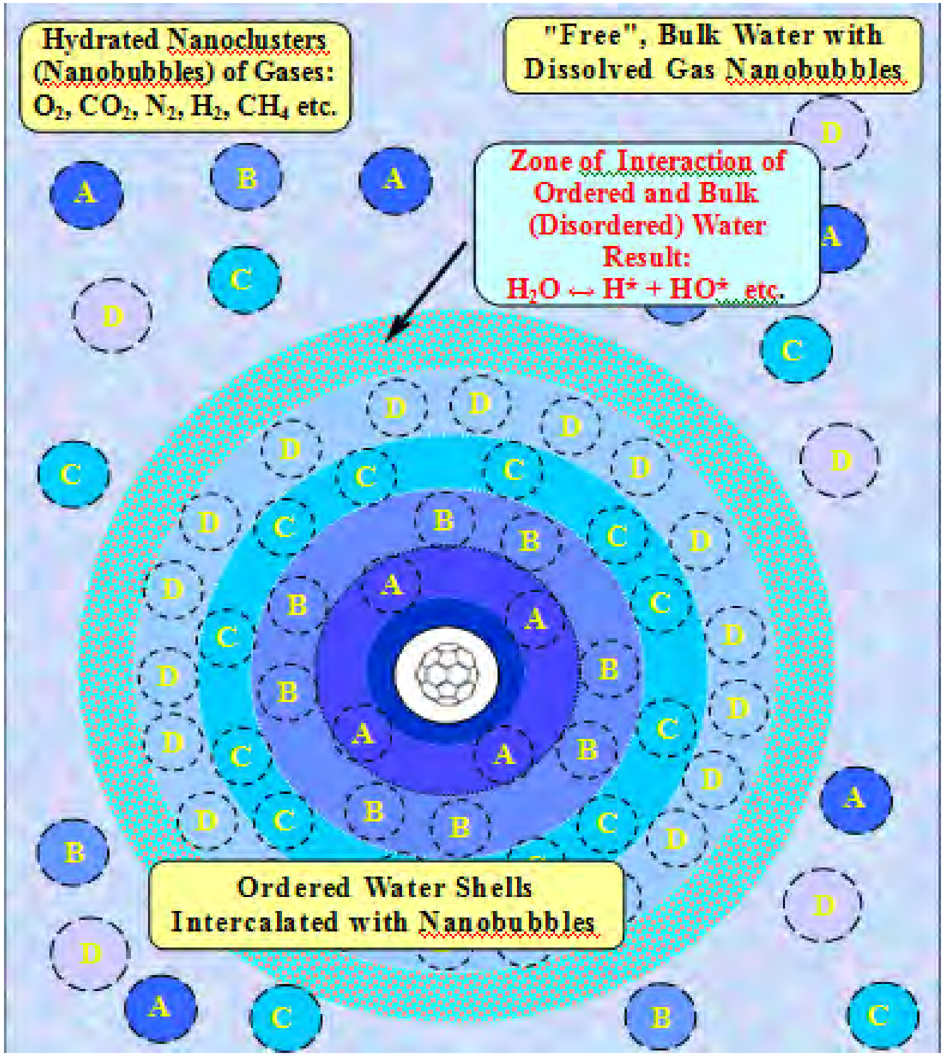
CO_2 Solubility in Water Ordered by C_{60}HyFn is More in 5 - 7 Time than in Pure Water

Earlier mentioned curves of C_{60}FWS titration by NaOH testify that in water ordered by C_{60}HyFn the CO_2 solubility is essentially above than in the pure, poorly ordered water





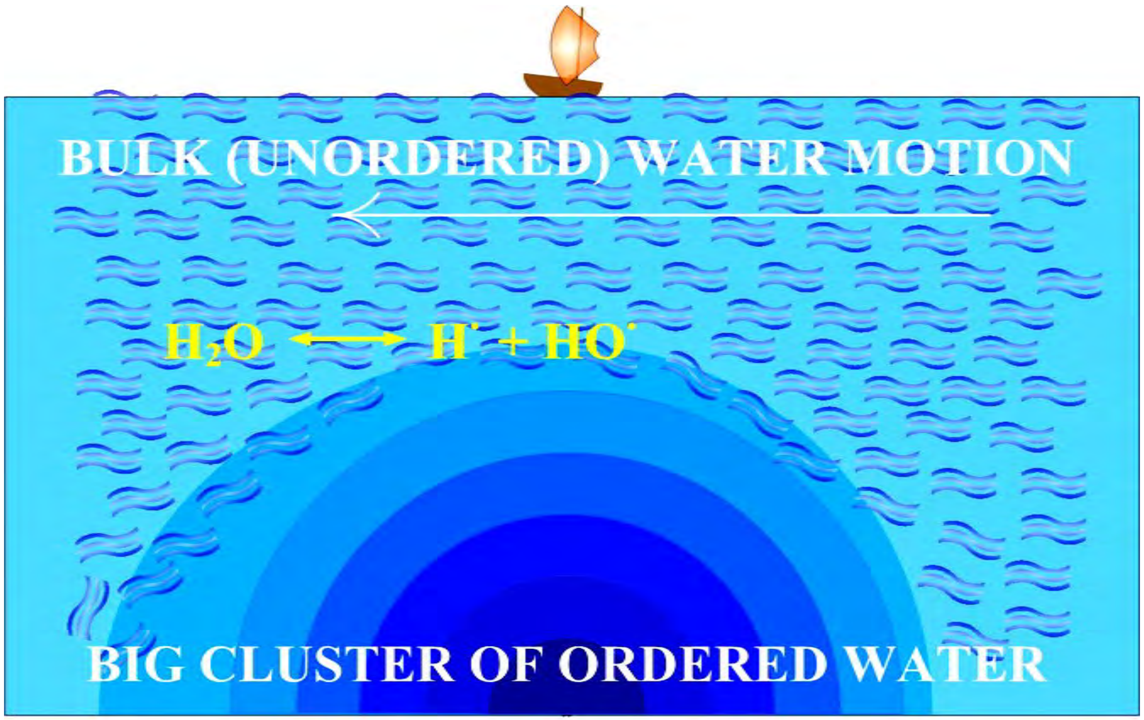
Model of Light-Scattering Structures in C_{60}HyFn Water Solutions (C_{60}FWS)



STRUGGLE of the ORDER AGAINST a DISORDER

or

Movements of Ordered Water Cluster in Bulk Water Can Stimulate on It Diffusive Surfaces the Dissociation of Water Molecules with Formation of Quickly Recombining H-, O-, C-, N-Containing Radicals.



$$(H_2O)_n(H_2O \leftarrow H\text{--}OH \rightarrow OH_2)(H_2O)_m \rightarrow (H_2O)_n(H_2O + H^{\bullet} + {}^{\bullet}OH + OH_2)(H_2O)_m$$

$$CO_2 + {}^{\bullet}OH \rightarrow HCO_3^{\bullet} \qquad O_2 + {}^{\bullet}H \rightarrow HO_2^{\bullet}$$

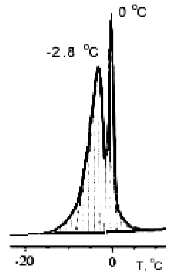
$$2\,{}^{\bullet}OH \rightarrow H_2O_2 \qquad 2H_2O_2 \rightarrow 2H_2O + O_2 \ldots \text{ etc.}$$

LET'S COMPARE THE THEORY and FACTS!!!

Parameters \ Objects	**Coherence Domain of Water (CDW)** by Emilio DelGiudice	**Hydrated C_{60} Fullerene (C_{60}HyFn)** by Grigoriy Andrievsky
Size (Diameter)	**100 nm**	Coherence Domain of Water Ordered by C_{60}HyFn (**C_{60}CDW**) – **40-160 nm (on the average ~100 nm !!!)** For diluted C_{60}HyFn solution.
Another Sizes	**Is CDW Exists with Size <<100 nm?**	**1.6-1.8 nm** for C_{60} Molecule With First Closely Bounded Water Shell and Fractal Cluster Nanostructures of C_{60}HyFn and Water Clusters of Higher Order (with the size approx. **3.4, 7.1, 10.9, 14.5, 18.1, 21.8, 25.4, 28.8, 32.4, 36.0 nm**)
“0.2 eV”	**FUNDAMENTAL BIO-PHYSICAL VALUE**	**Δ_{tr}H - Heat, Enthalpy of C_{60} fullerene HYDRATION**
Energy	**12.06 eV** Energy of Excited State of CDW	**15.5 eV** Estimated Energy of C_{60}HyFn Formation, Stabilization
For Comparison	**12.6 eV** - Energy of Water Ionization	

C_{60}HyFn and Biomolecules

LOW TEMPERATURE DIFFERENTIAL SCANNING CALORIMETRY (DSC) OF HYDRATED C_{60} FULLERENE AND VARIOUS HYDRATED BIO-MACROMOLECULES (COMPARISONS and SEARCH FOR ANALOGIES).



Two Typical Endothermic Peaks for DSC Traces of Wet Powder of HyFn Precipitated from C_{60}FWS.
(In This Case, Molar Ratio of H_2O/C_{60} = 27)

HYDRATED C_{60} FULLERENE

The DSC traces indicated below are taken from the book of G.M. Mrevlishvili "Low Temperature Calorimetry of Biolgical Macromolecules", Metsniereba, Tbilisi, 1984,

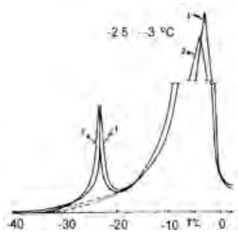


Fig.78. **Microcalorimetric Traces of "Ice-Water" Phase Transition and Eutectic Melting in System of *"Na-DNA - H_2O - NaCl"***
1 - Native DNA; 2 -Denatured DNA
(DNA - 1.35 mg; NaCl - 0.38 mg; H_2O - 1.07 mg).

***D N A* -** Deoxyribonucleic Acid, Na-Salt

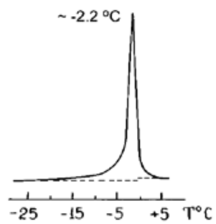


Fig. 42. **Microcalorimetric Trace of Phase Transition of Water in RNase Solution**
(C=45%; V=14.5 °/h) [ref. 248].

RIBONUCLEASE -
Enzyme, Globular Protein

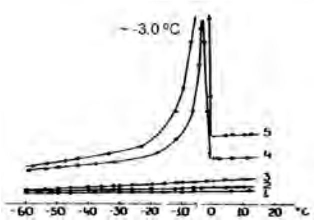


Fig.50. **Temperature Dependence of Collagen Heat Capacity at Various Content of Water** (W, g H_2O / g protein) **in the Region of "Ice - Water" Phase Transition**
(W: 1 -0.02; 2 - 0.15; 3 - 0.35; 4 - 0.66; 5 - 1.00) [ref. 49, 178].

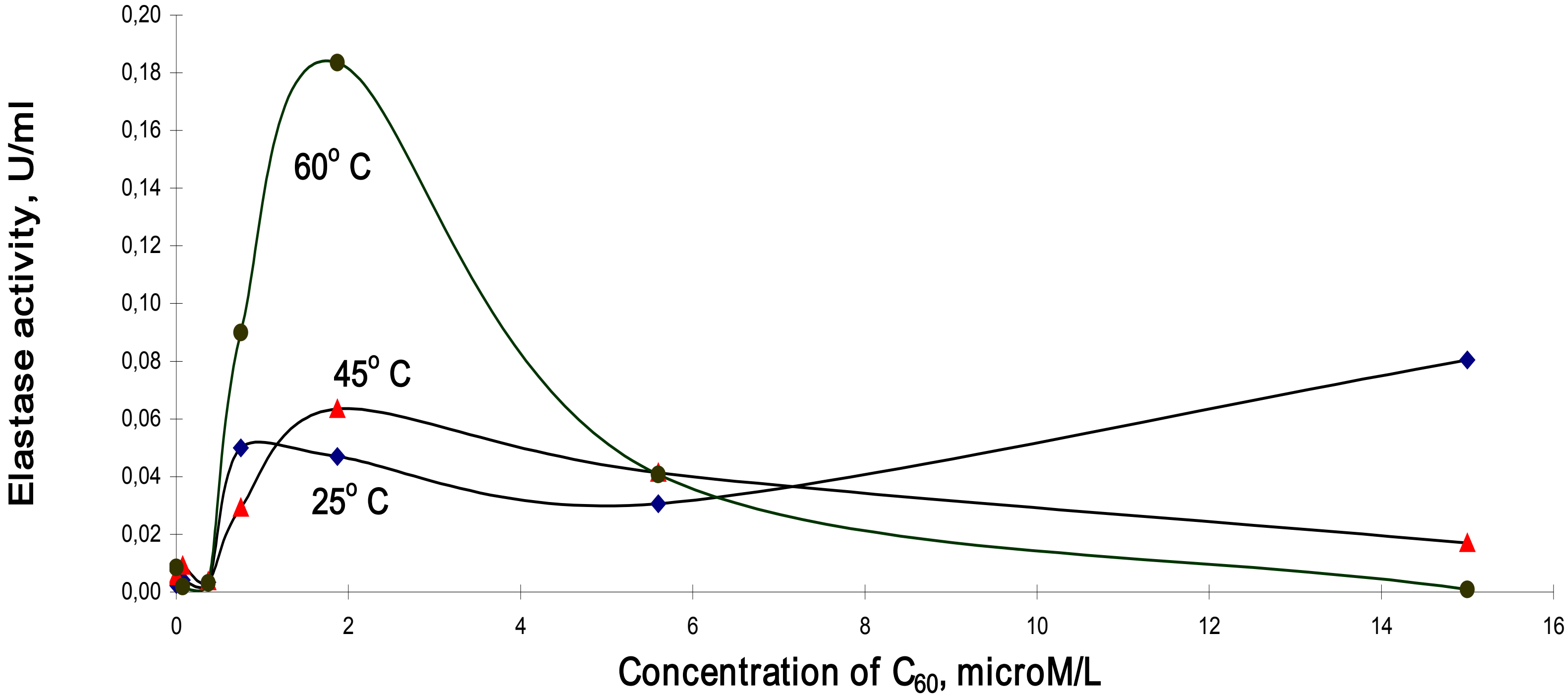
COLLAGEN -Fibrillar Protein

HYDRATION OF NUCLEIC ACIDS

Low Temperature Calorimetry Data
from the book of
Mrevlishvili G.M. "Low Temperature Calorimetry of Biological Macromolecules", "Mertsnierba", Tbilisi, 1984, 188 p.

	g H_2O/ g polymer	**mol H_2O/pair of nucleic bases**
DNA (fron T2 phag)	0.55	22
DNA (from calf thymus)	0.6	25
Poly-[d(A-T)]	0.7	28
Poly-[d(G-C)]	0.4	16
t-RNA	0.68	28 [277]
		24 (on average)

Dependence of the Elastase Activity from HyFn Concentration in Incubation Medium at 25°C and also after Temperature Treatment During 15 Minutes at 45° C and 60° C.



УФ-Вид (UV-Vis) спектры поглощения :

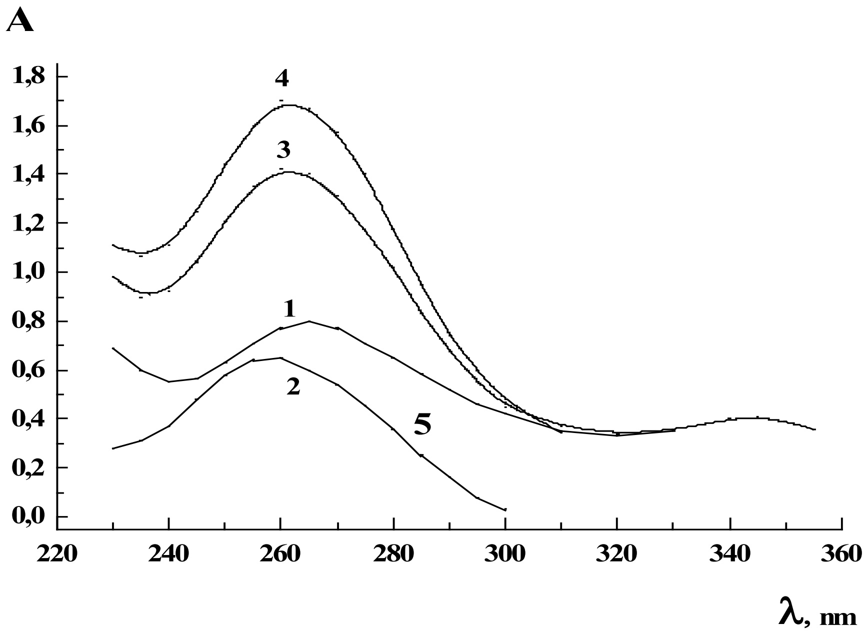
гидратированного C_{60} фуллерена (1 – C_{60}HyFn**);**

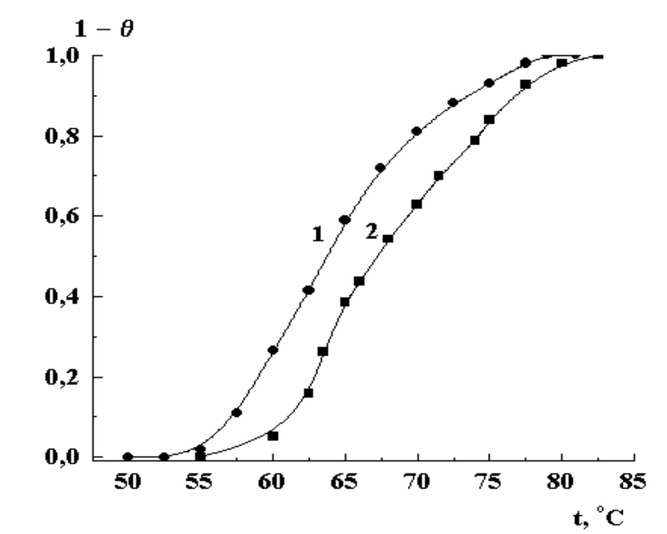
ДНК (2 - DNA**)**

и их смеси (C_{60}HyFn+DNA**) при T=20° C (**3**) и T=85° C (**4**)**

в 4 mM растворе NaCl.

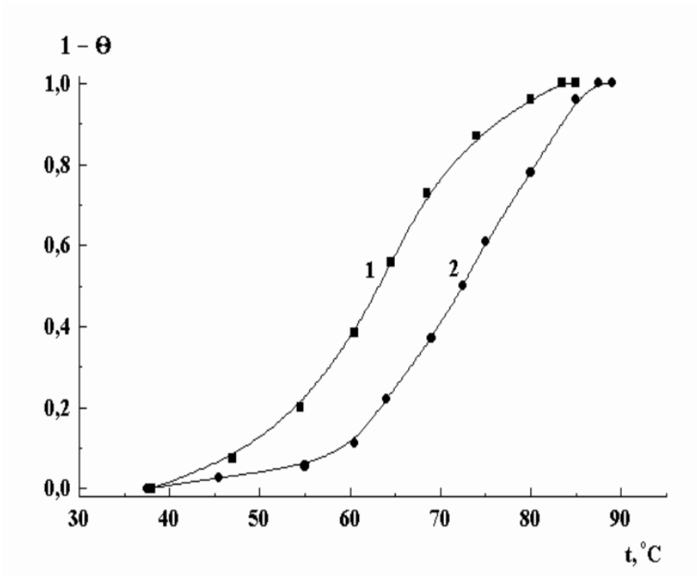
Кривая (5**) представляет разность поглощений между кривыми (**3**) и (**1**), и практически совпадает с экспериментально наблюдаемой кривой для чистой ДНК (**2**).**





Curves of melting of highly polymeric thymus DNA: without (1) and in the presence (2) of hydrated C_{60} fullerene

(P_{DNA}/C_{60} = 10, C_{NaCl} = 4 mM)



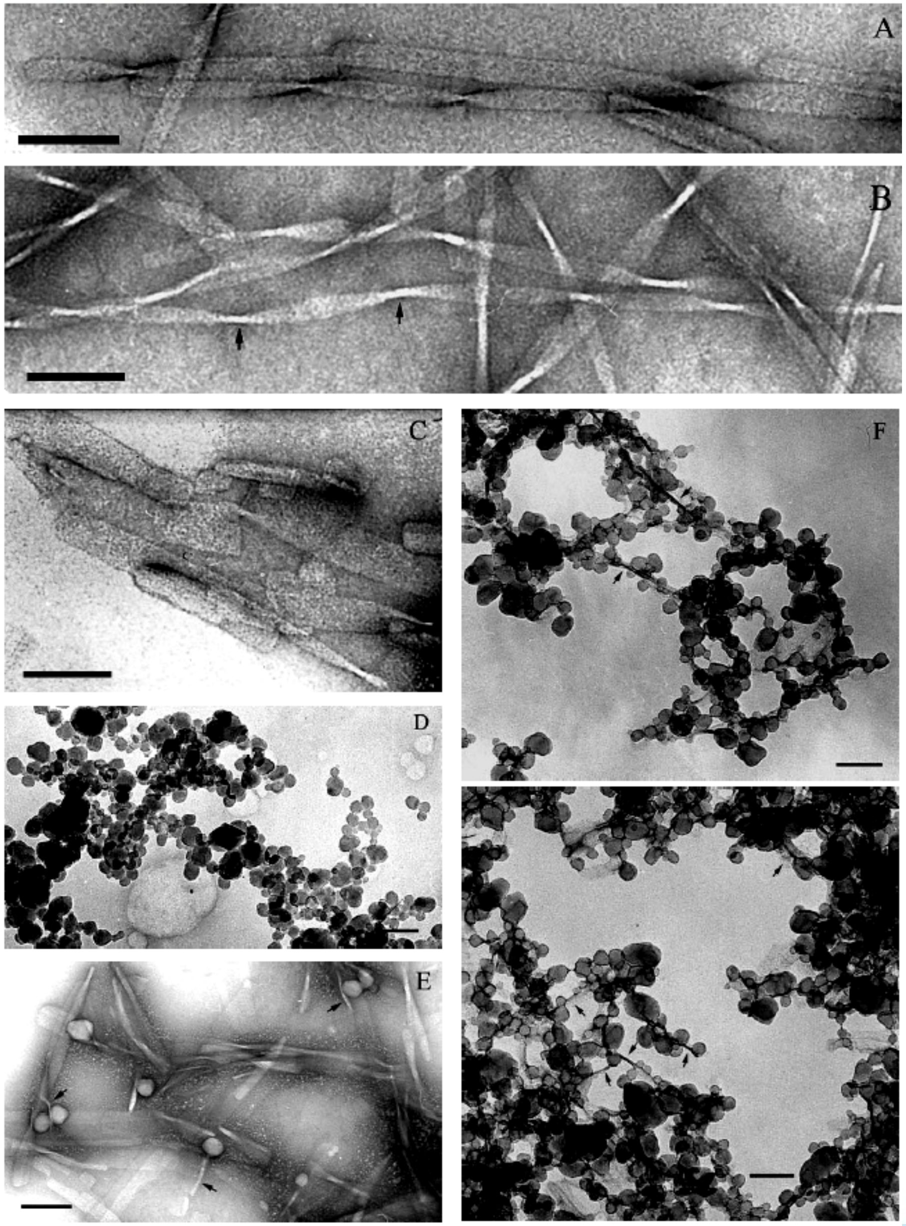
Curves of melting of thymus DNA: without (1) and in the presence (2) of hydrated C_{60} fullerene
The sample of DNA has preliminary been subjected to the process of denaturation in the saltless medium and the subsequent renaturation in the solution of 4 mM NaCl

Kruglova E., et al., Department of Biophysics, Institute of Radiophysics and Electronics NAS of Ukraine, Kharkov, 2001

HYDRATED FULLERENES (*HyFn*) PROTECT NATIVE STRUCTURES of HYDRATED BIOMOLECULES (*DNA, PROTEINS, ENZYMES…*) from DENATURING INFLUENCES and INCREASE the TEMPERATURE of their DENATURATION

(on 4-10^{o}C**).**

These Effects are Stipulated by
the Native Structure Stabilization of Biomolecules
due to Integration (Merging) of their Hydrated Layers
with the Similar Water Shells of HyFn.



ELECTRON MICROGRAPHS OF NEGATIVELY STAINED SAMPLES OF $A\beta_{25-35}$ PEPTIDE IN THE PRESENCE AND IN THE ABSENCE OF C_{60}HyFn.

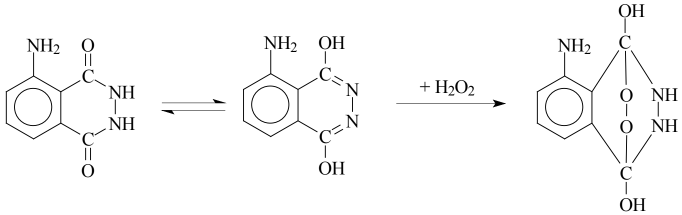
(**A, B**) Control helically twisted long ribbons and (**C**) sheet-like arrays of $A\beta_{25-35}$ peptide incubated for 24 hours at 37 C.
(**D**) Control spherical clusters of the C_{60} HyFn and their aggregates.
(**E**) Co-incubation of $A\beta_{25-35}$ peptide and the fullerene in molar ratio 1 : 0.17.
Shorter ribbons, sheet-like arrays and short narrow protofibrils (arrows) decorated with spherical clusters of the C_{60} HyFn are distinctly seen.
(**F , G**) Co-incubation of $A\beta_{25-35}$ peptide and the fullerene in molar ratio 1 : 2.5.
Short narrow protofibrils decorated with spherical aggregates of the C_{60} HyFn (arrows) become the major species in this case. Bar is 100 nm.

Ya. Podolski, Z. A. Podlubnaya, E. A. Kosenko, E. A. Mugantseva, E. G. Makarova, L. G. Marsagishvili, M. D. Shpagina, Yu. G. Kaminsky, G. V. Andrievsky, V. K. Klochkov. EFFECTS OF HYDRATED FORMS OF C_{60} FULLERENE ON AMYLOID β-PEPTIDE FIBRILLIZATION *IN VITRO* AND PERFORMANCE OF THE COGNITIVE TASK. J. of Nanoscience and Nanotechnology, 7, (4-5) (2007) 1479-1485

Hydrated fullerenes create in water medium the ordered, structurally heterogeneous environment in which chemical and biochemical processes go differently than in pure (unordered) water.

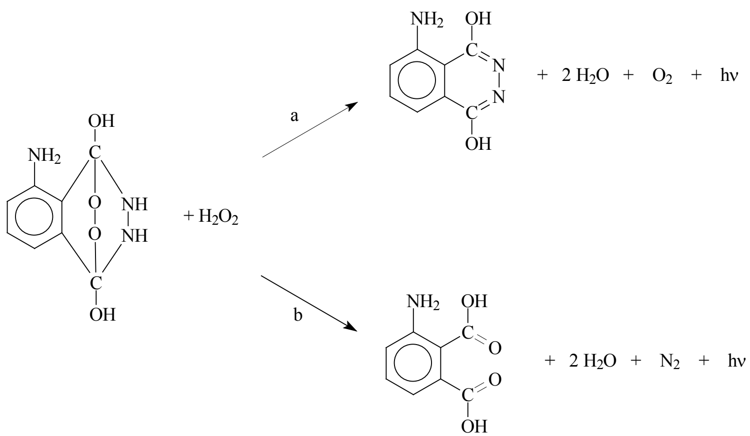
Гидратированные фуллерены создают в своем окружении упорядоченную, структурно гетерогенную водную среду, в которой направленность и кинетика химических и биохимических процессов отличается от таковых, происходящих в чистой (неупорядоченной) воде.

PROBABLE REACTIONS OF LUMINOL OXIDATION BY HYDROGEN PEROXIDE



LUMINOL **LUMINOL PEROXIDE**

Non-luminescent substance **Stromgly luminescent sudstance**



Weakly luminescent sudstance

[1] Drew H.D.K. Trans. Faraday Soc., 35 (1939) 207-215.

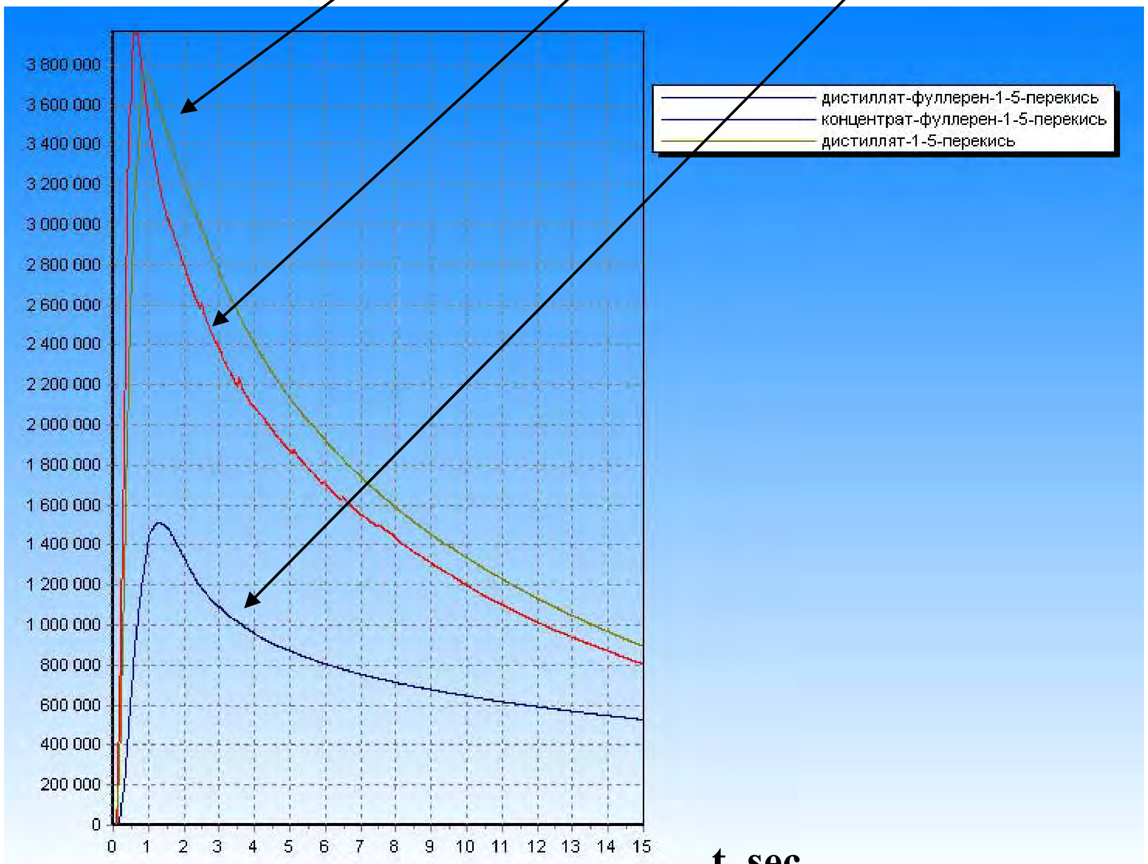
Kinetics of Luminol Reaction in Water Without and in the Presence of Hydrated C_{60} Fullerene

(luminometer “LIK", SRI “BINAR», Russia)

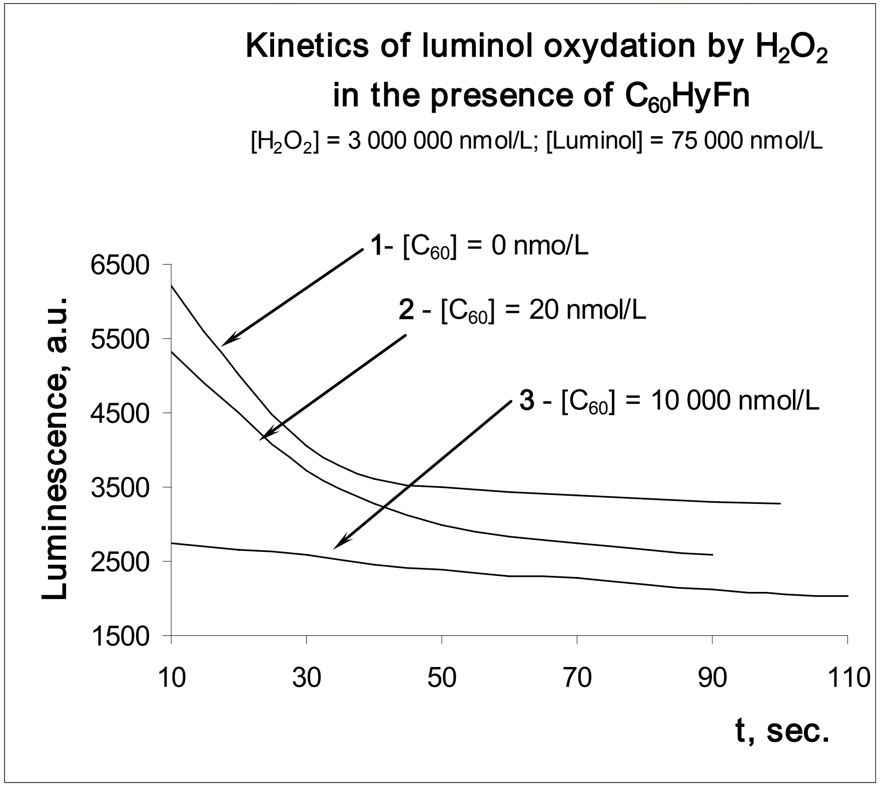
H_2O_2 3x10^{-3} М/л; Luminol 75x10^{-6} М/л

C_{60}HyFn concentration: *1* - 0 M; *2* - 5*10^{-9} M; *3* - 5*10^{-7} M.

Luminescence, a.u.



t, sec.



Curve №	C_{60} concentration, nmol/l	Molar ratio		% inhibition of reactions
		Luminol / C_{60}	H_2O_2 / C_{60}	
1	0	---	---	0%
2	20 (3)	3 700 (25 000)	150 000 (1 000 000)	15% in serum and in water
3	10 000 500	7,5 150	300 6000	44% in serum 61% in water

Free Radical Biology & Medicine 47 (2009) 786–793

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

ELSEVIER

Original Contribution

Peculiarities of the antioxidant and radioprotective effects of hydrated C_{60} fullerene nanostuctures *in vitro* and *in vivo*

Grigory V. Andrievsky [a,b], Vadim I. Bruskov [c], Artem A. Tykhomyrov [d,*], Sergey V. Gudkov [c]

[a] *Institute of Physiologically Active Compounds LLC, Kharkiv, Ukraine*
[b] *Institute of Scintillating Materials (ISMA), STC "Institute of Single Crystals," National Academy of Sciences, Kharkiv, Ukraine*
[c] *Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russian Federation*
[d] *Department of Biophysics and Biochemistry, National University of Dniepropetrovsk, Gagarin ave., 72, Dniepropetrovsk, Ukraine, 49010*

ARTICLE INFO

Article history:
Received 31 December 2008
Revised 30 April 2009
Accepted 12 June 2009
Available online 17 June 2009

Keywords:
Hydrated C60 fullerene
Hydroxyl radicals
Ionizing radiation
DNA damage
Antiradical activity
Antioxidant
Radioprotector
Free radicals

ABSTRACT

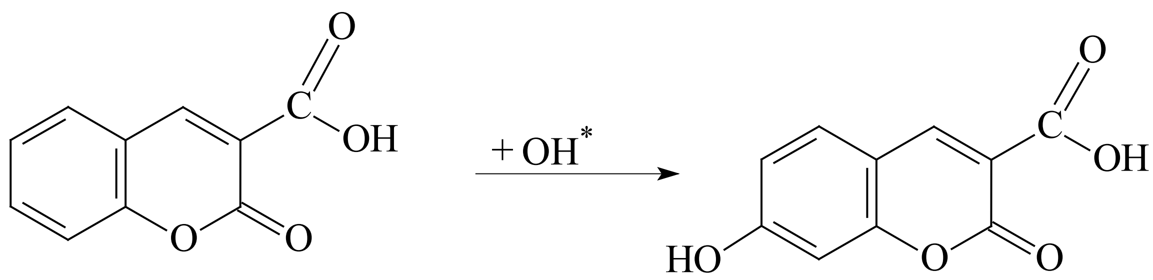
Aqueous solutions of highly stable supramolecular donor–acceptor complexes of chemically nonmodified pristine C_{60} fullerene molecules with H_2O molecules (hydrated C_{60} fullerene–C_{60}HyFn) and their labile nano-sized clusters were examined for their antioxidant effects on removal of hydroxyl radicals (·OH) and protecting DNA against oxidative damage induced by ionizing radiation *in vitro*. The suppressing influence of C_{60}HyFn on the formation of OH-radicals in water exposed to X-rays at doses of 1–7 Gy was assessed by determination of oxidation levels of coumarin-3-carboxylic acid. C_{60}HyFn demonstrates apparent antiradical activity *in vitro* in the range of concentrations of 10^{-11}–10^{-6} M. Paradoxically, the ·OH-removing efficacy of C_{60}HyFn was in reverse correlation with fullerene concentration. It was hypothesized that the antiradical action of C_{60}HyFn in water medium generally is due to a "nonstoichiometric" mechanism, supposedly to a hydrated free radical recombination (self-neutralization), which is catalyzed by specific water structures ordered by C_{60}HyFn. With the use of 8-oxoguanine as a marker of oxidative damage to DNA, it has been demonstrated that C_{60}HyFn in concentrations of 10^{-7}–10^{-6} M protects nucleic acids against radical-induced damage. The second part of the present study was aimed to evaluate the overall radioprotective efficacy of C_{60}HyFn in doses of 0.1 or 1 mg/kg b.w. injected intraperitoneally to mice either 1 h before or 15 min after lethal dose exposure of the X-ray (7 Gy) irradiation. Survival rate of the mice was observed at 30 day intervals after irradiation, while the weight gains of experimental animals were monitored as well. The most significant protective effect was demonstrated when 1 mg/kg dosage of C_{60}HyFn was administered before irradiation. The outcome of the substance testing is 15% survival rate of irradiated animals at 30 days of observation, and prevention of noticeable weight loss characteristic for radiation impact, versus unprotected control animals. In conclusion, results of the study obviate that the apparent protective action of C_{60}HyFn *in vivo* is determined by its considerable ability to decrease X-ray-generated reactive oxygen species. Based on the results and that neat C_{60} is nontoxic, actually in the hydrated form, without side effects and with sufficient radioprotective effects in low doses, C_{60}HyFn may be considered as a novel antioxidant agent, which substantially diminishes the harmful effects of ionizing radiation.

WATER RADIOLYSIS and CCA OXIDATION by MEANS of HYDROXYL RADICALS (OH^*)

$H_2O \xrightarrow{h\nu\ (\gamma)} H_2O^*$

$H_2O^* \rightarrow$ **OH^*** (~ 4.8 - 4.9 %)

$H_2O^* \rightarrow$ **$H^* + e^-_{hydr}$ + $H_2 + H_2O_2 + H_3O^+$ + ROS** (Reactive Oxygen Species) (~ 95 %)

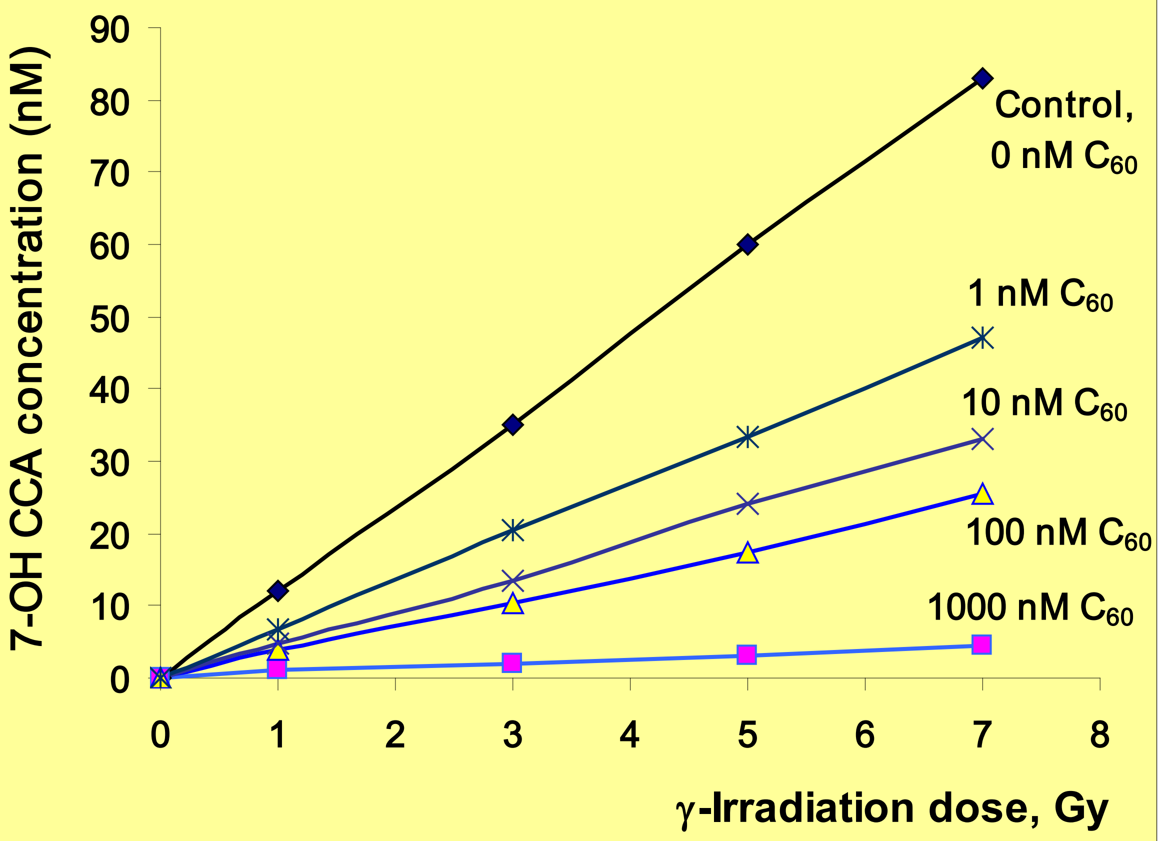


CCA
(coumarin-3-carboxylic acid)

7-OH CCA

Strongly luminescent substance

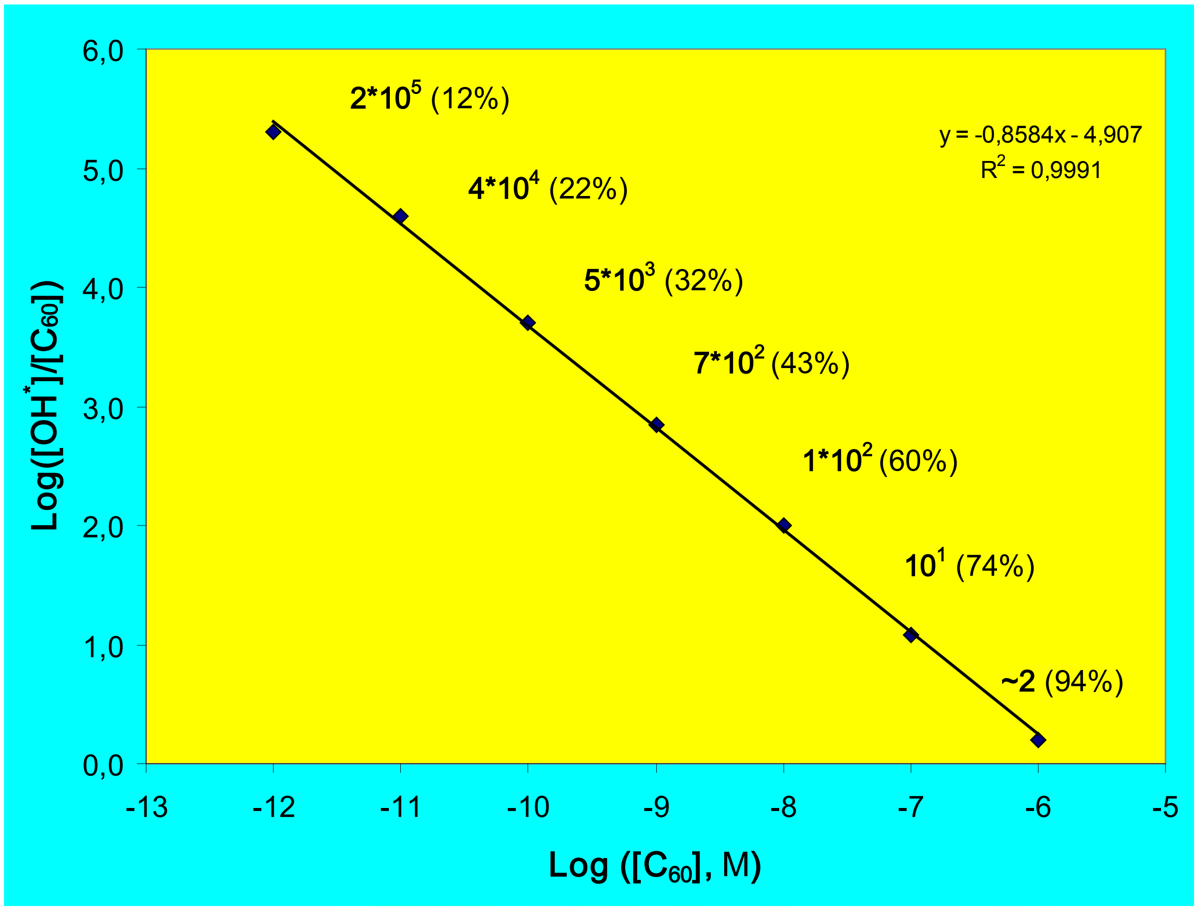
Neutralization of Hydroxyl-Radicals (OH^*) Formed by Means of γ-Rays Radiolysis of Water at Presence of Various C_{60}HyFn Concentrations



C_{60}HyFn Antiradical Efficiency (*[OH]*/[C_{60}]*) Increases When its Concentration are Decreased

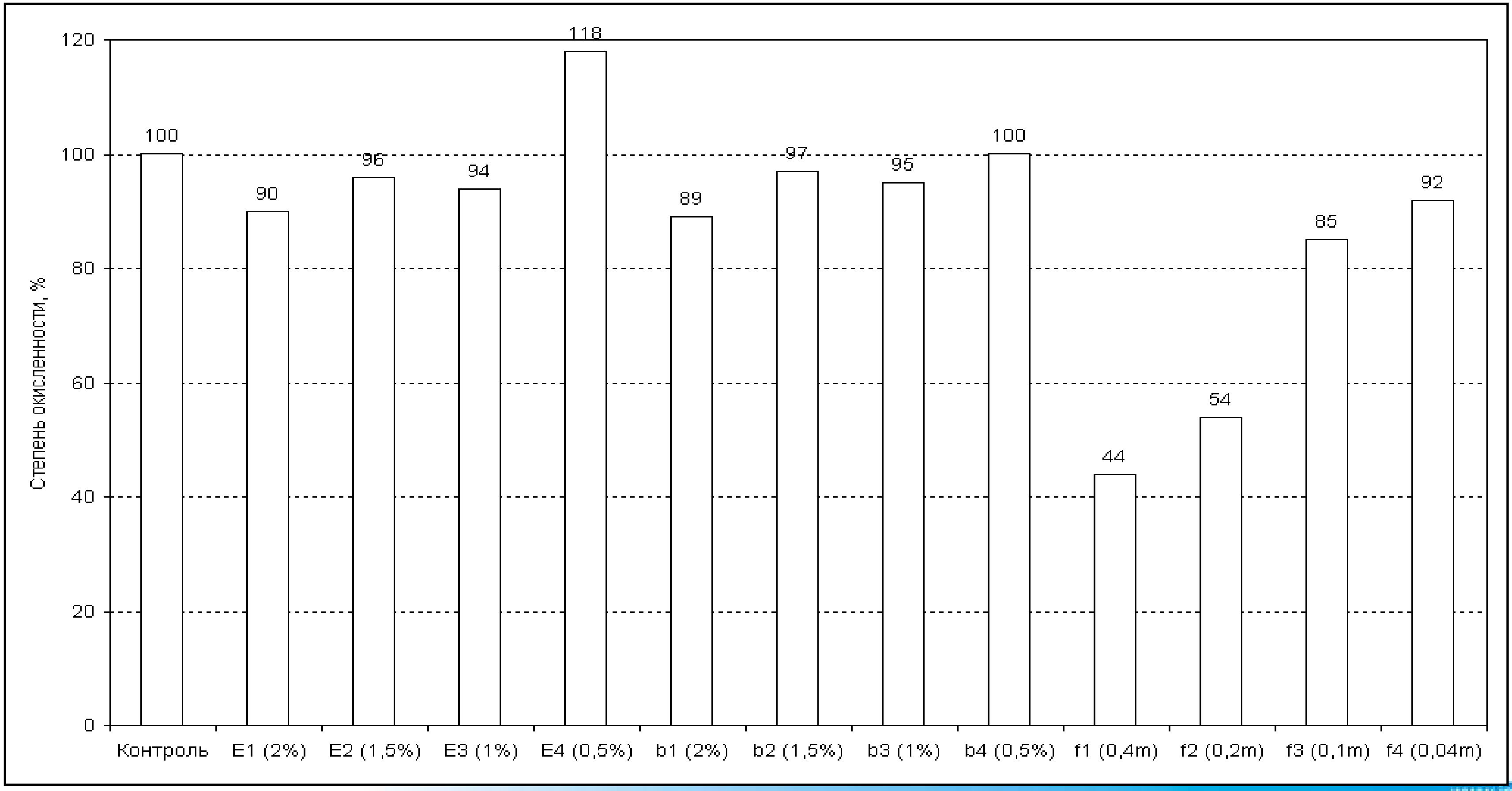
Absolute values of [OH*]/[C_{60}] ratio and % of OH* neutralized are indicated near dots of graph

(by estimation of the 7-OH-CCA formation at 7 Gy Dose of γ-irradiation)



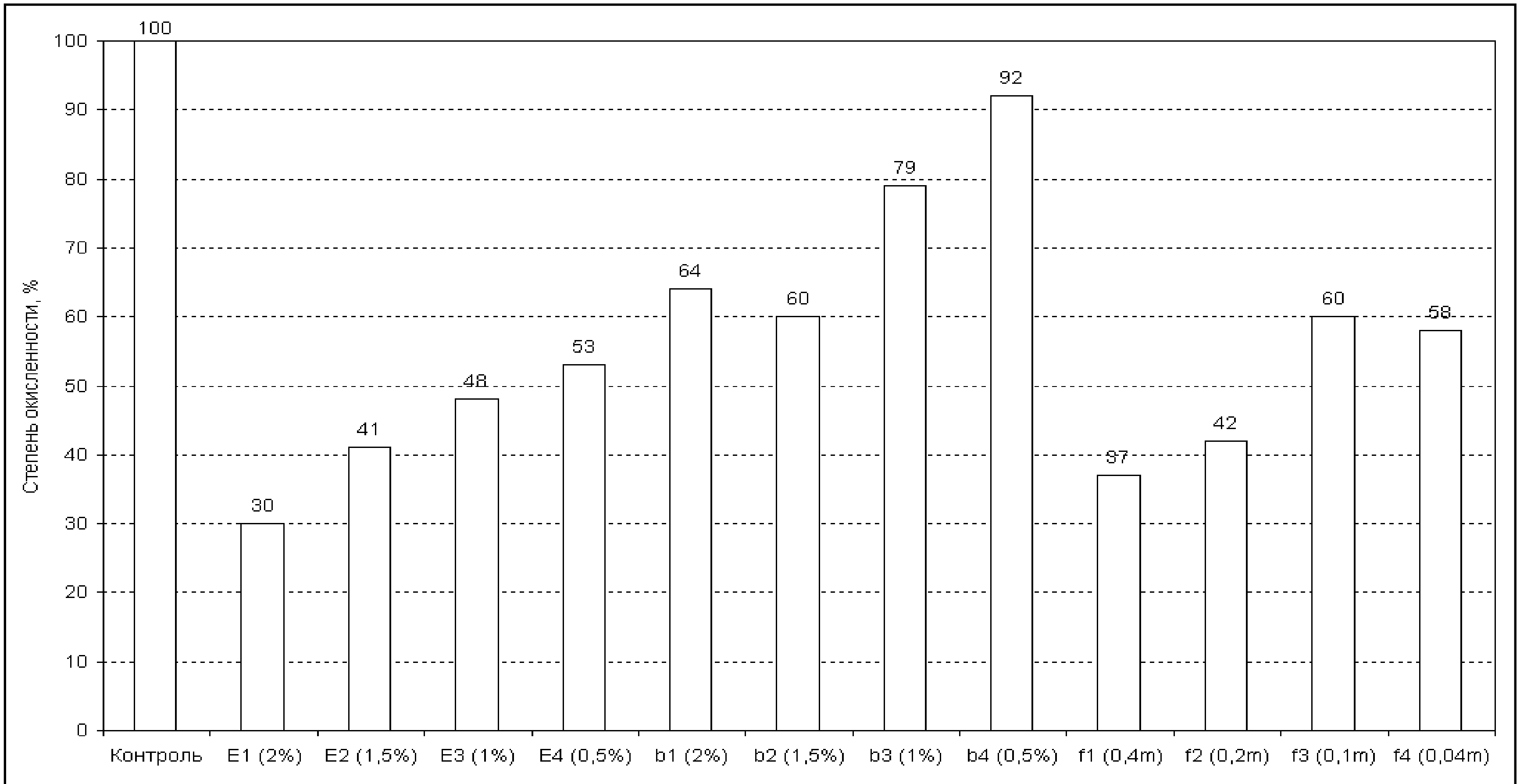
Сравнительная характеристика (по уровню MDA) различных антиоксидантов (витамин E, β-каротин, C_{60}HyFn) после γ-облучении (3 ч.) фосфолипидных дисперсий

(Важно, что, в одинаковых опытах, дозы C_{60} были в 1000-10 0000 раз меньшими, чем витамина E и β-каротина!!!)



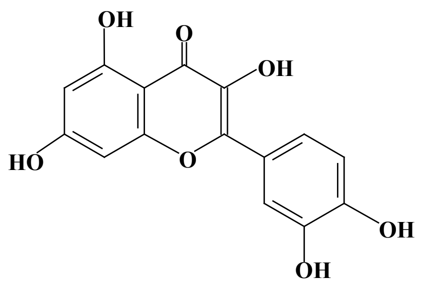
Сравнительная характеристика (по уровню MDA) различных антиоксидантов (витамин E, β-каротин, C_{60}HyFn) после обработки ультразвуком (10 мин, 25 кГц) фосфолипидных дисперсий

(Важно, что, в одинаковых опытах, дозы C_{60} были в 1000-10 0000 раз меньшими, чем витамина E и β-каротина!!!)



C_{60}HyFn Change Essentially the Kinetics and Accelerate (on ~50 %) the Auto-Oxidation of the Quercetin

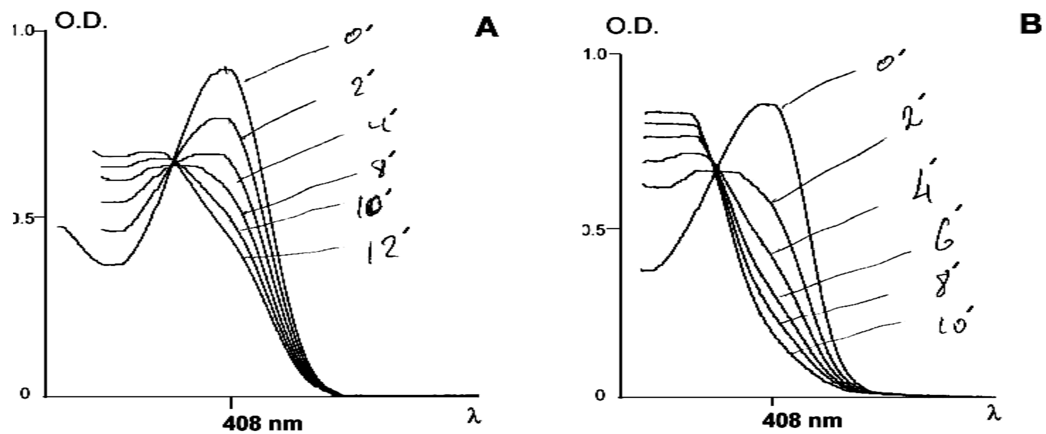
A - control in water (C_{Que} = 30 μM/l, pH=10);
B - at presence of C_{60}HyFn (Quercetin/C_{60} = 10)



QUERCETIN

- Free Radical

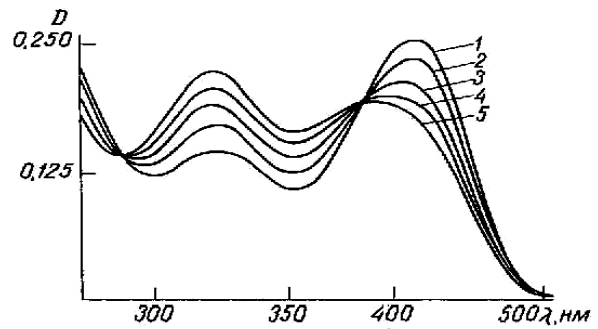
Initiator



Typical dynamics of adsorption spectra changes of Quercetin auto-oxidation in aqueous medium at pH=10

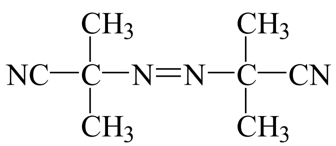
Динамика спектральных изменений, отражающая процесс аутоокисления кверцетина при pH 10,0 в присутствии ТМЭДА (0,8 мМ) и ЭДТА (0,08 мМ)

1 — спектр поглощения неокисленного кверцетина ($1{,}4 \cdot 10^{-5}$ М); *2*—*5* — спектры поглощения кверцетина после 5, 10, 15 и 20 мин окисления соответственно.



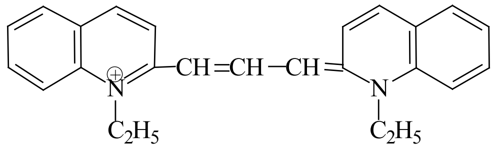
Исследование “окисляемости” жиросодержащих образцов косметических кремов

В эксперименте брали 3 г мазевой основы крема, содержащей ненасыщенные жирные кислоты, разбавленной ксилолом до общего объем образца = 5 мл. После добавления, средняя концентрация инициатора свободнорадикального окисления АИБН (2,2'-Azobisisobutyronitrile - (**AIBN**) - Free Radical Initiator) в испытуемом образце составляла около 10^{-2} моль/литр. Реакцию ингибирования свободнорадикального окисления наблюдали при 90 °C. Изначально, в образцах №3 и №2, мазевая основа содержала до 20 объ.% нашего антиоксидантного агента - "OW" с концентрацией ингибиторов, гидратированных фуллеренов (ГФ), $3*10^{-9}$ и $30*10^{-9}$ моль/литр, соответственно. Т.о. доля OW в испытываемых образцах составляла около 0,1, а молярное соотношение АИБН / ГФ для образца №3 составляло 10^{8} и для образца №2 – 10^{7}!!!

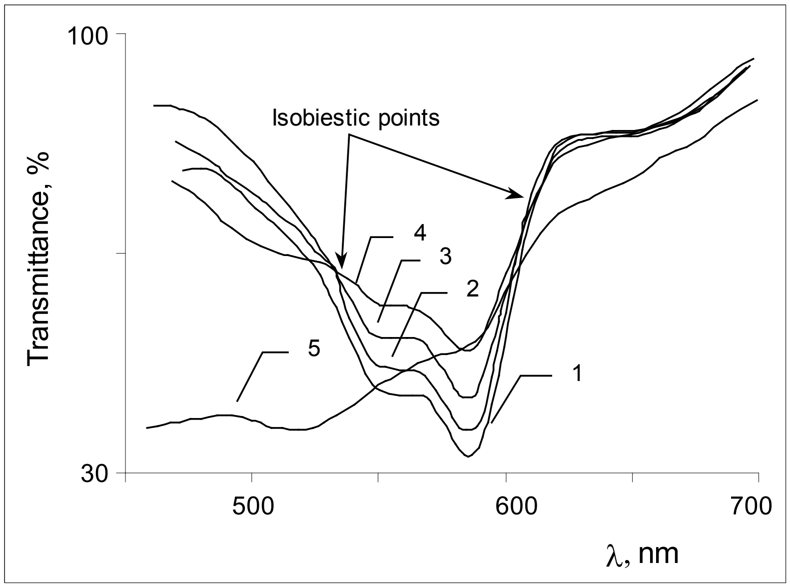


Обра-зец	Конц. [ГФ], моль/литр	[АИБН] / [ГФ]	Параметр окисляемости, α_i	% ингибирования $(\alpha_1 - \alpha_i) / \alpha_1 * 100\%$
№1	0	------	9,19	0%
№3	10^{-10}	10^{8}	7,79	15%
№2	10^{-9}	10^{7}	4,6	50%

UV-VIS SPECTRA OF **PINACYANOL**



AT VARIOUS CONCENTRATIONS OF **HYDRATED C_{60} FULLERENE** (HyFn)



1. **Pinacyanol** (2,7 μM/L) in H_2O.
2. **Pinacyanol** (2,7 μM/L) in FWS; **[C_{60}]** = 0,01 μM/L
3. **Pinacyanol** (2,7 μM/L) in FWS; **[C_{60}]** = 0,70 μM/L
4. **Pinacyanol** (2,7 μM/L) in FWS; **[C_{60}]** = 2,00 μM/L
5. **Pinacyanol** (2,7 μM/L) in FWS; **[C_{60}]** = 20,00 μM/L

In the presence of C_{60}HyFn the cationic dyes form stacking structures = H-aggregates

1. N.O. Mchedlov-Petrossyan, et al., INTERACTION BETWEEN CATIONIC DYES AND COLLOIDAL PARTICLES OF C_{60} HYDROSOL. Mendeleev Commun., 2 (1999) 63.
2. N.O. Mchedlov-Petrossyan, et al.,. INTERACTION OF POLYMETHINE DYES WITH FULLERENE C_{60} HYDROSOL. Sci. Appl. Photo., 43 (1) (2001) 1-13.
3. N.O. Mchedlov-Petrossyan, et al., INTERACTION BETWEEN COLLOIDAL PARTICLES OF C_{60} HYDROSOL AND CATIONIC DYES. Chem. Phys. Lett., 341, N 3-4 (2001) 237.

Fullerenes, Nanotubes, and Carbon Nanostructures, 18: 303–311, 2010

ISSN: 1536-383X print / 1536-4046 online
DOI: 10.1080/15363831003785257

Taylor & Francis
Taylor & Francis Group

The Acceleration of Blood Plasma Clot Lysis in the Presence of Hydrated C_{60} Fullerene Nanostructures in Super-Small Concentration

G. ANDRIEVSKY[1,2], D. SHAKHNIN[3], A. TRONZA[4], D. ZHERNOSEKOV[4] AND A. TYKHOMYROV[5]

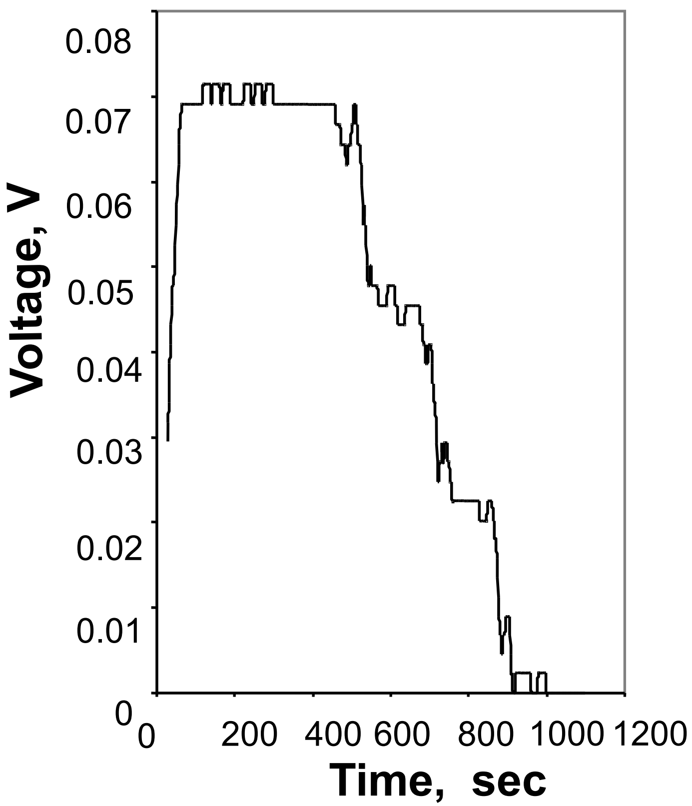
[1]Institute of Physiological Active Compounds LLC, Kharkiv, Ukraine
[2]ISMA NAS of Ukraine, STC "Institute for Single Crystals," Kharkiv, Ukraine
[3]Public International University of Human Development, Kyiv, Ukraine
[4]Palladin Institute of Biochemistry, NAS of Ukraine Kyiv, Ukraine
[5]Dniepropetrovsk State Agrarian University, Dniepropetrovsk, Ukraine

There are no systematic data about the influence of chemically nonmodified fullerenes on key enzymes of organism, in particular, proteases of haemostasis system. In this study we describe preliminary in vitro *data indicating the essential acceleration of blood plasma clot cleavage in the presence of nanostructures of hydrated C_{60} fullerene ($C_{60}HyFn$). The super-small concentration of $C_{60}HyFn$ (10^{-12}-10^{-14} M) applied in the experiment was comparable to that in which hydrated fullerene exhibits marked antioxidant and neuroprotective effects* in vivo. *The influence of $C_{60}HyFn$ on the kinetics of clot lysis, induced by tissue plasminogen activator (t-PA), has been explored* in vitro. *The incubation of t-PA samples in $C_{60}HyFn$ solution has increased the lysis rate 2.7 times and diminished the half-lysis time 1.6 times, compared with corresponding parameters for t-PA dissolved in distilled water. Taking in consideration that in this case C_{60} concentration (in mol) was by 2–4 orders less than concentration of enzymes participating in fibrinolysis, it has been concluded that biological activity of super-small $C_{60}HyFn$ concentrations (doses) was not directly related to the biological properties of C_{60} fullerene molecule itself but was mediated by specific and ordered water structures which C_{60} molecule organizes round itself. Also, we have suggested that the activation of fibrinolysis may occur due to the stabilization/protection of active conformation of protease molecules in the presence of specific water structures ordered by $C_{60}HyFn$. Applications of hydrated fullerenes can be a perspective approach for both usage of thrombolytic enzymes and for design of novel anticoagulants.*

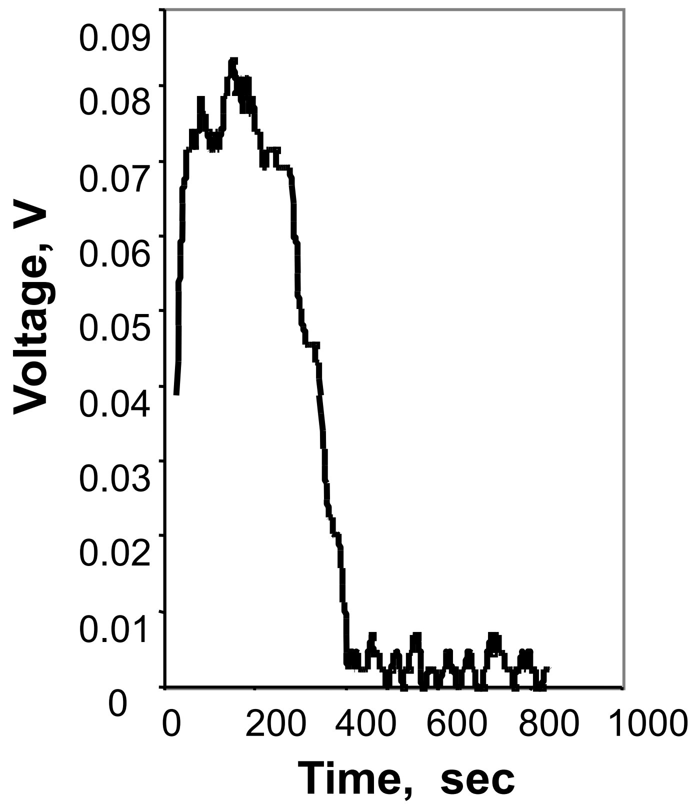
Keywords Blood clots lysis, fibrinolysis, hydrated C_{60} fullerenes, ordered water structures, tissue plasminogen activator

ACCELERATION OF FIBRIN CLOT LYSIS IN THE PRESENCE OF SMALL CONCENTRATIONS (*10^{-14}-10^{-12} Mol/L*) OF C_{60}HyFn - HYDRATED C_{60} FULLERENE NANOSTRUCTURES

*Note that concentration of fibrinolysis enzymes was in 10^6(!!!) time **MORE** then conc. C_{60}*



Control (in water)



In the presence of C_{60}HyFn

Some Biological and Bio-Medical Aspects of Multidimensional Activity of Hydrated C_{60} Fullerene

======================

Additional information about it see on our site:

http://www.ipacom.com/index.php/en/publications-about-c60hyfn/72

http://www.ipacom.com/index.php/en/publications-about-c60hyfn/70

http://www.ipacom.com/index.php/en/fullerenes-and-water-left/78

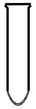
http://www.ipacom.com/index.php/en/production

http://www.ipacom.com/index.php/en/production-left/68

http://www.ipacom.com/images/Articles/results_trials_en.pdf

http://www.ipacom.com/images/Articles/annotation_en.pdf

BASIC BIOLOGICAL PROPERTIES OF AQUEOUS SOLUTION OF THE HYDRATED C_{60} FULLERENE (HyFn) DETECTED TO THE PRESENT DAY (*07/15/04*).



In Vitro **Experiments reviled that HyFn with C_{60} concentrations up to 100 μM/l:**

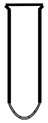
1. Are neither CYTITOXIC nor HEPATOTOXIC;
2. Do not INHIBIT CELLS' RESPIRATION;
3. STABILIZE CELL'S MEMBRANES;
4. Don't SUPPRESS MEMBRANE BONDED ATP-ase ACTIVITY;
5. INCREASE CATALYTIC ACTIVITY of ENZYMES (e.g., Serine Proteinases);
6. Don't SUPPRESS, but PROMOTE the GROWTH of MICRO-ORGANISMS (MICROBES, FUNGI ...) and IMPROVE the SURVIVAL RATE and VIABILITY of CELLS (e.g., SPERMATOZOA);
7. Do not AFFECT BLOOD COAGULATION;
8. POSSESS SIGNIFICANT ANTIOXIDANT PROPERTIES;

In Vivo **Experiments reviled that HyFn with C_{60} Total Doses up to 25 mg/kg of b.w.:**

1. Are NONTOXIC;
2. Are NOT IMMUNOGENIC;
3. INCREASE RESISTANCE of PLASMA MEMBRANES to DAMAGING FACTORS;
4. POSITIVELY INFLUENCE ACTIVITY of ADRENERGIC, GABA-, HYSTAMINIC- and, especially, SEROTONINERGIC SYSTEMS and, as a consequence, ENHANCE ADAPTOGENIC FUNCTIONS of ORGANISMS;
5. ACTIVATE ANTIOXIDANT and ENERGETIC SYSTEMS;
6. POSSESS ANTIHISTAMINIC and ALLERGY SUPPRESSING ABILITIES, i.e. ACT as ANTI-INFLAMMATORY AGENTS,
7. SHOW POSITIVE NEURO PROTECTING and NON-SPECIFIC ANALGESIC EFFECTS;
8. DEMONSTRATE ANTI-MALIGNANT, RADIOPROTECTIVE, ANTI-ATHEROSCLEROTIC (ANTI-ATHEROGENIC) PROPERTIES;
9. PROTECT AGAINST VIRAL INFECTION (e.g., INFLUENZA, etc..): ***(HyFn do not KILL VIRUSES and MICROBES, but do not ALLOW THEM to INCORPORATE into ORGANISM's CELLS!!!).***

ОСНОВНЫЕ БИОЛОГИЧЕСКИЕ СВОЙСТВА ВОДНОГО РАСТВОРА ГИДРАТИРОВАННОГО C_{60} ФУЛЛЕРЕНА (HyFn), ОБНАРУЖЕННЫЕ К НАСТОЯЩЕМУ ВРЕМЕНИ

(ноябрь 2006).



***In Vitro* эксперименты выявили, что HyFn, в концентрациях C_{60} вплоть до 100 μM:**

1. **Не цитотоксичны, в т.ч. не гепатотоксичны;**
2. **Не подавляют, а наоборот способствуют росту изолированных культур клеток (микробы, грибы и пр.); способствуют выживаемости и повышению жизнеспособности сперматозоидов в процессе «криоконсервирования – оттаивания»;**
3. **Не ингибируют клеточного дыхания;**
4. **Не подавляют каталитическую активность мембраносвязанных АТФ-аз;**
5. **Обладают антиоксидантными свойствами более выраженными, чем у ионола, витамина Е и β-каротина;**
6. **Стабилизируют клеточные мембраны и увеличивают их устойчивость в неблагоприятных условиях;**
7. **Не влияют на системы свертывания крови;**
8. **Повышают на 5-10°С температуру денатурации биомолекул (ДНК, альбумина, коллагена и т.п.);**
9. **Повышают каталитическую активность изолированных ферментов (напр., сериновых протеаз).**

In Vivo эксперименты выявили, что HyFn в широком диапазоне суммарных доз C_{60} (от супермалых и вплоть до 25 мг/кг веса тела):

1. **Не токсичны, не иммуногенны, не аллергенны;**
2. **Повышают устойчивость плазматических мембран к повреждающим факторам;**
3. **Оказывают положительное влияние на антиоксидантные и энергетические системы;**
4. **Обладают радиопротекторными свойствами за счет подавления избыточного уровня свободных радикалов;**
5. **Обладают сильным и долговременным антигистаминным и противоалергическим действием, т.е. способны работать, как противовоспалительные агенты;**
6. **Оказывают положительное влияние на активность адрено-, ГАМК-, гистамино- и, особенно, серотонин-эргических систем и, как следствие, повышают адаптогенные функции организма;**
7. **Обладают мощной гепатопротекторной активностью;**
8. **Оказывают выраженное нейропротекторное (в т.ч., при болезни Альцгеймера) и неспецифическое анальгетическое действие;**
9. **Не убивая раковые клетки, тормозят развитие опухолевых патологий;**
10. **Обладают антиатеросклеротическими (антиатерогенными) свойствами;**
11. **Способны защищать организмы от инфицирования вирусами (напр., вирусами гриппа) _(HyFn не убивают вирусы, но не позволяют им эффективно проникать внутрь клетки)_;**
12. **Улучшают и повышают репродуктивные (детородные) функции.**

The Accelerated Development of Wheat Seeds that Germinated During 6 Days on Diatomite Hydroponics after Once-Through Watering Them with C_{60}FWS – Aqueous Solution of Hydrated Fullerene (C_{60}HyFn) with C_{60} Concentration of 10^{-9} mol/L (~1 ng/ml) -- (A). In positive control (B) - Watering with Distilled Water. The Weight of A Germs is More than of B Germs by 60%.

Ускоренное развитие семян пшеницы, проросших в течение 6-ти дней на диатомитовой гидропонике после однократной поливки их C_{60}FWS – водным раствором гидратированного фуллерена (C_{60}HyFn) - с концентрацией C_{60} 10^{-9} Моль/л (~1 нг/мл) -- (А). В контрольном образце (В): – аналогичная поливка дистиллированной водой. Вес проростков А на 60% больше, чем у проростков В .

Ускоренный рост пшеницы после однократного полива C_{60}FWS (30 нМоль/л) (справа); контроль – на дистиллир. воде (слева)

Помидоры сорта «Волове серце», проращиваемые 5 дней на дист. воде (слева) и C_{60}FWS (30 нМоль/л) (справа)

FOOD INTAKE with SUPER SMALL DOSES of HYDRATED C_{60} FULLERENE (C_{60}HyFn) ACCELERATES ESSENTIALLY the INCREASE in WEIGHT of BROILER CHICKENS

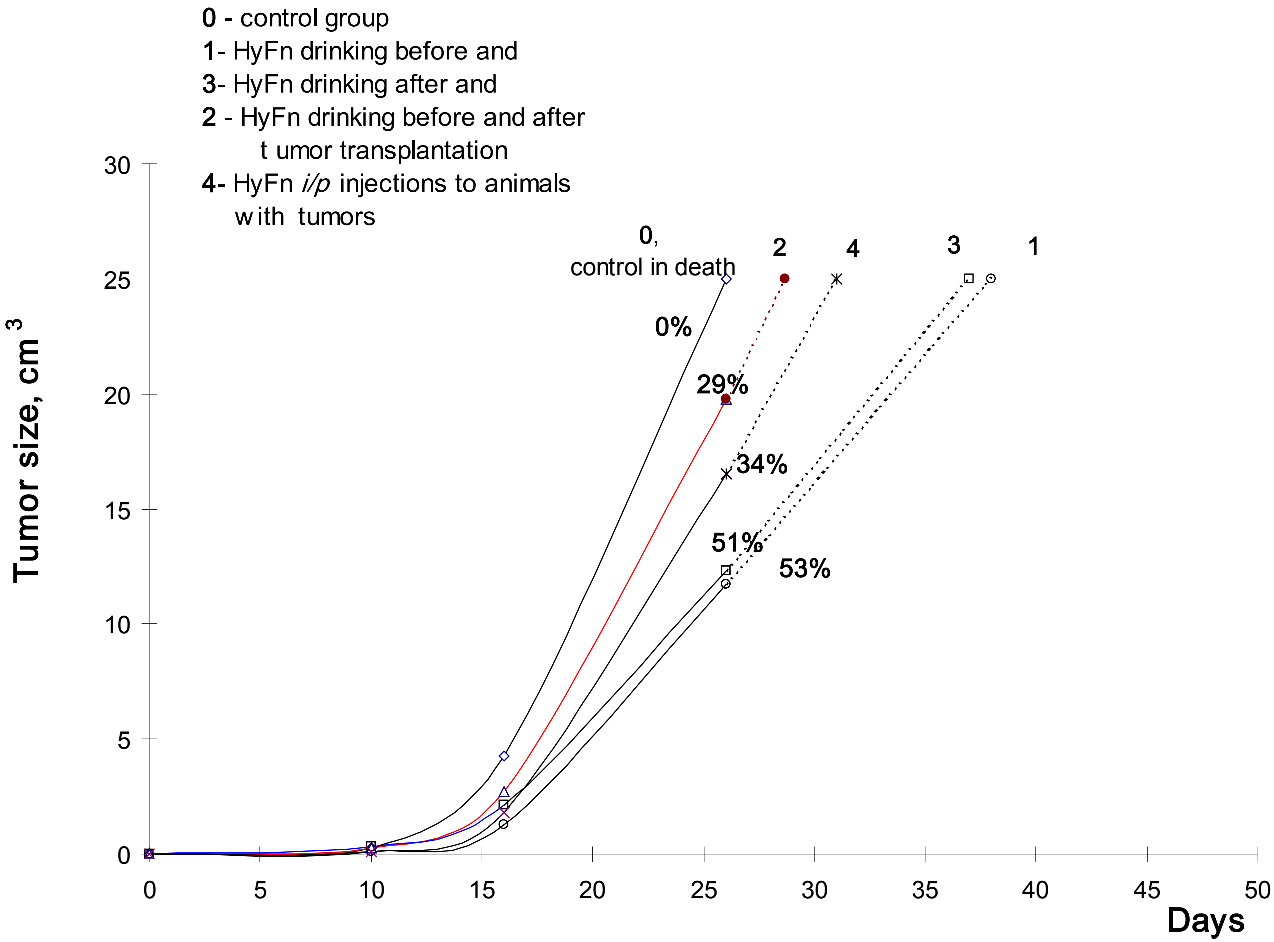
Group (n=10)	**Daily Drinking and Feeding by Birdseed**	**Mass of Chickens** ("Ross-308"), g	
		At the beginning of exp.	**After 36 days of exp. (% of overweight)**
1	**Water** (Control)	**53**	**1267** ±103
2	Water Solution of **Colloidal Composite of Fe, Cu, Mg and Ag Nanoparticles** (10^{-3} g/L)	**54**	**1263** ±165 (**+0%** as compared with group 1)
3	Birdseed with **Ultra Dispersed Fe_2O_3 Particles** (10^{-3} g/kg) and **Ascorbic Acid** (0.08 g/kg)	**54**	**1368** ±199 (**+8%** as compared with group 1 and 2)
4	**C_{60}HyFn** Water Solution with 10^{-9} M = 10^{-6} g/L of **C_{60}**	**55**	**1558**±141 (**+23% !!!** as compared with group 1 and 2)

Tumors Sizes (mm^3) and Tumor Growth Suppression (+%) in Mice (n = 10, C_{57}BL females) with Transplantable Ovarian Tumor During the Preventive Intake of Micro-Dozes of Hydrated C_{60} Fullerene (HyFn) and at the Treatment by Means of HyFn of the Experimental Cancer Pathology

	Groups of mice	Beginning of tumor trasplantation	After of 10 days		After of 16 days		After of 26 days	
0.	The control with transplanted tumours	0	266	**0%**	4267	**0%**	24970	**0%**
1.	HyFn drinking (12 days) befor trasplantation *Total dose of C_{60} = 1,8 μg (0,09 mg/kg)*	0 1,8 μg (0,036 mg/kg)	88 ----	**67%** ---	1283 ---	**70%** ---	11720 ---	**53%** ---
2.	HyFn drinking befor and after trasplantation *Total dose of C_{60} = 5,6 μg (0,28 mg/kg)*	0 1,8 μg (0,036 mg/kg)	273 +1,5 μg	**-3%**	2719 + 0,8 μg	**36%**	19753 + 1,5 μg	**21%**
3.	HyFn drinking after trasplantation *Total dose of C_{60} = 3,8 μg (0,19 mg/kg)*	0 --- ---	313 +1,5 μg	**-18%**	2118 + 0,8 μg	**50%**	12314 +1,5 μg	**51%**
4.	I/p injection of HyFn after trasplantation *Total dose of C_{60} = 16 μg (0,8 mg/kg)*	0 --- ---	118 + 6 μg	**56%**	1798 +6 μg	**58%**	16513 + 4 μg	**34%**

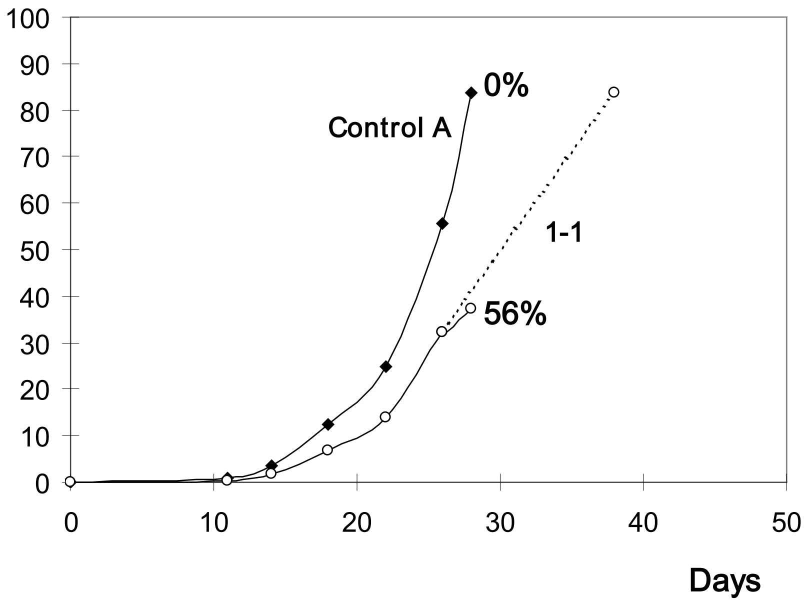
[C_{60}] = ~42 nM/L = 0,03 μg/ml

Tumors sizes (cm^3) and tumor growth suppresion (+%) in mice (n = 10, C_{57}BL females) with transplantable ovarian tumor during the preventive intake of micro-dozes of hydrated C_{60} fullerene (HyFn) and at the canaer treatment by means of HyFn.

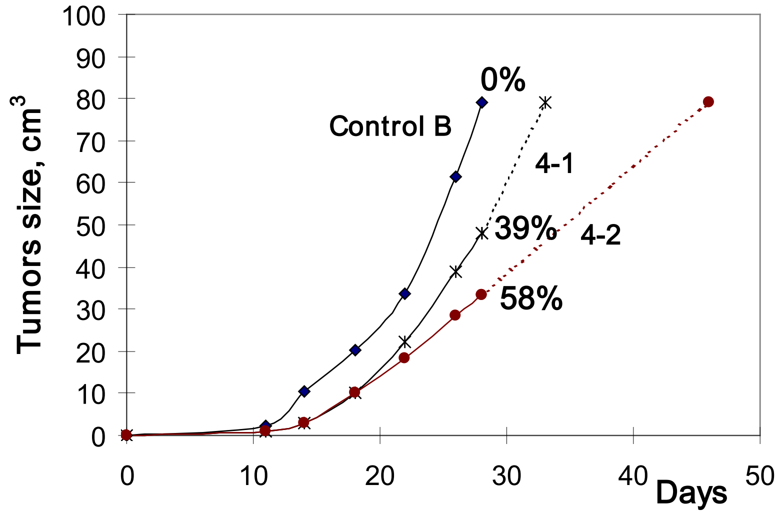


Sizes of Tumors (cm^3) and Suppression of Tumor Growth (%) in Rats (N = 10, ACI Males) with Transplantable Prostate Cancer During the Preventive Intake of Micro-Dozes of Hydrated C_{60} Fullerene (HyFn) and at the Treatment of the Experimental Cancer Pathology by Means of HyFn Injections

Suppression of tumor growth after drinking of HyFn (8 days before tumor transplantation). Total dose of C_{60} = 6μg (0.03 mg/kg)



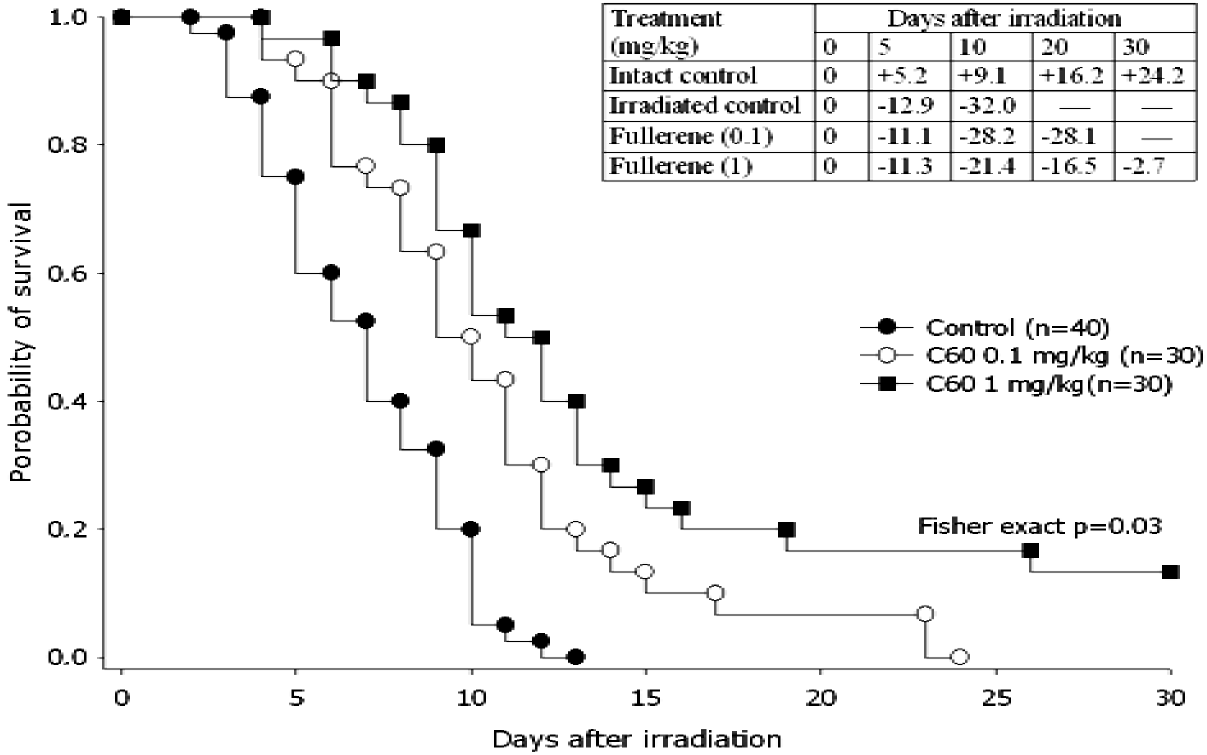
Suppression of tumor growth during the treatment of animals by i/v and i/p injections of HyFn; total dose of C_{60} = 40 μg (0.2 mg/kg) and 160 μg (0.8 mg/kg), correspondingly.



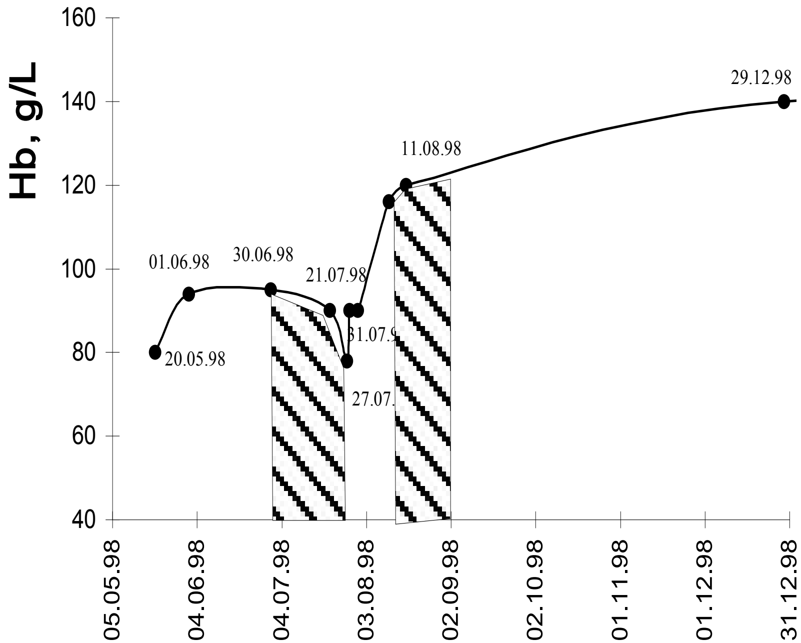
INFLUENCE OF C_{60}HyFn PRETREATMENT ON THE SURVIVAL RATE AND BODY MASS GAIN OF MICE IRRADIATED WITH A DOSE OF 7 GY.

(Effective radioprotective dose of C_{60}HyFn is less in 1-2 order than for C_{60} fullerenols and amifostine [Coll.Surf. B: Biointerface, 58, 2007, 39-43])

Treatment (mg/kg)	Days after irradiation				
	0	5	10	20	30
Intact control	0	+5.2	+9.1	+16.2	+24.2
Irradiated control	0	-12.9	-32.0	—	—
Fullerene (0.1)	0	-11.1	-28.2	-28.1	—
Fullerene (1)	0	-11.3	-21.4	-16.5	-2.7



Restoration of Blood Hemoglobin (Hb) Level After Laparoscopy (20.05.98**) and Course (**30.06-27.07.98 and 11.08-02.09.98**) of γ-Therapy (**61,4 Gy**) in the Patient with Rectal Adenocarcinoma ($T_3N_xM_1P_3$) under Treatment by Super Small Doses of Hydrated C_{60} Fullerenes (HyFn).**



Восстановление уровня гемоглобина крови (Hb) после лапароскопии (20.05.98**) и курса (**30.06-27.07.98 и 11.08-02.09.98**) γ-терапии (**61,4.Грэй**) у пациента с ректальной аденокарциномой ($T_3N_xM_1P_3$) при лечении сверхмалыми дозами гидратированных C_{60} фуллеренов (HyFn).**

HYDRATED C_{60} FULLERENES PREVENT PROCESS of EXPERIMENTAL ATHEROSCLEROSIS DEVELOPMENT and FACILITATE the ATHEROSCLEROTIC LESIONS REVERSION.

Gistological and histochemical methods were used for investigations of hydrated C_{60} fullerenes (HyFn) aqueous solutions on the process of experimental atherosclerosis development in animals.

During 5 months, four groups of animals (40 males of "Shinshilla" rabbits) were investigated, namely:

(A) **intact ones;**

(B) **rabbits, which received oral cholesterol load (200 mg/kg/day) during 5 months in accordance with the scheme:**
5 days of cholesterol diet, 2 days of rest;

(C) **rabbits, which received HyFn only (6 i/v injections for 3 months; Total dose of C_{60} = 0.6 mg/kg of b.w.);**

(D) **rabbits, which received oral cholesterol load during 5 months, and from the beginning of the fourth month of experiment, have been treated with HyFn (4 i/v injections for 2 months; Total dose of C_{60} = 0.4 mg/kg of b.w.).**

CONCLUSIONS (with photo on the next slide**)**

***1*.- On the Fourth Month of Cholesterol Diet (*Group B*), in Aorta Intima, in Heart, Liver and Kidneys Tissues of Animals the Multiple Lesions, Typical for Experimental Atherosclerosis, were Observed.**

***2*.- Intravenous Injections of HyFn to the Rabbits (*Group C*), which were on the Standard Diet, haven't Resulted in any Morphological and Ultrastructural Alterations in Cardiac Vessels and Another Organs.**

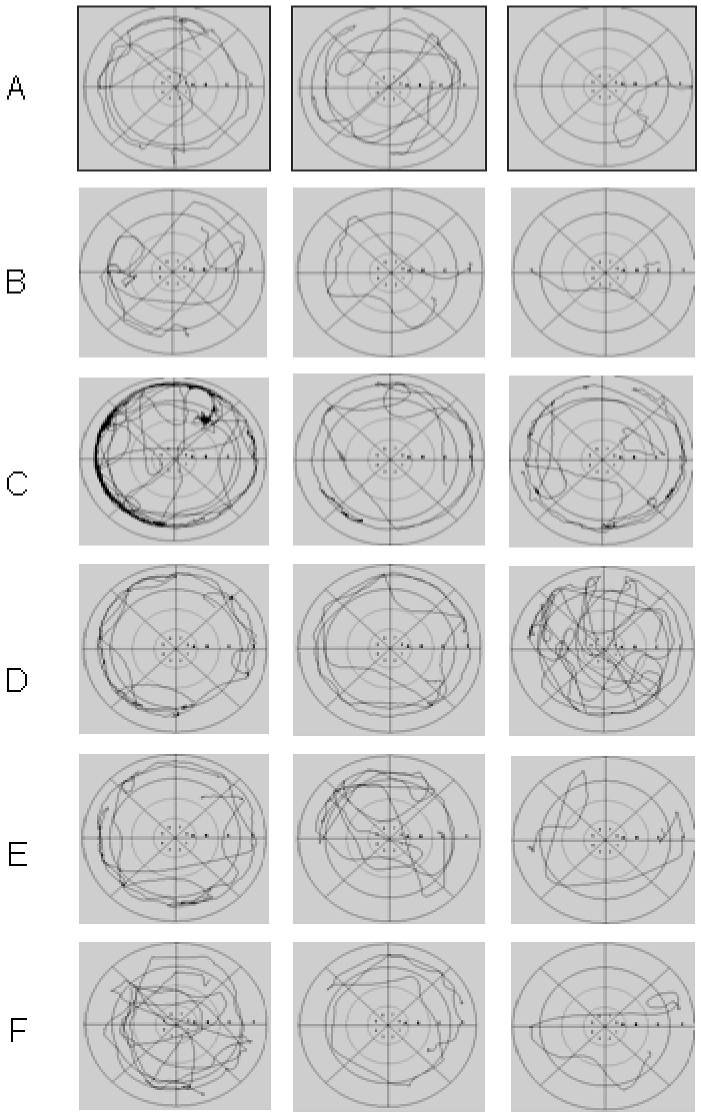
***3*.- Beginning from the Fourth Month of Experimental Atherosclerosis Development (*Group D*), Treatment with HyFn Stopped the Progression of it and Promoted the Reversion of Atherosclerotic Lesions.**

Aorta Intima of Intact Animals (Group A)

Multiple Lesions in Aorta Intima of Animals after Development of Experimental Atherosclerosis (Group B)

Involution (Regression) of Multiple Lesions in Aorta Intima of Animals with Experimental Atherosclerosis after i/v Treatment by Hydrated C_{60} Fullerenes (Group D)

C_{60} HyFn IMPROVED SIGNIFICANTLY THE PERFORMANCE OF THE COGNITIVE TASK IMPAIRED BY AMYLOID PEPTIDE $A\beta_{25\text{-}35}$



Examples of a computer display of the tracks covered by representative rats in the Morris watermaze experiments with $A\beta_{25\text{-}35}$ and C_{60}HyFn :

(**A, B**) the control rat after the i.c.v. injection of 0.9% NaCl (1st, 3d, and 5th trials);

(**C, D**) the rat after the i.c.v. injection of the aggregated $A\beta_{25\text{-}35}$;

(**E, F**) the rat after the i.c.v. injection of the C_{60}HyFn before $A\beta_{25\text{-}35}$.

(A, C, E) the first sessions and
(B, D, F) the second sessions, 72 h after the first session.

Conclusoins:

1. I.c.v. injection of a small C_{60}HyFn dose 3.6 nmol (2.6 μg)/ventricle did not affect the performance of the complicated cognitive task in control rats.
2. C_{60}HyFn at a dose of 7.2 nmol (5.2 μg)/ventricle improved significantly the performance of the probabilistic cognitive task in a normalcy.
3. Moreover, a single injection of C_{60}HyFn at a dose of 3.6 nmol (2.6 μg)/ventricle prevented the $A\beta_{25\text{-}35}$-induced impairment of the performance of the cognitive task.

I.Ya. Podolski, Z.A. et al.,. EFFECTS OF HYDRATED FORMS OF C_{60} FULLERENE ON AMYLOID β-PEPTIDE FIBRILLIZATION IN VITRO AND PERFORMANCE OF THE COGNITIVE TASK. J. of Nanoscience and Nanotechnology, 7, (4-5) (2007) 1479

ELSEVIER

Available online at www.sciencedirect.com
ScienceDirect

Toxicology 246 (2008) 158–165

TOXICOLOGY

www.elsevier.com/locate/toxicol

Nanostructures of hydrated C_{60} fullerene (C_{60}HyFn) protect rat brain against alcohol impact and attenuate behavioral impairments of alcoholized animals

Artem A. Tykhomyrov [a,*], Victor S. Nedzvetsky [a], Vladimir K. Klochkov [b], Grigory V. Andrievsky [b]

[a] National University of Dniepropetrovsk, 49050 Dniepropetrovsk, Ukraine
[b] ISMA NAS of Ukraine, STC "Institute for Single Crystals", 61001 Kharkiv, Ukraine

Received 21 November 2007; received in revised form 5 January 2008; accepted 7 January 2008
Available online 18 January 2008

Abstract

It is well known that chronic ethyl alcohol (EtOH) consumption is capable to injure brain cells and to cause essential abnormalities in behavioral characteristics of animals addicted to alcohol. In this work we for the first time have shown that administration of aqueous solutions of hydrated C_{60} fullerenes (C_{60}HyFn) with C_{60} concentration of 30 nM as a drinking water during chronic alcoholization of rats (a) protects the tissues of central nervous system (CNS) from damage caused by oxidative stress with high efficacy, (b) prevents the pathological loss of both astrocytes (the main cells of CNS) and astrocytic marker, glial fibrillary acidic proteins (GFAP) and, as consequence, (c) due to their adaptogenic effects, C_{60}HyFn significantly improves behavioral response and eliminates emotional deficits induced by chronic alcohol uptake. The wide range of beneficial biological effects, zero-toxicity, and efficacy even in super-small doses provide a rationale for the possible application of C_{60}HyFn for the treatment of alcohol-induced encephalopathy as well as alcoholism prophylaxis.

Keywords: Ethanol; Fullerenes; Hydrated C_{60} fullerene

Toxicology 282 (2011) 69–81

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Protective effects of nanostructures of hydrated C_{60} fullerene on reproductive function in streptozotocin-diabetic male rats

Ramazan Bal[a,*], Gaffari Türk[b], Mehmet Tuzcu[c], Okkes Yilmaz[c], Ibrahim Ozercan[d], Tuncay Kuloglu[e], Seyfettin Gür[c], Victor S. Nedzvetsky[f], Artem A. Tykhomyrov[g,**], Grigory V. Andrievsky[g], Giyasettin Baydas[h], Mustafa Naziroglu[i]

[a] Department of Physiology, Faculty of Medicine, Firat University, 23119 Elazig, Turkey
[b] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Firat University, 23119 Elazig, Turkey
[c] Department of Biology, Faculty of Science, Firat University, 23119 Elazig, Turkey
[d] Department of Pathology, Faculty of Medicine, Firat University, 23119 Elazig, Turkey
[e] Department of Histology and Embryology, Faculty of Medicine, Firat University, 23119 Elazig, Turkey
[f] National University of Dnepropetrovsk, 49050 Dnepropetrovsk, Ukraine
[g] Institute of Physiologically Active Compounds LLC, 58 Lenin Ave., 61072 Kharkov, Ukraine
[h] Bingol University Rectorate, Bingol University, Bingol, Turkey
[i] Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

ARTICLE INFO

Article history:
Received 14 September 2010
Received in revised form
28 November 2010
Accepted 6 December 2010
Available online 14 December 2010

Keywords:
Streptozotocin
Diabetes mellitus
Hydrated C_{60} fullerene
Testis
Oxidative stress
Apoptosis
Sperm characteristics
Fatty acid composition

ABSTRACT

Diabetes mellitus is a well-recognized cause of male sexual dysfunction and impairments of male fertility. Streptozotocin (STZ) is used for medical treatment of neoplastic islet β-cells of pancreas and producing of animal model of diabetes mellitus type 1 that is characterized by suppression of reproductive activity due to the hyperglycaemia-induced oxidative stress and histopathological alterations in testes. Seeking for the agents that could alleviate diabetes-induced damage to reproductive system is yet the important area of inquiry. The present study was designed to evaluate whether hydrated C_{60} fullerene (C_{60}HyFn), which is known to be powerful bioantioxidant, eliminate testicular dysfunction induced by STZ-diabetes in rats. Wistar strain male albino rats were divided into four groups of six animals each: (1) control group, (2) C_{60}HyFn-treated nondiabetic group, (3) STZ-diabetic group and (4) C_{60}HyFn-treated diabetic group. Once hyperglycaemia was induced by STZ, rats in the second and fourth groups were treated with C_{60}HyFn (in the form of drinking water) at the dose of 4 μg/kg daily for 5 weeks. In diabetic rats, relative weights of right cauda epididymis, seminal vesicles, prostate, sperm motility and epididymal sperm concentration were significantly less than those of control group, but which were restored in the fourth group treated with C_{60}HyFn ($p < 0.001$). In hematoxylin and eosin staining, marked histopathological changes including degeneration, desquamation, disorganisation and reduction in germinal cells, interstitial oedema and congestion were evident in the testis of diabetic rats, but C_{60}HyFn treatment resulted in recovery of histopathological changes and an increase in Johnsen's testicular score significantly ($p < 0.001$). C_{60}HyFn treatment restores the increased apoptosis induced by STZ-diabetes. In diabetic rats, levels of serum testosterone, testicular reduced glutathione (GSH) and alpha-tocopherol were significantly reduced and testicular lipid peroxidation level was increased ($p < 0.001$). Nevertheless, treatment of diabetic rats with C_{60}HyFn resulted in significant corrective effects on these parameters towards the control levels. C_{60}HyFn, applied alone, did not exert any toxic effects in testicular tissues. Furthermore, C_{60}HyFn treatment in diabetic and nondiabetic rats resulted in considerable elevations of some important polyunsaturated fatty acids. In conclusion, we have presented for the first time substantial evidence that administration of C_{60}HyFn significantly reduces diabetes-induced oxidative stress and associated complications such as testicular dysfunction and spermatogenic disruption.

Antioxidat Activity of Hydrated Fullerenes is stipulated their ability to regulate free-radica processes in aqueous medium by ‘wise’ manner.

Антиоксидантная Активность Гидратированных Фуллеренов обусловлена их способностью регулировать в водной среде свободно-радикальные процессы (реакции) «разумным» образом.

Antioxidative Properties of Hydrated C_{60} Fullerenes (HyFn)

Experimental Models (+ HyFn)	Concentrations and Total Doses of Hydrated C_{60} Fullerene	Index of Oxidative Activity (IOA)	Antioxidative Effect IOA Changes in Comparison with the Control Experiment (i.e., without HyFn addition)
IN VITRO	*µM/L*		
Rat Hepatocytes	1.0 - 8.0	*Spontaneous Chemoluminescence*	1.28 ↓
Human Erythrocytes	2.5	*TBARS* (*Thiobarbituric Acid Reactive Substances*)	1.12 ↓
Blood Serum From MCI Patients (POL induction with Fe^{2+} ions)	5.0 - 50.0	*TBARS* (*Thiobarbituric Acid Reactive Substances*)	4.00 ↓
IN VIVO	*mg/kg*		
Oxidative Stress (Rats. Induction by means of *i/p* injection of Co^{2+} ions)	0.20 (*i/p;* *once only*)	*Malonic Dialdehyde* *In Liver:* *In Blood Serum:*	In 3 h after HyFn injection: 2.30 ↓ 3.98 ↓
Experimental Atherosclerosis (Rabbits; Treatment)	0.4 (*4 x 0.1; i/v; 4 inject. for 8 weeks*)	*Spontaneous Chemoluminescence of Blood Serum*	In 10 weeks after treatment with HyFn 4.00 ↓
Acute Autoimmune Glomerulo-nephritis (Disease of Kidney) (Rats; Treatment)	0.35 (*i/p;* *once only*)	*In Blood Serum:* *GSH* (*H-Glutathione*) *SH-groups* *Conjugated Denes* *Malonic Dialdehyde* *SOD* (*Superoxidedismutase*) *In Brain:* *Histamine* *Serotonin*	In 1 day after HyFn injection: 2.00 ↑ 2.44 ↑ 1.46 ↓ 1.32 ↓ 1.52 ↓ 1.28 ↓ 2.51 ↑
Immune Deficiency with Burn Disease (Mice; Treatment)	0.45 (*in 0.09; i/v; once daily for 5 days*)	*In Brain:* *Histamine* *Serotonin* *Histamine of Skin Tissue*	In 10 days after HyFn / In 20 days after HyFn 2.17 ↓~ / 1.96 ↓ 1.00 -- / 2.24 ↑ 1.47 ↓ / "NORM!"

In numerous and multi-faceted experiments *in virto*, on modelling pathologies *in vivo*, and also by official pharmacological and clinical trials it has been unequivocally shown that hydrated C_{60} fullerene (C_{60}HyFn) in a wide range of doses, including super small, shows unique and "wise" antioxidant properties.

The reason of these is the properties of special structures of water which C_{60}HyFn organises and supports round itself.

In addition see on:
http://www.ipacom.com/index.php/en/fullerenes-and-water-left/78

В многочисленных и разноплановых экспериментах *in virto*, на модельных патологиях *in vivo*, а также официальными фармакологическими и клиническими испытаниями было однозначно показано, что гидратированный фуллерен C_{60} (C_{60}HyFn) в широком диапазоне доз, включая и сверхмалые, проявляет уникальные, «разумные» антиоксидантные свойства.

Причина тому – свойства особых структур воды, которые C_{60}HyFn организует и поддерживает вокруг себя.

Подробнее с этим можно ознакомиться на нашем сайте по ссылке:
http://www.ipacom.com/index.php/ru/fullerenes-and-water-left/78

Probable Scheme of Processes of Free Radical (*FR*) Absorbtion, Concentration and Recombination Under the Influence of the Ordered Water Structures Formed Around the Hydrated C_{60} Fullerenes (*HyFn*).

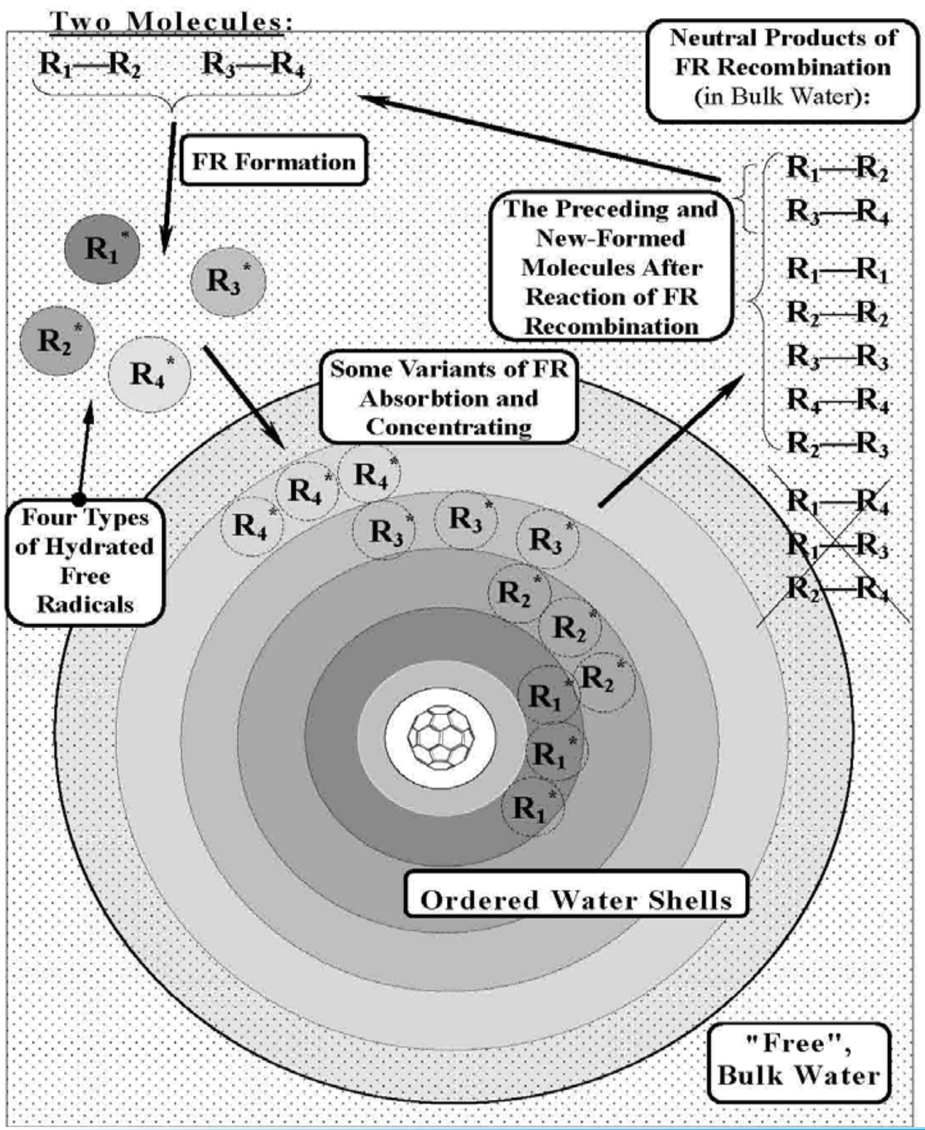
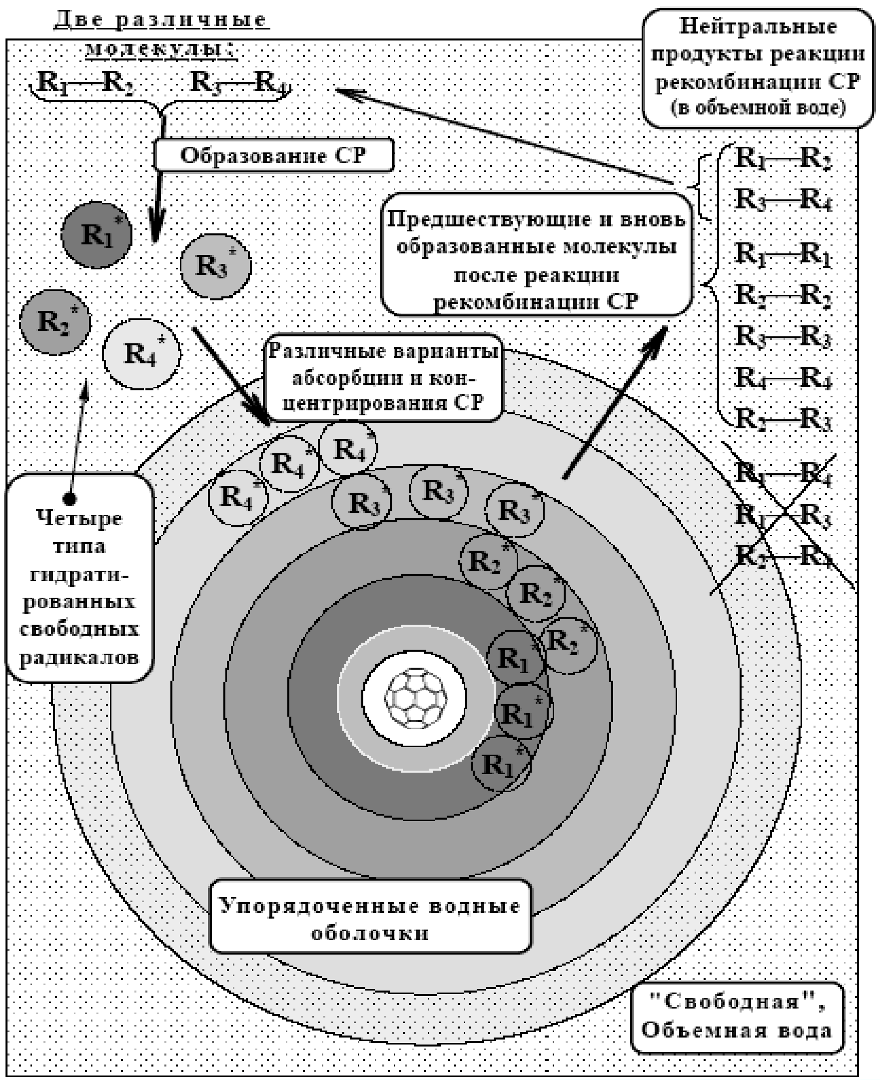


Рис.3 ***Вероятная схема процесса абсорбции, концентрирования и рекомбинации свободных радикалов (СР) под влиянием упорядоченных водных структур, сформировавшихся вокруг гидратированного C_{60} фуллерена (HyFn).***



It is important that hydrated C_{60} fullerene:

(a) does not suppress the natural level of free radicals in the organism, but becomes active only under conditions of their increased concentration. The more that free radicals form in the organism, the “more” active hydrated fullerene become to neutralize them;

(b) does not possess of prooxidant properties because:

--- it is not capable to stimulate the formation of strong oxidant - singlet oxygen ($^1O_2$). The reason for it is that the strong water shell surrounding C_{60} molecule does not allow O_2 molecule to come nearer and to contact directly with C_{60} surface so that subsequently the energy of photoexcitation of C_{60} molecule could be transferred to O_2 molecule with formation $^1O_2$.

--- it is not capable to capture from without the free electron with formation of C_{60}-anion radical ($C_{60}^{\bullet-}$), which subsequently could transfer its unpaired electron to O_2 molecule with formation of strong oxidant - superoxid-anion radical ($O_2^{\bullet-}$). The reason for it is that in donor-acceptor complex $C_{60}@\{H_2O\}_n$ the C_{60} molecule is already "saturated" by external electrons (from O-atoms of water molecules) and as consequence of it the hydrated C_{60} has lost its former electron-acceptor properties and is not able to form $C_{60}^{\bullet-}$.

Важно, что гидратированный фуллерен C_{60}:

(а) не подавляет естественного уровня свободных радикалов в организме, а становится активным лишь в условиях повышения их концентрации. И чем больше образуется свободных радикалов в организме, тем "активнее" гидратированный фуллерен их нейтрализует;

(б) не обладает свойствами прооксиданта, т.к.:

--- он не способен стимулировать образование сильного оксиданта - синглетного кислорода ($^1O_2$). Причина этому есть то, что прочная водная оболочка, окружающая молекулу C_{60}, не позволяет молекулам O_2 приблизиться и непосредственно контактировать с поверхностью C_{60} для того, чтобы в последующем энергия фотовозбуждения молекулы C_{60} могла бы передаться на молекулу O_2 с образованием $^1O_2$.

--- он не способен захватывать извне свободный электрон с образованием C_{60}-анион радикала ($C_{60}^{\bullet-}$), который в последующем мог бы передать свой неподеленный электрон на молекулу O_2 с образованием сильного оксиданта - супероксид-анион радикала ($O_2^{\bullet-}$). Причина этому есть то, что в донорно-акцепторном комплексе $C_{60}@\{H_2O\}_n$ молекула C_{60} уже «насыщена» внешними электронами (от О-атомов молекул воды) и, как следствие такого, она потеряла свои прежние электрон-акцепторные свойства, а следовательно и способность образовывать $C_{60}^{\bullet-}$.

VARIOUS SORT OF HYDRATED NANOPARTICLES WITH THE SIZES FROM 3 UP TO 30 nm CAN POSSESS BIO-ANTIOXIDANT PROPERTIES BUT WHICH, IN VIEW OF THEIR CHEMICAL NATURE, DO NOT ABLE TO PARTICIPATE DIRECTLY IN CHEMICAL REACTIONS WITH FREE RADICALS.

SUCH IS CONNECTED WITH THE NATURAL WATER ORDERED STRUCTURES, IN WHICH THE SIMILAR NANOPARTICLES ARE CAPABLE TO BE EMBEDED AND SUPPORT THEM.

The similar facts are found out for the following nanoparticles:

- **- hydrated C_{60} fullerene and it fractal associates with size 3.4, 7.1, 10.9, 14.5, 18.1, 21.8, 25.4, 28.8, 32.4, 36.0 nm** [*1*]**;**
- **- CeO_2 with size 3-5 и 7, 20, 30-50** nm [*2, 3*]**;**
- **- Y_2O_3 с размерами 6 и 12 нм** [*4*]**;**
- **- Ag with size ~ 15 nm** [*5*]**;**
- **- nanodimond with size 3.4-3.8 (2-10) nm** [*6*]**.**
- **- $BaTiO_3$ (?) with size 10-100 nm** [7]

[1] G.V. Andrievsky et al. *Fullerenes, Nanotubes and Carbon Nanostructures*, 13 (4), (2005) 363.
[2] S. Babu et al. *Chem. Phys.Letters*, 442 (2007) 405.
[3] B.A. Rzigalinski. *Nanomedicine*, 1 (4) (2006) 399.
[4] D. Schubert et al. *Biochem. Biophys. Res. Commun.*, 342 (2006) 86.
[5] J. Tian et al. *ChemMedChem*, 2 (1) (2006) 129.
[6] A.M. Schrand et al. *J. Phys. Chem. B*, 111 (1) (2007) 2.
[7] Y. Katsir et al., *J. Electrochem. Soc.*, 154 (4) (2007) D249.

Биоантиоксидатными свойствами обладают различного рода <u>Гидратированные Наночастицы с размерами</u> <u>от 3 до 30 нм</u>, но которые, ввиду их химической природы, не участвуют сами непосредственно в реакциях со свободными радикалами.

Такое связано с естественными упорядоченными структурами воды, в которые подобные наночастицы способны «вписываться» и поддерживать их.

***Подобные факты обнаружены для наночастиц*:**

- **- гидратированных фуллеренов C_{60} и их фрактальных ассоциатов с размерами 3.4, 7.1, 10.9, 14.5, 18.1, 21.8, 25.4, 28.8, 32.4, 36.0 nm** [*1*];
- **- CeO_2 с размерами 3-5 и 7, 20, 30-50** nm [*2, 3*];
- **- Y_2O_3 с размерами 6 и 12 нм** [*4*];
- **- Ag с размерами около 15 nm** [*5*];
- **- наноалмазов с размерами 3.4-3.8 (2-10) нм** [*6*].
- **- $BaTiO_3$ с размерами 10-100 нм** [7]

[1] G.V. Andrievsky et al. *Fullerenes, Nanotubes and Carbon Nanostructures*, 13 (4), (2005) 363.
[2] S. Babu et al. *Chem. Phys.Letters*, 442 (2007) 405.
[3] B.A. Rzigalinski. *Nanomedicine*, 1 (4) (2006) 399.
[4] D. Schubert et al. *Biochem. Biophys. Res. Commun.*, 342 (2006) 86.
[5] J. Tian et al. *ChemMedChem*, 2 (1) (2006) 129.
[6] A.M. Schrand et al. *J. Phys. Chem. B*, 111 (1) (2007) 2.
[7] Y. Katsir et al., *J. Electrochem. Soc.*, 154 (4) (2007) D249.

Каким должен быть идеальный "антиоксидант"?

В сентябре 2005 в своем письме академику РАН Скулачеву В.П. были высказаны мысли по поводу того, какими свойствами должен был бы обладать идеальный биоантиоксидант (БАО) и который, в частности,

(а) нейтрализовывал бы только избыток свободных радикалов и не затрагивал бы их уровень, минимально необходимый для нормального функционирования биологической системы;

(б) регулировал бы свободнорадикальные процессы на уровне как гидрофобных, так и гидрофильных компартментов биологической системы в целом;

(в) не изменял бы естественные состояния гидратных оболочек, непосредственно окружающих нормальные (нативные) биологические структуры и, более того, стабилизировал бы и то, и другое;

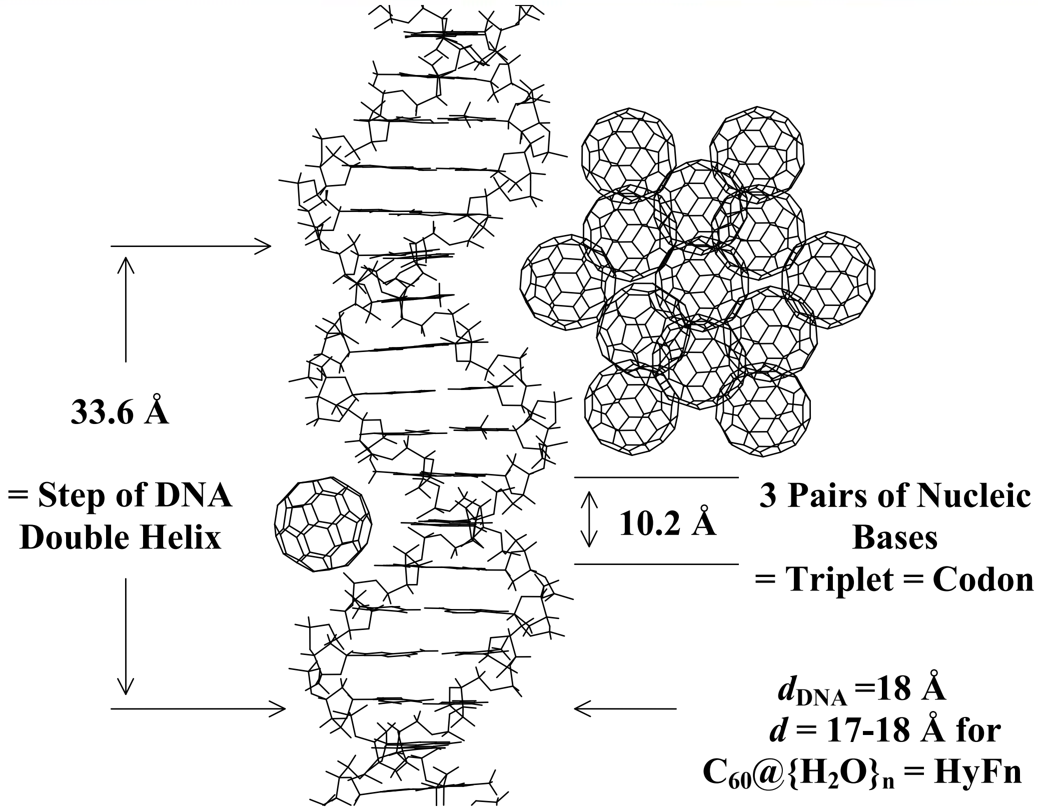
(г) не воспринимался бы организмом, как чужеродное вещество, т.е. был бы нетоксичным в целом, неиммуногенным и т.п., а на уровне клетки, не влиял бы на ее нормальный гомеостаз;

(д) имитировал бы работу ферментов антиоксидантной защиты, например, был бы СОД-миметиком;

(е) работал бы как своеобразный катализатор самонейтрализации свободных радикалов в очень малых дозах и в течение длительного времени (напр. дни, недели) после однократного введения в организм.

Как показали наши, более чем 16-и летние исследования, удовлетворить таким требованиям в настоящее время может только гидратированный фуллерен C_{60} ($ГФС_{60}$, $C_{60}HyFn$)

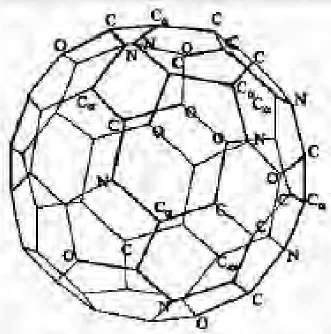
VARIOUS INTERESTING FACTS AND COINCIDENCE



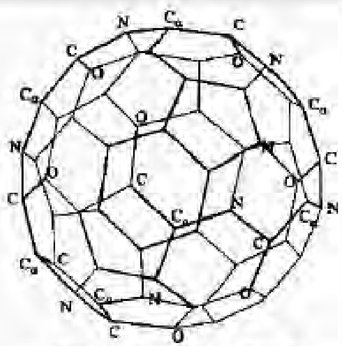
Correspondence of the DNA Segment Structure in *B*-Form to the Geometry of C_{60} Molecule (10 Å) and First Spherical Cluster of Hydrated $(C_{60})_{13}$ with Diameter of ~34 Å.

Scheme of Pattern Matching of Spatial Structure of Various Peptides with Matrix of C_{60} Fullerene Structure.

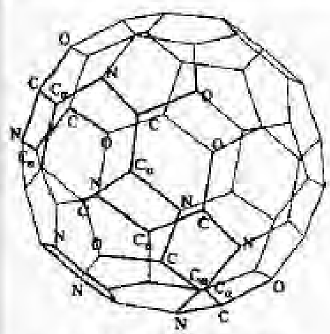
[by means of Computer Simulation in: "Biocompatibility of Fullerene C60 with Oligopeptides Using a Comparative Analysis of their Spatial Form". Baranov A.A., Esipova N.G. Biofizika 45 (2000) 801-808 (Russ)].



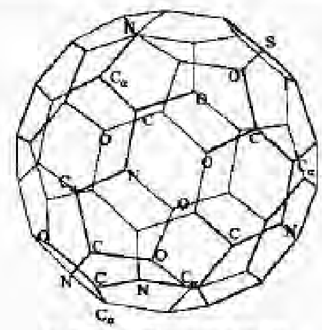
Ala-Ala-Gly-Ser-Ala-Ala-Gly



Gly-Ala-Gly-Trp-Gly-Gly-Ile



Gly-His-Gly-Ala-Ala-Gly



H-Tyr-Gly-Gly-Phe-Met-OH

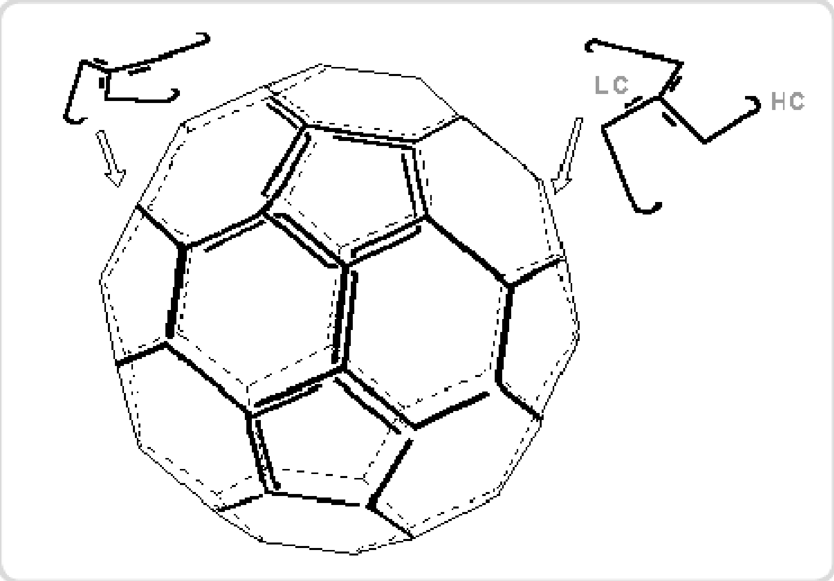
The Similar Amino Acid Sequences have been Identified in Structures of More than 280 Natural Proteins*, particularly in:

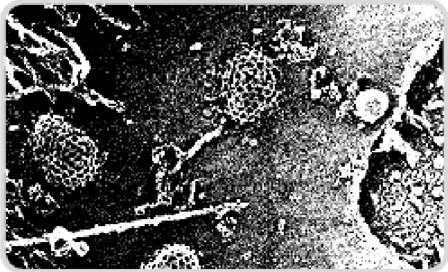
- Lethal (1) Discs Large-1 Tumor Suppressor Protein;
- Major DNA-Binding Protein;
- Glucan Endo-1,3-β-Glucosidase Precursor and Galactokinase (EC);
- DNA-Polymerase;
- Dehydrin, DHN1;
- Met-Enkephalin and Corticotropin-Lipotropin Precursor (Pro-Opiomelanocortin);

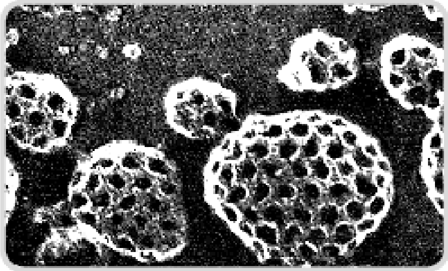
* - Data From Swiss-Prot (Protein Sequence Database, EMBL, Europian Bioinformatics Institute).

Diagrams of Clathrin Trimers (Triskelions) and Their Packing Arrangement in a Cage (HC, protein heavy chain; LC, protein light chain).

Reproduced from Harisson S.C. & Kirchhausen T., Cell, 33 (1983) 650 and cited by Wileman T., Harding C., Stahl P., Receptor-Mediated Endocytosis. (A Reviw Article), Biochem. J., 232 (1985) 1-14.







Intracellular Membranes Surface with Lipid Vesicles Coated by Clathrin Cages

Microbiology Comment

Fungal growth on buckminsterfullerene

Buckminsterfullerene (colloquially known as buckyballs) is a recently discovered C60 allotrope of carbon which is causing considerable interest in organic chemistry laboratories around the world, not least because it has considerable industrial potential as a catalyst and lubricant (1). It is a black powder which looks very like fine charcoal or soot.

Although the chemical and physical properties of buckminsterfullerene have been widely studied, little is known about the biological properties, if any, of this carbon allotrope. From its structure, however, it seems unlikely that it would be biologically unreactive. Despite this, a novel fullerene derivative has been found to block an enzyme found in human immunodeficiency virus (2).

We recently exposed buckminsterfullerene (Fluka, Chemica) in ultrapure water to the laboratory atmosphere (25 mg in 100 ml water, in chromic-acid-washed glass Petri dishes; 10 dishes per treatment) to see if contaminating fungi would grow on it in the absence of any added nutrients. The dishes were left for 7 d at room temperature (approx. 18–20 °C), followed by a further 7 d incubation with the Petri dish lids replaced.

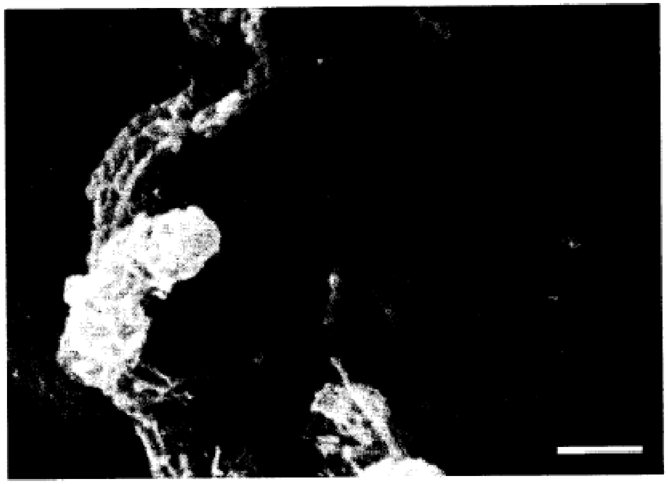


Fig. 1. Scanning electron micrograph showing a contaminant of a species of *Penicillium* growing on buckminsterfullerene in ultrapure water. Bar, 10 µm.

After this period, fungal mycelium was clearly seen (in all of the 10 Petri dishes) with the naked eye growing from a clump of floating buckminsterfullerene. The fungus failed to sporulate on buckminsterfullerene (Fig. 1), but grew well and sporulated on Czapek Dox agar (Oxoid); it was identified as a species of *Penicillium*.

The fungus could have originated from the laboratory air or, since the buckminsterfullerene was not sterilized, from the powder itself. No obvious signs of degradation of buckminsterfullerene due to fungal growth were observed, which is not surprising, since it is unlikely that a fungus could use this allotrope as a sole source of carbon. What is more likely is that the *Penicillium* grew oligotrophically on buckminsterfullerene using nutrients scavenged from the air. Fungi can grow in this way, apparently by scavenging combined forms of carbon and nitrogen from the atmosphere (4). Having said this, no fungi were seen growing in dishes incubated alongside those containing buckminsterfullerene but containing only ultrapure water. In addition, mycelium did not appear on activated charcoal when added to ultrapure water; a fact which suggests that mould growth on buckminsterfullerene was not merely due to it acting as a growth-encouraging surface. Buckminsterfullerene can absorb gases, such as hydrocarbons and ammonia (3) which could act as nutrient sources for microbial growth. As a result, it is likely that the fungus grew on air-borne nutrients absorbed by the particles, rather than by using the allotrope itself as a carbon source (it is also possible that, despite its stated purity, the sample of buckminsterfullerene used here contained organic contaminants).

These observations once again illustrate the marked ability of fungi to grow in the apparent absence of nutrients. They also suggest that buckminsterfullerene, even when not contaminated with other substrates, will become overgrown with moulds when stored under a moist condition – a possibility that should be borne in mind should buckminsterfullerene eventually be used in industry or medicine.

Buckminsterfullerene can have an interstellar origin and may have played a role in the origin of life. Fullerenes have been found in a crater near Sudbury, Ontario, Canada, formed some 1.5 billion years ago when a meteorite the size of Mount Everest fell to Earth (5). Buckerminsterfullerene deposited by meteorites may therefore have provided the organic carbon needed to get life started. One theory of the origin of life, panspermia, suggests that life came to this planet from outer space. The fact that fungi, and presumably also bacteria, can grow on buckyballs in the absence of added nutrients suggests the possibility that life may have travelled to Earth on buckminsterfullerene or else it may have helped extraterrestrial micro-organisms to grow once they had arrived on the planet.

▶ GUIDELINES

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

Approval for publication rests with the Editor-in-Chief, who reserves the right to edit letters and/or to make a brief reply. Other interested persons may also be invited to reply. The Editors of ***Microbiology*** do not necessarily agree with the views expressed in ***Microbiology* Comment**.

Contributions should be addressed to the Editor-in-Chief via the Editorial Office.

Milton Wainwright* and Abdullah M. Falih

Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, S10 2UH, UK.

*For correspondence. Tel: +44 114 768555 ext. 4410. Fax: +44 114 272 8697.

1. Culotta, E. & Koshland, D.E. (1991). Molecule of the year; buckyballs: a wide open field for chemists. *Science* 254, 1706–1709.

2. Lipkin, R. (1993). Novel buckyball blocks AIDS virus enzyme. *Sci News* 144, 87.

3. Stroh, M. (1992). Serendipity yields buckyball trap for gases. *Sci News* 141, 356–357.

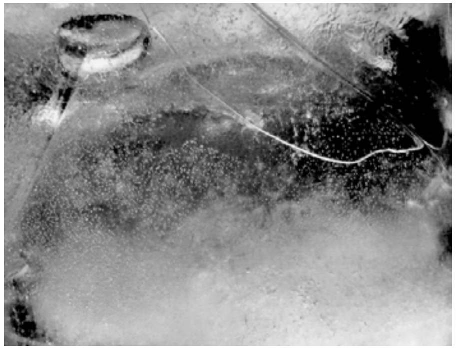
4. Wainwright, M. (1993). Oligotrophic growth of fungi – stress or natural state? In *Stress Tolerance of Fungi*, pp. 127–144. Edited by D. H. Jennings. New York: Marcel Dekker.

5. Zimmer, C. (1996). Buckyballs from space. *Discover* 17, 30.

IMAGES OF ACI FROM:

MINERAL WATER:

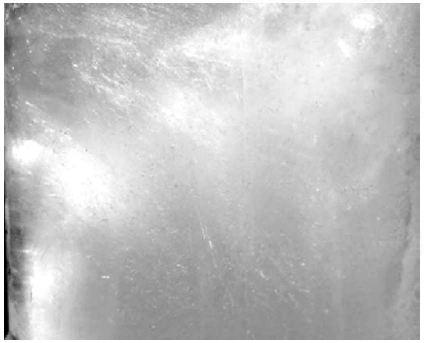
"ROGANSKAYA", Kharkov, Ukraine TDS 300 mg/L

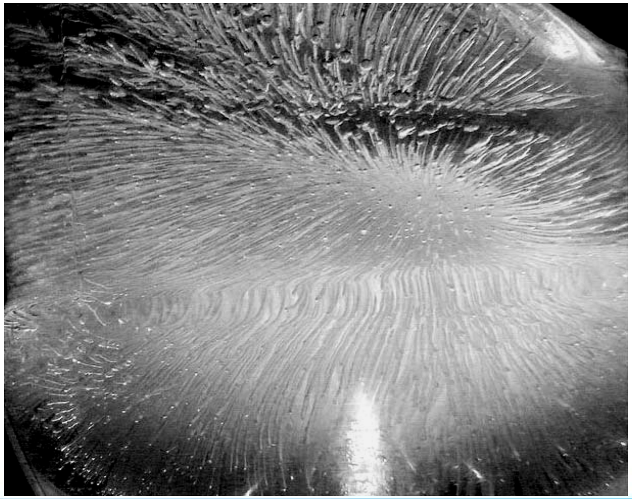


ALPINE WATER "EVIAN" TDS 270 mg/L

"MORSHINSKAYA", Ukraine TDS 80 mg/L

TAP WATER, Kharkov, Ukraine TDS 450-500 mg/L

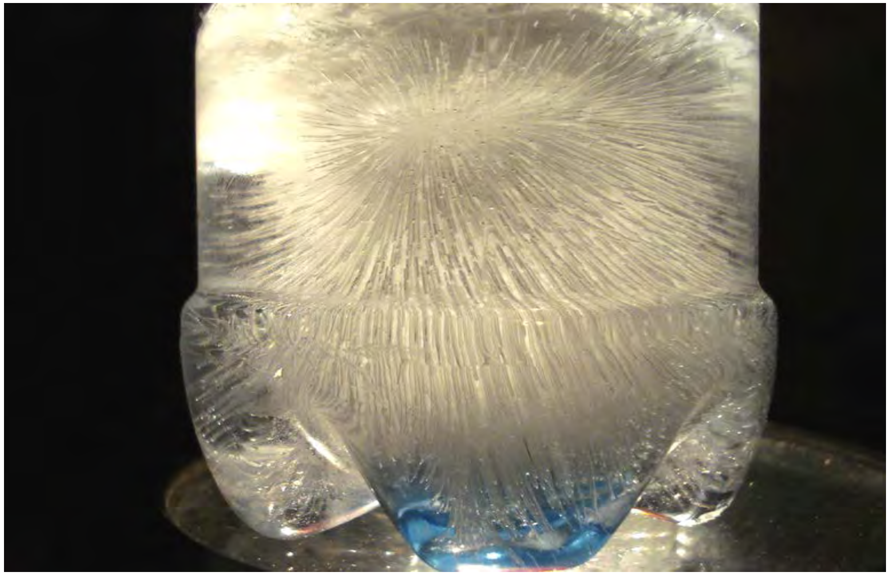


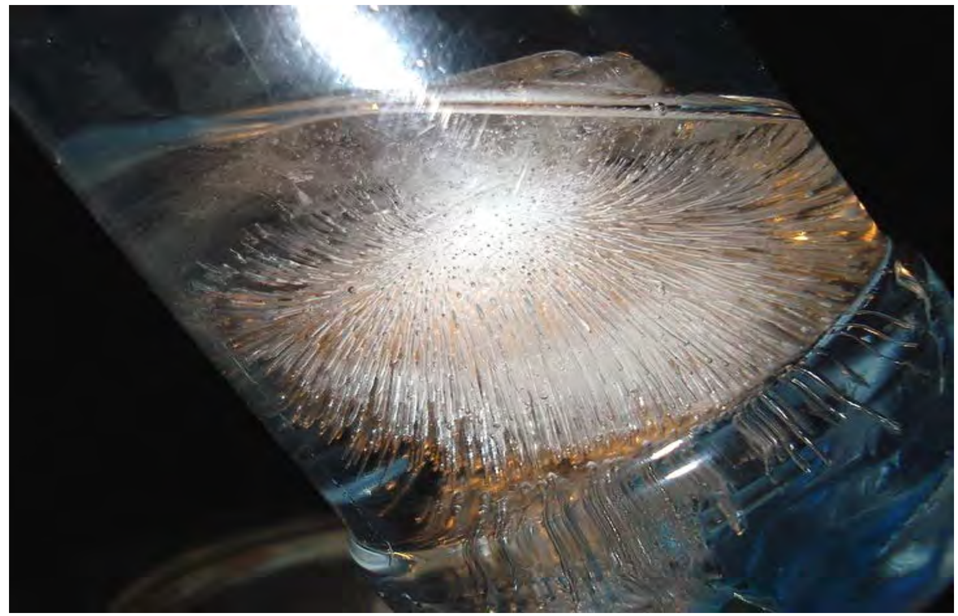


"FULLERENE WATER"

IPAC LLC, Kharkov, Ukraine TDS 0-2 mg/L

FULLERENE WATER ICE





In XX century, Nobel laureate Albert Szent-Gyorgyi stated that:

“BIOLOGY HAS FORGOTTEN CELL WATER, OR NEVER DISCOVERED IT.”

===

В 70-х годах XX века Нобелевский лауреат Альберт Сцент-Дьерди заявил о том, что:

“БИОЛОГИЯ ЗАБЫЛА КЛЕТОЧНУЮ ВОДУ, ИЛИ ВООБЩЕ НИКОГДА ЕЕ НЕ ОБНАРУЖИВАЛА.”

In the words of A. Szent-Gyorgyi,
«Life is water dancing to the tune of solids».

(Жизнь - это есть вода, танцующая
под мелодию твердых материй)

but in the words of G. Andrievsky,
«Life is the carbon-contained solids dancing
to the tune of ordered structures of water».

(Жизнь - это есть углеродсодержащие
твердые материи, танцующие под
мелодию упорядоченных структур воды)

In the words of A. Szent-Gyorgyi**,**

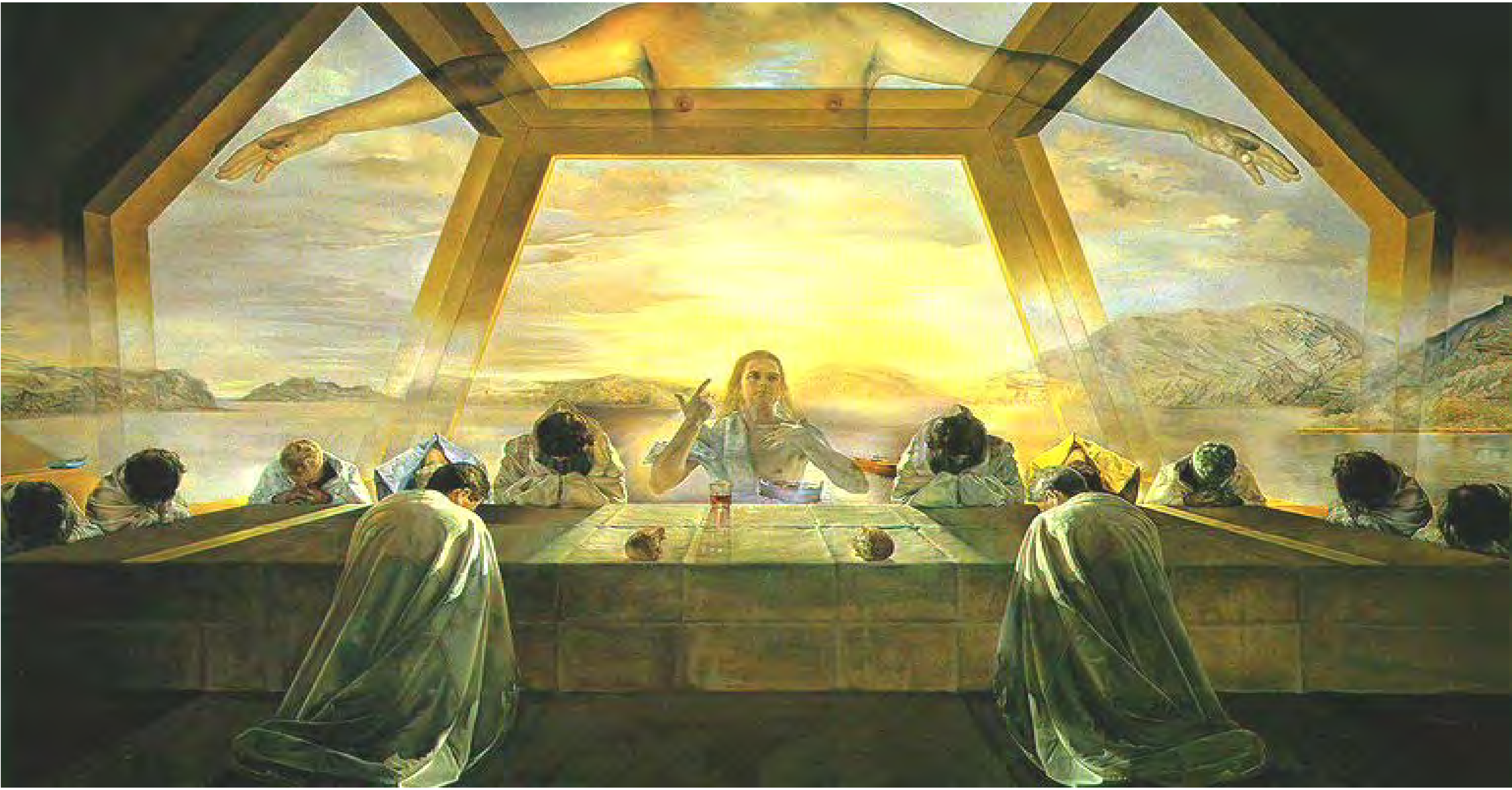
“Life is water dancing to the tune of solids”.

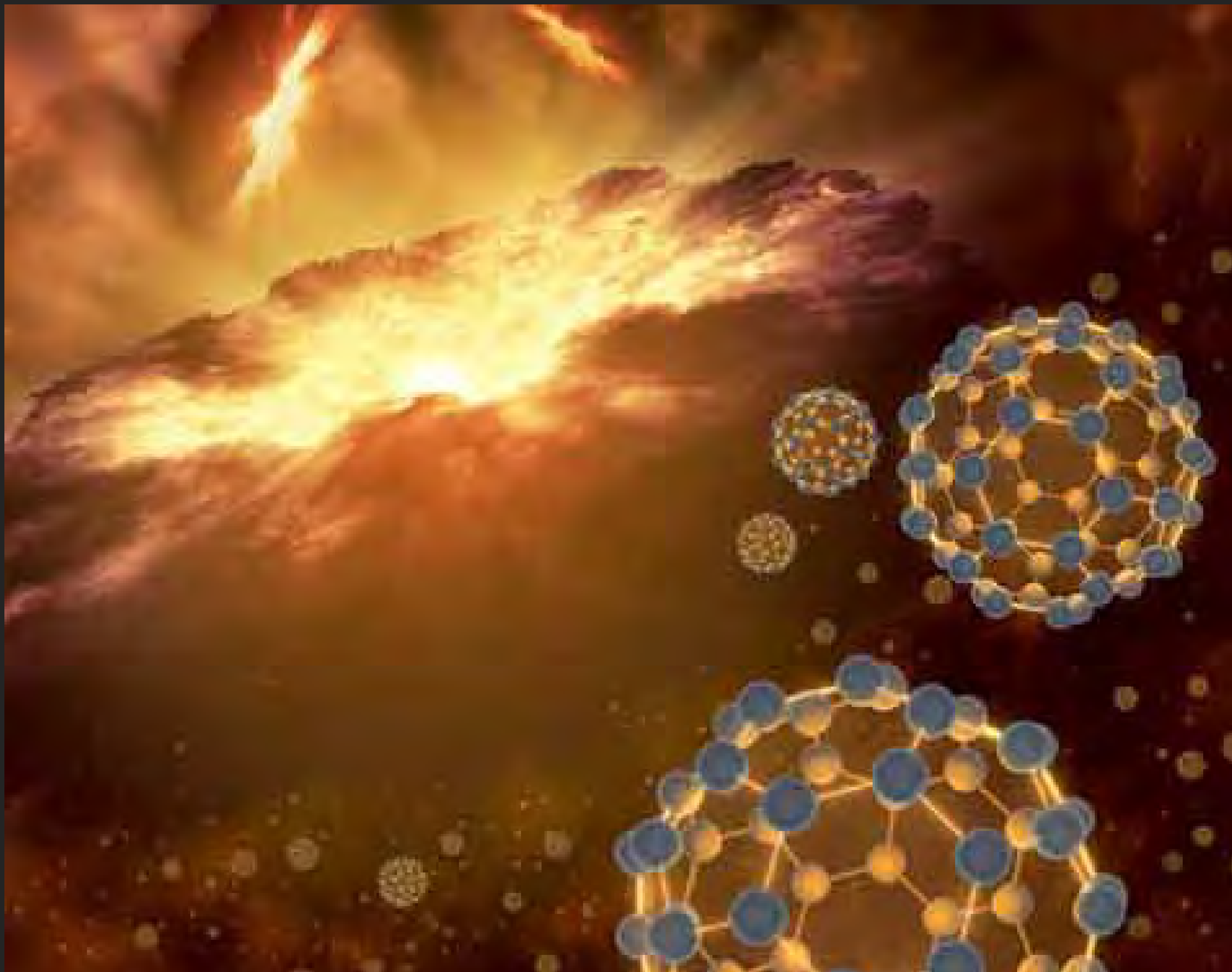
(Жизнь - это есть вода, танцующая под мелодию твердых материй)

but in the words of G. Andrievsky**,**

“Life is the carbon-contained solids dancing to the tune of ordered structures of water”.

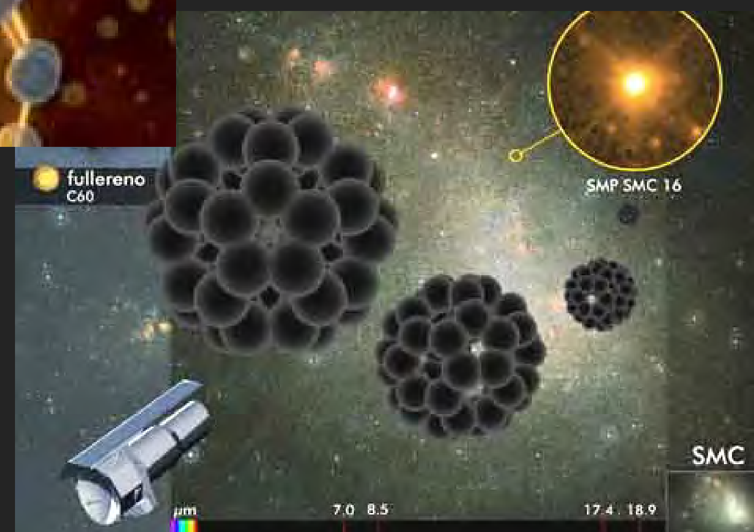
(Жизнь - это есть углеродсодержащие твердые материи, танцующие под мелодию упорядоченных структур воды)

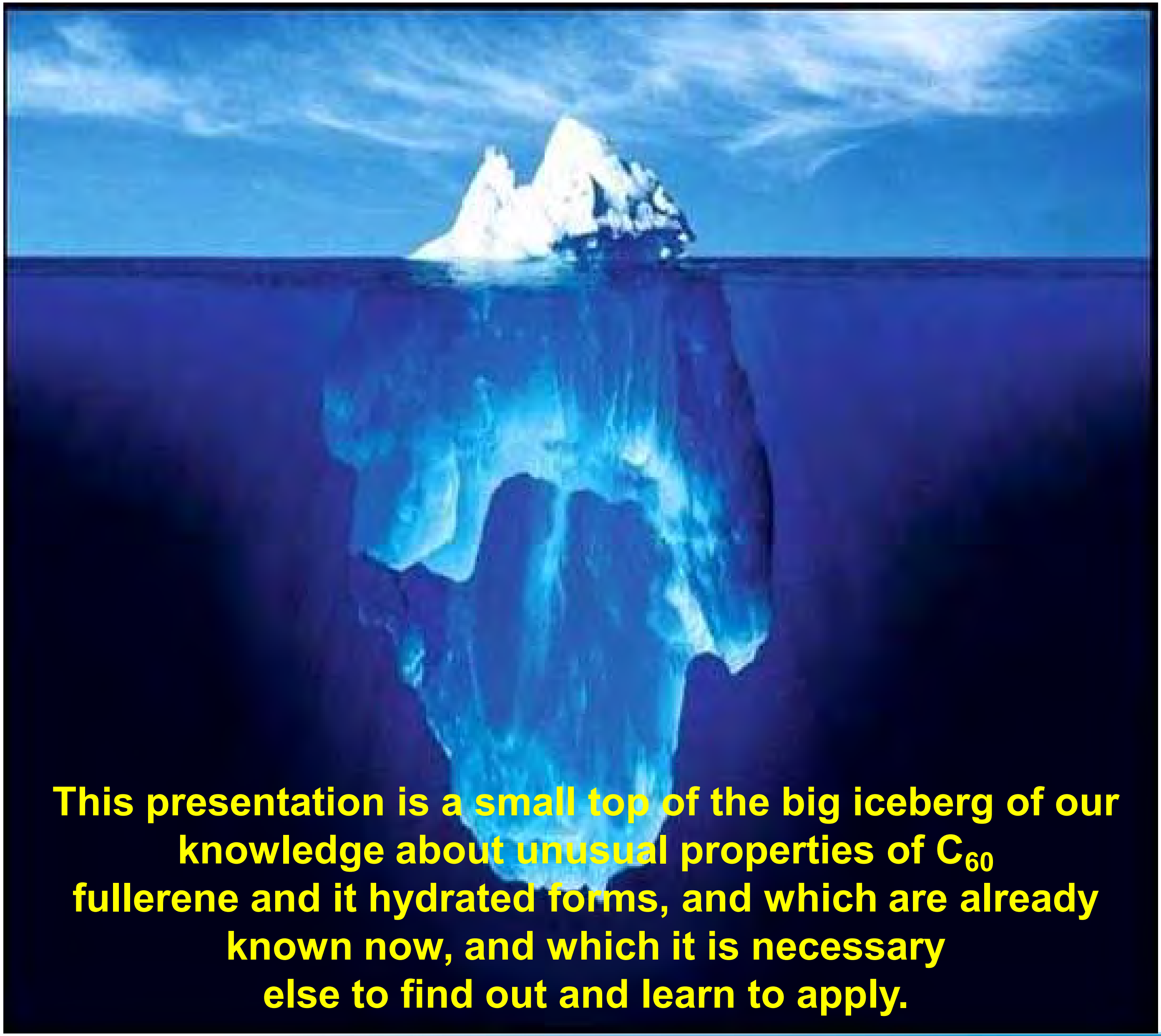




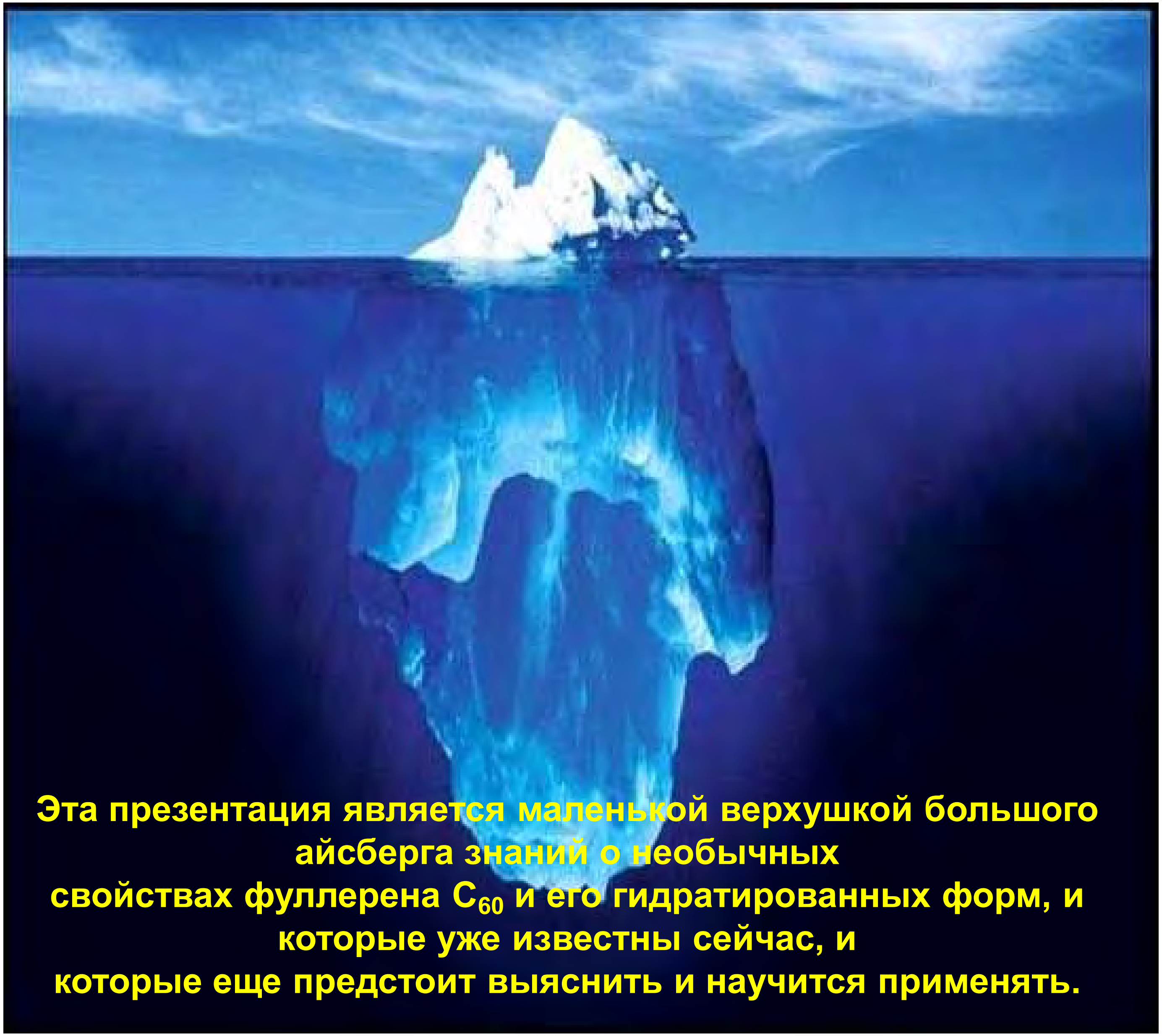
C_{60} Fullerenes

in Cosmos





This presentation is a small top of the big iceberg of our knowledge about unusual properties of C_{60} fullerene and it hydrated forms, and which are already known now, and which it is necessary else to find out and learn to apply.


Эта презентация является маленькой верхушкой большого
айсберга знаний о необычных
свойствах фуллерена C_{60} и его гидратированных форм, и
которые уже известны сейчас, и
которые еще предстоит выяснить и научится применять.

T H A N K S F O R Y O U R A T T E N T I O N !!!

And we are certain that fullerenes can still offer many remarkable surprises, both in the sphere of science and in life!!!

С П А С И Б О З А В Н И М А Н И Е !!!

И убеждены, что фуллерены еще способны принести немало удивительных сюрпризов и в науке, и в жизни!!!

“Reality does not speak to us objectively, and no scientist can be free from constraints of psyche and society. Most of us are not naive enough to believe the old myth that scientists are paragons of unprejudiced objectivity, equally open to all possibilities, and reaching conclusions only by the weight of evidence and logic of argument. We understand that biases, preferences, social values, and psychological attitudes all play a strong role in the process of discovery. However, we should not be driven to the opposite extreme of complete cynicism – the view that objective evidence plays no role, which perceptions of truth are entirely relative, and those scientific conclusions are just another form of aesthetic preference. <u>Science, as actually practiced, is a complex dialogue between data and preconceptions. The greatest impediment to scientific innovation is usually a conceptual lock, not a factual lack</u>.”

Harvard paleontologist - Stephen Jay Gould
in: NANO-STRUCTURED AQUEOUS SOLUTIONS AS A SUPPORT BASE TO OPTIMIZE COHERENT CELLULAR COMMUNICATION.

“Действительность не говорит с нами объективно, и никакой ученый не может быть свободен от ограниченности души и общества. Большинство из нас не достаточно наивны, чтобы верить старому мифу, что ученые являются образцами беспристрастной объективности, одинаково открты ко всем возможностям, и достигают выводов только за счет взвешивания очевидности и логических аргументов. Мы понимаем что предубеждения, предпочтения, социальные ценности и психологические ориенации вся это играет сильную роль в процессе научного открытия. Однако, нам не следует бросаться и в другую крайность полного цинизма, а именно в представление, что объективная очевидность не играет никакой роли, и что восприятие истины является полностью относительным, и те научные заключения является только лишь другой формой эстетического предпочтения. Наука, как фактически сложилось, является сложным диалогом между фактическими данными и предвзятыми мнениями. Обычно самое большое препятствие к научному новшеству это есть концептуальный (*понятийный*) замок (*стопор*), а не недостаток фактического материала.”

Harvard paleontologist - Stephen Jay Gould

in: NANO-STRUCTURED AQUEOUS SOLUTIONS AS A SUPPORT BASE TO OPTIMIZE COHERENT CELLULAR COMMUNICATION.