Research Communication

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Shilajit Divyarasayan: A Key Component of Expansion Of The Universe

Abstract **: Unified manifestations of the macrocosmic and microcosmic expressions on Earth are expressed by the co-occurrence of C_{60}-fullerene-dibenzo-hydroxy-á-pyrone conjugates (adducts) in meteorites (celestial debris), ammonites (190-200 mybp, extinct group of mollusks), and Shilajit (Multi-component naturally occurring organo-mineral substance. Shilajit is used in Ayurveda as a rejuvenator, energy transducer). Three key celesto-terestial compounds viz. minerals mainly composed of Iron [Fe^{n+}], C_{60}-fullerene, eicosapentaenoic acid and their equivalents and fullerene adducts are primarily responsible for the material expansion of the outer Universe. The biomimetic synthetic aspects of these expressions are now accomplished and reported in this article.**

Introduction : Asteroids are minor planets, those do not have planet-like disk. Millions of asteroids exist in interstellar space of the solar nabula. Although the composition of asteroids varied and poorly understood till today, individual asteroids are classified mainly in three groups: (i) C-type (carbon-rich, major carbon source of fullerene), (ii) M-type (metal-rich organo metallic) and (iii) S-type (silicate-rich).[1] Meteorites are extra-terrestrial debris that fall on earth-surface profusely (10^5 to 10^6 kg/day).[2] Metiorites in space interact with materials and produce varied types of organo-metallic compounds, which fall on earth.[3] Adducts of C_{60}-fullerene and 3-hydroxy-dibenzo-a-pyrone (DBP), are produced from their interactions.[4] Size is one parameter that differentiates asteroids (diameter greater than one meter) from metiorites (diameter less than one meter). Source materials of meteorites are cometory or astrodial.

Shilajit is a pale-brown to blackish-brown exudation of essentially humified ammonites of variable consistencies, found worldwide in sedimentary rocks. It is also found embedded in the interior of the rock sediments. From there, Shilajit is exposed, in association with clay minerals, after landslides, geological excavation or mountain road cutting.

In India, Shilajit is mainly collected from the Himalayas. Plate tectonic movement at the early stage of formation of Himalayas, caused the sea-bed to be pushed up. Repeated plate tectonics seem to produce sedimentary mountains where marine invertebrates such as ammonites were trapped in different racks and humified, mineralized following millions of years of interaction during humification.[4, 5-7]

Micro-bioactivities of Shilajit are primarily dependent on three micro chemical compounds.[8] These are (i) C_{60}-fullerene, (ii) DBP and (iii) mineralized organo-metal ions, e.g. Iron [Fe^{n+}], Copper [Cu^{n+}] and Zinc [Zn^{n+}] and combined adducts resulted thereof e.g. (i-iii).

This paper gives a succinct account of the products providing celesto-terestial substances found in asteroids, meteorites, ammonites and Shilajit. Comprehensive chromatographic (HPTLC), spectroscopic (UV, IR, and mass) evidence followed by cogent synthesis of adducts lend credence to the title subject.

Materials and methods: **Test samples:** Asteroid:[1,3,5] M/5 (2bybp), R/47 (3bybp) (Supplier: Geological Survey of India, Courtesy Dr. A.K. Pal (since retired), GSI, Kolkata, India), Ammonites: [9-11] *Perisphinctes*, *Asteroceras*, *Hildoceras* and *Graphoceras* (Supplier: Messrs Hindusthan Minerals and Natural History Samples Supply Co., Kolkata), Meteorites: [2, 3] Mercedes, (Common chondrite H5), Aldama [B], N.W. Africa (ordinary chondrite LL5) (Supplier: Messrs Impacta, Denever, Colorado, US), Shilajit [5, 7] Divyarasayan collected from different places of the globe. All analyzed samples have been preserved at the R & D centre of Natreon Inc., Branch Office, India (ISO – 9001:2015 certified centre). Interaction of representative members' viz. Asteroid M/5, Mercedes (Meteorite), Perisphinctes (Ammonite) and Shilajit Divyarasayan-I (Figure 1) are shown in this paper.

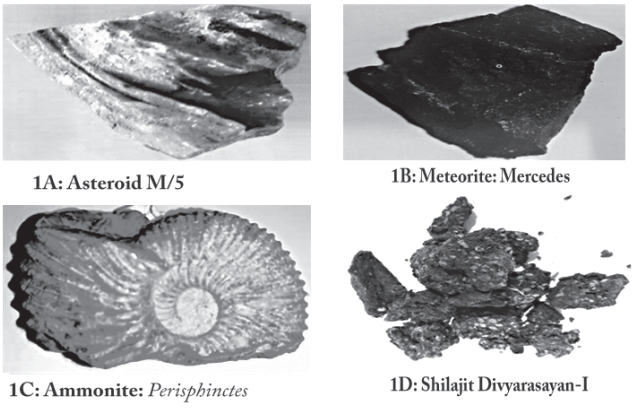


Figure 1. Four test Samples used for experiment: **1A**: Asteroid: M/5; **1B**: Meteorite: Mercedes; **1C**: Ammonite: *Perisphinctes* and **1D**: Shilajit Divyarasayan-I.

Synthesis of C_{60}-fullerene-3-hydroxy-dibenzo-a-pyrone adducts:

Details of operation of Scheme 1

1. C_{60}-fullerene (72 mg, 0.1 mmol, 1 equiv.), DBP(21mg, 0.1 mmol, 1 equiv.), I_2 (catalytic amount, 5 mol%.), 1,8-Diazabicyclo(5.4.0)undec-7-ene (DBU, catalytic amount, 10 mol%) and toluene: methanol (9:1, 10 mL) were taken in 50 mL standard joint conical flasks.
2. The reaction mixtures were chased with nitrogen and the resultant solutions were exposed to (i) direct sunlight (90h) and (ii) UV irradiation (2h).
3. The solvent was evaporated in *vacuo*.
4. Resultant mass was extracted with 50 mL of toluene thrice
5. The combined organic extract was evaporated to dryness, to obtain C_{60}-fullerene-3-hydroxy-dibenzo-a-pyrone adducts.

Synthesis of C_{60}-fullerene-3-hydroxy-dibenzo-a-pyrone-Fe(II) adduct:

Details of operation of Scheme 2

1. C_{60}-fullerene (72 mg, 0.1 mmol, 1 equiv.), DBP (21 mg, 0.1 mmol, 1 equiv), ferrous sulphate (15 mg, 0.1 mmol, 1 equiv.) and toluene: methanol: water (1:8:1, 10 mL) were taken in 50 mL standard joint conical flasks.
2. The reaction mixtures were chased with nitrogen and the resultant solutions were exposed to (i) direct sunlight (72h) and (ii) UV irradiation (2h).
3. The mixtures were spray dried to obtain C_{60}-fullerene-3-hydroxy-dibenzo-a-pyrone-Fe(II) adducts.

Extraction of C_{60}-fullerene-3-hydroxy-dibenzo-a-pyrone adducts:

In a typical experiment, 10 gm of Shilajit Divyarasayan-I was extracted with Toluene: Ethyl acetate (7:3) under reflux condition for 6h. The extract obtained thereof was spray dried. Similar procedure was followed for other three test samples. The extractive values obtained from analyses of samples are given in **Table 1**.

Extraction of C_{60}-fullerene-3-hydroxy-dibenzo-a-pyrone-mineralo adducts:

50 gm of Shilajit Divyarasayan-I was extracted with water under reflux condition for 2h. The extract obtained thereof was spray dried. Similar procedure was followed for other three test samples. The extractive values obtained from analysis of four test samples are given in **Table 1**.

Table 1. Extractive values of different test samples are given below.

Name of the material	Extractive value (% w/w) of Toluene: Ethyl acetate extract	Extractive value (% w/w) Aqueous extract
Shilajit Divyarasayan-I	0.12	27
Asteroid: M/5	0.01	7
Perisphinctes	0.09	12
Mercedes	0.07	9

Instrument used: HPTLC, IR, LCMS

HPTLC conditions for identification and estimation of adducts:

HPTLC was performed on Merck KGaA (1.05554.0007) pre-coated silica gel 60 F_{254} aluminium TLC plates. Synthesized adducts, e.g. C_{60}-fullurene-3-hydroxy-dibenzo-a-pyrone and C_{60}-fullurene-3-hydroxy-dibenzo-a-pyrone-Fe(II) adducts along with extracts of different experimental samples were applied in TLC plates. Plates were developed in a TLC chamber pre-saturated with toluene as mobile phase for C_{60}-fullerene-3-hydroxy-dibenzo-a-pyrone adducts and $CHCl_3$: MeOH (9:1) as mobile phase for C_{60}-fullerene-3-hydroxy-dibenzo-a-pyrone-Fe(II) adducts. Densitometric evaluation of the plates was performed at $ë_{max}$ 254 nm by Camag TLC Scanner 3 in absorption mode. The scanned data were processed by the Camag winCATS software, version 1.3.4. The plates were subsequently scanned to determine the UV reflectance spectra of each spot between 200 and 400 nm to identify the presence of adducts and equivalents in different samples. The estimation of adducts were carried out by the calculation of single point calibration, using peak area

and concentration of the samples, fitted in the equation given below.

% content =

$$\frac{\text{Area of the sample}}{\text{Area of the standard}} \times \frac{\text{Concentration of the Standard}}{\text{Concentration of the Sample}} \times 100$$

Amount of C_{60}-fullerene-3-hydroxy-dibenzo-α-pyrone adducts present in four test samples are listed in Table 2 and amount of C_{60}-fullerene-3-hydroxy-di-benzo-α-pyrone-Fe(II) adducts present in four test samples are enlisted in Table 3.

Table 2. Amount of C_{60}-fullerene-3-hydroxy-dibenzo-α-pyrone adducts.

Name of the material	Amount of adduct w.r.t. extract (% w/w)
Shilajit Divyarasayan-I	0.18
Asteroid: M/5	0.03
Perisphinctes	0.07
Mercedes	0.05

Table 3. Amount of C_{60}-fullerene-3-hydroxy-dibenzo-α-pyrone Fe(II) adducts.

Name of the material	Amount of adduct w.r.t. extract (% w/w)
Shilajit Divyarasayan-I	0.36
Asteroid: M/5	0.02
Perisphinctes	0.11
Mercedes	0.09

IR spectral analysis:

IR spectra were determined in Perkin Elmer FTIR spectrometer (model Spectrum Two). In a typical experiment 100 mg of KBr and 200iL methanolic solution (from 1mg/mL stock slution) of Toluene: Ethyl acetate extract of Shilajit Divyarasayan-1 was taken and oven dried at 105°C for 12h. %T (transmittance) was recorded against blank KBr pellet. Similar procedure was followed for all other test samples.

Mass spectrum of C_{60}-fullerene-3-hydroxy-dibenzo-a-pyrone adducts:

Toluene: Ethyl acetate extract of Mercedes was analyzed in FAB Mass in +ve ion mode to find out the desired mass of adduct.

Results and Discussions: Densitometric evaluation of standard fullerene at λ_{max} 254 nm (scanned between 200-400 nm) showed major 3 absorption maxima centered at

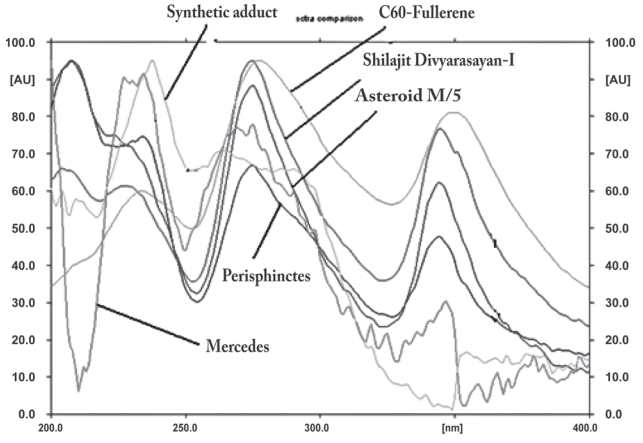


Figure 2. Reflectance Spectrum (UV) similarities between standard fullerene, Synthetic C_{60}-fullerene-3-hydroxy dibenzo-α-pyrone adduct and Toluene:Ethyl acetate extract of four different test samples at R_f=0.99 (Eluent, Toluene).

238, 279 and 350 nm. Comprehensive chromatographic and spectroscopic data of standard fullerene, synthetic adduct and different test samples were superimposable for toluene: ethyl acetate extract at R_f =0.99 (Eluent, Toluene, Figure 2). There is a decrease in sensitivity in absorption spectra was observed for densitometric evaluation of aqueous extracts containing C_{60}-fullerene-3-hydroxy-dibenzo-α-pyrone-Fe(II) adducts presumably because of chelation with metal ions. Superimposable spectroscopic data of synthetic C_{60}-fullerene-3-hydroxy-dibenzo-α-pyrone-Fe(II) with those of aqueous extracts of natural test samples were assigned at R_f=0.44 (Eluent, $CHCl_3$:MeOH (9:1), Figure 3).

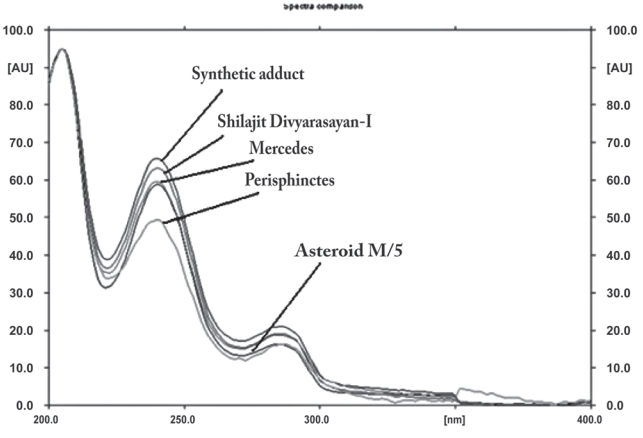
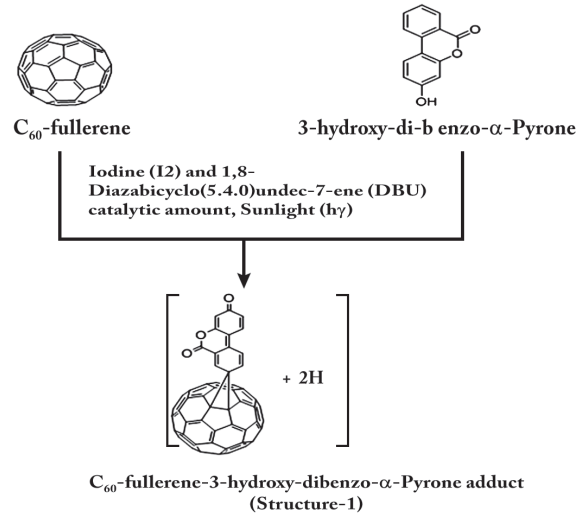


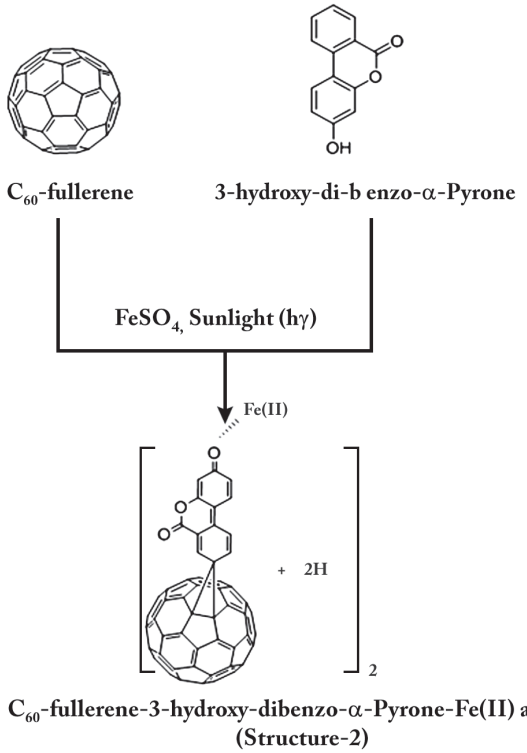
Figure 3. Reflectance Spectrum (UV) similarities between synthetic C_{60}-fullerene-3-hydroxy-di-benzo-α-pyrone- Fe(II) adducts and aqueous extracts of four different Shilajit samples observed at R_f=0.44 (Eluent, $CHCl_3$:MeOH (9:1)).

Presence of different carbonyl groups and characteristic feature of reduced fullerene (fullerane) moiety in structure-1 and structure-2 (Scheme 1 and Scheme 2) have been analyzed by IR spectral fineness.



Scheme 1. Synthesis of C_{60}-fullerene-3-hydroxy-dibenzo-α-pyrone (Details of operation).

Literature reported values for six member lactone ring, conjugated and aromatic carbonyls are strikingly superimposable. These signals appeared at 1735 cm^{-1}, 1680 cm^{-1} and 1670 cm ^{1}, respectively.[12]



Scheme 2. Synthesis of C_{60}-fullerene-3-hydroxy-dibenzo-α-pyrone (Details of operation).

Analysis of toluene: ethyl acetate extract of Shilajit Divyarasayan-I showed strong carbonyl bands in the range of 1670 to 1766 cm^{-1} with a peak centered at 1733 cm^{-1}. Literature report for low hydrogen exposed reduced fullerene (fullerane) showed a broad band centered at 2900 cm^{-1} and extending upto 3000 cm^{-1}.[13] In toluene: ethyl acetate extract of Shilajit Divyarasayan-I similar broad band centered at 2923 cm^{-1}, was observed. These peaks indicate the presence of different carbonyl function groups attached to fullerene moieties.

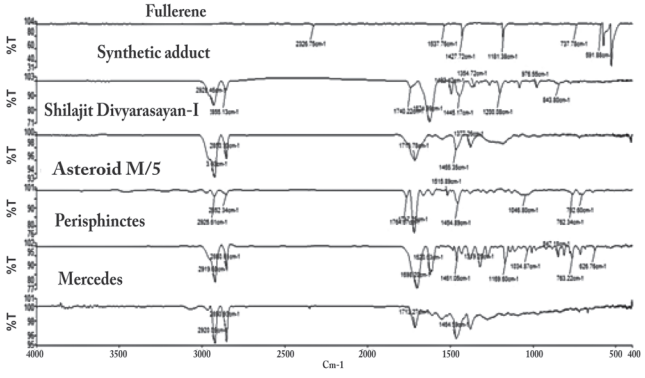


Figure 4. IR spectrum of synthetic C_{60}-fullerene-3-hydroxy-di-benzo-α-pyrone adduct, fullerene and Toluene:Ethyl acetate extracts four of different test samples.

Similarities (Figure 4) in tolune: ethyl acetate extracts of other test samples were observed: in Asteroid: M/5 carbonyl band appeared at a range of 1670 to 1770 cm^{-1} with the peak centered at 1747 cm^{-1} and the peak of fullerane appeared at 2929 cm^{-1}. For *Perisphinctes* carbonyl band appeared at a range of 1665 to 1766 cm^{-1} with the peak centered at 1698 cm^{-1} and peak of fullerane appeared at 2919 cm^{-1} and for Mercedes carbonyl band appeared at a range of 1670 to 1773 cm^{-1} with the peak centered at 1713 cm^{-1}; the peak of fullerane appeared at 2922 cm^{-1}.

Similar observations were recorded for dried aqueous extracts of different test samples (Figure 5) containing C_{60}-fullerene-3-hydroxy-dibenzo-α-pyrone-Fe(II) adducts. A shift, as well as decrease in sensitivities of carbonyl bands, in IR spectra of the test samples due to chelation with metal ions was observed. In Shilajit Divyarasayan-I carbonyl bands appeared in the range of 1659 to 1738 cm-

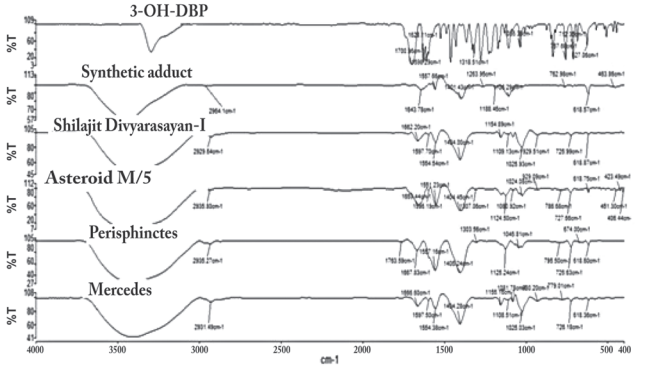


Figure 5. IR spectrum of synthetic 3-hydroxy-di-benzo-α-pyrone (3-OH-DBP), C_{60}-fullerene-3-hydroxy-di-benzo-α-pyrone)-Fe(II) adducts and aqueous extracts four of different test Shilajit samples.

[1] and reduced fullerene peak appeared at 2929 cm^{-1}. Close similarities (Figure 5) in aqueous extracts of other three test samples were observed.

The FAB-MS spectrum (Figure 6) yielded M^+ at m/z at 743.1 amu which was due to $[fullerene+Na]^+$. Another peak appeared at m/z 933 $[M+H]^+$ which was assigned to C_{60}-fullerene-3-hydroxy-dibenzo-a-pyrone (Structure 1).

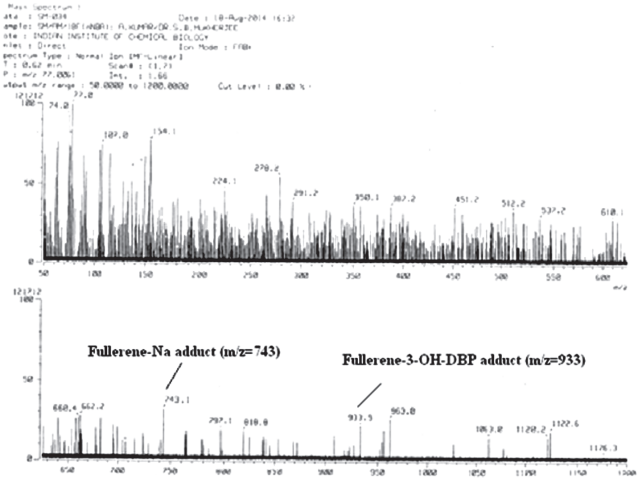
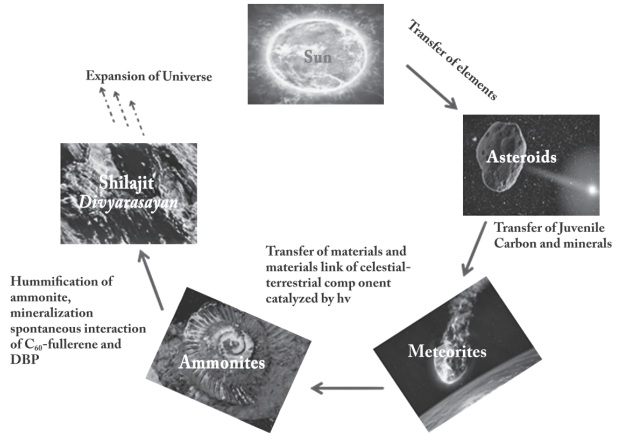


Figure 6. Mass spectra Toluene: Ethyl acetate extract of Meteorite: Mercedes, evident for fullerene-Na and C_{60}-fullerene-3-hydroxy-di-benzo-α-pyrone adducts.

On the basis of the above findings, one would conclude that, three types of obligatory- micro constituents of the outer Universe viz. C_{60}-fullerene, DBP and minerals, mainly composed of Iron $[Fe^{n+}]$, and the various combinations thereof would cause growth and maintenance of the outer Universe. These compounds would come to the earth by carriers like asteroids, common chondrites and carbonacious chondrites. When the contour of the Universe is saturated with the said bioactives, the inner equilibrium movement is perturbed and expansion of the order Universe would take place by a synchronous process.



Scheme 3. Sequence of material transformations leading to expansion of material and spiritual Universe.

These compounds when present in isolation are short lived while in the milieu of adducts (structure 1 and 2) would live unperturbed for eons. The adduct-compounds and their further transformed products are ceaselessly being produced and carried to different areas of the outer universe thereby enriching and expanding the boundaries of the Universe (Scheme 3).

It may be recalled that in The Vagbat Gita, Arjuna, in awe after his "Vishwaroop Darshan" exclaimed similar "Boundary-less expansion of the Universe." [14]

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SHIBNATH GHOSAL*,
A. V. MURUGANANDAM,
DEBKUMAR NANDI

Technical Advisor, Natreon Inc., Rishi Tech Park,
Premises no- 02/360, Action area 1D, New Town,
Kolkata -700 160
*Corresponding author.
Email: natindiainc@gmail.com

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