

## Life Outside Planet Earth: Biological and Stratospheric Samples and Their Relation to Interstellar Grains

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**Abstract:** Composition, shape, size, nature and formation of the interstellar grains has posed a real dilemma for astronomers in particular and scientist in general. This is evidence from the substantial efforts on the part of many scientists from many branches of science since the first recognition that grains exist some 70 years ago. Although many observational data have been obtained, great deal of theoretical calculations also made, especially during the 1960's and 1970's still there was little agreement among astronomers about the nature and composition of the interstellar grains. Studies of five interstellar dust particles analysed by mass spectrometers aboard the Stardust spacecraft showed heteroaromatic cross-polymers as high-impact break-up products. This material has structural strengths similar to bacterial cell walls. Air samples collected aseptically over tropical India at altitudes ranging from 20km to 41 km using cryosampler assemblies on balloons have shown evidence of living microbial cells at all these altitudes. Clumps of viable sells were found at the height of 41 km well above the local tropopause; a prima facia case for a space incidence of these microorganisms is established.

**Keywords:** *Biological, Stratospheric*

### Introduction

The wavelength dependence of interstellar extinction, which at visual wavelength has an average value of approx. 2 mag. kpc (Magnitude per kilo parsec, 1 kpc = 3.1 light year or  $3 \times 10^{21}$  cm.) in the direction close to the plane of the Milky Way galaxy, is the most direct observational test of any interstellar model. Combining this datum with other scattering and polarization observations leads to the result that the grains are strongly dielectric with radii about 10-5 cm and they make up a few percent by mass of all the interstellar matter [1].

Most of the interstellar medium is comprised of hydrogen and helium. The chemical elements Carbon, Nitrogen, Oxygen and Neon make up a total relative abundance to Hydrogen by number approximately 10-3. Magnesium, Silicon, Sulphur and Iron make a total relative abundance of about  $10^{-4}$  to  $10^{-5}$ .

A wide range of materials involving carbon, nitrogen, oxygen in various combinations with hydrogen, remains in the field of possible grain compositions. One dielectric type of such material is organic grain, made up of a mixture of organic molecules and/or bio chemicals. Sir Fred Hoyle and Professor Chandra Wickramasinghe back in the 1970's [2] were the first scientists establishing that most of the interstellar grains are organic/biological. Organic molecules have been detected in the interstellar medium by radio astronomers for some years. Molecules such as methanol at 834 MHz, formic acid at 1638.805 MHz and cyanodiacetylen [2], the total number of interstellar molecules discovered until now exceeds one hundred. These discoveries have paved the way for organic materials to share the field with the inorganic material as a major contributor to the interstellar material. The interstellar medium, in general, contains solid grains, radicals, molecules, ions, together with ultra violet photons have overall conditions in the dens molecular clouds that favour condensation of complex organics.

Amino acids such as alanine, glutamic acid, praline and glycine were detected in Murchison meteorite. It is essential that about  $3 \times 10^{14}$  gm of amino acids could have been deposited on Earth by meteoritic influx during the time from the origin of the Earth to the time when life is thought to have begun [3].

The average hydrogen mass density in the solar neighbourhood is  $2 \times 10^{-24}$  gm cm<sup>-3</sup>. The number density of hydrogen corresponding to this mass density is 1.2 atom cm<sup>-3</sup>. The maximum special density of the available grain forming material is  $2.6 \times 10^{-26}$  gm cm<sup>-3</sup>.

Observations of the material (gas and dust) lying between Earth and the centre of the galaxy show absorption of light, which is wavelength dependent. There is an absorption band at lambda wavelength 2.4 micron due to carbon monoxide and another broader absorption at 3.4 micron, which is due to CH stretching. The 2.4-micron absorption can be explained by the presence of CO in gaseous phase, the 3.4-micron absorption is much larger than be attributed to gaseous CH or other CH bands in other gaseous organic molecules. Hence the 3.4-micron absorption must be attributed to linkages present in solid particles apparently of organic nature. In the beginning of the 1980's our group in Cardiff (Hoyle, Wickramasinghe & Al-Mufti) started using completely different type of material to explain the extinction produced by the interstellar grains. This material was biological i.e. possibly bacteria. The first step was to find the refractive index (RI) of the postulated grain; bacteria under astronomical conditions. It is well known that under normal condition on earth, about two third of the volume of a bacterial cell is occupied by free water with RI of 1.39. A dry bacterium (removing all the water from the cell) has a RI of 1.167.

The available abundances of elements in the interstellar medium will be sufficient for this type of grain, carbon and oxygen atoms are equally abundant in biological material, and on the other hand oxygen atoms are

nearly twice as abundant as carbon in cosmic material. It is known also that most of the interstellar carbon is tied up in grains. From these two factors it follows that one oxygen atom is left over for every carbon atom present in biological material provided the grains are bacteria type.

There are vast numbers of bacterial species, making it very difficult to determine strictly weighted size distribution, therefore selection criteria is needed. Hoyle and Wickramasinghe [4] used a procedure to restrict the sample to spore-forming bacteria. Figure 1 shows the size distribution obtained. A question might be asked about the ability of bacteria surviving the harsh conditions in interstellar space, which is due to the belief that most biotic materials would have been destroyed at a temperature of 250°C. Microorganisms can survive as well as grow at temperatures down to -18°C [5], whilst survival alone occurs for freeze-dried microorganisms at temperatures of only a few degrees above absolute zero. Microorganisms can survive temperatures above 350°C and can grow at 250°C [6,7]. Survival and growth of bacteria under extreme conditions of high doses of radiation, low water activity, high values of acidity, very low pressure conditions, etc., are well known and documented in the literature.

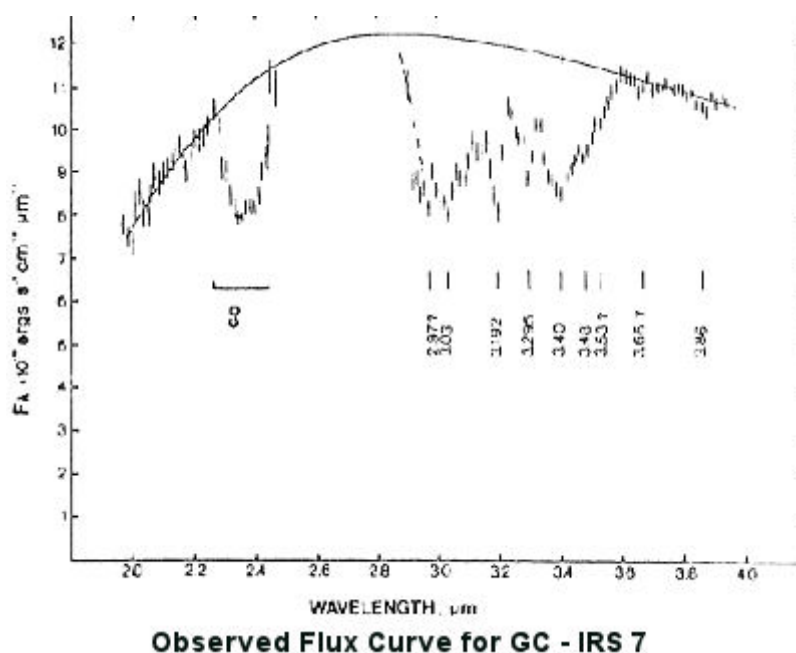


Figure 1: The laboratory spectra of biological material. A broad absorption band between 2.9 m and 4.0 m has been observed with indications that complex organic molecules are involved

We carried experiments to substantiate this; microorganisms were sealed inside potassium bromide discs using pressure of 7 tonnes per square cm, then this disc was placed inside a furnace with a temperature regulator for various lengths of time. Microorganisms such as *Escherichia coli*, blue-green algae, diatoms and dry yeast were used in the experiment, using temperatures as high as 400°C, well above what usually suggested [8]. A good example for this ability is the black smoker bacteria, which can live, grow and retain their viability at temperatures well above 250°C.

The other question related to this mater is the amount of small particles entering the Earth's atmosphere and the effect of ultra violet (UV) radiation on them. The amount of material entering the Earth's atmosphere is estimated at about 1000 tonnes per year, if only 1% of this mass is biological, this represent 10 tonnes per year in another word,  $10^{20}$  units per year. The UV radiation at a wavelength of 0.2 micron is damaging to the genetic material, this damage occurs by changing the bonding of the bases in such a way that they tend to stick together in pairs, specially when the base Thymine is involved. Bacteria posses a remarkable protective mechanism against any damage done by UV radiation. This is the enzymic repair mechanism where an enzyme is able to reverse the effect of the UV light with respect to the sticking of bases in pairs, returning them to independent operation; or in some cases snipping out a damaged structure and returning the bacteria to a viable condition. Microorganisms are variable in their UV resistance from species to species, and also within the same species, different strains can be considerably viable to the dose of UV light. For example, *Sarcina lutea*, *Bacillus globigii* spores and radio resistance *Micrococcus* are three different bacterial species, the tolerance of each strain exposed to UV light ranges to approximately 210, 3210 and 10700 erg.cm<sup>2</sup> respectively.

The other radiation abundant in space is X-Rays, which causes damage to microorganisms by generating photoelectrons within them, these photoelectrons then cause breaks in the DNA & RNA. This kind of damage is the same as that caused by high-speed particles generated by radioactivity. In many experiments carried out, it is noticed that the percentage of viable bacteria fell rapidly with increased dose, the dose ranging between 150 kilo rad and 2.5 mega rad [9-12]. Nasim and James [13] carried out experiments by exposing the bacterium *Micrococcus dariophilus* to radiation causing breaks in the DNA linkage of the order of 10000 breaks. The bacteria survived by the process of sniping and inversing base copying and managed to repair the whole of the damage caused by the radiation.

### **Earth Atmosphere**

The lower layer of the atmosphere is the Troposphere, extending from sea level to an altitude of between 8 km at the poles and 18 km at the tropics. The Troposphere contains about two third of the atmosphere by mass and is the layer of clouds and weather systems. It is a region heated from the ground by infrared radiation and convection. The temperature falls with increasing height to reach a minimum of about -55oC at the mid altitude and Polar Regions and to about -80°C at the equatorial region. The upper height of the Troposphere where these low temperatures are reached is called the Tropopause. All the weather changes are limited to this layer, which also has a constant atmospheric turbulence, the most familiar example of which is the "jet stream" which consists of a thin current of air that flows from west to east at a height of 10.5 km with wind speed of km hr<sup>-1</sup>.

The layer above the Tropopause is the Stratosphere, which is an atmospheric layer in which temperature is at first steadily decreasing with altitude before increasing to a maximum of 0oC at about 50 km, which marks the Stratopause. The origin of this rise in temperature from about 12 km, on average, is due to the degradation of a portion of the solar radiation to thermal energy through the agency of primary absorption by Ozone molecules. These molecules are themselves formed by the action of UV radiation on oxygen molecules. As the ozone layer absorbs the UV radiation, it heats up and the re-radiation in the Infra Red (IR) radiation, which produces the energy balance and hence the stable temperature profile. This temperature variation inhabits vertical air movements and makes the stratosphere a very stable region.

An extensive calculation for the altitudes between 30 and 100 km found that below latitude of 40o and 50o each point in the stratosphere is balanced by radiation alone, no vertical transfer of energy of any significance is required for stability. Because of the stratosphere's inverse gradient of temperature versus altitude, there will be no vertical adiabatic transfer of matter and energy [14].

The next layer above the stratosphere is the Mesosphere in which the temperature falls with height to reach about -90oC at the Mesopause, which is at the top of this layer at a height of 85 km. This layer is identified by a strong temperature decrease from the maximum temperature zone in its lowest part.

From this brief discussion, it seems that below 25 km altitude, horizontal transport is dominated over vertical process, and it is manifested by high velocity winds. So it is obvious that the convection currents in the atmosphere, which are responsible for carrying small particles high in the atmosphere, normally stops at the height of about 15km. Another reason for the improbability of carrying microorganisms upward from the Earth, under normal conditions, is gravity. Earth gravity has a significant downward pull on particles with the size of bacteria, particularly in the thin air above 50 km.

### **Interstellar Grains might be biological in origin**

Spectroscopic technique are used to observe three main types of molecular transitions, which are:

1. Electron Transition: Usually observed in the ultra-violet and the visible Regions of the spectrum
2. 2. Vibration-Rotation Transitions: Observed in the near infrared region of the spectrum.
3. 3. Pure Rotational Transitions: Observed in the far infrared and the microwave region of the spectrum.

The boundaries between the above transitions are only loosely defined, and indeed some overlapping does occur.

A molecule can be considered as a group of atoms bound together in a certain geometrical configuration by elastic bonds formed by the electron cloud around the atomic nuclei. The vibration of the atoms about the main position is due to these elastic bonds. To describe the movement of an object in space, six degrees of freedom are required, three for rotation and three for translation. Thus for a molecule of N atoms, six degrees of freedom are reserved for rotational and translational movements, leaving  $3N-6$  degrees for the movement of the atom inside the molecule, so the molecule will give rise to at least  $3N-6$  absorption bands somewhere in the infrared region. The degree of absorption is a measure of the quantity of molecular species present in a sample, where the frequency at which absorption bands are observed indicates the type of molecular group present in the molecule.

The laboratory spectra of biological material is largely unaffected by temperatures over the range considered, and it is also unaffected by the precise nature of the specimen as long as it is biological. The feature of the astronomical infrared spectrum near 3.4 m might be consistently attributed to biological materials. Observational results for the astronomical infrared source GC-IRS 7, this source is located at near the centre of our galaxy, the Milky Way, at a distance of 10 Kpc ( $3 \times 10^{22}$  cm). A broad absorption band between 2.9 m and 4.0 m has been observed with indications that complex organic molecules are involved. This spectrum is shown in Figure 1 [15]. The detection of the 3.4 m absorption band in several sources established the fact that the C-H band might be a common feature of the material of the interstellar grain. To observe the C-H band at 3.4 m, it is essential, that the water ice must be absent and the amount of visual extinction is very high. These conditions are satisfied along the line of sight to GC IRS 7 at a distance of 10 kpc.

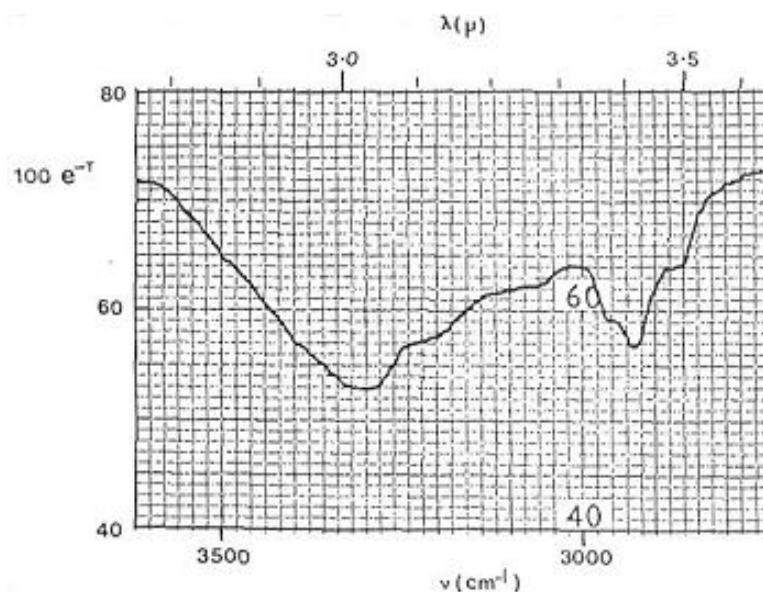


Figure 2: The infrared spectrum of the E. coli heated to 350°C at the wavelength 2.7-3.6 m.

To compare the spectrum of Figure 1 with that of a biological spectrum, *E. coli* bacteria were used. Using about 1.0 mg of dried bacteria mixed with 250 mg of KBr. The infrared spectrum of the *E. coli* heated to 350°C at the wavelength 2.7-3.6 m is shown in Figure 2. The transmittance values from this spectrum were used to calculate theoretical flux values for the bacterial model for comparison with the astronomical data.

Figure 3 shows the detail agreement between the spectrums of the source GC IRS 7 in the wave band 3.3-3.9 m. The maximum departures are no more than 2% with the astronomical observation. Comparing the values of visual extinction coefficient of 57000 cm<sup>2</sup> gm<sup>-1</sup> with the value of 750 cm<sup>2</sup> gm<sup>-1</sup> we find that the visual extinction is approximately 76 times larger than the extinction at 3.4 m, this gives us the value of 36.5 mag for the visual extinction to the source GC IRS 7.

The mass required to produce this effect is  $7.57 \times 10^{-4}$  gm.

The required average grain density along the  $3 \times 10^{22}$  cm line of sight from the Earth to the IRS 7 will be  $2.52 \times 10^{-26}$  gm cm<sup>-3</sup>.

The grain density needed to explain the observed extinction curve must be at least about  $5 \times 10^{-3}$  x of the hydrogen density; where is the density.

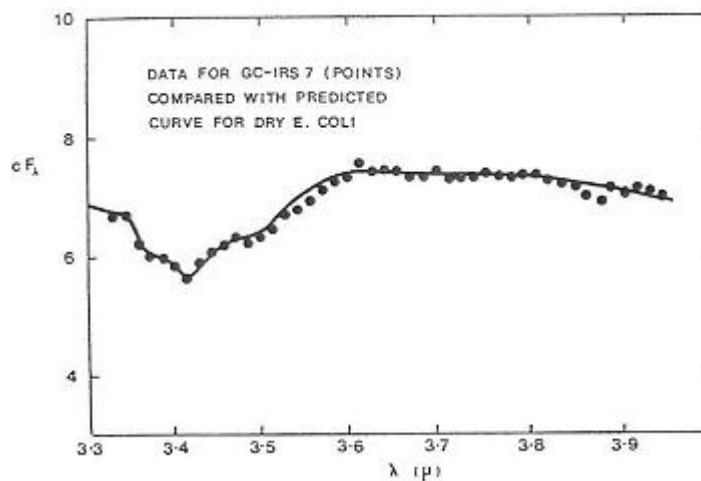


Figure 3: The detail agreement between the spectrums of the source GC IRS 7 in the wave band 3.3-3.9 m

On searching the literature for non-biological materials that might give a tolerable approximation to the astronomical data, none is found, except two cases where "least bad" fits were obtained. Khare and Sagan [16] produced one of these spectra; in their experiment where inorganic gases (CH<sub>4</sub>, C<sub>2</sub>H<sub>6</sub>, NH<sub>3</sub>, and H<sub>2</sub>S) and liquid water were irradiated with ultra violet light. The infrared spectrum obtained by this process shows a poor agreement to the astronomical observation. In 1979, Sagan and Khare [17] put forward the idea that complex organic solids produced from cosmically abundant molecules may explain the infrared spectra of the

interstellar dust. They produced a synthesised material called Tholin from two different processes. The first produced is called ultraviolet Tholins produced by near ultraviolet (2537 Å Hg lamp) photo dissociation of H<sub>2</sub>C and HCHO, using initial reactants of CH<sub>4</sub>, NH<sub>3</sub>, C<sub>2</sub> H<sub>6</sub>, H<sub>2</sub>O and H<sub>2</sub>S. The second product is called spark Tholin, first made by Miller [18], in which initial reactant CH<sub>4</sub>, NH<sub>4</sub>, and H<sub>2</sub>O are used and the energy source of irradiation is a high-frequency Tesla coil.

Moore and Donn[19], Butchart and Whittet [20] also produced materials from inorganic materials but the match of their spectra to the astronomical spectra were very poor.

### **Detection of Viable cells in the stratospheric region:**

A balloon launched in the early hours of 21<sup>st</sup> January 2001 from the National Scientific Balloon Facility of the Tata Institute of Fundamental Research at Hyderabad, India, carrying a scientific payload to sample the stratosphere under the most stringent aseptic conditions. The payload included a cryosampler manifold with fully sterilised evacuated stainless steel probes each with a 0.35 litre capacity and capable of withstanding a pressure in the range 10<sup>-6</sup> mb-600 bar. The evacuated probes were cooled in liquid neon to produce the cryopump action with sterilised valve fitted which can be opened at a pre-determined height, between 20 and 41 km, through ground station telecommand.

From each probe, in a sterile system, air was passed in a sterile in a laminar flow chamber; first through a 0.45 µm pore filters then through a 0.22 µm filter.

Approximately 4 mm<sup>2</sup> squares were aseptically cut from the filters and treated with either a fluorescent cationic carbocyanine or an anionic oxonol dye sensitive to membrane potential.

The cationic dyes penetrate the cell membrane of viable cells, but not of dead cells, whilst anionic dyes penetrate the membranes only of non-viable cells. Lloyd and Hayes [21] and Lopez-Amoros et al, [22], describe these techniques. Any viable living cells present in the sample would be expected to give rise to fluorescent spots when illuminated with UV light and could be identified under an epifluorescence microscope. Each such spot, depending on resolution, would represent either a single cell or a lump of cells. From this experiment, isolates treated with cyanine dye showed fluorescent spots in the form of lumps of 0.3-1.00 µm sized cells, the clumps themselves measuring 5-15 µm across. The detection of viable cells by this technique (not found in the sterile controls) is beyond doubt.

The use of anionic dyes revealed a comparable detection rate of dead or non-viable cells. On each of the micro pore examined, measuring 2 x 2 mm, a number N in the range 1<sup>-3</sup> microbial clumps were found. Since the air volume passing through each filter is about 80 litre NTP and the area of

the entire filter is 2000 mm<sup>2</sup> with N=3, the density of the microbial clumps at 25 km is estimated as:

$$[3 \times 2000 / 4] / 80 = 18.75 \text{ per litre at NPT}$$

The atmospheric temperature at 25 km is 0.025 bar, and then the estimated number density on microbial clumps at this altitude is 0.47 per litre.

At a height of 40 km the average number of clumps per 4 mm<sup>2</sup> of membrane filter, N =1, the NTP equivalent volume of air passing through 2000 mm<sup>2</sup> of this filter being 18.50 litre and the ambient air pressure for this altitude being 0.0025 bar, we can estimate a density of clumps about 0.068 per litre.

On the basis of the in fall of micron size clumps in a standard atmosphere, the steady-state number density of particles at 40 km is expected to be one tenth of that at 25 km [23], which is consistent with what is observed, within the accuracy of the measurement.

Since the local tropopause over the launch site was estimated at 16 km, the isolates are all above the level at which any terrestrial contamination can be expected, particularly at the 41 km altitude. With an average falling speed for 3 micron sizes clumps of 40 km of about 0.3 cm sec<sup>-1</sup> [24], the in fall rate of clumps, assuming number density of 0.068 per litre, over the entire Earth area of 5 x 10<sup>18</sup> cm would be:

$$\{0.068 \times 10^{-3}\} \times 0.3 \times 5 \times 10 \times 10^{18} \text{ per second}$$

Assuming an average of 100 individual bacteria cells each of mass 3 x 10<sup>-14</sup> gm in a clump we obtained a daily mass input of about a third of a tonne of biomaterial. A prima facia case for a space incidence of bacteria onto the Earth may have been established.

For the first time a material was found that could match the astronomical observations over a wide range of wavelengths without invoking artificial choices of size and shape, as in earlier theoretical models.

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