MICROSCOPIC CHARACTERIZATION OF PRIMORDIAL PHOTOAUTOTROPHIC SELF-SUSTAINING SUPRAMOLECULAR ASSEMBLIES "JEEWANU"

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ABSTRACT

The 1:2:1:1 Jeewanu mixture shows the photochemical formation of protocell-like microstructures 'Jeewanu' (1970). The optical and electron probe microscopic observations shows that 'Jeewanu' have distinct structural organization and were able to show some properties of biological orders.

The 'Jewanu mixture' satisfies the optimal conditions for self-assembly and self-organization and emergence of minimal protocell-like system.

Key Words : *Jeewanu, Protocell, Microstructures, Protocell-like model*

INTRODUCTION

The scientific assumption about origin of life suggested that life originated In hot springs on land or hydrothermalvent on the ocean floor **(1,2)** at mesoscopic level and specific physicochemical conditions,the self-assembgy and self-organization of prebiotic molecules in specific steric position led to the formation of protocell-like intities, capable of showing primitive characteristics of biological order **(3).**

Oparin**(4)** and Haldane **(5)** suggested "Molecular and chemical evolution hypothesis."Haldane **(5)** discussed a genetic view of life, that first life sere probably large molecule synthesized under the influence of suns radiation.

Early Earth possessed a variety of chemicals like hydrophobic amorphous silica **(6)**, mixture of inorganic and organic molecules, hygroscopic salts and bulk of water **(6).** Highly polar environment was responsible for the hydrophobic effect (7), liquid-liquid phase separation and promotes amphiphile self-assembly into micellis, membrane bound compartmentation and organized structural configuration.The first organic synthesis may have been driven by mineral-assisted photoactivation, allowing photo-dependent heterotrophic origins of life **(8).**All these reactions require an energy source with a geological setting that can fuel chemical reaction.

Conclusively, it can be said that the combination of prebiotically plausible organic molecules in a plausible primitive reducing environment would have led to the formation of a variety of complex, aggregated and organized structural configuration.In these process of shaping morphologies both inorganic and organic molecules playa a significant role.

So an attempt was made to microscopic characterization of photochemically synthesized protocell-like microstructure "Jeewanu" synthesized in sterilized aqueous mixture of some inorganic and organic substances using sunlight as a source of energy.

EXPERIMENTAL

Photochemical synthesis of protocell-like microstructure "Jeewanu"

The protocell-like microstructures "Jeewanu" were photochemicallysynthesixed in a sterilized aqueous mixture of some inorganic and organic substances using sunlight as a source of energy **(9).**The ultra-fast laser induced flesh photolysis observation have confirmed the photochemical formation of photoproducts “Jeewanu” on giving flesh of 10-9, 10-20 nano seconds **(10)**

Filteration – After appropriate hours of exposure in sunlight the exposed Jeewanumixtue was filtered on 0.2 pm. Pore size of nucleoporewhat man filter paper.The precipitate was collected and air dired using vacuum desiccator.

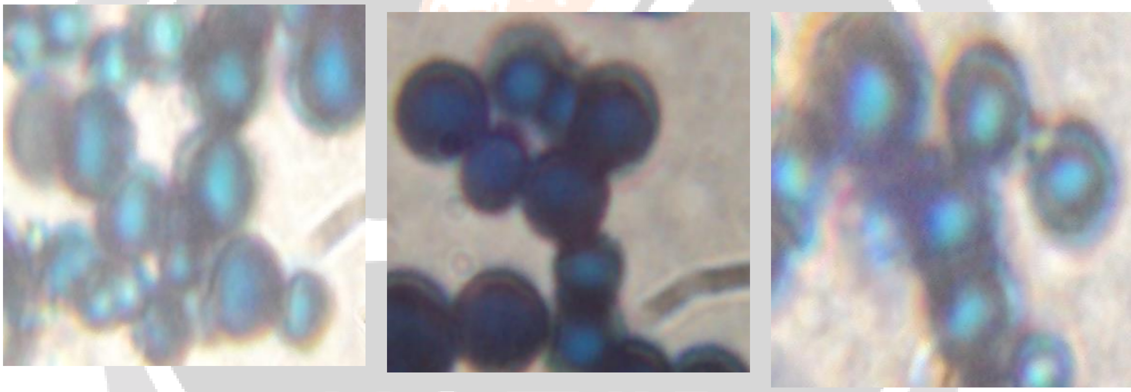
MICROSCOPIC CHARACTERIZATION

To obtain structural characterization, biomimic functions, high quality microscopic images and extract in depth structural information of “Jeewanu” a combination of instrumental tools (e. g. Optical microscopy, Phase contrast microscopy, Fluorescence microscopy, Confocal microscopy, Atomic force microscopy, Scanning electron and Transmission electron microscopy) have been employed.

The morphometric and photo micrographic investigation of Jeewanu was carried out under optical microscope using image analysis software (Magnus pro/ Olympus). The phase contrast microscopy, were conducted in Guru Ghasidas central university bilaspur (C.G.) India.The Electron microscopic studies (SCM and TEM), confocal microscopy, were conducted by the help of Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi, India.

1. Optical Microscopy

Optical microscope has been used to understand the dynamic behavior of “Jeewanu” without invasive sample fixing and staining. A smear of a drop of sunlight exposed “Jeewanu” were prepared and examined under different magnification.



A **B** **C**

Fig. Optical Micrograph of Photochemically formed “Jeewanu” (24 Hours exposure).
08 Hours Exposure.
12 Hours exposure.
24 Hours Exposure.

The optical microscopic observation revealed that the photochemically synthesized “Jeewanu” are spherical in shape and blueish in colour.They have a double walled boundary and an intricate internal structure.Their size varies from 0.5μ to 3.5μ in diameter.tThe presence of spherical budding vesicles of different size in suspension mixture.

2. Phase contrast microscopy

Phase contrast microscopy is based on different refractive index and thickness of sample.They have a special objective they can translate the phase differences of transmitted light in to visual amplitude or brightness, change and display the structural information in high contrast bright field image in grey back ground.It has a special objective that converts the phase difference of the transmitted light into visible amplitude or brightness, and displays structural information as a high-contrast bright-field image in gray background.Phase contrast micrographs of the sample were taken on a Nikon Inverted microscope with a 400 X magnification.

The phase contrast micrographs of Jeewanu shows that photoproducts were enclosed a thin surface membrane.A clear dark brownish-black mass is visible at central regionand intricate internal structural configuration that possess a differential refractive index. In various region of "Jeewanu". The extra central region showed the presence of relatively less dense brownish-black shades at different regions.The differential interference contrast microscopy revealed a 3 dimentional image of the sample.

3. Fluorescence microscopy

Florescent microscope is used in characterization of Integrity**(11),** distribution and localization of internal chemical substances**(12)**in photochemically synthesized "Jeewanu". Florescent microscopic view of "Jeewanu" provide the localization of molecules inside the protocell.

4. Confocal microscopy

Confocal microscopy was used to understand the 3D conformational characteristics and internal structure of photoproduct.

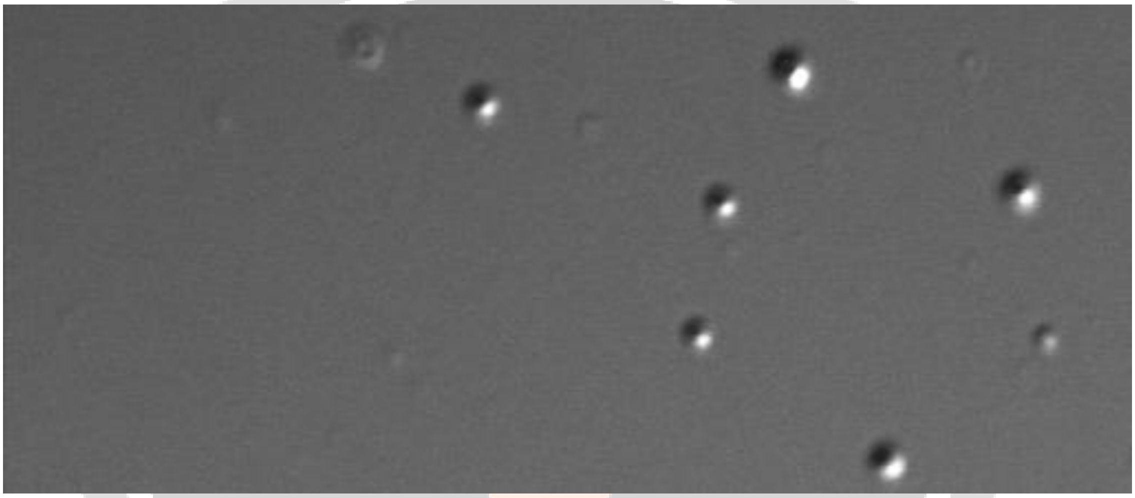


Fig. Confocal Micrograph of Photochemically formed "Jeewanu" (24 Hours exposure).

Confocal micrographs revealed the presence of circular and organized structural configuration.The distinction between central and extra central region clearly visible.The internal structure of Jeewanu appeared heterogeneous in nature.

The 3D conformation of sample revealed the anisotropic morphology.During confocal analysis, the presence of inorganic compounds in solution influence the complexity of Jeewanu.

5. Atomic force microscopy

Atomic force microscopy is a surface-influenced analysis technique that provides high resolution nanoscale image of the sample.

The AFM image provides a high resolution and 3dimentional image of the outer surface of "Jeewanu". The AFM studies revealed the organized structural configuration of "Jeewanu" at nanoscale.

6. Electron Microscopy

Illumination source used in electron microscope and detector have provide a versatile platform to investigate the thermodynamic and kinetic behavior of protocell across a range of scale. Electron microscope provide high resolution image of Jeewanu.

6.1 Scanning electron microscopy

Fig SEM micrograph of Jeewanu (24 hours exposure)

The scanning electron microscopic observations shows that Jeewanu are spherical in shape.Their size varies from 0.2µ to 3.5µ in diameter.The surface topology of microstructures appeared smooth.The large particles consists small elevations in their outer surface showed the transitional stage of budding.The separating junctions of the growing and budding units were clearly visible.The differences in the size of particles were showed that newer units come from parental unit which is larger in size by budding.

6.2 Transmission electron microscopy

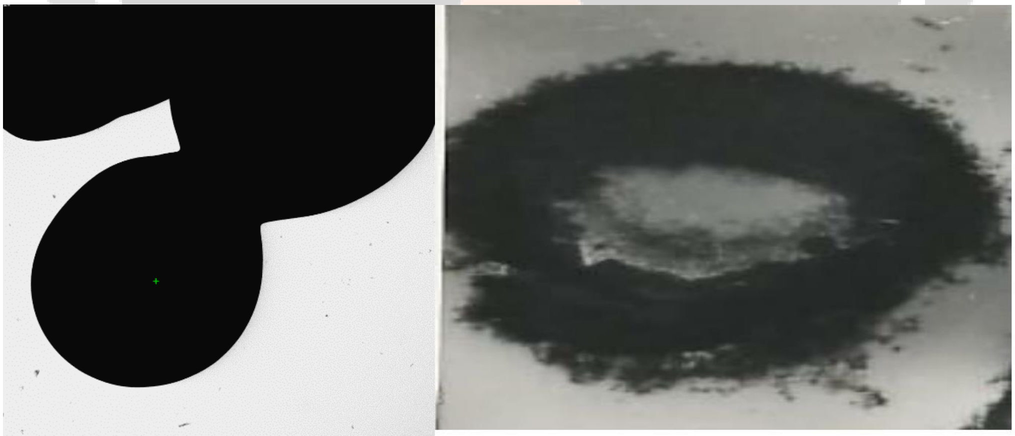


Fig TEM micrograph of Jeewanu (24 hours exposure) Curtsey Dr. V. K. Gupta.

The transmission electron micrographs of Jeewanu showed that the Jeewanu are spherical in shape.Jeewanu at different size are visible.Their size varies from 0.2µ to 3.5µ in diameter.Images showed that newer units come from larger older units.A larger unit (2.5µ to 3.0µ) contains small, tiny, spherical protuberance on their outer surface.The protuberances of different size are found.Separating junctions of multiplicating units were clearly visible. (Fig. no. 2.4.1)

CONCLUSION

- The phase contrast microscopic observations showed the heterogeneous nature of Jeewanu. The central and extra central region revealed differential refractive index.

- The confocal microscopic study revealed Jeewanu are spherical in shape and have a definite boundary wall. The internal structural organization of Jeewanu is anisotropic and heterogeneous in nature. Diversity in their size showed transient stages of their formation.
- The scanning electron microscopic investigation further confirms that "Jeewnu" are spherical in shape and their surface topology was smooth. They were capable of showing growth from within by actual synthesis of material and multiplication by budding.
- The transmission electron microscopic study of Jeewanu revealed structures that are possible intermediate in growth and division. The presence of definite boundary Wall.

This study offers detailed insights into the microscopic characterization of "Jeewanu" The protocell-like microstructures synthesized photochemically,. Utilizing advanced microscopy methods such as optical, phase contrast, fluorescence, confocal, atomic force, and electron microscopy, the investigation presents a comprehensive analysis of the structural and morphological properties of "Jeewanu". The results demonstrate that the Jeewanu'sare spherical in shape,bluesh in colour, size ranging from 0.2 to 3.5 micron in diameter, showcasing well-defined boundary walls and intricate internal configurations. Heterogeneous and anisotropic internal organization is emphasized by phase contrast and confocal microscopy, revealing distinct regions with varying refractive indices. Evidence from electron microscopy studies strongly indicates "Jeewanu" are able to show the properties of growth and reproduction. The presence of small protuberances on larger entities and visible separating junctions imply a budding mechanism for replication, supporting the notion that these structures could be early models of self-replicating systems.

This study makes a significant contribution to the realm of research on the origins of life by providing empirical data that enriches our comprehension of potential routes in prebiotic chemical evolution. Nonetheless, it is imperative to interpret these discoveries in light of existing theories on abiogenesis. Future research avenues might involve more intricate biochemical assessments of Jeewanu composition, exploration of their metabolic functions, and scrutiny of their reactions to diverse environmental circumstances. Such investigations would further clarify the importance of Jeewanu-like formations in early evolutionary scenarios and enhance our insight into their probable involvement in the genesis of cellular life. Ultimately, this study establishes a sturdy groundwork for forthcoming inquiries into prebiotic chemical systems. By elucidating the structural attributes and reproductive mechanisms of Jeewanu, this research offers valuable perspectives into our comprehension of the origins of life and sets the stage for additional exploration into the development of cellular complexity.

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