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Cytochemical characterisation of photochemically formed, self-sustaining, abiogenic, protocell-like, supramolecular assemblies "Jeewanu"

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ABSTRACT

Sunlight exposed sterilised aqueous mixture of some inorganic and organic substances reported by Bahadur and Ranganayaki provided an optimal physico-chemical environment for the self-assembly of molecules and photochemical formation of self-sustaining, abiogenic, protocell-like model 'Jeewanu'. They possess an ordered structural configuration. They are capable of showing multiplication by budding, growth from within. The histochemical findings showed that the acidic material-like substances were localised in the central region, basic material-like substances were localised in extra central region while phospholipid-like substances were present in outer limiting surface of Jeewanu. The presence of RNA-like material is significant. In plausible prebiotic atmosphere the synthesized protocell-like model similar to Jeewanu was photoautotrophic in nature. They have an ability to convert solar energy into vital activities or useful forms.

Keywords: Jeewanu, Self-sustaining, Supramolecular, Abiogenesis, Protocells

INTRODUCTION

Understanding the emergence of functional cells in prebiotic phase is one of the most challenging issues in the current research of origin of life (Szostak *et al*., 2001; Luisi, 2006; Deamer, 2009). Modern research of origin of life concerns with the environmental conditions where the molecular systems with properties of life were first appeared on the Earth. It was suggested that natural geothermal environment with aqueous phase and organic compounds provided an appropriate site where compounds were interacted with mineral surfaces to promote self-assembly, self-organization and polymerization reactions (Deamer *et al*., 2006). This process led to synthesis of polymers, key biomolecules and emergence of

complex supramolecular structures (Deamer, 2002; Hazen and Sverjensky, 2010). The synthesised structures have an ordered structural organization with properties of biological orders (Deamer, 2007).

All life forms are composed of molecules that are not themselves alive (Rasmussen *et al*., 2004). The transition from chemistry to biology reveals a series of stages of increasing molecular complexity (Mansy and Szostak, 2009).

The physico-chemical factors, mineral surfaces and inorganic matter played a decisive role in the assembly of first cell and origin of life (Orgel, 1997; Hazen *et al*., 2001; Pereira *et al*., 2011; Barnal, 1959). Mineral surfaces initiated the additional complexity in the origin of life scenario (Lasaga, 1990; Hazen, 2004).

The self-assembly of amphiphilic fatty acid molecules enhanced the assembly of membrane and vesicle (Budin *et al*., 2009). The amphiphilic membrane is permeable to molecules and polar nutrients such as nucleotides (Mansy *et al*., 2008). These molecules were initiated the self-assembly, polymerization and replication cycle. The continued cycle of such reactions would increase the internal osmotic pressure. This pressure can drive vesicle growth and division (Zhu and Szostak, 2009; Hanczyc *et al*., 2003; Chen and Szostak, 2004). This vesicle contains subsets of mixed components and nucleic acids (Budin *et al*., 2009). The synthesised structures were able to capture energy from environment and initiate primitive reactions associated with metabolism, growth and replication (Deamer, 2007).

The construction of a heterotrophic protocell capable of Darwinian evolution is a fundamental aspect of biological research. The synthesised protocell model will provided useful clues to the kind of molecules and the nature of the physico-chemical environment that may have co inspired to generate protocell on the early Earth (Mansy and Szostak, 2009).

Various types of protocell-like models have been prepared under plausible prebiotic atmosphere to explain transformation of lifeless materials into living systems (Zhu and Szostak, 2009 A, B; Lusi, 1998; Pohorille and Deamer, 2002; Rasmussen *et al*., 2003; Rasmussen *et al*., 2004).

A true protocell consists a self-replicating genome and a membrane compartment that can grow and divide (Zhu and Szostak, 2009 A; Hanczyc *et al*., 2003; Johnston and Unrau, 2001; Hanczyc and Szostak, 2004). The Chemoton concept of minimal living system postulated by Ganti emphasizes that a chemical super system comprising of three systems; a metabolic network, template replication and boundary system (Ganti, 1971). Stano *et al*. suggested that a cell having minimal and sufficient number of components to be considered as protocell (Stano *et al*., 2011).

Bahadur *et al*. observed photochemical formation of self-sustaining, autoreplicative, protocell-like, microstructures 'Jeewanu' in a sunlight exposed sterilised aqueous mixture of some inorganic and organic substances (Bahadur and Shrivastava, 1963; Bahadur *et al*., 1964; Bahadur and Ranganayaki, 1970; Bahadur, 1975; Bahadur *et al*., 1980). The abiogenically formed protocell-like microstructures 'Jeewanu' are capable of showing various properties of biological orders. Viz. multiplication by budding, grow from within and metabolic activities.

They are spherical in shape, blueish in colour have a double walled boundary and an intricate internal structure. The Jeewanu mixture have been analyzed for the presence of various compounds of biological interest viz. amino acids in free as well as in peptide combination, sugars as ribose as well as deoxyribose, nucleic acid bases as purines as well as pyrimidines, phospholipid-like material and RNA-like material (Bahadur, 1954; Bahadur and Shrivastava, 1963; Bahadur *et al*., 1964; Briggs, 1965; Bahadur, 1966; Bahadur and Ranganayaki, 1970; Ranganayaki *et al*., 1972, Singh, 1975; Gupta and Rai, 2013). The presence of various enzyme-like activities viz. phosphatase, ATP-ase, esterase, nitrogenase and Thiamine phosphatase-like activity have been detected in Jeewanu mixture (Bahadur and Shrivastava, 1963; Bahadur and Ranganayaki, 1970; Singh, 1973; Bahadur and Gupta, 1984; Gupta and Rai, 2015).

The EPR spectra of Jeewanu revealed the presence of ferredoxin-like material in them (Rao, 1978). Jeewanu have been found to catalyze photolytic decomposition of water. Studies using D_2O revealed that hydrogen produced in Jeewanu mixture is coming from splitting of water (Pal *et al*., 1986). Further studies using N^{15} and C^{14} confirmed that in Jeewanu mixture hydrogen produced by photolytic decomposition is utilized in photochemical fixation of CO_2 and N_2 (Smith *et al*., 1981).

The various findings concerning the work of Jeewanu reported by Bahadur at al. (Bahadur and Shrivastava, 1963; Bahadur *et al*., 1964; Bahadur, 1966; Bahadur and Ranganayaki, 1970; Bahadur *et al*., 1980; Smith *et al*., 1981) were confirmed by various workers and complete reviews of the work on Jeewanu were also published (Linda and Ponnamperuma, 1967; Grote, 2011). In light of Chemoton model of minimal living system, Ganti co inspired that Jeewanu is a promising model system to understand the origin, evolution and fundamentals of life (Ganti, 1971; 2003).

Therefore, an attempt was made to understand the nature of physico-chemical conditions which brought about the organization of molecules in specific stearic position and appearance of earliest living systems on the Earth or elsewhere.

The aim of the present investigation is to characterize the structural organization of Jeewanu, the abiogenic protocell and to suggest the nature of earliest living systems.

MATERIAL AND METHODS

Method of preparation of Jeewanu

The Jeewanu were prepared as follows reported by Bahadur and Ranganayaki (1970).

1. 4 % Ammonium molybdate (w/v)
2. 3 % Di-ammonium hydrogen phosphate (w/v)
3. Mineral solution -

It was prepared by dissolving 20 mg. each of potassium di-hydrogen ortho phosphate, sodium chloride, magnesium sulphate, potassium sulphate, calcium acetate, manganous sulphate and 50 mg. of ferrous sulphate in 100 ml. of distilled water.

The above solutions taken in conical flask were cotton plugged and sterilised in an autoclave at 15 lb. pressure for 30 minutes.

After cooling, 4% ammonium molybdate solution (1 volume), 3% di-ammonium hydrogen phosphate (2 volumes) and mineral solution (1 volume) were mixed in a sterilised conical flask. 36% Formaldehyde (1 volume) was aseptically added in the above solution.

The stock solution was divided into ten parts (numbered 1 to 10) and cotton plugged. The mixture no 1&2 were used as control. Mixture no. 1 was kept in dark and mixture no. 2 was covered with several folds of thick black paper.

The mixtures no, 2 to 10 were exposed to sunlight for varying periods of exposure viz. 0.30, 1.0, 4.0, 8.0. 16.0 24.0, 32.0 and 36 hours respectively.

The exposed mixtures were thoroughly shaken and a drop of suspension was aseptically mounted on a microscopic slide, covered with micro cover glass and examined under optical microscope at 1500X.

The microstructures synthesised in the exposed mixture were filtered from experimental mixture by decantation and dried in vacuum desiccators. The dried samples were transferred into sterilized eppendorf tubes, sealed and sent to Advanced Instrumentation Research Facilities, Jawaharlal Nehru University, New Delhi, India, for confocal and electron microscopic (SCM & TEM) investigations.

The air-dried samples of Jeewanu were fixed in 0.25% CrO_3 (aqueous) till blueish colour of microstructures were bleached (Bahadur *et al*., 1980). The fixed Jeewanu were thoroughly washed with distilled water. A drop of suspension was mounted on a glass slide; airs dried and were used for histochemical localisation of acidic and basic material-like substances in Jeewanu. Eosin Y and Eosin B were used for histochemical localisation of basic material-like substances while acidic material-like substances were studied by using Gention violet, Methyl green, and Giemsa stain (Bahadur *et al*., 1980; Kurnick, 1955; Shapiro and Mansy, 2007). The histochemical localisation of RNA-like activity and phospholipids-like substances were observed by using Pyronin Y and Sudan black B stain (Kurnick, 1955; Bahadur and Verma, 1981; Chiffelle, 1951).

RESULTS

The control mixtures no. 1&2 showed negative results. The mixtures remained colourless on exposure to sunlight (Figure 1). The other experimental mixtures exposed to sunlight became blueish in colour in few seconds of exposure. The blueish colour of the mixtures deepened due to progression of photochemical reaction on further exposure (Figure 2). These observations showed that the synthesis of Jeewanu is strictly a photochemical outcome.

The morphological study of Jeewanu of different exposures by optical microscopy showed that they are spherical in shape, blueish in colour. They have a definite double walled boundary and an intricate internal structure. Their size varies from 0.5µ to 3.5µ in diameter.

The presences of spherical budding vesicle of different size were first seen at 1.0 hour of exposure. The size of buds was also increased on further exposure. The numbers of Jeewanu on per microscopic view were also increased exponentially in relation with the duration of exposure.

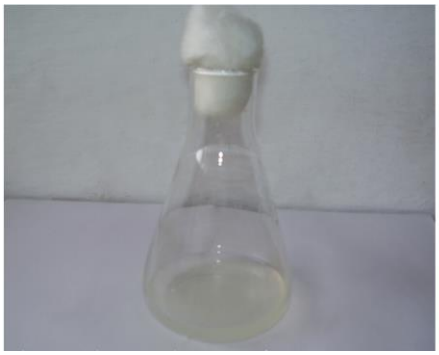
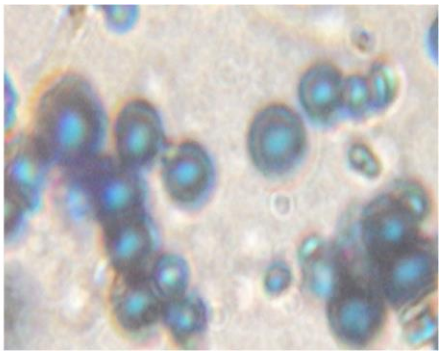


Fig. 1

Fig. 2



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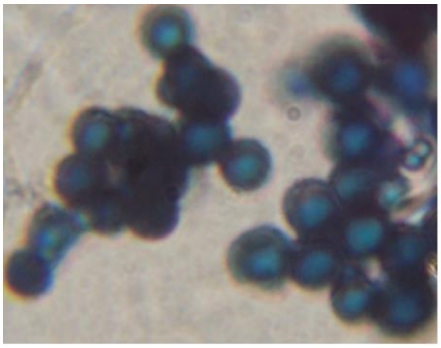

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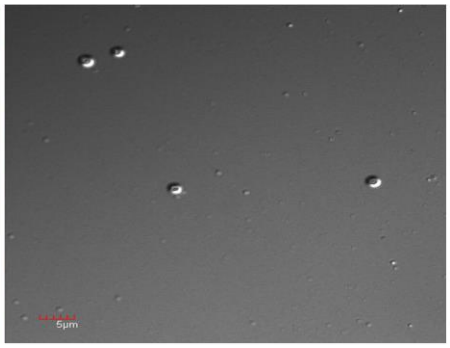

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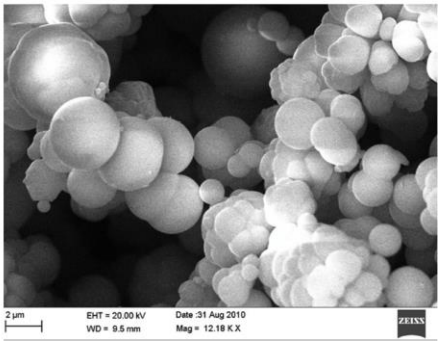

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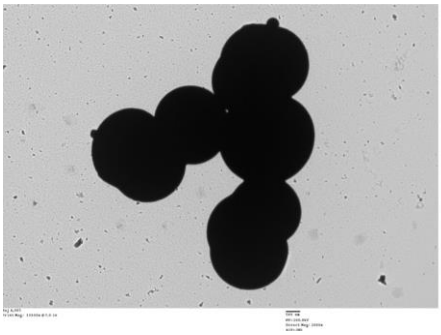

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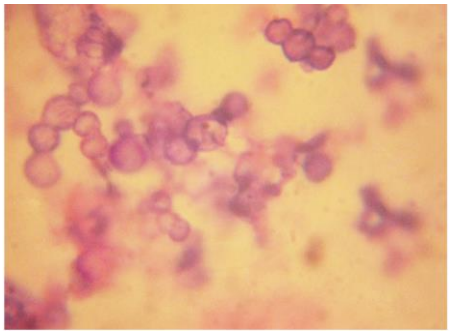


Fig. 8

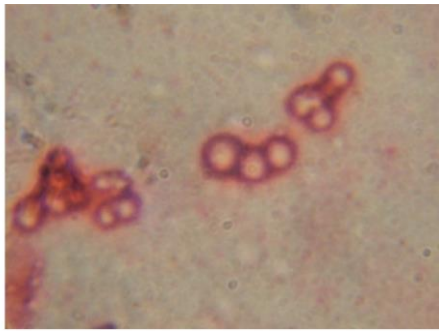


Fig. 9

Figure 1: Control mixture showing no photochemical reaction.

Figure 2 : Mixture of Jeewanu exposed to sunlight for 15 minutes showing blueish coloration due to photochemical reaction.

Figure 3 & 4 Micrographs of Jeewanu (8 and 24 hours of exposure) showing a definite spherical shape, multiplication by budding and growth from within (1500X).

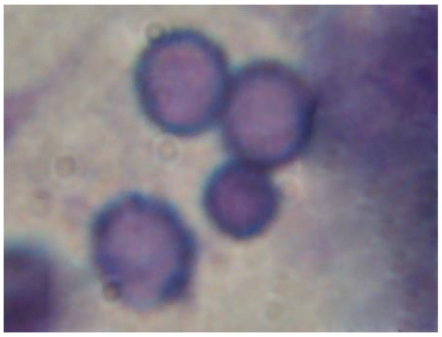
Figure 5 :Confocal micrograph of Jeewanu (24 hours exposure) showing anisotropic nature of Jeewanu.

Figure 6 : Scanning electron micrograph (SCM) of Jeewanu (24 hours exposure) showing smooth surface topology and various morphological characteristics.

Figure 7 : Transmission electron micrograph (TEM) of Jeewanu (24 hours exposure) showing their heterogeneous structural organization.

Figure 8 : Jeewanu stained pinkish with Eosin Y showing histochemical localisation of basic material-like substances in the extra central region (1500X).

Figure 9 :Jeewanu stained bright red colour with Eosin B showing histochemical localisation of basic material-like substances in the extra central region (1500X).



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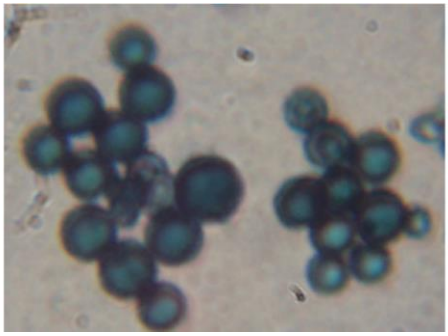

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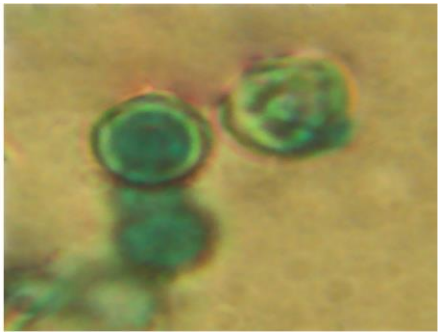

Fig. 12

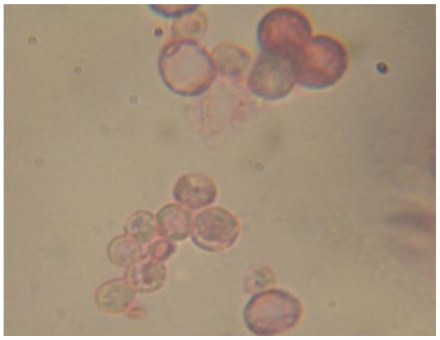

Fig. 13

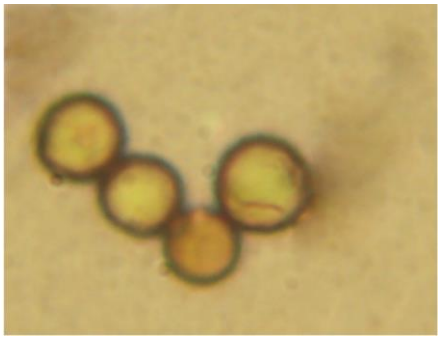

Fig. 14

Figure 10: Jeewanu stained violet with Gention violet showing histochemical localisation of acidic material-like substance in the central region (1500X).

Figure 11: Jeewanu stained violet with Giemsa showing histochemical localisation of acidic material-like substances in the central region (1500X).

Figure 12: Jeewanu stained greenish with Methyl green showing histochemical localisation of acidic material-like substances in the central region (1500X).

Figure 13: Jeewanu stained bright red colouration with Pyronin Y showing histochemical localisation of RNA material-like activity diffused throughout the microstructure (1500X).

Figure 14: Jeewanu stained brilliant black and brownish black colouration with Sudan black B showing a definite boundary wall and histochemical localisation of phospholipid-like substance (1500X).

Jeewanu were capable of showing multipli-cation by budding, grow from within by actual synthesis of material and metabolic activities. (Figure 3&4). The presence of spherical budding vesicles of different size was showed their different stages of transition. The confocal (Figure 5), electron microscopy (SCM & TEM) (Figure 6&7) of Jeewanu showed that they posses an anisotropic morphology, smooth surface topology, have a definite boundary wall with heterogeneous internal structure.

The histochemical investigations showed the presence of acidic and basic material-like substances in the Jeewanu. The pinkish and bright red colour of Eosin Y and Eosin B (Figure 8,9) in the extra central region of the microstructures showed the presence and localisation of basic material-like substances while the central region stained violet with Gention violet (Figure 10) and Giemsa (Figure 11), greenish with Methyl green (Figure 12) showed the presence and localisation of acidic-like material in the central structure.

The diffused bright red colouration of Pyronin Y diffused throughout the microstructures showed the localisation of RNA-like activity (Figure 13). On staining with Sudan black B the appeared brilliant black colouration (Figure 14) of outer limiting boundary showed the presence of definite boundary wall, made up of phospholipid-like material.

DISCUSSION AND CONCLUSION

The emergence of first cell on the Earth was the culmination of long history of prior chemical and physical process (Schrun *et al*., 2010). The physical and chemical conditions for the synthesis of functional biostructures have been discussed by various workers (Deamer *et al*., 2006). The suitable physico-chemical conditions were essential for self-assembly of molecules

which led to produce supramolecular complex structures with certain properties of biological order. Such structures were able to capture energy available in the environment and initiate primitive reactions associated with metabolism, growth and replication (Deamer, 2007).

Sunlight is a primary weakest, appropriate and abundant source of energy for the synthesis of molecules of biological interest (Hull, 1960; Bahadur, 1964). This initiates primitive reactions associated with metabolism growth and replication. The products were incorporated into molecular systems and initiated photoautotrophic energy transductions in the primordial conditions.

Bahadur and Ranganayaki reported that sterilized aqueous mixture of some inorganic and organic substances showed photochemical formation of an ordered structural configuration 'Jeewanu', capable of showing various properties of biological order viz. multiplication by budding and growth from within (Bahadur and Ranganayaki, 1970).

The histochemical localization of acidic and basic material-like compounds of biological interest in Jeewanu revealed that it possesses an ordered configuration.

The diffused RNA-like activity throughout Jeewanu showed that The RNA monomers are photochemically synthesised in Jeewanu mixture. The primitivity of RNA in the plausible prebiotic atmosphere has been discussed (Joyce and Orgel, 2006).

The self organization of photochemically formed micellar moieties led to encapsulation and appearance of a definite boundary wall in Jeewanu. The staining of Jeewanu with sudan black B showed its nature of constitution and similarity with primordial biological systems.

The permeation property of limiting membrane of Jeewanu possibly allowed vesicle volume to increase significantly during growth and resulting in maintenance of spherical vesicular shape. The diversity in the size of Jeewanu showed various transient stages of their formation. This property gradually regulated the passage of molecules which resulted in increased internal architectural complexity of Jeewanu.

The photochemical formation of membrane bounded protocell-like model Jeewanu with complex internal structure and properties of biological orders showing self-assembly of molecules and transformation of lifeless material in to living system. The formation of Jeewanu also supports 'Metabolism-first theory' which claimed that early catalytic cycles first evolved in solution and became encapsulated inside lipid vesicle latter on (Pereira *et al*., 2011).

It is quiet probable that the earliest energy transducing systems were possibly a photoautotroph similar to Jeewanu (Smith, 1982). They have an ability to convert solar energy into useful forms..

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