

# **Effect of NaCl Addition to the PEM on the Functional Properties of BMJ24 Jeewanu Prepared under Oxygenic Conditions (PUOC) in Water and in Phosphate Buffer of pH 6, 7 and 8 under Oxygenic Conditions**

Deepa Srivastava

Assistant Professor, Department of Chemistry, S.S.Khanna Girls' P.G. College, Constituent College of Allahabad University, Allahabad, Uttar Pradesh, India

**ABSTRACT:** The cell as we know today would not have been synthesized under natural conditions, to begin with, because the cell as we know it today has acquired this shape by evolution during all the 3.2 billion years, after it was synthesized for the first time.

According to Bahadur and Ranganayaki the earliest living cells was very simple in structure and were full of the properties of biological order.

Bahadur in 1967 suggested that the way in which the systems organize themselves with properties of biological order is very important feature of the study of origin of life. Jeewanu, the autopoietic eukaryotes were prepared by Bahadur in 1967.

It was observed that when a mixture of Jeewanu and water is exposed to sunlight, water is split up into H<sub>2</sub> and O<sub>2</sub>. But due to the nitrogenase like activity of Jeewanu, the produced hydrogen is utilized in the fixation of nitrogen.

The hydrogen produced by the splitting of water is also utilized in the reduction of inorganic carbon.

Khare prepared boron molybdenum Jeewanu in 1989 which showed significant water splitting.

NaCl is a common constituent of the living cells.

An attempt has been made in this paper to study the effect of NaCl addition to the PEM on the functional properties of BMJ24 Jeewanu prepared under oxygenic conditions (PUOC) in water and in phosphate buffer of pH 6, 7 and 8 under oxygenic conditions.

**KEYWORDS:** Jeewanu, pH, BMJ24, PEM, NaCl, blue colour intensity, autopoietic, eukaryotes, exposure, Mineral solution, sunlight, PUOC, phosphate buffer, oxygenic condition

## **I. INTRODUCTION**

The cell as we know today would not have been synthesized under natural conditions, to begin with, because the cell as we know it today has acquired this shape by evolution during all the 3.2 billion years, after it was synthesized for the first time. Bahadur and Ranganayaki showed that the earliest living cells was very simple in structure and were full of the properties of biological order. (Bahadur, K and Ranganayaki, S, 1964).[1] Jeewanu, the autopoietic eukaryotes were prepared by Bahadur in 1967.

To tackle the problem of origin of life, the knowledge of the synthesis of the earliest cells under natural conditions is essential. The basis of the modern approach to the problem of 'Origin of life' is based on the Molecular or the Chemical Evolution Theory given by Haldane (1929)[2] and Oparin (1924)[3]. Bahadur in 1967 suggested that the way in which the systems organize themselves with properties of biological order is very important feature of the study of

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 10, October 2016

origin of life. (Bahadur, 1967)[4]. The problem of origin of life is investigated basically in order to find out the natural condition under which the replicating, self-sustaining systems were produced. (Blum, 1961, Bahadur and Ranganayaki, 1966)[5, 6].

The origin of life can be approached the best way if life and the living systems are considered in the light of functional properties. (Bahadur and Ranganayaki, 1980)[7].

It was observed that when a mixture of Jeewanu and water is exposed to sunlight, water is split up into  $H_2$  and  $O_2$ . But due to the nitrogenase like activity of Jeewanu, the produced hydrogen is utilized in the fixation of nitrogen. Kumar in 1981 estimated the fixed nitrogen of the exposed mixture and thus confirmed the fixation of nitrogen by Jeewanu chemically. (Kumar, 1981)[8]. The hydrogen produced by the splitting of water is also utilized in the reduction of inorganic carbon. (Smith et al, 1981)[9]. They observed that on exposure to light from a mercury lamp, the aqueous mixture of Jeewanu,  $NaHCO_3$  and water shows the appearance of  $^{14}C$  in the organic material.

When the Jeewanu water mixture is kept in Warburg's flask, which is attached to the manometer filled with mercury and the entire apparatus is exposed to sunlight and kept in shade after some time, it is observed that the pressure in the flask is changed. Three reactions take place in the flasks: (i) splitting of water (ii) fixation of nitrogen (iii) loss of fixed nitrogen and the change in the pressure of the flasks might be due to the resultant of the gases evolved and fixed by these reactions. Reaction (i) is photochemical and stops as soon as light is cut off. Reaction (ii) and (iii) are ionic in nature in presence of molybdenum Jeewanu which has nitrogenase like material and takes place both in light and shade. Although third reaction is slower than the second reaction and takes place all the time during the nitrogen fixation but can be observed in shade only after all the hydrogen set free is utilized in the fixation of nitrogen and is topped as the pressure of the Warburg's flask increases.

In light, Jeewanu splits water molecule to hydrogen and oxygen and this increases the pressure in the Warburg's flasks. The hydrogen produced is very active and combines with nitrogen of the air of the overhead space of the mixture of Warburg's flasks. In presence of molybdenum Jeewanu having nitrogenase like activity, the ionic reaction of combination of hydrogen and nitrogen takes place both in light and shade. The increase in pressure due to the water splitting is completely stopped in shade and so the combination of hydrogen and nitrogen is more prominently observed in shade.

When kept in shade, a decrease in the pressure of the flasks is observed.

Yogesh Khare (1989) prepared boron molybdenum Jeewanu and reported that addition of sodium borate in the PEM in which Jeewanu are prepared, decreased the size of the Jeewanu and increased their number. (Khare, Y, 1989)[10]. It has further been observed that the BMJ Jeewanu shows significant water splitting.

Bahadur and Ranganayaki (1970) have observed that the organo- molybdenum microstructures are able to split water into hydrogen and oxygen in presence of sunlight and fix molecular nitrogen.[11]

Sodium chloride is widely distributed in nature. About 2.8% sodium chloride is found dissolved in sea water and it is a common constituent of the living cells.

It was observed that addition of sodium chloride in the PEM of BMJ24 Jeewanu, produced particles of larger size and the hydrogen ion formation increased with period of exposure. (Srivastava, D., 1991)[12].

Effect of variation in the concentration of mineral solution, formaldehyde and ammonium molybdate on pH and colour intensity of the PEM of 1.531211 SMJ38 Jeewanu before and after exposure to sunlight was studied by Srivastava, D.[13] - [17]

Effect of irradiation of 1.5312211SMJ29 Silicon Molybdenum Jeewanu PEM with clinical mercury lamp and sunlight on the morphology of the silicon molybdenum Jeewanu was studied by Srivastava. [18]

Effect of addition of Methanol and Ammonium Molybdate to (0+15):30:10:20:10:10 SMJ8 Jeewanu on the morphology, pH and colour intensity of the PEM of the Jeewanu both before and after Exposure to Sunlight up to a total of 32 Hours was studied by Srivastava, D. [19,20]

In this paper, an attempt has been made to the study of the effect of NaCl addition on the functional properties of BMJ24 (PUOC) in water and in phosphate buffer of pH 6, 7 and 8 under oxygenic conditions.

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 10, October 2016

## II. RELATED WORK

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## III. EXPERIMENTAL RESULTS

The following solutions were prepared:

- 1) 4% (w/v) ammonium molybdate
- 2) 3% (w/v) diammonium hydrogen phosphate
- 3) **Mineral solution:** This was prepared by dissolving 20.0 mg each of potassium sulphate, calcium acetate, magnesium sulphate, zinc sulphate, manganese sulphate and 10.0 mg of copper sulphate in 80 ml of double distilled water. One salt was dissolved completely before adding another salt. In a separate test tube, 50 mg of ferrous sulphate was dissolved in 10.0 ml of distilled water. To this, 0.1 ml of  $6\text{NH}_2\text{SO}_4$  was added to avoid hydrolysis. Both solutions were mixed. The total volume of the mineral solution was made to 100 ml by adding distilled water.
- 4) 36% formaldehyde
- 5) 3% (w/v) sodium chloride solution,
- 6) 5% (w/v) sodium borate solution

Each solution, except formaldehyde, was sterilized in an autoclave at 15 lbs for 15 minutes.

Two clean, dry and sterilized conical flasks of 250 ml capacity were taken and labeled from 1 and 2.

To each flask, 15 ml of ammonium molybdate, 30 ml of diammonium hydrogen phosphate, 10 ml mineral solution, 20 ml of 36% formaldehyde and 10 ml of sodium borate were added. Then, to flask 2, 10 ml sodium chloride was also added. The total volume of flask 1 was 85 ml and the total volume of the flask 2 was 95 ml.

The flask number 1 and 2 were cotton plugged. Each flask was shaken carefully and exposed to sunlight for a total of 24 hours giving 4 hours exposure daily. Thus, in the mixture of flask 1 and 2, the Jeewanu were prepared under oxygenic conditions (PUOC). After 4 hours, 8 hours, 12 hours, 16 hours, 20 hours and 24 hours of exposure, the pH and the colorimetric readings of flask 1 and 2 were recorded. Then, after this, contents of each flask was filtered, dried and weighed and the yield was recorded.

## PROCEDURE

Nine clean and dry Warburg's flasks of total volume 14.5 ml and bottom area 9.5 sq.cm. were taken and labeled as 1 to 9. Flask 1 was treated as control having only 5.0 ml distilled water. 0.3 ml distilled water was kept in the side lobe. Flask number 2, 3, 4 and 5 were filled with 20.0 mg of 1.531201 BMJ24 (PUOC) Jeewanu without NaCl in its PEM and 4.0 ml distilled water respectively. In the remaining flasks labeled 6, 7, 8 and 9, 20.0 mg of 1.531211 BMJ24 (PUOC) Jeewanu with NaCl in its PEM and 4.0 ml of distilled water was added.

In the side lobe of each flask 0.3 ml of distilled water was added. Then, in flask 2 and 6, 1 ml distilled water, in flask 3 and 7, 1 ml phosphate buffer solution of pH 6, in flask 4 and 8, 1 ml phosphate buffer solution of pH 7, in flask 5 and 9, 1 ml phosphate buffer solution of pH 8 were added respectively. These flasks were attached to their respective manometers filled with mercury. The whole apparatus was then exposed to sunlight for half an hour. The pressure changes of the Warburg's flasks were recorded after every five minutes. Then the whole apparatus was brought in shade for half an hour, readings were taken at every five minutes intervals. The whole process was repeated twice in a day.

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 10, October 2016

The readings of the control flask having water alone were subtracted from each reading at that time to account for the pressure difference due to the change in the temperature.

## OBSERVATIONS

**TABLE – 1**

Effect of sodium chloride on the amount of solid material formed in the two PEM.

Flask no.	Type of PEM of BMJ24	Amount of solid material formed in the PEM in g
1	PEM without NaCl (PUOC)	0.7702
2	PEM with NaCl (PUOC)	0.7734

The readings of the Warburg's flasks were as follows:

**TABLE – 2**

Pressure changes in Warburg's flasks (in cm of mercury), containing 1.531201 BMJ24 (PUOC) without NaCl in the PEM and 1.531211 BMJ24 (PUOC) with NaCl in the PEM, in water and in phosphate buffer of pH values 6, 7 and 8 under oxygenic conditions.

Time in minutes	1.531201 BMJ24 (PUOC) without NaCl				1.531211 BMJ24 (PUOC) with NaCl			
	water	pH of phosphate buffer			water	pH of phosphate buffer		
		6	7	8		6	7	8
Sunlight								
5	+2.4	+1.7	+1.6	+1.8	−0.3	−0.7	+1.6	+2.1
10	−1.1	−2.0	+2.7	+2.1	−1.4	−1.7	+3.1	+2.6
15	−3.7	−2.6	+3.2	+2.4	−4.2	−1.8	+3.1	+3.0
20	−4.1	−2.0	+3.7	+3.4	−3.9	−0.4	+3.7	+2.7
25	−4.8	−2.3	+4.3	+3.0	−3.4	−0.5	+4.8	+2.2
30	−4.8	−3.7	+4.1	+3.6	−3.5	−1.2	+4.9	+1.8
Shade								
5	−6.7	−5.6	+1.1	−3.9	−5.1	−3.2	+1.0	+0.7
10	−6.7	−6.3	+0.5	−4.2	−5.6	−4.1	+0.3	−0.2
15	−6.3	−6.7	+0.2	−4.4	−5.5	−4.6	−0.1	−0.8
20	−6.6	−7.1	+0.2	−4.4	−6.0	−4.9	−0.4	−0.8
25	−6.8	−7.2	0.0	−4.5	−6.0	−4.0	−0.5	−0.9
30	−7.3	−7.4	0.0	−4.5	6.3−	−4.1	−0.6	−1.0
Sunlight								
5	−6.7	−5.5	+1.0	−4.6	−3.7	−2.3	+2.0	−0.3
10	−6.7	−4.8	+2.9	−4.7	−3.8	−2.1	+3.4	−0.2
15	−5.7	−5.1	+3.0	−4.7	−3.5	−2.4	+3.8	0.0
20	−4.8	−4.8	+2.9	−4.5	−3.2	−2.1	+3.7	+0.2
25	−4.7	−5.0	−3.6	−4.6	−5.8	−1.6	+3.0	−0.4
30	−4.9	−4.8	−3.9	−4.6	−5.8	−1.6	+2.8	+0.1
Shade								
5	−5.9	−7.2	−7.3	−4.2	−8.0	−4.4	−1.1	+0.0
10	−6.5	−7.3	−7.6	−4.4	−8.5	−4.7	−1.8	−0.2
15	−6.6	−7.3	−6.5	−4.6	−8.7	−4.9	−2.0	−0.3
20	−7.1	−7.4	−7.1	−4.6	−9.1	−5.1	−1.6	−0.5
25	−7.3	−7.5	−7.3	−4.6	−9.3	−5.1	−1.9	−0.7
30	−7.3	−7.4	−7.8	−4.4	−9.3	−5.1	−2.1	−0.6

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 10, October 2016

## IV. CONCLUSION

It has been observed from the data given above that in the case of Boron Molybdenum Jeewanu (BMJ) prepared under oxygenic conditions (PUOC) without NaCl, the fixation of molecular nitrogen increased significantly. In shade, the nitrogen is fixed in the form of diimine  $\text{NH}=\text{NH}$  which shows decrease in pressure in shade. The nitrogenase like property in the molybdenum Jeewanu has already been observed. (Smith, A, E, Folsome, C and Bahadur, K, 1981). In presence of Jeewanu, the hydrogen set free due to the splitting of water in sunlight reacts with the hydrogen of the overhead space of Warburg's flasks. This reaction is ionic and in presence of Jeewanu which has nitrogenase like material in it, take place, both in sunlight as soon as the hydrogen is produced and also in shade till all the hydrogen produced by the water splitting is consumed.

In presence of sunlight, water is split up into hydrogen and oxygen by the Jeewanu and this causes an increase in the pressure of the Warburg's flasks. If this increase is more than the decrease in the pressure of the Warburg's flasks caused by the fixation of the nitrogen of the overhead space of the Warburg's flask, increase in the pressure of the Warburg's flask is observed in sunlight. When the exposure to sunlight is stopped, the photolytic splitting of water stops and there is no increase in the pressure of the Warburg's flask. Thus a decrease in the pressure in the Warburg's flask is observed in shade due to the fixation of nitrogen as diimine. The decrease in the pressure of the Warburg's flask stops when all the hydrogen of the Warburg's flask is consumed. If there is loss of nitrogen in the mixture, it is observed as increase in the pressure in the Warburg's flask after the fixation of nitrogen has stopped even in shade.

In the case of Boron Molybdenum Jeewanu, it has been observed that when the NaCl was present in the PEM of these Jeewanu, the Jeewanu produced show a little more water splitting and less nitrogen fixation.

When phosphate buffer of pH 6, 7 and 8 was added to the mixture of Jeewanu and water, the Jeewanu produced in the PEM having NaCl showed more water splitting in all the three cases, and less nitrogen fixation, as compared to the Jeewanu having no NaCl in its PEM. However, maximum water splitting, on exposure to sunlight, was shown by the mixture having phosphate buffer of pH 7 and 1.531211 BMJ24 Jeewanu prepared under oxygenic conditions (PUOC) with NaCl in the PEM. The maximum nitrogen fixation was shown by the mixture having no phosphate buffer and 1.531211 BMJ24 Jeewanu prepared under oxygenic conditions with NaCl.

It was further observed that when phosphate buffer of higher pH value was added, the mixture showed more pronounced water splitting and less nitrogen fixation as indicated by the pressure changes in the Warburg's flasks under oxygenic conditions.

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Vol. 5, Issue 10, October 2016

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