International Journal of Scientific Research and Reviews

Montmorillonite Cation Incorporated Synthesis of Amino acids from Methane-Ammonia and Water vapour under Primordial Conditions

Bhatt Hemlata[1*] and Pant Chandra Kala[2]

[1]Department of Chemistry, SSJDWSSS Govt. Post Graduate College, Ranikhet (Almora) Uttarakhand-263645, India
[2]Chemical Laboratory, DSB Campus, Kumaon University Nainital, Uttarakhand-2636001
DOI - https://doi.org/10.37794/IJSRR.2023.13105

ABSTRACT

A study was conducted to investigate the reaction of methane-ammonia water vapour in primordial conditions with and without the presence of green catalyst montmorillonite and cation-exchanged montmorillonite, which resulted in the formation of significant number of amino acids. The study identified the resulting products through the use of chromatographic and spectroscopic techniques. The formation of lysine, aspartic acid, glycine, glutamic acid, alanine, valine and leucine was reported from the above experimental solutions.

KEYWORDS

Synthesis, Montmorillonite (MT),Primitive conditions, Spectroscopy

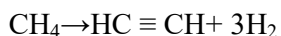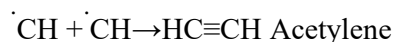***Corresponding author**
Dr. Hemlata Bhatt
Department of Chemistry
SSJDWSSS Govt. Post Graduate College, Ranikhet, Almora
Uttarakhand-263645, India
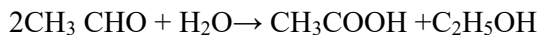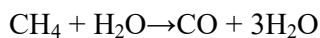Email: hemlatabhatt2021@gmail.com,
Mob No. 9411517236

INTRODUCTION

Manyresearchers[1-4] have reported the formation of amino acids and other bio-monomers from primordial gases, such as methane, acetylene, ethylene, ammonia, ammonium acetate, hydrogen and water, using various energy sources. It is now accepted that the growth of life processes has been closely associated with the growth and evolution of Earth's crust, hydrosphere, and atmosphere. Oparin's[5]conclusion was that the majority of the carbon present on the early Earth was initially composed of hydrocarbons, ammonia, water vapour, and noble gases. Gradually, the first atmosphere of the Earth was lost by diffusion, and a secondary atmosphere, still reduced, was formed by the outgassing of the Earth's interior. This theory was later supported byUrey[6], Kuhn[7], Bernal[8], and others[9].

As the primitive Earth's atmosphere was hot enough, and its weak gravitational forces allowed lighter gases, such as hydrogen, to escape into the upper atmosphere, strong reducing agents like hydrogen were lost, resulting in a less reducing and finally oxidizing atmosphere.Lemmon[10]provided a potent argument in favour of a reduced primitive atmosphere and suggested that when mixtures of CH_4, NH_3 (or N_2), and water, the principal constituents of the Earth's presumed early atmosphere, were subjected to available energy sources, such as ultraviolet[11,12] and ionising radiation, many biologically important compounds (amino acids, sugars, and nucleic acid bases) were formed.

Various energy sources on primitive Earth activated components of the atmosphere to generate organic molecules via chemical reactions. Heat[13,14]was also extremely effective in promoting the abiotic formation of simple organic compounds. Sufficiently high temperatures may have existed near the sites of volcanic activity on the primitive Earth. Ammonia was formed by the direct combination of nitrogen and hydrogen in the upper layer of the atmosphere at a few earlier stages of the existence of the Earth. Gradually[14], the crust and atmosphere of the primitive Earth cooled, and its temperature reached 1000°C or lower. The highly reactive radicals of carbon and hydrogen played a significant role in the formation of simple hydrocarbon derivatives, both saturated and unsaturated outlined as:

$\overset{.}{C}H + \overset{.}{C}H \rightarrow HC \equiv CH$ Acetylene

$\overset{.}{C}H_2 + \overset{.}{C}H_2 \rightarrow CH_2 = CH_2$ Ethylene

$\overset{.}{C}H_2 + \overset{.}{C}H_2 \rightarrow CH_4 + \overset{.}{C}$ Methane

$CH_4 \rightarrow HC \equiv CH + 3H_2$

The atmosphere's super-heated steam reacted with hydrocarbons, producing carboxy and hydroxy derivatives as:

$CH_4 + H_2O \rightarrow CO + 3H_2O$

$CO + H_2O \rightarrow HCOOH$ Formic acid

$HC \equiv CH + H_2O \rightarrow CH_3CHO$ Acetaldehyde

$2CH_3 CHO + H_2O \rightarrow CH_3COOH + C_2H_5OH$

Miller[15,16] and his team investigated the potential contribution of ammonia to chemical evolution. They concluded that on primitive Earth, ammonia existed mainly as NH_4^+, which was present in solution or bound to clay minerals and covalently linked with organic compounds through photon bonding. Meanwhile, Tayler and colleagues[17]reported on the decomposition of ammonia, which can give rise to its derivatives through the formation of NH_2 free radicals.

$NH_3 \rightarrow NH_2 + \overset{.}{H}$

$\overset{.}{NH_2} + \overset{.}{NH_2} \rightarrow H_2N\text{-}NH_2$ Hydrazine
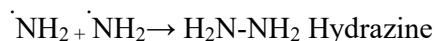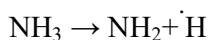
The presence of hydrogen in the primitive atmosphere might be helpful to preserve ammonia from photochemical destruction.

$\overset{.}{NH_2} + H_2 \rightarrow NH_3 + \overset{.}{H}$ Ammonia

The next biologically important reaction afforded amines, amides and ammonium salts in the biological era of the Earth by the reaction of ammonia with simple hydrocarbons and other derivatives as reported by Elgin et al[18]

$CH_4 + NH_3 \rightarrow CH_3NH_2 + 2\overset{.}{H}$

$CO + NH_3 \rightarrow HCONH_2$
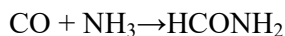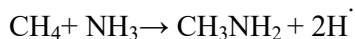
Bernal's[19]hypothesis suggests that clays and metal oxides (such as silica and alumina) may act as catalysts for the controlled synthesis of protein and nucleic acid monomers in significant amounts. However, further research is needed to explore the role of these substances, both with and without cations, in this process. Here we attempt to investigate the possible thermal synthesis of AA from Met, Amm and H_2O_{vapour} under simulated wetting-drying conditions that may be present near the boundaries of the hydrosphere and lithosphere in the primitive sea.

EXPERIMENTAL PROCEDURE:

Preparation of solution

During the investigations, an eco-friendly medium and green catalystwereutilised. Sterilised double distilled water was consistently used as the solvent in all experiments. In instances where necessary, the vapour of the triple distilled water was allowed to pass through the reaction

vessels. Great care was taken to ensure the samples purity was maintained throughout the experiment.

To prepare methane, a mixture of fused sodium acetate and soda lime (BDH/E Merck) in proportions 1:3w/w was heated in a Pyrex vessel. The gases produced were then passed through various traps containing ammoniacal solutions of anhydrous calcium chloride and concentrated H_2SO_4 flasks before use. To prepare ammonia gas, liquor ammonia of Qualigens (AR grade) was slowly heated in a sand bath. In this paper, we will use abbreviations such as Met, Amm and H_2O_{vapour} for methane, ammonia and water vapour respectively forthe reaction system. Great care was taken to ensure the purity of the gases used in the experiments. All gases were bubbled continuously (at 23±2mmHg pressure) through Pyrex glass reaction vessels (containing 10ml water to generate water vapour) kept on a hot plate at 90±5°C. The pH was maintained at 9.5±0.5. Calcium (II) chloride, magnesium (II) chloride, and nickel (II) chloride were obtained from BDH Co., USA. All chemicals were of analytical grade and used without further purification. Solutions were prepared in triply distilled water. Montmorillonite (MT) was purchased from E. Merck Chemical Company Mumbai and the purity of <2μm samples was tested by X-ray diffraction measurement after purification by water sedimentation. Calcium, magnesium, and nickel homo-ionic clays were prepared using the saturation method with 50ml of the 1M concentration of each metal chloride such as $CaCl_2$, $MgCl_2$, and $NiCl_2$. Excess salts were percolated out by washing and centrifugation procedures were separated until chloride ion was no longer detected by silver nitrate[20]. Finally, divalent cation exchanged forms thus obtained were driedat 45°C for 48 hours. The dried product was then ground and sieved to 125μm particle size.

Ultra-violet-spectra

Ultraviolet spectra of the various reaction mixtures or elutes of some products were determined in an aqueous solution using a Jasco-V-Series spectrophotometer

Infrared spectroscopy (IR)

IR spectra of reaction concentrates were recorded in a Perkin Elmer 881($4000-600cm^{-1}$) spectrophotometer.

High-Performance Liquid Chromatography(HPLC)

For HPLC, a 10μl sample was injected into the SHIMADZU SPD-10A Dual 1 UV-VIS detector with a C_{18} column. The mobile phase used was acetonitrile: water (65:35 v/v) with 1% H_3PO_4 (pH 2.8) at a temperature of 23°C, and the flow rate was set to 1.0 ml/min. The UV detector

was set to monitor at 254nm to detect amino acids. To compare the results, retention times of the reference amino acids, run in the same HPLC column under identical conditions, were used as the standard.

Source and procedure under primordial conditions:

Hot plates and heating metal (Ambassador with a temperature range of 0-100°C) were used to heat the reaction vessels fitted with air condensers (120 cm long, plugged with sterilized cotton). Heating was continued carefully until the last drop of solution was dried, and 5 ml of deionized water was added to start a new cycle. Each cycle was heated for 8-10 hours under primordial conditions.

Solvents for Paper chromatography(PC)

n-butanol - acetic acid -water (4:1:1v/v)

n-butanol - acetic acid -water (4:1:5v/v upper layer)

n-butanol - acetic acid -pyridine-water (15:3:10:12v/v)

RESULTS:

Heating was carried out for varying periods up to 250h, separately in different flasks and the heated concentrates were analysed periodically after 50h, 100h, 150h, 200h and 250h respectively for amino acids. Preliminary physico-chemical investigations of the heat-initiated products are recorded in Table 1 and illustrated in Fig.1and plotted in 1a,revealing the formation of amino acids. PC of the reaction concentrate of methane- ammonia and water vapour heated up to 50h showed the formation of four ninhydrin-positive products (I-IV). Out of these, product II identified as aspartic acid formed an appreciable amount. On prolonging the duration of heating up to 100h, the formation of three new products (V-VII) was observed and simultaneously the number of products I, II, III and IV was increased. When the duration ofheating was extended up to 150h, the amounts of all the resulting products (I-VII) appreciably increased and were characterised as lysine (I), aspartic acid (II), glycine (III), α-alanine (IV), α-aminobutyric acid (V), valine (VI), and leucine (VII). Heating the reaction mixture further for 200h, upon PC, showed a decrease in quantity andthe number of resulting products. 250h of heating, however, showed a remarkable decrease in the quantity of aspartic acid (II) and glycine (III). Other products lysine, α -alanine, α -aminobutyric acid, valine and leucine also disappeared on the paper gram.When we heated the reaction system for several hours, we got the best result at 150h and then all the reactions were

done in the same period. The reaction products I to VII formed in appreciable amounts were identified and characterized by chemical, chromatographic and spectral methods.

Finally, the results were confirmed by HPLC as shown in Fig. 2of the reaction concentrate of methane, ammonia and water vapour heated up to 150h. 10μl sample injected in SHIMADZU SPD-10A Dual 1 UV-VIS detector with C_{18} column, mobile phase acetonitrile: water (65:35 v/v) with 1% H_3PO_4 (pH 2.8), at temperature 23°C, flow rate 1.0 ml/min, UV detector at 254nm showed peaks corresponding to aspartic acid (2.52min), glycine (8.81min), α -alanine (15.78min), valine (17.65min), α -aminobutyric acid (21.44min), leucine (21.85min) and lysine (26.44min). Results were compared with standard AA run in the same HPLC column under similar conditions.

The UV absorption spectrum of the sample showed an absorption maximum at around 220nm shown in Fig.3indicating the formation of aminoacids. The IRspectrum of similar absorption frequencies in regions of 3300-3100cm^{-1}(N^+H_3;asym),1650-1550cm^{-1}(asymstret. COO$^-$),1430-1380cm^{-1}(sym. stret. of COO$^-$),1635-1550cm^{-1} due to N-H bending(asym) in $NH_3^+$and 1550-1505cm^{-1} due to N-H bending(sym) in NH_3^+,(C-NStret),2972-2900cm^{-1} due to C-H stret(asym) in CH_3,2885-2840 cm^{-1} due to C-H stret(sym) in CH_3 as shown in fig. 4 depicted the formation of amino acids.

Asimilar experimental solution of Met, Amm andH_2O_{vapour} was heated up to 150h with MT and cation exchanged MT under fluctuating wetting-drying conditions, overall, seven ninhydrin-positive products appeared on the chromatogram. All these products were identified as lysine (I), aspartic acid (II), glycine (III), glutamic acid (IV), α-alanine (V), valine (VI), and leucine (VII) by usual chromatographic methods. In the presence of MT, seven products were formed. However, a new amino acid identified as glutamic acid (IV) was formed in good amounts. In contrast,a-aminobutyric acid formed in the absence of clay was not detected on the chromatogram. Out of these resulting amino acids lysine and aspartic acid were formed in appreciable amounts, glycine in moderate amounts, valine in poor amounts, andα -alanine and leucine in traces. In the presence of calcium-exchanged clay identical range of products was formed. the quantity of α -alanine,valine and leucine was relatively increased while the quantity of lysineand glutamic acid decreased.Presence of magnesium-incorporatedMT in the above reaction system showed a slight decrease in the amount of all the resulting amino acids. When the reaction system was heated in the presence of nickel-exchangedMT, an increase in the amount of aspartic acid, glycine, glutamic, α-alanine and leucine was observed. A comparative analysis of the number of AA formed in the presence and absence of MT from a reaction system

of Met, Ammand H_2O_{vapour} heated up to 150h has shown the following order:MT-Ni^{2+}> MT-Ca^{2+}> MT-Mg^{2+}>MT

where MT stands for montmorillonite, results are recorded in Table 2 and illustrated in Fig 5 and plotted in 5a.

This paper discusses the significant contribution of primordial gases such as Met, Amm and H_2O_{vapour}in formingamino acids and other important constituents of N-bases. The role of clays in chemical evolution, as suggested by Bernal[19], has been partially confirmed by experimental research. The present investigations have shown that MT with or without divalent cations, played a significant role in the prebiotic synthesis of amino acids. Although in our studies[21], the bio-monomers were formed in minute amounts, the formation of these biologically significant molecules is meaningful because the chemical evolution process leading to the origin of life took a long time to complete in the primitive era of the Earth.

ACKNOWLEDGEMENT

Table No. 1:Periodic analysis of the synthesis of amino acids from methane, ammonia and water vapour under primordial conditions

Reaction System CH$_3$-NH$_3$-H$_2$O(v)	Duration of Heating(h)	Product of thermal reaction(mg/l)						
		I	II	III	IV	V	VI	VII
	50	1.24	9.22	3.27	1.31	-	-	-
	100	1.31	9.56	5.24	3.93	1.31	3.93	4.58
	150	6.97	1.24	1.02	4.48	1.96	7.22	5.49
	200	1.93	10.4	3.34	3.94	-	2.04	-
	250	-	3.03	1.04	-	-	-	-
1. R$_f$ values (%) using								
(i)n-BAW,4:1:5 v/v upper layer		8	12	18	30	45	51	58-
(ii)n-BAPW,15:3:10:12, v/v		35.0	40.0	42.0	46.0	49.8	65.0	70.0
2. Colour with reagents								
(i) Ninhydrin		V	V	RV	V	V	V	PV
(ii) Isatin		PBr	dB	P	BV	Bp	Brp	Bp
(iii) Folin's		GBr	VR	GY	G	BG	gBr	GY
3. Solubility in								
(i)Ether		Ins	Ins	Ins	Ins	Ins	Ins	Ins
(ii)Water		S	S	S	S	S	S	S
4.UV-Fluoresence(254nm)		BW	BW	BW	BW	BW	BW	BW
5. Amino acids overlapped in co-chromatography		Lys	Asp	Gly	ala	ABA	Val	Leu
6.R$_f$ value (%) of DNP amino acids		70	14	38	47	-	69	73
5. Amino acids identified		Lys	Asp	Gly	ala	ABA	Val	Leu

V, Violet; R, Red; P, pink; Br, brown; d, dull; B, blue; p, purple; G, green; Y, yellow; g, grey; Ins, Insoluble; S, soluble; Ss, sparingly soluble; Lys, lysine; Asp, aspartic acid; Gly, glycine,Glu, glutamic acid; ala, α -alanine; ABA, α- amino butyric acid, Val, valine; Leu, leucine;

Table No.2: Synthesis of AA from methane, ammonia and water vapour in the presence and absence of MT and divalent cations heated up to 150h

Composition of Reaction system	Product of Thermal reaction (Quantity in mg/l)						
	I	II	III	IV	V	VI	VII
CH_3-NH_3-H_2O(v)MT	9.30	8.71	2.58	4.90	T	1.96	T
CH_3-NH_3-H_2O(v) Ca^{2+}	3.24	8.71	2.59	3.80	3.89	3.92	2.58
CH_3-NH_3-H_2O(v) Mg^{2+}	2.58	7.99	2.40	2.59	3.58	3.89	3.60
CH_3-NH_3-H_2O(v) Ni^{2+}	1.24	8.89	6.90	4.27	6.99	3.61	3.61
Characteristics							
1. Rf value (%) with							
(i)n-BAW,4:1:5v/v upper layer	12	18	25	31	43	65	70
(ii)n-BAPW,15:3:10:12, v/v	8	10	23	28	40	60	63
2. Colour with Reagents							
Ninhydrin	V	V	RV	V	V	V	PV
Isatin	PBr	dB	P	BV	Bp	Brp	Bp
Folin's	GBr	VR	GY	G	BG	gBr	GY
3. Solubility							
Ether	Ins	Ins	Ins	Ins	Ins	Ins	Ins
Water	S	S	S	S	S	S	S
4. Amino acids overlapped in co-chromatography	Lys	Asp	Gly	Glu	Ala	Val	Leu
5. Amino acids identified	Lys	Asp	Gly	Glu	Ala	Val	Leu

Glu, glutamic acid, MT, Montmorillonite and all abbreviations as such mentioned in the above table
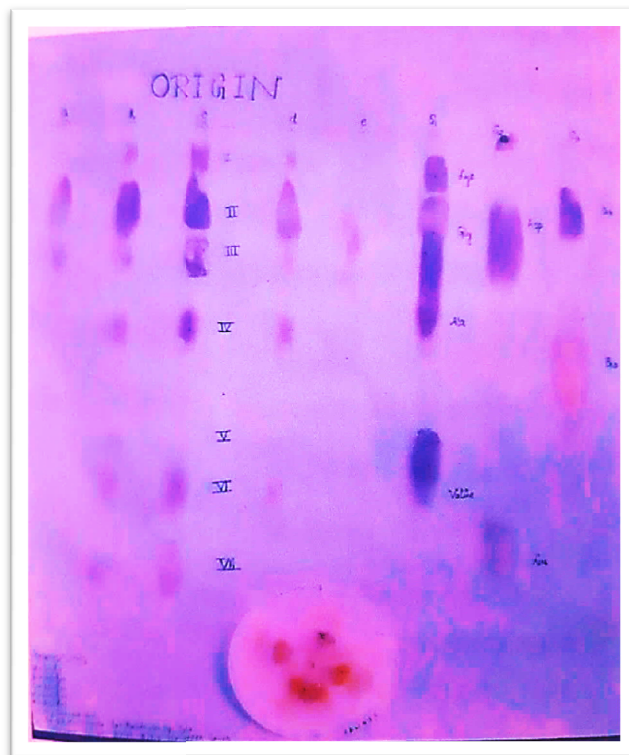

Fig. 1 Chromatogram of amino acids from the experimentalsolution ofMet-Amm -H_2O $_{vapour}$heated periodically up to50-250h
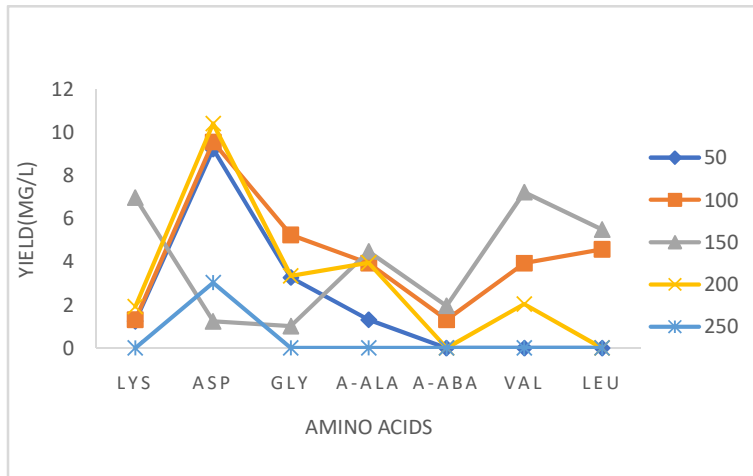
Fig.1a, Yield (mg/l) of amino acids from the of Met-Amm -H$_2$O$_{vapour}$ heated periodically up to 50 - 250h
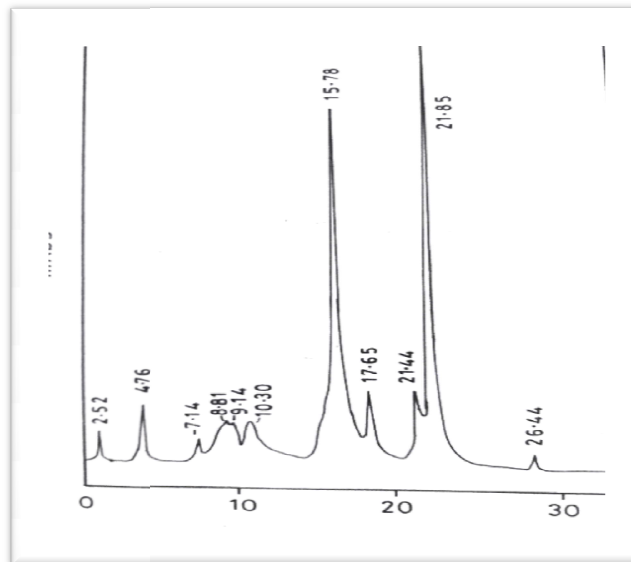

Fig.2HPLC of reaction concentrate of Met-Amm -H$_2$O$_{vapour}$ system heated upto 150h
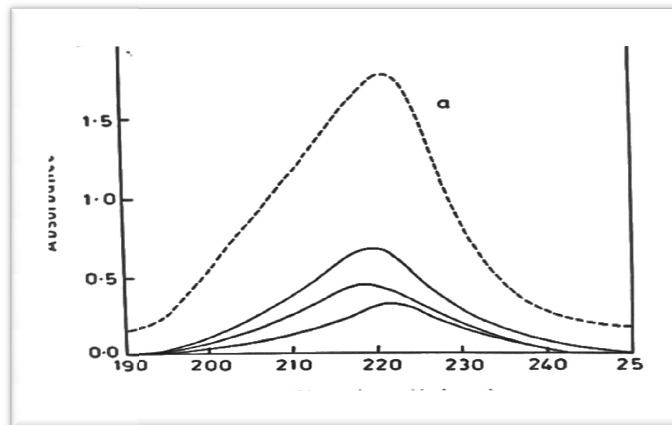

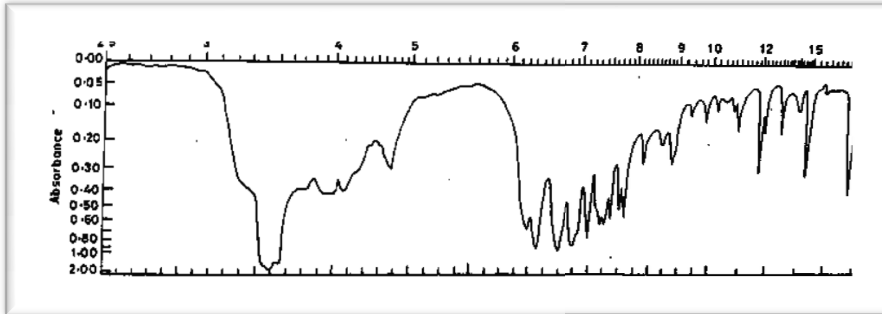**Fig.3Absorption spectra of reaction concentrate of Met-Amm-H$_2$O$_{vapour}$**

Fig.4IR absorption spectra of the Met-Amm-H$_2$O $_{vapour}$water vapour system shows frequencies in regions of 3000-2900cm^{-1}
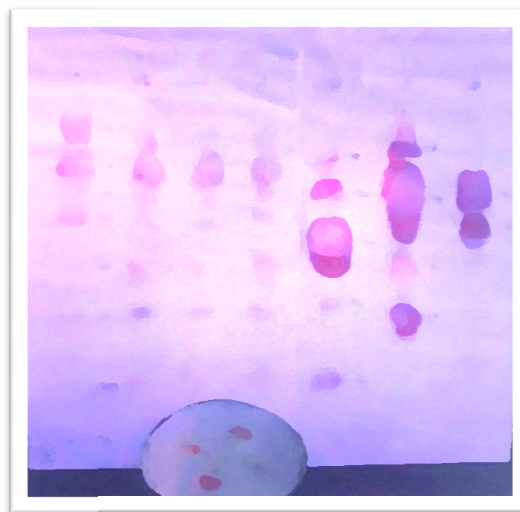

Fig.5Chromatogram showing the formation of amino acid from Met -Amm - H$_2$O $_{vapour}$ heated up to 150h in the presence of MT and cation exchanged MT
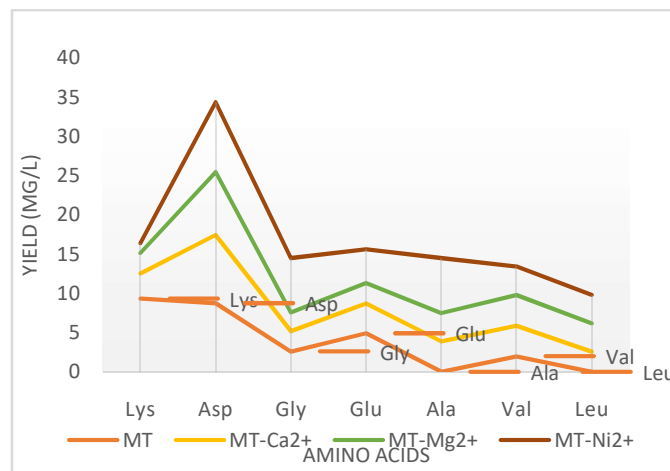

Fig. 5aYield(mg/l) of amino acid from the experimental reaction concentrate   in the presenceof MT and their incorporated cations.

REFERENCES

1. S.L.Miller,Science,1953,117,528.
2. C. K.Pathak, J.N.Pant and H.D.Pathak,1978,J.Brit.Inter.Soc.,London,32,223-227.
3. H. Bhatt, N. Pandey and C. K. Pant, 2019,IOSR, Journal of Applied Chemistry, Volume 12, Issue 8, Ser. I, e-ISSN: 2278-5736,67-73
4. H. Bhatt, C. K. Pant, 2022, International Journal of Scientific Research and Reviews, 11(4), 86-93, ISSN: 2279–0543.
5. A. I. Oparin, 1938, The Origin of life,2nd ed, Dover New York 270pp.
6. H. C. Urey,1952, The Planets: The Origin and Development, Yale Univ. Press, New Haven.
7. W. Kuhn, 1956, Chem. Ber., 89, 303.
8. J. D. Bernal, 1961,Nature,190,129-131.
9. S.L. Miller and L. E.Orgel,1974,The Origin of Life on Earth,Prentice Hall,Inc.Englewood Cliffs,New Jersy.
10. R.M.Lemmon,1970,Chem.Evol.,Chem.Rev.,70,95-109.
11. O.N. Perti,K.Bahadur and H.D.Pathak,1962,Ind.J.Appl.Chem.,25,90.
12. C.Saganand B. N. Khare,1971,Science,173,417.
13. K.Harada and S.W.Fox,1964, Science,143,384.
14. J. G. Lawless and C. D. Boynton, 1973, Nature, 243, 405-407.
15. S. L. Miller and H. C. Urey,1959,Science, 130, 245.
16. S. L. Miller and J. L. Bada,1988, Nature,334, 609.
17. H. S. Taylor and H. J. Emeleus, 1931, J. Amer. Chem.Soc.,53,562.
18. J. G. Elgin, and H.S. Taylor, 1929, J. Amer.Chem.Soc.,92,2059.
19. J. D. Bernal, 1951,Nature, The Physical Basis of life Routledge and Kegan Paul, London.
20. B. K. G. Theng, 1974, The Chemistry of clay organic reactions, Wiley, New York, p 158-186.
21. H. Bhatt, N. Pandey, C. K. Pant, 2021, International Journal ofInnovative Research in Technology, Volume 8, Issue 3, ISSN: 2349-6002.