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# Thermal Synthesis of Amino acids From the Aqueous Solution of Ammonium Acetate in Presence and Absence of Silica and Alumina Under Drying - Wetting Cycles of Primitive Earth

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***Abstract:*** *The action of heat on the aqueous solution of ammonium acetate as well as in presence of silica and alumina at 95 ± 5ºC under prebiotic drying-wetting conditions has been investigated for the plausible formation of amino acids. The reaction products were analyzed and characterized by chromatographic ( PC and HPLC ) and spectroscopic methods. Reaction system heated up to 100hrs, showed the best formation of lysine, aspartic acid, serine, glycine, glutamic acid, α-alanine, β-amino butyric acid, valine, isoleucine and leucine. In presence of silica and alumina accelerated the formation of amino acids and three new products were observed in the concentrate of the reaction system. Thus, the study is an attempt to explore the possible role of metal oxides in the prebiotc formation of amino acids, which constitute the main matrix of the living systems.*

***Keywords****: Amino acid, Ammonium acetate, Chromatographic techniques, Metal oxides, Primitive*

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## I. Introduction

The most significant outcomes of chemical evolution are the origin of biologically important macromolecules such as proteins and nucleic acids, origin of life and environment to sustain life on the earth. According to theory put forward by Academician A. I. Oparin[1-2], the abiotic synthesis of simple organic molecules and complex molecular compounds is one of the stages in the origin of life on the earth. The increasing complexity of organic compounds thus formed was due to a variety of environmental factors one of which it seems, may have been the interaction of such molecules with the surfaces of minerals and rocks composing the outer stones shell of the primordial earth. Silica and aluminum compounds and related minerals were probably among the major components of the primitive earth lithosphere. The possible role of such compounds as a catalyst in chemical evolution of first bio-organic compounds on the primitive earth has been proposed[3-4]. Earlier, Bujdak and coworkers[5], Basiuk et al[6],Yanagawa and Kobayashi[7], Rohlfing et al[8] have suggested that clays and silicon oxide which were in abundance on the earth crust in remote abiotic times might have catalyzed oligomerization reactions of amino acids leading to the evolution of proteins. Parida et al[9] have been reviewed that silica has a well characterized surface and can be modified to a wide range of functionality owing to the presence of active hydroxyl groups. It has been observed that a variety of organic compounds such as alcohols, aldehydes, acids, their salts, amides and other compounds could have formed by the excitation of primordial gases and water, under primitive earth conditions. Amongthese, ammonium acetate formed by interaction of acetic acid and ammonia might have been an effective precursor for the formation of bio-monomers as reported by few isolated workers using X - rays[10], B - rays[11], UV - light[12] and γ - radiation[13] as the source of energy. Harada et al[14] studied the thermal behavior of amino acids from a point of view of prebiotic synthesis under a large variety of conditions.

However, thermal synthesis of biomolecules from ammonium acetate under periodic conditions are lacking in literature. Although, in early stages of formation of earth, the temperature was very high due to frequent volcanic activity and high energy UV - radiations directly coming from the sun in absence of ozone layer and thus, might have been a major source of energy for abiogenic synthesis of biomolecules from primary substances of abiotic origin. A relevant source of acetic acid was methyl cyanide formed by photolysis of acetylene and ammonia might have formed ammonium acetate, which under prebiotic thermal condition could have converted into acetamide whereas Kotake et al[15] have been reported methyl cyanide, cyanoacetylene and aminonitriles as reaction intermediates during discharge experiments some of the reactions may as follows:

$HC{\equiv}CH + NH_3 \rightarrow CH_3C{\equiv}N$
$CH_3C{\equiv}N + H_2O \rightarrow CH_3COOH$

$CH_3COOH + NH_3 \rightarrow CH_3COONH_4$
$CH_3COONH_4 \rightarrow CH_3CONH_2$
$CH_3CONH_2 \rightarrow CH_3C{\equiv}N$

Therefore, keeping in view the significance of ammonium acetate as a precursor of biomolecules, attempts have been made to synthesize amino acids constituents by the action of heat on aqueous solution of ammonium acetate in the presence and absence of silica and alumina under wetting/ drying conditions presumed to be available near lithosphere-hydrosphere boundary of primitive sea beach environment that may throw some light on the process of chemical evolution leading to the evolution of life on earth.

## II. Material and Methods

**2.1 Preparation of Solution:** - All the experiments were carried out in aqueous medium. Hence, it is desirable to sterilize the reaction systems prior to heating, as there is every possibility of microbial contamination of the solutions during the course of experimentation, which may render the natural course of reaction defectively. The reaction vessels, heated solutions and other applications were therefore, sterilized prior to their use in experiments. Sterilized double distilled water was used as the solvent in every experiment wherever necessary the vapour of the double distilled water was allowed to pass through the reaction vessels. Every care was taken to ensure the purity of the samples employed.

0.1M solution of ammonium acetate of Sigma - Aldrich Company (0.5ml each, pH 4.6 ± 0.6) was heated in reaction vessels - Kjeldahl flasks (100ml) in the presence and absence of silica and alumina (50 mg each). Samples of reaction concentrates were analyzed for the possible formation of amino acids using chromatographic technique on Whatman No.01 paper both by uni - and two - dimensional chromatography was using various solvent systems as discussed below. Amino acid spots were visualized with ninhydrin as well as with isatin and comparison of their $R_f$ values with authentic amino acid as well as of their DNP derivatives.

**2.2 Blank solution:** - In every experiment an identical solution heavily wrapped in several folds of black cloth and paper was kept along side of the reaction vessels in small Pyrex flasks without heating. Such solutions were used as controls in all cases studied. The control solutions tested and analyzed in the same manner as that of the experimental solutions.

**2.3 Source and procedure of heating under wetting - drying conditions**

Hot plates and heating mantles (Ambassador, temp. range 0 - 100$^o$C ) were used for heating the reaction solutions in Pyrex / Borosil glass reaction vessels fitted with air condensers ( 120 cm length, plugged with surgical sterilized cotton). Heating was continued till the solutions were dried and 5ml of water was resuspended for the new cycle to start. Thus, heating was done for 8 - 10 hrs / day in each cycle under drying / wetting conditions.

**2.4 Procedure for taking out experimental solution**

Portions of heated samples were taken out from reaction vessel under asceptic conditions with the help of sterilized measuring cylinders of different measurements. The solutions were then taken out within an inoculating chamber. The samples of solutions were instantly transferred to borosil vials. Anterior to chromatographic analysis, the heated samples were concentrated in vacuum evaporator. Then the concentrates were analyzed chromatographically and also by chemical methods.

**2. 5 Solvent systems for paper chromatography**

2.5.1 One - dimensional chromatography
(i) n - butanol - acetic acid - water ( 4 : 1 : 1 v/v)
(ii) n - butanol - acetic acid - water ( 4 : 1 : 5 v/v, upper layer)
(iii) n - butanol - acetic acid – pyridine - water ( 15 : 3 : 10 : 12 v/v)
(iv Phenol - water (80: 20 w/v)
2 .5 .2 Two - dimensional paper chromatography
First run: Phenol - water - ammonia (80: 20: 3 w/v)
Second run: n-butanol - acetic acid - water (4: 1:5 v/v upper layer)

**2.6 Spectral studies**

Identify of resulting products was confirmed by UV - IR spectroscopic methods, comparing their spectra with standard samples.

UV- spectra : ultraviolet absorption spectra of the various reaction mixtures or elutes of some resulting products were determined in aqueous or alcoholic as well as in acidic medium using JASCO UV / VIS - 550 series spectrophotometer. Distilled water and methanol were used as a reference.

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

**2.6 Colorimetric estimation of amino acids**

The quantitative determination of amino acids was carried out by colorimetric methods. This method is based on the principle that the intensity of coloration is directly proportional to the concentration. Estimation was carried out by comparison of color intensity of the unknown compound with that of a reference solution employing photochemical colorimeter MK - III.

**2.7 High performance liquid chromatography**

HPLC analysis in SHIMADZU SPD – 10 A Dual 1 UV - VIS detector with $C_{18}$ column ; 10 µ sample was injected in the column using mobile phase acetonitrile : water ( 70:30 v/v), pH 2.8 adjusted with 0.1 % $H_3PO_4$ at temperature 28ºC with flow rate 1.0 ml / min.

## III. Results and Discussion

0.1M aqueous solution of ammonium acetate was heated for 200 hrs and the reaction concentrates were analyzed periodically after 50 hrs, 75 hrs, 100 hrs, 150 hrs and 200 hrs, revealed the formation of amino acids on the paper chromatogram. Reaction system heated upto 50 hrs have shown the formation of four ninhydrin positive products (III, IV, VI and X). Out of which, products IV and X formed in moderate amount were identified as glycine and valine respectively. 75 hrs of heating resulted in the formation of nine ninhydrin positive products on the paper chromatogram and the quantity of product appreciably enhanced. On prolonging the duration of heating upto 100 hrs the number of resulting products increased and overall, twelve ninhydrin positive spots were appeared. The products were further identified as lysine ( I ), aspartic acid ( II ), serine ( III ), glycine (IV ), glutamic acid, α-alanine, α-amino butyric acid ( VIII ), valine ( X ), isoleucine ( XI ) and leucine ( XII ) by comparison of their Rf values, colour with different reagents and other physico-chemical characteristics. However, the identity of products VII and IX could not be ascertained. Further, prolonging the duration of heating upto 150 hrs, revealed the formation of identical products while the quantity of products I, VI, VII, IX and XII was relatively decreased. Heating the reaction solution upto 200 hrs showed a considerable decrease in the number of resulting products. Thus, time lapse studies have shown that the best yield of amino acid was obtained at 100 hrs of heating from reaction system of ammonium acetate and water vapor under periodic wetting-drying conditions shown in fig.1 and recorded in table 01. The formation of amino acids was also identified on the basis of UV and IR spectra of the reaction concentrate heated upto 100 hrs. Control solution analyzed parallel to reaction system failed to synthesize amino acids.

The above reaction sample (heated upto 100 hrs) was further analyzed by HPLC, which showed twenty one peaks. Out of which, peaks corresponding to retention time 1.800, 2.070, 4.376, 5.129, 6.244, 6.434 and 8.604 min were identified as aspartic acid, glutamic acid, glycine, α-alanine, valine, isoleucine, leucine and lysine respectively by comparison with the retention time of standard amino acids injected in the same HPLC column under identical conditions (fig 2). UV- absorption spectrum of the sample showed band in between 190 - 210 nm region with a shoulder band between 250 - 270 nm. The band between 190-210 nm is attributed to mixture of amino acids ( fig 3).The Infrared absorption ( IR ) spectrum of similar sample showed absorption frequencies in regions of 3200 – 3000 $cm^{-1}$ ($N^+H_3$; asym. stret.), 1600 – 1580 $cm^{-1}$ ($N^+H_3$; asym. bending,), 1600-1560 $cm^{-1}$ ($COO^-$ asym. stret.), 1580 - 1400 $cm^{-1}$ ( $COO^-$ sym. stert.), 1295 - 1090 $cm^{-1}$ ( $N^+H_3$ in plane bending) (fig. 4).

The similar reaction system of ammonium acetate and water vapour was heated for 100 hrs in presence of silica and alumina separately, formation of twelve amino acids was observed on the paper chromatogram. Chromatographic analysis of reaction concentrates of ammonium acetate and water vapor in presence of silica revealed the formation of twelve ninhydrin positive products. Of these products, III, V, VII, VIII, and X to XII were characterized as serine, glutamic acid, α- aminobutyric acid, α-alanine, β-alanine, valine, isoleucine and leucine respectively (fig 5a). Presence of alumina during heating accelerated the thermal process particularly the amount of lysine ( I) serine, glycine ( I ), glutamic acid, threonine ( VI ), α-alanine, β-alanine, proline (IX ) and isoleucine was enhanced and results are recorded in table no 02 and illustrated in fig (5b). Formation of amino acids a comparative study of reaction concentratse of ammonium acetate and water vapour in presence of silica and alumina was shown in fig 6.

On the basis of the results discussed above it was observed that two new amino acids, identified as threonine and proline were formed in addition to the amino acids detected in absence of any catalyst. Thus, in the presence of silica and alumina accelerated the formation of hydroxyl as well as heterocyclic amino acid and their catalytic effect among themselves was found as follows:

Alumina > Silica > $CH_3COONH_4$ - $H_2O$ (v)

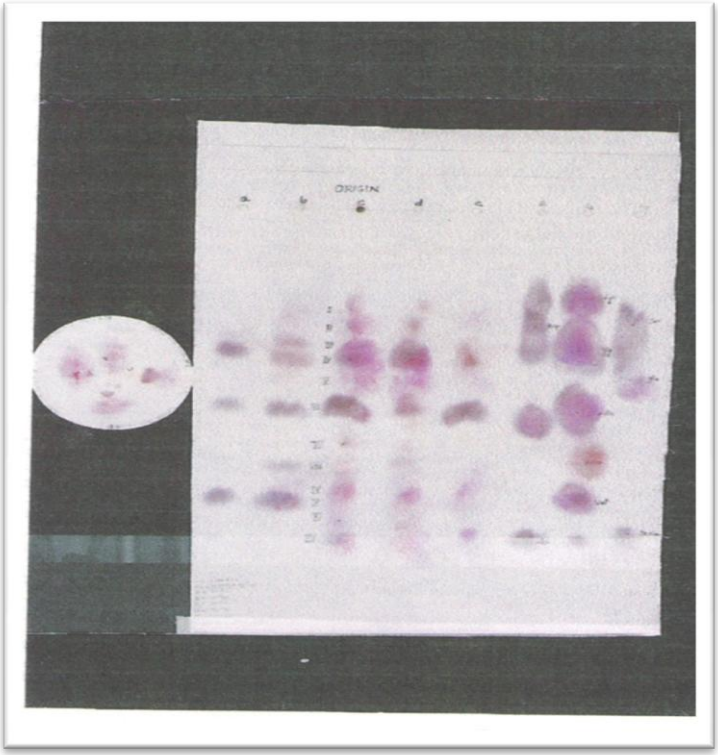

**Fig. 1** Chromatogram showing the formation of amino acids from reaction concentrate of ammonium acetate-water vapour heated from 50 – 200 hrs (a-e) at 95 ± 5$^{o}$C under wetting drying conditions

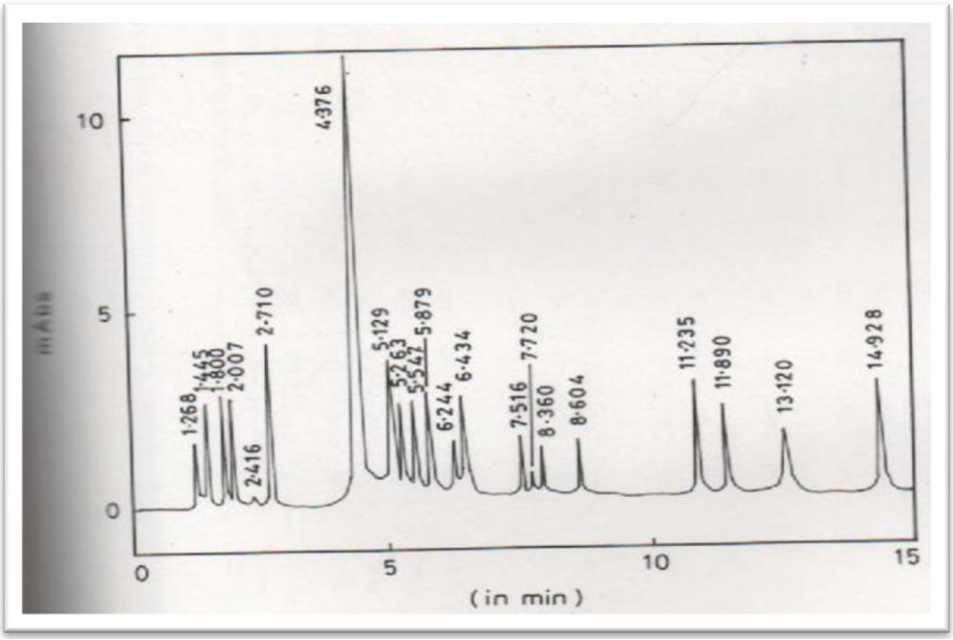


**Fig. 2** HPLC of reaction concentrate of ammonium acetate - water vapour heated up to 100 hrs under wetting-drying conditions

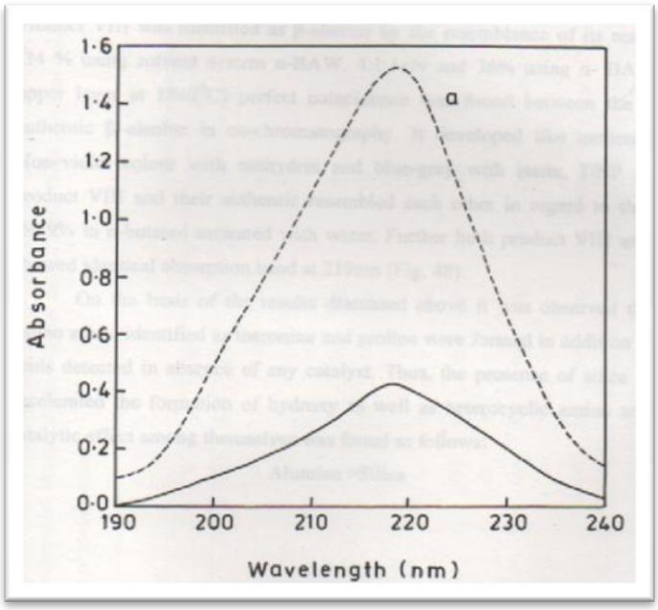


**Fig. 3** UV absorption spectra of reaction concentrate of ammonium acetate- water vapour heated up to 100hrs

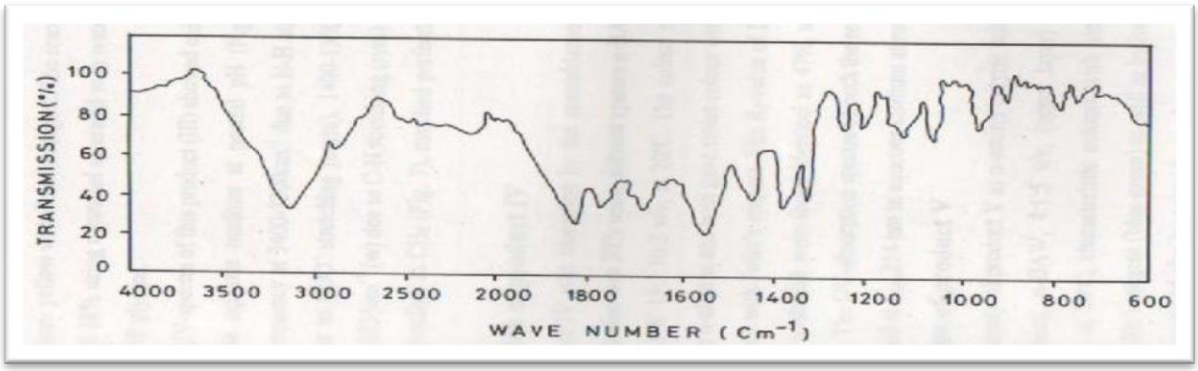


**Fig. 4** IR absorption spectra of reaction concentrate of ammonium acetate - water vapour heated upto 100 hrs

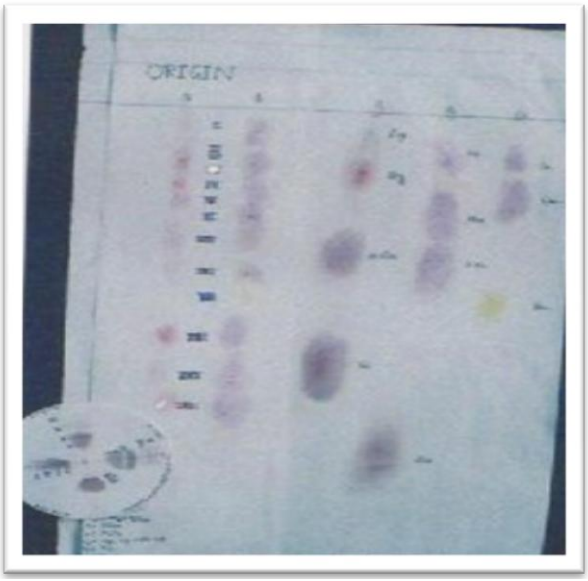


**Fig. 5** Chromatogram showing the formation of amino acids from reaction concentrates of ammonium acetate and water vapour heated upto100 hrs (a-b) with silica and alumina

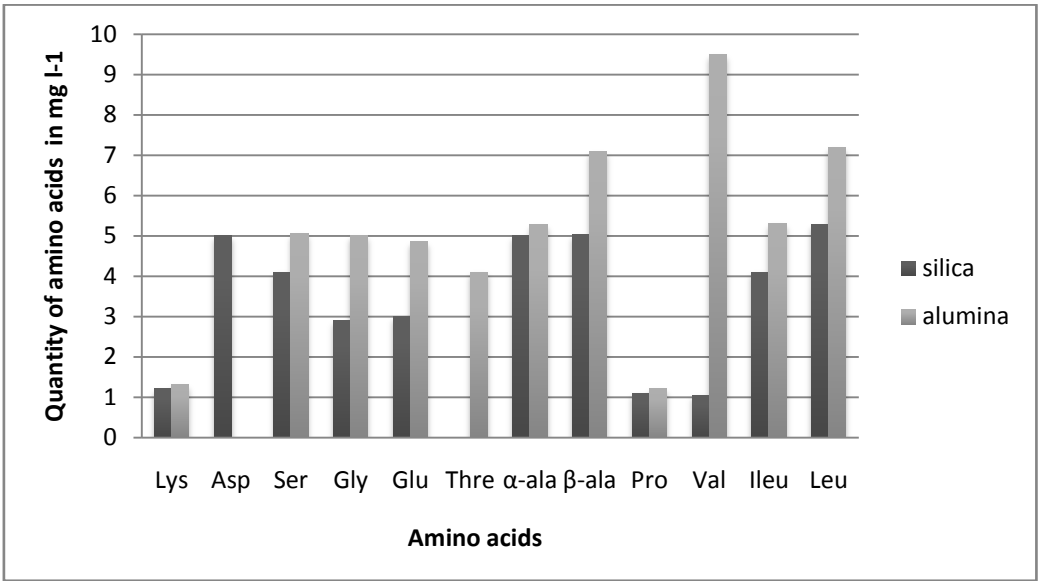


**Fig.6.** Formation of amino acids from reaction concentrates of ammonium acetate – water vapour in presence of silica and alumina a comparative diagram

**Table no 1**- Heat induced synthesis of amino acids from reaction system of ammonium acetate-water vapour at $95 \pm 5^0$C under wetting - drying cycles

| **Reaction system** | **Duration of heating** | **Number of products(mgl$^{-1}$)** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **I** | **II** | **III** | **IV** | **V** | **VI** | **VII** | **VIII** | **IX** | **X** | **XI** | **XII** |
| $CH_3COONH_4$+$H_2O$(V) | 50hrs | - | - | 1.10 | 4.29 | - | 3.20 | - | - | - | 4.19 | - | - |
| | 75hrs | 0.52 | 0.26 | 1.01 | 5.50 | T | 5.80 | - | 1.04 | + | 9.49 | - | - |
| | 100hrs | 1.21 | 2.90 | 7.09 | 7.19 | 1.24 | 10.5 | 4.86 | 0.91 | 5.30 | 1.17 | 1.05 | 5.67 |
| | 150hrs | 0.66 | 4.80 | 6.70 | 9.49 | 1.22 | 4.10 | 0.65 | 0.52 | 2.93 | 0.39 | 0.79 | 2.90 |
| | 200hrs | + | + | 6.89 | 2.93 | T | 1.31 | - | - | 0.65 | T | - | 0.56 |
| 1. Rf% at 232±$^0$C using | | | | | | | | | | | | | |
| (i)n-BAW,4:1:5v/v upper layer | | 17 | 20 | 21.5 | 23.9 | 26 | 30 | 34 | 36 | 39 | 41 | 45 | 50 |
| (ii)Phenol –water 80:20w/v | | 30 | 32 | 36.9 | 40 | 49 | 55 | 58.5 | 60 | 63 | 66.9 | 70 | 75 |
| 2.Colour with: | | | | | | | | | | | | | |
| (i)Ninhydrin | | V | V | V | PB | V | V | V | V | V | V | V | V |
| (ii)Isatin | | PBr | dB | O | P | PB | BV | RV | BV | BV | Brp | RV | Bp |
| (iii)Folin's reagent | | GBr | VR | GBr | GY | BG | G | GB | gBR | - | gBr | GY | GY |
| 3.Solubility in | | | | | | | | | | | | | |
| (i) Ether | | Ins | Ins | Ins | Ins | - | Ins | Ins | - | Ins | Ins | Ins | Ins |
| (ii) Dilute HCl | | S | S | S | S | S | S | S | S | S | S | S | S |
| 4.UV-Fluorescence at 254 nm | | BW | BW | BW | BW | - | BW | BW | - | BW | BW | BW | BW |
| 5. Amino acid Overlapped in co-chromatography | | Lys | Asp | Ser | Gly | Glu | α-Ala | - | α - ABA | - | Val | Ileu | Leu |
| 6.Amino acids identified | | Lys | Asp | Ser | Gly | Glu | A-Ala | - | α - ABA | - | Val | Ileu | Leu |

V,violet; P, pink; Br, brown; B , blue,; d , dull; O, orange; R, red; G, green; g, grey; Lys, lysine; Asp, aspartic acid; Ser, serine; Gly, glycine; α-Ala , α,-alanine; α - ABA α,- aminobutyric acid; Val, valine; Ileu, isoleucine; Leu, leucine; Ins, insoluble; S, soluble; BW, bluewhite

**Table no 2** Quantity ( $mgl^{-1}$ ) and physicochemical characteristics of the amino acids from aqueous solution of ammonium acetate heated upto 100 hrs, at 95 ± $5^0$C in presence of metal oxides

| Reaction system comprised | Number Of Products Quantity ($mgl^{-1}$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII |
| $CH_3COONH_4$-$H_2O$(v)-Silica | 1.21 | 0.05 | 4.10 | 2.90 | 2.99 | T | 5.01 | 5.03 | 1.10 | 10.5 | 4.10 | 5.28 |
| $CH_3COONH_4$-$H_2O$(v)-Alumina | 1.31 | T | 5.05 | 5.00 | 4.86 | 4.10 | 5.28 | 7.09 | 1.21 | 9.49 | 5.30 | 7.19 |
| 1.$R_f$ (%)values at 18 ± 2°C | | | | | | | | | | | | |
| n-BAW,4:1:1v/v | 8 | 12 | 16 | 20 | 24 | 26 | 30 | 34 | 38 | 40 | 45 | 49 |
| n-BAW,4:1:5v/v upper layer | 13 | 15 | 19 | 22 | 26 | 30 | 34 | 36 | 41 | 45 | 49 | 56 |
| 2. Colourwith (i) Ninhydrin | V | V | V | RV | V | V | V | V | V | V | V | V |
| (ii) Isatin | PBr | dB | O | P | PB | BrV | BV | gB | B | BrP | RV | Bp |
| 3. Solubility in Dilute HCl | S | S | S | S | S | S | S | S | S | S | S | S |
| 4. Amino acids overlapped in co-chromatography | Lys | Asp | Ser | Gly | Glu | Thre | α-Ala | β-Ala | Pro | Val | Ileu | Leu |
| 5. Amino acids Identified | Lys | Asp | Ser | Gly | Glu | Thre | α-Ala | β-Ala | Pro | Val | Ileu | Leu |

Abbreviations are same as above table

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