

## Separation and identification of amino acid by paper chromatography

Experiment No: 11

9-1-2019

Separation and identification of amino acid by paper chromatography.

### Apparatus:

Test tubes, test tubes rack, capillary tube, thread and chromatographic tank.

### Chemicals:

amino acids eg. glutamic acid, aspartic acid, glycine, vaniline, ninhydrin soln.

### mobile phase:

n butanol : acetic acid : H<sub>2</sub>O

4 : 1 : 1

12 : 3 : 5

n butanol : methyl ethyl ketone : H<sub>2</sub>O

2 : 2 : 1

### Theory:

amino acids are colourless  
So 2% solution of ninhydrin

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Chemicals:

Ninhydrin solution, Amino acids  
e.g. glycine, valine, aspartic and glutamic acid.

Mobile Phase:

Isopropyl Alcohol : 2/1 NH<sub>3</sub>  
2 : 1

n-butanol : acetic acid : H<sub>2</sub>O

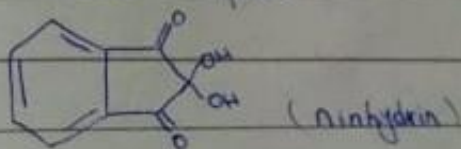
4 : 1 : 1

12 : 3 : 5

n-butanol : methyl ethyl  
ketone : H<sub>2</sub>O

2 : 2 : 1

is used as locating agent and give pink color spots



### Procedure:

solution of amino acids were prepared and applied on Pencil line at filter paper with capillary tube. poured mobile phase in tank and hanged paper with thread. After 70% flow of solvent front, paper was dried and sprayed with ninhydrin to locate colourless spots.

### Result:

R<sub>f</sub> value of mixture found 0.12 which show it contain valine amino acid.

### Discussion:

Amino acid is basic unit of protein

# Identification and calculation:

NO	Amino acid	R <sub>f</sub> value
1	glycine	0.182
2	valine	0.118
3	glutamic acid	0.74
4	aspartic acid	0.23
5	mixture	0.12

Chromatogram?

10/12/20