

Fractionation of amino acid essay sample



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In the separation and purification of a single protein or amino acids, a solution containing the desired analyte is passed through a column of ion exchange media, and the eluent checked to ensure that the desired component of mixture has bound to the column. The column is then washed with 2 – 5 column volumes of low ionic strength buffer to remove any unbound material which has simply adhered to the column. There are four steps of mechanism, firstly is the selective adsorption of the molecules to be separated by the ion exchange resins followed by release of the exchangeable group from the matrix. Next is the elution of the absorbed molecule by specific eluants and regeneration of the matrix by recharging with the original exchangeable groups (James, 1972).

Since amino acids do not absorb light in the visible region therefore are not coloured and cannot be seen with the “ naked” human eye. In this case, the visualization reagents must be used to identify this compound. Amino acids react with a dye called ninhydrin to form a highly conjugated aromatic derivative which absorbs light in the visible portion of the spectrum.

Furthermore, ninhydrin amino acid derivative became blue-violet color and easily visible. For example, determination of tryptophan in proteins and feed stuff has been carried out by ion exchange chromatography with the use of ninhydrin in amino acid estimation (Khan, 2008). Besides, Ninhydrin reacted to decarboxylate the amino acids, and yield an intensively blue-purple coloured product with having absorption maximum at 570nm and the mechanism shown below:

The Sakaguchi test is a specific qualitative test for the detection of a specific type of protein with the amino acid containing the guanidinium group, and

has been used for determining trace amount of arginine in biological materials. Besides, amino acids are ampholytes with pH-dependent net charges. For example, arginine has the highest isoelectric point ($pI = 10.8$) among amino acids commonly found in grape juice. The result shown a red solution formed due to the mechanism of Sakaguchi test. In acidic solution, Arg^{2+} and Arg^+ can be absorbed by strong cation-exchange resins and eluted from the resin by alkaline solution. Alpha naphthol and sodium hypobromite/chlorite react with the aforementioned compound to form red-orange complexes. Arginine is removed from the column by an alkaline solution, usually sodium hydroxide since it may interfere with the Sakaguchi method (Li et al, 2008). Conclusion

The reaction with ninhydrin reagent shows a positive test when there is a change in colour (primary amines will give a blue product). In addition, alpha naphthol and sodium hypochlorite react with the aforementioned compound form red-orange complexes.

References

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