# Amino Acid Analyzer

Amino acid analysis is a technique based on ion exchange liquid chromatography, used in a wide range of application areas to provide qualitative and quantitative compositional analysis.

The basic principle of operation is the continuous flow chromatography procedure to produce fully automatic, high speed, sensitive analyses. This is somestimes referred to as classical amino acid analysis.

The sample containing a mixture of amino acids is loaded onto a column of cation-exchange resin. Buffers of varying pH and ionic strength are then pumped through the column to separate the various amino acids. The column temperature is accurately controlled and can be varied, as necessary, to produce the required separation. The column eluent is mixed with the ninhydrin reagent, this mixture being passed through the high temperature reaction coil. In the reaction coil ninhydrin reacts with the amino acids present in the eluate to form coloured compounds. The amount of coloured compound produced is directly proportional to the quantity of amino acid present in the eluate.

From the reaction coil, the eluate/ninhydrin mixture is fed to the photometer unit where the amount of each coloured compound is determined by measuring the amount of light absorbed. The light absorption is measured at two wavelengths, 570nm and 440nm, because amino acids produce coloured compounds which absorb light with a wavelength of 440nm(yellow color by proline and hydroxyproline), whereas other amino acid coloured compounds absorb light at 570nm(purple color).

The photometer output is connected either to a two channel chart recorder which plots the amino acid concentrations as a series of peaks or to an appropriate integration system. The retention time of the peak on the chart identifies the amino acid, the area under the peak indicating the quantity of amino acid present. As an amino acid analyser is a comparative instrument, a calibration analysis must be performed before commencing a series of analyses, to produce a standard trace for comparison purposes.

After each sample analysis, the column is regenerated by pumping a strong base through the column followed by buffer 1 which equilibrates the column prior to the next analysis.

All amino acid analysis systems are completely automatic, all functions of the analyser are controlled by the software. Various analytical standard programs are supplied with the instrument, these programs contain all the information required to perform the analysis.





