For lipids, add ethanol to extract (dissolve) any lipids present (lipids are soluble in alcohols). Pour the solution into water in a clean test tube. If lipids are present, a cloudy white emulsion will form near the top of the water.

## Quantitative methods to determine the concentration of a chemical substance in a solution

To include colorimetry and the use of biosensors (an outline only of the mechanism is required).

A colorimeter measures the percentage absorbance/transmission of light by a coloured solution. The more concentrated a solution is the more light it will absorb and the less light it will transmit.

A cuvette filled with distilled water is first place in the colorimeter to calibrate it and used after every measurement taken. The solutions are first filtered to remove precipitate and placed in cuvettes. The colorimeter will measure the % transmission/absorbance. A calibration curve can be plotted to show the results.



Biosensors determine the presence and concentration of molecules.

Molecular recognition – A protein or DNA is immobilised to a surface. This will interact with, or bind to, the analyte (compound under investigation).

Transduction – This interaction causes a change in the transducer. The transducer detects changes and produces a response.

Display – A visible, qualitative or quantitive signal is produced.

## The principles and uses of paper and thin layer chromatography to separate biological molecules / compounds *To include calculation of retention (Rf) values.*

Chromatography allows us to separate substances in complex mixtures.

In paper chromatography, the stationary phase is paper. The mobile phase may either be an aque up heter-based) liquid or a non-aqueous organic (carbon-based) solvent.

Thin layer chromatography (TLC) is similar to paper chromatography but osteal or paper, the stationary phase is a thin layer of an inert (unreactive) substance (e.g. silica) supported of a nine to reactive surface (e.g. a glass plate).

TLC tends to produce more useful chromatograms than paper chromatography, which show greater separation of the components in the mixture - and are therefore tasks to analyse.

Rf values = <u>Distance traval to py component</u> Distance travelled by solvent

Rf values are always less than 1.

## Practical investigations to analyse biological solutions using paper or thin layer chromatography For example the separation of proteins, carbohydrates, vitamins or nucleic acids.

A pencil line, about 2cm from the bottom, is drawn on the chromatography plate/paper. The solution is spotted using a capillary tube and allowed to dry before being spotted again (If it is unknown, the other known solutions are also spotted at a distance from the unknown spot). The plate/paper is then placed in a solvent about 1cm above the bottom of the plate/paper. It is left in the solvent until the solvent has reached about 2cm from the top. The plate/paper is left to dry.

Colours may be used to make colourless solutions clearer e.g. Ninhydrin spray reacts with amino acids turning them a purple/brown colour.