

Separation Techniques – Partitioning of Indicators

Background: A two-phase solvent system is made up of a polar and a non-polar solvent. Based on the differential solubility of the indicator added to such a two-phase system, and the subsequent partitioning of the chemical in one of the phases, we can determine whether the indicator is polar or non-polar.

This principle of partitioning can be used to separate components of a mixture using Partition chromatography wherein the mobile phase could be a mixture of solvents differing in their polarities and the stationary phase is also some solvent bound to a matrix such as paper.

In this experiment, we will be able to determine the nature of different indicators depending on their affinity for a particular solvent in a two phase solvent system. We will be using diethyl ether and distilled water as the two solvents to form the two-phase solvent system. Methyl orange, Sudan red and Thymol blue will be used as the indicators.

Requirements:

Category	Particulars
Chemicals	<ul style="list-style-type: none"> • Acetone • Diethyl ether • Distilled water • Pre-made Methyl orange stain solution • Pre-made Sudan red (IV) stain solution • Pre-made Thymol blue stain solution
Apparatus/Facilities	<ul style="list-style-type: none"> • Plastic droppers • Micropipette (1000 μL) and tips • Test tubes • Test tube stand • Tip discard beaker containing tap water



Procedure:

1. Add 2 mL of distilled water to a test tube.
2. Then to the same test tube, add 2 mL of diethyl ether.
3. The test tube now contains a two-phase solvent system with diethyl ether and water forming the upper and lower phases, respectively.
4. Add a drop of methyl orange to the two-phase solvent system and shake the tube well. Based on its polarity, methyl orange will dissolve and be partitioned into one of the two phases.
5. Repeat the procedure for Sudan red and Thymol blue stain solutions in fresh tubes for each indicator, and record your observations.

Points for Discussion:

1. Identify the polar and non-polar stains based on their differential solubility in the two-phase solvent system.
2. Which of the three indicators can be used for staining waxy tissue in a plant leaf cross-section for microscopic observation?