1.14.2 Paper chromatography

In paper chromatography the stationary phase is a sheet of paper of suitable texture and thickness, which may sometimes be impregnated with a liquid phase that is immiscible with the mobile phase.

Chromatographic separations on paper are usually considerably slower than on thin-layer plates and the method is, in general, not so versatile as thin-layer chromatography since the degree of variation of the stationary phase is much more restricted. Neither is it possible to use the variety of corrosive detection reagents that is commonly employed when the adsorbent is an inorganic material coated on a glass plate. Nevertheless, paper chromatography is still a useful technique and certain very effective separations that were originally devised using paper have never been successfully transferred to the thin-layer plate. For semi-quantitative and quantitative work it is considerably easier and more effective to cut out a required area of paper and to elute a separated component than it is to remove completely the powder layer for elution, as is necessary with thin-layer chromatography.

The concepts of R_f and R_r values referred to in the discussion on thin-layer chromatography apply equally well to paper chromatography. Because of the nature of the adsorbent it is possible to carry out paper chromatography in either a descending or an ascending mode.

Recommended procedure

Descending paper chromatography

The apparatus consists of a glass chamber of suitable dimensions to accommodate the chromatographic paper used, ground at the top to take a closely fitting glass lid. The lid has a central hole about 1.5 cm in diameter closed by a heavy glass rod or a stopper. In the upper part of the chamber is suspended a solvent trough with a device, usually a glass rod, for holding the chromatographic paper. On either side of the trough, parallel to and slightly above its upper edges, are two glass guide rods to support the paper in such a manner that no part of it is in contact with the walls of the chamber. The chromatographic paper consists of suitable filter-paper, cut into strips of sufficient length, and of any convenient width between 2.5 cm and the length of the trough; the paper is cut so that the mobile phase runs in the direction of the grain of the paper.

Method . Place in the bottom of the chromatographic chamber a layer 2-3 cm deep of the stationary phase specified in the monograph. Close the chamber, and allow to stand for 24 hours at constant temperature. All operations during which the paper is exposed to the air should preferably be carried out at a relative humidity of about 50%. Maintain the chamber under these conditions throughout the subsequent procedure. Draw a fine pencil line horizontally across the paper at such a distance from one end that when this end is secured in the solvent trough and the remainder of the paper is hanging freely over the guide rod the line is a few centimetres below the guide rod and parallel to it. Using a micropipette, syringe, or other suitable means, apply to a spot on the pencil line the volume of the solution specified in the monograph. If the total volume to be applied would produce a spot more than 10 mm in diameter, apply the solution in portions, allowing each to dry before the next application. When more than one chromatogram is to be run on the same strip of paper, space the solutions along the pencil line at points not less than 3 cm apart. Insert the paper in the chamber, close the lid, and allow to stand for 90 minutes. Introduce into the solvent trough, through the hole in the lid, a sufficient quantity of the mobile phase specified in the monograph, close the chamber and allow development to proceed for the prescribed distance or time. Remove the paper from the chromatographic chamber and allow to dry in air. The paper should be protected from bright light during the development and drying processes.

Ascending paper chromatography

The apparatus consists of a glass chamber of suitable dimensions to accommodate the chromatographic paper used, ground at the top to take a closely fitting glass lid. In the top of the chamber is a device that suspends the chromatographic paper and is capable of being lowered without opening the chamber. In the bottom of the chamber is a dish to contain the mobile phase into which the paper may be lowered. The chromatographic paper consists of suitable filter-paper, cut into strips of sufficient length and not less than 2.5 cm wide; the paper is cut so that the mobile phase runs in the direction of the grain of the paper.

Method . Place in the dish a layer 2-3 cm deep of the mobile phase specified in the monograph. If specified in the monograph, pour the stationary phase between the walls of the chamber and the dish. Close the chromatographic chamber and allow to stand for 24 hours at constant temperature. Maintain the chamber at this temperature throughout the subsequent procedure. All operations during which the paper is exposed to the air should preferably be carried out at a relative humidity of about 50%. Draw a fine pencil line horizontally across the paper 3 cm from one end. Using a micropipette, syringe, or other suitable means, apply to a spot on the pencil line the volume of the solution specified in the monograph. If the total volume to be applied would produce a spot more than 10 mm in diameter apply the solution in portions, allowing each to dry before the next application. When more than one chromatogram is to be run on the same strip of paper, space the solutions along the pencil line at points not less than 3 cm apart. Insert the paper into the chamber, close the lid, and allow to stand for 90 minutes. Lower the paper into the mobile phase specified in the monograph, and allow development to proceed for the prescribed distance or time. Remove the paper from the chamber and allow to dry in air. The paper should be protected from bright light during the development and drying processes.