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A trough for descending paper chromatography with a solvent gradient

While many descriptions of gradient generating systems can be found in the scientific literature, most of them are intended for use in column chromatography, and very few are designed for application to paper chromatography. In the devices proposed for ascending[1], horizontal[2], and descending[3,4] paper chromatography, an excess of solvent is used to establish the gradient, and only a small fraction of it serves for the development of the chromatogram. A disadvantage of such systems is that a lot of solvent is wasted, and that the change in solvent composition must be carefully matched to the flow rate through the paper if reproducible results are to be obtained. Moreover the need for equipment such as magnetic stirrers or chromatographic pumps may limit the number of separations which can be carried out simultaneously.

In the trough described below, the production of the gradient is controlled only by the flow of solvent through the paper and no excess solvent is used. The system is completely analogous to gradient generators with an open mixing chamber[5] which are frequently used for column chromatography. The difference lies in the shape and size

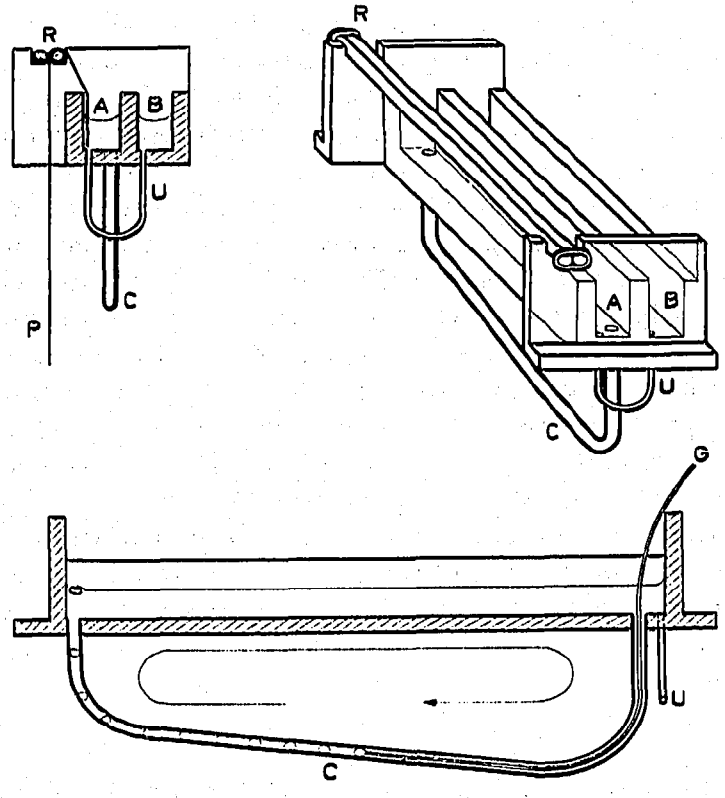

Fig. 1. Perspective view, transverse section and longitudinal section of the trough. The paper sheet P is supported by glass rods R and its edge is folded against the front wall of compartment A, which contains the solvent of lowest eluting power. As the chromatography proceeds the level in A sinks, and solvent of high eluting power from compartment B arrives by connection tube U. Efficient mixing of the two solvents is obtained by bubbling nitrogen, arriving by the gas supply tube G, through circulation tube C. The bubbles carry a stream of liquid with them, resulting in a circulation of solvent in the direction of the arrow.

of the solvent containers. Mixing is obtained by a stream of gas bubbles, and several separations may be carried out simultaneously by supplying gas to different tanks.

Apparatus and procedure

The trough was constructed from 5 mm thick Perspex plate and glass tubing. A solution of Perspex in dichloroethane was used to seal the Perspex parts together and a commercial cement (Stabilit, Henkel & Cie, Düsseldorf) to seal glass to Perspex.

The details of the construction are illustrated in Fig. 1. The trough consists of two parallel oblong compartments, one of which (A) serves as mixing chamber and the other (B) as reservoir. Both compartments are 1 cm wide and 2 cm deep, while the length is 2 cm in excess of the width of the chromatogram. Short troughs for 15 cm wide strips and long ones for 46.3 cm wide sheets were constructed. A U-shaped tube (U) of 1 mm internal diameter connects both compartments and a circulation tube (C) of 2.5 mm internal diameter connects both extremities of compartment A.

Separations are carried out as follows. After application of the substances the paper strip or sheet (P) is clamped between 2 glass rods (R) kept together by two pieces of rubber tubing, with the starting line 1 cm below the rods. The upper edge is folded inside compartment A. It may be gently pressed against the front wall by inserting a few pieces of polythene tubing, slightly thicker than 1 cm, between the paper and the back-wall. A polythene gas-supply tube (G) of 1 mm external diameter is introduced half way along circulation tube C and connected with a cylinder of nitrogen provided with a sensitive pressure gauge. The trough is placed in the chromatographic tank and the paper allowed to equilibrate with the solvent vapour. Solvent is then introduced through a hole in the tank lid into compartment B. Aqueous solutions fill the connection tube U but do not flow over into compartment A because of the water-repellant surface of the Perspex. Siphoning only starts from the moment solvent is introduced into compartment A. Organic solvents, on the contrary, start flowing over slowly but immediately so that compartment A must be filled promptly after compartment B. The gas valve is opened and the pressure adjusted so that a regular stream of bubbles through tube C is obtained. The bubbles, which should follow each other at a few mm distance, carry small cylinders of liquid between them, and an efficient circulation of solvent results. As the chromatography proceeds the solvent level in compartment A sinks and solvent from compartment B arrives by connection tube U. It is sucked up in circulation tube C and diluted homogeneously over compartment A. At the end of the run, the paper is taken out of the tank between the rods R and allowed to dry.

The volume of the gradients which may be produced range from about 1 to 3.5 ml per cm width of the paper chromatogram. There is a small dead volume of solvent remaining in tubes U and C after the run, but this is not more than 1 to 4% of the total gradient, depending on its volume.

Applications and discussion

The trough was used in this laboratory to separate ribooligonucleotides on DEAE-cellulose paper with linear salt concentration gradients. The separations were comparable to similar ones obtained on columns but were simpler to carry out and presented advantages such as high sensitivity of detection and easy recovery of separated material. Detailed results will be reported later. Wet DEAE-cellulose paper is difficult to remove from a tank without damage but this problem was solved by

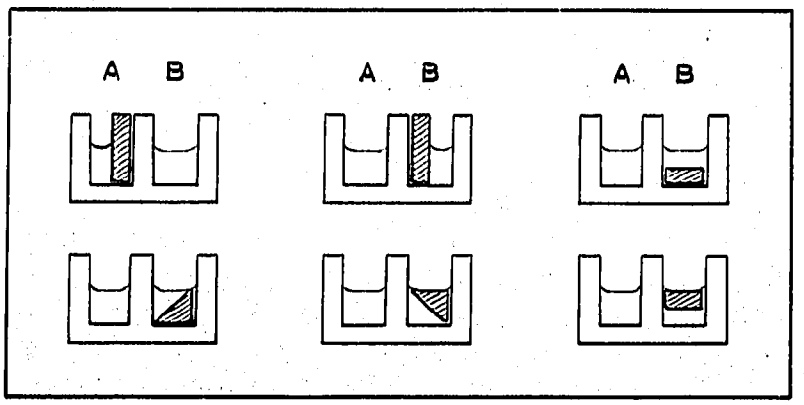

Fig. 2. Some possibilities for producing non-linear gradients. By placing adapters of different section in mixing chamber (A) or reservoir (B), convex (left), concave (middle), or discontinous (right) gradients are obtained. The adapters should be the length of the compartment but be shaped so as to leave the orifices of tubes C and U (Fig. 1) free.

clamping the paper between two rods instead of using the single anti-siphon rod of conventional equipment.

The trough can be used for any type of separation on paper with an ionic strength-, pH-, or polarity gradient. The use of solvents which dissolve Perspex may require a different construction material. As illustrated in Fig. 2, non-linear gradients may easily be produced by placing adapters of simple forms in one of the two compartments. The trough may be constructed for use with large paper sheets with a view to two-dimensional separations or combinations of chromatography and electrophoresis.

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