A SIMPLIFIED FRACTION COLLECTOR FOR GRADIENT ELUTION CHROMATOGRAPHY

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Gradient elution, as contrasted to the "step-wise" elution technique, is best executed with the aid of a fraction collector. This instrument is becoming an essential part of the equipment of many research laboratories, but unfortunately, the high prices of the apparatus available commercially are very often a deterrent to this acquisition. A relatively simple and inexpensive fraction collector has been constructed in this laboratory, and, although it does not have all the advantages which the commercial apparatus offers, it has found wide application in both the protein and virus fields.

DESCRIPTION OF THE APPARATUS

In Fig. 1 is given a fairly detailed sketch of the fraction collector. A is an Erlenmeyer flask of 250 c.c. capacity connected via a capillary tube and stopcock to a similar flask B which contains a tiny bar magnet. B is held firmly on a magnetic stirrer C.

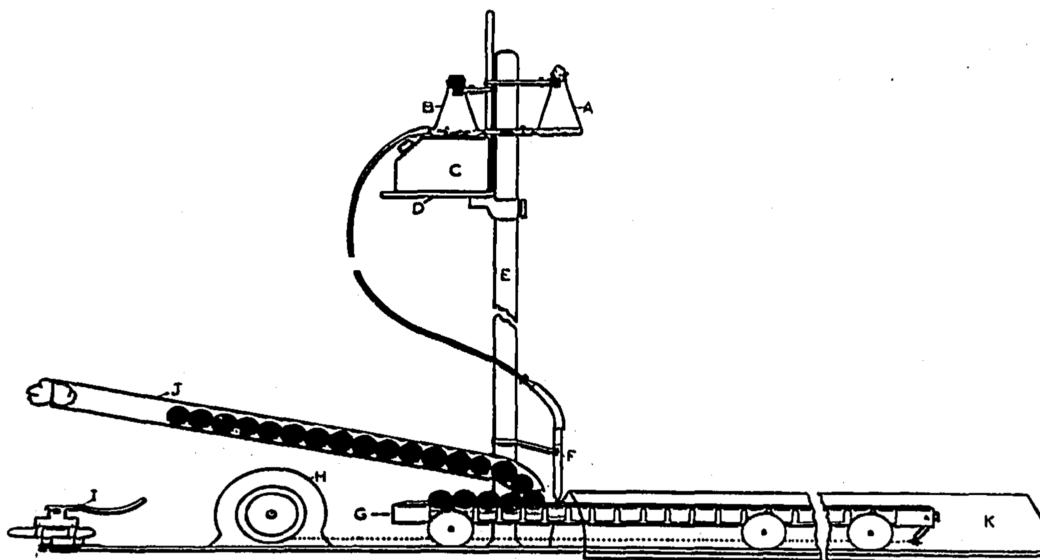

Fig. 1. The fraction collector. (For details see text).

C is supported on a metal platform D, the altitude level of which can be varied along a rigid metal rod E (2 m in length) and which is fixed onto a heavy metal plate.

A latex tube of 1 mm bore joins B onto the ion exchange column F. G is a perspex bar, of dimensions 2 × 2 × 100 cm, into which 50 cavities of uniform capacity have been drilled. The upper 2 mm of the cavities were drilled in such a way that they overlap slightly to produce sharp ridges between the cups. The perspex bar is supported by three pairs of meccano wheels which fit into grooves cut into a perspex sheet of dimensions 5 × 200 cm. The perspex carriage or "collector bar" is pulled along the tracks by a cotton thread which is wound up on a pulley attached to the shaft of an electric clock motor H. By attaching pulleys of different diameters onto the shaft, the speed by which the collector bar is pulled along the tracks may be varied. When the front of the carriage reaches the end of the tracks it is stopped automatically when it pushes over a mercury switch I, which, in turn disconnects the current supply to the motor.

A means is provided for minimizing the time of exposure of the samples collected in the cups to the atmosphere. A glass tube J, 100 cm long and 2 cm internal diameter, has its end bent slightly and its periphery cut to form an elipsoidal orifice, so that when the tube is mounted on a stand the elipsoidal orifice will be parallel to the surface of the collector bar. Prior to mounting, the tube is filled with glass marbles of approximately 15 mm diameter and autoclaved. The tube is mounted above the collector bar in such a way that each cavity is covered by a marble as it moves past the ion exchange column. The tube is held obliquely in order to reduce the weight of the column of marbles on the collector bar.

The salt or pH gradient, whichever it may be, is produced by means of the arrangement of flasks A and B and the magnetic stirring mechanism in a similar manner to that described previously by POLSON AND CRAMER[1]. The gradient curve is established from refractive index measurements on droplets taken from the various samples collected. An Abbe refractometer is well suited for the purpose.

Sterilization

Autoclaving as a means of sterilization of the collector bar was avoided on account of the harmful effect which excessive heating has on perspex. The cavities were therefore sterilized by washing with a 10 % formalin solution followed by thorough rinsing with cold sterile distilled water. Prior to use the bar is kept under the perspex hood K shown in Fig. 1.

RESULTS

Turnip yellow mosaic virus (TYMV)

2 ml of a 1 % virus prepared according to the method of MARKHAM AND SMITH[2] suspension in 0.01 *M* phosphate buffer of pH 7.2, to which was added a trace of rabbit haemoglobin, was adsorbed onto a DEAE anion exchange column and eluted by a gradient of sodium chloride in 0.01 *M* phosphate buffer. The haemoglobin served as a control for the process. The virus antigen contents of the different fractions collected were assayed by the double gel diffusion technique described by POLSON[3], after they had been dialysed against saline and their volumes adjusted to 2 ml.

In Fig. 2 the results of the experiments are given. The percentages of original virus in the different cups are plotted as ordinates against the sample number as abscissae. The salt gradient curve is shown as a separate line running obliquely across the diagram. It will be noticed that the virus antigen was eluted as a single component

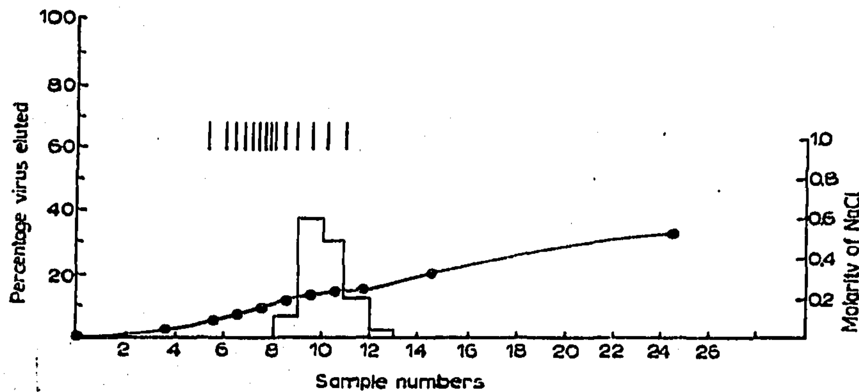

Fig. 2. Elution diagram of TYMV from a DEAE column. The ordinates present the percentage of original virus antigen present in each sample. The position of the haemoglobin is indicated by vertical stripes, sample 8 containing the highest concentration. The oblique line presents the sodium chloride concentration of the fractions as determined by light refraction measurements. The bulge in the line between sample 9 and 12 is due to the contribution of refractive index by the virus antigen.

from the column and that there was only very slight tailing on the elution diagram. In Fig. 3 is given a photograph of the gel precipitin apparatus taken during the formation of the precipitin bands in the test on the antigen contents of the fractions. Preliminary tests indicated that well defined precipitin bands may be produced by TYMV suspensions containing as little as 10^{-5} g per ml. It therefore follows that the virus contents of the fractions which failed to produce bands contained less than this amount of antigen.

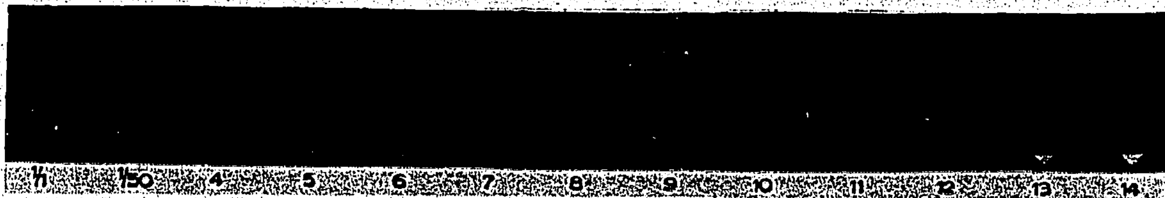

Fig. 3. Double gel precipitin tests on the different samples collected. The photograph is a composite picture of two separate perspex apparatuses, the break occurring between tubes 10 and 11. The samples were placed in the top compartments of the apparatus and the antiserum in the bottom cups. 0.5 % neutral agar in saline was placed in the central cavities. The first two tubes are standards showing bands formed by the original antigen and by the antigen diluted 1 in 50 respectively. The remaining tubes are numbered with the appropriate sample numbers and the second series of precipitin bands are formed by the antigen in the fractions.

MEF_1 strain of type II poliovirus

Suckling mouse-adapted MEF_1 virus was partially purified by three cycles of high and low speed ultracentrifugation of 10 ml of a 10 % brain suspension in the manner commonly used. The final pellet was resuspended in 2 ml, 0.01 *M* phosphate buffer

of pH 7.2 and, after final clarification, was subjected to DEAE adsorption elution chromatography. A trace of haemoglobin was again used as a control. The fractions obtained were titrated in 3–4 weeks old mice using six mice per tenfold dilution. The animals were observed for 3 weeks. In Fig. 4 the results of an experiment are

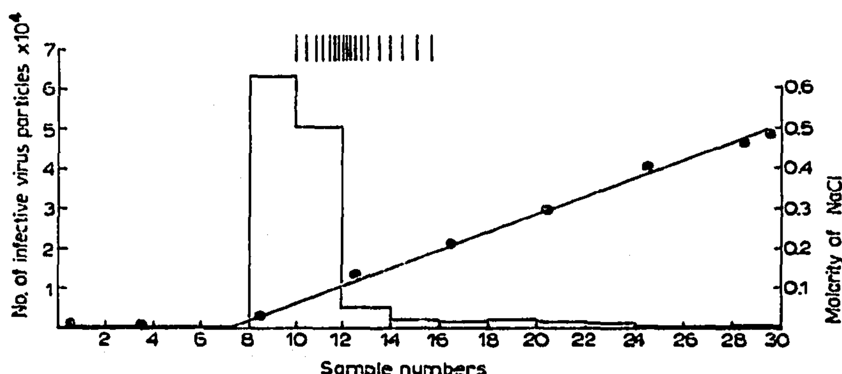

Fig. 4. Elution diagram of MEF_1 strain of Type II poliovirus. The ordinates present the titres of the different samples expressed as number of infective particles per 0.03 ml. The peak of the haemoglobin, the position of which is indicated by vertical strips, appeared in samples 12 and 13. The oblique line presents the NaCl concentration of the fractions as determined by refractive index measurements. The amount of crude virus placed on the column was too small to have any effect on the refractive index.

given. It will be seen from the elution diagram that, unlike TYMV, this virus did not behave as a single entity, but that it showed a main sharp peak which was eluted before the haemoglobin and a broader second region of activity which made its appearance after the haemoglobin had moved out of the column. Adsorption elution chromatography has been done previously on poliovirus using ECTEOLA cellulose anion exchanger by HOYER *et al.*[4].

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SUMMARY

An inexpensive collector is described which is easy to construct and to operate. The rate of flow is regulated and the fractions collected in cups drilled into a perspex bar. The cups are shaped in such a way that there is slight overlapping of their peripheries to form sharp ridges between them. The volumes of the fractions may be varied by altering the hydrostatic pressure in the adsorption column or by varying the speed with which the perspex bar is pulled past the adsorption column. The eluting gradient curves were established from refractive index measurements on droplets of the different fractions. The method is demonstrated on two viruses, TYMV and on the suckling mouse-adapted MEF_1 strain of Type II poliovirus. TYMV behaved as a single entity whereas MEF_1 poliovirus showed evidence of heterogeneity.

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