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A STUDY OF EXTRACTION COLUMNS FOR AQUEOUS POLYMER TWO-PHASE SYSTEMS

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SUMMARY

The design and performance of two types of columns for use with aqueous polymer two-phase systems is described. In one of the columns the lower phase is stationary and the upper phase is mobile, while in the other column the two phases move towards each other. Studies on the separation of nucleic acids, proteins and particles showed that the columns function efficiently.

INTRODUCTION

Macromolecules have been found to partition in a reproducible manner in a phase system composed of dextran, poly(ethylene glycol) and water together with various salts and buffers[1]. By changing the ionic composition or pH, the macromolecules can be transferred from one phase to the other. In some instances two batch extractions are sufficient to achieve satisfactory separation, while in other instances a multistage procedure is necessary. Counter-current distribution and column extractions are two such useful techniques. Owing to the small difference in the densities of the phases and their high viscosities, the polymer phase systems have a long separation time compared with conventional phase systems. ALBERTSSON[2] described a thin-layer counter-current distribution apparatus with which only a short separation time is required owing to the small depth of the phases. In this paper, the construction of two types of columns designed for use with polymer two-phase systems and experiments carried out with these columns are described. In the construction of the extraction columns, special precautions were taken to shorten the separation time. One of the columns is a modification of a column described by ROMETSCH[3] and SCHEIBEL AND KARR[4]. In this column, both upper and lower phases move towards each other through alternating mixing and settling chambers. The other type of column described has a stationary lower phase while the upper phase moves through the mixing and settling chambers.

CONSTRUCTION

Principle

Two types of columns were studied, which are referred to as type I and type II. Fig. 1 is a schematic drawing of a type I column, which consists of chambers

stacked on top of each other with a stirrer in every second chamber (A). The chambers are separated from each other by a sieve plate (C) with holes that are sufficiently large to allow free passage of the phases, but sufficiently small to prevent mixing in the alternate chambers (B). The lower phase, which is pumped into the column at the top (D), is mixed in each of the chambers with the upper phase, which is pumped into the column at the bottom (F). The lower phase is sucked off at E and the upper phase at G. The sample can either be pumped in continously or as a zone dissolved in one of the phases in the injection chamber (H) or in one of the phase inlets.

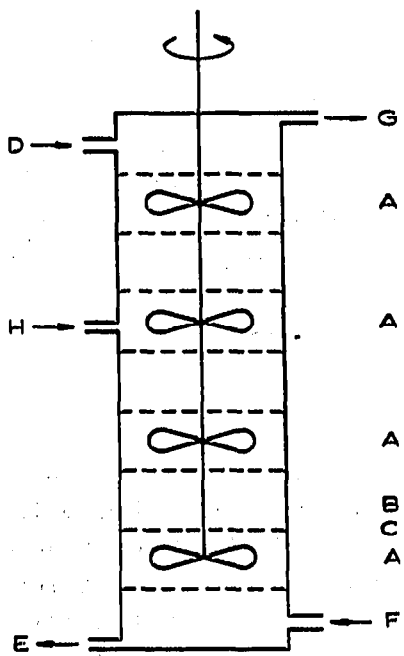


Fig. 1. Type I column. The lower phase, pumped in at D, is mixed with the upper phase, pumped in at F, in the chambers A. The upper phase is sucked off at G, and the lower phase at E.

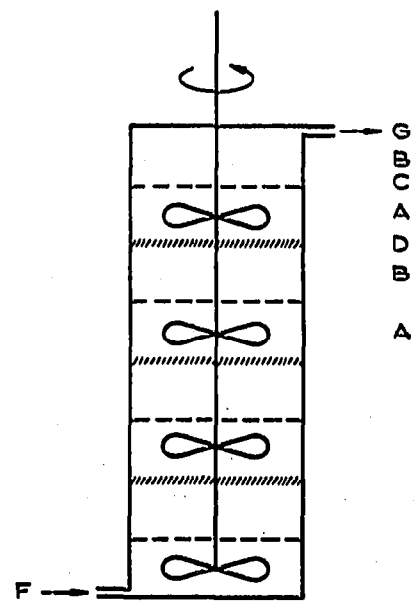


Fig. 2. Type II column. The upper phase, pumped from below into the column at F, is mixed with the lower phase in the chambers A. The upper phase is sucked off at the top of the column, while the lower phase remains stationary in the column.

Fig. 2 is a schematic drawing of a type II column, which consists of the same type of chambers as in the type I column. The sieve plate below the A-chambers is replaced by a sieve plate (D) with holes that are sufficiently small to prevent the viscous bottom phase from passing through the plate to the chamber below. The upper phase, pumped in from below the column, is mixed with the lower phase in each of the A-chambers. The phase mixture from an A-chamber enters the B-chamber above through a sieve plate (C), where the two phases separate; the upper phase moves into the mixing chamber above through a sieve plate (D) and the lower phase re-enters the mixing chamber (A). Hence, the lighter phase entering the column at F passes through the column with successive mixings and settlings until it leaves the column at G. The heavier phase is stationary in each chamber. The sample dissolved in the upper phase is injected as a zone in the upper phase inlet.

Details

Details of a type I column are shown in Fig. 3. The column consists of the following main parts: A, the lower outlet and inlet chamber; B, the upper outlet and inlet chamber; C, mixing and settling chambers; and D, spindle fitted with stirrers.

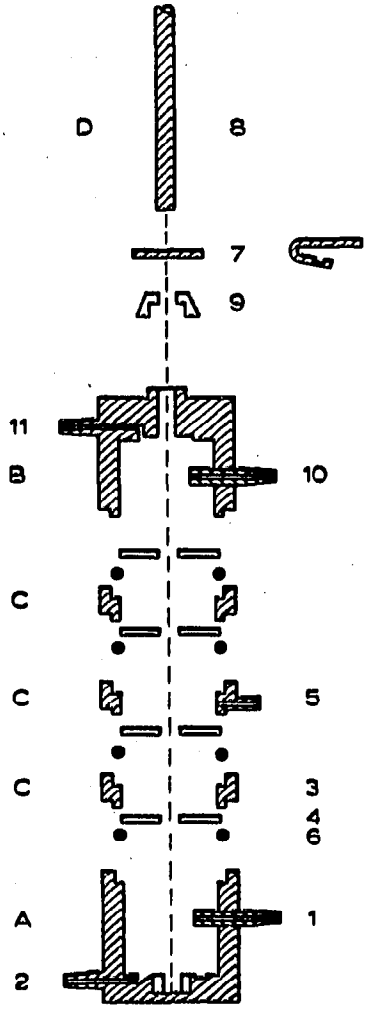


Fig. 3. Details of type I column (actual size × 3/2). For explanation, see text.

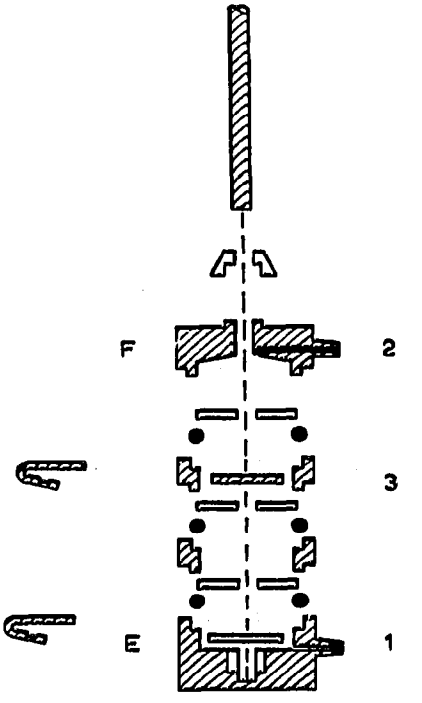


Fig. 4. Details of type II column (actual size × 3/2) For explanation, see text.

The different parts are held together with two clamps. Chamber A has a connection for the inlet (1) of the upper phase, which is situated halfway up the chamber so as to prevent the upper phase from following the lower phase out of the column at the lower phase outlet (2). Above chamber A are alternate mixing and settling chambers (3) separated by sieve plates (4). One of the mixing chambers is fitted with an inlet (5) for the injection of the sample. To avoid leakage from the column, O-ring gaskets (6) are placed between the chambers. In the mixing chambers, the mixing is accomplished by stirrer (7) mounted on a spindle (8), which passes down the length of the column. The stirrer is a bent rod, which can be clamped on the spindle. At the top of the column is the upper outlet and inlet chamber, B. The spindle passes through

the chamber, which is fitted with a Teflon gasket (9) to prevent leakage from the spindle entry. The inlet (10) for the lower phase is placed halfway down chamber B so as to prevent the lower phase from following the upper phase out of the column at the upper phase outlet (11).

Fig. 4 shows details of a type II column. The column consists of the same parts as a type I column, with the following exceptions. The inlet (1) for the upper phase is placed in the bottom of chamber E and the outlet (2) for the upper phase is placed in the top of chamber F. A stirrer is placed in every second chamber beginning with chamber E. The last chamber with a stirrer is the chamber below F. Below each mixing chamber is placed a porous Vion plate (3), which prevents the lower phase from passing down through the column but does not prevent the upper phase from passing through.

MATERIALS AND METHODS

The polymers used were Dextran 500, Pharmacia Fine Chemicals, Uppsala, Sweden, and poly(ethylene glycol) (Carbowax 6000), Union Carbide Chemical Co., New York, U.S.A. Dextran 500 had a weight-average molecular weight of 518000. The molecular weight of poly(ethylene glycol) was about 6000–7500. The phase system used contained 7 % Dextran 500 and 4.4 % Carbowax 6000.

The source of the DNA used was calf thymus. The phycoerythrine phycocyanine mixture was a gift from Prof. S. HJERTÉN, Uppsala, Sweden.

All the chemicals used were of analytical grade. The water used was distilled twice in a quartz apparatus.

For pumping the phases, an ordinary peristaltic pump was used (Stålprodukter, Uppsala, Sweden).

The UV absorbance was measured in some experiments with an absorptiometer at 254 nm (Uvicord Absorptiometer, LKB-Produkter AB, Stockholm, Sweden). In the other experiments, the concentration of the dissolved substance in the two phases was measured in a Zeiss PMQ II spectrophotometer. Before measurements were made in the spectrophotometer, the phase to be measured was diluted with buffer so as to diminish light scattering.

Type I columns

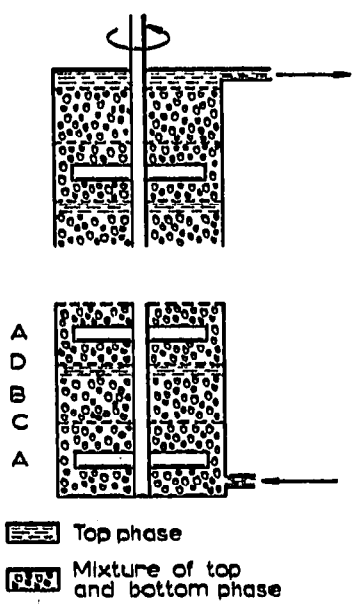
A typical experiment is carried out in the following manner. The lower phase is pumped into the column at the bottom with an automatic syringe and, after half the column has been filled, the upper phase is pumped into the column, also at the bottom. The filling must be carried out with great care in a dry column so as to prevent the formation of air bubbles. When the filling is completed, the motor that is used to drive the stirrers is started so that the stirrers rotate at 60–80 r.p.m. When a clear layer of upper phase is observed in the upper outlet and inlet chamber, the peristaltic pumps are started with a maximum pumping rate of 0.12 ml/min. The sample, dissolved in one of the phases, can now be applied as a zone or continuously to either the injection chamber or one of the inlets. The sample is pumped into the injection chamber at 0.01 ml/min.

Partition of biological particles often occurs between one of the phases and the interface, in which event a mixture consisting of a major proportion of one of the phases and a small amount of the other must be withdrawn, so that particles

sticking to the interface will leave the column. This can be achieved by increasing the pumping rate of the lower phase, so that a small amount of the upper phase will leave the column with the lower phase. The pumping rates used in this type of experiments were 0.12 ml/min for the upper phase and 0.20 ml/min for the lower phase.

Type II columns

The column is filled with a homogeneous mixture consisting of 50 % of upper phase and 50 % of lower phase, by using an automatic syringe. To prevent the formation of air bubbles in the column, it is advisable to remove the dissolved air in the phase system before pumping it into the column. After several minutes, when the two phases have settled in the column, each mixing chamber is filled with the lower phase and each settling chamber with the upper phase. The sample dissolved in the upper phase is then injected into the upper phase inlet with an ordinary syringe. When the sample enters the chamber, it will displace the heavier phase into the settling chamber above, where the heavier phase will displace the lighter phase into the mixing chamber above. This process is repeated throughout the column. When the sample has been applied, the mixing is started and the upper phase is pumped into the column at a rate of 0.15 ml/min. When the upper phase enters mixing chamber A, it is mixed with the lower phase and the phase mixture thus formed moves into settling chamber B through sieve plate C. The phase mixture settles and the upper phase moves through sieve plate D and enters the next mixing chamber, and the process is repeated throughout the column, as illustrated in Fig. 5.



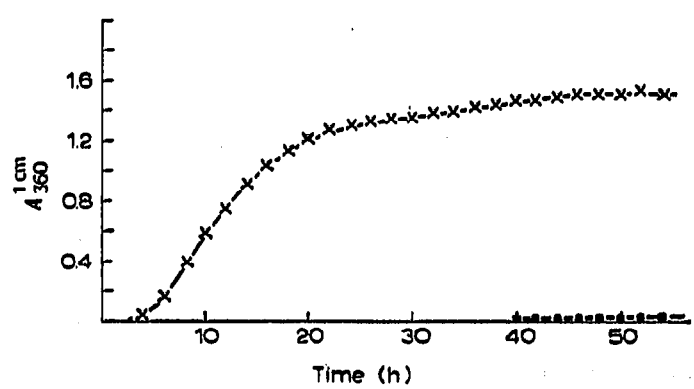


Fig. 5. Principle of type II column. The upper phase pumped in from below is mixed with the lower phase in chamber A. The phase mixture thus formed will displace the upper phase in chamber B to the chamber A above. The process is repeated along the length of the column and the upper phase is sucked off at the top of the column.

Fig. 6. Partition of DNP-methionine in type I column. ×—×, Concentration of DNP-methionine leaving the column in the upper phase and ●—●, concentration of DNP-methionine leaving the column in the lower phase. The rates of pumping the phases are in the ratio 13:1, the upper phase having the higher rate. The ionic composition in the phase system was 5 m*M* NaH_2PO_4 and 5 m*M* Na_2HPO_4. The temperature during the experiment was 23°.

RESULTS

To determine the number of theoretical plates in the type I column, DNP-methionine dissolved in the upper phase was pumped continuously into the injection chamber placed in the middle of the column. When a constant concentration of DNP-methionine in the lower phase relative to the upper phase was obtained (Fig. 6), the experiment was stopped and the number of theoretical plates was calculated according to ROMETSCH[3]. In a column consisting of 10 mixing and 10 settling chambers, the number of theoretical plates was 31.

To study the behaviour of macromolecules, native and denatured DNA were used as models. A zone of heat-denatured and native DNA was injected into the middle of the column. In the system used, native DNA having a high K-value (K = concentration in upper phase/concentration in lower phase) left the column with the upper phase while denatured DNA having a low K-value left the column with the lower phase (Fig. 7).

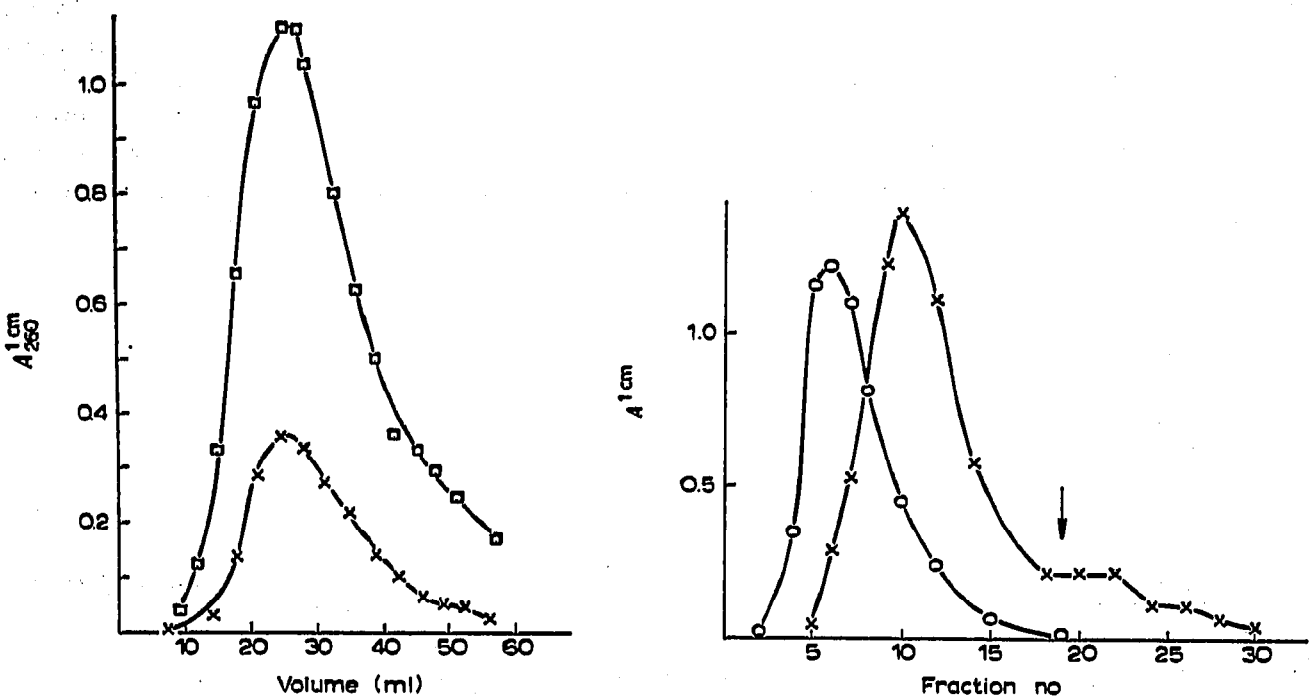


Fig. 7. Separation of a mixture of native DNA and heat-denatured DNA by using type I column. □—□, Native DNA leaving the column in the upper phase and ×—×, denatured DNA leaving the column with the lower phase. The ionic composition of the phase system was 5 mM NaH_2PO_4 and 5 mM Na_2HPO_4. The experiment was carried out at 23°.

Fig. 8. Separation of a mixture of *E. coli* G 11 A1 and *Chlorella pyrenoidosa* by using type I column. The bacteria leave the column with the lower phase (×—×) while the green algae leave the column with the upper phase (○—○). The arrow indicates the content of green algae in the lower phase when the column is emptied of the phase system. The temperature during the run was 23°.

Fig. 8 shows a separation of green algae (*Chlorella pyrenoidosa*) from bacteria (*Escherichia coli* K 12, strain G 11 A1). In a phase system with the ionic composition 5 mM KH_2PO_4 and 5 mM K_2HPO_4, the algae will prefer the upper phase while the bacteria mostly stick to the interface. When the column is run as described above,

the bacteria will leave the column with the lower phase and the algae with the upper phase. Microscopic studies of the two phases showed no contaminating bacteria among the algae in the upper phase and no contaminating algae among the bacteria in the lower phase.

Fig. 9 shows an extraction experiment with a type II column with DNP-methionine as the sample. The theoretical curve was calculated according to BRENNER *et al.*[5] and it can be seen that it coincides well with the experimental curve. The number of theoretical plates calculated according to the usual formula for chromatography[6] was found to be 73 in a column consisting of 50 mixing and 50 settling chambers.

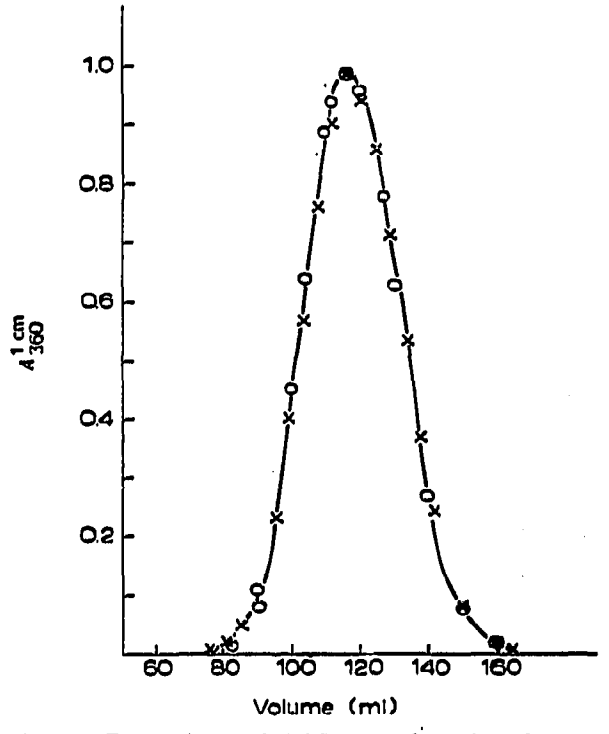


Fig. 9. Partition of DNP-methionine in type II column. ×—×, DNP-methionine leaving the column with the upper phase and o—o, the theoretical curve. The ionic composition of the phase system was 5 m*M* NaH_2PO_4 and 5 m*M* Na_2HPO_4. The experiment was carried out at 23°.

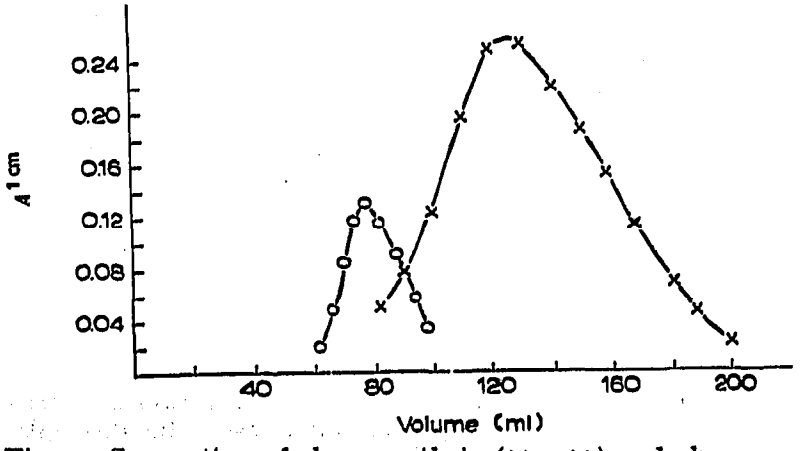


Fig. 10. Separation of phycoerythrin (×—×) and phycocyanin (o—o) by using type II column. The experiment was carried out at 23°. (For details of phases see text.)

The separation of two proteins, phycocyanin and phycoerythrin from red algae, is shown in Fig. 10. In a phase system with the ionic composition 5 mM KH_2PO_4, 5 mM K_2HPO_4 and 0.75 M KCl, phycoerythrin has a partition coefficient of 0.9 and phycocyanin 2.3. The peak on the left represents phycocyanin leaving the column first, well separated from phycoerythrin, the peak on the right.

In a phase system with the ionic composition 5 mM NaH_2PO_4 and 5 mM Na_2HPO_4, native DNA has a partition coefficient above 10 while denatured DNA has a partition coefficient below 0.1. This means that native DNA will follow the

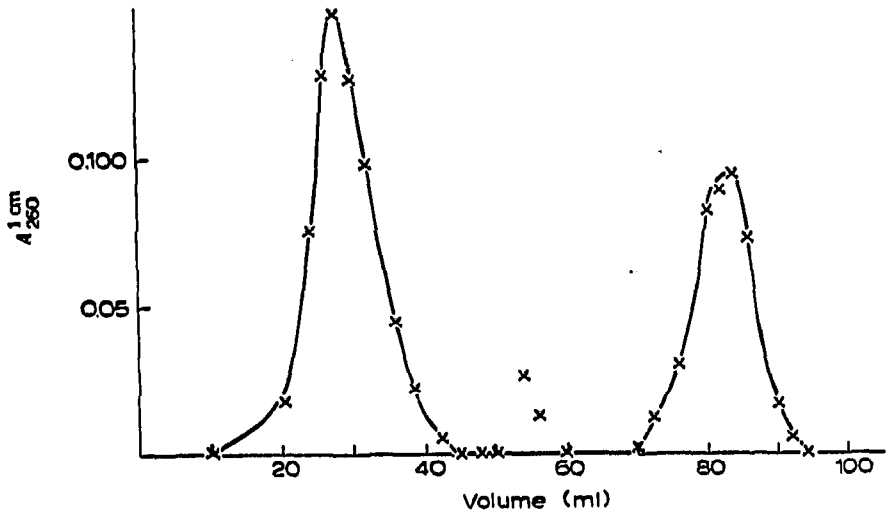


Fig. 11. Separation of native DNA and heat-denatured DNA by using type II column. The peak on the left represents native DNA, which leaves the column first. The peak on the right represents heat-denatured DNA. The temperature during the experiment was 23°. (For details of phases see text).

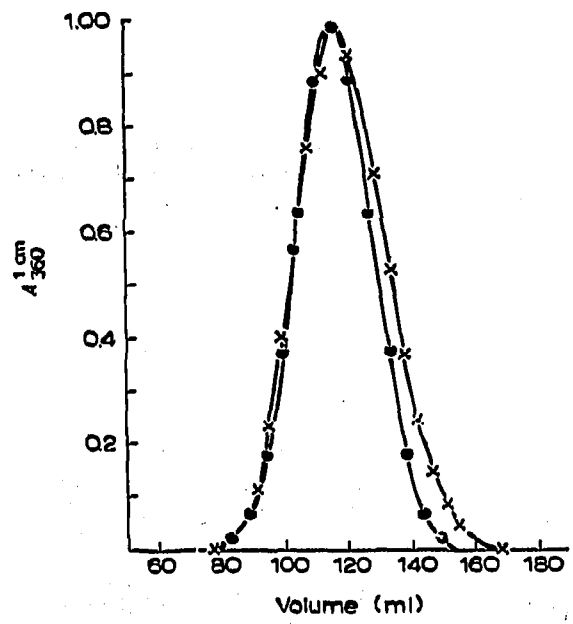


Fig. 12. Partition of DNP-methionine by using type II column. ×—×, Concentration of DNP-methionine leaving the column with the upper phase. The experimental curve coincides with the theoretical curve calculated according to BRENNER *et al.*[5]. ●—●, Theoretical curve calculated according to HECKER[7]. (For details of phases see text.)

front out of the column and denatured DNA will not move at all. To elute denatured DNA, the partition coefficient must be changed so that denatured DNA prefers the upper phase. This can be achieved by pumping in an upper phase with the ionic composition 10 m*M* Na_2HPO_4 (Fig. 11).

The efficiency of the type II column compared with counter-current distribution can be illustrated by comparing the theoretical curves calculated according to HECKER[7] and BRENNER *et al.*[5]. Fig. 12 shows the results of a column experiment compared with the two theoretical curves. It can be seen that the curve marked with closed circles, showing the theoretical curve calculated according to HECKER[7] for counter-current distribution run with a single withdrawal, is narrower than the one marked with crosses, calculated according to BRENNER *et al.*[5].

The efficiency of the type II column compared with counter-current distribution can be calculated to be 88 %.

DISCUSSION

The work with the columns described here has so far involved test experiments. However, their performance indicates that these columns would be useful in various separation problems. The separation of two classes of particles can easily be performed with the type I column while the type II column is not satisfactory for this purpose. In the settling chambers of the type II column, the particles tend to aggregate, but no such phenomena could be detected in the type I column.

The type II column proved to be best for the separation of macromolecules with closely related *K*-values. The time required for a satisfactory separation in the type I column is longer and the sample is therefore more diluted. If, instead, samples with widely different *K*-values are to be separated continuously, the type I column is recommended. The type I column can be run for several days without any detectable change in the proportions between the two phases in the column, while in the type II column carry-over of the lower phase with the upper phase out of the column occurs.

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