# AN ELECTRICAL ANALOGY TO THE GEL FILTRATION PROCESS

TORVARD C. LAURENT AND ESTAN P. LAURENT

*Department of Medical Chemistry, University of Uppsala, Uppsala, and
Department of Telegraphy and Telephony, Royal Institute of Technology, Stockholm (Sweden)*

## INTRODUCTION

Gel filtration is a type of partition chromatography in which substances are separated largely on the basis of molecular size[1,2]. It has been suggested that this is due to a decreased available volume for other molecules in the gel network, and LAURENT AND KILLANDER[3] have shown that most experimental data are compatible with such a mechanism. Their theoretical treatment of gel filtration makes it possible to predict the elution position of a substance for the ideal case, but it does not, in general, permit calculation of the shapes of the elution curves. Therefore an analog computer has been constructed which permits analysis of the effect on the elution pattern of available volume, equilibration time, and flow rate. The computer is built according to the principle that a chromatographic column can be hypothetically subdivided into a number of plates, and that the solute moves in the liquid phase from plate to plate. The solute is assumed to attain some degree of equilibrium, partial or complete, between the liquid phase and the gel phase of each plate. Each plate is simulated by an electrical circuit containing two condensors and a resistor. A somewhat similar picture of a chromatographic process has been proposed by GOLAY[4].

The picture of dividing a chromatographic column into a number of plates is common in the theoretical treatment of chromatography[5–9]. The plates defined in this paper are equivalent to conventional "theoretical plates" if the resistance in the circuit is zero. When a finite resistance is used, the analog will give a kinetic description of the chromatographic process with local non-equilibria on the column similar to that described in the "rate theories"[8–11].

## ELECTRICAL ANALOGY

It is assumed that the chromatographic bed can be represented by ninety-six (or $n$ times ninety-six) plates of equal height. The liquid column in the bed is furthermore assumed to move stepwise along the bed at intervals of $\Delta t$ seconds, each step corresponds to the height of one plate. A solute, present within a plate, is distributed between the liquid and gel phases during the time interval.

The electrical analogy of each plate is a circuit of the type shown in Fig. 1. The condensors $C_G$ and $C_L$ correspond to the gel and liquid phases, respectively. Their capacitances are proportional to the volumes of the phases which are available to the solute. When charged, their charges represent, respectively, the amounts of solute
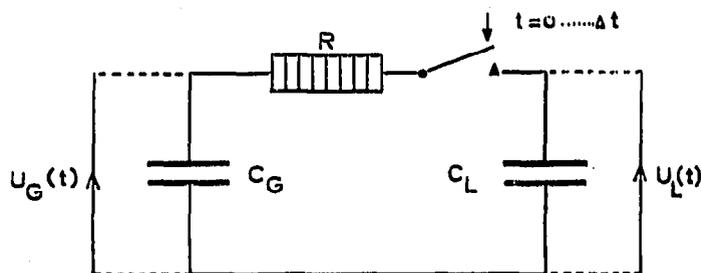
Fig. 1. Electrical analogy to the distribution process in a plate of the chromatographic column.

present in each phase. The voltages, $U_G$ and $U_L$, correspond to the concentrations of solute.

The condensors will discharge over the resistance, $R$, when the circuit is closed. The voltages, $U_G$ and $U_L$, will change during the time $\Delta t$ according to the relation:

$$U_L\,(\Delta t) - U_G\,(\Delta t) = (U_L\,(0) - U_G\,(0))\cdot e^{-\frac{\Delta t}{T}} \qquad (1)$$

where $T$ is the time constant:

$$T = R\frac{C_G\cdot C_L}{C_G + C_L} \qquad (2)$$

Since charge is conserved:

$$C_G U_G\,(0) + C_L U_L\,(0) = C_G U_G\,(\Delta t) + C_L U_L\,(\Delta t) \qquad (3)$$

These three relations determine $U_G\,(\Delta t)$ and $U_L\,(\Delta t)$.

The gel phase in the column is represented by 96 equal condensors, $C_G'$, $C_G''$ ..., and the liquid phase by one condensor, $C_L$, which may be connected successively to the gel condensors by a sliding contact. Representation of the liquid phase by a single condensor instead of a row of condensors sliding parallel to the gel condensors simplifies the construction. Each movement of the contact arm along the gel condensors corresponds to the transport along the gel bed of a volume of liquid equal to that in one plate. Any amount of liquid can be transported along the bed by repeating the procedure an appropiate number of times. The voltage retained by the condensor $C_L$ after the sliding contact has passed all gel condensors is proportional to the concentration of solute in the liquid when it emerges from the column. The voltage is recorded by a recording voltmeter.

A particular solute introduced into a gel bed is distributed between the liquid and gel phases in a characteristic way. The partition at equilibrium between the gel phase and liquid phase is equal to the ratio $C_G/C_L$. It can be varied by varying $C_L$.

A diffusion equilibrium will not be reached in each plate if the diffusional flux between the gel phase and liquid is slow or if $\Delta t$ is very short. This is analogous to a high value of the resistance, $R$ (Fig. 1). By varying $R$, one can obtain various concentration distributions, which can be expressed as fractions of the initial concentration differences. Thus, a variation of $R$ represents either a change in flow rate or a change in the time constant for diffusional exchange between gel and liquid.

## CONSTRUCTION OF COMPUTER

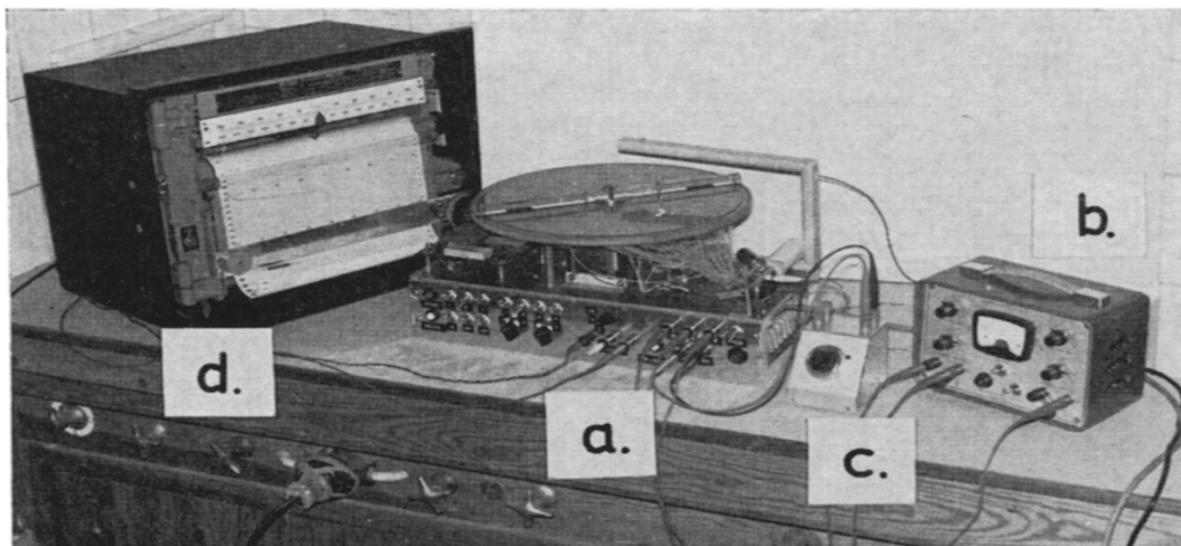An illustration of the instrument is shown in Fig. 2. The main parts are: (a) a con-

Fig. 2. View of the analog computer.

nection block containing all the condensors with a distributor on top; (b) a power supply; (c) a variable resistor; and (d) a recording voltmeter.

A general circuit diagram is shown in Fig. 3. The distributor, D, effects the connections between the condensors. It consists of a wiper arm mounted on a driving shaft which passes through the centre of a circular plate. The wiper arm is rotated at 2.2 revolutions per second by a synchronous motor. The connections to the 96 condensors (0.96 $\mu$F $\pm$ 0.5 % each) are arranged in a semicircle at the edge of the distributor. The condensors, designated $C_G'$, $C_G''$ ... etc., are connected with each end of the wiper shaft through sliding contacts.
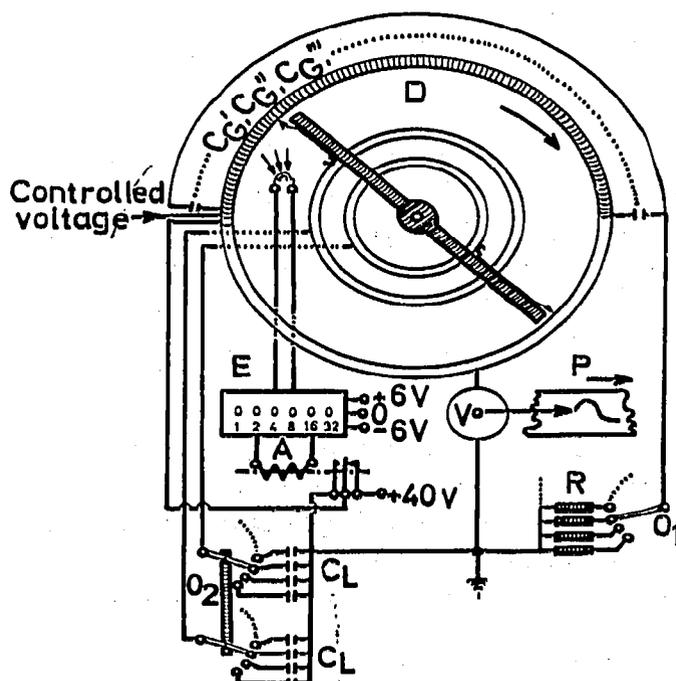


Fig. 3. General circuit diagram of the analog computer.

There are two sets of condensors corresponding to $C_L$. These two sets are so designed (see appendix I) that their capacitances can be varied between 0.27 and 9.12 $\mu$F. Each set of $C_L$ is connected by a sliding contact to one side of the wiper arm and thereby to $C_G$. The value of $C_L$ is varied by adjusting a bank of switches on the front panel.

Before the arm passes $C_G'$, it will obtain an electrical impulse from a power supply (see appendix II) which is connected to the first contact in the semicircle. The number of times that the wiper shaft carries a new charge to $C_G'$ is regulated by a relay, A, and an electronic counter, E (see appendix III). The electronic counter can be preset on the front panel for a certain number of impulses (1 to 63). More than 63 impulses can be introduced if the counter is short circuited. The voltage of the charging impulse can be varied if desired, by the use of a special arrangement (see appendix IV).

After the wiper shaft has passed the last $C_G$ condensor, it connects $C_L$ with the recording volt meter, P, which records the voltages on a moving paper strip. $C_L$ is then automatically discharged before the shaft starts a new revolution.

R is a variable resistance (0–100 k$\Omega$) on the line between $C_G$ and $C_L$. It corresponds to R in Fig. 1.

The computer is operated in the following way: the capacitance of $C_L$, the resistance of R and the number of pulses to be introduced are selected. The recording volt meter and the motor that drives the wiper shaft are started. The first pulse is obtained by pushing the "start" button, which connects the power supply with the first contact in the semicircle. The starting time is recorded manually on the recording strip. The voltmeter then records the elution curve. When the operation is over, the instrument is discharged and the counter is returned to the zero position by pressing the reset button.

### COMPARISON OF GEL FILTRATION AND ITS ELECTRICAL ANALOGY

The following symbols are used[3]:

$V_0$ = void volume or total volume of liquid phase in the gel bed.

$V_x$ = total volume of gel phase.

$V_t = V_0 + V_x$ = total volume of gel bed.

$K_{av}$ = the fraction of the gel phase available to the solute.

$K_{av} \cdot V_x$ = total volume available to the solute in the gel phase.

$\varepsilon$ = equilibration factor for the distribution of solute between the gel phase and the liquid phase. It is equal to the change in the difference in solute concentration between the gel and liquid phases of each plate, divided by the total initial concentration difference.

*Application of material*

The volume of material applied to the column corresponds to the number of times that $C_L$ is recharged before passing $C_G'$. The electronic impulse counter can be set at any value between 1 and 63, representing a range of sample sizes from 1–66 % of the void volume or approximately 0.3–20 % of the total volume of the column. If the counter is short circuited, a larger volume can be introduced.

*Elution curve*

The elution curve recorded graphically begins at the void volume of the column. The

movement of the paper strip corresponds to.the flow of liquid from the column and the recorded voltage is proportional to the concentration of solute. Thus, the area under each peak is proportional to the total amount of substance in the effluent.

*Void volume, $V_0$*

Although the void volume is not recorded in the elution diagram, it can be calculated easily. Each half turn of the wiper shaft moves $1/96$ of the liquid phase through the column. The liquid phase or void volume will therefore have emerged completely after 48 turns; the void volume corresponds to the distance the recording strip has moved during this time.

*Total volume of gel bed, $V_t$*

The total volume cannot be determined directly. Experiments on Sephadex G-200 gels[3] showed that the void volume in this case was in the order of 29–30 % of the total volume, *i.e.* the total volume is approximately 3.3 times that of the void volume.

*Fraction of the gel phase available to the solute, $K_{av}$*

The fraction of the gel phase available to solute molecules is determined by their sizes. However, the analog computer is not programmed with $K_{av}$, but with the ratio of the total available volume of the gel phase to the void volume:

$$\frac{C_G}{C_L} = \frac{K_{av} \cdot V_x}{V_0} = \frac{K_{av} (V_t - V_0)}{V_0} \sim \frac{K_{av} \cdot 0.7 V_t}{0.3 V_t} \sim 2.3 \, K_{av} \tag{4}$$

The analog computer cannot be programmed with $K_{av}$ unless the exact relationship between $V_t$ and $V_0$ is known.

*Equilibration factor, $\varepsilon$*

The equilibration factor is represented in the computer by the variable resistance $R$. It can be calculated from eqns. (1) and (2):

$$\varepsilon = 1 - \frac{U_L(\Delta t) - U_G(\Delta t)}{U_L(0) - U_G(0)} = 1 - e^{-\frac{\Delta t}{T}} \tag{5}$$

$\Delta t$ is the time of contact between the wiper shaft and each $C_G$ condensor. The latter is 1.4 msec in the instrument and the equilibration factor becomes:

$$\varepsilon = 1 - e^{-\frac{0.0014 \, (C_G + C_L)}{R \cdot C_G \cdot C_L}} \tag{6}$$

In the gel filtration process, the equilibration factor is a function of the flow rate, the free diffusion rate of the solute, the size and shape of the gel grains and the sieve action of the polymer in the gel grain.

### DISCUSSION

The theoretical treatment of various types of chromatography is extremely complex and the process is far from completely understood (see *e.g.* refs. 5–11). The great

success achieved in making analogies between electrical and hydrodynamical processes in general[12] induced us to determine whether an electrical analog computer for gel filtration could be constructed. Two assumptions were made; namely, that a chromatographic column could be divided hypothetically into plates and that the distribution of solute between the gel and liquid phases could be represented by charge transfer in the circuit shown in Fig. 1. The first assumption is often used in the theoretical treatment of chromatography. The second amounts to the assumption that the solute encounters resistance to diffusion only in the boundary between the gel and liquid phases, rather than in the gel phase itself. It seems to be a reasonable approximation.

The number of plates was restricted to be ninety-six for practical reasons only. An increase in the number of plates will in general increase the resolution between two components[5] and experiments with gel filtration have sometimes given resolutions comparable to 5000 plates or more[2]. A 4 × 40 cm column of Sephadex G-200, 200–270 mesh, operated at a flow rate of 25 ml/h, has a resolution for albumin corresponding to 500 plates. The selection of ninety-six plates for the analog does not, however, limit its applicability. An elution curve can be fed back into the computer by a special device (appendix IV), and the effective length of the column can thereby be increased many times by simulating the recycling operation employed experimentally[13]. Obviously, the recycling behaviour of parts of elution curves can also be studied with the analog.

The analog gives a simplified picture of the gel filtration process. It does not account for certain factors, such as longitudinal diffusion and turbulent flow. However, the former is generally considered to be negligibly small and the latter is very difficult to estimate.

When the equilibration factor is 1, i.e. when complete equilibrium is obtained in each plate during the process, the analog describes a counter current distribution experiment. In principle, one should be able to obtain nearly statistical distribution curves on a column with ninety-six plates[5]. Such curves are demonstrated in Fig. 4.

The ideal case of complete equilibrium is seldom obtained in practice and the analog makes possible a systematic analysis of the effects of dis-equilibrium. Non-ideal gel filtration behavior is shown most often when very large molecules (low values
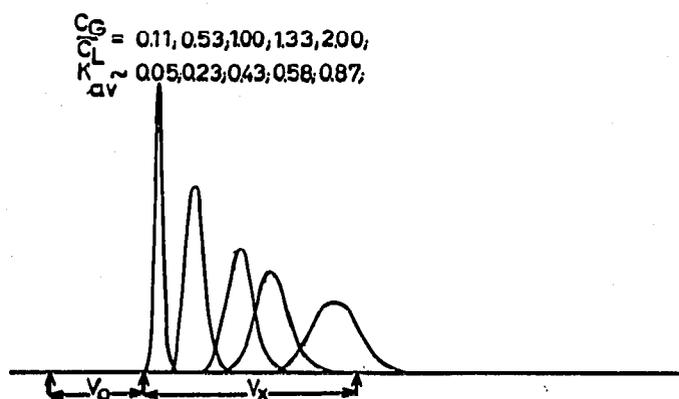


$\frac{C_G}{C_L}$ = 0.11, 0.53, 1.00, 1.33, 2.00;

$K_{av} \sim$ 0.05, 0.23, 0.43, 0.58, 0.87;

Fig. 4. Elution diagrams of substances with various partitions ($C_G/C_L$) between the gel and liquid phases. $K_{av}$ values have been calculated on the assumption that $V_0$ = 0.3 $V_t$. $\varepsilon$ = 1. Eight electrical impulses were used, corresponding to approximately 2.5 % of the total volume of the column.

of $C_G/C_L$) are studied at high flow rates. An example of how a decreasing equilibration factor influences the elution pattern is shown in Fig. 5. A more detailed comparison of experimental results with those obtained in the analog will be given in another paper.
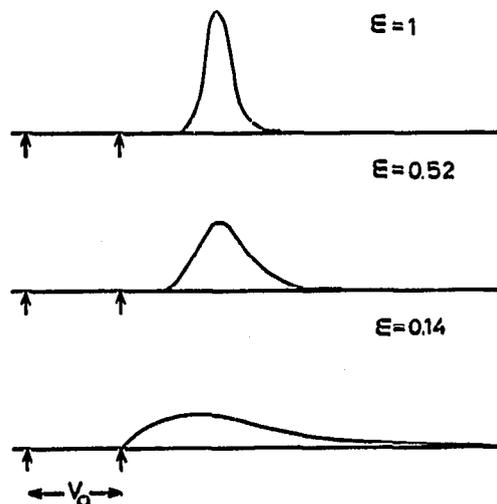


Fig. 5. Elution diagram of substances with partitions $(C_G/C_L) = 1$ but with varying equilibration factors. Eight impulses were used.

The analog computer described was built in order to extend the theoretical treatment of gel filtration presented in a preceding paper[3]. However, it may also be used to reproduce other kinds of chromatography or counter current distribution. The analog may be useful for the design of experimental conditions in various types of separations.

After this paper had been completed, the authors became aware that, simultaneously, VINK[14] had described chromatography in a similar fashion. He did not make an electrical analog, but, starting from equations equivalent to eqns. (1), (2) and (3), calculated two elution patterns for a column divided into thirty plates. His numerical approach is suitable for digital computers.

### ACKNOWLEDGEMENTS

### SUMMARY

An electrical analog computer has been constructed to study the process of gel filtration. The gel phase is represented by a series of condensors and the liquid phase by one condensor connected to a wiper shaft which moves along the row of condensors. The time for diffusion equilibrium between the phases is regulated by a resistor. The elution curve for a solute can be simulated if the partition coefficient and the time factor for the equilibration of the substance between the gel and liquid phases are known.

## APPENDIX I

The condenser, $C_L$, is composed of a number of condensors which have different capacitances and can be connected in series or parallel in different combinations. This is shown schematically in Fig. 6. The object was to obtain a satisfactory number of
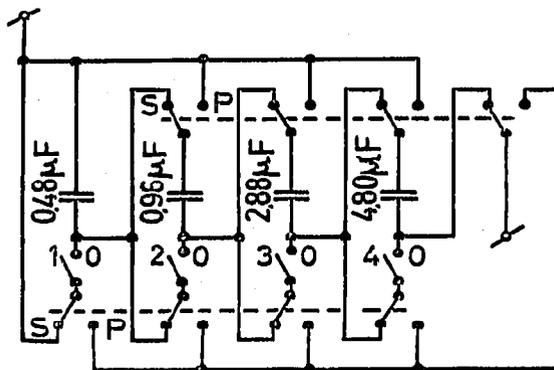


Fig. 6. Condensor ($C_L$) change-over switch.

possible variations from a minimum number of condensors and switches. The switches are set with the aid of a table, which shows $C_G/C_L$ and $C_G \cdot C_L/(C_G + C_L)$ for various combinations.

## APPENDIX II

The D.C. voltages needed are obtained from a 220 V A.C. source through a power supply unit, which is shown schematically in Fig. 7. It is essential that the D.C.
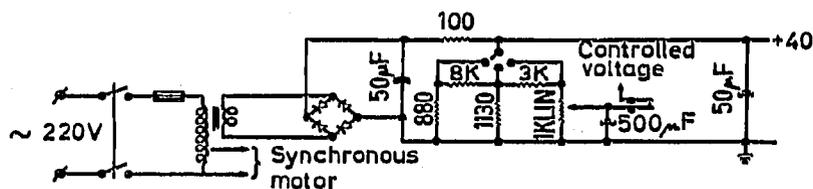


Fig. 7. Power supply circuit.

voltages are kept constant and that the charge impulses do not interfere with the electronic counter. The protection has been obtained with 50 $\mu$F electrolytic condensors.

## APPENDIX III

Fig. 8 shows a block diagram of the counter and Figs. 9, 10 and 11 are the circuit diagrams of the various units.

The relay is activated in its original position, which is obtained by setting the switches 1–6 (Fig. 10) in zero position and pressing the hand-operated reset button. The relay becomes locked by the clamping circuit (Fig. 11). The number of impulses wanted (1–63) is set by the switches 1–6 in Fig. 10. The relay is released when the "start" button is pressed.

The recharge voltage is connected through relay contact 1 when the relay is non-excited (Fig. 11). The counter is reset through relay contact 2.
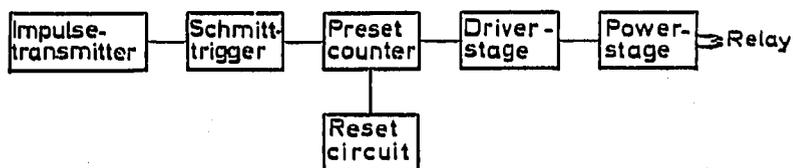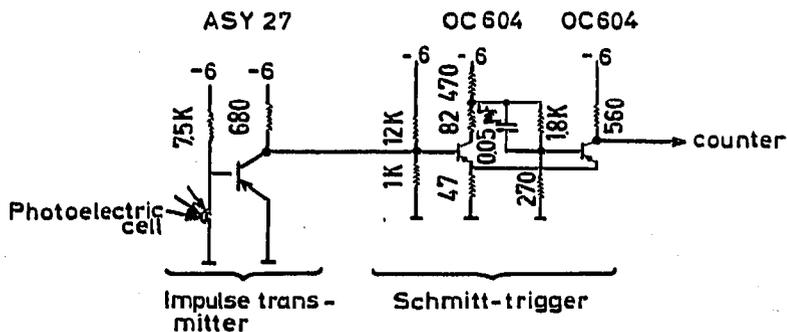
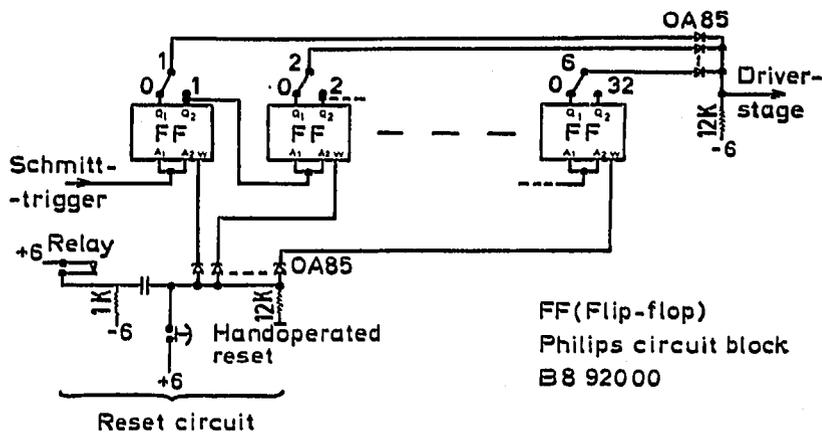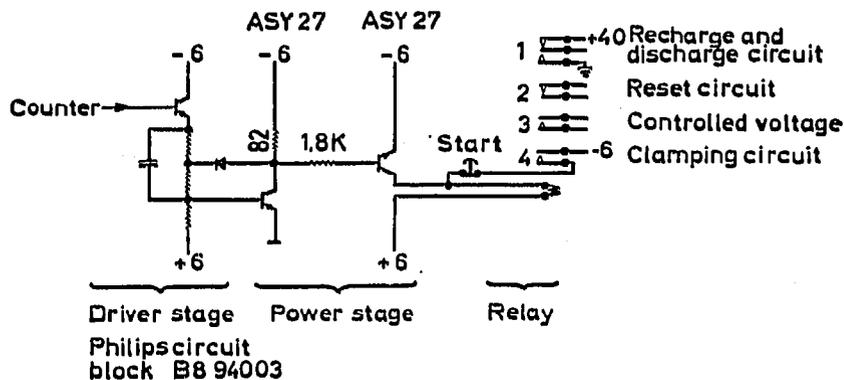Fig. 8. Block diagram of the counter.

Fig. 9.

Fig. 10.

Fig. 11.

Figs. 9–11. Circuit diagrams of parts of the electronic counter.

The Schmitt-trigger, which gives the square waves to the preset counter is controlled by impulses generated by a photoelectric cell circuit. When the preset number of impulses has been transmitted, the relay becomes excited and locks.

## APPENDIX IV

$C_L$ can be charged with a voltmeter-controlled voltage instead of a constant voltage before it is discharged to the condensors $C_G'$, $C_G''$ ... etc. The voltage can be varied manually in such a way that the charge voltage has the same curve form as the recently obtained elution curve. In this way the recording voltmeter will record a new elution curve that corresponds to 192 plates instead of 96. This procedure can be repeated as many times as desired. The preceding elution curve is followed manually on the recording paper strip with a needle which is mechanically linked to a potentiometer.

## REFERENCES

1 J. PORATH, *Advan. Protein Chem.*, 17 (1962) 209.
2 P. FLODIN, *Dextran Gels and Their Applications in Gel Filtration*, Dissertation, Uppsala, 1962.
3 T. C. LAURENT AND J. KILLANDER, *J. Chromatog.*, 14 (1964) 317.
4 M. J. E. GOLAY, in V. J. COATES, H. J. NOEBELS AND I. S. FAGERSON (Editors), *Gas Chromatography*, Academic Press, New York, 1958.
5 A. J. P. MARTIN AND R. L. M. SYNGE, *Biochem. J.*, 35 (1941) 1358.
6 B. GLUECKAUF, in *Ionic Exchange and Its Applications*, Society of Chemical Industry, London, 1955.
7 E. HELFFERICH, *Ionenaustauscher*, Band 1, Verlag Chemie GmbH, Weinheim/Bergstr., 1959.
8 R. A. KELLER, G. H. STEWART AND J. C. GIDDINGS, *Ann. Rev. Phys. Chem.*, 11 (1960) 347.
9 H. W. HABGOOD, *Ann. Rev. Phys. Chem.*, 13 (1962) 259.
10 J. C. GIDDINGS, *J. Chromatog.*, 2 (1959) 44.
11 J. C. GIDDINGS, *J. Chromatog.*, 5 (1961) 46.
12 T. LAURENT, *Vierpoltheorie und Frequenztransformation*, Springer Verlag, Berlin, 1956.
13 J. PORATH AND H. BENNICH, *Arch. Biochem. Biophys.*, Suppl. 1 (1962) 152.
14 H. VINK, *J. Chromatog.*, 15 (1964) 488.