

## A Contribution to the Theory of Chromatography

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When the adsorption isotherm is linear, the isochrones in elution chromatography are Gauss'ian curves moving along the column at a rate proportional to the flow rate; simultaneously the standard deviation increases proportionally to the square root of the flow rate. Consequently the isoplanes can only in some cases be approximated by Gauss'ian curves, whereas a better approximation is obtained by considering them as Gauss'ian functions of the square root of the eluted volume. As it is very difficult to decide whether or not a series of experimental values fit a certain type of non-linear curve, attention is called to the method of Bliss according to which the problem concerning the normal distribution is converted into an investigation of a linear dependency. The results are tested experimentally in ion-exchange chromatography of chloride ions.

In the theoretical treatment of elution chromatography Vermeulen and Hiester<sup>1</sup> have given a solution of the general case in which the adsorption isotherm is non-linear and the flow rate is too great to obtain equilibrium. Furthermore, several authors have given approximate solutions which in many cases may be satisfactory. All these solutions are, however, rather inconvenient as it is very difficult to make a series of experimental values fit a non-linear curve in such a way that the parameters of the function are determined most accurately. The following elucidation is founded on the plate theory applied by Martin and Synge<sup>2</sup> and Mayer and Thompkins<sup>3</sup>.

Consider the column as divided in segments by planes perpendicular to the axis of the column. Each segment contains  $v$  ml liquid and they are numbered 0, 1, 2, . . . p . . . The process is started by pouring  $v$  ml solution of the substance in question on the top of the column, and when  $v$  ml are drawn off from the bottom, the substance is now in segment No. 0. It is assumed that the adsorption isotherm is linear; then the fraction  $\alpha$  of the solute in the liquid phase and the fraction  $1-\alpha$  in the solid or stationary phase are independent of the concentration. When adsorption equilibrium has been established,  $v$  ml eluant are poured on the top of the column and the whole liquid phase is displaced one segment. The  $v$  ml which are hereby drawn off from the bottom of the column are collected as the first fraction, these being numbered 1, 2 . . . n . . . When equilibrium has again been established the next displace-

ment of one segment takes place. Let the total quantity of solute in the  $p$ 'th segment after  $n$  fractions of  $v$  ml have passed the column be  $q_{n,p}$  measured in units of the originally introduced quantity. Then the condition of material balance is

$$q_{n,p} = \alpha q_{n-1,p-1} + (1-\alpha) q_{n-1,p} \quad (1)$$

When  $n$  fractions have passed, the total quantity of substance is distributed in the  $n+1$  segments from  $p = 0$  to  $p = n$ , while for  $p > n$  and  $p = -1$  (pure eluant entering the column)  $q_{n,p} = 0$ . From these data and  $q_{0,0} = 1$ ,  $q_{n,p}$  can be calculated for all values of  $n$  and  $p$  by successive application of (1). It is however simpler to substitute

$$q_{n,p} = Q_{n,p} \alpha^p (1-\alpha)^{n-p} \quad (2)$$

by which (1) becomes

$$Q_{n,p} = Q_{n-1,p-1} + Q_{n-1,p} \quad (3)$$

Employing the conditions for  $q_{n,p}$  above (2) yields

$$Q_{0,0} = 1, Q_{n,-1} = 0 \text{ and } Q_{n,n+1} = 0$$

and as (3) is identical with the recursion formula for the binomial coefficients (2) is converted into

$$q_{n,p} = \binom{n}{p} \alpha^p (1-\alpha)^{n-p} \quad (4)$$

In statistics the probability in the binomial distribution, *i.e.* the probability that an event happens  $p$  times out of  $n$ , the probability of the single event being  $\alpha$ , is given by (4). It can be shown<sup>4</sup> that when  $n$  increases (4) converges against the normal frequency function

$$q_{n,p} \rightarrow \frac{1}{\sigma} \varphi\left(\frac{p-\mu}{\sigma}\right) \quad (5)$$

with the mean value  $\mu = n\alpha$  and the standard deviation  $\sigma = \sqrt{n\alpha(1-\alpha)}$ . Here and in the following the Gauss'ian frequency function and distribution function are designated  $\varphi(x)$  and  $\Phi(x)$ , respectively:

$$\varphi(x) = \frac{1}{\sqrt{2\pi}} e^{-\frac{x^2}{2}} \text{ and } \Phi(x) = \int_{-\infty}^x \varphi(t) dt$$

Measured in units of the initial concentration the concentration in the liquid phase is

$$c = \frac{\alpha \cdot q_{n,p}}{v}$$

Introducing  $V = pv$  and  $U = nv$

(5) becomes

$$c = \frac{\alpha}{\sqrt{U\alpha(1-\alpha)}v} \varphi\left(\frac{V-\alpha U}{\sqrt{U\alpha(1-\alpha)}v}\right) \quad (6)$$

which gives the concentration as a function of the two variables  $V$ , the distance from the top of the column to the regarded point measured in ml of interstitial volume and  $U$ , the eluted volume of liquid.

As the liquid in practice moves continually the above deduction is valid only for infinitely small quantities of substance. Actually the experiment is

started by pouring a finite quantity, say  $A$  ml solution on the top of the column. The concentration  $c = f(V, U)$  is then the result of a superposition of an infinite number of infinitely small quantities, the first and last of which have moved  $V-A$  and  $V$  ml along the column, respectively. The total amount of solute that has accumulated in a differential segment with abscissa  $V$  when  $U$  ml of eluate have passed is given by

$$A \cdot c = \int_{V-A}^V f(V, U) dV = \int_{V-A}^V \frac{\alpha}{S} \varphi(t) dV,$$

in which

$$S = \sqrt{U\alpha(1-\alpha)} v \text{ and } t = \frac{V-aU}{S}$$

$$\text{As } dt = \frac{dV}{S} \text{ and } t_A = \frac{V-A-aU}{S}$$

$$A \cdot c = \alpha \int_{t_A}^t \varphi(t) dt = \alpha(\Phi(t) - \Phi(t_A))$$

According to the mean value theorem

$$\Phi(t) - \Phi(t_A) = \varphi\left(\frac{V-a-aU}{S}\right) \left(\frac{V-aU}{S} - \frac{V-A-aU}{S}\right)$$

in which  $0 < a < A$

$$\text{Consequently } c = \frac{\alpha}{S} \varphi\left(\frac{V-a-aU}{S}\right)$$

When  $A \ll V$ ,  $a$  may be neglected, and (6) is valid for finite quantities.

The discussion of this formula is divided in two parts: 1°.  $U = U_0 = \text{constant}$  (*isochrones*). In this case the standard deviation is also a constant and  $c$  is a Gauss'ian function of  $V$ , the mean value being  $a U_0$  and the standard deviation  $S_0 = \sqrt{U_0\alpha(1-\alpha)} v$ . When  $U$  increases this distribution of the solute moves through the column with a rate proportional to that of the eluant,  $V_{\max}$  being equal to  $a U$ ; simultaneously the curve is flattened out,  $S$  being proportional to  $\sqrt{U}$ . 2°.  $V = V_0 = \text{constant}$  (*isoplanes*). This is the case encountered in practice, the eluate being collected in fractions and the concentrations of solute herein determined in some conventional way. Equation (6) is written

$$c = f(U) = \frac{1}{\sqrt{U \frac{1-\alpha}{\alpha} v}} \varphi\left(\frac{V_0/\alpha - U}{\sqrt{U \frac{1-\alpha}{\alpha} v}}\right) \quad (7)$$

This is not a normal distribution, the "standard deviation" containing the variable  $U$ . If, however,  $U$  is great compared with  $\sqrt{Uv(1-\alpha)/\alpha}$ ,  $U_0$  may, as a first approximation, be substituted for  $U$  in this expression and (7) takes the form of a Gauss'ian equation:

$$c \approx f_1(U) = \frac{1}{s} \varphi\left(\frac{U_0 - U}{s}\right) \quad (8)$$

in which  $U_0 = V_0/\alpha$  and  $s = \sqrt{U_0 v(1-\alpha)/\alpha}$   
 A great ratio between  $U_0$  and  $s$

$$\frac{U_0}{s} = \sqrt{\frac{V_0}{v} \frac{1}{1-\alpha}}$$

means that the longer the column and the greater  $\alpha$  the better is the approximation. It should be noticed that in the normal distribution 95 % of the area lies between the values  $U_0 \pm 2s$  and 99.7 % lies between  $U_0 \pm 3s$ .

When necessary, a better approximation may be obtained by the following transcription of (7):

$$c = \frac{1}{\sqrt{U}} \cdot \frac{1}{\sqrt{\frac{1-\alpha}{\alpha} v}} \varphi \left( \frac{(V\bar{U}_0 - V\bar{U})(V\bar{U}_0 + V\bar{U})}{\sqrt{U} \sqrt{\frac{1-\alpha}{\alpha} v}} \right)$$

By substituting  $\sqrt{U_0}$  for  $\sqrt{U}$  in the sum in the numerator and in the denominator in  $\varphi$  we get

$$c \approx f_2(U) = \frac{1}{2\sqrt{U}} \cdot \frac{1}{\frac{1}{2}\sqrt{\frac{1-\alpha}{\alpha} v}} \varphi \left( \frac{\sqrt{U_0} - \sqrt{U}}{\frac{1}{2}\sqrt{\frac{1-\alpha}{\alpha} v}} \right) \quad (9)$$

If a function  $g(U)$  is normally distributed:

$$F(U) = \varphi \left( \frac{g(U) - g(U_0)}{\sigma} \right)$$

the frequency function becomes

$$f(U) = F'(U) = \frac{1}{\sigma} g'(U) \varphi \left( \frac{g(U) - g(U_0)}{\sigma} \right)$$

and as  $\frac{d\sqrt{U}}{dU} = \frac{1}{2\sqrt{U}}$  (9) indicates that  $c$  is a Gauss'ian function of  $\sqrt{U}$ , the mean value and standard deviation of which are  $\sqrt{U_0}$  and  $\frac{1}{2}\sqrt{\frac{1-\alpha}{\alpha} v}$ , respectively.

The result of an experiment consists of a table giving the concentration of the solute as a discontinued function of the eluted volume. Even if a continuous curve is drawn by a recording instrument, measuring some physical property of the flowing solution, that varies with concentration, it is necessary to determine from the curve the concentration for some discrete values of the volume. When the fractions or intervals of  $w$  ml each are numbered 1, 2 . . .  $m$  . . . the problem to solve is whether the stair-shaped curve, which is a result of a plot of  $c$  against  $m$ , approximates a Gauss'ian curve, and next to determine the mean value and standard deviation thereof. According to statistical methods<sup>6</sup> this is done in the following way: The expressions

$$H(m) = \frac{\sum_a^m wc}{\sum_a^m wc} = \frac{\sum_a^m c}{\sum_a^m c} \quad (10)$$

are calculated. Here the first and last eluted fraction in which the concentrations are above the analytical error are numbered  $a$  and  $b$ , respectively. Thus  $H(m)$  means the fraction of the total amount of solute contained in the eluted fractions from number  $a$  to  $m$  inclusive, and it should be valid:

$$H(m) \approx \Phi\left(\frac{m-\mu}{\sigma}\right)$$

in which  $\mu$  and  $\sigma$  are the mean value and standard deviation, respectively. Consequently the inverse function

$$\Phi^{-1}(H(m)) = \frac{m-\mu}{\sigma}$$

and this equation represents a straight line having the slope  $\frac{1}{\sigma}$  and intersecting the abscissa axis in  $\mu$ . The values of  $\Phi^{-1}(x)$ ,  $0 < x < 1$  are called quantiles; as a rule quantiles + 5 called probits are tabulated<sup>7</sup> in order to avoid negative values.

If the points  $(m, \Phi^{-1}(H(m)))$  sufficiently well fit a straight line,  $c = H(m)$  can be regarded as a Gauss'ian function, the constants of which are determined by the line. As

$$\frac{d\Phi^{-1}(x)}{dx} \rightarrow \infty \text{ for } x \rightarrow 0 \text{ and } x \rightarrow 1$$

the points near the ends of the line are rather uncertain and may be neglected. According to (8)

$$w \cdot \mu = U_0 = V_0/\alpha \text{ and } w \cdot \sigma = s = \sqrt{U_0 \frac{1-\alpha}{\alpha} v}$$

and thus  $\alpha$  and  $v$  can be calculated.

If the line is curved it is tried whether the points  $(\sqrt{m}, \Phi^{-1}(H(m)))$  give a straight line.

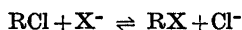
In this case the constants are related to the constants of (9) by

$$\sqrt{w} \cdot \mu = \sqrt{U_0} = \sqrt{V_0/\alpha} \text{ and } \sqrt{w} \cdot \sigma = \frac{1}{2} \sqrt{\frac{1-\alpha}{\alpha} v} \quad (12)$$

from which  $\alpha$  and  $v$  may be calculated.

## EXPERIMENTAL

The process investigated was the elution of chloride ions through columns containing Amberlite IRA 400 loaded with some other anion, preferably nitrate, the eluant being a solution of a salt containing the same anion:



When 1 ml of the column filling contains  $\beta$  ml liquid with a total concentration of  $l$  mequiv./ml, and  $1-\beta$  ml resin with an exchange capacity of  $r$  mequiv. the mass action law for small amounts of chloride gives

$$K_c = \frac{[\text{RX}][\text{Cl}^-]}{[\text{RCl}][\text{X}^-]} \approx \frac{[\text{Cl}^-]}{[\text{RCl}]} \frac{r}{l(1-\beta)}$$

The ratio of the total amount of chloride in the liquid phase to that in the resin is

$$\frac{a}{1-a} = \frac{\beta [\text{Cl}^-]}{(1-\beta) [\text{RCl}]} = K_c \cdot \frac{\beta l}{r}$$

$$K_c = \frac{a}{1-a} \cdot \frac{r}{\beta l} \quad (13)$$

The substitution of  $[\text{RX}]$  and  $[\text{X}^-]$  by  $r/l-\beta$  and  $l$ , respectively, is justified by the fact, that in the experiments performed in this work  $[\text{X}^-]$  varied between 0.1 and 1, while the maximum chloride concentrations measured in the adsorption bands were between  $10^{-3}$  and  $10^{-2}$ . Three types of column systems were used.

I. The column consisted of a glass tube of 27 mm inner diameter and 100 cm length. The lower end of the tube was narrowed down to the diameter of the outlet tube (inner diameter 1–2 mm, outer 6–7 mm). The filling was Amberlite IRA 400 ( $\text{NO}_3$ ) resting on a glass wool plug. The top of the tube was provided with a three hole rubber stopper, through which were inserted a tube for the eluant, a funnel tube for introducing the chloride solution and a tube for letting out air. The eluant reservoir was a Mariotte bottle, the level of which was adjustable, and a flowmeter was inserted in the line. Immediately after the outlet tube the eluate passed an enlargement of the tube containing a silver-silver chloride electrode and a Radiometer K 9001 reference electrode (saturated  $\text{K}_2\text{SO}_4$   $\text{Hg}_2\text{SO}_4/\text{Hg}$ ; 650 mV referred to the normal hydrogen electrode). The silver-silver chloride electrode was made by electrolysis with a silver wire  $50 \times 0.5$  mm as anode a 0.1 M sodium chloride solution with about 5 mA for two minutes. The potential of this cell was measured by a Radiometer PHM 22 and recorded by a Rec. Ia, from the same firm. As it appeared difficult to keep the flow rate constant the eluate after passage of the measuring cell ran to a siphon pipet, the emptying of which operated a relay. This switched the potentiometer to measure the potential of a fixed cell, and as the drainage of the siphon pipette lasted about half a minute, the pen came to rest and put a mark on the record for each fraction eluted.

II. The second type of column was very similar to type I, but it was provided with ten silver-silver chloride electrodes arranged as seen in Fig. 1. In order to prevent the electrolyte solution from creeping along the silver wires, where they pass through the rubber tubings, the central tube was filled with silicone oil. The recording of potentials from all ten electrodes was arranged by means of a selector from an automatic telephone exchange. The selector had eleven positions, and it was operated by minute pulses from the main electric clock of the institute; in this way the ten electrodes were successively connected to one terminal of the potentiometer, while the reference electrode constantly was connected to the other one. In the eleventh position of the selector the terminals of the potentiometer were short-circuited in order to record zero potential. It was thus always easy in the rather confused diagram on the paper strip to identify the marks corresponding to any of the electrodes.

III. The third column system consisted of ten single columns 17 mm inner diameter and 150 cm long. From the outlet tube the eluate passed an electrode system similar to that described in I and next a pump that transported it to the top of the next column. The pumps were magnetically operated pipette rubber bulbs attached to a side tube between two glass valves. The lumina of the measuring cell, valve housings and connecting tubes were chosen as small as practicable in order to avoid mixing. As the time elapsed between the passage of the adsorption band by two consecutive measuring cells was considerably greater in this case, the selector was operated by five minute pulses.

The electromotive force of the measuring cell is related to the chloride concentration by

$$E = 650 - 222 + 58 \log [\text{Cl}^-] \text{ mV,}$$

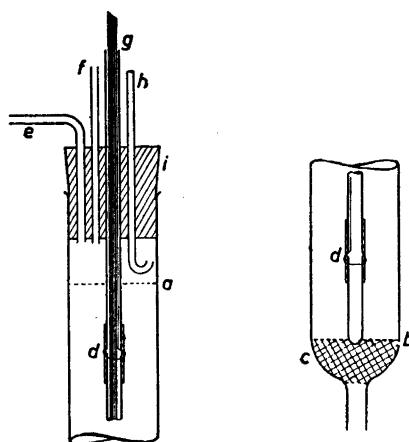


Fig. 1.

- a-b* ion exchange resin  
*c* glass wool plug  
*d* rubber tubing connecting the parts of the central tube. A silver wire is pricked through the rubber tubing and laid around it. Inside the wire is soldered to a copper wire leading to the selector.  
*e-f* tubes for introducing chloride solution, and eluant  
*g* central tube with ten electrode wires  
*h* electrolytic bridge of the reference electrode  
*i* rubber stopper

the standard potential of the silver-silver chloride electrode being 222 mV at room temperature. Now it proved difficult to keep the standard potential of the cell equal to the theoretical value; it could differ somewhat for different electrodes, and for the same electrode it could alter with time. Another potential also proved to alter with the treatment and age of the electrode, *viz.* the potential in a chloride-free solution. This was as a rule about 200 mV corresponding to a chloride concentration of  $10^{-4}$ .

Because of these facts and as equation (10) contains ratios of concentrations, the potential curves were treated as follows. The horizontal part of the curve immediately before and after the band passage  $a-1$  and  $b+1$ , *cf.* eqn. (10) were interconnected by a straight line; as a rule this was parallel to the abscissa axis and only rarely the difference exceeded 10 mV. At appropriate intervals the ordinate differences between this line and the curve were determined, and this potential difference is related to the chloride ion concentration  $c$  and the fictive zero concentration  $c_0$  by

$$E = 58 \log (c/c_0)$$

from which

$$c - c_0 = c_0(\text{antilog } (E/58) - 1)$$

The difference  $c - c_0$  was taken as a measure of the chloride concentration in the fraction in question; and as the absolute value was irrelevant the expression  $\text{antilog } (E/58) - 1$  was inserted for  $c$  in (10). This procedure was justified by the following experiment: 5 ml 1 M potassium chloride were poured on the top of column I and eluted by 0.5 M potassium nitrate. The fractions were titrated according to Volhard, and the expressions  $\text{antilog } (E/58) - 1$  with  $E$  values referring to the midpoints of the fractions were calculated. The ratios of corresponding values were determined, and the mean error was found to be 3 %.

The interstitial volume of the ion-exchanger was determined by washing down a column with 0.1 M nitric acid until the eluate had the same concentration as the entering

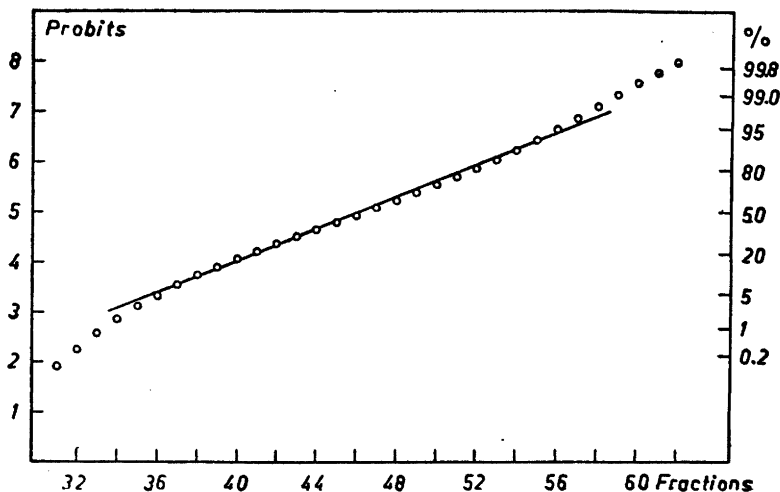


Fig. 2. Column I. 5 ml 1 M potassium chloride eluted with 0.5 M potassium nitrate at 2.3 ml/min. Probits versus fractions of 20.5 ml each.

solution. The acid level was adjusted to the surface of the ion-exchanger, and the acid was rinsed out with distilled water and titrated. After removal of the ion-exchanger the volume of the glass wool plug and the buret tip was determined in the same manner. The capacity of the resin was determined in the same column; the nitrate ions were displaced by chloride ions, a 2 M sodium chloride solution being washed through until the eluate gave no reaction for nitrate with sulphuric acid and brucine. After washing out with distilled water the chloride ions were removed by means of 0.5 M potassium nitrate and titrated. The capacity was referred to the nitrate resin. The mean values of duplicate determinations gave the following results: column I and II:  $\beta = 0.74$  and  $r = 1.55$ ; column III:  $\beta = 0.75$  and  $r = 1.45$ .

## RESULTS

Fig. 2 shows a characteristic diagram of an experiment. As mentioned, the first and last points of the line are rather uncertain; furthermore  $H(m)$  is not a stochastic variable, every value containing the errors of the preceding ones. In most cases the lines show a systematic curvature, probably as a result of these facts and also because eqn. (8) is an approximation to eqn. (7), and this, in addition, is derived on assumptions, the validity of which are questionable. The determination of the mean value, the abscissa for probit 5, is only slightly affected hereof, but the estimation of the standard deviation, which is the reciprocal of the slope is made somewhat uncertain. In order to obtain comparable results from the different experiments it was decided to exclude all points, the ordinates of which were less than 3 and greater than 7. This means that the constants of the distribution are determined mainly by the central 95 % of the material as indicated by the  $\Phi$  values shown at the right hand of the diagram. The straight line has the constants  $U_0 = 46.1$  fractions and  $s = 6.29$  fractions. The standard deviation of the observed ordinates from the



corresponding ones of the line between the ordinates 3 and 7 amounts to 0.07. In some other experiments the deviations were calculated and found to be of the same order of magnitude.

A series of five experiments were performed under identical circumstances to try the reproducibility of the procedure. In column I 5 mmoles of potassium chloride were eluted by means of 0.5 M potassium nitrate at a rate of 2.24 ml/min  $\pm$  3 %. The mean errors of the 5 sets of values of  $U_0$  and  $s$  were found to be 1.5 % and 4 %, respectively.

Tables 1 and 2 show the results of some experiments made to determine the permissible limits to flow rate and initial concentration. It is seen that if the velocity is less than 3.3 ml/min and the quantity of chloride does not exceed 5 mmoles, the constants of the distribution are independent of these two variables. The cross section of column I was 6.0 cm<sup>2</sup> and as the interstitial volume amounted to 74 % of the total volume, the limiting velocity corresponded to 0.8 cm/min. Furthermore the chloride being applied as a 1 M solution occupied 1.2 cm of the column height. These conditions were observed in all the following experiments.

In column II a series of experiments were performed with different concentrations of the eluant, the chloride concentration being recorded at ten places in the column. In two of the experiments some of the electrodes were in disorder, wherefore the corresponding isoplanes could not be calculated. The results are shown in Tables 3—6. In column system III two experiments were carried out to investigate the moving of the adsorption band along a very long column. The travelled distances in the experiments, the results of which

Table 1. 5 ml 1 M potassium chloride eluted with 0.5 M potassium nitrate in column I.

ml/min	$U_0$ ml	$s$ ml
1.28	964	121
2.32	930	125
3.34	925	123
4.74	934	139
7.15	947	152
12.72	936	161
18.45	947	181

Table 2. Potassium chloride eluted with 0.5 M potassium nitrate in column I. Flow rate: 2.2 ml/min  $\pm$  10 %.

1 M KCl ml	$U_0$ ml	$s$ ml
2	926	133
5	930	125
10	968	126
20	977	130
40	1032	159

Table 3. 0.2 mmoles potassium chloride eluted with 1.0 M potassium nitrate.

$V_0$ ml	$U_0$ ml	$s$ ml	$\alpha$	$s^2/V_0$
38.4	60.9	6.98	0.631	1.27
64.9	100	9.27	0.648	1.33
91.2	136	11.8	0.669	1.52
118	178	13.1	0.662	1.46
144	218	14.8	0.660	1.52
170	260	18.6	0.656	2.03
197	298	18.4	0.660	1.73
223	339	19.7	0.658	1.74
249	383	20.5	0.651	1.69
276	425	21.8	0.648	1.72
Mean value			0.654	1.60
Mean error			1.5 %	14 %

Table 4. 0.2 mmoles potassium chloride eluted with 0.5 M potassium nitrate.

$V_0$ ml	$U_0$ ml	$s$ ml	$\alpha$	$s^2/V_0$
38.4	84.2	11.2	0.465	3.23
64.9	135	13.6	0.481	2.85
118	247	21.5	0.478	3.92
144	313	28.4	0.460	5.60
170	362	32.6	0.469	6.25
223	478	28.2	0.467	3.57
249	549	28.6	0.454	3.29
276	606	38.6	0.455	5.40
Mean value			0.466	4.26
Mean error			2 %	30 %

Table 5. 0.2 mmoles potassium chloride eluted with 0.2 M potassium nitrate.

$V_0$ ml	$U_0$ ml	$s$ ml	$\alpha$	$s^2/V_0$
64.9	262	31.7	0.248	15.7
118	481	58.9	0.246	29.5
144	599	73.6	0.240	38.1
170	731	85.9	0.233	43.4
223	927	82.5	0.240	30.9
249	1050	69.3	0.238	19.1
276	1170	99.5	0.236	36.3
Mean value			0.240	30.4
Mean error			2 %	33 %

Table 6. 0.2 mmoles potassium chloride eluted with 0.1 M potassium nitrate.

$V_0$ ml	$U_0$ ml	$s$ ml	$\alpha$	$s^2/V_0$
36.5	258	31.8	0.142	27.6
62.8	468	49.0	0.134	38.2
89.0	650	64.5	0.137	46.6
116	840	78.2	0.138	52.6
142	1060	83.0	0.135	48.6
168	1270	100	0.133	59.6
195	1450	106	0.135	57.5
221	1660	118	0.133	63.3
248	1880	121	0.132	59.8
274	2080	129	0.132	60.5
Mean value			0.135	51.4
Mean error			2 %	22 %

Table 7. 2 mmoles potassium chloride eluted with 0.5 M potassium nitrate.

$V_0$ ml	$U_0$ ml	$s$ ml	$\alpha$	$s^2/V_0$
266	525	34.4	0.507	4.43
529	1062	50.8	0.498	4.88
793	1584	62.2	0.501	4.87
1060	2130	74.7	0.498	5.26
1315	2643	85.8	0.498	5.58
1576	3163	96.4	0.498	5.89
1806	3653	99.3	0.494	5.46
2045	4135	105.0	0.495	5.38
Mean value			0.499	5.22
Mean error			1 %	9 %

Table 8. 2 mmoles potassium chloride eluted with 0.2 M potassium nitrate.

$V_0$ ml	$U_0$ ml	$s$ ml	$\alpha$	$s^2/V_0$
266	945	54.7	0.282	11.8
529	1782	80.0	0.296	12.1
793	2736	125	0.290	19.7
1060	3720	134	0.285	17.0
1315	4661	167	0.282	21.3
1576	5567	161	0.283	16.4
1806	6394	158	0.284	13.9
2045	7251	163	0.282	13.1
2304	8157	186	0.282	15.5
2570	9117	185	0.282	13.3
Mean value			0.285	15.4
Mean error			2 %	21 %

Table 9.  $K_c$  and  $v$  are calculated from the results in Tables 3–8 by means of formulas (13) and (11).

Column	[KNO <sub>3</sub> ]	$K_c$	$v$
II	1.0	3.96	1.92
II	0.5	3.66	1.73
II	0.2	3.31	2.30
II	0.1	3.27	1.09
III	0.5	3.85	2.59
III	0.2	3.85	1.75
Mean value		3.65	1.90
Mean error		8 %	27 %

Table 10. Column II with the ion-exchanger in the acetate form. 1 mmole potassium chloride eluted with 1.0 M sodium acetate.  $w = 20.18$  ml per fraction.  $a$  and  $v$  are calculated according to (12),  $v = 0.65$  ml. From (13)  $K_c = 0.100$ .

$V_0$ ml	$\mu$	$\sigma$	$a$
86.2	9.45	0.449	0.0478
113	10.75	0.484	0.0482
139	12.15	0.466	0.0467
165	13.34	0.358	0.0460
192	14.50	0.413	0.0451
218	15.65	0.391	0.0441
244	16.61	0.383	0.0438
271	17.64	0.353	0.0432
Mean value		0.412	0.0456
Mean error		12 %	4 %

Table 11. Column II with ion-exchanger in the acetate form. 1 mmole potassium chloride eluted with 0.5 M sodium acetate.  $w = 20.47$  ml per fraction. Calculated as in the preceding table,  $v = 0.87$  ml and  $K_c = 0.116$ .

$V_0$ ml	$\mu$	$\sigma$	$a$
26.4	6.59	0.606	0.0297
52.7	9.59	0.606	0.0280
79.1	11.82	0.610	0.0277
105	13.66	0.611	0.0276
132	15.46	0.642	0.0269
158	17.26	0.638	0.0259
185	18.50	0.640	0.0263
211	19.94	0.622	0.0259
237	21.19	0.638	0.0269
264	22.5	0.635	0.0254
Mean value		0.625	0.0270
Mean error		3 %	5 %

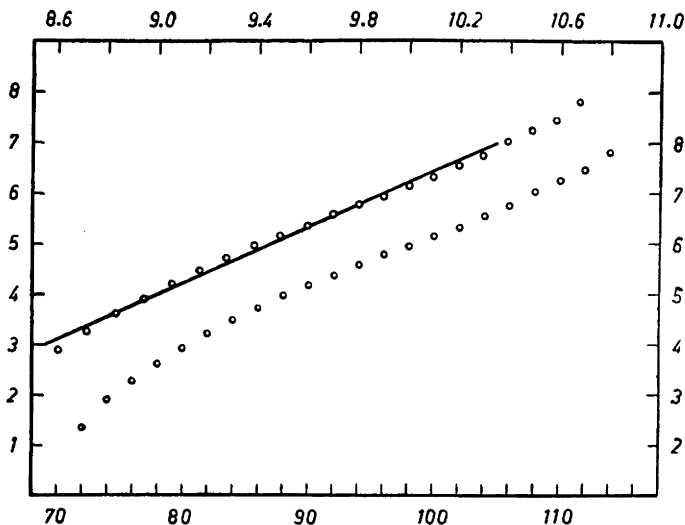


Fig. 3. Column II with the resin in the acetate form. 1 ml 1 M potassium chloride eluted with 1 M sodium acetate at 0.94 ml/min. 20.2 ml per fraction. Lower curve: Probits versus fractions, coordinates indicated at the right hand and at the bottom. Upper curve: Probits versus the square root of fraction numbers, coordinates indicated at the left hand and at the top.

are shown in Tables 7 and 8, were 12 and 15 m, respectively. A summary of these six experiments are seen in Table 9. The value of the equilibrium constant is in good agreement with the value 3.8 which Wheaton and Bauman<sup>8</sup> found for the chloride-nitrate equilibrium on Dowex 1, which is also a strong base anion exchange resin.

Finally two experiments were made to try if the second approximation (9) was satisfactory when eqn. (8) could not be used, and that is the case if  $\alpha$  is small enough. As acetate ions are much weaker adsorbed by the ion-exchanger than chloride ions, the resin in column II was converted into the acetate form and sodium acetate was used as the eluant. In the graphical representations the points ( $m, \Phi^{-1}(H(m))$ ) gave curved lines while the points ( $\sqrt{m}, \Phi^{-1}(H(m))$ ) rather satisfactorily gave straight lines. One example is given in Fig. 3, and the entire results of the two experiments appear in Tables 10 and 11. In this case the agreement of the equilibrium constant with the value 0.17 found by Wheaton and Bauman<sup>8</sup> is not so good, but it should be borne in mind, that although Dowex 1 and Amberlite IRA 400 are of the same type there may be differences in their properties.

The validity of formula (8) requires the constancy of  $\alpha$  and  $s^2/V_0$  shown in the two last columns of Tables 3—8. It has been mentioned that the mean error of  $s$  is somewhat greater than that of  $U_0$ , and consequently the mean error of the last ratio is expected to be considerably greater than that of  $\alpha$ . The experimental results indicate, however, these methods of treating elution curves to be a rather good approximation and a valuable tool in designing

columns for chromatographic separations. It should be emphasized that all experimental values constituting the isoplane are used in calculating the parameters. The calculating work is rather easy, but it can be further simplified by plotting  $H(m)$  directly against  $m$  on "probability paper".

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## REFERENCES

1. Vermeulen, Th. and Hiester, N. K. *Ind. Eng. Chem.* **44** (1952) 636.
2. Martin, A. J. P. and Synge, R. L. M. *Biochem. J. London* **35** (1941) 1385.
3. Mayer, S. W. and Tompkins, E. R. *J. Am. Chem. Soc.* **69** (1947) 2866.
4. Hald, A. *Statistiske Metoder*, Det private Ingeniørfond, København 1948, p. 553.  
Kendall, M. G. *The Advanced Theory of Statistics*, Charles Griffin and Co. London 1947, Vol. I, p. 103.
5. Hald, A. *Statistiske Metoder*, Det private Ingeniørfond, København 1948, p. 154;  
Kendall, M. G. *The Advanced Theory of Statistics*, Charles Griffin and Co. London 1947, Vol. I, p. 16.
6. Hald, A. *Statistiske Metoder*, Det private Ingeniørfond, København 1948, p. 115;  
Bliss, C. I. *Ann. Appl. Biol.* **22** (1935) 134.
7. Fischer, R. A. and Yates, F. *Statistical Tables*, Oliver and Boyd, London 1948, p. 50.
8. Wheaton, R. M. and Bauman, W. C. *Ind. Eng. Chem.* **43** (1951) 1088.

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