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SEPARATION MECHANISM AND DETERMINATION OF ACIDIC COMPOUNDS BY ION-EXCLUSION LIQUID CHROMATOGRAPHY WITH ELECTROKINETIC DETECTION*

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SUMMARY

It was found that in ion-exclusion chromatography both strong acids ($pK_a < 2.5$) and weak acids ($pK_a > 6.5$) were eluted unseparated, the first at the beginning and the latter at the end of the elution. The retention volumes of the remaining acids ($2.5 < pK_a < 6.5$) were found to be proportional to their pK_a values. This kind of behaviour is described in terms of appropriate equations. The dead and inner volumes of the chromatographic column were determined from the observed dependence of retention volumes on the pK_a values of the analysed acids. The monitoring of the acids was performed using electrokinetic detection. The detectabilities of the acids were found to be inversely proportional to their dissociation constants.

INTRODUCTION

The ion-exclusion method in liquid chromatography has already been applied to the separation of ionic from non-ionic compounds, and also to the separation of mixtures of acids with widely different structures[1–7]. The separation of acids on a cation-exchange resin in the hydrogen form is governed by the Donnan membrane equilibrium[2]. Anions are repulsed (excluded) from the negatively charged resin, whereas non-ionic compounds (undissociated acids) can enter the resin network. Therefore, a dependence of the retention volumes, V_R, of acids on their pK_a values ($pK_a = -\log K_a$ where K_a is the dissociation constant of the acid) is expected. Such a dependence was found by Tanaka *et al.*[5].

In this work we have studied the problem of the separation of acids in ion-exclusion chromatography in more detail. The monitoring of the acids was performed using the earlier elaborated[8,9] electrokinetic detection. Following Tanaka *et al.*[5], we determined the dead volume, V_0, and the inner volume, V_i, of the chromatographic column and calculated the distribution coefficients, K_d, of the analysed acids. The

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observed dependence of K_d (and conversely of V_R) on the pK_a values of the analysed acids is explained in terms of appropriate equations derived from the Donnan membrane equilibrium condition. The experimental K_d values are in good agreement with those calculated from the equations.

EXPERIMENTAL

An IChF-PAN T-302 high-performance liquid chromatograph (Warsaw, Poland) with a 5-μl open-loop injection valve and a stainless-steel column (150 × 4 mm I.D.) slurry packed with LiChrosorb KAT (E. Merck, Darmstadt, F.R.G.) was used. The column was thermostated at 22°C. An electrokinetic detector[8,9] with a PTFE capillary (100 × 0.2 mm I.D.) was used. The streaming potential, E, and the streaming current, I, were measured using a Z.R. M. Kasprzak-Unitra (Warsaw, Poland) T-219 electrometer and recorded on a Sefram (Paris, France) T-PE recorder. The solutions of the acids were prepared by dissolving the analytical-reagent grade free acids in redistilled water. The mobile phase (water) was degassed prior to chromatographic measurements.

RESULTS AND DISCUSSION

Retention volumes of acids

The retention volumes of 39 acids at a concentration of 10^{-3} M are presented in Table I. Amino acids (Nos. 34–39) are included at the end of the table because they are characterized by nearly the same dissociation constants and retention volumes. The strong, completely dissociated acids (pK_a = −10 to 2.5) were eluted together at a retention volume of 0.80 cm^3. Assuming that they are completely excluded, this is the value of the dead volume of the chromatographic column (Fig. 1). The retention volumes of the weaker acids (pK_a = 2.5–6.5) were found to be proportional to their pK_a values. The weakest acids (pK_a = 6.5–8.5) were eluted together at a retention volume of 2.8 cm^3. Assuming total permeation of these acids into the resin, the inner volume of the column is given by the difference between the retention volumes of the strong and the weak acids.

The values of the distribution coefficient, K_d, and the capacity factors, k', were calculated from the following equations:

$$K_d = \frac{V_R - V_0}{V_i} \tag{1}$$

and

$$k' = \frac{V_R - V_0}{V_0} \tag{2}$$

The results are presented in Table I, where it can be seen that the distribution coefficients lie in the range 0–1 from the strong to the weak acids, respectively. This and the dependence of the retention volume on pK_a indicate that the ion-exclusion mechanism predominates in the separation of acids.

TABLE I

EFFECT OF pK_a VALUES ON CHROMATOGRAPHIC PARAMETERS (RETENTION VOLUME, V_R, DISTRIBUTION COEFFICIENT, K_d, AND CAPACITY FACTOR, k') OF THE ANALYSED ACIDS

Concentration of acids: 10^{-3} *M*. Column: 150 × 4 mm I.D. Support: LiChrosorb KAT. Mobile phase: water. Flow-rate: 10 μl/s. Temperature: 22°C.

No.	*Acid*	pK_a	V_R (cm^3)	K_d	k'
1	Perchloric	−10	0.80	0	0
2	Hydrochloric	−6.1	0.84	0.02	0.05
3	Sulphuric	−3.0	0.78	0	0
4	Nitric	−1.4	0.84	0.02	0.05
5	Iodic	0.80	0.87	0.03	0.09
6	Oxalic	1.27	0.88	0.04	0.10
7	Maleic	1.95	0.89	0.05	0.11
8	*o*-Nitrobenzoic	2.17	0.76	0	0
9	Chloroacetic	2.87	0.96	0.08	0.20
10	Phthalic	2.94	0.94	0.07	0.16
11	Salicylic	3.00	1.05	0.13	0.31
12	Fumaric	3.02	0.94	0.07	0.18
13	Tartaric	3.03	0.92	0.06	0.15
14	Citric	3.13	0.93	0.07	0.16
15	*p*-Nitrobenzoic	3.44	1.10	0.15	0.38
16	*m*-Nitrobenzoic	3.49	1.02	0.11	0.28
17	Terephthalic	3.54	0.96	0.07	0.18
18	Formic	3.75	1.10	0.15	0.38
19	Lactic	3.86	1.03	0.12	0.29
20	Benzoic	4.20	1.45	0.33	0.82
21	Ascorbic	4.21	1.20	0.20	0.50
22	Succinic	4.21	1.17	0.19	0.46
23	Methoxyphenylacetic	4.32	1.44	0.32	0.80
24	*p*-Acetylsalicylic	4.57	1.14	0.17	0.43
25	Acetic	4.76	1.51	0.35	0.89
26	Isobutyric	4.81	1.66	0.45	1.11
27	Valeric	4.84	1.84	0.52	1.30
28	Propionic	4.87	1.66	0.43	1.08
29	Caproic	4.88	2.09	0.65	1.61
30	Carbonic	6.45	2.86	1.03	2.58
31	*p*-Nitrophenol	7.10	2.80	1.00	2.50
32	*o*-Nitrophenol	7.23	2.76	0.98	2.45
33	*m*-Nitrophenol	8.40	2.80	1.00	2.50
34	Histidine	1.77	0.88	0.04	0.10
35	Arginine	2.10	0.88	0.04	0.10
36	Tyrosine	2.20	0.81	0.01	0.01
37	Valine	2.29	0.76	0	0
38	Alanine	2.35	0.78	0	0
39	Tryptophan	2.38	0.78	0	0

However, in separation of the acids other mechanisms also play a role, such as "partition" on the reversed phase. As an example, let us consider monocarboxylic (acetic–caproic) acids. Although characterized by nearly the same pK_a values (Fig. 1, Table I), they are eluted at different retention volumes in order of increasing length of their aliphatic chains. As another example, it can be noted that salicylic and ace-

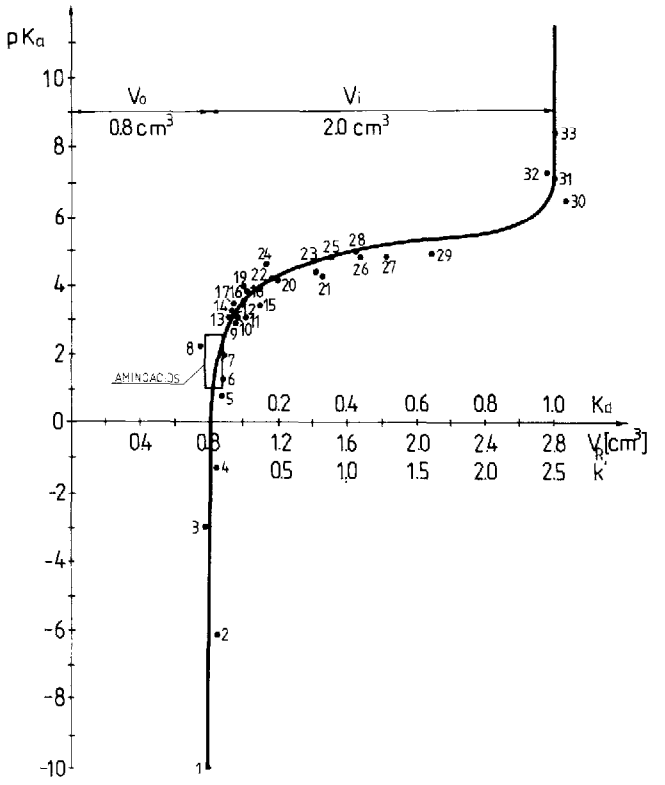


Fig. 1. Influence of the pK_a values of the analysed acids on their retention volumes, V_R, distribution coefficients, K_d, and capacity factors, k'. The numbers correspond to the acids listed in Table I. Stainless-steel chromatographic column (150 × 4 mm I.D.), slurry packed with LiChrosorb KAT, 10 μm. Mobile phase: water. Flow-rate: 10 μl/s. Sample volume: 5 μl. Electrokinetic detector with PTFE capillary (100 × 0.2 mm I.D.).

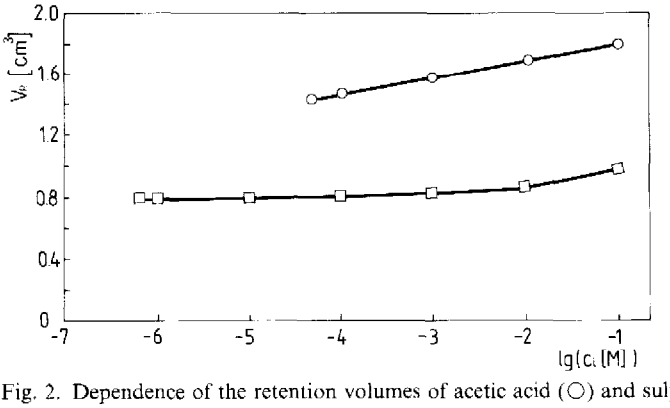


Fig. 2. Dependence of the retention volumes of acetic acid (○) and sulphuric acid (□) on their concentrations. Other conditions as in Fig. 1.

tosalicylic acids, and also phthalic and terephthalic acids, are eluted at nearly the same retention volumes, although their pK_a values are different. This is obviously due to the similarity of their structures.

It was observed that with pure water used here as the mobile phase, the retention of acids depends on their concentration (Fig. 2). The more dilute the acid, the more it dissociates and therefore the faster is its elution.

Repetitive injections of acids yielded their retention volumes with a relative standard deviation (R.S.D.) of better then 2%.

Discussion of the mechanism of the separation of acids in ion-exclusion chromatography

Let us consider any acid, HR, which dissociates according to

$$HR = H^+ + R^- \tag{3}$$

Both the dissociated and the undissociated form of the acid may exist both in the mobile and in the stationary phase (denoted by the subscripts M and S, respectively). The separation of ions in ion-exclusion chromatography is ruled by the Donnan membrane equilibrium mechanism. In a thermodynamic equilibrium the chemical potentials of the acid on both sides of a membrane must be equal. With dilute solutions, when the activities of the species can be replaced by their concentrations, the above equilibrium condition assumes the form

$$[H^+]_M[R^-]_M[HR]_M = [H^+]_S[R^-]_S[HR]_S \tag{4}$$

In both phases the equilibrium of reaction 3 may be described by the dissociation constant of the acid:

$$K_a = \frac{[H^+]_M[R^-]_M}{[HR]_M} = \frac{[H^+]_S[R^-]_S}{[HR]_S} \tag{5}$$

The electroneutrality conditions in both phases can be expressed as follows:

$$[H^+]_M = [R^-]_M \tag{6}$$

$$c_2 = [H^+]_S + [R^-]_S \tag{7}$$

where c_2 is the concentration of dissociated functional groups in the stationary phase.

The total amount of the acid in the injected sample is given by the product of the concentration and volume of the injected sample, $c_i v_i$, and is equal to the sum of the concentrations of the dissociated and undissociated forms of the acid, in both the mobile and stationary phases, multiplied by the peak volume, V_P:

$$c_i v_i = V_P([R^-]_M + [HR]_M + [R^-]_S + [HR]_S) \tag{8}$$

V_P is given by

$$V_P = \frac{(2\pi)^{1/2} V_R}{N^{1/2}} \tag{9}$$

TABLE II

DISTRIBUTION COEFFICIENTS, K_d, AND RETENTION VOLUMES, V_R, CALCULATED FROM EQN. 15 FOR THE ASSUMED RATIO c/K_a (SAMPLE CONCENTRATION AT THE PEAK MAXIMUM TO DISSOCIATION CONSTANT OF ANALYSED ACID)

Parameter	c/K_a									
	10^{-4}	10^{-3}	10^{-2}	10^{-1}	10^{0}	10^{1}	10^{2}	10^{3}	10^{4}	10^{5}
K_d	10^{-4}	$9.97 \cdot 10^{-4}$	$9.71 \cdot 10^{-3}$	$7.87 \cdot 10^{-2}$	0.333	0.667	0.870	0.960	0.986	0.996
V_R	0.800	0.802	0.819	0.957	1.467	2.133	2.544	2.714	2.772	2.791

where N is the number of theoretical plates of the column. Substituting eqn. 9 into eqn. 8 we obtain

$$c = [R^-]_M + [HR]_M + [R^-]_S + [HR]_S \tag{10}$$

where c is the concentration of the sample at the peak maximum.

The distribution coefficient can be described by

$$K_d = \frac{[R^-]_S + [HR]_S}{[R^-]_M + [HR]_M} \tag{11}$$

From eqn. 11 combined with eqns. 5, 6 and 10, it follows that

$$K_d = \frac{c - [R^-]_M - [R^-]_M^2/K_a}{[R^-]_M + [R^-]_M^2/K_a} \tag{12}$$

In the above equation $[R^-]_M$ is unknown. It can be obtained as the solution of the quartic equation derived on the basis of eqns. 5–10. If

$$c \ll c_2 \tag{13}$$

the quartic equation can be reduced to a quadratic equation. Then $[R^-]_M$ can be easily obtained as a solution of the quadratic equation and eqn. 12 assumes the form

$$K_d = \frac{4c + K_a - \sqrt{K_a^2 + 8K_a c}}{4c - K_a + \sqrt{K_a^2 + 8K_a c}} = \frac{2c + K_a - \sqrt{K_a^2 + 8K_a c}}{2c - 2K_a} \tag{14}$$

Eqn. 14 describes the dependence of the distribution coefficient on the concentration and the dissociation constant of the analysed acids. Then their retention volumes can be calculated from eqn. 1. It is worth noting that, according to eqn. 14, K_d does not depend on c_2. Eqn. 14 can be further transformed to the form

$$K_d = \frac{4\frac{c}{K_a} + 1 - \sqrt{1 + 8\frac{c}{K_a}}}{4\frac{c}{K_a} - 1 + \sqrt{1 + 8\frac{c}{K_a}}} = \frac{1 + 2\frac{c}{K_a} - \sqrt{1 + 8\frac{c}{K_a}}}{2\frac{c}{K_a} - 2} \tag{15}$$

which shows that K_d and V_R depend on only one experimental magnitude, the ratio c/K_a. In Tables II and III and Fig. 3, both the experimental and calculated dependences of K_d on c/K_a are presented. It can be seen that the values of K_d calculated from eqn. 15 are in good agreement with the experimental values.

Application of ion-exclusion chromatography to the analysis of acids

It can be seen from Fig. 1 that acids with pK_a values ranging from 2.5 to 6.5 can be separated on the column. We have performed the separations of three groups

TABLE III

CONCENTRATIONS OF ANALYSED ACIDS AT THE PEAK MAXIMUM, c, AND THE RATIO c/K_a

The numbers correspond to the acids listed in Table I.

No.	$c \cdot 10^5$ *(M)*	c/K_a
1	7.48	$7.48 \cdot 10^{-15}$
2	7.12	$5.66 \cdot 10^{-10}$
3	7.67	$7.67 \cdot 10^{-8}$
4	7.12	$2.84 \cdot 10^{-6}$
5	6.88	$4.34 \cdot 10^{-4}$
6	6.80	$1.27 \cdot 10^{-3}$
7	6.72	$5.99 \cdot 10^{-3}$
8	7.87	$1.17 \cdot 10^{-2}$
9	6.23	$4.62 \cdot 10^{-2}$
10	6.37	$5.55 \cdot 10^{-2}$
11	5.70	$5.70 \cdot 10^{-2}$
12	6.37	$6.67 \cdot 10^{-2}$
13	6.51	$6.97 \cdot 10^{-2}$
14	6.44	$8.68 \cdot 10^{-2}$
15	5.44	$1.50 \cdot 10^{-1}$
16	5.87	$1.81 \cdot 10^{-1}$
17	6.23	$2.16 \cdot 10^{-1}$
18	5.44	$3.06 \cdot 10^{-1}$
19	5.81	$4.21 \cdot 10^{-1}$
20	4.13	$6.54 \cdot 10^{-1}$
21	4.97	$8.09 \cdot 10^{-1}$
22	5.12	$8.30 \cdot 10^{-1}$
23	4.16	$8.49 \cdot 10^{-1}$
24	5.25	1.95
25	3.96	2.28
26	3.61	2.33
27	3.25	2.25
28	3.61	2.67
29	2.86	2.17
30	2.86	$2.07 \cdot 10$
31	2.14	$2.69 \cdot 10^{2}$
32	2.17	$3.68 \cdot 10^{2}$
33	2.14	$5.37 \cdot 10^{3}$

of acids: (i) nitrobenzoic, (ii) dicarboxylic and (iii) substituted benzoic acids (Figs. 4–6). As an example of a practical application we determined benzoic acid in mustard. Benzoic acid is added to many industrial food products as a preservative. Its separation from other ionic compounds (non-ionic substances are not detected by the electrokinetic detector) in mustard (2.56 g in 25 cm^3 of water) is presented in Fig. 7. This method has the advantage that an aqueous extract with no further preparation is required for the analysis.

Characteristics of the electrokinetic detector

It has been found that the detectability of the electrokinetic detector is pro-

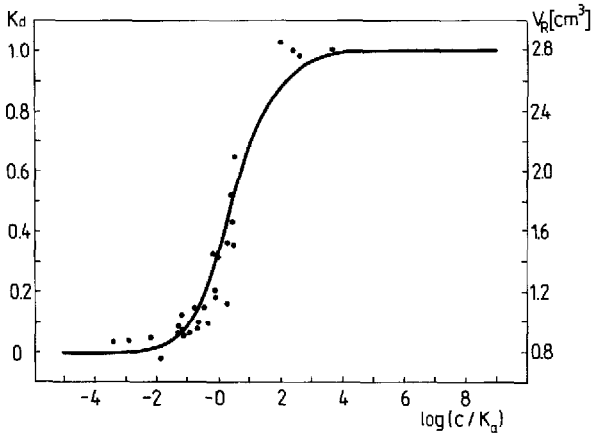
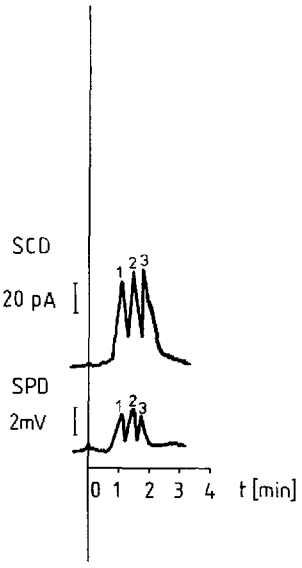


Fig. 3. Distribution coefficient, K_d, and retention volume, V_R, as a function of the ratio of sample concentration at the peak maximum to the dissociation constant, c/K_a. Comparison of experimentally obtained K_d and V_R values (points) with those calculated using eqn. 15 (line).

portional to the pK_a value of the analysed acid (Table IV). The detectability of the streaming potential detector for the strong acids (pK_a < 0) was $1.5 \cdot 10^{-12}$ mole ($3 \cdot 10^{-7}$ *M*) and for the very weak acids (*e.g.*, glucose, pK_a = 12.4) $1.0 \cdot 10^{-7}$ mole ($2 \cdot 10^{-2}$ *M*). Repetitive injections yielded heights of the chromatographic peaks differing by less than 2% for the streaming potential detector and 3% for the stream-



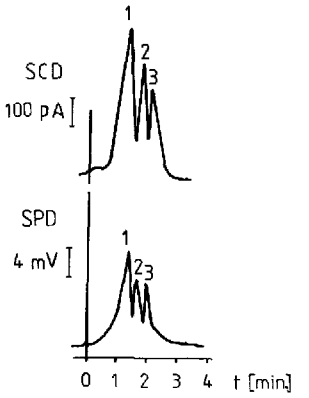
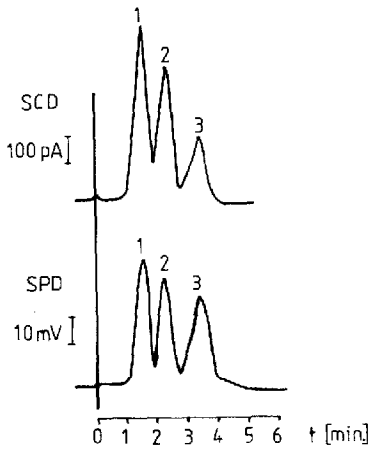


Fig. 4. Separation of 10^{-3} *M* (1) *o*-, (2) *m*- and (3) *p*-nitrobenzoic acids. Streaming potential (SPD) and streaming current detection (SCD). Other conditions as in Fig. 1.

Fig. 5. Separation of 10^{-3} *M* dicarboxylic acids. 1 = oxalic; 2 = succinic; 3 = tartaric. Other conditions as in Fig. 1.



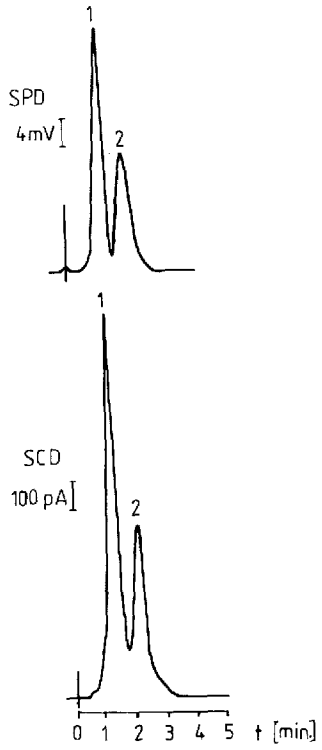


Fig. 6. Separation of 10^{-3} *M* *p*-aminobenzoic (1), benzoic (2) and *p*-methylbenzoic (3) acids. Other conditions as in Fig. 1.

Fig. 7. Separation of benzoic acid (2) from other ionic components (1) in mustard. Other conditions as in Fig. 1.

TABLE IV

COMPARISON OF DETECTABILITIES OF SOME ACIDS MEASURED BY STREAMING CURRENT DETECTOR (SCD) AND STREAMING POTENTIAL DETECTOR (SPD)

Acid	*Detectability*			
	SPD		*SCD*	
	c (M)	*n (mole)*	*c (M)*	*n (mole)*
Sulphuric	$3 \cdot 10^{-7}$	$1.5 \cdot 10^{-12}$	$7 \cdot 10^{-7}$	$3.5 \cdot 10^{-12}$
Formic	$2 \cdot 10^{-5}$	$1.0 \cdot 10^{-10}$	$7 \cdot 10^{-5}$	$3.5 \cdot 10^{-10}$
Acetic	$5 \cdot 10^{-5}$	$2.5 \cdot 10^{-10}$	$1 \cdot 10^{-4}$	$5.0 \cdot 10^{-10}$
Propionic	$8 \cdot 10^{-5}$	$4.0 \cdot 10^{-10}$	$5 \cdot 10^{-4}$	$2.5 \cdot 10^{-10}$
Carbonic	$1 \cdot 10^{-3}$	$5.0 \cdot 10^{-9}$	$4 \cdot 10^{-3}$	$2.0 \cdot 10^{-8}$
o-Nitrophenol	$1 \cdot 10^{-3}$	$5.0 \cdot 10^{-9}$	$4 \cdot 10^{-3}$	$2.0 \cdot 10^{-8}$
Glucose	$2 \cdot 10^{-2}$	$1.0 \cdot 10^{-7}$	$5 \cdot 10^{-2}$	$2.5 \cdot 10^{-7}$

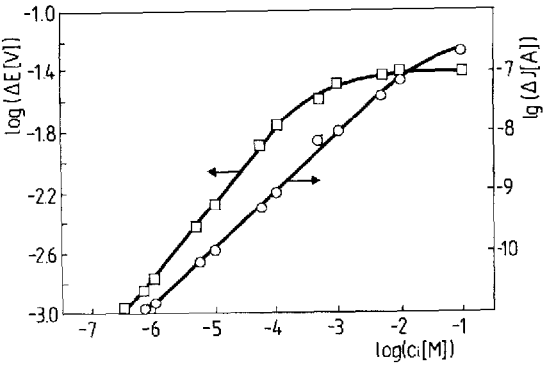


Fig. 8. Calibration graphs for sulphuric acid obtained using streaming potential (□) and streaming current (○) detectors. Other conditions as in Fig. 1.

ing current detector.

The dependence of the response, S, of the detector (*i.e.*, the recorded changes of the streaming potential or the streaming current) on the concentration can be represented as follows:

$$S = kc^n \tag{16}$$

or

$$\log S = \log k + n \log c \tag{17}$$

where k is the sensitivity and n the response index of the detector. The k and n values (obtained from eqn. 17 and Fig. 8) and, for comparison of the two types of the electrokinetic detectors, the detectabilities, D_L, and linear dynamic ranges, LDR, are presented in Table V. It can be seen that the streaming current detector is characterized by a linear response ($n = 1$) and a higher LDR, but the detectability is better for the streaming potential detector. Finally, it can be noted that the separation conditions (pure solvent as the mobile phase) in ion-exclusion chromatography are especially advantageous for electrokinetic detection.

TABLE V

COMPARISON OF THE TWO TYPES OF THE ELECTROKINETIC DETECTION, STREAMING POTENTIAL (SPD) AND STREAMING CURRENT (SCD)

The symbols are described in the text.

Detector	*k*	*n*	D_L *(mole)*	*LDR*	*R.S.D. (%)*
SPD	$2.6 \cdot 10^6$ V/mole	0.5	$1.5 \cdot 10^{-12}$	2	2
SCD	2 A/mole	1.0	$3.5 \cdot 10^{-12}$	4	3

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