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TRACE ANALYSIS BY INJECTION-GENERATED GRADIENT ION-PAIR CHROMATOGRAPHY WITH MICRO-BORE COLUMN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

K. ŠLAIS*, M. KREJČÍ and D. KOUŘILOVÁ
Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, 611 42 Brno (Czechoslovakia)

SUMMARY

Injection-generated gradient ion-pair chromatography in which the counter ion is introduced into the column simultaneously with a sample is applied to trace analysis. The theory of this method is based on adsorption isotherms of the counter ion, sorbed on a reversed octadecyl silica phase. Expressions for the minimum quantity of the counter ion required for sampling and for the characteristic counter-ion concentration at the moment of solute elution are derived. The resulting elution of the sample in the concentration gradient of the counter ion can be influenced by the quantity and nature of the injected counter ion. The method described makes it possible to decrease the minimum detectable concentration of the solute in the sample by at least two orders of magnitude in comparison with conventional isocratic elution. The method also takes advantage of small-bore columns.

INTRODUCTION

In trace analysis, the minimum detectable concentration of a solute is restricted by the column parameters on the one hand and by the detector characteristics on the other. It can be decreased (at identical characteristics of the column and the detector) only by use of enrichment techniques.

Sample enrichment on the chromatographic column is one of the simplest techniques. The technique of injecting the samples dissolved in a non-eluting solvent[1,2] is particularly suitable for microcolumns[3], with which substantially lower minimum detectable solute concentrations can be reached at a high mass sensitivity of the analysis.

Generally, situations can occur in which the solvent cannot be chosen so as to be a weaker mobile phase for the sample than the liquid used for the separation. Under these circumstances, the solute can be injected, dissolved in a non-eluting solvent, if the sample is treated so as to obtain a time-limited change in the sorption properties of the column.

In reversed-phase chromatography, ionic compounds are often not retained significantly in the column, even when the weakest mobile phases (aqueous solutions of buffers) are used. In this case, the solute can be injected, dissolved in a non-eluting

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solvent, provided that a dynamic ion exchanger is formed by the sorption of a counter ion at the stationary phase. For instance, injection of the counter ion immediately before the solute injection, followed by washing of both the sample and the counter ion from the column by a mobile phase gradient with an increasing concentration of the organic component, has been described[4]. Another procedure is simultaneous injection of the solute and the suitable counter ion[5].

In this work, we propose a theoretical description of the co-elution of the solute and the counter ion injected simultaneously into the column. Relationships are derived for the determination of the required minimum amount of counter ion injected, for the retention volume of the solute, depending on the retention behaviour of the counter ion, and for the characteristic counter-ion concentration at the moment of solute elution. The conditions under which the solute is eluted in the concentration gradient of the counter ion are also described. The required concentrations of the counter ion in the sample are low enough that the effect of solute enrichment substantially surpasses the effect of sample dilution in the counter ion.

A detection system that meets the demands of the gradient technique should be chosen for the proposed technique. Selected solutes, catecholamines, are detected at high sensitivity with an amperometric detector, which does not respond to the aromatic sulphonic acids used as counter ions. The concentration course of the elution of counter ions was studied with a spectrophotometric detector.

THEORETICAL

Reversed-phase ion-pair chromatography has been applied to separations of ionizable compounds. If the solute is perfectly dissociated and the formation of ion pairs in the mobile phase can be neglected, ion exchange is the main reason for the retention of the solute. It is effectuated between the mobile phase and the layer of counter ion, adsorbed on an hydrophobic sorbent.

The equation describing ion exchange can then be simply written[6–13]

$$(PC)_s + S_m \rightleftarrows (PS)_s + C_m \tag{1}$$

where $(PC)_s$ is the adsorbed counter ion P at the surface of the sorbent, together with the electrostatically bonded co-ion C, S_m is the solute in the mobile phase, $(PS)_s$ is the counter ion adsorbed with the solute at the surface of the sorbent, and C_m is the co-ion in the mobile phase. The equilibrium constant for the ion exchange, K_{ie}, is then:

$$K_{ie} = \frac{[(PS)_s]\,[C_m]}{[(PC)_s]\,[S_m]} \tag{2}$$

As has been shown repeatedly[6–8,12], in a certain concentration range (up to a surface concentration of *ca.* 2 μmol/m^2)[6], the capacity ratio of the solute, k, can be considered to be directly proportional to the surface concentration of the adsorbed counter ion, $[(PC)_s]$,

$$k = \varphi S_a \cdot \frac{[(PS)_s]}{[S_m]} = \frac{w_s S_a}{V_m} \cdot \frac{[(PC)_s]}{[C_m]} \cdot K_{ie} \tag{3}$$

where φ is the ratio of phases, expressed by the relationship

$$\varphi = w_s/V_m \tag{4}$$

where V_m is the column dead-volume, w_s is the mass of the sorbent in the column and S_a is the specific surface of the sorbent. When the ionized solute and the counter ion are simultaneously injected, the solute moves in the column in a zone of variable counter-ion concentration. Fig. 1a shows the situation schematically. If a sufficient amount of counter ion is injected into the column together with the solute, the solute is retained at the head of the column. The counter ion will move along the column as a tailing zone with a sharp front. The counter-ion concentration in the stationary phase will gradually decrease at the head of the column and thus the solute capacity ratio will decrease simultaneously to such an extent that the solute will start moving along the column.

The situation at the tail end of the column can then be illustrated by Fig. 1b. The concentration of the counter ion, will reach a maximum, $[P_m]_{max}$, at the corrected elution volume, V'_p, and after that the counter-ion concentration will decrease gradually. The solute will be eluted somewhere in the range of the decreasing counter-ion

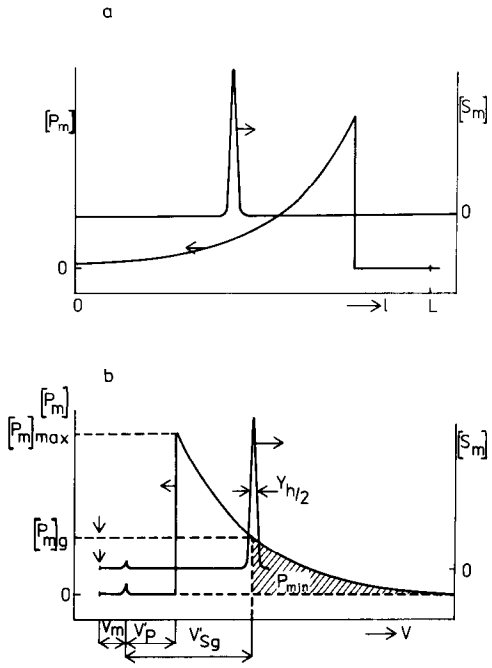

Fig. 1. Diagram of the elution of ionized solute in the counter-ion zone. a, Course of the concentration as a function of the distance, l, from the column inlet; b, course of the concentration at the column outlet as a function of the mobile phase volume passed, V. Symbols: L = column length; $[P_m]$ = counter-ion concentration in the mobile phase; $[S_m]$ = solute concentration in the mobile phase; $[P_m]_{max}$ = counter-ion concentration at the counter-ion zone front; $[P_m]_g$ = counter-ion concentration at the moment of the solute elution; $Y_{h/2}$ = solute peak width at half-height; V_m = column dead-volume; V'_P = corrected elution volume of the counter-ion zone maximum; V'_{Sg} = corrected elution volume of the solute in the counter-ion zone.

concentration. Let us designate the corrected elution volume of the solute which is eluted in the zone of the counter ion as V'_{Sg}. The magnitude of this elution volume can be derived on the basis of the theory of gradient elution. If the capacity ratio of the solute changes, depending on the mobile phase volume that passes through the column, V, it will be true that[14]:

$$\int_0^{V'_{Sg}} \frac{dV}{V_m k} = 1 \tag{5}$$

To achieve solute elution in the gradient of decreasing counter-ion concentration, it is necessary that the adsorption isotherm of the counter ion be convex. It thus becomes necessary to describe the elution of the counter ion in terms of the theory of non-linear chromatography. Published theories of non-ideal non-linear chromatography[15–18] permit the description of minor deviations from the linear adsorption isotherm. For large deviations, zone elution can be described only by neglecting the diffusion contributions to the zone spreading, *i.e.*, with the use of the model of ideal non-linear chromatography[19–22].

The relationship between the corrected retention volume of the counter ion, V'_P, and the surface concentration, $[(PC)_s]$, can then be expressed by[19,21]

$$\frac{V'_P}{w_s S_a} = \frac{d[(PC)_s]}{d[P_m]} \tag{6}$$

where $[P_m]$ is the concentration of the counter ion in the mobile phase. In order to solve eqn. 6, the analytical expression of the adsorption isotherm, which can generally be formulated as

$$[(PC)_s] = f([P_m]) \tag{7}$$

must be known. The elution volume of the solute eluted in the counter-ion zone, V'_{Sg}, can be calculated by solving eqns. 3, 5–7.

For the presence of the solute in the counter-ion zone during the entire migration time of the solute through the column (*viz.*, Fig. 1b) it is necessary that:

$$V'_P \leqslant V'_{Sg} \tag{8}$$

The minimum amount of counter ion, P_{min}, injected together with the solute, that is required to ensure this condition can be calculated from the relationship

$$\int_0^{[P_m]_{max}} [P_m]\, dV = P_{min} \tag{9}$$

where $[P_m]_{max}$ is the counter-ion concentration in the mobile phase at the counter-ion zone front (see Fig. 1) and it is simultaneously true that $V'_P = V'_{Sg}$. At the same time,

the relationship between $[P_m]$ and the eluted volume, V, is obtained by solving eqns. 6 and 7.

In a particular case, the calculation of V'_{Sg} and of the required amount of counter ion, P_{min}, depend on the analytical expression for the adsorption isotherm of the counter ion (eqns. 7).

In certain concentration ranges, counter-ion adsorption is sometimes described[9,10,13] by the Langmuir isotherm. The Freundlich isotherm was also used repeatedly to evaluate the experimental data[11,23]. Using the Freundlich isotherm in the form

$$[(PC)_s] = a[P_m]^b \tag{10}$$

where a and b are constants, the relationships for the retention volume of the solute eluted in the counter-ion zone

$$V'_{Sg} = (w_s S_a a)\left(\frac{K_{ie}}{[C_m]}\right)^{1-b}\left(\frac{1}{1-b}\right)^{1-b} \cdot b^b \tag{11}$$

and for the minimum amount of counter ion necessary to obtain the described effect

$$P_{min} = \left(\frac{1}{b} - 1\right)^b (1-b)\, a S_a w_s \left(\frac{[C_m]}{K_{ie}}\right)^b \tag{12}$$

have been described in Appendix I.

Experiments have produced isotherms[7,8] that corresponded to none of the commonly used types of the analytical expressions of isotherms. An exponential dependence of the decrease in concentration of the compound eluted in the mobile phase[24] was also used to describe the experiments. For the counter-ion elution this dependence has the following form

$$[P_m] = [P_m]_0 \cdot \exp(-V'_P/V_t) \tag{13}$$

where $[P_m]_0$ and V_t are constants, V_t representing the eluent volume that is required to wash the column so as to obtain an e-fold decrease in the concentration of the eluted compound at the column outlet. With the use of eqn. 13, expressions for V'_{Sg} and P_{min} will be in the form (*viz.*, Appendix II):

$$V'_{Sg} = 3.7\, V_t \log\left[1.22\left(\frac{[P_m]_0}{[C_m]} \cdot K_{ie} + 0.696\right)\right] \tag{14}$$

$$P_{min} = [P_m]_0\, V_t \left[1.22\left(\frac{[P_m]_0}{[C_m]} \cdot K_{ie} + 0.696\right)\right]^{-1.61} \tag{15}$$

EXPERIMENTAL

A Model 8700 piston pump (Spectra-Physics, San José, CA, U.S.A.) with a splitter was applied. A sampling six-port valve with a loop volume[3] of 100 μl and a four-port sampling valve with a 1-μl internal loop have been described earlier[25,26]. A 150 × 0.7 mm I.D. glass column in a metallic holder (CGC, Laboratory Instruments, Prague, Czechoslovakia) was connected to the sampling valve. An EMD-10 amperometric detector (Laboratory Instruments) with an internal cell volume of 4 nl was connected directly to the column outlet. The polarization voltage of the platinum working electrode was 1.0 V. A Model 769Z spectrophotometric detector (Kratos, Ramsey, NJ, U.S.A.) with a cell volume of 0.5 μl was connected via a capillary, 0.2 mm I.D., following the electrochemical detector. The column was packed by the slurry technique[27] with Separon SI-C18 (Laboratory Instruments) reversed phase, particle diameter 10 μm. Its specific surface area, S_a = 243 m^2/g, was measured by the nitrogen dynamic desorption method[28]. The amount of sorbent in the column was 47.3 mg and, the dead-volume, V_m = 50.6 μl.

Purchased chemicals were adrenalin (A), noradrenalin (NA) (Sigma, St. Louis, MO, U.S.A.), sodium perchlorate monohydrate, EDTA (Lachema, Brno, Czechoslovakia) and perchloric acid p.a. (Merck, Darmstadt, F.R.G.). The sodium salts of sulphonic acids, *o*-xylenesulphonate (OX), *p*-xylenesulphonate (PX), 1-naphthalenesulphonate (AN) and 2-naphthalenesulphonate (BN) were prepared by the authors by sulphonation of the respective hydrocarbons. The mobile phase was 0.1 *M* sodium perchlorate, 0.001 *M* perchloric acid, 0.001 *M* EDTA in distilled water. The stock solutions of the solutes (10^{-3} *M*) were stabilized by 0.1 *M* sodium bisulphite; 10^{-6} *M* solutions of catecholamines were used for injection.

Measurements

Solutions of catecholamines containing various amounts of aromatic sulphonates were injected into the microcolumn. Counter-ion elution was monitored with the UV detector, and the shapes and retentions of the catecholamine peaks were recorded by the electrochemical detector. The adsorption isotherms of the counter ions were evaluated from the UV detector records by the method of elution of a characteristic point (ECP) and from the elution volumes of the peak maxima[19,21]. The relationships between the peak heights of the catecholamines and their concentration in the samples were determined at the optimum concentration of *o*-xylenesulphonate in the sample. The minimum detectable concentrations of catecholamines in the sample were determined from these relationships. The injected amount of the counter ion in the sample, the measured concentration of the counter ion at the moment of elution of the catecholamines and the respective elution volumes were compared with the calculated values.

RESULTS AND DISCUSSION

Isotherms of OX, PX, AN and BN on the C_{18} reversed phase are illustrated in Fig. 2. They were measured by the ECP method[19,21] in the concentration range $7 \cdot 10^{-4}$ to 2 mol l^{-1}. The retention volume of the counter-ion peak maximum depends on the concentration or on the amount of counter ion injected into the column (Figs.

3–6). At low amounts, *e.g.*, up to $5 \cdot 10^{-8}$ mol PX, the isotherm can be considered linear and, consenquently, the reduced elution volume of the counter ion, V'_P, is practically constant, as seen from Figs. 3 and 4 for OX and PX. At higher values of P, the isotherms become convex, and, as a result, V'_P decreases. At higher values of P the dependence of V'_P on [P] is logarithmic. At the counter-ion concentrations where V'_P decreases, eqn. A13 (Apendix II) holds for the counter-ion surface concentration, $[(PC)_s]$, and so does eqn. 13 for the counter-ion elution. At higher concentrations of the counter ion, the dependence of the reduced elution volume of the counter ion on the injected amount, P, can be expressed by eqn. A2 (Appendix I). The values of the constants r and s in eqn. A2 are presented in Table I. Fig. 7 illustrates the relationship between V'_P and P for $P > 1$ μmol. The nature of the curves in Fig. 7 suggests that their courses can be described by eqn. 10, and thus conform to the Freundlich isotherm. The values of the coefficient b in eqn. 10, calculated from eqns. A2–A4 (Fig. 7), differ insignificantly from those determined as the tangents to the isotherms (ECP) at high concentrations (Table I).

The values of the characteristic counter-ion concentration, $[P_m]_g$, found for the smallest amount of counter ion required, P_{min}, are used to calculate the ion-exchange constants, K_{ie}, for the exchange of the catecholamines with Na^+. They are listed in Table I.

If the anion adsorbed in the stationary phase is responsible for the surface sorption activity, then the ion-exchange constant, K_{ie}, should be independent of the hydrocarbon skeleton of the molecule of the sulphonated compound used as a counter ion. If the condition in eqn. 8 is fulfilled, then solutes will be eluted from the column at a certain characteristic outlet concentration of the given counter ion in the

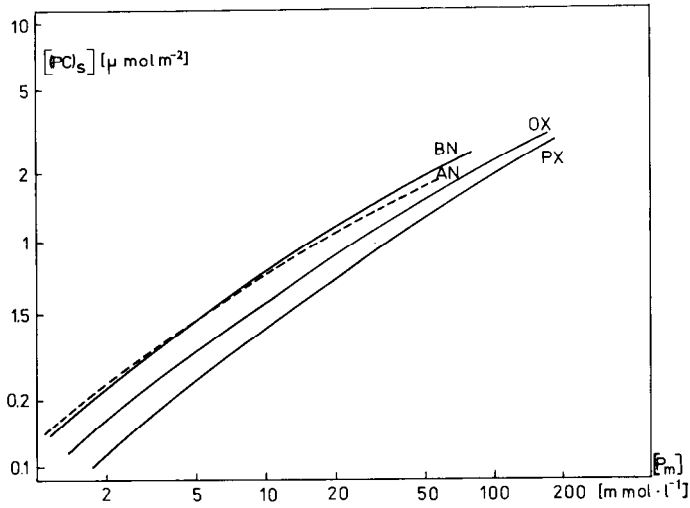

Fig. 2. Adsorption isotherms of counter ions measured by the method of the elution of a characteristic point[19,21]. Conditions: stationary phase, Separon SI–C18, d_p = 10 μm, S_a = 243 m²/g, CGC column (150 × 0.7 mm I.D.); mobile phase, 0.1 *M* sodium perchlorate, 0.001 *M* perchloric acid, 0.001 *M* EDTA in distilled water. Counter ions: OX = *o*-xylenesulphonate; PX = *p*-xylenesulphonate; AN = 1-naphthalenesulphonate; BN = 2-naphthalenesulphonate. $[(PC)_s]$ = Surface concentration of adsorbed aromatic sulphonate.

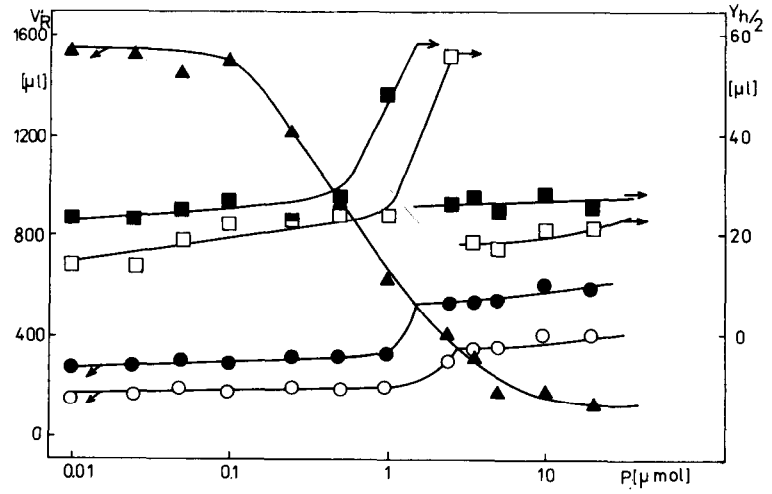

Fig. 3. Relationship between the corrected retention volumes (V'_R) of OX and catecholamines and the injected amount of OX. Sample volume: 0.1 ml. ▲—▲, Corrected retention volume of OX ($V'_R \equiv V_P$); ○—○ and ●—●, corrected retention volumes of NA and A, respectively ($V'_R \equiv V'_{S_g}$); □—□ and ■—■, $Y_{h/2}$ (see Fig. 1) of NA and A, respectively. Conditions: EMD-10 electrochemical detector; platinum working electrode, +1.0 V; Kratos UV detector with a cell volume of 0.5 μl; for other conditions see Fig. 2.

mobile phase, $[P_m]_g$, or in the stationary phase, $[(PC)_s]_g$, regardless of the amount of counter ion injected into the column. The values of $[P_m]_g$ and $[(PC)_s]_g$ depend on the steepness of the counter-ion concentration gradient and, thus, on the adsorption isotherm. Table II gives the measured values of $[P_m]_g$ and $[(PC)_m]_g$ for the counter ions studied. Average values of K_{ie}, calculated according to eqn. A9, are 2.2 ± 7% relative and 5.1 ± 5% relative for noradrenalin and adrenalin, respectively, with all

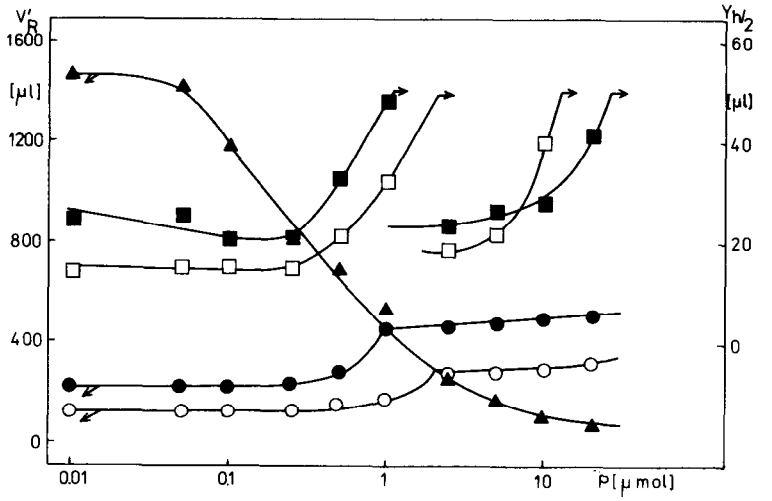

Fig. 4. Relationship between the corrected retention volumes of PX and catecholamines and the injected amount of PX. For conditions and symbols see Fig. 3, except ▲—▲, corrected retention volume of PX.

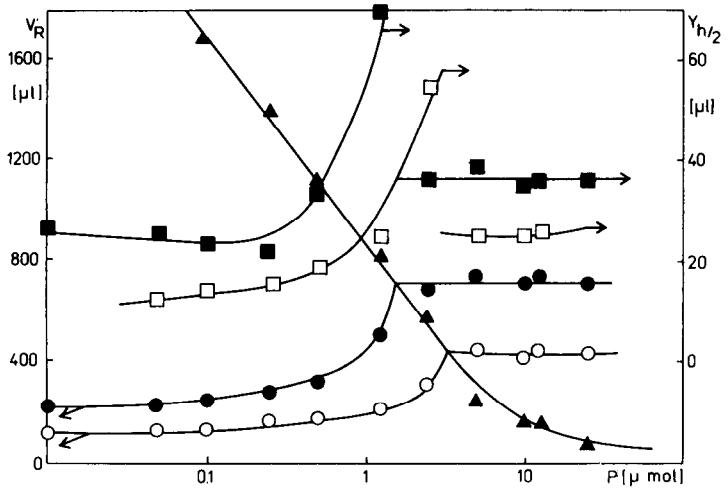

Fig. 5. Relationship between the corrected retention volumes of AN and catecholamines and the injected amount of AN. For conditions and symbols see Fig. 3, except ▲—▲, corrected retention volume of AN.

four counter ions studied. Table I lists the values of K_{ie} calculated according to eqn. A20 (the isotherm is described by an exponential function). With the use of this description of the isotherm, the values of K_{ie} obtained are obviously almost double those calculated according to the Freundlich isotherm. The standard deviation is also higher. The mean values of K_{ie} for adrenalin compared with the values measured by various authors by the technique of isocratic ion-pair chromatography are presented in Table III. An exact comparison of data is impossible. Nevertheless, the K_{ie} values,

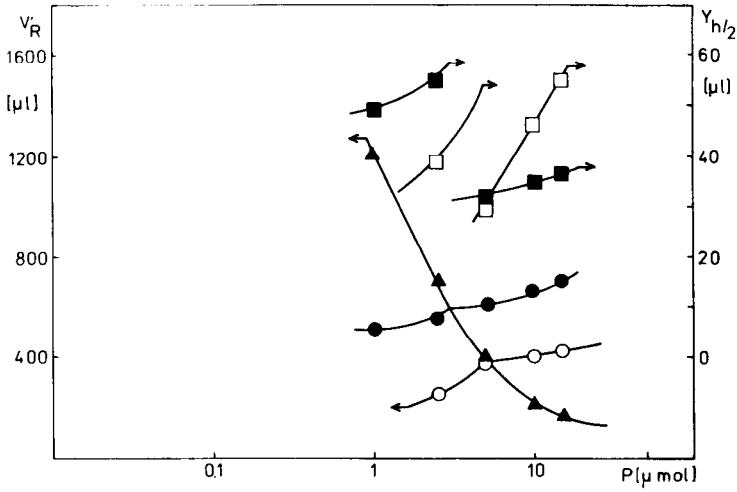

Fig. 6. Relationship between the corrected retention volumes of BN and catecholamines and the injected amount of BN. For conditions and symbols see Fig. 3, except ▲—▲, Corrected retention volume of BN.

TABLE I

PARAMETERS OF EQUATIONS CHARACTERIZING COUNTER-ION ELUTION

$[C_m]$ = 0.1 *M* Na^+; for the other conditions see Fig. 3. Experimental values of $[P_m]_g$ and P_{min} were were taken for the calculation.

Counter ion	*Constants of eqn. A2 from Fig. 7*		*Constants of eqn. 10*				*Constants of eqn. 13*		*K_{ie} for A and NA according to*			
	r (μl)	*s*	*Using elution volume maxima*		*Using elution curve by ECP method*		$[P_m]_0$ (mM)	V_t (μl)	*Eqn. A9**		*Eqn. A20*	
			a (μmol/m²)	*b*	*a* (μmol/m²)	*b*			*NA*	*A*	*NA*	*A*
OX	0.63	0.50	5.6	0.67	8.9	0.63	23	152	2.2	5.3	4.7	10.3
PX	0.14	0.59	5.0	0.63	8.9	0.65	21	121	2.0	4.8	3.2	9.9
AN	0.006	0.87	2.4	0.53	7.6	0.51	33	152	2.3	5.3	3.7	8.2
BN	0.046	0.75	4.2	0.57	9.8	0.54	19	226	1.9	4.9	2.9	9.2

* Values of *a*, *b* determined from elution volume maxima.

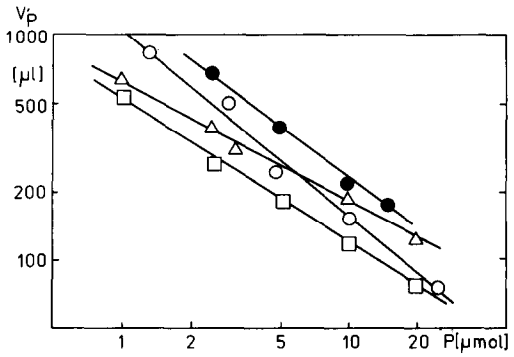

Fig. 7. Relationship between the corrected retention volumes, V_P', of aromatic sulphonates and their injected amounts at high P. The values are taken from Figs. 3–6. Symbols: △—△, OX; □—□, PX; ○—○, AN; ●—●, BN.

measured by the technique of solute elution in the elution zone of the counter ion, lie in the same range as the distribution constants, measured by the technique of isocratic ion-pair chromatography. The differences in K_{ie} values, arising from different descriptions of the isotherm, are ascribed to the fact that, in the course of solute migration in the column, the zone shape and, thus, also the character of the

TABLE II

COUNTER-ION CONCENTRATIONS, MEASURED IN THE MOBILE AND THE STATIONARY PHASES, $[P_m]_g$ AND $[(PC)_s]_g$, RESPECTIVELY, AT THE POINT OF CATECHOLAMINE ELUTION

For conditions see Fig. 3.

Counter ion	*P (µmol)*	V_R		$[P_m]_g$ *(mM)*		$[(PC)_s]_g$ *(µmol/m²)*	
		NA	*A*	*NA*	*A*	*NA*	*A*
OX	20.0	450	640	10.4	4.3	0.53	0.30
	10.0	456	654	10.1	3.8	0.53	0.28
	5.0	410	588	9.8	4.1	0.51	0.29
	3.5	396	580	9.7	4.2	0.51	0.30
	2.5	341	586	–	4.2	–	0.30
PX	20.0	380	563	15.0	4.1	0.57	0.21
	10.0	353	542	14.0	4.1	0.54	0.21
	5.0	342	532	14.0	4.5	0.54	0.23
	2.5	337	513	12.0	4.9	0.50	0.24
AN	25.0	476	756	14.0	5.0	0.87	0.46
	12.5	502	787	13.0	5.0	0.83	0.46
	10.0	466	742	12.0	4.4	0.80	0.43
	5.0	493	791	11.0	4.4	0.76	0.43
	2.5	360	735	–	4.7	–	0.45
BN	15.0	475	740	18.0	6.9	1.00	0.58
	10.0	450	710	18.0	6.9	1.00	0.58
	5.0	428	653	15.0	7.0	0.96	0.59

TABLE III

VALUES OF CONSTANTS K_{ie} AND φK_{ie} FOR ION EXCHANGE OF ADRENALIN SODIUM ION

Isocratic elution except where indicated otherwise

K_{ie}	φK_{ie} (g/cm^3)	*Counter ion*	*Adsorbent*	S_a (m^2/g)	*Mobile phase*	*Ref.*
9		Butanesulphonate	ODS Hypersil	173	0.05 *M* Phosphate buffer (pH 2.1)	8
1.5		Octyl-, decyl-, dodecyl sulphate	ODS Hypersil	105	0.02 *M* Phosphate buffer (pH 6) in water–methanol (80:20), 0.05 *M* Na^+	6
10	12.5	Hexanesulphonate	LiChrosorb RP-18		0.05 *M* Phosphate buffer (pH 3.00)	12
17	21	Octane sulphonate	LiChrosorb RP-18		0.05 *M* Phosphate buffer (pH 3.00)	10
4.9	6.3	Dodecyl sulphate	Partisil ODS		0.01 *M* Sodium phosphate buffer (pH 3.0) + 0.5% (v/v) 2-propanol	10
6.7	6.3	2-Naphthalenesulphonate	Separon SI-C18	243	0.1 *M* Sodium perchlorate + 0.001 *M* perchloric acid + 0.001 *M* EDTA	29
4.9	4.6	2-Naphthalenesulphonate	Separon SI-C18		0.1 *M* Sodium perchlorate + 0.001 *M* perchloric acid + 0.001 *M* EDTA*	eqn. A9
9.2	8.7	2-Naphthalenesulphonate	Separon SI-C18		0.1 *M* Sodium perchlorate + 0.001 *M* perchloric acid + 0.001 *M* EDTA*	eqn. A20

* Gradient of counter ion.

counter-ion isotherm, vary from one isotherm type to another. Neither type can be excluded due to the fact that, in the actual range of concentrations of the counter ion, the counter-ion adsorption isotherm is of a transitional character between the two types considered. This is supported by the fact that the value of K_{ie}, determined from an isocratic experiment[29], lies between the values calculated by the two different procedures for the description of gradient elution.

To accomplish the elution of the solute in the concentration gradient of the counter ion, we require a certain minimum amount of the counter ion, P_{min}, which we must introduce into the column simultaneously with the solute. The magnitude of P_{min} for the cases studied can be read from the graphs in Figs. 3–6. Table IV presents the values of P_{min} calculated according to eqn. 12 or 15. Whereas the values calculated according to eqn. 12 are approximately twice those found experimentally, the values according to eqn. 15 are only about half. Since P_{min} depends on the solute ion-exchange constant, K_{ie}, the reason for the discrepancy between the experimentally determined and calculated values of P_{min} is probably identical with to that we mentioned in connection with the difference in the values of the ion-exchange constants.

Depending on the nature of the isotherm and the amount of counter ion introduced, not only the solute retention volume but also the peak width at half-height, $Y_{h/2}$, will vary. From Figs. 3–6 one can verify that neither the solute elution volume nor the peak width at half-height change markedly providing the amount of the counter ion is such that the conditions in eqn. 8 are fulfilled. If this condition is not fulfilled, the solute will be eluted, for a certain part of its migration, before the counter-ion peak maximum. Under these circumstances, the solute is eluted in the increasing concentration gradient of the counter ion, which leads to a considerable broadening of the solute peak, *i.e.*, to an increase in $Y_{h/2}$. If the amount of the counter ion injected is substantially smaller than that required by eqn. 8, only an enrichment of the solute will result. However, the zone of solute will migrate along the column independently of the counter-ion zone. For solute enrichment, the amount of counter ion required is less than 10^{-8} mol in the case of the counter ions studied and the experimental conditions given.

Of the counter ions studied, *o*-xylenesulphonate proved to be the most suitable in the given chromatographic system of injection-generated gradient elution. An ex-

TABLE IV

COMPARISON OF THE EXPERIMENTALLY DETERMINED AND CALCULATED MINIMUM REQUIRED AMOUNT OF THE COUNTER ION, P_{min}.

Counter ion	*NA*				*A*			
	$[P_m]_g$ *(mM)*	P_{min} *(μmol)*			$[P_m]_g$ *(mM)*	P_{min} *(μmol)*		
		Eqn. A10	*Eqn. A18*	*Found*		*Eqn. A10*	*Eqn. A18*	*Found*
OX	10	3.36	1.5	3.0	4.2	1.9	0.65	1.5
PX	12	3.7	1.7	2.1	4.9	1.8	0.60	1.0
AN	11	8.3	1.95	3.4	4.7	4.6	0.73	1.6
BN	15	9.0	4.1	5.0	5.0	4.3	1.13	3.0

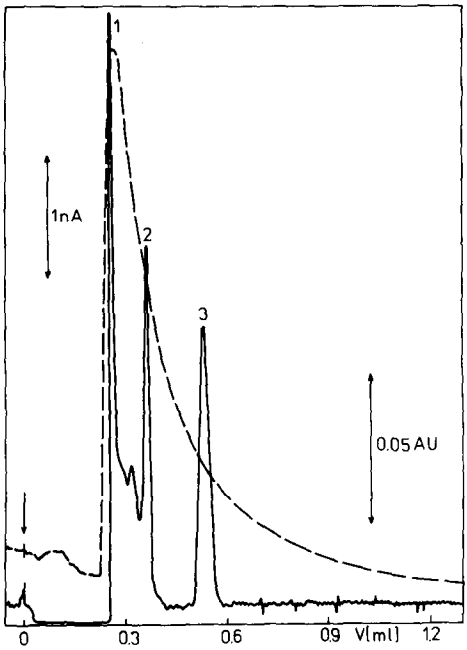

Fig. 8. Example of catecholamines elution in the zone of OX. ----, UV detector trace 280 nm; ——, electrochemical detector trace. Samples: 1 = OX (0.05 *M*); 2 = NA ($1 \cdot 10^{-7}$ *M*); 3 = A ($1 \cdot 10^{-7}$ *M*). Flow-rate = 2.5 μl/s. For other conditions see Fig. 3.

ample of a chromatogram obtained by this technique is shown in Fig. 8. Calibration of this technique with the electrochemical detector yielded the minimum detectable concentration of catecholamines in the sample, *ca.* $5 \cdot 10^{-9}$ *M*. The advantages of the described procedure can be evaluated from Table V. It is obvious that the addition of a suitable counter ion to the sample prior to its injection into the column provides

TABLE V

COMPARISON OF CHROMATOGRAPHIC SEPARATION OF CATECHOLAMINES BY INJECTION WITHOUT AND WITH THE COUNTER ION

For conditions see Fig. 3. Counter ion: 0.05 *M* OX.

	Sample volume (μl)	*Retention volume (μl)*	*Peak width, $Y_{h/2}$ (μl)*	*Minimum detectable concentration in sample (M)*
NA				
Without counter ion	1	107	8.2	$4 \cdot 10^{-7}$
With counter ion	100	375	19.5	$3.5 \cdot 10^{-9}$
A				
Without counter ion	1	200	15.9	$9 \cdot 10^{-7}$
With counter ion	100	555	28.0	$5 \cdot 10^{-9}$

an opportunity for substantially increasing the injected sample volume. This will also substantially decrease the minimum detectable solute concentration, in the present case, by more than two orders of magnitude.

The injection-generated gradient of the dynamic stationary phase is advantageous for trace analysis, enabling greater utility of microcolumn chromatography. Hence, microcolumns of diameter for which the technique of independent gradient generation has not yet been elaborated can be used.

APPENDIX I

Substituting eqn. 10 into eqn. 6, we obtain the relationship between V'_P and the counter-ion concentration in the mobile phase:

$$V'_P = w_s S_a ab[P_m]^{(b-1)} \tag{A1}$$

Substituting eqn. A1 into eqn. 9 and integrating, we obtain the relationship between V'_P and P

$$V'_P = rP^{-s} \tag{A2}$$

where

$$r = (w_s S_a ab)^{1/b} \left(\frac{b}{1-b}\right)^{\frac{b-1}{b}} \tag{A3}$$

and

$$s = \frac{1-b}{b} \tag{A4}$$

Relationships A2–A4 make it possible to calculate the constants of a and b of the Freundlich isotherm from the measured relationship between V'_P and P. We obtain the relationship for the calculation of V'_{Sg} by applying the following procedure. Substituting eqn. 10 into eqn. A1 we obtain:

$$V'_P = w_s S_a ab \left\{\frac{[(PC)_s]}{a}\right\}^{\frac{b-1}{b}} \tag{A5}$$

Substituting eqn. A5 into eqn. 3, we obtain the relationship between the solute capacity ratio and the eluent volume passed:

$$k = \frac{w_s S_a}{V_m} \cdot \frac{K_{ie}}{[C_m]} \cdot a \left(\frac{V'_P}{w_s S_a ab}\right)^{\frac{b}{b-1}} \tag{A6}$$

Hence, by substitution of eqn. A6 into eqn. 5 we obtain:

$$\int_0^{V'_{Sg}} \frac{[C_m]}{K_{ie}} (w_s S_a a)^{\frac{1}{b-1}} \left(\frac{V'_P}{b}\right)^{\frac{b}{1-b}} \cdot dV = 1 \tag{A7}$$

If we consider that at the moment when the solute leaves the column (*viz.*, Fig. 1b)

$$V'_P = V'_{Sg} \tag{A8}$$

we obtain, by integrating eqn. A7 under the condition in eqn. A8, the expression for the calculation of the solute retention volume in the counter-ion zone in the form of eqn. 11. We further obtain the counter-ion concentration in the mobile phase at the column outlet at the moment of the solute elution, $[P_m]_g$, by substituting eqn. A1 into eqn. 11, and considering the condition required by eqn. A8:

$$[P_m]_g = \frac{[C_m]}{K_{ie}} \cdot b\,(1 - b) \tag{A9}$$

Relationship A9 also permits the calculation of the equilibrium ion-exchange constant, K_{ie}, provided that the counter-ion concentration in the mobile phase at the moment of the solute elution, $[P_m]_g$, is known. According to the ion-exchange model described in the Theoretical section, K_{ie} should not be dependent on the counter ion used[6]. Further, $[P_m]_g$ is not, according to eqn. A9, dependent on the amount of counter ion injected, P. We obtain the minimum amount of counter ion, P_{min}, required for the fulfillment of the condition in eqn. A8, by substituting eqns. A2–A4 into eqn. 11, which leads to eqn. 12.

If concentration $[P_m]_g$ is known, we can use the relationship obtained by the substitution of eqn. A9 into eqn. 12 in order to calculate P_{min}:

$$P_{min} = (1 - b)\left(\frac{1}{b^2}\right)^b a S_a w_s ([P_m]_g)^b \tag{A10}$$

APPENDIX II

If the dependence of the counter-ion concentration in the mobile phase, $[P_m]$, on the eluted volume of the mobile phase is described by eqn. 13, a simple equation is valid between the sampled amount and the concentration at the zone maximum:

$$P = [P_m]\, V_t \tag{A11}$$

Substitution of eqn. 13 into eqn. 6 and subsequent integration result in a relationship between the counter-ion concentration in the stationary phase and the volume passed:

$$[(PC)_s] = \frac{[P_m]_0\,(V'_P + V_t)}{w_s S_a} \cdot \exp\left(-\frac{V'_P}{V_t}\right) \tag{A12}$$

Substituting relation eqn. 13 into eqn. A12, we obtain the adsorption isotherm equation:

$$[(PC)_s] = [P_m]\frac{V_t}{w_s S_a}\left(1 + \ln\frac{[P_m]_0}{[P_m]}\right) \tag{A13}$$

To get an idea of the shape of such an isotherm, let us assume $V_t/(w_s S_a) = 1$ and $[P_m]_0 = 1$ in arbitrary units. Such an isotherm can then be illustrated by Fig. A1. It follows from eqn. A13 and Fig. A1 that this isotherm can be approximated by the Freundlich adsorption isotherm in the range of approximately one order of magnitude. An analogous shape had been found earlier in the case of the adsorption of counter ions in ion-pair chromatography[7,8].

Substituting eqns. A12 and 3 into eqn. 5 and rearranging we obtain:

$$\int_0^{V'_{Sg}/V_t} \frac{\exp\,(V'_P/V_t)\,\mathrm{d}(V'_P/V_t)}{V'_P/V_t + 1} = [P_m]_0 \cdot \frac{K_{ie}}{[C_m]} \tag{A14}$$

The integral at the left-hand side of eqn. A14 can be solved by expansion into a series

$$\int_{-\infty}^{x} \frac{e^x}{x+1} \cdot \mathrm{d}x = \frac{1}{e} \int_{-\infty}^{x} \frac{e^{x+1}}{x+1} \cdot \mathrm{d}x =$$

$$\frac{1}{e}\left[\gamma + \ln(x+1) + x + 1 + \frac{(x+1)^2}{2 \cdot 2!} + \frac{(x+1)^3}{3 \cdot 3!} + \ldots\right] \tag{A15}$$

where γ is the Euler constant, 0.5772. A graph of the dependence of the integral on the left-hand side of eqn. A15 on x is presented in Fig. A2. It is obvious that for $1 < x < 5$ the integral can be approximated by an exponential function:

$$\int_{-\infty}^{x} \frac{e^x}{x+1} \cdot \mathrm{d}x \approx 0.81 \cdot 10^{\frac{x}{3.7}} \tag{A16}$$

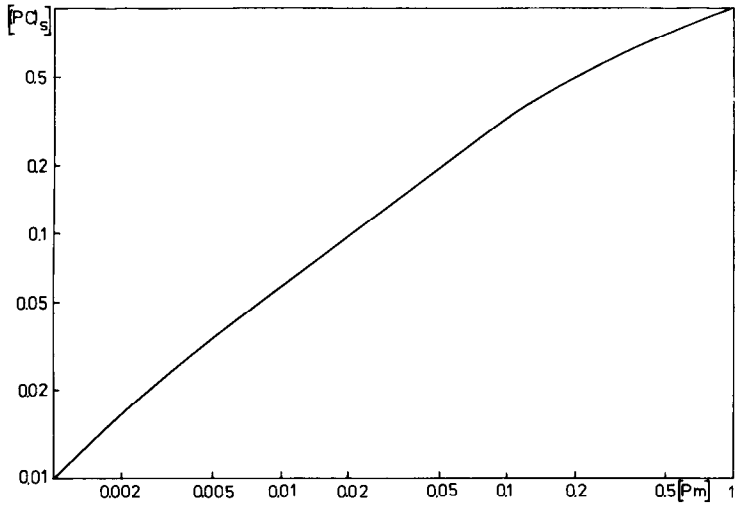

Fig. A1. Dependence of the counter-ion adsorption for an exponential decrease in the concentration in the mobile phase, on the eluent volume passed. Values on axes are in relative units.

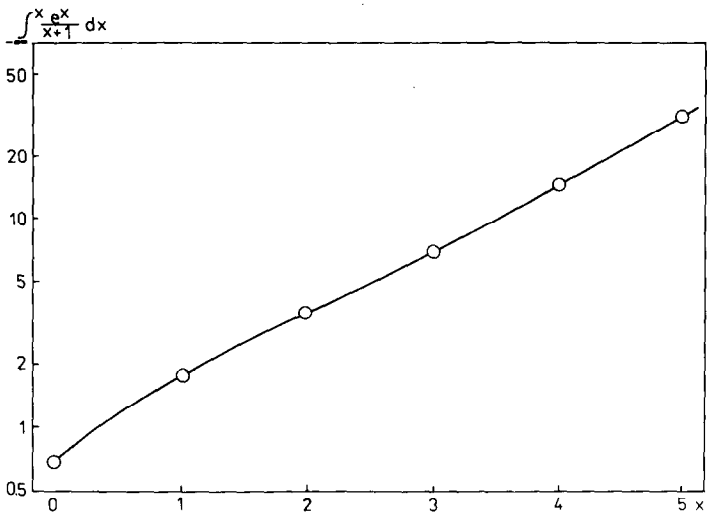

Fig. A2. Dependence of the exponential integral according to eqn. A15 on the value of x.

For $x = 0$, the integral equals 0.696 according to eqn. A15 and, hence, for $1 < x < 5$:

$$\int_0^x \frac{e^x}{x+1} \cdot dx \approx 0.81 \cdot 10^{\sqrt[3.7]{x}} - 0.696 \tag{A17}$$

If this relationship is used, then eqn. 14 for the elution volume of the solute in the counter-ion zone follows from eqns. A14 and A17. We can then obtain from eqns. 14 and 13 an expression having the form of eqn. 15 for the minimum amount of counter ion that must be injected into the column, P_{min}. If the counter-ion concentration in the mobile phase at the moment of the solute elution, $[P_m]_g$, is known, the required amount of the counter ion can then be calculated simply by using eqn. A11:

$$P_{min} = [P_m]_g V_t \tag{A18}$$

Combining eqns. A18 and 15, we obtain the expression for the calculation of $[P_m]_g$:

$$[P_m]_g = [P_m]_0 \left\{1.22 \left(\frac{[P_m]_0}{[C_m]} \cdot K_{ie} + 0.696\right)\right\}^{-1.61} \tag{A19}$$

Eqn. A19 can be arranged into an expression that is suitable for the calculation of K_{ie} from the measured values of $[P_m]_g$, $[P_m]_0$ and $[C_m]$:

$$K_{ie} = \left\{\left(\frac{[P_m]_g}{[P_m]_0}\right)^{-0.622} \cdot 0.81 - 0.696\right\} \frac{[C_m]}{[P_m]_0} \tag{A20}$$

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