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Separation of simple ions by gel chromatography

I. Simple model of separation for single-salt systems

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ABSTRACT

The chromatographic separation of simple ions by a polymer gel in water was modelled as a liquid–liquid partition process. The model consists of two "homogeneous" phases, a mobile phase of pure eluent and a stationary (gel) phase of a structureless concentrated polymer solution with a few electric charges fixed within it. Thermodynamic considerations and a simplifying approximation yielded a simple description of single-salt systems, which accounted for the chromatographic behaviour of simple ions at low and high concentrations in a systematic fashion. The introduced notion of "intrinsic distribution coefficients" of ions proved to be useful. The main mechanism of the separation of simple ions is suggested to be the difference in the thermodynamic stabilities of individual ions in the two phases, rather than involving size-exclusion and adsorption effects.

INTRODUCTION

Gel chromatography, so designated by Determann[1], is a method for separating molecules into different species by use of a polymer gel. Its basic principle is generally considered to be a size-exclusion effect exerted by the porous gel matrix. This form of liquid chromatography has developed into a particularly useful tool for separating macromolecules according to their size. In the field of synthetic polymers, it is more commonly termed gel permeation chromatography (GPC)[2].

It is also known that certain non-electrolytic polymer gels are capable of separating simple ions in an aqueous medium[3,4]. The basic mechanism of simple-ion separation has been assumed to be a GPC effect arising from the size difference of

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(hydrated) ions. However, a number of examples exsist that cannot be explained in terms of the size effect or the size effect alone.

According to Yoza[3], possible "side effects" include ion exclusion, adsorption, counter-ion effects and others. Ion exclusion is a Donnan effect exerted by a usually small number of ionic charges often incorporated in the (basically) neutral gel matrix for aqueous use. Pecsok and Saunders[4] and Kadokura *et al.*[5] have discussed this problem on a quantitative basis. "Side effects" that cause retardation of a sample solute in the column for a longer period than expected for its size have been often ascribed to chemical or physical adsorption. In some instances, adsorption or sorption[4] has been claimed to be a major mechanism, rather than a side effect, of simple-ion separation by a polymer gel[4,6–8]. The counter-ion effect has been phenomenologically treated by Saunders and Pecsok[6]. More recently, Shibukawa *et al.*[9] modelled simple-ion separation as a combined effect of size exclusion and partition, the latter involving the counter-ion effect and all other possibly operative effects.

These previous results seemed to us to suggest that the major mechanism of separation might be neither size exclusion nor adsorption. We then attempted to model the gel chromatography of simple ions like a liquid–liquid partition process: our model consists of two "homogeneous" phases, a mobile phase of pure eluent and a stationary phase of a polymer gel through which simple ions are assumed to migrate freely. We assume no particular structure for the gel phase, viewing it as if it were a concentrated polymer solution. Hence, in this model, ions are partitioned between the two phases according to their intrinsic thermodynamic stability in each phase. The counter-ion effect is automatically taken into account by postulating electrical neutrality in the two phases. To simplify the theoretical formulation and to make direct comparisons of theory and experiment feasible, we introduce the assumption that the activity coefficients of the individual ions are equal in the two phases. This is admittedly a crude approximation, but at the cost of rigour it provides a simple and new insight into the problem. It will be shown here that the model is capable of systematically describing experimental results for single-salt systems with fair precision. The simplicity of the model is particularly useful for treating more complicated systems like mixed-salt systems, which will be the subject of a separate report[10].

THEORETICAL

We let a cation 1 of the type K^{+z_1} and an anion 2 of the type A^{-z_2} be distributed between a mobile phase of volume V_m and a stationary (gel) phase of volume V_g, and assume V_m and V_g to be constant (Fig. 1). In Fig. 1, N_i and N_i' denote the total numbers of ion i (= 1 or 2) in the mobile and the gel phases, respectively. Hereafter a prime denotes the gel phase, and numbers are mole-based. As most polymer gels for aqueous use are more or less electrically charged, we assume that a total N_g' of *negative* charges are fixed in the gel phase.

The chemical potential μ_i of each ion in each phase is given by

$$\mu_i = \mu_i^0 + RT \ln a_i + (-1)^i F z_i \psi \quad (1)$$

$$\mu_i' = \mu_i'^0 + RT \ln a_i' + (-1)^i F z_i \psi' \quad (2)$$

Mobile Phase (m)	**Stationary Phase (g)**
volume: V_m	**volume:** V_g
cations: N_1	**cations:** N_1'
anions: N_2	**anions:** $N_2' + N_g'$

Fig. 1. Schematic representation of the model (see text for details).

where μ_i^0 and $\mu_i'^0$ are the standard chemical potentials, a_i and a_i' are the activities, z_i is the charge number of the ion, ψ and ψ' are the electrostatic potentials and R, T and F are the gas constant, the absolute temperature and the Faraday constant, respectively. At equilibrium $\mu_i = \mu_i'$, and we have

$$\Delta\mu_1^0 + RT \ln (a_1'/a_1) - Fz_1\Delta\psi = 0 \tag{3}$$

$$\Delta\mu_2^0 + RT \ln (a_2'/a_2) + Fz_2\Delta\psi = 0 \tag{4}$$

with

$$\Delta\mu_i^0 \equiv \mu_i'^0 - \mu_i^0 \tag{5}$$

$$\Delta\psi \equiv \psi' - \psi \tag{6}$$

We now introduce the already-mentioned approximation that the activity coefficients of the ions are equal in the two phases, *i.e.*,

$$a_i'/a_i = c_i'/c_i \tag{7}$$

where c_i and c_i' are the concentrations given by

$$c_i = N_i/V_m \tag{8}$$

$$c_i' = N_i'/V_m \tag{9}$$

Eliminating $\Delta\psi$ from eqns. 3 and 4 and using eqns. 7–9, we obtain

$$k_1^{1/z_1}k_2^{1/z_2} = (k_1^0)^{1/z_1}(k_2^0)^{1/z_2} \tag{10}$$

with

$$k_i \equiv N_i'/N_i \tag{11}$$

$$k_i^0 \equiv (V_g/V_m) \exp (-\Delta\mu_i^0/RT) \tag{12}$$

We shall call k_i as given by eqn. 11 the "distribution coefficient". It is more common to define the distribution coefficient by c_i'/c_i, but we prefer our definition for the sake of simpler descriptions and easier correlation with experiments (see below). If we write $c_i'/c_i \equiv K_i$, we have the relationship

$$K_i = (V_m/V_g)\, k_i \tag{13}$$

The conditions of electrical neutrality are

$$z_1 N_1 = z_2 N_2 \tag{14}$$

$$z_1 N_1' = z_2 N_2' + N_g' \tag{15}$$

Eqns. 10–12, 14 and 15 lead to

$$(k_2 + g_2)^{1/z_1} k_2^{1/z_2} = (k_{12}^0)^{(1/z_1 + 1/z_2)} \tag{16}$$

$$k_1 = k_2 + g_2 \tag{17}$$

with

$$g_2 \equiv N_g'/(z_2 N_2) \tag{18}$$

$$k_{12}^0 \equiv (k_1^0)^{z_2/(z_1+z_2)} (k_2^0)^{z_1/(z_1+z_2)} \tag{19}$$

Thus eqns. 16 and 17 give k_1 and k_2 as a function of k_1^0, k_2^0 and g_2.

When the number of gel-fixed charges is negligibly small in comparison with the number of free charges, or in the limit of $g_2 \rightarrow 0$, we have

$$k_1 = k_2 = k_{12}^0 \tag{20}$$

Eqns. 19 and 20 are the central relationship to be examined in this paper. Referring to eqn. 12, we see that k_i^0 is a constant related to the difference in the standard chemical potentials of the individual ions in the two phases, and we may properly call k_{12}^0 the "intrinsic" distribution coefficient of the ion (in a particular system). k_{12}^0 as defined by eqn. 19 is the distribution coefficient of a 1–2 salt, *i.e.*, a salt comprising ions 1 and 2, in the absence of the fixed-charge effect. Saunders and Pecsok[6] have empirically given the distribution coefficient of a 1–2 salt as a charge-weighted algebraic mean of the individual cation and anion contributions. Eqn. 19 indicates that the arithmatic mean may be preferable theoretically.

When the fixed-charge effect is important, or g_2 is non-zero, k_1 and k_2 become dependent on solute concentration. As we have assumed the fixed charges to be negative or $g_2 > 0$, it generally holds that $k_1 > k_{12}^0 > k_2$ (see eqns. 16 and 17). This has been discussed by Pecsok and Saunders[4] and more extensively by Kadokura *et al.*[5]. Their theoretical results regarding the fixed-charge effect are essentially the same as ours, but our equations describe the effect in a more general form.

EXPERIMENTAL

Chromatographic-grade chemicals and highly deionized water were used throughout. The gel column used was a glass tube, 300 mm × 15 mm I.D., packed with Sephadex G-10 gel (Pharmacia). It was placed concentrically in a larger glass tube with thermostated water circulated through the gap to maintain the system at 20.0 ± 0.5°C. An ERMA Model ERC-7520 differential refractometer was used as a detector. A flow-rate of 1 ml/min was maintained by use of a peristaltic pump.

The void volume of the column was estimated using the conventional GPC mode with a series of poly(ethylene glycol) (PEG) samples (Wako). The exclusion limit of the column was of the order of a few hundreds in PEG molecular weight, and thus we regarded the elution volume of the PEG with a nominal molecular weight of 20 000 as equal to the void volume, which was 14.6 ml. In the following discussion, we shall identify this volume with the mobile phase volume V_m. In the context of our model, the gel phase volume V_g may be given by the bed volume minus V_m, which is about 28 ml, but this value itself is of no direct importance in the following analysis. With conventional notation, the elution volume V_e can be represented by

$$V_e = V_m + KV_g \tag{21}$$

with K defined as previously. Eqns. 11, 13 and 21 give

$$k = (V_e - V_m)/V_m \tag{22}$$

i.e., k is formally independent of V_g. This is one of the reasons why we prefer k to K or otherwise defined distribution coefficients. Of course, the value of V_g will become important if one wishes to go into greater detail than we do here.

Chromatographic runs with salt samples were carried out by the equilibrium saturation mode of Sebille *et al.*[11], *i.e.*, we used the sample solution as the eluent and injected a small amount of pure water as a "probe". The amount of water injected ranged from 0.05 to 0.2 ml. The higher the sample concentration, the smaller was the amount of water sufficient for sample detection. Usually, passage of two to three column volumes of the sample solution was sufficient to bring the column system to equilibrium prior to each run.

RESULTS AND DISCUSSION

Concentration dependence and fixed charge effect

The equilibrium saturation mode has the advantage over the conventional mode that it allows us to determine the distribution coefficient as a function of concentration with little theoretical and experimental ambiguity[5,11]. First, once the column system has been equilibrated with the sample eluent, the composition of the mobile phase is always equal to that of the eluent. Second, owing to the usually small size of the pure solvent injected as a probe, the internal equilibrium is little perturbed.

Fig. 2 shows the chromatograms for sodium chloride solutions obtained using this mode. There is a set of negative peaks showing a lack of the salt. These peaks, which are fairly sharp and symmetrical, correspond to the elution volumes of the anion

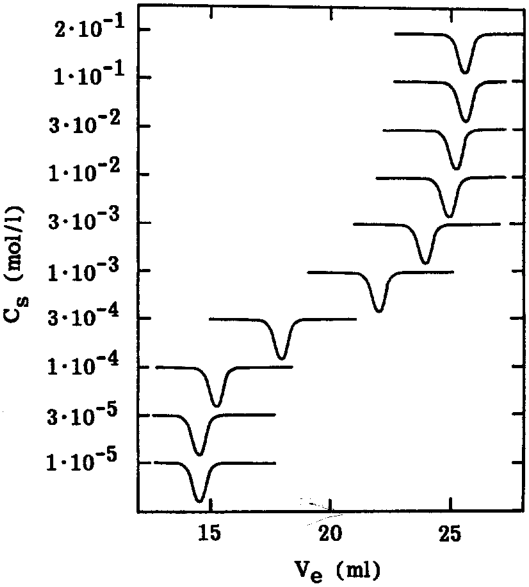

Fig. 2. Chromatograms for Cl^- at different NaCl concentrations, C_s.

Cl^- but not of the cation Na^+, as our gel has negative fixed charges or cation-exchanging ability[8]. An additional experiment is necessary to observe cation elution volumes[5]. Each baseline level corresponds to the salt concentration in the mobile phase, C_s, which in this example is equal to the Cl^- concentration in that phase, *i.e.*, $C_s = N_2/V_m$. When C_s is sufficiently smaller than the fixed-charge density, the sample salt can hardly enter the gel phase because of the relatively high population of the counter ions bound in the gel phase, and hence it is eluted with V_e close to V_m. On the other hand, when C_s is large enough, the fixed charges have no important effect, and the sample salt, freely distributed between the two phases, is eluted at the position V_e^0 characteristic of the salt. At intermediate concentrations, V_e takes intermediate values between V_m and V_e^0.

In Fig. 3, values of k_2 for sodium chloride and sodium sulphate solutions are plotted against C_s. The points are experimental values and the curves show eqns. 16 and 18 with $N_g'/V_m = 7.0 \cdot 10^{-4}$ mol/l and $k_{12}^0 = 0.75$ (NaCl) or 0.35 (Na_2SO_4). The good agreement between theory and experiment suggests the validity of the present treatment and also confirms the previous work[5]. Moreover, the new finding that the elution behaviour of the two salts with k_{12}^0 values very different from each other can be described in terms of the common number of fixed charges N_g' suggests that size exclusion is not the main mechanism of separation in these systems; if salts of different sizes penetrate the gel phase to different extents, the effective numbers of fixed charges which they "feel" should be different.

We carried out chromatographic runs for some salts at higher concentrations, *e.g.*, $C_s \approx 1$ mol/l, and observed that V_e is substantially the same as those in the 0.1 mol/l range. This implies that the present model, despite the crude approximation of equal activity coefficients, works unexpectedly well at least in a practically important range of concentrations.

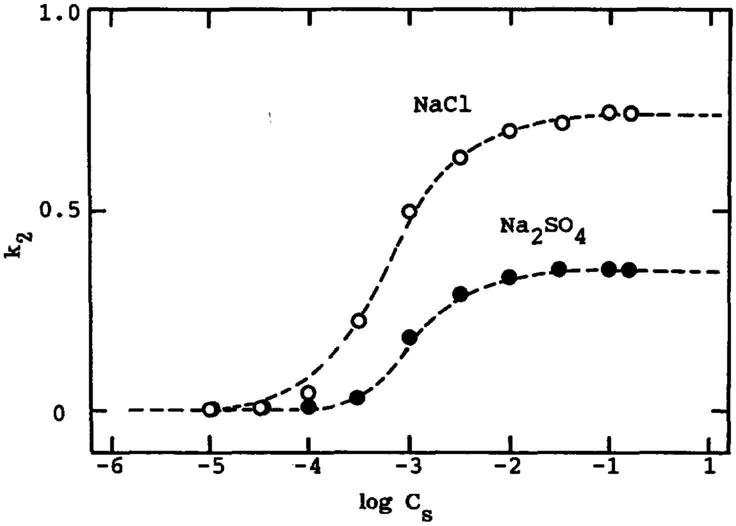

Fig. 3. Anion distribution coefficient k_2 as a function of salt concentration C_s (in mol/l).

Behaviour at high concentrations

We now turn to the main problem. As we have seen, the fixed-charge effect becomes unimportant at concentrations higher than about 0.1 mol/l (Fig. 3). Thus we regarded k_2 at $C_s = 0.2$ mol/l as equal to k_{12}^0. Values of k_{12}^0 thus determined for various sodium and chlorine compounds are listed in Table I. It can be seen that k_{12}^0 differs among sodium compounds and also among chlorides.

According to eqn. 19, k_{12}^0 is given by the arithmetic mean of the intrinsic distribution coefficients k_1^0 and k_2^0 of the individual ions. Hence, knowing the value of k_i^0 for a standard ion, *e.g.*, Cl^-, we can calculate the k_1^0 values of various cations from the k_{12}^0 values of the corresponding chlorides by use of eqn. 19, and $k_{Na^+}^0$ value so calculated and the experimentally determined k_{12}^0 values of the sodium salts may subsequently be used to evaluate the k_2^0 values of various anions. However, there is no

TABLE I

DISTRIBUTION COEFFICIENTS OF SIMPLE ELECTROLYTES IN A SEPHADEX G-10 COLUMN

Salt	k_{12}^0	*Salt*	k_{12}^0	*Salt*	k_{12}^0
NaF	0.563	$AlCl_3$	0.563	KCl	0.792
NaCl	0.750	$BaCl_2$	1.01	LiCl	0.764
NaBr	0.917	$CaCl_2$	0.840	$MgCl_2$	0.694
NaI	1.48	$CdCl_2$	0.910	NH_4Cl	0.771
Na_2SO_4	0.354	$CoCl_2$	0.771	$NiCl_2$	0.750
$NaNO_2$	0.883	$CuCl_2$	0.778	$PbCl_2$	1.59
$NaNO_3$	1.01	$CsCl_2$	0.771	RbCl	0.759
NaSCN	1.87	$FeCl_3$	0.632	$ZnCl_2$	0.944
NaOH	1.43	HCl	0.924		

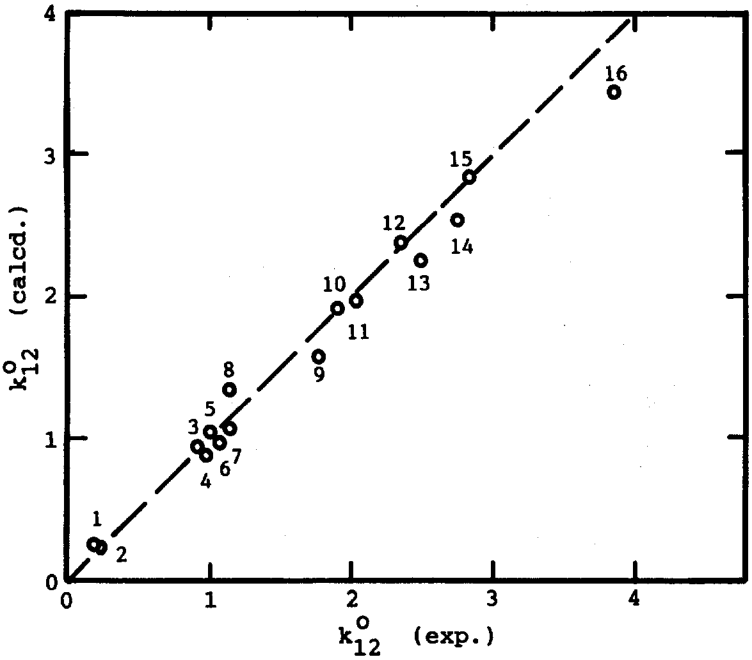

Fig. 4. Comparison of experimental and calculated distribution coefficients k_{12}^0 for (1) $CuSO_4$, (2) $MgSO_4$, (3) LiBr, (4) $Al(NO_3)_3$, (5) $LiNO_3$, (6) KBr, (7) KNO_3, (8) $Cd(NO_3)_2$, (9) KI, (10) NH_4SCN, (11) KSCN, (12) $Pb(NO_3)_2$, (13) CdI_2, (14) BaI_2, (15) $Ca(SCN)_2$ and (16) $Ba(SCN)_2$.

direct method for determining k_i^0, as an ion never behaves independently of its counter ion. We propose to assume k_{Cl^-} to be unity. It can be easily shown that results such as those given in Fig. 4 are independent of the choice of the standard. The relative orders of k_i^0 among anions and among cations, respectively, are also independent of this choice. Comparison of a cation k_1^0 and an anion k_2^0 is obviously meaningless, however.

TABLE II

INTRINSIC DISTRIBUTION COEFFICIENTS OF SIMPLE IONS (SEPHADEX G-10)

It is assumed that $k^0_{Cl^-} = 1$.

Anion	k_2^0	*Cation*	k_1^0	*Cation*	k_1^0
SO_4^{2-}	0.140	Al^{3+}	0.100	Ca^{2+}	0.593
F^-	0.563	Fe^{3+}	0.160	Cs^+	0.594
Cl^-	(1)	Mg^{2+}	0.334	NH_4^+	0.594
NO_2^-	1.38	Ni^{2+}	0.423	K^+	0.627
Br^-	1.49	Co^{2+}	0.445	Cd^{2+}	0.806
NO_3^-	1.81	Cu^{2+}	0.471	Zn^{2+}	0.841
OH^-	3.64	Na^+	0.563	H^+	0.854
I^-	3.89	Rb^+	0.576	Ba^{2+}	1.04
SCN^-	6.21	Li^+	0.584	Pb^{2+}	4.01

Table II lists the values of k_i^0 determined in this way. Of all the cations listed, k_1^0 is the smallest for Al^{3+} and the largest for Pb^{2+}, and of all the anions listed, k_2^0 is the smallest for SO_4^{2-} and the largest for SCN^-.

We can now use eqn. 19 and the k_i^0 values in Table II to predict the k_{12}^0 value of an arbitrary 1–2 salt. Fig. 4 shows a comparison of the predicted and experimental values of k_{12}^0 for various non-sodium, non-chloride salts. The agreement is generally satisfactory.

Comparison with other gel systems

We have seen that the present model describes well the experimental results for the Sephadex G-10 system. Fig. 5 compares the k_2^0 values of typical anions for Sephadex G-10 with those for two other gels, Sepharon 300 (Pharmacia) and Extex HG-7 (Japan Exlan, Okayama, Japan). Sepharon is based on a water-soluble polymer, poly(hydroxyethyl methacrylate). The chemical structure of Extex is unreported; it has a low affinity towards water and excellent mechanical strength. The Sepharon data were taken from Borăk[12] and the Extex data were obtained by us using the same procedure as for the Sephadex system. Despite the differences in the physico-chemical properties of the gels, linear relationships are observed in Fig. 5, which supports the general validity of the present model.

To conclude, the main mechanism of simple-ion separation by a polymer gel in water is suggested to be the difference in the thermodynamic stabilities of the individual ions in the mobile and gel phases. Both size-exclusion and adsorption concepts are basically inconsistent with the experimental results. The trend in the gel chromatographic separation of ions is in general agreement with that in their water–oil partition behaviour[13], which supplements our conclusion.

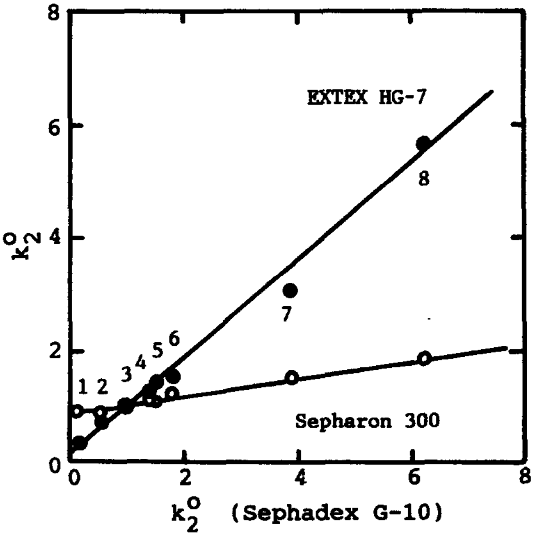

Fig. 5. Comparison of gels with respect to the anion distribution coefficients k_2^0 of (1) SO_4^{2-}, (2) F^-, (3) Cl^-, (4) NO_2^-, (5) Br^-, (6) NO_3^-, (7) I^- and (8) SCN^-; $k_{Cl^-}^0$ is assumed to be unity in all instances.

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