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GEL CHROMATOGRAPHIC BEHAVIOUR OF ELUENT IONS ON SEPHADEX G-10 AND EVIDENCE FOR ELECTRONEUTRALITY IN THE GEL PHASE

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SUMMARY

Methods for determining the distribution coefficient, K_d , of sodium, hydrogen, chloride and hydroxide ions in the eluent were examined by using refractometric and potentiometric detection and a radiotracer method on Sephadex G-10 with a 0.1 M sodium chloride eluent at several pH values. The K_d values of sodium and chloride ions obtained by using their radiotracers were considerably different from those by both refractometry and potentiometry. It was demonstrated by the use of the K_d values of sodium and chloride ions (radiotracer method) and that of hydrogen ion (refractometry) that electroneutrality for the eluent ions holds in the gel phase over the range pH 1.5–3.

INTRODUCTION

The mechanism of the separation of inorganic ions on tightly cross-linked gels is more complicated than that of organic compounds and has not yet been clarified, although several approaches have been devoted to the interpretation of the behaviour of small inorganic ions in aqueous gel systems¹. Among them, it has been reported that the volumetric distribution coefficients of sample ions may be correlated chiefly with the properties of the counter ions contained in the eluent^{2–6}, which is usually regarded as acting as a masking agent for the electrostatic interaction between the sample ions and the charge arising from the ionic dissociation of groups fixed to the gel matrix⁷. Hence, it is very important for studies on the mechanism of the separation of inorganic compounds to obtain detailed information about the behaviour of the eluent ions.

We have previously reported that the K_d values of alkali metal and halide ions⁸ and tetraalkylammonium ions⁹ vary significantly depending on the pH of the eluent on Sephadex G-10, although these ions do not transform into any other form or species over the range pH 1.5–12 investigated. Moreover, the K_d values of sodium and chloride ions used as an eluting agent are considerably different from each other over this wide pH range, as shown in Fig. 1^{8,10}. The pH dependence of the K_d values of

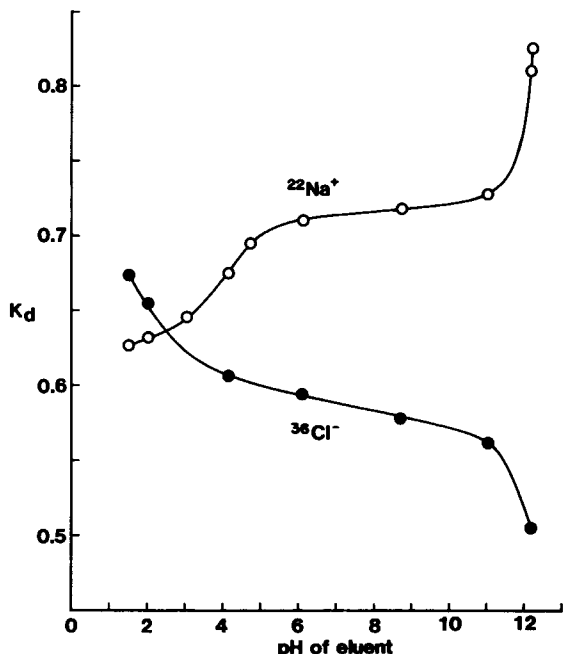


Fig. 1. pH dependence of K_d values of eluent ions obtained by the radiotracer method^{8,10}. Gel: Sephadex G-10. Eluent: 0.1 M NaCl at various pH values adjusted with HCl or NaOH. Temperature: 20°C.

these ions was interpreted speculatively in terms of the variation in the charge of the gel phase owing to the dissociation of carboxyl and hydroxyl groups fixed to the gel matrix.

Volumetric parameters, such as K_{av} and K_d , of eluting agents have usually been obtained by monitoring the concentration distribution of eluent salts and ions by means of an appropriate chemical analysis^{11,12}, an atomic absorption flow detector¹³, an ion-selective electrode detector¹⁴ or a differential refractometer⁵. In general, it is believed that the K_{av} or K_d values of eluent cations are identical with those of eluent anions.

Therefore, this work was undertaken in order to establish a method for determining the true K_d values of the eluent ions on Sephadex G-10 with a 0.1 M sodium chloride eluent at several pH values and for evaluating the charge balance of the eluent ions in the gel phase. It was found that a radiotracer method, rather than refractometry and potentiometry, gives valid K_d values for the eluent ions, and that electroneutrality holds in the gel phase over the range pH 1.5–3, provided that the contribution of hydrogen ion added to adjust the pH of the eluent is taken into account.

EXPERIMENTAL

Sample solutions

All reagents used were of guaranteed reagent grade, unless otherwise stated. Sodium chloride solutions of concentrations 0.1, 0.2 and 0.3 M at various pH values

were used as sample solutions (see Figs. 2, 3 and 4). The sample solution contained ^{22}Na or ^{36}Cl (New England Nuclear, Boston, MA, U.S.A.) as a radiotracer to obtain the K_d values of eluent ions in each instance. Dextran T-2000 (Pharmacia, Uppsala, Sweden; 0.04%) and tritiated water (New England Nuclear) were employed as standard materials with $K_d = 0$ and 1, respectively.

Eluents

Eluents used were 0.1 M sodium chloride solutions at pH 1.51, 1.83, 2.37, 2.84, 12.05 and 12.50.

Column and elution procedure

Sephadex G-10 (Pharmacia; dry particle size, 40–120 μm) was packed into a column (Pharmacia, K16/100) with flow adaptors at both the top and bottom, as described previously¹⁵. The dimensions of the gel bed were 73 \times 1.6 cm. The eluent was passed through the column before use until the pH of the effluent was identical with that of the eluent.

A 1-cm³ volume of the sample solution was introduced on to the column with a line sample injector of the loop-valve type (Seishin Pharmaceutical, Tokyo, Japan; VMU-6). The elution was allowed to proceed at a constant flow-rate of 60 cm³/h under a hydraulic pressure and at a constant temperature of $20 \pm 0.5^\circ\text{C}$. The effluent from the column was monitored continuously with a differential refractometer (Showa Denko, Tokyo, Japan; Shodex RI, SE-11) and then collected in *ca.* 1-cm³ fractions with an Ultrac 7000 fraction collector (LKB, Bromma, Sweden). Some fractions were chosen arbitrarily and their volumes were measured so that the fraction volume could be determined accurately. The dead volume from the cell of the refractometer to the fraction collector (0.59 cm³) was taken into account for correction of the elution volumes determined by the potentiometric and radioactivity measurements.

The radioactivities of tritiated water, $^{22}\text{Na}^+$ and $^{36}\text{Cl}^-$ of the effluent collected in each fraction were measured as previously¹⁶. The concentrations of hydrogen, sodium and chloride ions of the effluent were determined by means of a digital potentiometer (Orion Research, MA, U.S.A.; 701A) equipped with a combined electrode of 91-02, 96-11 or 96-17 type.

The procedure for calculating K_d values was as reported previously¹⁶. Marsden^{17,18} reported that the K_d value of tritiated water is 1.091 on Sephadex G-10 owing to an exchange reaction of the hydrogen atoms between the tritiated water and the hydroxyl and/or carboxyl groups of the gel matrix. This isotopic exchange should be taken into account for the evaluation of positive and negative charges in the gel phase. However, the correction of the K_d values was neglected in this study, because the pH dependence of the K_d value of tritiated water was not examined. Even if the factor of 1.091 was introduced, the charge balance in the gel phase would hold over the range pH 1.5–3 within the relative error of 2%.

RESULTS AND DISCUSSION

Identification of elution peaks

Figs. 2a and d show typical chromatograms obtained by means of a differential

refractometer with a 0.1 M sodium chloride eluent at pH 1.51 on a Sephadex G-10 column. Peaks 1 and 2 appeared in a positive direction when the concentrations of sodium chloride and hydrogen chloride in the sample solution were larger than those of the eluent, respectively, and *vice versa*. The K_d value of peak 1 and 2 (the values shown in parentheses), however, was almost invariable in each instance. These facts

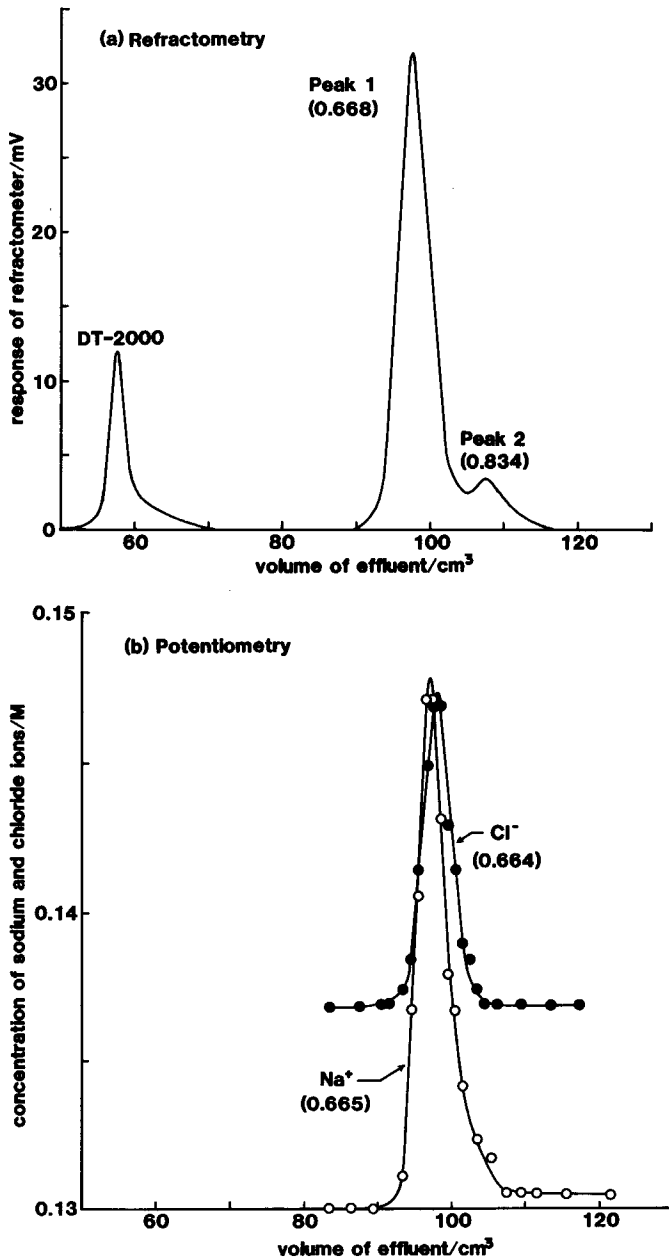


Fig. 2.

suggest that peak 1 corresponds to the variation in the concentration of sodium chloride owing to the introduction of the sample solution, and that peak 2 corresponds to the variation in the concentration of hydrogen chloride.

This was confirmed by the potentiometric detection method using sodium, chloride and hydrogen ion-selective electrodes. Namely, the K_d values of sodium and

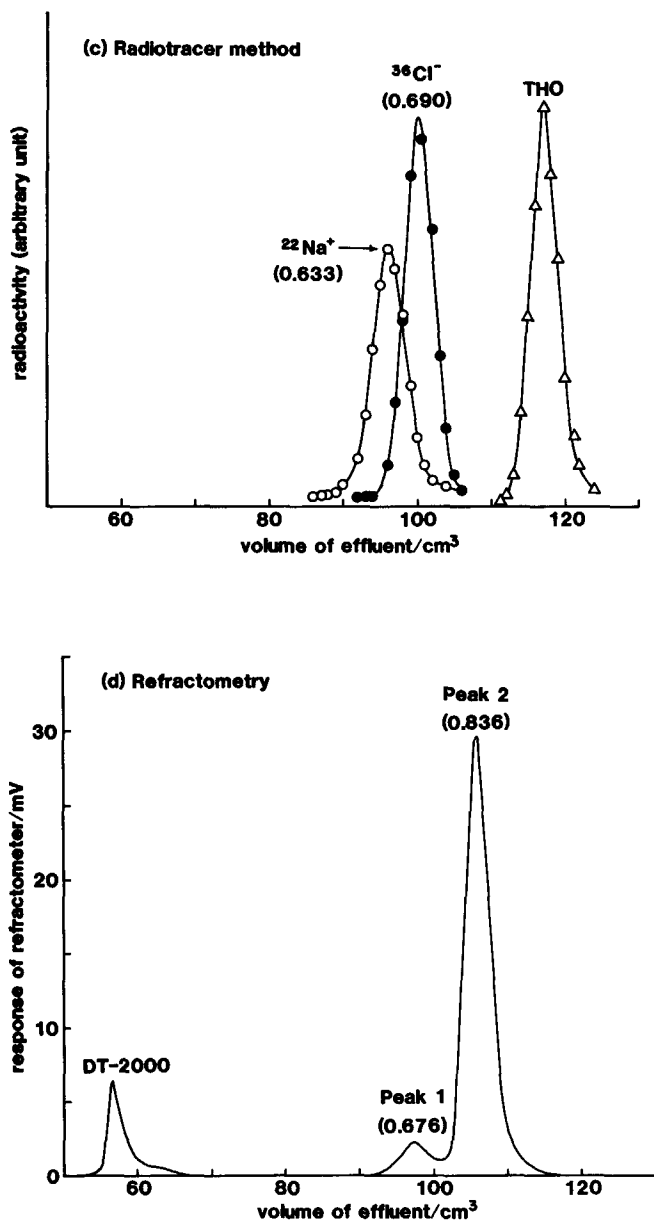


Fig. 2.

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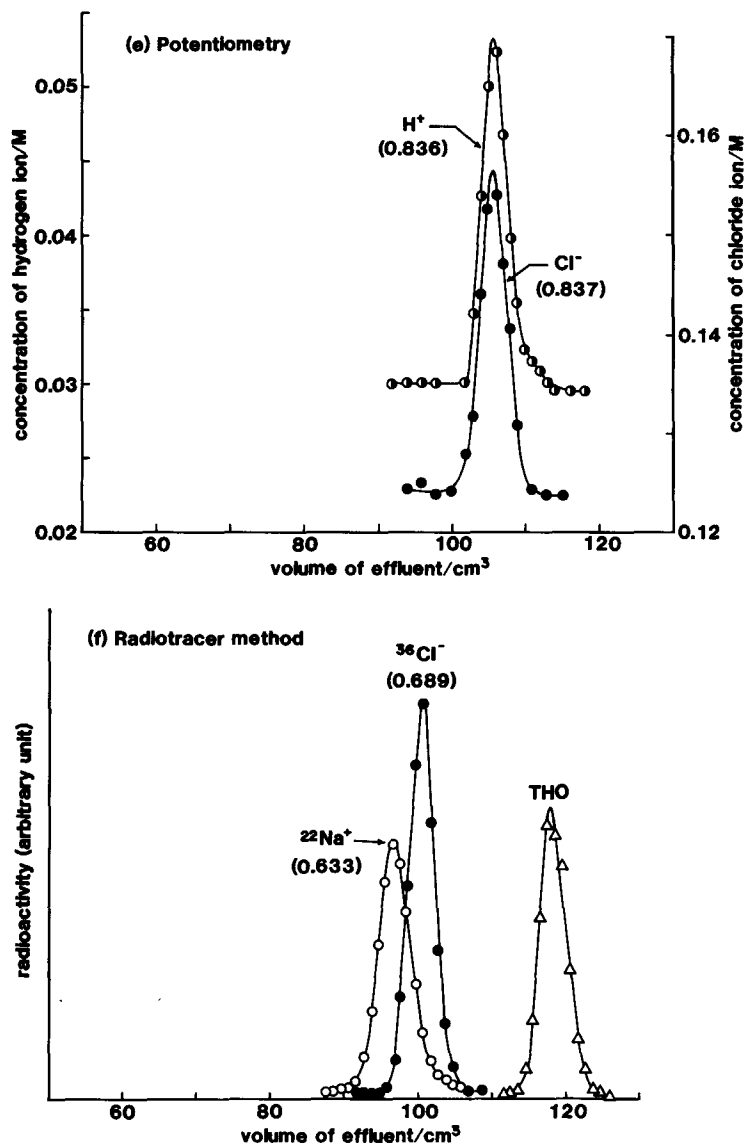


Fig. 2. Chromatograms obtained by refractometry, potentiometry and radiotracer methods with an acidic eluent. Gel: Sephadex G-10, 73 × 1.6 cm. Eluent: 0.1 M NaCl at pH 1.51. Sample: (a), (b), (c), 0.2 M NaCl at pH 1.51; (d), (e), (f), 0.1 M NaCl at pH 0.90. Temperature: 20°C. Attenuation of refractometer: 8. THO = Tritiated water.

chloride ions in Fig. 2b are identical with each other and with those of peak 1 in Fig. 2a and 2d within experimental error. The K_d values of hydrogen and chloride ions in Fig. 2e are also identical with those of peak 2 in Fig. 2a and 2d. The higher baseline for chloride ion than for sodium ion in Fig. 2b is attributable to the higher concentration of chloride ion in the eluent owing to the pH adjustment with hydrogen chloride. The small peak 1 or 2 in Fig. 2d or 2a always appeared in spite of careful adjustment

of the sodium chloride concentration and of the pH in the sample solution. Yóza *et al.*¹⁹ and Deguchi and co-workers^{11,12,14} have already reported similar phenomena, *i.e.*, when the sample solution prepared by dissolving electrolytes in the eluent was chromatographed on Sephadex G-15, the peak of the eluting agent appeared at the elution volume corresponding to its K_d value because of the exclusion of the eluent ion from the sample zones.

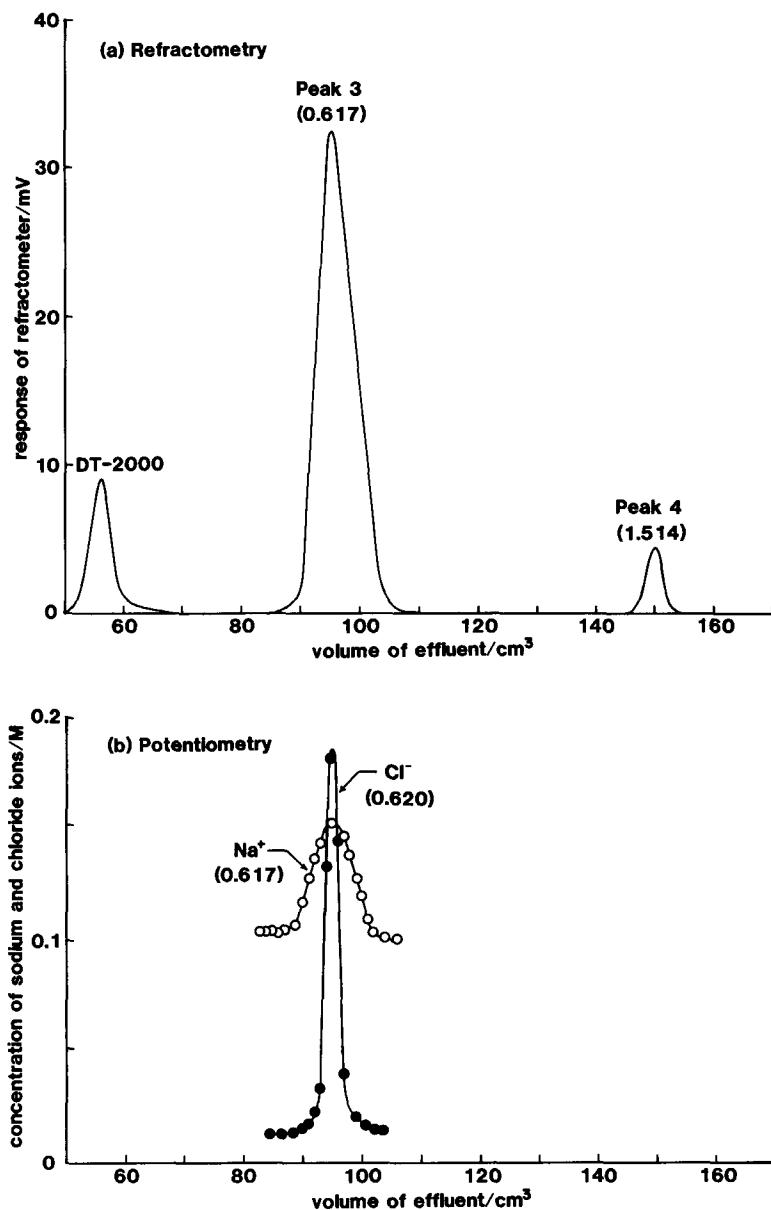


Fig. 3.

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In contrast to the cases with refractometric and potentiometric detection, the K_d values of $^{22}\text{Na}^+$ and $^{36}\text{Cl}^-$ in the sample solution were obviously different from each other beyond experimental error, as mentioned in the Introduction.

Fig. 3 shows chromatograms with a 0.1 M sodium chloride eluent at pH 12.50. Peaks 3 and 4 were identified as elution peaks of sodium chloride and sodium hydroxide, respectively, by using ion-selective electrodes, as in the case with the acidic eluent.

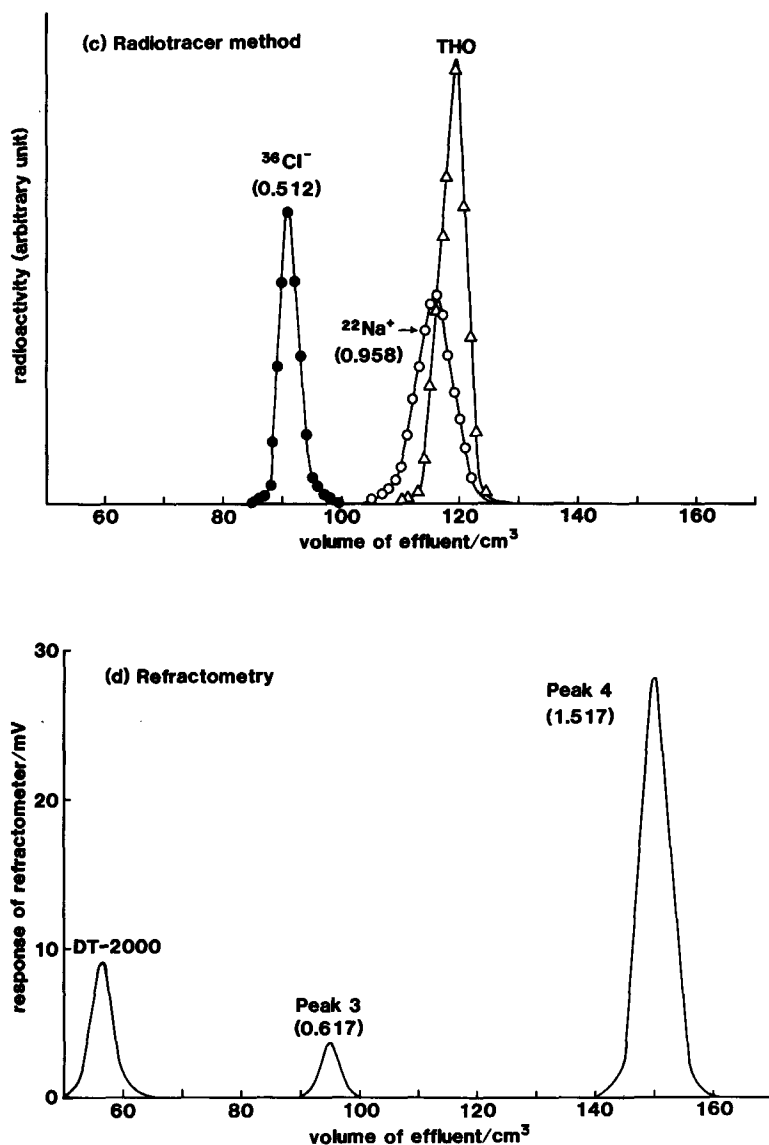


Fig. 3.

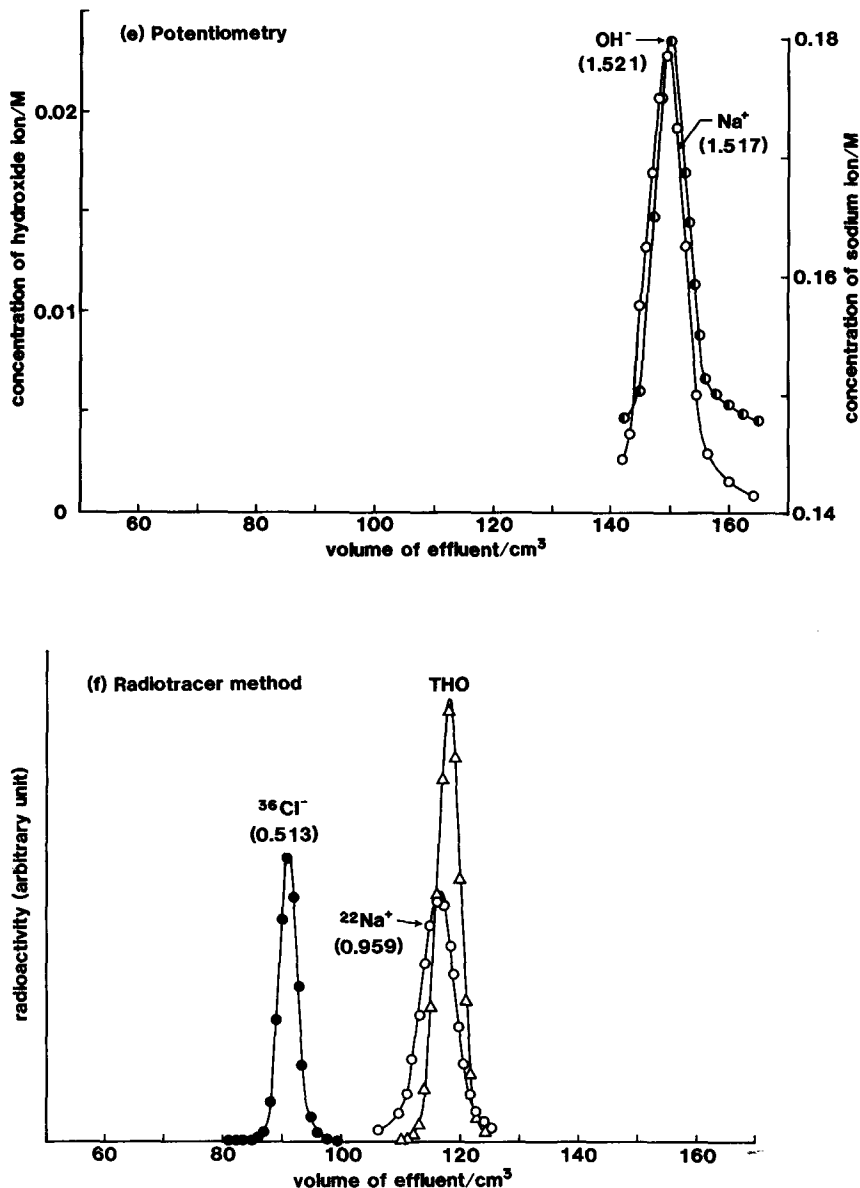


Fig. 3. Chromatograms obtained by refractometry, potentiometry and radiotracer methods with a basic eluent. Gel: Sephadex G-10, 73×1.6 cm. Eluent: $0.1 M$ NaCl at pH 12.50. Sample: (a), (b), (c), $0.3 M$ NaCl at pH 12.50; (d), (e), (f), $0.1 M$ NaCl at pH 13.35. Temperature: 20°C . Attenuation of refractometer: 8. THO = Tritiated water.

With the basic eluent, the K_d value of $^{22}\text{Na}^+$ increased and that of $^{36}\text{Cl}^-$ decreased so significantly that the difference between them became nine times larger than that with the eluent at pH 1.51. This will be discussed in terms of electroneutrality in the gel phase in the following section.

Electroneutrality in gel phase

Concerning only the behaviour of $^{22}\text{Na}^+$ and $^{36}\text{Cl}^-$, the positive charge does not seem to balance with the negative charge in the gel phase of Sephadex G-10 as shown in Fig. 1, except at pH 2.3, where the K_d values of these ions are identical. Hydrogen and hydroxide ions added to adjust the pH of the eluent must contribute to the charge balance in both the mobile and gel phases, too.

Since the K_d value is generally accepted as a partition coefficient of the solute between the mobile and gel phases in gel chromatography, it is expressed as

$$K_d = C_g/C_m \quad (1)$$

where C_g and C_m denote the concentrations of the solute in the gel and mobile phases, respectively. When the solute is the same as the eluting agent, C_g is obtained according to eqn. 2

$$C_g = K_d \times C \quad (2)$$

because C_m can be regarded as approximately constant and equal to the concentration of the eluent, C , in dynamic experiments using a gel column.

With a sodium chloride eluent at any pH adjusted with hydrogen chloride or sodium hydroxide, the positive and negative charges in the gel phase can be written as follows

$$\text{Positive charge} = K_{d(\text{Na}^+)} \times C_{\text{Na}^+} + K_{d(\text{H}^+)} \times C_{\text{H}^+} \quad (3)$$

$$\text{Negative charge} = K_{d(\text{Cl}^-)} \times C_{\text{Cl}^-} + K_{d(\text{OH}^-)} \times C_{\text{OH}^-} \quad (4)$$

provided that the gel matrix possesses no electric charge. $K_{d(\text{Na}^+)}$ and $K_{d(\text{Cl}^-)}$ are the distribution coefficients of sodium and chloride ions determined by using their radio-tracers and C_{Na^+} , C_{Cl^-} and C_{OH^-} the concentrations of respective ions contained in the eluent. The radiotracers for hydrogen and hydroxide ions are not available. Therefore, their K_d values, $K_{d(\text{H}^+)}$ and $K_{d(\text{OH}^-)}$, were assumed to be equal to the K_d values of hydrogen chloride and sodium hydroxide, respectively, obtained by refractometric detection, when both the composition and pH of the sample solution are identical with those of the eluent. The elution peaks of hydrogen chloride and sodium hydroxide, however, are undetectable in such a case. Accordingly, the dependence of the K_d values of hydrogen chloride and sodium hydroxide on their concentrations, *i.e.*, on the pH of the sample solution, was examined, as shown in Fig. 4, where their K_d values are represented as $K_{d(\text{H}^+)}$ and $K_{d(\text{OH}^-)}$. The $K_{d(\text{H}^+)}$ and $K_{d(\text{OH}^-)}$ values at the pH values of the eluent were obtained from the plots of Fig. 4. The above assumption proved to be valid since the positive and negative charges in the gel phase balance over the range pH 1.5–3, where the gel matrix seems to possess no electric charge.

Table I gives the results of elucidating the electroneutrality in the gel phase by using the K_d values and the concentrations of the eluent ions. The $K_{d(\text{Na}^+)}$ and $K_{d(\text{Cl}^-)}$ values in this Table are somewhat different from those shown in Fig. 1 at corresponding pH values, because gels of different lot numbers were used in each series of experiments.

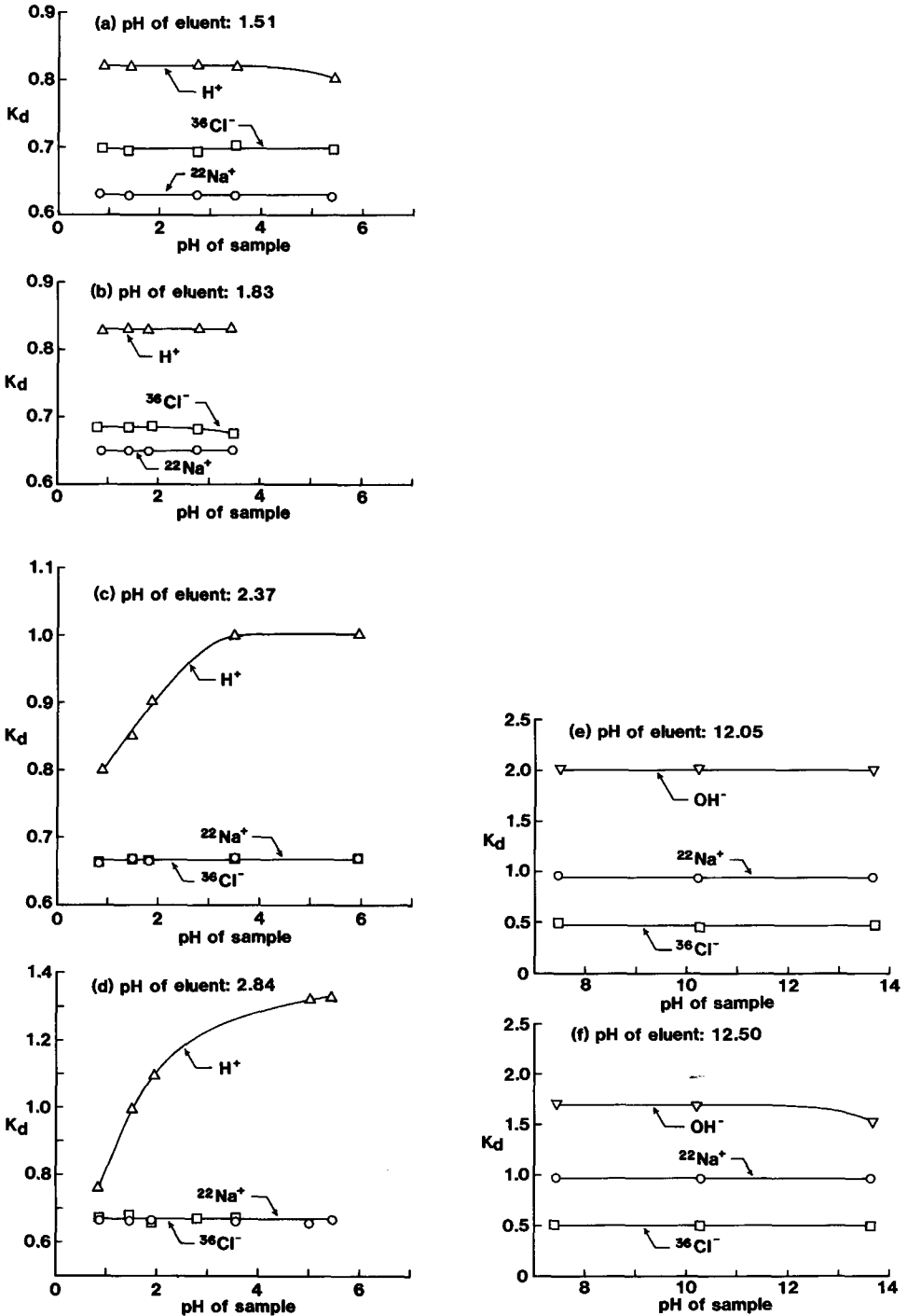


Fig. 4. Dependence of K_d values of eluent ions on pH of sample solutions. Gel: Sephadex G-10, 73×1.6 cm. Eluent: 0.1 M NaCl at pH 1.51, 1.83, 2.37, 2.84, 12.05 and 12.50. Sample: 0.1 M NaCl at various pH values shown in this figure. Temperature: 20°C.

TABLE I

EVALUATION OF ELECTRONEUTRALITY IN GEL PHASE

Gel: Sephadex G-10. Eluent: 0.1 M NaCl at various pH values. Temperature: 20°C. The K_d values of NaCl and of H^+ and OH^- were obtained by refractometry, those of Na^+ and Cl^- by the radiotracer method. C = Coulomb; M = molar concentration.

Eluent pH	K_d					Positive charge (10^{-20} CM)	Negative charge (10^{-20} CM)
	NaCl	Na^+	Cl^-	H^+	OH^-		
1.51	0.664	0.636	0.686	0.833	—	1.45	1.44
1.83	0.673	0.651	0.683	0.838	—	1.25	1.26
2.37	0.674	0.660	0.665	0.952	—	1.12	1.11
2.84	0.674	0.660	0.660	1.230	—	1.08	1.07
12.05	0.656	0.872	0.543	—	2.067	1.55	1.24
12.50	0.615	0.964	0.512	—	1.578	2.30	1.62

With eluents in the low pH region, the values of the positive and the negative charges agree well with each other within experimental error. Moreover, as shown in Fig. 5, the charge of the gel phase increases linearly with the ionic strength of the eluent in the gel phase, I , which was calculated according to:

$$I = \frac{1}{2} [K_{d(Na^+)} \times C_{Na^+} + K_{d(H^+)} \times C_{H^+} + K_{d(Cl^-)} \times C_{Cl^-} + K_{d(OH^-)} \times C_{OH^-}] \quad (5)$$

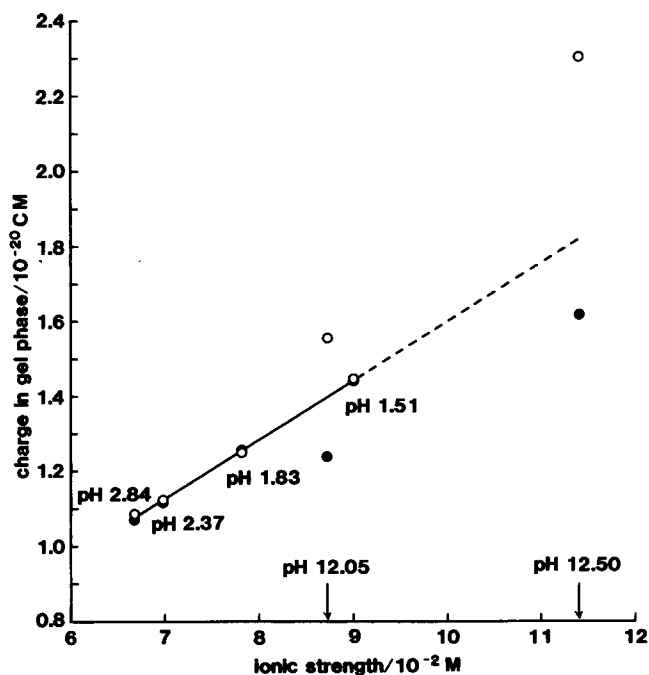


Fig. 5. Relationship between charge of eluent ions and ionic strength in gel phase. Data taken from Table I. O, Positive charge; ●, negative charge.

These facts strongly support the approach used in this study for elucidating the electroneutrality in the gel phase in gel chromatography.

In contrast, the positive charges at pH 12.05 and 12.50 were markedly larger than the negative charges. These points for the positive charge deviate above and those for the negative charge below the linear plot of the charge *vs.* *I* in Fig. 5. This suggests that the negative charge of the gel matrix should be taken into account in evaluating the charge balance in the high pH region, as discussed later.

The pH dependence of the K_d values of $^{22}\text{Na}^+$ and $^{36}\text{Cl}^-$ can be explained in terms of the variation in the charge of the gel phase. In the acidic region the K_d value of hydrogen ion is considerably larger than those of sodium and chloride ions, as shown in Table I. Therefore, Na^+ tends to be repelled and Cl^- to be attracted by the gel phase. The carboxyl groups of the gel matrix may dissociate above this pH region. The negative charge increases markedly above pH *ca.* 11 possibly due to the adsorption of hydroxide ions²⁰ and/or to proton dissociation from hydroxyl groups of the gel matrix¹⁶. In fact, preliminary experiments revealed that the pH profile of the cation exchange capacity of a Sephadex G-10 column resembles closely that of the K_d value of $^{22}\text{Na}^+$ in Fig. 1 (ref. 21). Hence, the variation of charge of the gel phase with pH may cause the pH dependence of the K_d value of sodium and chloride ions.

The K_d values of both $^{22}\text{Na}^+$ and $^{36}\text{Cl}^-$ differ appreciably from those of sodium chloride obtained by refractometric detection at corresponding pH values, as shown in Table I. We may speculate that after the sample is introduced on to a gel column, sodium chloride including $^{22}\text{Na}^+$ and $^{36}\text{Cl}^-$ equilibrates instantaneously on a plate between the mobile and gel phases according to plate theory. When the gel phase carries an electric charge, the Donnan equilibrium also should hold between two phases. For example, on a gel with negative charge, the concentration of sodium ions in the gel phase may be larger than that of chloride ions. Such an equilibrium is successively attained in each plate due to the movement of the mobile phase. Then the elution peak obtained by refractometric detection arises according to the concentration distribution of sodium chloride, regardless of the sample or the eluent, since the cold sodium and chloride ions in the sample solution cannot be differentiated from those in the eluent. On the other hand, the elution peaks of sodium and chloride ions of the sample, represented by $^{22}\text{Na}^+$ and $^{36}\text{Cl}^-$, appear at different positions from that of cold sodium chloride because of the Donnan effect mentioned above.

In conclusion, the K_d value of an eluent ion should be determined by the use of its tracer, at least on Sephadex G-10 which is tightly cross-linked and has dissociable groups fixed to the gel matrix.

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