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On the Mechanism of Gel Chromatography of Inorganic Salts*

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Summary

Sorption isotherms, spectroscopic studies, and chromatographic retention data were used to elucidate the mechanisms involved in the separation of strong electrolytes on polyacrylamide gels. For the most part Bio-Gel P-2 was investigated but some results on the dextran gel Sephadex G-10 are also discussed. On P-2 the primary mechanism is not sieving or exclusion on the basis of size but rather a weak physical sorption of the cations to the gel and probably a hydrogen-bond interaction between the anions and the amide hydrogens. Molecules and ions which are not sorbed on the gel are partly excluded from a fraction of the internal solvent volume which is involved in hydration of the gel matrix and is firmly bound to it. When chromatographing solutions at low sample concentrations on some polyacrylamide gels, K_D falls off drastically. This effect is more pronounced in Sephadex than in Bio-Gel and it has been interpreted as resulting from a Donnan exclusion of anions which arises from a small number of anionic groups attached to the gel matrix. Equations relating elution to the various mechanisms are developed.

In recent years both hydrophobic and hydrophilic gels have been used as stationary phases for the separation of a wide variety of mixtures. Molecules of various sizes are fractionated by a molecular sieving process which depends upon the size of pores within the swollen gel matrix. Current knowledge on the theory and applications of gel chromatography has been the subject of several recent reviews (*1–3*).

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Gel chromatography is not without side effects. To separate molecules on the basis of size or to determine molecular weights with this technique, side effects such as sorption and ion exclusion are to be avoided. (The term sorption is used here in lieu of the term adsorption because the latter implies a defined surface or phase interface on which the interaction can take place. It is unclear whether or not a surface as such exists in these gels.) In some cases specific interactions have been put to good use. The occurrence of nonsteric interaction between certain small molecules and dextran gels was first demonstrated by Gelotte (*4*) and more recently by Janson (*5*) and Eaker and Porath (*6*). Polyacrylamide gels are also capable of sorption of some organic molecules (*7*).

Ion exclusion is manifested in the tendency of certain solutes to be eluted in volumes much smaller than would be expected by their size. These solutes are apparently excluded from the gel by an ionic mechanism. Ion exclusion of various solutes from Sephadex has been attributed to a small number of fixed carboxyl groups which are present in the gel matrix (*4,6*). The effect is most apparent when distilled water or low ionic strength buffers are used as eluents because in these media the electrostatic forces operate over long distances and can influence charged species of any size. Neddermeyer and Rogers suggested that ion exclusion is of major importance in the separation of inorganic salts on Sephadex (*8*). They found that even very small ions are completely excluded and skewed bands occur when distilled water is the eluent. These effects largely disappear when an eluent of ionic strength of 0.01 or more is used.

The separation of strong electrolytes on the polyacrylamide Bio-Gel P-2 was reported by Saunders and Pecsok (*9*). Recently, Henry and Rogers (*10*) and others (*8,11*) have reported separations of inorganic substances on Sephadex G-10 and G-25. The latter have found that in addition to the ion-exclusion effect described above, ion size is also a major factor influencing the separations. The present authors suggested that the mechanism involved in the separation of strong electrolytes on P-2 is sorption, but little discussion of this was presented at that time (*9*). The purpose of this investigation was to elucidate the extent to which steric exclusion, ion exclusion, sorption, and diffusion influence the chromatographic behavior of inorganic salts on Bio-Gel P-2.

From an interpretation of sorption isotherms and spectroscopic

results, we conclude that diffusion and steric exclusion mechanisms are absent and that sorption is primarily involved. Ultraviolet and nuclear magnetic resonance results indicate that a weak physical sorption of the cations and a direct ion-dipole hydrogen bond to the anions are probably involved in the retention of salts on Bio-Gel P-2. Ion exclusion may be important in some lots of this gel, but it has always been smaller than in Sephadex. A certain portion (~20%) of the internal solvent was found to be firmly bound water hydrating the gel matrix and is unavailable to nonsorbed molecules and ions. Equations are developed which relate the retention of electrolytes to various mechanisms.

EXPERIMENTAL

Chemicals

All chemicals used were either reagent grade or the purest grade available. Bio-Gel P-2 (Bio Rad Laboratories, Richmond, Calif.), 100–200 mesh lot numbers 3403 and 45683, and Sephadex (Pharmacia, Piscataway, N.J.) lot numbers 9493 (G-10) and 9917 (G-25), were used after rinsing with several volumes of deionized water. An uncross-linked polyacrylamide solution was prepared by dissolving 35 g of acrylamide in 1.0 liters of air-free water and adding first 1.00 ml of 3-dimethylaminopropionitrile (Eastman) and then 1.0 g of ammonium persulfate. The mixture was kept free from air at 24°C for 18 hr and then reduced to a thick syrup on a rotary evaporator over steam. Evaporation of droplets of this syrup on microscope slides, placed a few centimeters above a hot plate, facilitated an estimation of the polymer concentration (4.8% w/w).

Apparatus

The 25-cm column and other chromatographic apparatus have been previously described (9). Two modifications of this system were made. First, the detector, column, and eluent tube immediately preceding the column were jacketed for operation of the system at elevated temperatures. Second, the eluent was degassed before entering the system by a continuous reflux apparatus. The reflux apparatus employed a Beckman metering pump operated at a slightly higher in-flow than required by the pump supplying the column. Degassing was necessary to prevent bubbles from forming

in the packing when the column was operated above 35°C. Injection volumes were always 0.10 ml unless otherwise noted.

Sorption isotherms were measured using a Radiometer CD2M conductivity meter with a Raytheon voltage stabilizer. The scale could be read to ±0.2 mm using the parallax mirror, and the system was stable for several hours within the readability. The conductivity cell was a Radiometer CDC 114 pipet-type cell, which was modified by covering the opening with a small piece of 400-mesh nylon net held in place with a small neoprene O-ring. When this assembly was placed in a gel slurry, only the solution around the gel was drawn into the cell. Thus the concentration of the solution in equilibrium with the gel could be measured without interference from the gel itself.

A Varian A60 proton NMR spectrometer, a Varian 4200-A Wide-line NMR spectrometer, and a Cary 11 spectrophotometer were used in this study.

Procedures

Sorption isotherms were measured by pipeting 10.00 ml of deionized water into a 30-ml beaker with 3.00 g of dry gel which had been washed with copious quantities of deionized water and dried as previously described (9). After allowing the gel to swell for 30–60 min, the background conductivity was recorded and the gel slurry was titrated with small increments of various salt solutions. After the addition of each increment, readings were taken repeatedly until they remained constant for at least 2 min. Even with rapid stirring it usually took 5 to 7 min to reach equilibrium. After the titration was complete in some runs, the slurry was allowed to remain with stirring for about 1 hr to ensure that equilibrium had been reached. In no case did the final values vary measurably. To calibrate conductivity versus concentration, a duplicate experiment was run without gel. The amount of solute sorbed per gram of dry gel, Q, was calculated from

$$Q = \frac{C_T V - C_0(10 + V)}{a} \tag{1}$$

where C_T is the titrant concentration, V is the volume of titrant added, C_0 is the equilibrium concentration measured, and a is the dry weight of the gel.

The ion-exchange capacity of both Sephadex and Bio-Gel is

quite low. However, Sephadex was found to have carboxyl groups sufficient enough to be measured by back titration. The titration technique was not successful with Bio-Gel because of the extremely low capacity. In all cases the gels were equilibrated with large quantities of dilute (0.01 *N*) HCl, washed free of all excess acid, and dried under vacuum at 80°C for 6 hr. One-gram samples of Sephadex were equilibrated with 25.00 ml of 0.002 *M* NaOH overnight, after which 5.00-ml aliquots were titrated to a phenolphthalein end point with 0.002 *M* HCl. Blanks were run and care was taken to avoid errors from CO_2. The difference in titer between blank and sample was used to calculate the amount of carboxyl groups. With Bio-Gel a 10-ml portion of 1 *M* NaCl was adjusted to pH 7 with a Leeds and Northrup 7674 pH meter. Then about 3 g of P-2 was allowed to equilibrate with the salt solution, and the ion-exchange capacity was computed from the decrease in pH value resulting from the release of acid. Final pH values were usually about 6.0 so that the ionizable groups (assumed to be carboxyl) were probably entirely dissociated. CO_2 was removed by bubbling N_2 through the solution before making the measurements.

Several alcohols, glycols, and ketones were chromatographed. Because these solutes are not detected using the conductivity monitor, 0.2-ml fractions of the emerging solution were collected on a spot plate and tested with one of two solutions. For alcohols and glycols a 35% solution of ceric ammonium nitrate in 2*N* HNO_3 was used. Ketones were detected using 2 drops of a solution prepared by dissolving 2 g of 2,4-dinitrophenylhydrazine in 1000 ml of ethanol containing 10 ml of concentrated HCl. In this way elution volumes could be determined to ±0.3 ml.

RESULTS AND DISCUSSION

Steric Exclusion

Information on the properties and molecular weight ranges of the various Bio-Gels P-2 to P-300 is limited. P-2 is the most highly cross-linked gel of the series and thus has the smallest pore size, which in turn gives it the lowest molecular weight range. The manufacturer gives the effective molecular weight range of 200 to 2000, but the details of how this range was determined are uncertain. It would be useful to determine if the sizes of hydrated ions are comparable to the molecules used to determine the molecular weight range

(probably sugars or glycols). That is, it would be useful to determine whether or not "molecular" sieving should be expected with inorganic salts. It is difficult to compare the radius of hydrated ion to the molecular weight of an organic molecule; however, Smith and Kollmansberger (*12*) have shown that molecular *volume* is a more fundamental parameter to use for calibration of gels which separate small molecules. Triethylene glycol (MW 150) has a molecular volume of 220 Å^3, while glucose (MW 180) is approximately 200 Å^3 at 20°C. The volume of hydrated ions can be computed from the values for the effective radii of the hydrated ions given by Kielland (*13*). All the ions we have studied have effective volumes less than 120 Å^3, except magnesium (260 Å^3) and aluminum (380 Å^3). Thus, for the most part, the volume of the hydrated ions studied is at the lower limit of the gel range; therefore, little or no exclusion should be expected.

The distribution coefficient in gel chromatography, K_D, is given by

$$K_D = \frac{V_e - V_o}{V_i} \tag{2}$$

where V_e is the elution volume, V_o the outer volume between the gels beads, and V_i the inner volume of water imbibed within the gel. When only steric exclusion is operating, K_D is equal to the fraction of the inner volume which is available to a solute molecule and as such is limited to values from zero to 1 only. When sorption or other side effects occur, K_D becomes a complex distribution coefficient. A K_D value above unity is unequivocal evidence for sorption (*6*). All K_D values for the strong electrolyte salts previously reported (*9*) were greater than unity except the fluorides of the alkali metals. The latter had K_D values greater than 0.80 under all conditions studied in that paper. As will be discussed later, this partial exclusion of fluorides probably results from the exclusion from the water of hydration of the gel polymer strands, which is unavailable to all nonsorbed molecules and ions, rather than exclusion on the basis of the size of the ion.

One other fact suggests that the size of the ion is of little importance. It has been shown (*9*) that the K_D value of many salts can be predicted by the empirical equation

$$K_D = \frac{s\Phi_c + t\Phi_a}{s + t} \tag{3}$$

where s and t are the charges on the anion and cation, respectively, and Φ_c and Φ_a are selectivity parameters for the cation and anion, respectively. The Φ_c values for the different alkali metals vary by a factor of only 1.3 and tend to increase with hydrated radii. Thus, for a given anion, K_D tends to *increase*, not decrease, with size of cation. Furthermore, the Φ_a values for the halides vary by a factor of 3.5 but their hydrated radii are essentially constant. Thus, to a first approximation, K_D given by Eq. (3) is essentially a sorption coefficient. The remainder of this paper is devoted to the elucidation of some of the characteristics of the mechanism of this sorption.

Water of Hydration

A salt which is neither excluded nor sorbed should have an elution volume equal to $V_o + V_i$. Sodium and potassium fluorides are certainly not strongly sorbed and probably not sorbed at all. The K_D values of these salts on P-2, lot number 45683, are about 0.80, approximately equal to the values for acetone (0.81) and ethanol (0.77). Methanol and glycerol have slightly higher K_D values (0.89, 0.87) probably resulting from a slight sorption. Certainly acetone and ethanol are not excluded on the basis of size. Thus partial exclusion of small molecules probably results from a small amount of firmly bound water which hydrates the gel matrix. The concept of a firmly bound water of hydration is not at all new. Lathe and Ruthven (*14*) reported that wet starch contains 0.25 g of water of hydration per gram. Gelotte (*4*) found an upper limit of 0.8 for K_D of small nonsorbed molecules on Sephadex G-25, and Eaker and Porath (6) have calculated the "limiting" K_D value for Sephadex G-10, G-15, G-25, and G-50 as 0.75, 0.83, 0.90, and 0.95, respectively. To consider the consequences of this hydration, we define V_f as the free internal volume and V_b as the (hydration) bound internal volume; thus

$$V_i = V_f + V_b \tag{4}$$

Combining Eq. (4) with Eq. (2) and noting that for small molecules $V_e \rightarrow V_o + V_f$, we find that

$$\lim_{\mathrm{MW}\rightarrow 0} K_D = \frac{V_f}{V_i} \tag{5}$$

where MW is the solute molecular weight. Thus for Bio-Gel P-2

the alkali fluorides, acetone, and ethanol indicate that the limiting K_D value is about 0.80.

Sorption Isotherms

Sorption isotherms have been determined for several salts on Bio-Gel P-2. A calibration curve of the isotherm apparatus is illustrated by line B in Fig. 1. The ordinate is the measured concentration and the abscissa is the theoretical concentration after correction for dilution. When gel is present in the cell and sorption occurs, less than the theoretical concentration is measured because some of the salt is fixed to the gel, and a curve such as A in Fig. 1 results. In other cases, as will be described below, slopes greater than that of line B are observed, as indicated by line C. The amount of salt sorbed can be calculated from the vertical distance between

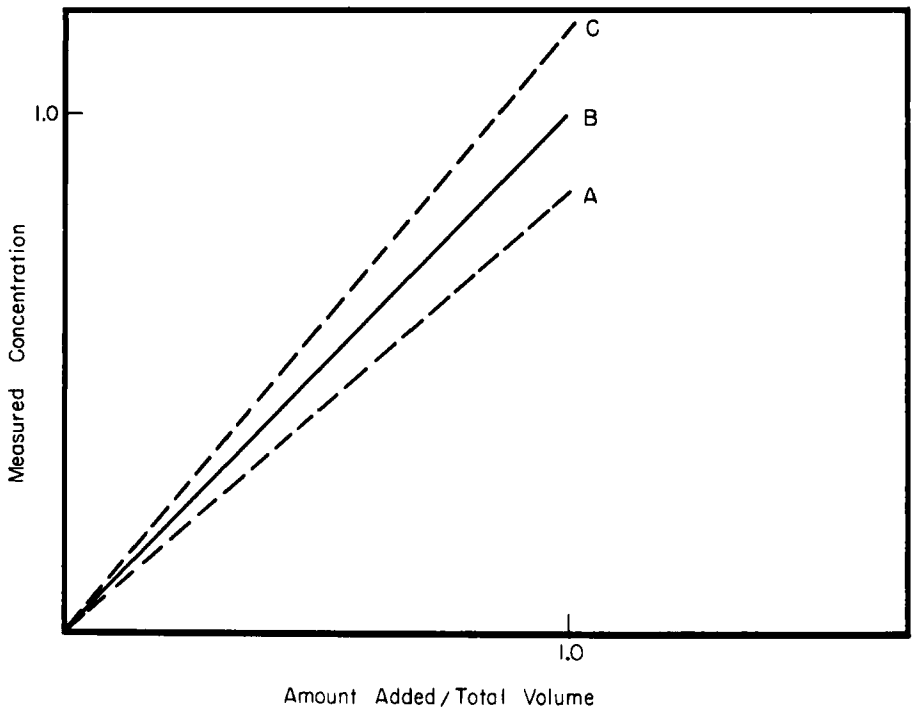


FIG. 1. Effect of sorption and exclusion on the measured concentration versus the calculated concentration. A, Sorption effect; B, calibration line; C, exclusion effect.

TABLE 1

Sorption Isotherm on Bio-Gel P-2 ($T = 24°C$, Bio-Gel P-2 lot #45683)

$CaBr_2$			NaF		
V,[a] ml	C_0, $M \times 10^2$	A_s, (mM/g) $\times 10^2$	V,[b] ml	C_0, $M \times 10^2$	A_s, (mM/g) $\times 10^2$
0.10	0.14	0.19	0.12	0.25	−0.04
0.20	0.31	0.28	0.20	0.42	−0.10
0.30	0.47	0.38	0.30	0.63	−0.16
0.50	0.76	0.66	0.50	1.05	−0.34
0.70	1.08	0.80	0.70	1.44	−0.47
1.00	1.46	1.29	1.00	2.00	−0.67
2.00	2.68	2.57	2.00	3.60	−1.07
3.00	3.77	3.60	3.00	4.90	−1.23
5.00	5.46	5.93	5.00	7.11	−2.21
7.00	6.88	7.54	7.00	8.69	−2.58
10.00	8.58	9.27	10.01	10.42	−2.78

[a] Titrant concentration: 0.199 M.
[b] Titrant concentration: 0.200 M.

the calibration and the measured sorption line or with Eq. (1). The results of two typical isotherm titrations are given in Table 1.

In the sorption isotherm system, when the 3 g of gel swells, from 40 to 50% of the initial 10-ml volume is imbibed within the gel phase. As discussed above, nearly all this water should be available to the ionic solutes under consideration; however, the part of the water hydrating the gel, V_b, is not available to nonsorbed ions. Thus an important consequence of the hydration layer in the isotherm system is that it effectively reduces the volume of solvent available to the solute. Thus when an aliquot of a nonsorbed salt, such as the alkali fluoride, is added to the system, there is less solution available to dilute the titrant than if the gel were not present. As a result the measured equilibrium concentration is higher than the theoretical line, and line C in Fig. 1 results. In addition, since Q of Eq. (1) is a measure of the solute removed, whenever K_D is less than unity no solute is removed, and Q becomes negative. In these instances Q is a measure of the amount of solute *excluded* from the gel. Typical plots of the two kinds of sorption isotherms are shown in Fig. 2 and 3. It is not clear whether or not the bound water is effective in ob-

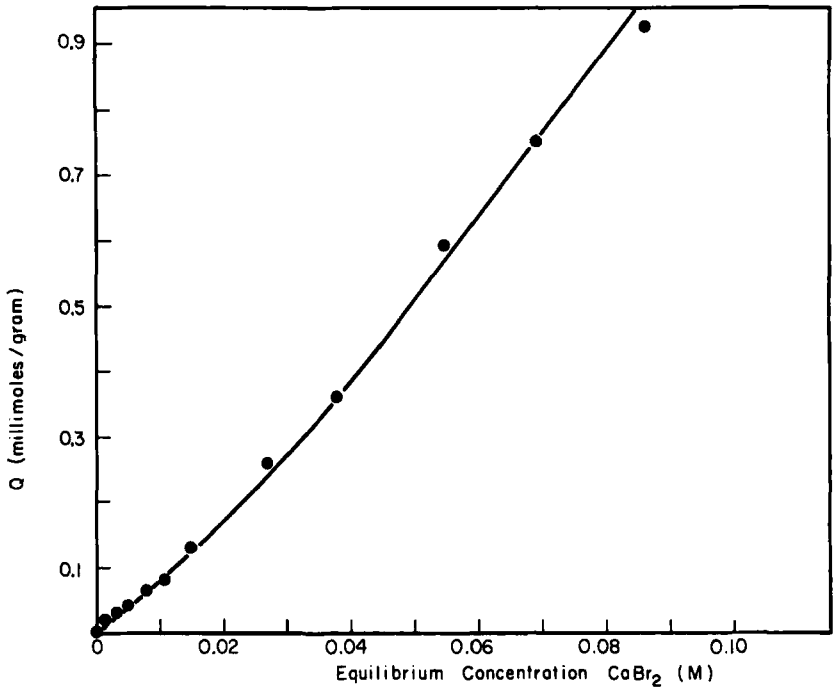


FIG. 2. $CaBr_2$ *sorption isotherm.*

structing the approach of a solute when sorption occurs. It may be that when an ion interacts with a sorption site, some bound water is displaced.

It would be useful to interpret the shape of the sorption isotherm; however, the data are subject to rather large errors arising from the extremely small magnitude of the sorption. The greatest difference in slopes between the calibration and sorption curves of the type shown in Fig. 1 was only 15% ($CaBr_2$ isotherm); usually 3–5% differences were measured. Even though the systems were stable, this kind of difference amounts to only 2-mm variation on the meter pointer position. Thus the isotherm shapes should be interpreted with care. It can be stated, however, that no large deviations from linearity were found, and in no case was any strong sorption observed. Even in the chromatographic experiments here and in the previous paper (9), K_D is seldom greater than 3 and is usually less than 2, which means that sorption normally accounts for less than one-half of the total elution volume.

The data from the sorption isotherms can be used to calculate K_D values for the solutes using Eq. (6), originally derived by Ackers (*15*):

$$K_D = \frac{(C_T V/C_0) - V_o}{V_i} \tag{6}$$

where C_T is the concentration of the titrant, V is the volume of titrant added, and C_0 is the concentration of solution in equilibrium with the gel. The problem is to determine how the total solvent volume in the cell is distributed between the inner (V_i) and outer (V_o) volumes. This was done by using the water regain, W_r, defined by

$$V_i = W_r a \tag{7}$$

where W_r is given in milliliters per gram. V_i is determined from Eq. (7) and V_o is equal to the remainder of the solution. Problems associated with the determination of W_r have been discussed pre-

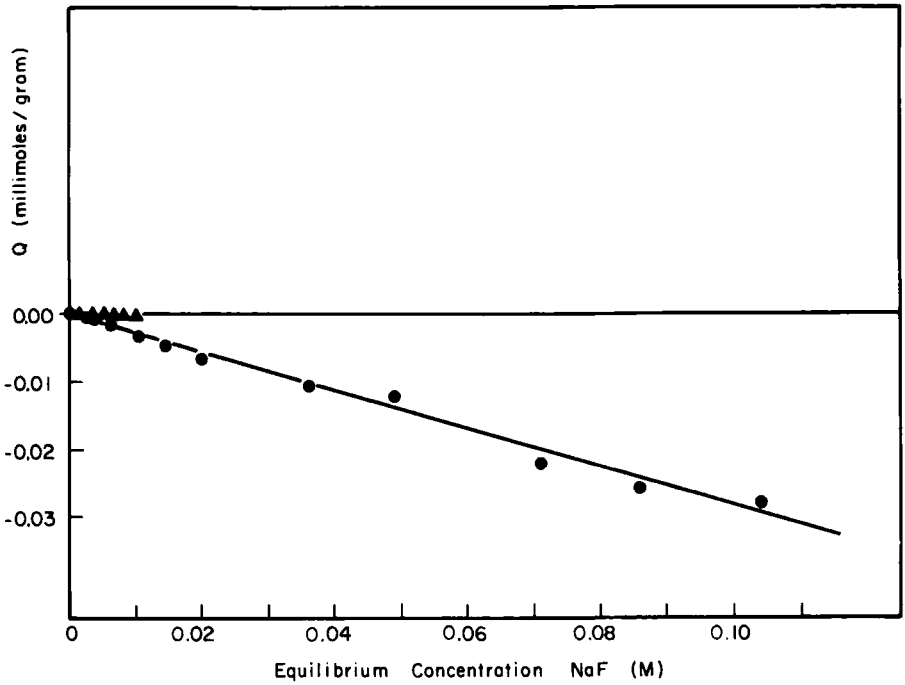


FIG. 3. NaF sorption isotherm.

viously and a new method of finding V_i under chromatographic conditions in the column has been developed (*9*). Because we wish to compare the column K_D values to those from the static isotherm K_D values, an average W_r value determined from seven columns was considered to be the most appropriate. The value used was 1.24 ml/g at 24°C for P-2 lot 45683. Equation (6) then becomes

$$K_D = \frac{(C_T V/C_0) - (10 + V - W_r a)}{W_r a} \tag{8}$$

Obviously static K_D values are related to the slope of the sorption isotherm, so that it is difficult to select the proper concentration range of the isotherm which will give a meaningful comparison with the chromatographic results. This will be discussed more fully later but it turns out that the 10^{-2} to 10^{-3} M range is the best to compare to the chromatographic results because of dilution on the column. The K_D values for several isotherms averaged for the given concentration ranges as well as the chromatographic values are given in Table 2. It can be seen that the 10^{-2} to 10^{-3} M range numbers give reasonable agreement with chromatographic and calculated [Eq. (3)] results.

One conclusion that can be drawn from Table 2 is that no diffusion-controlled mechanism operates, because if diffusion were important, then the static values would tend to be significantly larger than the chromatographic values (*15*). In addition, NaF and $CaBr_2$ at two concentrations were chromatographed at five flow rates from 0.1 to 2.0 ml/min. That equilibrium is attained is thus

TABLE 2

Comparison of K_D Values on Bio-Gel P-2 at 24°C

Method	Concentration, M	K_D				
		NaF	NaCl	Na_2SO_4	KF	$CaBr_2$
Static[a]	10^{-4}–10^{-3}	0.6	1.4	—	1.0	2.1
Static[a]	10^{-3}–10^{-2}	0.88	1.35	1.01	0.79	1.79
Static[a]	10^{-2}–10^{-1}	0.76	1.25	0.91	0.81	1.78
Chromatographic[b]	5×10^{-2}	0.91	1.27	1.07	0.88	2.06
Calculated	5×10^{-2}	0.88	1.22	1.02	0.83	2.08

[a] Lot #45683.
[b] Lot #3403.

confirmed by the fact that no variation in K_D with flow rate was observed.

Spectroscopic Results

To investigate the interaction of metal ions with P-2, an uncrosslinked polyacrylamide was prepared. The thick syrupy solution had a concentration of 4.8% (w/w). Separate portions of $NiCl_2$ and $Ni(en)_3Cl_2$ (en denotes ethylenediamine) were dissolved in this polymer solution and the ultraviolet and visible spectra of these two solutions were recorded from 800 to 200 mμ against a pure water reference. When the intensity at every wavelength was compared to identical solutions made up in pure water, no differences were observed down to 350 mμ. Below 350 mμ the polyamide solution absorbance increased rapidly until it was off scale. This UV absorbance was traced to the edge of the amide carbonyl absorption. Thus the nickel absorption remained entirely unchanged. Next, to facilitate a higher concentration of amide groups, 3 *M* acetamide was chosen as a model for the gel network. The gel structure is essentially a carbon backbone with —$CONH_2$ groups on alternate carbons so that we believe acetamide is an adequate model for the gel. Again, $NiCl_2$ spectra in this amide–water solvent did not change from that in pure water except for the carbonyl absorption below 350 mμ. Veturello and Burdese (*16*) investigated the absorption spectra of copper and cobalt adsorbed on activated alumina. They also found no difference in the spectra from that in dilute aqueous solutions and concluded that chemical interaction between the adsorbed ions and Al_2O_3 was absent. Thus, as in the case of alumina, it appears that Ni^{2+} is attracted to the gel by a weak physical sorption which probably does not involve any penetration into the first coordination sphere of the metal. In any case no chemical bonding is indicated. It should be recalled that Φ_c for copper and nickel are nearly identical to the Φ_c values for all the alkaline earths. Thus, although the transition metals might be expected to be involved in rather specific interactions such as complex formation, it appears that all dipositive metals studied are sorbed to approximately the same extent on Bio-Gel. In addition, the univalent metals also lack selectivity and have Φ_c values about half that of the dipositive ions. It appears then that the cations are sorbed with their solvent shells intact by a mechanism which depends primarily on their charge.

To investigate further the extent of the interaction, a series of

wide-line NMR experiments involving ^{23}Na were carried out. Jardetzky and Wertz (*17*) found that the NMR line width is a sensitive tool for investigating weak complexes of ^{23}Na. Rechnitz and Zamochnick (*18*) found a linear correlation between line width and formation constants and were able to measure formation constants down to as low as 0.2 with this technique. Several solutions containing 2.0 *M* NaBr and acetamide varying from 0 to 6.0 *M* were prepared. The line width was constant (0.05 G) within experimental error in all these solutions. Apparently there is no interaction of alkali metals with the gel. These ions have the smallest Φ_c values of all the metals tested, which tends to support this conclusion.

Hinton and Amis (*19*) have recently reviewed NMR studies of ions in pure and mixed solvents. It has been found that in dioxane–water–salt solutions practically no shift of the dioxane proton resonance occurs. The data were interpreted as "conclusive" evidence that the cations were selectively solvated by water with no indication of solvation of either ionic species by dioxane (*20*). Conversely, Hinton and Amis (*21*) have recently measured the shift in the proton resonances of N-methylacetamide–water–aluminum chloride mixtures. In their words, "PMR studies of aqueous N-methylacetamide have shown conclusively that the organic component of the solvent mixture competes with water in solvating the ions present." Thus a shift in organic proton resonance in organic–water–salt mixtures is conclusive evidence of interactions and a lack of shift is conclusive evidence of no organic–salt interaction.

In light of the above results, an investigation of proton resonance shifts in various acetamide–water–salt mixtures was undertaken to lend more insight into the mechanism. Initially, sodium perchlorate was chosen as a test salt because its effect on the proton resonance of water is large and because perchlorate has a rather large Φ_c value. Table 3 shows the chemical shift of all protons in 2.00 *M* aqueous acetamide solutions containing from 0 to 3 *M* $NaClO_4$. The shifts were measured relative to a *t*-butanol internal standard which was present in all samples (2.5% v/v). The water protons vary linearly with salt concentration and even though acetamide is present in high concentration, the change in shift relative to pure water with increasing salt concentration is quite close to that predicted from the 40-Mc work of Hindman (*22*). Apparently the presence of the amide does not interfere with the salt–water interactions. The amide gave a single sharp CH_3 peak about 45 Hz downfield of *t*-

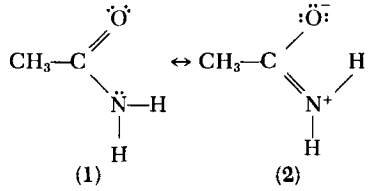
TABLE 3

Proton NMR of Sodium Perchlorate–Acetamide–Water Mixtures

Salt concn., *M*	Amide concn., *M*	δ Relative to *t*-butanol, Hz			
		CH_3	H_2O	NH_a [a]	NH_b [a]
0.00	2.00	45.7	213.0	331	380
0.60	2.00	45.4	207.2	325	368
1.20	2.00	45.8	200.4	328	362
2.10	2.00	45.8	191.5	325	359
3.00	2.00	46.1	184.8	325	350

[a] ±5 Hz.

butanol and a very broad partially resolved NH_2 doublet 350 Hz downfield of the standard. The latter doublet arises from the partial double-bond character of the amide bond.



(1) (2)

The NMR assignments of the amide protons in this compound have not been made to our knowledge, but in dimethylacetamide (*23,24*) and in formamide (*25*), the protons *trans* to the carbonyl are farther downfield. The resonance frequency of the downfield proton in this and other salt–acetamide mixtures changes more than the upfield proton. This effect may be steric in origin. The various proton shifts can be more easily visualized in Fig. 4. Table 4 gives the chemical shifts in other salt–amide solutions. Although these shifts are smaller, they show the same kind of effect exhibited by sodium perchlorate. Because the N—H protons are so strongly affected, a series of experiments with N,N-dimethylacetamide was run for comparison. The results are listed in Table 5. Even in 4.0 *M* $NaClO_4$ solutions, containing 1 *M* amide, no significant variation in the resonance frequency is observed. The different behavior of these two amides suggests the N—H protons are directly involved in an interaction with these salts, probably through hydrogen-bond formation.

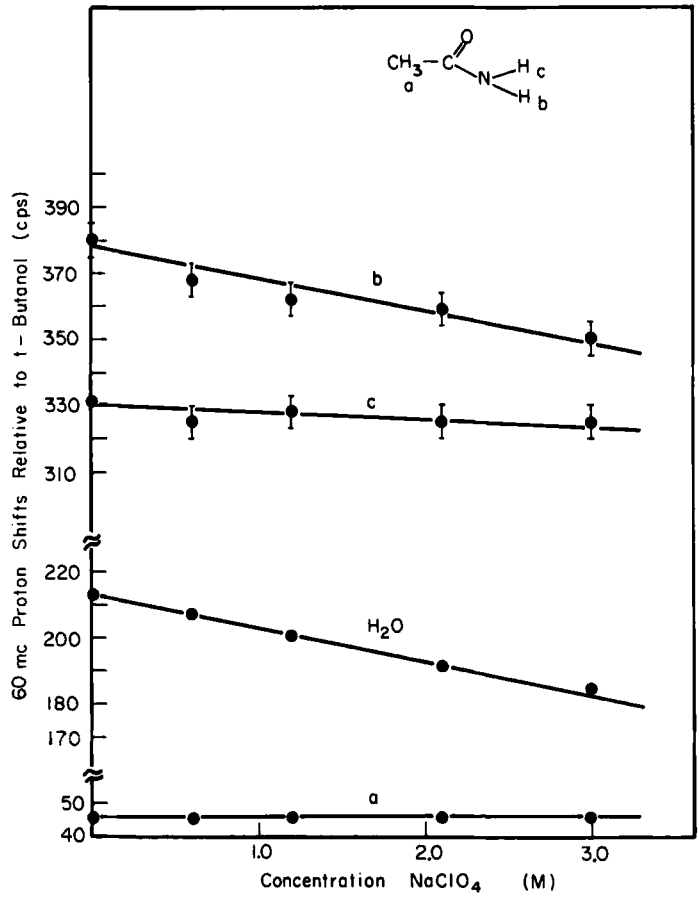


FIG. 4. Chemical shifts of acetamide and water protons in solutions containing various amounts of sodium perchlorate. a, Methyl protons; b, amide proton *trans* to the carbonyl; c, amide proton *cis* to the carbonyl.

The wide-line NMR and chromatographic experiments previously discussed strongly indicate little or no interaction of alkali metals occurs. Other evidence suggests that dipositive metals are only weakly sorbed with their hydration spheres intact, so that it is doubtful that the cations are responsible for proton NMR resonance frequency shifts. The amide protons may interact directly with the anions because of the partial positive charge on the nitrogen atom in resonance form **2**, leading to hydrogen-bond formation.

TABLE 4

Chemical Shift of Acetamide and Water Protons in 4.00 *M* Acetamide Solutions Containing Various Salts

Salt	Salt concn., *M*	δ Relative to *t*-butanol, Hz			
		CH_3	NH_a	NH_b	H_2O
—	0.00	45.1	335.9	376.1	212.9
KF	2.5	46.0	—	—	222.9
NaCl	2.5	46.8	333.3	375.3	199.4
$CaCl_2$	2.5	47.4	333.4	376.2	211.7
NaBr	2.5	46.6	332.3	372.0	194.8
NaI	2.5	46.8	329.0	363.5	187.0
KCl	2.5	46.2	336.1	376.5	200.3
$Al(NO_3)_3$	0.5	46.7	332.4	375.2	237.0
Standard deviation from salt-free solution		1.7	4.1	6.0	18.4

N,N-Dimethylacetamide shows no proton resonance frequency shifts because the hydrophobic character and steric interactions of the methyl groups probably prevent close approach of any ion, and these protons do not form hydrogen bonds. Thus amide protons in the gel may compete with water in solvating the anions. This type of interaction would be expected to be more selective than the weak physical sorption of cations. Indeed, as previously indicated, anion selectivity is nearly a factor of 5 greater for anions of the same

TABLE 5

Chemical Shifts of H_2O/Salt/N,N-Dimethylacetamide Solutions at 25°C

Salt	Salt concn., *M*	Amide concn., *M*	Chemical shift relative to *t*-butanol			
			C—CH_3	*trans* N—CH_3	*cis* N—CH_3	H_2O
	0.00	1.0	50.9	100.2	109.7	214.2
	0.00	2.0	51.2	100.2	110.1	214.7
$NaClO_4$	2.00	2.0	50.6	99.8	109.2	193.9
$NaClO_4$	4.00	1.0	50.2	99.4	108.4	178.3
NaF	2.00	2.0	51.7	100.4	109.8	220.7
NaF	3.00	1.0	51.5	100.5	109.9	220.7

charge than for cations of the same charge. Finally, compounds of amides with alkali metal salts (*26,27*) and alkaline-earth salts (*28,29*) in the solid state are well known. In an X-ray study of $NaBr \cdot (CH_3CONH_2)_2$, Piret et al. (*30*) found N—H · · · Br hydrogen bonds. It is not unlikely that the same kinds of electrostatic forces which operate in the solid state can also compete to some extent in aqueous solution, particularly since the concentration of amide groups in P-2 is about 8.5 *M*.

Sorption Elution Equation

It has been stated that K_D is a complex distribution coefficient when sorption occurs. It would be useful to separate the various components of K_D. Equation (2) can be rearranged to give the elution as a function of column parameters:

$$V_e = V_o + K_D V_i \tag{9}$$

With only slight modification of the definitions of K_D and V_i, Eq. (9) becomes the general equation for elution in gas chromatography:

$$V_R^0 = V_m + KV_L \tag{10}$$

where V_R^0 is the corrected retention volume, V_m is the mobile phase volume, V_L is the volume of stationary phase, and K is the bulk partition coefficient of the solute. Martin (*31*) and Martire et al. (*32*) extended Eq. (10) to include liquid surface adsorption:

$$V_R^0 = V_m + KV_L + K_a A \tag{11}$$

where K_a is the surface adsorption coefficient and A is the liquid surface area. A nearly analogous situation exists in gel chromatography where the normal steric exclusion mechanism may be accompanied by a sorption effect. However, one large difference does exist. If a molecule is partly excluded by a steric mechanism, then part of the gel matrix "surface" will be excluded as well, and the sorption will be reduced. This problem can be resolved by introducing F', the fraction of the matrix available for sorption. Equation (9) in analogy to Eq. (11) and considering Eq. (4) then becomes

$$V_e = V_o + FV_f + F'K_s S \tag{12}$$

where F is the fraction of the free inner volume (V_f) available to a solute, K_s is the sorption coefficient, and S is the effective surface

area of the gel. K_D was not used in Eq. (12) because it is defined by Eq. (2) and is a combination distribution coefficient. When loosely cross-linked gels are used, V_b becomes negligible with respect to V_f and V_f then equals V_i. If in addition K_s is zero, then Eq. (12) reduces to Eq. (9) with $F = K_D$. F should be considered as strictly a size parameter, while F', a function of F, serves to adjust the last term when the solute is partly excluded. For separations of simple ionic salts, all the inner volume and surface area is available giving $F = F' = 1$. The relation between F and F' is a function of the geometry of the system and at present is unknown, except when they are both unity.

The variation of V_i and V_o with temperature in a chromatographic column has been measured. The results are given in Fig. 5. Apparently the gel swells and V_i increases at elevated temperatures; however, the total volume of the bed remains constant so that V_o decreases. It is not known how V_f and V_b vary with temperature. The K_D values of 10 salts have been measured at several tempera-

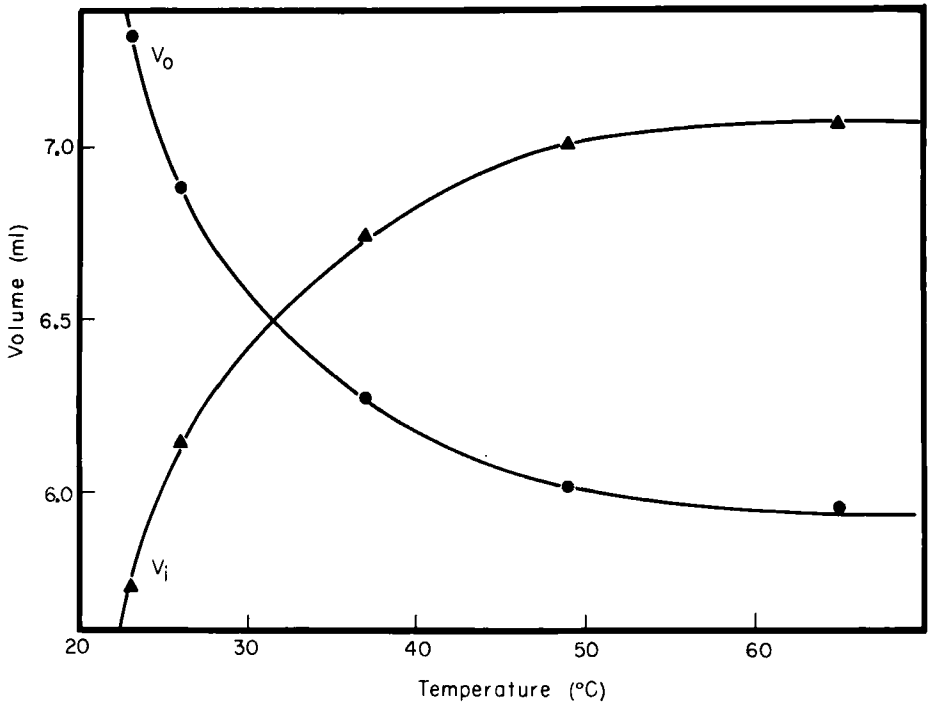


FIG. 5. The variation of V_i and V_o with temperature.

TABLE 6

Variation of K_D with Temperature

	K_D					
Salt[a]	23°[b]	26°	37°	49°	60°	65°
NaCl	1.27	1.26	1.18	1.14	—	1.10
NaI	1.76	1.68	1.49	1.33	1.22	1.26
Na_2SO_4	1.07	1.05	1.00	0.99	—	0.82
KCl	1.26	1.24	1.18	1.12	1.02	1.05
KI	1.63	1.65	1.49	1.37	1.37	1.16
K_2SO_4	0.99	1.00	0.97	0.92	0.85	0.85
$MgCl_2$	1.53	1.46	1.35	1.39	1.17	1.18
$MgSO_4$	1.29	1.41	1.20	1.03	—	0.94
$BaCl_2$	1.64	1.72	1.56	1.41	—	1.32
BaI_2	2.60	2.48	2.19	2.03	—	1.70

[a] Injected concentration: 5×10^{-2} M.

[b] Previously reported. Gel Lot #3403. See Ref. 9. All others Lot #45683.

tures. The results are listed in Table 6. It can be seen that K_D diminishes with temperature in every case. Because $F = F' = 1$ for these salts, Eq. (12) can be rearranged to read

$$K_s = \frac{V_e - V_o - V_f}{S} \tag{13}$$

If a variable related to S can be found, then an estimate of the heat of sorption ΔH_s can be determined from the usual relationship:

$$\frac{d \log K_s}{d(1/T)} = \frac{-\Delta H_s}{2.3R} \tag{14}$$

where R is the gas constant and T is the absolute temperature. Normally for rigid solid adsorbents the weight of the adsorbent (a) is used as a measure of the area. However, in this system the gel structure is not rigid and S probably changes with temperature. It has been found that V_i gives less scattered plots of $1/T$ versus log K_s than the gel weight, when substituted in Eq. (13) for S. Figure 6 shows a plot of log K_s versus $1/T$ for barium iodide.

It must be cautioned that the heat of sorption is subject to several errors. First, it is unclear whether or not V_b is available to sorbed

ions. Second, the variation of V_b with temperature is uncertain (the room temperature value was arbitrarily chosen as constant at all temperatures). Finally, another source of error results from the very small degree of sorption. For many salts V_e is very nearly equal to $V_o + V_f$ so that K_s is determined by the difference between two large numbers. Thus only the most strongly sorbed salts can be expected to give meaningful results and even then the errors in the value of V_b suggest the heats should be considered only as rough approximations. From the slope of Fig. 6 the heat of sorption of BaI_2, the most strongly retained salt investigated, was computed to be -3 ± 1 kcal/mole. Barium chloride and sodium iodide gave values of -3 ± 1 and -4 ± 1 kcal/mole, respectively.

The enthalpy of physical adsorption is usually considered to be less than 5 kcal/mole and the hydrogen bonds postulated for the anion interactions are usually about -5 kcal. Thus -3 to -4 kcal are reasonable values considering the kinds of interactions postulated and thus tend to support the mechanisms proposed.

Ion Exclusion

The authors' previous paper (9) indicated there was a concentration effect on K_D in which the latter tended to rise slightly at low

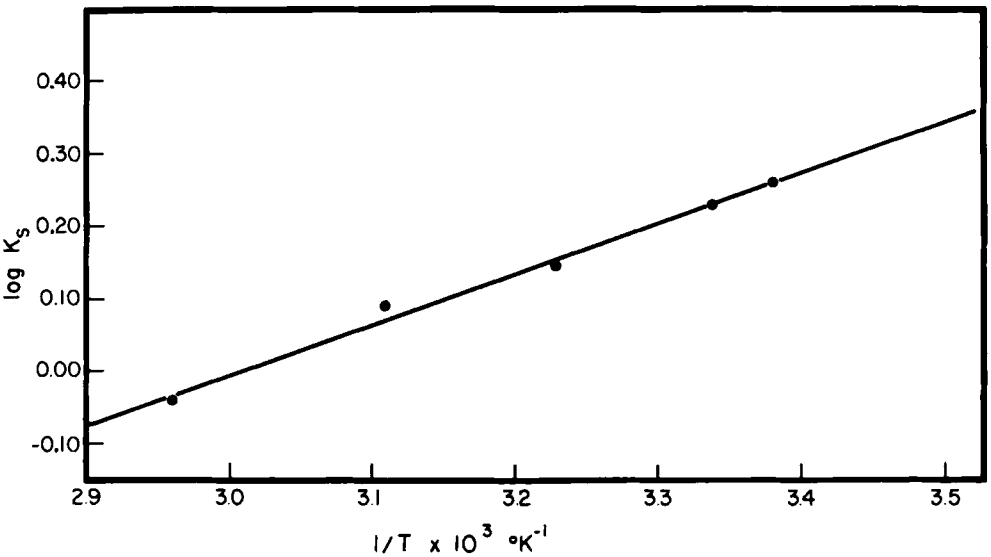


FIG. 6. Log K_s versus $1/T$ for BaI_2.

injected concentrations. In the present study the range of concentrations has been expanded with $CuBr_2$ as a solute. The gel lot number was the same as previously used (3403). The results are shown in Fig. 7. The K_D value continues to rise at low concentrations, while at high concentrations it levels off to a constant value. The formation of $CuBr_4^{2-}$ complex might be expected to alter the K_D value of $CuBr_2$ at concentrations greater than $\sim 0.5\ M$. The constancy of K_D at high injected concentrations can be explained by considering the dilution and subsequent dissociation of the $CuBr_4^{2-}$ complex which occurs as a result of band spreading on the column. A 3 M $CuBr_2$ sample was eluted with a concentration at peak maximum of less then 0.06 M in our system. This amount of band spreading is twice that for concentrations normally employed ($<0.5\ M$).

After the above $CuBr_2$ experiments were completed, our supply

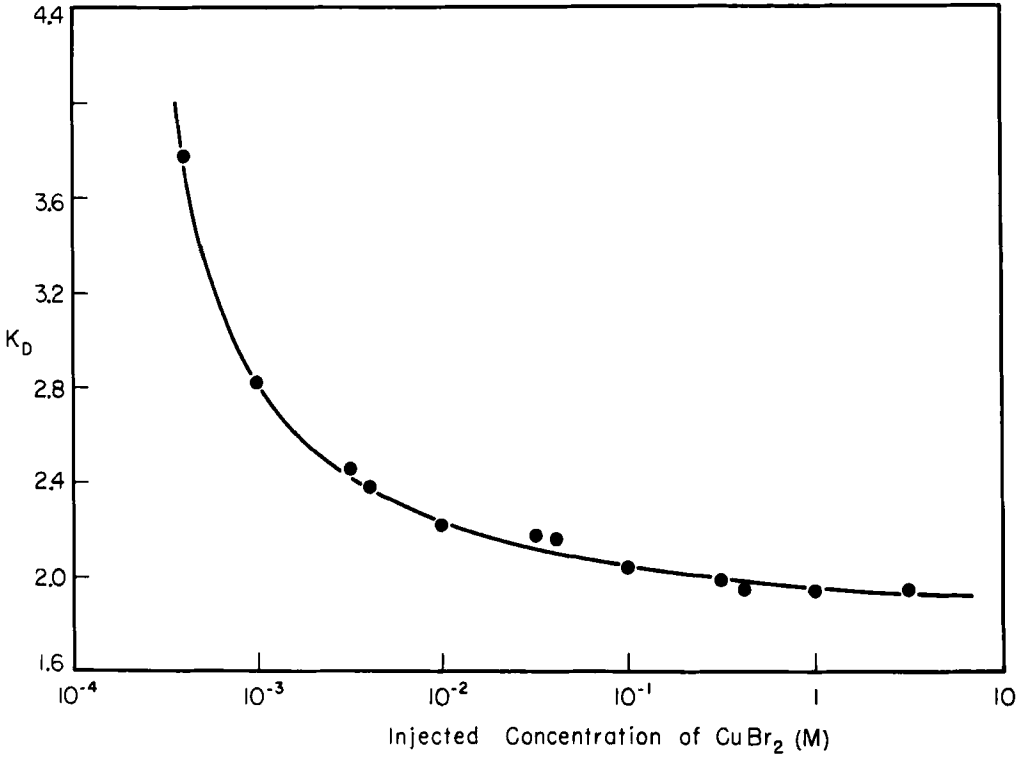


FIG. 7. Variation of K_D of $CuBr_2$ on Bio-Gel P-2 Lot 3403.

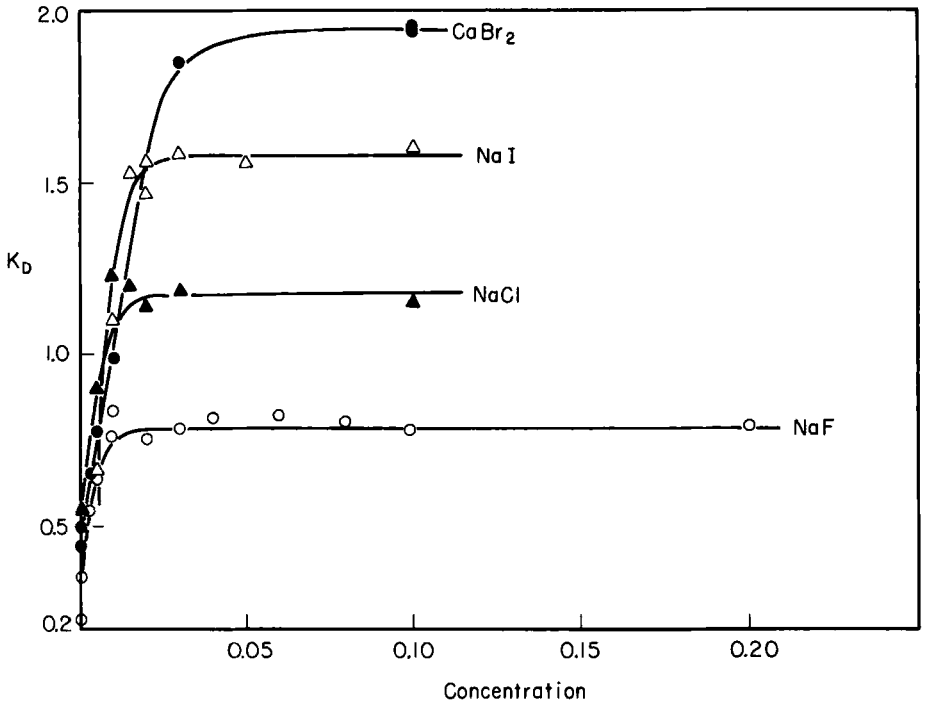


FIG. 8. Variation of K_D with solute concentration on Bio-Gel P-2 Lot 45683.

of gel lot number 3403 was exhausted and lot number 45683 was investigated for chromatographic concentration effect. Although the K_D values remained essentially the same as the previous gel at higher concentrations, below 0.03–0.05 *M* the K_D values dropped off drastically. The results are shown in Fig. 8. A similar effect has been reported to occur with Sephadex G-10 columns (8), so a series of experiments on G-10 was carried out for comparison. Figure 9 shows the results of the G-10 experiments, with the Bio-Gel results included for comparison. The sudden drop-off must be caused by an exclusion of the salts from the stationary phase. The exclusion effect on G-10 is a good deal stronger than on P-2. Ionic exclusion on Sephadex has been explained on the basis of Donnan effect (33) exclusion of the anions resulting from the small number of fixed carboxyl groups in the gel (6,8). No attempt has been made to relate it quantitatively to the concentration effect on K_D. Let us consider that the gel has a number of fixed monovalent anions (such as

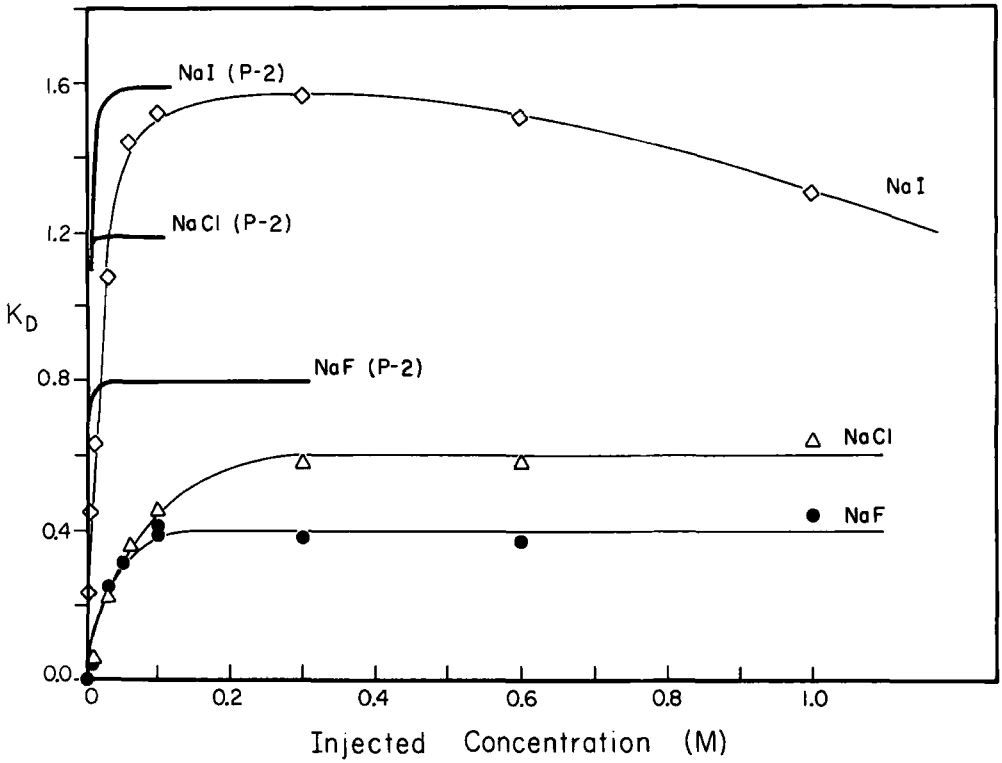


FIG. 9. Variation of K_D with solute concentration on Sephadex G-10 and Bio-Gel P-2.

carboxylate) with counter ions M^+ at concentration C_1. Now if a nonsorbed, nonsterically excluded salt MA (alkali fluorides meet the requirements) with initial concentration C_2 is allowed to equilibrate with the gel, then the Donnan condition for equilibrium will be

$$\gamma_{\pm}'^2 (M')(A') = \gamma_{\pm}^2 (M)(A) \tag{15}$$

where $\gamma_{\pm}$ is the mean ionic activity coefficient and the prime designates the gel phase. Let us assume that $\gamma_{\pm}' = \gamma_{\pm}$; then let x be the amount of salt transferred into the gel to reach equilibrium. Since V_f is the volume into which the salt is transferred, Eq. (15) becomes

$$\frac{V_f C_1 + x}{V_f} \frac{x}{V_f} = \left(\frac{C_2 V_o - x}{V_o}\right)^2 \tag{16}$$

Rearranging and solving for x we have

$$x = \frac{-[(C_1/V_f) + (2C_2/V_o)] \pm \sqrt{[(C_1/V_f) + (2C_2/V_o)]^2 + 4[(1/V_f^2) - (1/V_o^2)]C_2^2}}{2[(1/V_f^2) - (1/V_o^2)]} \quad (17)$$

K_D can be computed from the ratio of concentration of the anion in the two phases:

$$K_D = \frac{(A')}{(A)} \quad (18)$$

Since the amount of A which enters the gel is given by x, K_D can be expressed as

$$K_D = \frac{x/V_i}{(C_2V_o - x)/V_o} = \frac{xV_o}{C_2V_oV_i - xV_i} \quad (19)$$

Before Eq. (17) can be solved for x, we must determine what value of C_1 to use and how to compare C_2 to the injected concentration. The results of ion-exchange capacity determination gave a value of 2.3 μ-equivalents/g for G-10, while P-2 had only 0.02 μ-equivalents/g. If we assume that all the counterion was located in V_f, we can calculate C_1 from

$$C_1 = \frac{I}{W_r}\frac{V_i}{V_f} \quad (20)$$

where I is the ion-exchange capacity. For Sephadex G-10 a W_r value of 0.83 ml/g was measured at 24°C using the column-weighing method. Other values for the calculation are listed in Table 7.

The choice of an average C_2 concentration to compare to the injected concentration C_s is more difficult as virtually all concentrations from C_s to zero are encountered as the band passes along the

TABLE 7

Column Parameters

Gel	W_r, ml/g	V_i, ml	V_f, ml	V_o, ml	C_1, M
Sephadex G-10 (lot 2026)	0.83	5.82	2.81	6.90	6.0×10^{-3}
Bio-Gel P-2 (lot 45683)	1.24	6.35	5.02	7.16	1.5×10^{-4}

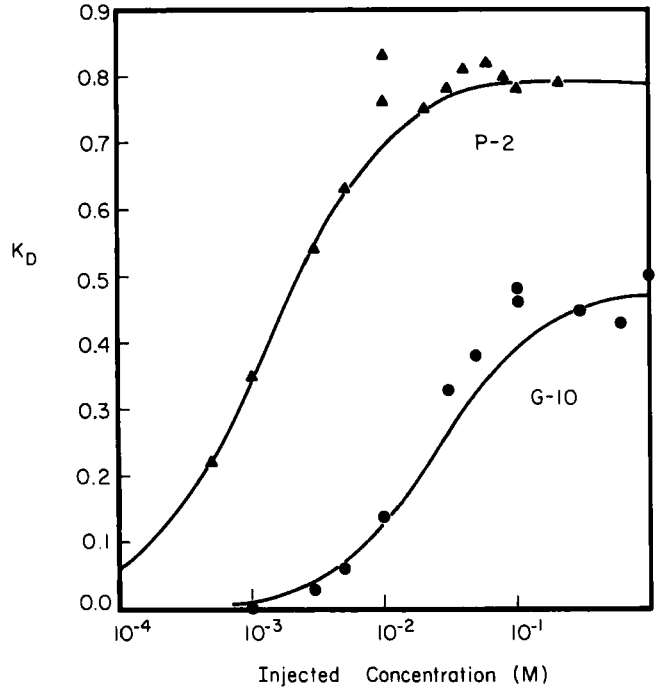


FIG. 10. The calculated and experimental variation of K_D with solute concentration.

column. For lack of a better approximation of the correct average initial concentration, the following method was used. The sodium fluoride peak width at half-height was considered to be the zone width. Injected 0.1-ml NaF samples were eluted with zone widths of 1 ml (Sephadex) or 2.0 (Bio-Gel), indicating a 10- or 20-fold dilution as the zone emerged from the end of the column. One-half of this dilution, i.e., a five- to tenfold dilution, was considered the average value of the concentration along the column and that which should be equated with C_2. That is, K_D calculated from a value of C_2 should be compared to the chromatographic experiment with C_s 5 or 10 times larger than C_2 for Sephadex or Bio-Gel, respectively. Although this is a gross approximation, it will suffice until a more rational approach can be made.

Using these approximations and assumptions, K_D was calculated using Eq. (19) for several C_2 values and plotted in Fig. 10 using the appropriate C_s. The lines in Fig. 10 correspond to the

solution of Eq. (19) with $C_1 = 6.0 \times 10^{-3}$ M for Sephadex and $C_1 = 1.5 \times 10^{-4}$ M for Bio-Gel, while the points are experimental. The agreement of the experimental points with the calculated line are better than expected for Sephadex; the value of C_1 used in the calculation was exactly equal to that calculated using the experimental I and Eq. (20). However, the value of C_1 used in the P-2 calculation agrees only poorly with the experimental value calculated from Eq. (20). The experimental value of C_1 was 2×10^{-5}, while the value needed to fit the data points in Fig. 10 was 7.5 times larger. The lack of agreement in the Bio-Gel system may be a result of errors in measuring the exchange capacity or possibly a heterogeneous distribution of fixed ionic groups. Considering the assumptions made and the extremely low level of fixed ionic groups in Bio-Gel,

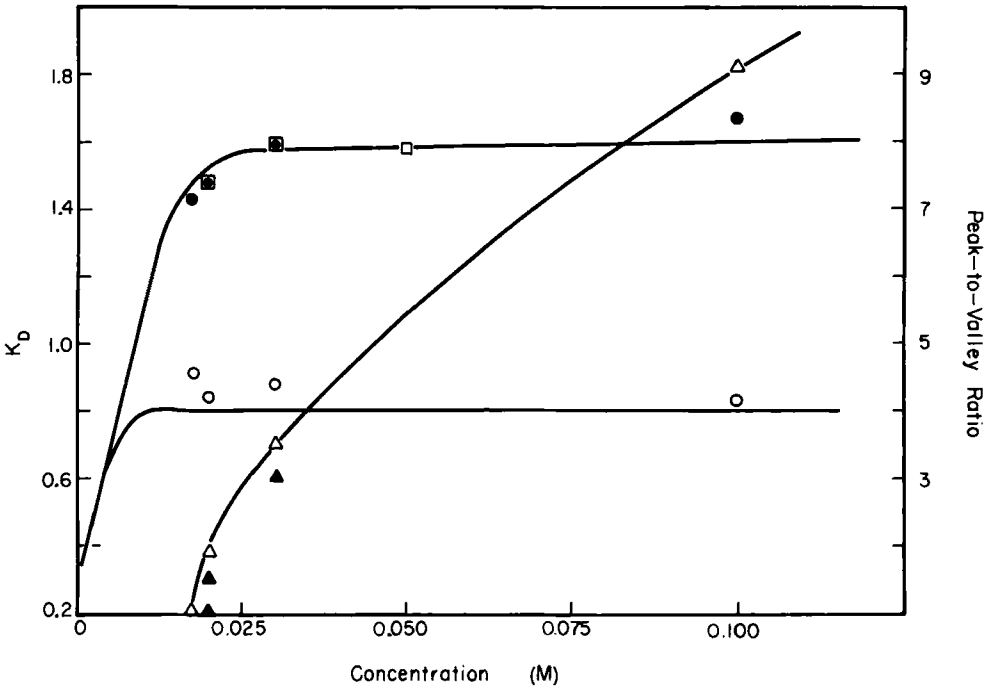


FIG. 11. Separation of mixtures of NaI and NaF. The lines correspond to those shown in Fig. 8. ●, K_D of NaI peak in equimolar mixtures; ○, K_D of NaF peak in equimolar mixtures; □, K_D of NaI peak in mixtures containing 0.1 M NaF; △, peak-to-valley ratio in equimolar mixtures; ▲, peak-to-valley ratio in mixtures containing 0.1 M NaF.

we believe the results can be considered as strong supporting evidence for the existence of Donnan effect exclusion in both gels.

The effect of Donnan exclusion on sorbed molecules appears in Figs. 8 and 9 to be more pronounced, because as part of the gel becomes unavailable, the surface available for sorption is reduced. Thus the elution volume is reduced in two ways. This more pronounced exclusion of sorbed molecules has an important consequence in the separation of mixtures. If, for example, an equimolar mixture of an iodide and a fluoride is to be separated, at high concentrations they will elute normally; however, as their concentration is reduced, the iodide will begin to move into the fluoride zone, reducing the resolution. To test the effect on the separation of mixtures, many experiments were carried out using P-2. Figure 11 is typical of the results. The lines are the concentration versus K_D curves taken from Fig. 8, while the points refer to various mixtures. The Δ points are the peak-to-valley ratios. As can be seen, the peaks merge completely when C_s is about 0.2 M. Thus the existence of even a trace quantity of fixed ionic groups in some gels can have a dramatic effect on elution and resolution and should be avoided if at all possible.

One solution has been advanced by Neddermeyer and Rogers (*8*) which simply involves using an eluent with an ionic strength high enough to swamp out Donnan exclusion. Normally this will require 0.01 to 0.05 M eluents for G-10 and lower for P-2. One difficulty with these eluents is that simple conductivity detectors cannot be used because of the high background. However, differential conductivity (*34*) may solve this problem.

Acknowledgment

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List of Symbols

A	area of the liquid surface in gas–liquid chromatography
a	dry weight of gel in grams
C_1	concentration of fixed ionic groups in the gel
C_2	concentration of nonsorbed salt before equilibrium with the gel

C_0	measured equilibrium concentration
C_s	concentration of sample injected into the chromatograph
C_T	concentration of titrant
F	fraction of the free inner volume available to a nonsorbed molecule
F'	fraction of the gel matrix surface available to a molecule for sorption
G-10, G-25	Sephadex G-10, G-25, a highly cross-linked dextran gel with water regain values of 1.0 and 2.5 ml/g, respectively
ΔH_s	enthalpy of sorption, kcal
I	ion-exchange capacity of the gel in milliequivalents per dry gram
K	bulk partition coefficient in gas–liquid chromatography
K_a	surface adsorption coefficient
K_D	overall distribution coefficient defined by Eq. (2)
K_s	sorption coefficient for solutes interacting with the gel
P-2	Bio-Gel P-2, a highly cross-linked polyacrylamide gel with a water regain of about 1.2 ml/g
Q	quantity of solute sorbed on the gel matrix
R	gas constant, 1.99 cal/mole-°K
S	effective surface area of the gel
s	charge on the anion equal to the cation-weighting factor in Eq. (3)
t	charge on the cation equal to the anion-weighting factor in Eq. (3)
V	volume of titrant added to the sorption isotherm system
V_b	volume of bound water in the gel
V_e	elution volume of a solute in gel chromatography
V_f	free inner volume of water in the gel
V_i	total volume of water in the gel equal to $V_b + V_f$
V_L	volume of the liquid phase in gas–liquid chromatography
V_m	volume of the mobile phase in gas–liquid chromatography

V_o outer or mobile phase volume in gel chromatography
V_R^0 corrected retention volume of a solute in gas–liquid chromatography
W_r water regain in milliliters per gram
x quantity of solute transferred into the gel
Φ_a empirical parameter for the anion
Φ_c empirical parameter for the cation
$\gamma_\pm$ mean ionic activity coefficient exterior to the gel
$\gamma'_\pm$ mean ionic activity coefficient inside the gel

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