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SELECTIVITY OF POLYACRYLAMIDE AND DEXTRAN GELS FOR SIMPLE CATIONS AND ANIONS*

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SUMMARY

Selectivity of Bio-Gel P-2 and P-100, and Sephadex G-10 gels for simple cations was shown by column chromatography of alkali and alkaline earth chlorides and nitrates, and Mn^{2+}, Tl^{+}, and Ag^{+} nitrates. When eluted with water, some of the cations were completely separated. Anion selectivity was demonstrated by chromatography of F^{-}, Cl^{-}, NO_3^{-}, and SO_4^{2-}, as sodium salts. Electrolyte behavior on these gels is discussed in terms of hydrated ionic size, adsorption, and chemical interaction.

INTRODUCTION

Polyacrylamide gels and dextran gels are used primarily for separating macromolecules of different size, and for desalting solutions of macromolecules. They have been successfully applied to the estimation of molecular weights[1-5] and molecular radii[3,6-10], particularly in the molecular weight range of 10,000 to 200,000. More recently, highly cross-linked gels with small pore sizes, on the order of hydrated ion sizes, have become available. Studies of electrolyte behavior on these gels have been very limited, although technical literature[11] mentions fractionations of alkali halides.

Observation of the separation of metal ions on columns used for desalting transfer ribonucleic acid solutions led to this study of electrolyte behavior as related to ion size. Both polyacrylamide and dextran gels were found to exhibit selectivity for several small cations and anions. The separation mechanism appears to involve chemical reactivity as well as size of the solute species.

EXPERIMENTAL

Materials

Reagent grade chemicals were purchased from the usual sources.

Polyacrylamide gel materials, Bio-Gel P-2 and P-100, with molecular weight exclusion limits of about 1600 and 100,000, respectively, were obtained from Bio-Rad Laboratories and from Calbiochem.

Sephadex G-10, a dextran gel with a molecular weight exclusion limit of about

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700, and Blue Dextran 2000, a water-soluble polymer with an average molecular weight of 2,000,000, were purchased from Pharmacia Fine Chemicals, Inc.

Procedures

The dry gel material was stirred into distilled water and allowed to swell for at least 24 h. The gel was then poured into a column of measured volume and washed with several column volumes of water. The columns were jacketed for temperature control. The void volumes of the columns were determined by measuring the elution volumes of Blue Dextran 2000. In each run, the salt solution was added to the top of the column and allowed to drain into the bed. The salts were then eluted with distilled water. In some cases a pump was used to maintain a constant flow rate. Fractions of approximately 5 ml were collected, and the volumes were measured. Effluent fractions were analyzed for metal ions by flame spectrophotometry, and for anions by standard techniques.

RESULTS

Polyacrylamide gel chromatography

A solution containing Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+} chlorides was chromatographed on a 2.5 × 223 cm column of Bio-Gel P-2. The cations were eluted with water, and the order of appearance in the effluent fractions was K^+, Na^+, Li^+, Mg^{2+}, Ca^{2+} (Fig. 1a). Certain pairs of ions, for example Na^+ and Ca^{2+}, were completely separated, and all were sufficiently resolved to indicate a definite order of selectivity of the gel for the ions.

The same elution order for Na^+, Li^+, Mg^{2+}, and Ca^{2+} was observed on a columr of Bio-Gel P-100 (Fig. 2); however, the peaks were not separated as well as on the Bio-Gel P-2 column. Although all the ions should be able to permeate the P-100 gel equally well, they still showed different retentions on the column. As expected, the peaks are all shifted to significantly higher elution volumes, near a column volume.

Chromatography of a solution containing Na^+, Cs^+, Mn^{2+}, Tl^+, and Ag^+ nitrates on a Bio-Gel P-2 column gave essentially complete separation of some of these cations (Fig. 1b). The alkali metal ions were eluted first, completely separated from the Mn^{2+} and Tl^+, which were eluted together. The elution volume of Ag^+, eluted last, was greater than a column volume.

The higher elution volume of Na^+ as the nitrate salt, compared with the chloride, suggested anion selectivity. This anion selectivity was demonstrated by chromatography of F^-, Cl^-, SO_4^{2-}, and NO_3^- (as sodium salts) on a Bio-Gel P-2 column (Fig. 1c). The order of elution was F^-, SO_4^{2-}, Cl^-, NO_3^-. The Cl^- and NO_3^- were almost completely separated from each other and from F^- and SO_4^{2-}. The F^- and SO_4^{2-} were resolved into distinct peaks, but they were not separated.

Tetramethylammonium iodide was chromatographed in solution with K^+ and Li^+ iodides on a 2.5 × 240 cm column of Bio-Gel P-2 at room temperature. These cations were well resolved; the elution order was $N(CH_3)_4^+$, K^+, Li^+. The elution volumes, measured as volume at maximum concentration, were 0.75, 0.87, and 0.95 (fraction of a column volume) for $N(CH_3)_4^+$, K^+, and Li^+, respectively. The elution volumes for K^+ and Li^+ were about 0.13 column volume higher in the iodide solution than in the chloride solution, indicating the selectivity for iodide over chloride.

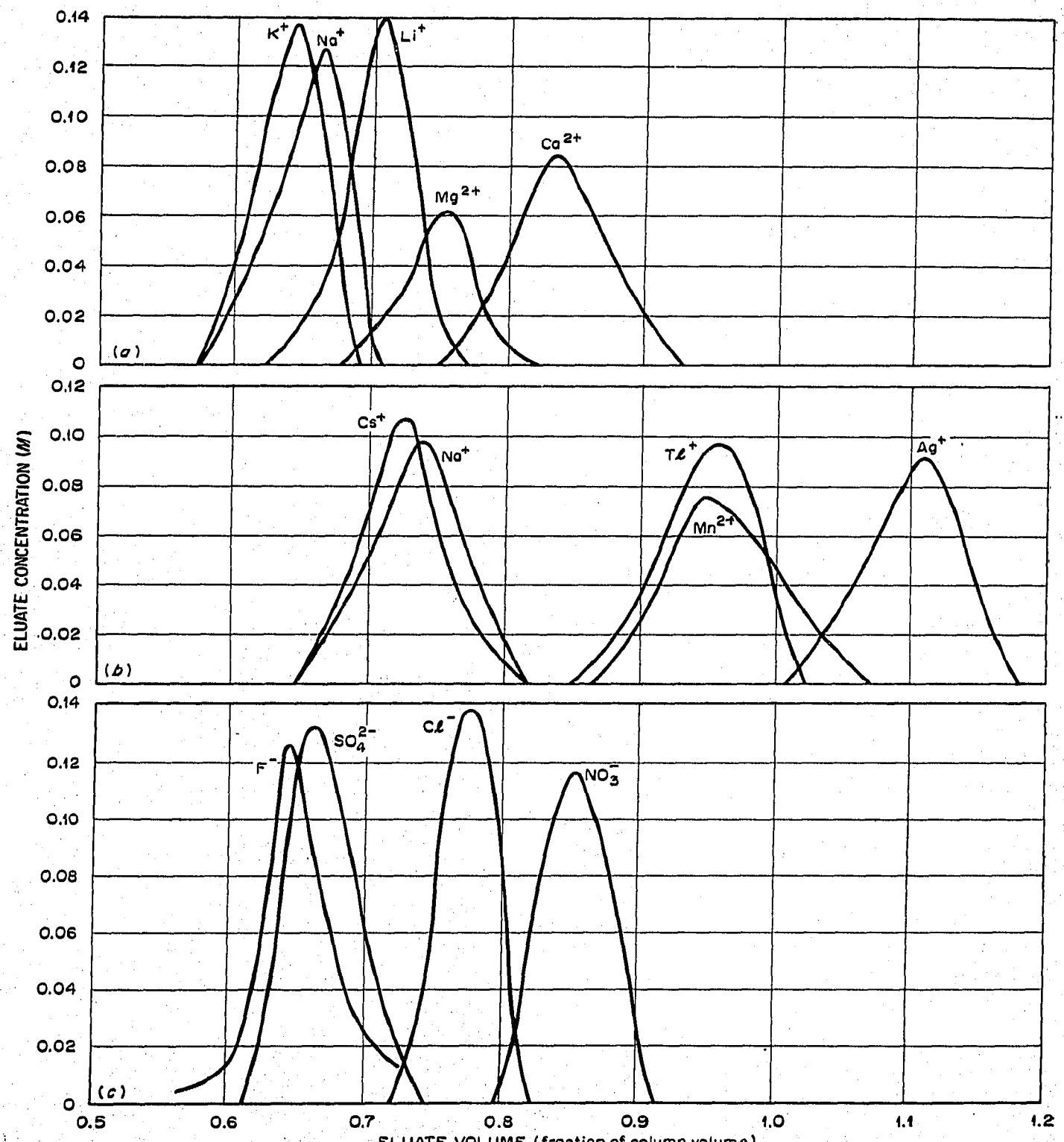

Fig. 1. Polyacrylamide gel chromatography of simple ions. Bio-Gel P-2 eluted with water. (a) 0.05 *M* Mg^{2+}, 0.1 *M* each Li^+, Na^+, K^+, Ca^{2+}, as chlorides, in 100 ml of solution; elution rate, 2 ml/min; temperature, 5°; column, ~ 2.5 × 223 cm; column volume, 1141 ml. (b) 0.1 *M* each Na^+, Cs^+, Tl^+, Ag^+, Mn^{2+}, as nitrates, in 100 ml of solution; elution rate, 2 ml/min; temperature, 5°; column, ~ 2 5 × 223 cm; column volume, 1141 ml. (c) 0.1 *M* each F^-, Cl^-, NO_3^-, SO_4^{2-}, as sodium salts, in 14 ml of solution; elution rate, 0.5 ml/min; temperature, 23°; column, ~ 1.0 × 235 cm; column volume, 210 ml.

Sephadex Gel chromatography

Selectivity by Sephadex G-10 for simple cations and anions is shown in Fig. 3.

A solution of Li^+, Na^+, Mg^{2+}, and Ca^{2+} chlorides was chromatographed on a 1.0 × 235 cm column of G-10 at room temperature. The Mg^{2+} was eluted first, partially separated from Li^+, Na^+, and Ca^{2+}, which eluted together (Fig. 3a).

A solution of Na^+, Cs^+, Tl^+, and Ag^+ nitrates was chromatographed under similar conditions (Fig. 3b). The alkali metals were eluted first. Elution of Tl^+ required more than a column volume. A small amount of Ag^+ was eluted with the Tl^+. Most of the Ag^+ was retained on the column, and would not elute with water.

The anions, SO_4^{2-}, F^-, Cl^-, and NO_3^- (sodium salts) were well resolved on a Sephadex G-10 column (Figure 3c). The elution order was SO_4^{2-}, F^-, Cl^-, NO_3^-.

DISCUSSION

The total column volume, or bed volume, may be expressed as:

$$V_t = V_o + V_i + V_g = V_a + V_g$$

where V_o is the void or interstitial volume, available to all solutes; V_i is the additional volume within the gel; $V_a = V_o + V_i$ is the total aqueous volume, available to solutes small enough to enter the pores; V_g is the volume of the gel matrix inaccessible to the

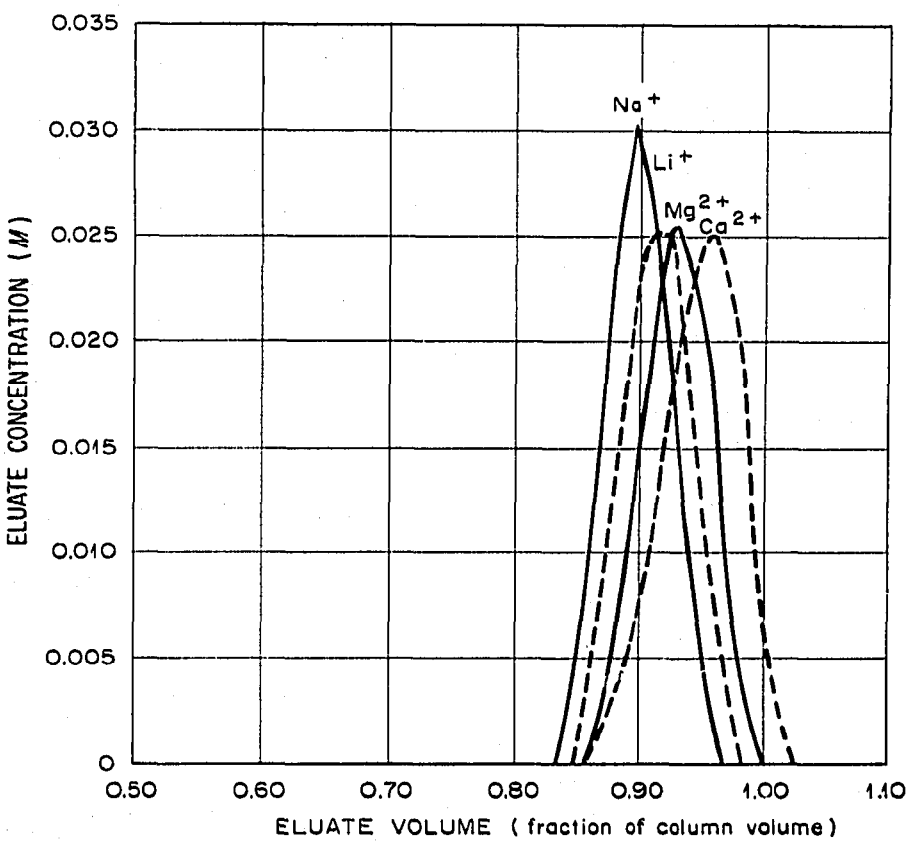

Fig. 2. Polyacrylamide gel chromatography of simple ions. Bio-Gel P-100 eluted with water. 0.1 *M* each Li^+, Na^+, Mg^{2+}, Ca^{2+}, as chlorides in 20 ml of solution; elution rate, 0.6 ml/min; temperature, 23°; column, ~ 2.5 × 211 cm; column volume, 1079 ml.

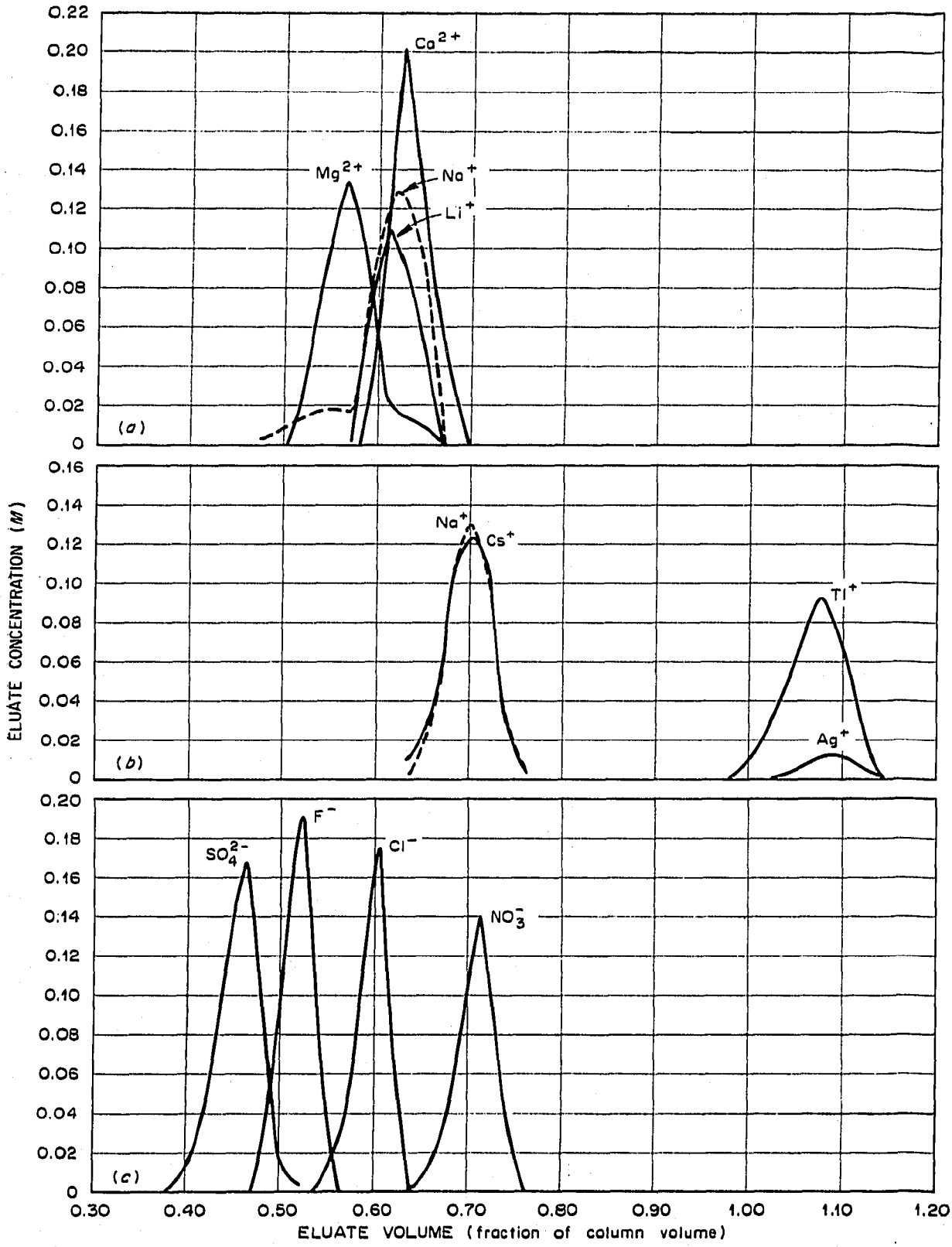

Fig. 3. Dextran gel chromatography of simple ions. Sephadex G-10 eluted with water. (a) 0.1 *M* each Li^+, Na^+, Mg^{2+}, Ca^{2+}, as chlorides in 15 ml of solution; elution rate, 0.45 ml/min; temperature, 23°; column, ~ 1.0 × 235 cm; column volume, 210 ml. (b) 0.1 *M* each Na^+, Cs^+, Tl^+, Ag^+, as nitrates in 15 ml of solution; elution rate, 0.45 ml/min; temperature, 23°; column, ~ 1.0 × 235 cm; column volume, 210 ml. (c) 0.1 *M* each F^-, Cl^-, NO_3^-, SO_4^{2-}, as sodium salts in 15 ml of solution; elution rate 0.45 ml/min; temperature, 23°; column, ~ 1.0 × 235 cm; column volume, 210 ml.

solute. The elution volume, V_e, is the effluent volume at maximum concentration of solute. Three types of behavior may be considered to describe the elution position of the ions from the gels. First, if the freely mobile ions have complete access to the inner volume of the gels, then the elution volume would equal V_a. Secondly, partial exclusion from the gels would result in elution volumes between the void volume (generally about 0.4 V_t) and V_a. Thirdly, interaction of the ions with the gels might cause the elution volume to be greater than V_a. Each of these types of behavior has been observed on both gels, at least in the case of cations. It appears, therefore, that the selectivity of the gels for cations and anions is based on chemical reactivity or physical adsorption as well as size. The chemical reactivity probably includes both ion exchange and complexation of some ions, for example, Ag^+, with the gels.

It is generally agreed[9,12–14] that separation of macromolecules by gel permeation chromatography is based on size differences of the solute molecules, although different parameters have been used to characterize this "size" and quantitatively relate it to column behavior. Several investigators have successfully correlated the elution volumes of large, organic solutes, especially proteins, and their molecular weights[2–5,8,15] and their Stokes radii[3,6–10]. It is difficult to apply these parameters to electrolytes. Molecular weights of dissociated salts are ambiguous. Stokes radius has doubtful meaning when applied to ions with radius less than about 5 Å in aqueous solution, since the water cannot be considered a continuous medium and the hydrated ions are not sufficiently larger than the water molecules to satisfy viscous flow conditions[16–18].

There is considerable disagreement in the literature as to the *absolute* size of hydrated ions, but there is general agreement on the *relative* sizes of many hydrated ions. Attempts to correlate the elution volumes of the ions studied with reported values[17,18] for hydrated radius and other size-related parameters, for example, diffusion coefficients, equivalent conductance, and apparent molal volume, have met with little success. Indeed, if ion size is the only determining factor in the selectivity, then the ions should show similar behavior on both the polyacrylamide and dextran gels, with the larger ions being eluted first. The anions do show similar, though not exactly the same, behavior. The cations apparently do not. According to NIGHTINGALE[18], the sizes of the hydrated anions decrease in the order SO_4^{2-}, F^-, NO_3^-, Cl^-, and the sizes of the cations decrease in the order Mg^{2+}, Ca^{2+}, Li^+, Na^+, K^+ and Mn^{2+}, Na^+, Ag^+, Tl^+, Cs^+. Clearly, the elution orders are not consistent with the larger ions being eluted first. Cation selectivity was observed even on Bio-Gel P-100, which presumably includes solutes with molecular weights as high as 100,000, so that differences among these ion sizes should be negligible. Calculation of the diameter of cation–anion pairs by adding hydrated ionic sizes gives values which are in the range estimated for the pore sizes of P-2 and G-10 gels, permeable to solutes with molecular weights up to about 1600 and 700.

The data illustrate that in addition to size considerations, charge effects and chemical interaction cannot be neglected when considering solute behavior on these gels.

The gels offer a unique method for separating certain cations and anions in which the only added reagent is water. Additional variables to be studied which may influence the separations are temperature, pH, ionic strength, and presence of other cations and anions.

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