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CHROMATOGRAPHIC BEHAVIOR OF ALKALINE EARTH METAL IONS ON SEPHADEX G-15 COLUMNS

NORIMASA YOZA AND SHIGERU OHASHI

Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka (Japan)

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SUMMARY

Alkaline earth metal ions were chromatographed on Sephadex G-15 columns with two different eluents, *viz.*, a 0.1 *M* sodium chloride solution and a 0.1 *M* sodium chloride–0.01 *M* hydrochloric acid solution. Separation of barium ions from the other alkaline earth metal ions was almost complete. The peak skewing of the elution curves of the alkaline earth metal ions, excepting magnesium, was ascribed to two factors: (1) the multicomponent character of the metal ions in solution and (2) the polyfunctional character of the gel phase due to incomplete penetrating ability of the background electrolyte into an inner part of the gel phase. Elution order could be explained in terms of the different sizes of the hydrated metal ions.

INTRODUCTION

Chromatography on Sephadex (cross-linked dextran) columns has been extensively developed as a useful technique for the separation of organic macromolecules and the estimation of their molecular weights. The principle of this method has been explained in terms of a sieving effect¹ in the gel phase, *i.e.*, the preferential penetration of smaller chemical species into the porous gel structure with the exclusion of larger ones. Some recent investigations have demonstrated that chromatography on a Sephadex column, as well as on a column packed with Bio-Gel²⁻⁴ (cross-linked polyacrylamide), is useful not only for the investigation of inorganic polymers such as polyphosphates⁵⁻⁷ and polymeric ferric hydroxide^{8,9}, but also for the separation of metal ions^{4,6,10} and their complexes^{10,11}. Although there are many arguments in the literature as to the separation mechanism of inorganic species on the columns, the sieving effect due to size difference of solutes appears to be the main determining factor.

This paper describes the behavior of alkaline earth metal ions chromatographed on Sephadex G-15 columns with two kinds of eluent, *viz.*, a 0.1 *M* sodium chloride solution and a 0.1 *M* sodium chloride–0.01 *M* hydrochloric acid solution. The results gave elution profiles which could be explained in terms of the size difference of the hydrated ions.

EXPERIMENTAL

Sample solution

All reagents used were of guaranteed grade from Wako Chemicals, unless otherwise stated. Sample solutions for magnesium, calcium, strontium and barium ions were prepared by dissolving their chlorides in a solution of the same composition as that of the eluent used for the column operation. Concentrations of the sample solutions were 0.01 *M*. Sample solutions for sodium ions were prepared by mixing trace amounts of radioactive sodium-22 (Radiochemical Center, Amersham, Great-Britain) in a 0.1 *M* sodium chloride solution and a 0.1 *M* sodium chloride-0.01 *M* hydrochloric acid solution, respectively.

Two standard solutions of Blue Dextran 2000 (Pharmacia Fine Chemicals, 0.2 %) and tritiated water (Radiochemical Center, used as tracer) were also prepared by a similar method.

Eluents

Eluent I. 0.1 *M* sodium chloride solution.

Eluent II. 0.1 *M* sodium chloride-0.01 *M* hydrochloric acid solution.

Preparation of the Sephadex column

Sephadex G-15 (Pharmacia Fine Chemicals, particle size 40-120 μ) was suspended in the solution to be used as eluent and allowed to swell for two days. Undesirable fine particles were eliminated by the repeated treatment of suspension, settling and decantation. A slurry of the prepared gel was poured into a column, (consisting of a glass tube, 1.5 \times 60 cm, with a porous polystyrene disc at the bottom). A third of the column volume should be filled with eluent before pouring the gel. After the gel had settled by allowing 500 ml of eluent to flow through the column, excess gel above the desired height of the Sephadex bed was removed, and a disc of filter paper (Toyo Roshi 5B) was placed on top of the bed as a stabilizer. When the neutral eluent was used 30 ml of a 0.01 *M* EDTA solution (disodium dihydrogen ethylenediaminetetraacetate, in the eluent) was passed through the column to remove adsorbed impurities, and the bed was then washed again with 100 ml of the eluent before use.

For convenience the columns operated with eluents I and II are called column I (bed volume: *ca.* 1.5 \times 56.5 cm) and column II (*ca.* 1.5 \times 57.0 cm), respectively.

Procedure

One ml of the sample solution or standard solution was placed on the column bed just as the last layer of eluent soaked into the bed. Then the eluent was applied when the last portion of the sample solution vanished into the bed. The effluent was collected in fractions of 1 ml, under a flow rate of 20-30 ml/h, with a Toyo Kagaku Drop Count Fraction Collector.

The amounts of solutes in the fractions were determined as follows:

Blue Dextran: measurement of absorbance at 620 m μ .

Alkaline earths: EDTA titration.

Sodium-22: radioactivity measurement with a G.M. counter.

Tritium: radioactivity measurement with a liquid scintillation counter.

RESULTS AND DISCUSSION

Standardization of column

In the molecular-sieve chromatographic column operation the elution volume of a given solute, V_e , is characterized by eqn. (1),

$$V_e = V_0 + K_d \cdot V_i \quad (1)$$

where V_0 is the void volume outside the gel particles and V_i the internal volume within the gel phase. The distribution coefficient or molecular-sieve coefficient, K_d , is defined as a fraction of the internal volume available for the distribution of the solute if the column is operating under the equilibrium conditions.

Many equations have been proposed by earlier investigators who successfully correlated the K_d values and the sizes of the macromolecules on assumptions of hypothetical structures for the gel matrixes, *viz.*, conical pores¹², rigid rods¹³, cylindrical pores¹⁴, and a mixture¹⁵ of cones, cylinders and crevices. To avoid the complexity of calibration parameters the present paper adopted the conception more recently proposed by ACKERS¹⁶, in which no definite geometrical structure was assumed for a given gel. There is an assumption that an individual microregion within the gel may be characterized by the radius R of the largest solute molecule which can enter into the microregion in question, and the frequency of sizes of such a microregion follows a normal distribution curve.

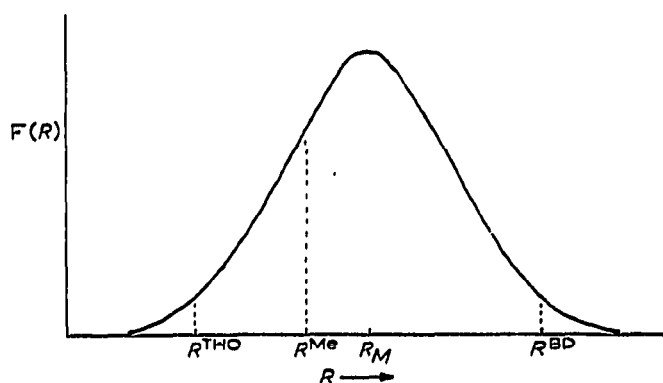


Fig. 1. Distribution of pore sizes. R^{BD} = radius of Blue Dextran, R^{THO} = radius of tritiated water.

For convenience of the interpretation the feature mentioned above is graphically represented by Fig. 1, in which a constant R_M is the radius at the position of the maximum frequency. The distribution coefficient of a given metal ion, K_d^{Me} , with a radius R^{Me} can be represented by eqn. (2),

$$K_d^{Me} = \int_{R^{Me}}^{\infty} F(R) dR = \int_{R^{Me}}^{\infty} \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(R - R_M)^2}{2\sigma^2}} \cdot dR \quad (2)$$

where σ is the standard deviation of size distribution, which in addition to R_M , is concerned with the separation efficiency or selectivity of the column.

The K_d^{Me} value can be determined experimentally by an approximation according to eqn. (3),

$$K_d^{\text{Me}} = \frac{V_e^{\text{Me}} - V_0}{V_t} \doteq \frac{V_e^{\text{Me}} - V_e^{\text{BD}}}{V_e^{\text{THO}} - V_e^{\text{BD}}} \quad (3)$$

where V_e^{Me} is the elution volume of the metal ions, and V_e^{BD} and V_e^{THO} represent the elution volumes of Blue Dextran (molecular weight = 2,000,000) and tritiated water, which were used as standard materials of $K_d = 0$ and 1, respectively. Experimental results in Figs. 2 and 3 show that there is no remarkable difference between column parameters which were obtained by procedures either with eluent I or II, both of which gave approximate volumes of 37% for V_0 , 46% for V_t and 17% for the gel matrix. This fact proves that the geometry of the gel matrix does not significantly change under the experimental conditions considered here.

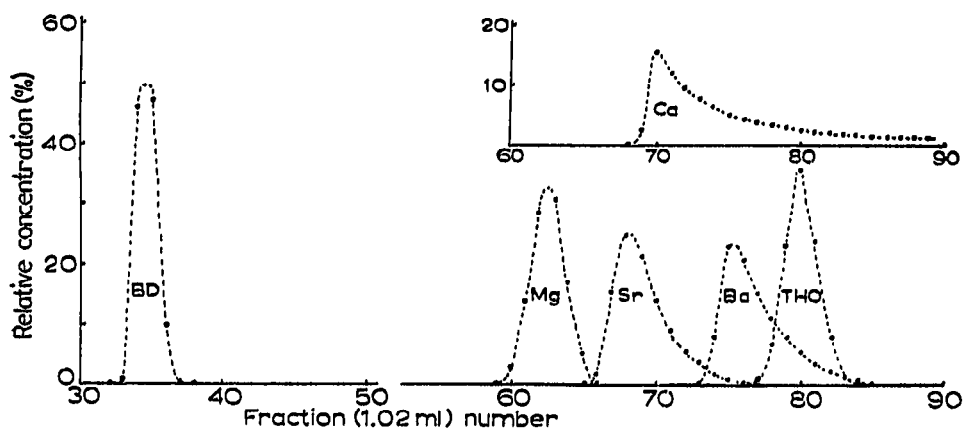


Fig. 2. Elution curves of alkaline earth metal ions chromatographed individually on column I.

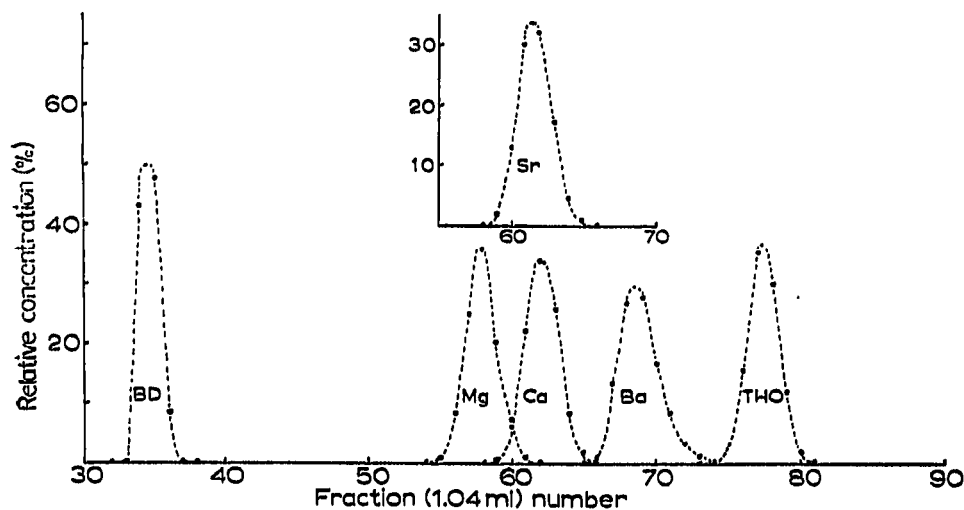


Fig. 3. Elution curves of alkaline earth metal ions chromatographed individually on column II.

Although it is convenient to use tritiated water as a standard material for the determination of the value of V_0 plus V_t , two questions remain to be answered in the future regarding the meaning of its elution position as obtained by the radioactivity measurement of tritium. One question is whether the associated form of the water molecules^{17,18} is small enough to penetrate into most parts of the internal volume, as defined mathematically; and the other question is whether or not there is interaction between the tritiated water and the gel matrix, such as an isotope exchange reaction between the hydrogen atoms, which, if there is, may result in the appearance of the elution peak for tritiated water at a position greater than the true value of V_0 plus V_t .

Separation

Elution profiles of alkaline earth metal ions chromatographed individually on Sephadex G-15 columns with eluents I and II are shown in Figs. 2 and 3, respectively, in which the relative amount of solute in each fraction is plotted on the ordinate. When the neutral eluent I is used it seems to be impossible to obtain complete separation of a mixture of all alkaline earth metal ions because of the considerable tailing, especially for calcium ions, of the elution curves of the metal ions, except magnesium. However, the separation of magnesium and barium ions is almost complete as shown in Fig. 4.

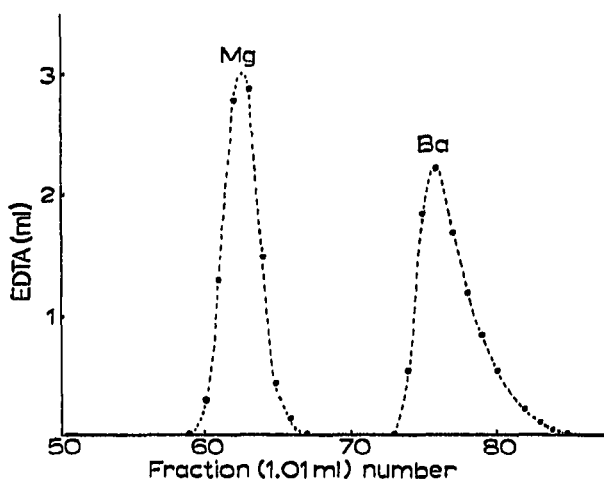


Fig. 4. Elution curve of a mixture of magnesium and barium ions chromatographed on column I.

Fig. 3 shows that the efficiency of separation was considerably improved by use of the acidic eluent II instead of eluent I owing to less skewing of the elution curves. As the result of the increased symmetry of the elution curves of calcium and strontium it became easier to separate barium ions from the other alkaline earth metal ions as shown in Figs. 5 and 6.

Peak skewing

The elution curves for the magnesium ions on both columns I and II are symmetrical, while calcium, strontium and barium ions chromatographed on column I show unsymmetrical elution curves with sharp front edges. However, the degree of peak skewing decreases on using the acidic eluent II in place of eluent I and only slight skewing of the elution curve of barium ions remains as shown in Fig. 3. It is thought

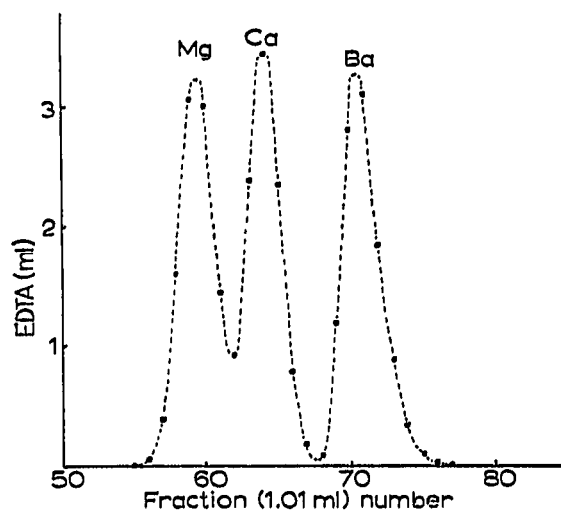


Fig. 5. Elution curve of a mixture of magnesium, calcium and barium ions chromatographed on column II.

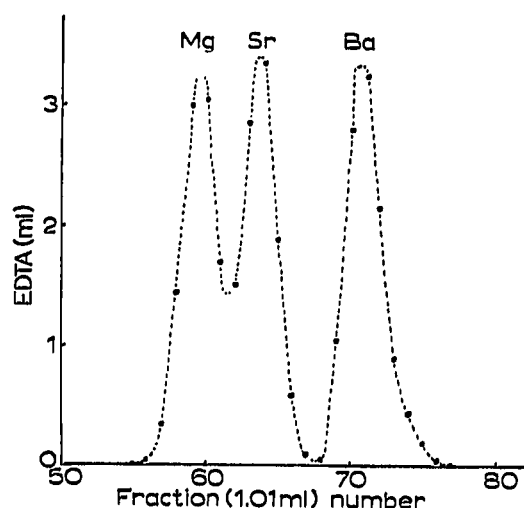
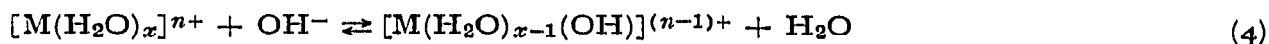


Fig. 6. Elution curve of a mixture of magnesium, strontium and barium ions chromatographed on column II.

that there are two probable causes of the tailing of the elution curves mentioned above.

The first is the existence of a minor component which is in equilibrium with a major component, *i.e.*, a hydrated metal ion which has a different chromatographic behavior to that of such as a less-hydrated metal ion. Since the chloride ion in the eluent is a poor complexing agent for the alkaline earth metal ions, the only equilibrium system which can be supposed to be in existence is the following hydrolytic reaction¹⁷ of the hydrated metal ion which is dependent on pH of the solution.



If the stability constants of the hydroxo complexes of alkaline earth metal ions in eqn. (4) increase with increasing atomic weight of the element, the above equation appears to be a reasonable explanation for the symmetrical elution curves of the magnesium ions in both eluents I and II and less peak skewing of the other three metal ions in acidic eluent II than in eluent I. However, the reported data¹⁹ have indicated that an ion with a larger atomic weight shows a smaller stability constant. It has also been shown that the stability constants of the hydroxo complexes of all alkaline earth metal ions are too small to produce observable quantities of hydroxo complexes.

Although peak skewing, therefore, cannot be simply correlated with eqn. (4), there is still the possibility that there may be an equilibrium reaction which is dependent on the pH of the solution and includes an intermediate chemical species, such as a hypothetical distorted form of the hydrated metal ion that is different from the two chemical species in eqn. (4). However, the present state of knowledge^{17,18} concerning the structures of hydrated metal ions and their reactions with solvents is too complicated to get a generally accepted conclusion. This problem will have to be

solved in the future, because it may influence the behavior of the metal ions on a Sephadex column.

In addition to the problem of the multicomponent character of metal ions in solution, the second factor in question is the polyfunctional character of the gel phase. Such an effect may be caused by the incomplete penetrating ability of the background electrolyte into the inner part of the gel phase. It will be examined by discussing the elution behavior of the alkaline earth metal ions on columns I and II and by correlating their elution positions with those of sodium ions. The relative elution orders on both columns can be represented by the increase in the elution volumes or K_d values.

Column I: $Mg^{2+} < Na^+ \approx Sr^{2+} < Ca^{2+} < Ba^{2+}$

Column II: $Mg^{2+} < Ca^{2+} \approx Sr^{2+} < Na^+ \approx Ba^{2+}$

For example, let us consider the elution of barium ions with a solution of sodium chloride as eluent, *i.e.*, background electrolyte. Since the distribution coefficient of sodium ions, K_d^{Na} , is smaller than that of barium ions, K_d^{Ba} , the available volume for barium ions within the gel phase, V_i^{Ba} , is represented by:

$$V_i^{Ba} = V_i \int_{R^{Na}}^{\infty} F(R) dR + V_i \int_{R^{Ba}}^{R^{Na}} F(R) dR \quad (5)$$

The first term on the right hand side of eqn. (5) corresponds to the region into which both sodium and barium ions can penetrate, while there is the inner region corresponding to the second term into which only the barium ions can penetrate. In the elution process barium ions are thus distributed over these two regions at different ionic strengths resulting from the presence (phase I) or absence (phase II) of the background electrolyte.

It has been commonly observed in chromatographic processes of inorganic compounds^{4,6} that, in addition to the sieving effect due to the size differences in the solutes, the contribution of side effects such as ion exchange and adsorption becomes more predominant when pure water with no background electrolyte is used as an eluent. Hence the chromatographic behavior of barium ions, which are in contact with phases I and II in the elution process, may be influenced by both the sieving effect and the side effects. The latter effects occurring in phase II may cause peak skewing of the elution curve of barium ions.

Since magnesium ions, which have a smaller K_d value than sodium ions, are only in contact with phase I even when a neutral or acidic eluent is used, the peak skewing due to the side effects does not occur. Symmetrical elution curves for calcium and strontium ions in eluent II can also be explained for the same reason. The elution behaviors of both strontium ions in eluent I and barium ions in eluent II are more or less influenced by the side effects, because the distributions of these ions with K_d values nearly equal to those of sodium ions include boundary regions between phases I and II. It is also expected that calcium and barium ions in eluent I are considerably influenced by the side effects.

At the present state of our knowledge peak skewing can be discussed in terms of two assumptions, *viz.*, (1) minor component ions in equilibrium with hydrated metal ions may be more adsorptive than the hydrated metal ions on to Sephadex gel, and (2) both ions may be more adsorptive on to phase II than phase I. Either or both of (1) and (2) can influence the chromatographic behavior of the metal ions to give peak skewing. However, there still remain many questions because of the difficulty of direct verification of these assumptions. For example there is the question of which factor contributes more predominantly, and why peak skewing for calcium ions in eluent I is more marked than for other alkaline earth metal ions.

Relationship between K_d values and ionic sizes.

If one tries to correlate the K_d value and the ionic size of a solute it must first be recognized that the column only operates under the equilibrium condition when only the sieving effect is operative, and that it is very difficult to determine the contribution of side effects such as adsorption and ion exchange.

If there are no side effects, the following condition must be satisfied, $0 \leq K_d \leq 1$. Almost all the elution curves of the alkaline earth metal ions in Figs. 2 and 3 satisfy the above condition, though that for the calcium ions in Fig. 2 shows exceptional tailing, which extends to far behind the elution position of tritiated water and can apparently be ascribed to the adsorptive property of the ions.

On the other hand it should be also noted that the appearance of an elution peak in the region of $0 \leq K_d \leq 1$ does not necessarily mean the absence of side effects. However, magnesium ions, with symmetrical elution curves, appear to be influenced by only negligibly slight side effects. This is also confirmed by the fact¹⁰ that the concentration dependence of the elution behavior of magnesium ions is contrary to that which is more commonly observed in adsorption chromatography²⁰.

Thus we can now consider a symmetrical elution curve which appears in the range of $0 \leq K_d \leq 1$ as a convenient criterion for the absence of the side effects. Some cases which satisfy the above conditions can be selected here to discuss the relationship between K_d values and ionic sizes. The elution curve for magnesium ions in

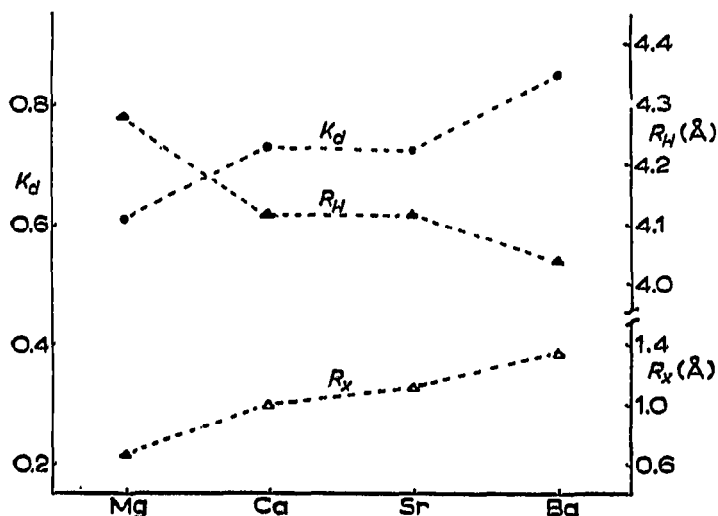


Fig. 7. Comparison between K_d values and sizes of alkaline earth metal ions. R_C = radius of crystal ion, R_H = radius of hydrated ion, K_d = eluted with eluent II.

Fig. 2 and the curves for magnesium, calcium and strontium ions in Fig. 3 belong to this category. Barium ions in Fig. 3 appear to be more or less influenced by side effects, but the elution position may be not far from that under ideal conditions where only the sieving effect operates.

Although there are not enough data to discuss the relationship mentioned above systematically, K_d value obtained from Fig. 3 and eqn. (1) can be correlated with the ionic sizes²¹ of the alkaline earth metal ions. In Fig. 7, the K_d values, the radii of the crystal ions, R_X , and the radii of the hydrated ions, R_H , are plotted on the ordinates with respective scales. The fact that the K_d values increase with the decrease of the radii of the hydrated ions in contrast to the reverse relationship between the K_d values and the radii of the crystal ions is of importance. It is also significant that both calcium and strontium ions with identical R_H values behave almost identically as shown in Figs. 5 and 6. These results suggest that if one can obtain a systematic relationship between radii of various hydrated metal ions and their K_d values it could be used as a calibration curve for the estimation of unknown ionic radii, such as has been commonly applied to the estimation of molecular sizes of organic macromolecules¹.

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