Determination of Apparent Equilibrium Constants of the Exchange Reactions of Sodium, Potassium, Calcium, and Magnesium with Amberlite IR-120

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Apparent equilibrium constants for the exchange reactions between a strong sulfonic acid resin and mixed solutions of sodium, potassium, calcium, and magnesium chlorides were determined. By using these constants and a predetermined mole fraction of ions which is desired on a resin, it is shown how to calculate a regenerating solution composition which will equilibrate the resin with the desired mole fraction. Application of the procedure with respect to a cationic system similar to that existing in milk is considered.

Recent reports (*2–4*) have shown that most of the radiostrontium can be removed from milk by treatment with cation exchange resins such as Dowex 50 W. Easterly *et al.* (*2*) and Nervik *et al.* (*4*) used resins in the single ion forms of sodium and/or calcium. Migicovsky (*3*) used resins charged with a mixed solution of the chlorides of calcium, potassium, and sodium, in which the proportion of the cations were the same as in milk. He also used a mixture of these three resin forms, each separately charged with its respective cation.

With the complex ionic system in milk, treatment with a single ion resin results in changes in the cationic composition. The results with the resins charged with a mixed solution of cations indicated no significant change in the three cations studied. However, treatment with a mixture of the three resin forms did show changes (*3*). These studies were carried out in batch systems and at the normal pH of milk.

The availability of the major cations in milk—calcium, potassium, sodium, and magnesium—for exchange with an ion exchange resin depends on a complex system of equilibria involving these ions and the various complexes in milk, which in turn are affected by such factors as individual variations in milk composition, pH, and heat treatment. The extent of exchange of the individual ions also depends on the ratio of these on the resin. To place a specific ratio of ions on the resin, it is necessary to know how the ions are distributed between the aqueous and resin phases.

In the present work, the apparent equilibrium constants for the exchange reactions between resin and aqueous solutions containing the above-mentioned cations are determined for a series of total concentrations of the chlorides of these ions. These constants may be used to calculate the required solution composition for charging the resin to a desired composition. The following equilibria are involved:

$$2KR + Ca^{+2} \leftrightharpoons CaR_2 + 2K^+ \quad (1a)$$

$$2KR + Mg^{+2} \leftrightharpoons MgR_2 + 2K^+ \quad (1b)$$

$$NaR + K^+ \leftrightharpoons KR + Na^+ \quad (1c)$$

$$2NaR + Ca^{+2} \leftrightharpoons CaR_2 + 2Na^+ \quad (1d)$$

$$2NaR + Mg^{+2} \leftrightharpoons MgR_2 + 2Na^+ \quad (1e)$$

$$MgR_2 + Ca^{+2} \leftrightharpoons CaR_2 + Mg^{+2} \quad (1f)$$

where R represents the anionic part of the resin.

Experimental

Reagent and Materials. The salts used were C. P. grade chlorides of calcium, potassium, sodium, and magnesium. The ion exchange resin was Amberlite IR-120, having a mesh size range of 16 to 50. Standard solutions for flame photometric analyses for Ca, K, Na, and Mg were obtained from Fisher Scientific Co.

Analysis. Analyses for calcium, potassium, sodium, and magnesium were made by flame spectrophotometry as described by Wenner (*8*).

Ion Exchange Column Procedure. Glass columns, 21 mm. in diameter and 60 cm. in length, were filled to a height of 30.5 cm. with resin. The resin was then backwashed and allowed to settle. The resin was initially put in the H-form by passing 6*N* HCl downflow through the resin, then washing thoroughly with distilled water. Salt solutions of total ionic strengths from 0.16 to 3.0 containing calcium, potassium, sodium, and magnesium chlorides in mole fraction ratios of about that existing in normal milk were passed downflow through the resin columns at a rate of 0.30 ml. per minute per gram of dry resin. (The moisture content of this resin was obtained, and the volume of resin was corrected to grams of dry resin. The flow rate was expressed on this basis.) Preliminary work included variations in the amount of solution passed through the column to determine the quantity necessary to establish equilibrium. The pH of the charging solutions was adjusted to 6.6, although variations from pH 6.6 to 7.0 had no effect on the equilibrium.

After equilibrium was established, the columns were rinsed thoroughly with distilled water. The cations were eluted with 6*N* HCl. Analyses for each ion in the eluate and in the aqueous charging solution were made. These data were used for calculation of the apparent equilibrium constants.

Results and Discussion

To show that conditions of apparent equilibrium were established the following conditions were met. Essentially all of the hydrogen ions were replaced by cations of the salt solution. This was shown when the pH of the effluent became constant and equal to the pH of the influent solution (Figure 1); the concentrations of the effluent cations ceased to change with increasing volume of solution passed through the column. This was determined by analyses. The analytical concentrations of the ions in the equilibrated solution and on the resin after equilibration are given in Table I. The volume of regenerant contained eight times the number of meq. of salts as the resin in the column through which it was passed.

The equilibria involved in these studies may be expressed in terms of a concentration equilibrium constant, or selectivity coefficient. For example, Equation 1c may be represented by the expression:

$$K_{K^+}^{Na^+} = \frac{(K^+)_R(Na^+)}{(K)(Na^+)_R} \quad (2)$$

where $(K^+)_R$ and $(Na^+)_R$ are concentrations in the resin, and (Na^+) and (K^+) are concentrations in the aqueous phase. The constants calculated herein

Table I. Concentration of Ions in Solution and on Resin After Equilibration

Ionic Strength, μ	Concentration in Solution, Mmole/Liter				Amount of Resin, Mmole/Column			
	Na	K	Ca	Mg	Na	K	Ca	Mg
0.16	20	47	27	4.5	3.46	19.53	44.10	3.15
0.21	39	94	50	9.0	5.84	28.67	46.36	3.30
0.57	73	170	97	14.0	7.59	35.88	51.06	3.76
0.72	168	172	92	32.0	13.49	26.98	33.37	8.88
1.02	150	355	160	25.0	5.89	34.72	48.98	3.70
1.05	270	252	109	63.0	15.60	34.80	24.90	11.80
1.12	148	360	185	36.5	8.16	39.40	46.50	3.40
1.14	145	345	188	37.0	7.02	33.90	36.85	3.97
1.55	337	322	188	132.0	15.37	33.06	33.64	9.80
2.29	290	700	355	80.0	10.67	46.97	42.70	4.16
2.98	700	645	355	157.0	18.60	42.60	34.80	9.45

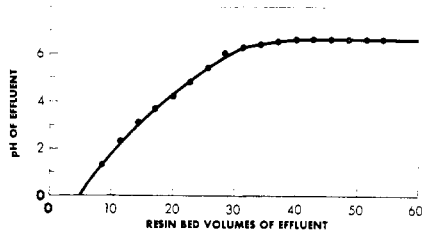

Figure 1. The change in effluent pH with volume of mixed salt solution passed through a column of Amerlite IR-120

(pH of salt solution, 6.6)

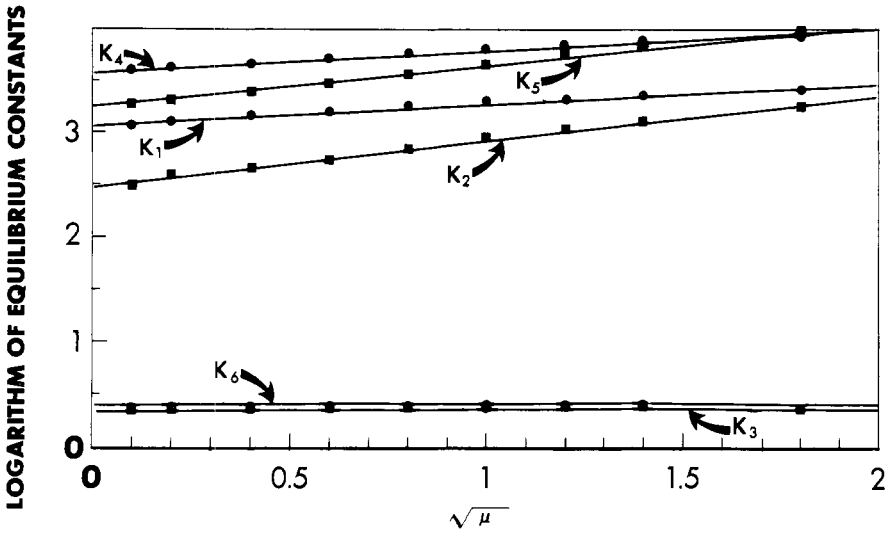

Figure 2. Effect of ionic strength on apparant equilibrium constants for exchanges represented by Equations 1a through 1f

(K_1 corresponds to Eq. 1a, K_2 to Eq. 1b, etc.)

are expressed in terms of millimoles per liter for the ions in solution and mole fractions for the ions on the resin. Equation 2 then becomes:

$$K_{K^+}^{Na^+} = \frac{(X^+)_R(Na^+)}{(K^+)(X_{Na^+})_R} \quad (3)$$

This is the form used for calculating the apparent equilibrium constants.

The uni-divalent exchanges, e.g., Equation 1a, has the form:

$$K_{Ca^{+2}}^{K^+} = \frac{(X_{Ca}{}^{+2})_R(K^+)^2}{(Ca^{+2})(X_{K^+})_R{}^2} \quad (4)$$

The logarithms of the apparent equilibrium constants as calculated above for the six equilibria (Equation 1a to 1f) are plotted against $\sqrt{\mu}$ in Figure 2 (where μ is expressed in terms of millimoles per liter instead of molality). The constants for uni-univalent and di-divalent exchanges are independent of μ, whereas the uni-divalent exchanges increased with μ, i.e., the reverse reactions are favored by increasing the ionic strength in the following exchanges:

$$2KR + Mg^{+2} \leftrightharpoons MgR_2 + 2K \quad (5a)$$

$$2KR + Ca^{+2} \leftrightharpoons CaR_2 + 2K^+ \quad (5b)$$

$$2NaR + Mg^{+2} \leftrightharpoons MgR_2 + 2Na^+ \quad (5c)$$

$$2\ NaR + Ca^{+2} \leftrightharpoons CaR_2 + 2Na^+ \quad (5d)$$

This is expected according to theory for binary systems as shown by Selke (*6*).

The problem is a complex one in that the ionic equilibria involves five cations in the aqueous and in the resin phases. The behavior of the ions in both phases is not ideal, but since the equilibria increase in the manner predicted by the Debye-Hückel limiting law, the results are meaningful. Hence, the constants for some exchanges are reproducible only at constant ionic strength.

More sophisticated treatment of exchanges involving aqueous salt solutions and resins have been given by several workers (*1*) in order to account for variations in equilibrium constants with concentration. The thermodynamic equilibrium constant for the exchange A^+ (aq.) + BR $\leftrightharpoons$ AR + B^+ (aq.) may be represented by:

$$K_a = \frac{(A_{B^+})(A_{AR})}{(A_{A^+})(A_{BR})}$$

where A_{A^+} and A_{B^+} are activities in solution, and A_{AR} and A_{BR} represent the activities on the resin. If the activity of the ions on the resin is set equal to the mole fraction, the equilibria may be represented by:

$$K = \frac{(A_{B^+})(X_{AR})}{(A_{A^+})(X_{BR})}$$

the true K_a could be calculated if A_{B^+} and A_{A^+} were known, but these are not known with any degree of accuracy in a concentrated salt solution. The Debye-Hückel relationship fails in high concentrations.

Calcium ions have a greater affinity for the resin than magnesium, potassium, or sodium ions (*5*). At low concentrations and ordinary temperatures, for a given valence, the extent of exchange increases with increasing atomic number. At higher concentrations, the differences between the relative exchange of ions of difference valences dimish (e.g., calcium ions *vs.* sodium ions). This may be caused by rate phenomenon since sodium ions may form ion pairs at high concentrations, thereby diminishing its exchange rate relative to that of calcium. The calcium may also form ion pairs, and the effective concentration of the calcium ion would be diminished because of the presence of the species $Ca(H_2O)_6{}^{+2}$ $Ca(H_2O)_5Cl^+$, and $Ca(H_2O)_4\ Cl_2$, as suggested by Strelow (*7*). The second species, being monovalent, may decrease the difference in affinity of calcium and sodium for the resin.

The apparent equilibrium constants may be used in charging a resin with a desired mole fraction ratio since the ionic strength of the solution may be chosen. An example of this follows: The desired mole fraction of ions on the resin was chosen to be $X_{Na} = 0.10$, $X_{Ca} = 0.41$, $X_K = 0.44$, and $X_{Mg} = 0.05$. The ionic strength of the charging solution was chosen to be 0.366. The millimoles of ions per liter is given by

$$\mu = \frac{1}{2}\ [C_{Na^+} + C_{K^+} + 4C_{Ca^{+2}} + 4C_{Mg^{+2}} + C_{Cl^-}]$$

where C is expressed in millimoles per liter and μ in millimoles, but

$$C_{Cl^-} = C_{Na^+} + C_{K^+} + 2C_{Ca^{+2}} + 2C_{Mg^{+2}}$$

Table II. Comparison of Mole Fractions of Cations Obtained on Amberlite IR-120 Ion Exchange Resins After Equilibrating with Solutions with a Composition Calculated to Give a Resin with the Desired Mole Fraction of Cations

Ion	Mole Fraction Desired	Mole Fraction Obtained (Column) I	II	III
Na	0.10	0.113	0.101	0.103
K	0.41	0.461	0.418	0.450
Ca	0.44	0.390	0.428	0.398
Mg	0.05	0.036	0.052	0.049

Therefore;

$$\mu = C_{Na^+} + C_{K^+} + 3C_{Ca^{+2}} + 3C_{Mg^{+2}}$$

From Figure 2, the following equilibrium constants are obtained for reactions 1a through 1f, respectively: 1.20×10^2; 5.81×10^2; 2.34; 7.10×10^3; 3.20×10^2; and 2.35. Since millimoles are used, $\mu = 366$, or

$$366 = C_{Na^+} + C_{K^+} + 3C_{Ca^{+2}} + 3C_{Mg^{+2}}$$

$$K_{K^+}^{Na^+} = \frac{[X_{K^+}]_R[Na^+]}{[X_{Na^+}]_R[K^+]} = 2.34 \qquad (3)$$

Substituting the desired mole fraction in equation 3,

$$\frac{0.44[Na^+]}{0.10[K^+]} = 2.34$$

$$[Na^+] = 0.533[K^+]$$

By successive substitutions, a quadratic equation is obtained, the solution of which yields the concentrations desired in the charging solution. For example, considered here these values are:

Na = 79 millimoles per liter
K = 149 millimoles per liter
Ca = 36 millimoles per liter
Mg = 9.6 millimoles per liter

Table II shows the mole fractions obtained for three different columns which were charged with a solution having this composition.

This method of determining apparent equilibrium constants involving exchange reactions between a strong sulfonic acid resin and a solution of potassium, sodium, calcium, and magnesium chlorides is shown to be a function of ionic strength. If the equilibrium constants and the mole fraction of ions which is desired on the resin are known, it is possible to calculate the solution composition which will equilibrate the resin with the desired mole fraction. This procedure has application for charging a resin so that it will be in equilibrium with milk when it is desirable to remove specific cations, such as strontium-90 or other cationic radionuclides, while leaving the other components unchanged, or for similar research on other liquids. In applying this procedure to milk systems, the equilibrium ratio of cations on resin with those in milk must first be determined. At the normal pH of milk, about $^2/_3$ of calcium is bound, and therefore the total cationic ratio in milk is not a measure of the equilibrium values obtained for salt solutions. A future paper will consider the cationic composition of milk as affected by ion exchange resins.

Literature Cited

(1) Boyd, G. E., Schubert, J., Adamson, A. W., *J. Am. Chem. Soc.* **69,** 2818 (1947).
(2) Easterly, D. G., Demott, B. J., Cragle, R. G., *J. Dairy Sci.* **43,** 137 (1960).
(3) Migicovsky, B. B., *Can. J. Biochem. Physiol.* **37,** 1287 (1959).
(4) Nervik, W. E., Kalkstein, M. I., Libby, W. F., University of California Radiation Laboratory, Berkeley, Calif., UCRL-2674 (1954).
(5) Schubert, J., *J. Phys. & Colloid Chem.* **52,** 340 (1948).
(6) Selke, W. A., "Ion Exchange Technology," Chapt. 4, pp. 57–60, F. C. Nachod and J. Schubert, Ed., Academic Press, New York, 1956.
(7) Strelow, F. W., *Anal. Chem.* **32,** 1185 (1960).
(8) Wenner, V. R., *J. Dairy Sci.* **41,** 761 (1958).

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MUSHROOM CULTURE

Factors Affecting the Growth of Morel Mushroom Mycelium in Submerged Culture

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During the past decade, there has been considerable interest in the submerged culture production of mushroom mycelium for food and fodder. The potential value of mushroom mycelium as a source of protein has been pointed out in reviews by Block (*2*), and Robinson and Davidson (*17*). In addition, the mycelia of certain species of mushrooms have desirable flavors which make them more attractive as food than other microorganisms such as algae and yeasts proposed as sources of protein.

Gray and Bushnell (*10*) have studied the biosynthetic activities of a number of Ascomycetes and Basidiomycetes which included various species of mushrooms. Species of a number of genera of mushrooms have been grown successfully in submerged culture (*3, 5, 11–13, 15, 16, 19–21*). However, only *Agaricus campestris* (*11, 13, 19*), *A. blazei* (*2*), *Lepiota rachodes* (*19*), *Coprinus comatus* (*8*), and *Tricholoma nudum* (*15, 16*) of the Basidiomycetes, and *Morchella* (*17, 20, 21*) species of the Ascomycetes have been reported to have a satisfactory flavor. Of the *Morchella* spp. that have been studied in the authors' laboratory, *Morchella hortensis*, *M. esculenta*, and *M. crassipes* were found to have a desirable aroma and flavor. Some of the factors affecting the growth of these organisms in submerged culture have been investigated, and the results of this work are presented in this paper.

Materials and Methods

Cultures. The stock cultures of *Morchella crassipes* and *M. esculenta* were obtained by culturing spores from the ascocarps of those organisms as collected from natural habitats. These cultures were maintained on a synthetic medium and on a tryptone–glucose–yeast extract medium (TGYE) whose compositions are shown in Table I. Slants were inoculated with a portion of the mycelium, at least 0.5 sq. cm., to minimize the probability of selecting variant types. The synthetic agar cultures were incubated for 6 days and the TGYE cultures for 4 days at 25° C. and then were stored at 4° C. Fresh transfers were made every 2 weeks.

Cultural Procedures. The media used for shake flask cultures are shown in Table II. Glucose was autoclaved separately to prevent excessive browning which occurred when all ingredients were autoclaved together. Flasks were in-