in a closed chamber containing a few crystals of iodine. The presence of the lipids (Table I) is usually revealed within a few minutes as characteristically colored zones (brown or yellow). The precise coloration appears to vary with the acidity or alkalinity of the dry paper. Inspection under ultra-violet light facilitates the detection of feebly staining compounds.

Alternatively, the dried papers may be sprayed or dipped in a 0.2% solution of iodine in petroleum ether or diethyl ether and the excess iodine allowed to volatilize in a gentle draught of air.

The use of iodine has been previously reported for the detection of certain steroids[4,5].

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Correlation of ion exchange column and equilibration experiments under non-ideal conditions

In devising separative procedures involving ion exchange chromatography, an investigator is ordinarily faced with the problem of monitoring effluent from the column. Since many experiments may be necessary to delimit conditions and to check on completeness of recovery, apparatus for automatic monitoring of column effluent is necessary for efficient operation. This represents a severe disadvantage, particularly for the investigator who desires to consider only a single separation in the course of other work and who may not have the necessary monitoring equipment readily available. The problem would be somewhat simplified if the initial experiments could be performed using a batchwise equilibration technique with these results applied to design of a column separation.

Procedures for correlating column and equilibration experiments have been presented; however, they refer to exchange reactions involving only monovalent ions in which the influent ion is present only in trace concentrations, allowing the approximation of ideal behavior both in solution and resin phases to be made. For practical purposes it is often necessary to perform separations of polyvalent ions in concentrations too high to be considered ideal. This paper reports the application of data obtained in shaking experiments to column experiments involving divalent ions in concentrations up to 0.5 M without any attempt to calculate activity coefficients, to determine to what extent failure of ideal behaviour would distort the results. The exchange of lead(II) nitrate in 2 N nitric acid with a cation resin in the hydrogen from has suitable elution characteristics.

Apparatus and reagents

Ion exchange resin. Dowex 50-X12, 100–200 mesh, analytical grade cation resin was used. This resin was obtained from Bio-Rad Laboratories, Berkeley, Calif., and had been washed repeatedly with acid and alkali and finally converted to the hydrogen form. It contained approximately 45% water and had a capacity of 5.01 mequiv. per oven-dried gram. The void space in water averaged 48%, the density 0.46 g/ml.

Standard lead solution. Lead solutions were prepared from analytical reagent grade $Pb(NO_3)_2$ and were standardized by precipitation of $PbSO_4$ or by amperometric titration with $K_2Cr_2O_7$ in acetic acid–sodium acetate buffer, using a D. M. E. at an applied voltage of zero *vs.* S. C. E.

Other reagents. Other reagents, with one exception, were analytical grade and were used without further purification. Gelatin, for maximum suppression in polarographic determination of lead, showed no blank.

Polarograph. A Sargent Model XXI, pen-recording polarograph and a Sargent "Ampot" amperometric titrimeter were used for lead determinations. Polarographic determinations were carried out using a controlled head of mercury above the D. M. E. and controlled temperature to allow use of a calibration curve.

Experimental

Equilibration experiments. Equilibrations were performed by shaking 1 g (wet basis) resin samples with 25-ml volumes of solutions of initially known composition for one hour. Longer equilibration periods up to 24 h showed no significant change. Resin moisture content was determined for each series of experiments by drying separate samples at 110° for 48 h. Supernatant solutions were analyzed for lead polarographically and for acid by titration. The number of equivalents of lead and hydrogen on the resin at equilibrium was determined by difference. All experiments were performed at 22° ambient temperature.

Column experiments. The columns used varied from 2.0 to 7.6 ml volume, and from 2.3 to 10.5 cm in length. In each case, the volume of influent lead solution was 1 ml, followed by elution with 2.00 N HNO$_3$. Flow rate, determined by the resistance of the column packing, varied from 1.5 to 3.2 cm/min. Columns were prepared by introducing resin samples in a water slurry to exclude air, but without other precautions to avoid channeling. Void space was determined by measuring the volume of water required to elute chromate ion from a column of known volume. Elution of lead from columns was followed polarographically, utilizing a cell that permitted introduction of the polarograph capillary directly into the effluent stream[1]. Data from column experiments showed no significant abnormal variation that could be attributed to variation in column dimensions or flow rate; accordingly, they are presented without reference to these factors.

Discussion

KRAUS AND MOORE[2] describe the movement of a band on an exchange column in terms of an elution constant: $E = dA/V$, where d is the distance moved by the band, A is the cross-sectional area of the column and V is the volume of elutrient. If correction is made for the void space between the particles of resin, the experimentally determinable E may be related to the distribution coefficient, D: $E = 1/(D + i)$, where i is the void fraction of the column. In performing a shaking experiment, the distribution of metal between solution and resin phases may be described by the coefficient K_d (moles per gram of resin (dry basis)/moles per ml of solution) which is related to D: $D = K_d \rho$, where ρ is the density of the resin.

The distribution coefficient for a polyvalent ion in an exchange reaction is a function of the concentration of the ion, even under ideal conditions. Therefore, correlation of column and shaking experiments involving a two-to-one exchange requires that the concentration of the influent ion in the aqueous phase of the resin be estimated, taking into consideration influent concentration, column dimensions, void fraction of the resin and the spreading of the band as it moves along the column. This estimation can be made by considering the exchange as an equilibrium reaction. For the exchange reaction:

$$\text{Pb}^{++} + 2\,\overline{\text{H}^+} = \overline{\text{Pb}^{++}} + 2\,\text{H}^+,$$

(symbols carrying bars indicate ions held on the resin), the equilibrium quotient, K_c: $K_c = (\overline{\text{Pb}^{++}})(\text{H}^+)^2/(\text{Pb}^{++})(\overline{\text{H}^+})$, may be expressed in terms of molar concentrations of ions in solution and of equivalent fractions of ions in the resin phase. If K_c remains constant with changing concentration of lead, distribution coefficients for the actual conditions of the column may be calculated. To check the validity of the approximation, a series of equilibrations was performed, holding the weight of resin, volume of solution and concentration of nitric acid constant and varying
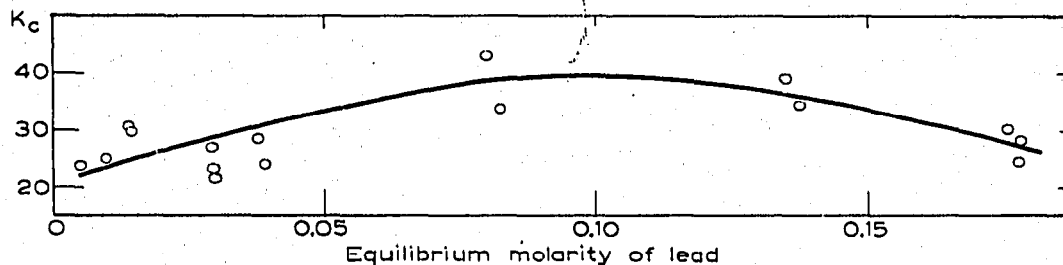

Fig. 1. Effect of change of Pb(II) concentration on K_c.
25 ml of 2.00 N HNO$_3$, 0.55 g resin (dry basis).

the amount of lead. The results shown in Fig. 1 indicate that only a small change in K_c occurs when the concentration of lead is changed. On this basis, a K_c value of 30 was used to calculate the distribution coefficients of the second column of Table I. These calculations were made by considering a 1-ml segment of the ion exchange column, which would contain 0.48 ml of the aqueous phase and 0.46 g (dry basis) of resin, to be filled with the influent solution of specified concentration. Calculated values may be compared with analogous D values obtained by direct column measurement, shown in the third column of Table I. These values are, with one exception, averages of results of several experiments. To indicate the magnitude of experimental error, absolute values of

TABLE I

DISTRIBUTION COEFFICIENTS FOR LEAD(II) ON DOWEX 50 IN 2.00 HNO_3

Initial lead molarity	D calc. from equilibration measurements	D from column measurements	Av. deviation of column measurements
0.004	8.6	9.0	—
0.025	8.5	7.6	0.8
0.05	8.3	8.5	0.7
0.10	8.0	8.4	0.1
0.20	7.4	7.2	0.4
0.30	6.9	6.2	0.3
0.40	6.1	5.5	0.2
0.50	5.6	5.4	0.8

deviation are shown. The agreement between results for column and equilibration experiments over a fairly wide range of concentrations indicates that one should be able to devise ion exchange separation procedures on the basis of shaking experiments without having to resort to monitoring the effluent from columns.

For a column of 1 cm^2 cross-sectional area and 10 cm length, the distribution coefficients of Table I correspond to elution volumes varying from 57 to 94 ml. This illustrates the fact that when a separation is based on successive elution of ions with only slightly different characteristics, e.g., separation of magnesium from calcium by elution with strong acid, the effect of change of concentration may become important.

Summary

To evaluate the correlation of ion exchange column operation with batchtype equilibration operation under non-ideal conditions, the system $Pb(NO_3)_2$–HNO_3–Dowex 50 was studied. Distribution coefficients based on equilibration experiments were calculated and compared with distribution coefficients obtained by direct measurement from column operation. Sufficiently close agreement was obtained between the two types of experiments to suggest the feasibility of making preliminary investigation of ion exchange chromatographic separations by simple shaking experiments, rather than by more involved column experiments.

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