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GEL PERMEATION CHROMATOGRAPHY OF TRANSITION METAL IONS USING BIO-GELS

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SUMMARY

Batch equilibrations of copper, palladium and platinum salts with Bio-Gel P-2 yielded adsorption isotherms of the Langmuir type. Distribution coefficients, equilibrium constants and enthalpy data, determined from elution profiles and adsorption isotherms, showed the interaction of palladium and platinum complexes with the gel to be much stronger than that of copper salts.

Kinetic data were obtained from breakthrough curves of these salts through Bio-Gel P-2. It was found that in all instances adsorption proceeds at a much higher rate than desorption. The rate-determining step in the chromatographic process was found to be the detachment of solute molecules from the functional groups of the gel.

INTRODUCTION

Transition metal ions are known to migrate through polyacrylamide gels (Bio-Gels) in discrete bands with sharply defined contours. The distribution coefficients of these elements with tightly crosslinked Bio-Gels are invariably greater than unity[1] and thus much higher than those of the pre-transition metal ions, which indicates that specific interactions take place with the functional groups of the gel matrix.

It is particularly striking that the anionic complexes of the second- and third-row transition metals interact with Bio-Gels much more strongly than first-row transition metal salts. The purpose of this work was to examine and compare the migration patterns of a first-row transition metal salt ($CuCl_2$), a second-row anionic complex (K_2PdCl_4) and a third-row anionic complex in two oxidation states (K_2PtCl_4 and K_2PtCl_6).

In order to obtain the necessary structural information, adsorption isotherms based on equilibrium measurements were used as a background for determining equilibrium constants and enthalpy data, while kinetic data (obtained from breakthrough curves) were used to elucidate the mechanism of adsorption and desorption.

EXPERIMENTAL

Materials

Bio-Gel P-2 and P-4 (Bio-Rad Labs., Richmond, Calif., U.S.A.), 200–400 mesh, were left to swell for 24 h in 1 *N* hydrochloric acid. In the course of that period, the liquor was decanted several times in order to remove any fines, and when swelling was completed the gel was deaerated with the aid of a water pump.

All of the salts and acids used in the preparation of the eluent solutions were of reagent grade. The metal complexes K_2PtCl_4, K_2PtCl_6 and K_2PdCl_4 were used as supplied by BDH, Poole, Great Britain. Solutions of metal complexes were prepared in 1 *N* hydrochloric acid.

Determination of column parameters and distribution coefficients

The columns, 30.0 × 1.0 cm glass tubes fitted with sintered-glass discs, were wet-packed with gel to a total bed volume (V_t) of 30 ml.

The column void volume, V_0, was determined as described elsewhere[2] with the aid of Blue Dextran (Pharmacia, Uppsala, Sweden), the molecular weight of which is above 10^6 and which is completely excluded from the stationary phase.

The inner volume, V_i, of the gel beads was determined by injecting 0.1 *N* sodium chloride solution and eluting it with distilled water. The concentration of the salt in the effluent was determined by potentiometric titration with 0.1 *N* silver nitrate solution.

The column parameters thus determined were as follows: for Bio-Gel P-2, $V_t = 30$ ml, $V_0 = 11.80$ ml and $V_i = 13.62$ ml; for Bio-Gel P-4, $V_t = 30$ ml, $V_0 = 11.3$ ml and $V_i = 15.1$ ml.

Distribution coefficients, K_D, for the metal complexes in hydrochloric acid solution were calculated from the equation

$$K_D = \frac{V_e - V_0}{V_i} \tag{1}$$

where V_e is the elution volume of the metal complex.

The columns were loaded with 4-ml portions of $5 \cdot 10^{-3}$ *M* solutions of the three metal complexes and of $5 \cdot 10^{-3}$ *M* solutions of $CuCl_2 \cdot 6H_2O$, and eluted with 1 *N* hydrochloric acid. The flow-rate was maintained at 18 ml/h.

For determining the dependence of K_D on the concentration of the K_2PtCl_4 solution, $2.4 \cdot 10^{-3}$, $4.8 \cdot 10^{-3}$ and $7.2 \cdot 10^{-3}$ *M* K_2PtCl_4 solutions were eluted with 1 *N* hydrochloric acid.

Breakthrough curves

For the breakthrough experiments, columns of dimensions 20.0 × 1.0 cm were used. The total bed volume was 16.45 ml and the flow-rate was maintained at 24 ml/h.

The columns, packed as described earlier, were continuously loaded with $5 \cdot 10^{-3}$ *M* solutions of K_2PtCl_4, K_2PtCl_6, K_2PdCl_4 and $CuCl_2 \cdot 6H_2O$. Feeding was continued until the metal concentration in the eluent was equal to that in the feed solution. K_2PtCl_4 solutions of concentration $2.5 \cdot 10^{-3}$ and $5 \cdot 10^{-4}$ *M* served for

determining the concentration dependence of the breakthrough curve shape, while the temperature dependence of the breakthrough curves was established for K_2PtCl_4 in a heated column at 22°, 33°, 41° and 51.5°.

Determination of adsorption isotherms by batch experiments

A 1.0-g amount of dry Bio-Gel P-2 was left to swell for 24 h in 5 ml of 1 *N* hydrochloric acid in 50-ml erlenmeyers flasks fitted with glass stoppers. Batches of Bio-Gel P-4 were prepared in the same way, except that the gel was swollen in 10 ml of the solvent.

To the swollen gels were added 5-ml portions of complex solutions with metal concentrations in the range 5–60 ppm. These batches were left to stand for a few days at room temperature and shaken occasionally. When equilibrium was reached, 1.0-ml aliquots were taken from each batch and the equilibrium metal concentration in solution was determined.

The amount of metal complex adsorbed on the gel was calculated from the equation[3]

$$Q_i = Q_t - V_0 C_0 \tag{2}$$

where

Q_i = amount adsorbed on the gel at equilibrium (mmole/g);
Q_t = total amount of metal complex;
C_0 = equilibrium concentration of the complex in solution (mole/l); and
V_0 = batch void volume.

Batch experiments were also carried out at elevated temperatures (in the range 30–50°) in order to determine enthalpy data. The batches (prepared as described above) were placed in a thermostat shaker (New Brunswick Scientific Co.) for 24 h and aliquot portions were then taken as described above.

The batch void volumes, V_0, were again determined with 0.1% Blue Dextran solution and found to be 7.8 and 8.03 ml per gram of gel for Bio-Gel P-2 and P-4, respectively.

Measurements

Metal concentrations in the effluent fractions and in the batch aliquots were determined with a Perkin-Elmer 403 atomic-absorption spectrophotometer fitted with a hollow-cathode lamp.

RESULTS AND DISCUSSION

An insight into the nature of the interaction between Bio-Gels and transition metal ions was gained from the thermodynamic and kinetic measurements. For the thermodynamic study, three types of experiments were performed: batch equilibrations, elution profiles and temperature-dependence experiments, and they are dealt with in that sequence below.

Batch equilibrations

Batch equilibrations were carried out at 22° for four different salts. The

graphical correlation of Q_i (solute concentration in the gel phase in millimoles per gram) *versus* C_0 (solute equilibrium concentration in the contacting solution in moles per litre) is presented in Fig. 1.

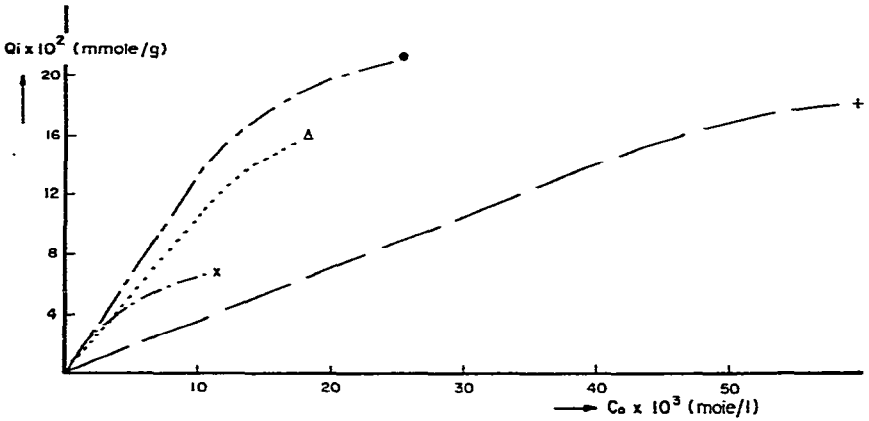


Fig. 1. Equilibrium adsorption isotherms of transition metal complexes on Bio-gel P-2. ● = K_2PdCl_4; △ = K_2PtCl_4; × = K_2PtCl_6; + = $CuCl_2 \cdot 6H_2O$.

The convex shape common to all of these graphs conforms with the Langmuir adsorption isotherm[4], which can also be plotted in a linear form by applying the following equation to the equilibrium condition:

$$Q_i = \frac{KaC_0}{1 + KC_0} \tag{3}$$

where a is the total adsorption capacity (mmoles/g) and K is the Langmuir equilibrium constant [(mole/l)$^{-1}$]. The linear form of the Langmuir equation is

$$\frac{1}{Q_i} = \frac{1}{a} + \frac{1}{KaC_0} \tag{4}$$

and Fig. 2 shows a linear plot for the four salts dealt with in Fig. 1.

From the slope and the intercept of the straight lines obtained, the equilibrium constant, K, was calculated. The results are summarized in Table I.

Elution profiles

Further evidence that the adsorption was of the Langmuir type was obtained from the elution profiles of these salts using 1 *N* hydrochloric acid as eluent (the same as in the equilibrium measurements). Fig. 3 shows the elution profile of K_2PtCl_4; elution profiles of the other three salts were established in the same manner.

The sharp-front boundary of the curve and its tailing off towards the end of the elution is characteristic of the Langmuir isotherm. Distribution coefficients (K_D) were calculated from the elution, void and inner volumes of these curves, and are summarized in Table I.

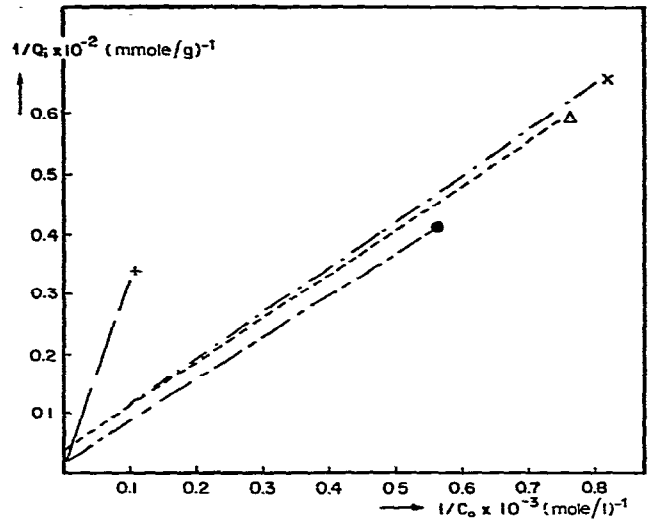


Fig. 2. Linear form of equilibrium adsorption isotherms illustrated in Fig. 1 (eqn. 4). ● = K_2PdCl_4; △ = K_2PtCl_4; × = K_2PtCl_6; + = CuCl ·6H O

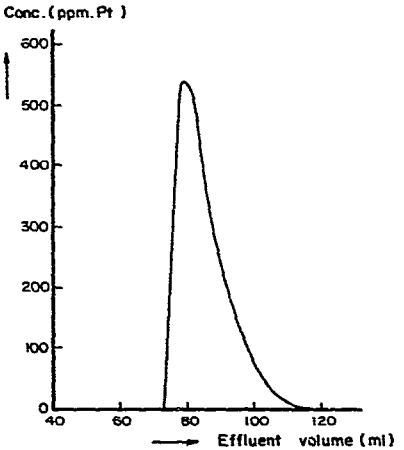


Fig. 3. Elution profile of K_2PtCl_4 on Bio-Gel P-2 using 1 *N* HCl as eluent.

TABLE I

EQUILIBRIUM AND DISTRIBUTION CONSTANTS ON BIO-GEL P-2 AT 22°

Solvent: 1 *N* hydrochloric acid.

Compound	$K[(mole/l)^{-1}]$	K_D
$CuCl_2 \cdot 6H_2O$	6.7	1.65
K_2PdCl_4	28.8	5.5
K_2PtCl_4	55	6.6
K_2PtCl_6	52	7.4

These results indicate that the interaction of platinum and palladium with the gel is much stronger than that of copper. Further, there is no difference between the strengths of interaction of $PtCl_4^{2-}$ and $PtCl_6^{2-}$. The smaller values of K_D compared with K indicate that non-equilibrium conditions exist in the column. K_D was also found to decrease as the solute concentration increased, which also supports the assumption of Langmuir adsorption.

Temperature dependence

Batch experiments with the four salts listed in Table I were carried out at 22°, 33°, 41° and 51.5°. A graphical example of the effect of temperature on solute concentration in the gel and solution phases is given for K_2PtCl_6 in Fig. 4.

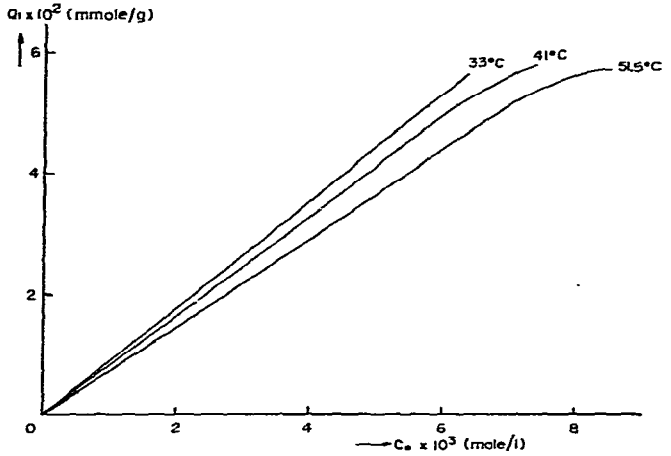


Fig. 4. Equilibrium adsorption isotherms of K_2PtCl_6 on Bio-Gel P-2 at elevated temperatures.

The convex shape of the Langmuir adsorption type is preserved at elevated temperatures. It is interesting that the elution profiles of these solutes on Bio-Gel P-2 showed a marked decrease of the tailing-off phenomenon as the temperature increased.

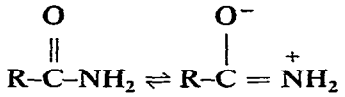
From the temperature dependence data of Q_1 and C_0, the equilibrium constant at different temperatures could be calculated from eqn. 4, and ΔH could then be determined from the graphical correlation of $\ln K$ *versus* $1/T$. These results are presented in Table II.

TABLE II

EQUILIBRIUM CONSTANTS AND ENTHALPY DATA

Temperature (°C)	*K [(mole/l)$^{-1}$]*			
	$CuCl_2 \cdot 6H_2O$	K_2PdCl_4	K_2PtCl_4	K_2PtCl_6
22	6.7	28.8	55	52
33	6.6	20	25	28
41	6.2	16.7	18.2	18.2
51.5	5.8	10	16.6	15.4
ΔH (kcal/mole)	−1.4	−6.0	−7.4	−7.8

The enthalpy data in Table II indicate an exothermic solute–gel interaction that is much stronger with the anionic complexes of palladium and platinum than with copper(II) chloride. The strength of the interaction of platinum and palladium with the gel is in the range 5–10 kcal/mole, which is known to be the energy range for weak electrostatic interactions[5]. Pecsok and Saunders[6] stated that the amide ligand in Bio-Gels can exist simultaneously in two resonance forms:



It is therefore reasonable to assume that the gel phase can act as a charged double layer and attract the anionic complexes by weak electrostatic interactions.

Kinetic measurements and breakthrough curves

From results in equilibrium systems it was deduced that the adsorption is of the Langmuir type, a Langmuir-type isotherm being assumed to exist at equilibrium when the rate of adsorption is determined by chemical effects rather than controlled by diffusion. The chromatographic process, described by Thomas[7], shows the difference between adsorption and desorption rates, and the ratio of the rate constants is the equilibrium constant:

$$K = \frac{k_a}{k_d} \tag{5}$$

where k_a is the adsorption rate constant (ml/mmole·min) and k_d is the desorption rate constant (min^{-1}).

The numerical values of K have already been determined from the linear form of the Langmuir isotherm for the four salts considered (Table I). The adsorption rate constant, k_a, for these salts can be derived from the slope of a saturation (breakthrough) curve at a low flow-rate, as expressed in the equation

$$\text{Slope at mid-point} = \frac{1}{4} \cdot \frac{k_a C_0}{F} \tag{6}$$

where C_0 is the solute concentration passing through the column and F is the flow-rate (0.4 ml/min).

Fig. 5 shows a breakthrough curve of K_2PtCl_4 in 1 N hydrochloric acid on Bio-Gel P-2. From the slope at the mid-point of that curve, k_a can be accurately determined. Similar curves were obtained for K_2PtCl_6, K_2PdCl_4 and $CuCl_2$. Once k_a has been determined experimentally, k_d can be calculated with the aid of eqn. 5 using the K values obtained from equilibration experiments (Table I). The results are summarized in Table III.

In order to characterize fully the migration mechanism inferred from the kinetic data, one must determine whether pore diffusion or chemical interaction is the rate-determining step. Several arguments appear to support the assertion that the latter mechanism is rate determining, as follows. The adsorption process can be regarded as consisting of two steps:

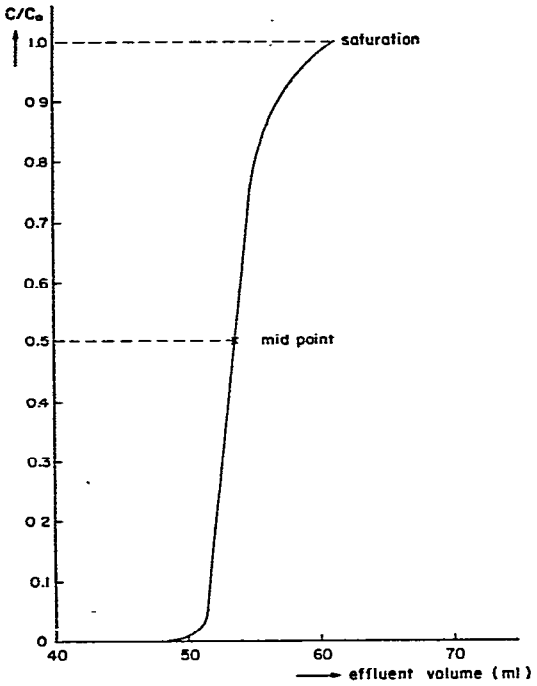


Fig. 5. Breakthrough curve for K_2PtCl_4 on Bio-Gel P-2.

TABLE III

EQUILIBRIUM COEFFICIENTS AND RATE CONSTANTS ON BIO-GEL P-2 AT 22°

Solvent: 1 *N* hydrochloric acid.

Compound	K_{eq} $[(mole/l)^{-1}]$	k_a $(ml/mmole \cdot min)$	k_d (min^{-1})
$CuCl_2 \cdot 6H_2O$	6.7	77	11.6
K_2PdCl_4	28.8	77	2.7
K_2PtCl_4	55	78	1.4
K_2PtCl_6	52	78	1.5

(1) solute diffusion into the inner volume of the gel beads and

(2) capture of solute molecules by the functional groups of the gel matrix.

Desorption, on the other hand consists of:

(3) release of solute molecules by the gel matrix, followed by

(4) diffusion of solute molecules out of the gel into the mobile phase.

As can be seen from Table III, adsorption proceeds at a much higher rate than desorption and is equal for all solutes. Hence there is no selectivity in the adsorption process of small molecules, and the rate-determining step should be sought in the desorption process.

Hall *et al.*[8] stated that, when pore diffusion is the rate-controlling step, the shape of the breakthrough curve is strongly affected by variations in the concentration

of the influent solute. Breakthrough curves obtained with solute concentrations twice and ten times lower than the $5 \cdot 10^{-3}$ *M* solutions used in the main experiments showed no change in the slope of the curves, which supports the assumption of a non-diffusional mechanism.

Rate constants for Bio-Gel P-4, determined with K_2PtCl_4 at 22°, were as follows: $k_a = 118.5$ ml/mmole·min, which is higher than k_a on Bio-Gel P-2, thus indicating a faster diffusion of small molecules into a matrix of higher inner volume and $k_d = 2.6$ min^{-1}, showing a slightly weaker electrostatic interaction between the functional groups of the gel and the anionic complex of the platinum.

Whether chemical bond breaking (step 3) is rate determining is best ascertained by measuring rate constants at elevated temperatures. These measurements were carried out for K_2PtCl_4 and are summarized in Table IV.

TABLE IV

RATE CONSTANTS OF K_2PtCl_4 ON BIO-GEL P-2 AT ELEVATED TEMPERATURES

Temperature (°C)	$K_{eq.}$ *[(mole/l)$^{-1}$]*	k_a *(ml/mmole·min)*	k_d *(min^{-1})*
22	55	77.8	1.4
33	25	83	3.3
41	18.2	94.8	5.2
51.5	16.6	119.5	7.2

It can be seen from Table IV that k_d is strongly influenced by temperature whereas k_a, which is probably diffusion controlled, is not so sensitive to temperature. The calculation of activation energies on the basis of these data showed that E_a for desorption is 11.3 kcal/mole, whereas E_a for adsorption was only 2.9 kcal/mole. These results lead to the conclusion that the detachment of the solute molecules from the functional groups of the gel is the slowest of the four steps in this chromatographic process.

CONCLUSION

An attempt was made in this work to determine experimentally some fundamental thermodynamic and kinetic parameters in gel permeation chromatography. Most of the theoretical work in gel permeation chromatography so far has been directed towards the determination of separation factors and similar parameters connected with the dynamics of chromatography. It was therefore felt to be necessary to determine equilibrium and rate constants in order to obtain further structural information. The measurements described, it is suggested, can also be used to gain a better understanding of the interaction of small molecules with Biogels.

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