CLINICAL CHEMISTRY OF THE TIN-INDIUM GENERATOR

P. Penkoske,* E. J. Potchen and M. J. Welch
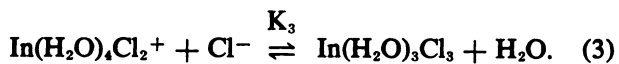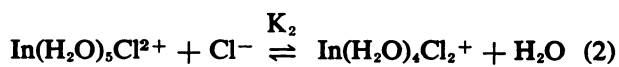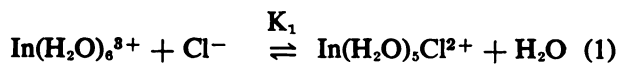
Edward Mallinckrodt Institute of Radiology,
Washington University School of Medicine, St. Louis, Missouri

T. J. Welch

Lindenwood College, St. Charles, Missouri

Indium-113m obtained by the elution of a ^{113}Sn-^{113m}In generator is gaining increasing importance as a general-purpose isotope used in clinical nuclear medicine. Selective scanning agents have been prepared for visualizing the liver, spleen, heart, lung, bone, bone marrow and brain (1–4).

Indium is obtained by eluting an alumina or zirconium oxide column with dilute (0.05 M) hydrochloric acid; the various scanning preparations are prepared by altering the chemical composition of the eluate. Although hydrochloric acid is used as the eluent, the chemical form of the indium eluted is uncertain. Indium (III) forms the following complexes with chloride ion:

$$In(H_2O)_6{}^{3+} + Cl^- \overset{K_1}{\rightleftharpoons} In(H_2O)_5Cl^{2+} + H_2O \quad (1)$$

$$In(H_2O)_5Cl^{2+} + Cl^- \overset{K_2}{\rightleftharpoons} In(H_2O)_4Cl_2{}^+ + H_2O \quad (2)$$

$$In(H_2O)_4Cl_2{}^+ + Cl^- \overset{K_3}{\rightleftharpoons} In(H_2O)_3Cl_3 + H_2O. \quad (3)$$

If one assumes the extreme literature values for the equilibrium constants (5), the eluate composition can vary greatly. Table 1 shows extreme published values of the equilibrium constants K_1 and K_2 together with the eluate composition using 0.05 M hydrochloric acid as the eluting solution (the formation of complex ions higher than $In(H_2O)_4Cl_2{}^+$ has been ignored). This work was undertaken to define the equilibrium constants and therefore to define more clearly the generator eluate composi-

tion as a prerequisite to understanding the chemical manipulations necessary for preparing organ-specific radiopharmaceuticals.

The equilibrium constants were determined by the batch ion-exchange method previously used by Schufle and Eiland (6), and the values of the equilibrium constants obtained in this way were verified by separating the chloride species using an ion-exchange column.

The data from the batch ion-exchange method were analyzed using Schufle and Eiland's simplification of the method of Fronaeus (7) using the following notation:

ϕ = distribution coefficient

$$= \frac{(In^{3+})_R + (InCl^{2+})_R + \cdots}{(In^{3+}) + (InCl^{2+}) + (InCl_2{}^+) + \cdots}$$

$$= \frac{\% \text{ activity in resin}}{\% \text{ activity in solution}} \times \frac{\text{volume solution}}{\text{mass resin}} \quad (4)$$

where $(In^{3+})_R$, $(InCl^{2+})_R$, etc., are the equilibrium concentrations of the metal ion and complex ions in the resin and (In^{3+}), $(InCl^{2+})$, etc., are the equilibrium concentrations in solution (for simplicity the coordinated water molecules have been left from $In(H_2O)_6{}^{3+}$, etc.). The distribution of each complex between resin and solution is given by

$$d_0 = \frac{(In^{3+})_R}{(In^{3+})}; \quad d_1 = \frac{(InCl^{2+})_R}{(InCl^{2+})};$$

$$d = \frac{(InCl^{2+})_R}{(In^{3+})_R (Cl^-)} = \frac{d_1 B_1}{d_0}.$$

B_n = formation constant on the nth complex =

$$\frac{(InCl_n{}^{3-n})}{(In^{3+})(Cl^-)^n}.$$

TABLE 1. ELUATE COMPOSITION USING EXTREME LITERATURE VALUES

K_1	K_2	% In^{3+}	% $InCl^{2+}$	% $InCl_2{}^+$
332	6.5	4.4	72.1	23.5
26	28	24.2	31.5	44.2
26	6.5	36.8	47.9	15.5
332	28	2.4	40.6	57.1

$$\phi_1 = \frac{(d_0/\phi) - 1}{(Cl^-)} \qquad (5)$$

$$f = \frac{d_0/\phi[B_1 - d)(Cl^-) - 1] + 1}{(Cl^-)^2} \qquad (6)$$

$$X = \sum_0^n B_n(Cl^-)^n \text{ where } B_0 = 1.$$

If $\phi = \dfrac{d_0[1 + d(Cl^-)]}{X}$, then $f = B_1\phi_1 - X_2$ where

$X_2 = B_2 + B_3(Cl^-) + B_4(Cl^-)^2$. From Eqs. 4 and 5

it can be shown that $\displaystyle\lim_{(Cl^-)\to 0} \frac{1}{\phi} = \frac{1}{d_0}$ and

$$\lim_{(Cl^-)\to 0} \phi_1 = B_1 - d.$$

From a plot of $1/\phi$ vs (Cl^-), $1/d_0$ can be obtained by extrapolation to the zero concentration of (Cl^-). The quantity $(B_1 - d)$ can be obtained by extrapolation to zero concentration of (Cl^-) on a graph ϕ_1 vs (Cl^-). Using these values one can evaluate the function f for various (Cl^-) values by substitution into Eq. 6.

At low values of (Cl^-)

$$X_2 = B_2 + B_3(Cl^-) \qquad (7)$$

so from Eqs. 5 and 7 the following relationship can be obtained:

$$\Delta f = B_1\Delta\phi_1 - B_3\Delta(Cl^-). \qquad (8)$$

Thus from a graph of $\Delta f/\Delta(Cl^-)$ vs $\Delta\phi_1/\Delta(Cl^-)$, B_1 is obtained from the slope of the line and B_3 as the intercept. Using the value obtained for B_1 the value of X_2 can be calculated for the various concentrations of (Cl^-) by using Eq. 5. From Eq. 7 a graph of X_2 vs (Cl^-) should give a straight line of slope equal to B_3 and intercept equal to B_2.

EXPERIMENTS

Determination of equilibrium constants. The equilibrium constants were determined by the batch ion-exchange method under conditions of excess chloride and excess indium.

Chloride in excess. Ten milliliters of a $1.0 \times 10^{-3}M$ or $1.0 \times 10^{-6}M$ solution of indium (III) containing ^{113m}In (half-life 1.7 hr) as a tracer and various chloride concentrations varying from $1.0 \times 10^{-3}M$ to $1.0 \times 10^{-1}M$ were shaken mechanically with 0.5 gm of oven-dried Dowex 50 W –X 8 resin. The ionic strength of the solution was maintained at 0.16 M using sodium perchlorate and the pH maintained at 2.31. Solutions were shaken for $\frac{1}{2}$ hr because comparative experiments showed equilibrium established after this time. Since indium has been known to adsorb on glass surfaces, a blank

consisting of the identical indium (III) and chloride solution but no resin was also shaken and counted. After shaking, distribution coefficients were determined by radioanalysis of aliquots of the blank solution and the equilibrium solution in contact with resin. The amount of indium on the resin was computed by taking the difference between the activity
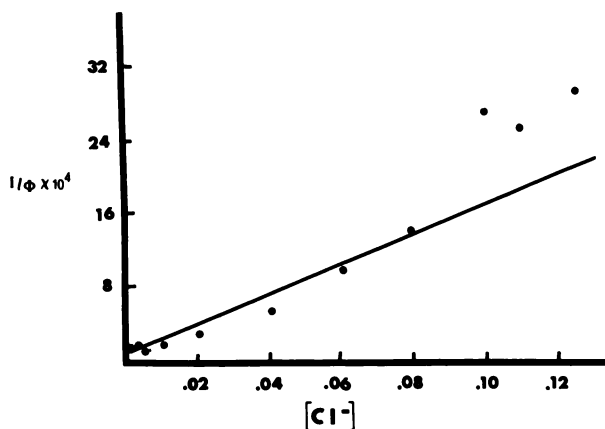

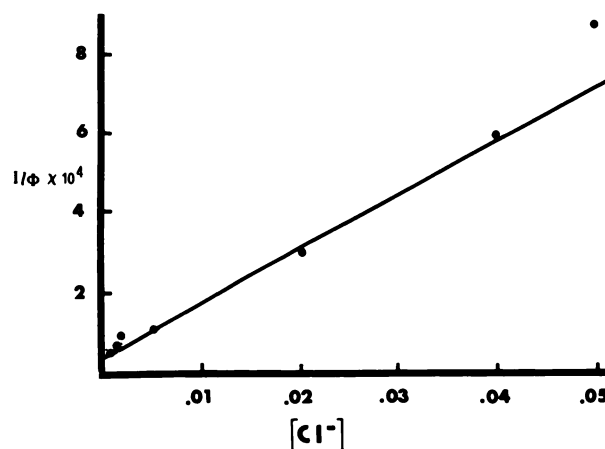**FIG. 1.** Plot of $1/\phi$ against (Cl^-) for case where chloride ion is in excess.

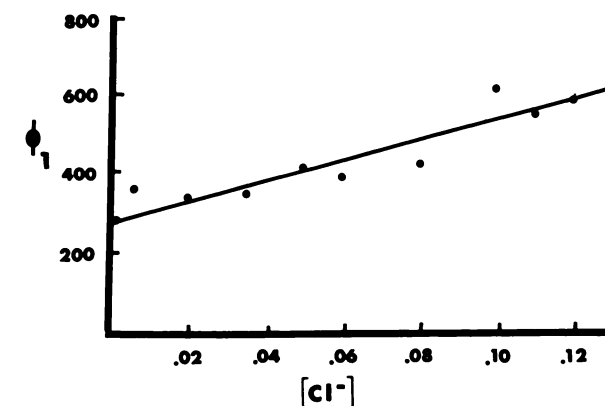**FIG. 2.** Enlargement of lower section of Fig. 1.

FIG. 3. Plot of ϕ_1 against (Cl^-).

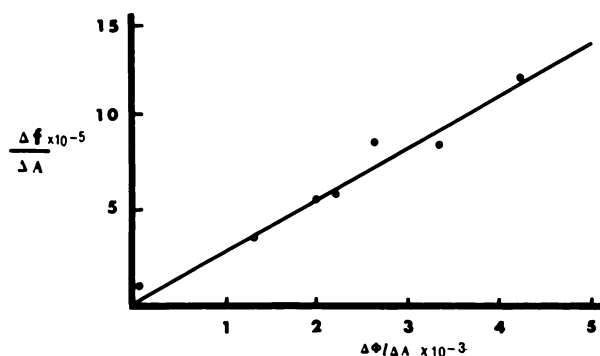in the original solution (represented by the blank) and the activity in the final solution in contact with the resin.

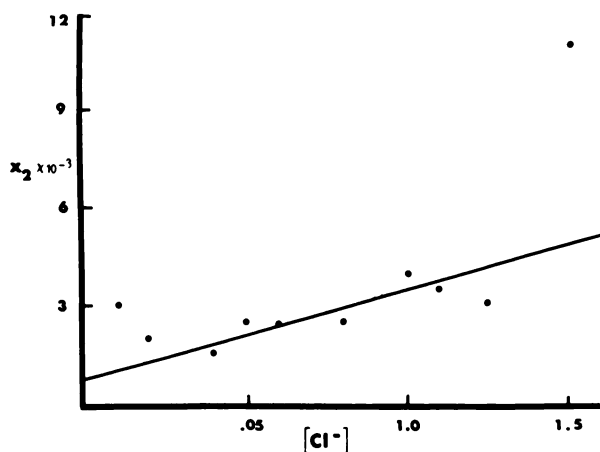**FIG. 4.** Plot of $\Delta f/\Delta A$ against $\Delta\phi/\Delta A$.
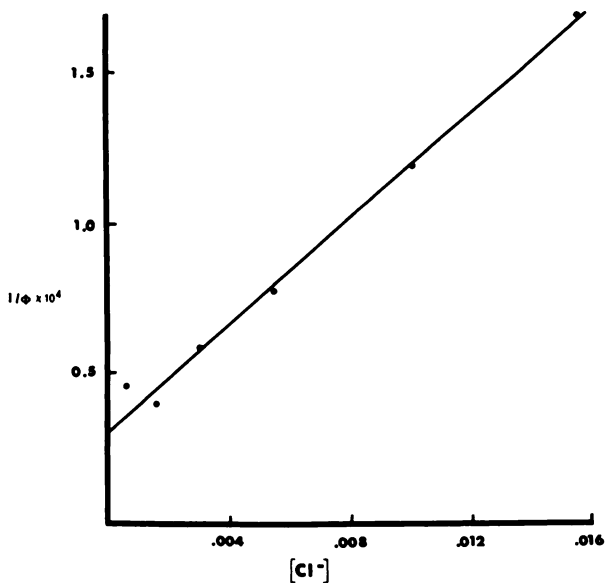

FIG. 5. Plot of X_2 against (Cl⁻).

FIG. 6. Plot of $1/\phi$ against (Cl⁻) for case where indium is in excess.

Indium in excess. Ten milliliters of a 1.5 × 10⁻²M indium (III) solution containing ¹¹³ᵐIn and chloride concentrations varying from 6.0 × 10⁻⁴M to 1.55 × 10⁻²M was shaken with 0.5 gm of oven-dried Dowex 50 W –X 8 resin. The ionic strength was adjusted to 0.16 M by the addition of perchloric acid, and the pH was 1.12. Solutions were analyzed as in the preceding section.

Chromatography. A 30 × 1.5-cm column of Amberlite CG-50 was prepared by rinsing with 2 M HCl and washing with 0.01 M HClO₄ until the pH was below 3.0 and the eluate was chloride-free. A solution consisting of 10 ml of a 1.0 × 10⁻³M indium solution containing ¹¹³ᵐIn in 0.05 M perchloric acid or 10 ml of a 1.0 × 10⁻³M indium solution in 0.05 M perchloric acid and 1.0 × 10⁻¹ in chloride was passed through the column. The eluate was collected in 7-ml portions, and the activity was correlated with that of a standard composed of 0.1 ml of sample solution diluted to 100 ml. Decay corrections were made by measuring the standard at various intervals in the counting of the sample.

RESULTS

Equilibrium constants: chloride in excess. The ϕ was evaluated from the experimental data using Eq. 1. From a graph of $1/\phi$ vs (Cl⁻) (Figs. 1 and 2; enlargement of lower section of Fig. 1) values of ϕ_1 for the various chloride concentrations were obtained by using Eq. 5 and $d_0 = 2.5 \times 10^4$. These values were plotted against their corresponding chloride concentrations (Fig. 3). Extrapolation to zero chloride concentration gave $B_1 - d = 278\ M^{-1}$.

Values of f were calculated using Eq. 6 and from the graph of $\Delta f/\Delta Cl$ vs $\Delta\phi_1/\Delta Cl$; B_1 was obtained from the slope of the line and B_3 as the intercept (Fig. 4).

Substitution of this value of B_1 into $f = B_1\phi_1^-X_2$ permitted the calculation of the various X_2's which when plotted against their corresponding chloride concentration gave a line with slope equal to B_3 and a zero intercept equal to B_2 (Fig. 5).

From these calculations

$$B = K_1 = 277\ M^{-1}$$

$$B_2 = 800 = K_1K_2 \quad K_2 = 2.88\ M^{-1}$$

$$B_3 = 2,600 = K_1K_2K_3 \quad K_3 = 3.7\ M^{-1\cdot}$$

Equilibrium constants: indium in excess. When the indium (III) concentration is in excess of the chloride concentration, it is assumed that no complex except the monochloro complex is formed and the amount of complex ion exchanged on the resin is much smaller than the amount of In(H₂O)₆³⁺ on the resin.

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Equation 4 for the distribution coefficient becomes

$$\phi = \frac{(In^{3+})_R}{(In^{3+}) + (InCl^{2+})}.$$

One can substitute $(In^{3+})_R = d_0(In^{3+})$ and $(InCl^{2+}) = B_1(In^{3+})(Cl^-)$.
Therefore

$$\phi = \frac{d_0(In^{3+})}{[In^{3+} + B_1(In^{3+})(Cl^-)]} = \frac{d_0}{1 + B_1(Cl^-)} \quad (9)$$

rearranging:

$$\frac{1}{\phi} = \frac{1}{d_0} + \frac{B_1(Cl^-)}{d_0}. \quad (10)$$

Figure 6 shows a plot of $1/\phi$ vs (Cl^-). The intercept is $1/d_0$ and the slope is B_1/d_0. B_1 obtained in this manner is 286 M^{-1}. Since the curve in Fig. 6 is linear (to a good approximation), Eq. 10 seems to be valid in this region.

Column chromatography. Using column chromatography it was possible to obtain two peaks from indium chloride solutions, one peak being eluted with 0.01 M $HClO_4$, the other with 0.05 M $HClO_4$. At very low chloride concentrations the activity in the first peak was very small but increased with increasing chloride concentration. It appears that the first peak contains $In(H_2O)_5Cl^{2+}$, $In(H_2O)_4Cl_2^+$, etc., the second peak $In(H_2O)_6^{3+}$.

If $1.0 \times 10^{-3}M$ indium (III) in $HClO_4$ containing $7 \times 10^{-4}M$ chloride was added to the column, the separation obtained is shown in Fig. 7. With the excess indium it is unlikely that ions above $In(H_2O)_5Cl^{2+}$ would be obtained.

Comparing the peak activities to the activity in the sample:

$In(H_2O)_5Cl^{2+} = 16.5 \pm 2\%$ (average of three experiments)

$In(H_2O)_6^{3+} = 82 \pm 5\%$ (average of three experiments)

Therefore $K_1 = \dfrac{(InCl^{2+})}{(In^{3+})(Cl^-)} = 287\ M^{-1}$.

This value for K_1 is in very good agreement with 277 M^{-1} and 286 M^{-1} obtained by the batch method.

As the chloride ion concentration was increased, the size of the first peak increased. With 0.1 M chloride concentration the two peaks were:

$In(H_2O)_5Cl^{2+} + In(H_2O)_4Cl_2^+$ etc. $= 94 \pm 3\%$

$In(H_2O)_6^{3+} = 5.5 \pm 3\%$.

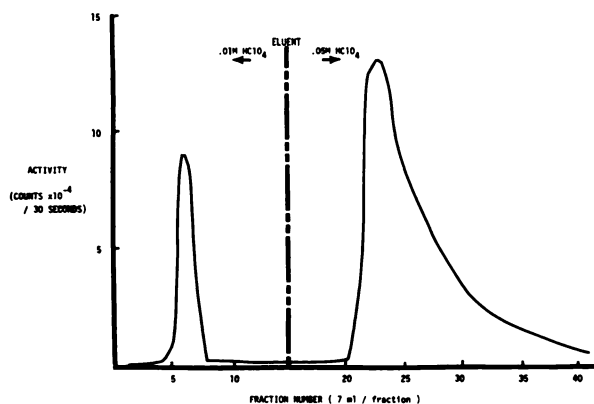Using the equilibrium constants obtained by batch ion-exchange method would expect this mixture to contain:

FIG. 7. Separation of $In(H_2O)_6^{3+}$ from chloride-containing species.

$In(H_2O)_6^{3+} = 2.55\%$

$In(H_2O)_5Cl^{2+} = 70.0\%$

$In(H_2O)_4Cl_2^+ = 20.4\%$

$In(H_2O)_3Cl_3 = 7.0\%$.

DISCUSSION

The values obtained for the equilibrium constants using the batch ion-exchange method agree very well with the separations of the ionic species obtained using column chromatography.

The low value obtained for K_2 is in agreement with the column separation of the 0.1 M chloride mixture. A higher value of K_2 would predict a much lower In^{3+} concentration than experimentally observed.

The equilibrium constants obtained in this way predict the following ionic composition of the eluate of the tin-indium generator using 0.05 M chloride (HCl) as the eluting solution:

$In(H_2O)_6^{3+} = 5.85\%$

$In(H_2O)_5Cl^{2+} = 80.5\%$

$In(H_2O)_4Cl_2^+ = 11.65\%$

$In(H_2O)_3Cl_3 = 2.00\%$.

Addition of further chloride which has been advocated for blood-pool scanning preparations will tend to give a greater percentage of the higher chloride states.

Attempts to analyze the generator eluate by direct column chromatography were unsuccessful, because the chloride-containing peak "tails" for a long time. This tailing is presumably caused by an interaction between the walls of the chromatographic column and the carrier-free indium.

Preparations used clinically for spleen and liver scanning were also separated by column chromatography; the analysis varies considerably from one sample to another, the average values obtained being
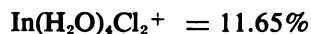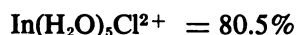
Peak 1 :   4 ±   1.6%

Peak 2 : 60 ± 13%.

Although it is impossible to identify these products, it seems that the major portion of the activity behaves as an ionic species, not as small particles which would be expected to remain on the column.

SUMMARY

Indium-113m is a promising, inexpensive, new broad-spectrum scintiscanning agent. The chemical nature of the indium as obtained from the tin-indium generator has been identified and found to be

$$In(H_2O)_6{}^{3+} \quad = \quad 5.85\%$$

$$In(H_2O)_5Cl^{2+} \quad = 80.5\%$$

$$In(H_2O)_4Cl_2{}^{+} \quad = 11.65\%$$

$$In(H_2O)_3Cl_3 \quad = \quad 2.00\%.$$

This information is an essential prerequisite to meaningful chemical manipulation of the eluate in preparation of organ specific radiopharmaceuticals. Based upon this data, simplified methods using prepared vials are being developed for brain, kidney, lung, liver, spleen, bone marrow and blood-pool scanning agents.

ACKNOWLEDGMENT

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