Determination of Net Ionic Charge on Tc-99m DTPA and Tc-99m EDTA by a Column Ion-Exchange Method

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The net charge on a stable complex ion is conveniently measured at tracer levels by studies of ion-exchange equilibrium. Previous applications to radiopharmaceuticals have used a batch equilibrium method, but such measurements are affected by any radiochemical impurities present. Since technetium pharmaceuticals are often heterogeneous, it is of value to have a technique that is directly applicable to a mixture of different species. Such a method is presented here: a column method to determine the net charge on the technetium-99m complex of diethylenetriaminepenta-acetic acid (DTPA), and also on the principal component in a mixture of species formed by reducing pertechnetate in the presence of ethylenediaminetetra-acetic acid (EDTA). The net charge was calculated from the effect of eluent concentration on retention time. The net charge on Tc-DTPA was found to be −2 at both pH 4.6 and 7.0. The net charge on the Tc-EDTA complex, measured at pH 7.0 only, was also −2.

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Since few chemical properties can be measured at tracer concentrations, the chemical structure of most technetium pharmaceuticals remains unknown. One property measureable at tracer concentrations is net ionic charge; Owunwanne and others (*1,2*) have recently measured the net charge on several technetium pharmaceuticals by an ion-exchange method. Ionic charge is a major determinant of biological distribution (*3*); its establishment helps to identify the oxidation state of technetium and to limit the number of possible structures that need be considered.

Owunwanne and colleagues measured the partition of activity between ion-exchange resin and ambient solution by a batch equilibrium method, calculating net charge from the variation of distribution coefficient with electrolyte concentration (*1,2*). Batch methods have generally been preferred for investigating solution chemistry, since they facilitate attainment of equilibrium, but for radiopharmaceutical applications the alternative column method (*4*) has advantages. Since equilibrium conditions presumably do not obtain in vivo (*5*), it is necessary to study species that are stable kinetically but not thermodynamically, and for this the column method is well suited. Furthermore, many technetium pharmaceuticals are radiochemically impure; they contain multiple components in addition to "free pertechnetate" and "hydrolyzed-reduced technetium" (*6–10*). This poses no problem with the column method, since charge can be determined from the variation of chromatographic retention time with electrolyte concentration for each separate component; the presence of multiple components, or of an irreversibly bound component, does not affect the measurement. With the batch equilibrium method, on the other hand, all components present are included in the measured distribution coefficient. The resulting error in the batch method can be neglected under certain circumstances, but in general the column method appears to be a more convenient tool for the study of heterogeneous mixtures.

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In this article we demonstrate the measurement of net electrical charge on technetium-99m complexes of diethylenetriaminepenta-acetic acid (H_5DTPA) and ethylenediaminetetra-acetic acid (H_4EDTA) using a column ion-exchange method. Tc-DTPA has been widely used as an imaging agent for kidney and brain; Tc-EDTA has recently been proposed as an agent for renal imaging (*11*).

MATERIALS AND METHODS

Labeling procedure. Technetium-99m chelates were prepared from solutions containing 0.1 *M* of the appropriate ligand (H_5DTPA or Na_2H_2EDTA) adjusted to pH 7.0 with NaOH. Five milliliters of this solution were de-aerated for 10 min with nitrogen; 1 μl of 0.5 *M* $SnCl_2$(in 2 *M* HCl, stored over metallic tin) was then added; and the solution was mixed and sealed in a vial under nitrogen. After 10 min, 2.5–25 mCi $Na^{99m}TcO_4$ in 0.1–0.5 ml 0.15 *M* NaCl (from a commercial generator) was added; the solution was mixed and allowed to stand for 10 more min before use. Chromatograms of a commercially available clinical Tc-99m DTPA preparation were so similar to those of the above Tc-99m DTPA preparation that we have no reason to doubt the chemical identity of the products, though peak times were not compared under standardized conditions. As usual in the preparation of technetium pharmaceuticals, an unknown amount of Sn(IV) was present as an impurity, and an unknown amount of Tc-99 as well, up to perhaps ten times the amount of Tc-99m, depending on when the generator was last milked.

Chromatographic procedure. Separations were performed on a 1- × 21-cm column of fibrous diethylaminoethylcellulose* (DEAE-cellulose). Eluent flow was maintained at 0.3 ml/min by means of a peristaltic pump. Preliminary experiments showed that the bed volume was stable over the concentration range employed, and that peak times could be reproduced without hysteresis after equilibration of the column with ten volumes or less of eluent. A small fraction (<5%) of the applied activity was retained permanently on the column, corresponding to the component commonly called "hydrolyzed-reduced technetium." To prevent column degradation by impurities in the eluent, the column was protected by a precolumn of the same material. No bacteriostat was used, and no effort was made to exclude oxygen. An advantage of this method is that even though Tc-EDTA was partially oxidized to pertechnetate by the oxygen present, this could be tolerated without affecting the results.

Sample volumes of 100 μl containing 50–500 μCi of Tc-99m were applied to the column by means of a sample loop constructed from two two-way, four-port valves. A crystal scintillation detector was used, shielded except for a pinhole over which the eluent passed. The elution profile was recorded by means of a logarithmic ratemeter and strip-chart recorder. Though chromatographic elution profiles are seldom displayed on a logarithmic scale because peak areas are then not proportional to total activity, we found this scale advantageous in the present study. Minor peaks are not missed because of inappropriate gain settings, and the amount of activity injected is not critical. With a logarithmic scale, an ideal Gaussian peak becomes parabolic; real peaks, which can often be represented as the convolution of a Gaussian with an exponential, then have a parabolic leading edge and a linear trailing edge (see Fig. 1). In the present work, only retention times were measured, for which the logarithmic display proved quite convenient.

Theory. It can be shown (*12*) that:

$$V_i' = V_i - V_o - V_D = K_i\bar{V}, \qquad (1)$$

where V_i' is the "corrected" retention volume for species i; V_i the measured retention volume for species i; V_o the column void volume; V_D the dead space outside of the column; K_i the molar partition coefficient between exchanger and eluent for species i; and $\bar{V}$ the volume of ion exchanger (excluding void volume). Quantities referring to the exchanger phase will be designated by bars: thus c_i represents the concentration (moles/l) in the mobile phase, $\bar{c}_i$ represents the concentration in the exchanger for species i, and $K_i = \bar{c}_i/c_i$.

At equilibrium, the electrochemical potential for each species is the same in the mobile and exchanger phases (*14*); $\therefore \mu_i = \bar{\mu}_i$, where

$$\mu_i = \mu_i^\circ + RT \ln(\alpha_i c_i) + z_i F\phi. \qquad (2)$$

Here μ_i° is the chemical potential in the reference state of unit activity; R the gas law constant; T the absolute temperature; α_i the activity coefficient; z_i the net charge on species i; F the Faraday constant; and ϕ the electrical potential of the phase. Equilibrium can reasonably be assumed, since analytical columns are usually operated under local equilibrium conditions to optimize resolution. (With resins the flow rate may need to be lowered or the column temperature raised to achieve local equilibrium, but this is not normally necessary with ion-exchange celluloses). Setting electrochemical potentials equal for exchanger and mobile phases, and rearranging, one obtains

$$K_i = \frac{\alpha_i}{\bar{\alpha}_i} e^{-\Delta\mu_i - z_i\Delta\phi}, \qquad (3)$$

where

$$\Delta\mu_i = \frac{\bar{\mu}_i^\circ - \mu_i^\circ}{RT}$$

and

$$\Delta\phi = \frac{F}{RT}(\bar{\phi} - \phi).$$

Equation 3 applies to any species i. If written once for the species of interest, i, and again for a reference species, j, of known charge z_j, the two equations then combined with Eq. 1 and $\Delta\phi$ eliminated, one obtains

$$\ln(V_i') = \frac{z_i}{z_j}\ln(V_j') + \left(1 - \frac{z_i}{z_j}\right)\ln(\bar{V}) + \left(\frac{z_i}{z_j}\Delta\mu_j - \Delta\mu_i\right) + \ln\left[\frac{\alpha_i}{\bar{\alpha}_i}\left(\frac{\bar{\alpha}_j}{\alpha_j}\right)^{z_i/z_j}\right] \quad (4)$$

No assumptions have been made about the nature of the supporting electrolyte or its invasion of the exchanger, so that Eq. 4 holds for mixed electrolytes and in the presence of invasion.

To apply Eq. 4, observe that all terms on the right, with the exception of the first, vary only slowly with concentration. A plot of ln V_i' against ln V_j' therefore approximates a straight line with slope z_i/z_j. The third term is constant by definition of standard state, but concentration-dependence of the second and fourth terms will introduce a small error into the measured value of z_i/z_j. The second term can be eliminated by suitable choice of reference species, since it vanishes when $z_i = z_j$, but it is small in any event: if the exchanger shrinks 10% with a tenfold increase in electrolyte concentration, the calculated error is only 0.04 charge units. The changes of activity coefficients with electrolyte concentration will tend to cancel in the fourth term, particularly if $z_i = z_j$. Errors should thus be minimized by choosing a reference species with charge equal to the species of interest, but in the cases studied they proved small enough to be ignored even without this recourse.

The foregoing treatment is thermodynamically rigorous for a two-phase system, but one must verify by experiment that the two-phase model fits the physical situation. For example, since cellulose is a fairly good adsorbent, it is conceivable that the system might best be represented by a three phase combination of mobile phase, adsorbent phase, and ion-exchange phase. Another possibility is that the binding sites are heterogeneous, corresponding to multiple ion-exchange phases each having different parameters. Such complications do not ordinarily arise with polystyrene resin exchangers, but we have been unable to find in the literature any detailed quantitative studies using DEAE-cellulose, and were therefore compelled to confirm this experimentally.

RESULTS

Chromatography showed only a single component in the Tc-DTPA preparation, but multiple fractions in Tc-EDTA. Elution profiles are shown in Fig. 1, on a semilog scale, which exaggerates minor peaks. The principal secondary peak, identified by retention time, was that for pertechnetate (last peak to right, Fig. 1); the other peaks on the trace for Tc-EDTA could represent

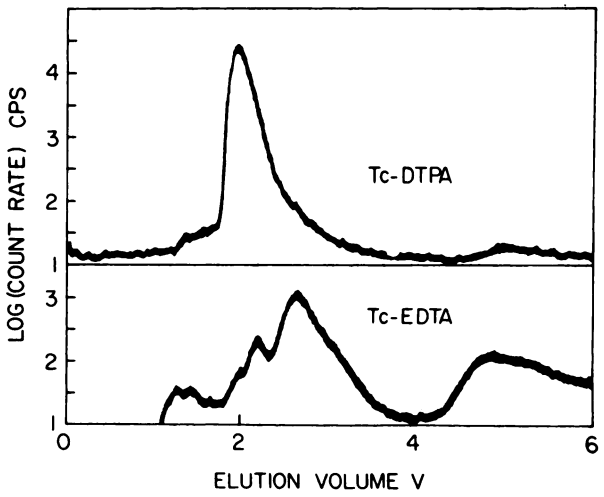

FIG. 1. Chromatographic elution profiles for Tc-DTPA and Tc-EDTA. Uncorrected retention volume is expressed as multiple of bed volume. Conditions: DEAE-cellulose column eluted with 0.15 *M* NaCl, 0.005 *M* NaH_2PO_4, 0.005 *M* Na_2HPO_4 at pH 7.0.

different EDTA complexes of technetium or complexes of technetium with trace impurities in the reagent-grade EDTA. The presence of secondary peaks is desirable for present purposes, since it permits us to demonstrate the usefulness of the column method in studying heterogeneous preparations, though for clinical use a different labeling procedure might be preferred.

The chromatographic retention is plotted against eluent concentration in Fig. 2. Retention volumes are expressed as multiples of the total bed volume for the chromatographic column, which renders them independent of column geometry. In order to calculate net charge, the retention volume must be corrected by the sum of void volume and extracolumn dead space (Eq. 1). This quantity was determined by measuring the retention

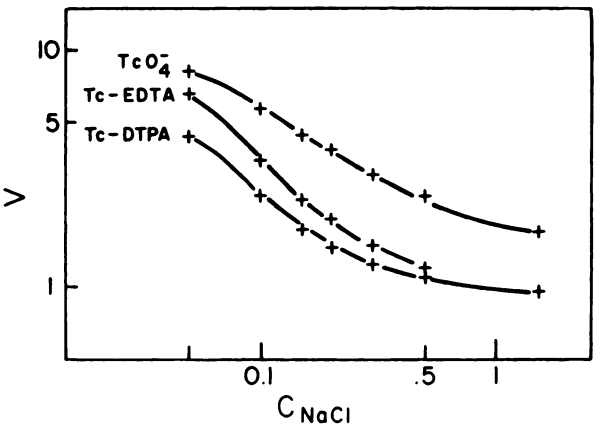

FIG. 2. Effect of electrolyte concentration on chromatographic retention of TcO_4^-, Tc-EDTA, and Tc-DTPA. Uncorrected retention volume (in multiples of bed volume) is plotted, log-log, against NaCl concentration (moles/l). Conditions: DEAE-cellulose column eluted with 0.005 *M* NaH_2PO_4, 0.005 *M* NaH_2PO_4, and variable NaCl concentration, at pH 7.0.

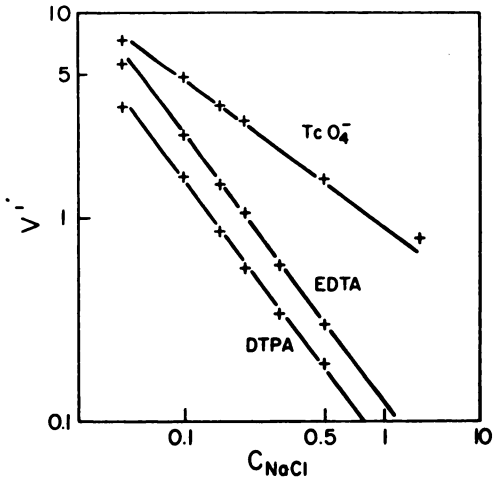

FIG. 3. Effect of electrolyte concentration on corrected retention volume of TcO_4^-, Tc-EDTA, and Tc-DTPA. Corrected retention volume (in multiples of bed volume) is plotted, log-log, against NaCl concentration (moles/l). Conditions as in Fig. 2.

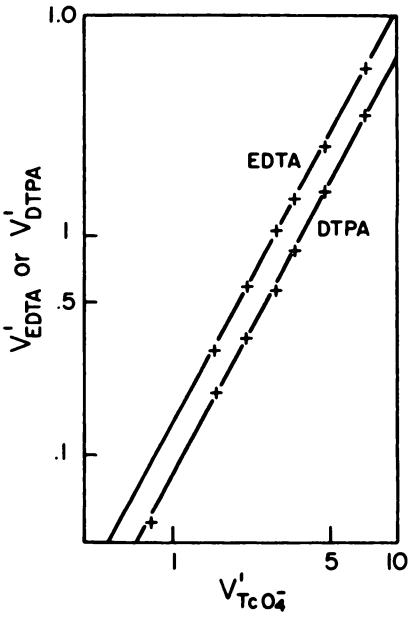

FIG. 4. Corrected retention volume for Tc-EDTA and Tc-DTPA plotted, log-log, against that for TcO_4^-. Conditions as in Fig. 2.

time for several cationic species, using a low salt concentration to ensure Donnan exclusion from the exchanger phase. The change of void volume with salt concentration was assumed negligible. Corrected retention volumes are plotted in Fig. 3.

The data in Fig. 3 are analogous to those obtained by the batch equilibrium method of Owunwanne and others (*1,2*) and thus permit estimation of net charge; the slope of a curve will equal the charge if the effects of the phosphate buffer and of exchanger invasion can be neglected. The presence of the buffer will cause the curves to be concave downward at low concentrations; invasion will cause them to be concave upward at high concentrations; and in the midregion both effects tend to decrease the magnitude of the measured slope. The midregion slope of the TcO_4^- curve in Fig. 3 is −0.72; of the Tc-DTPA curve, −1.33; of the EDTA curve, −1.33. Knowing the charge on pertechnetate, and observing that the slope for the other two curves is about twice that for pertechnetate, it can reasonably be inferred that the net charge on both Tc-DTPA and Tc-EDTA is −2. The deviation from theoretical values is substantial, but is in the direction predicted. Better accord with theory is achieved using Eq. 4, as shown in Fig. 4. The least-squares slope of the curves, which is the negative of the charge on the species, is 1.75 ± 0.15 for Tc- DTPA and 1.92 ± 0.07 for Tc-EDTA. (The error limits are 95% confidence limits calculated by simple linear regression; they serve as an index of experimental scatter but do not include the systematic errors discussed under Materials and Methods. The single point at highest salt concentration was omitted from the regression because of its sensitivity to small errors in the void volume.) The indicated net charge for both species is thus −2.

As an additional check, the data were also interpreted by the method of Marcus and Coryell, as modified by Marcus and Eliezer (*14*). If one uses our V′ values in place of the D values of Marcus, and neglects the change in exchanger volume with eluent concentrations, then the calculated log D° will differ only by an additive constant. The resulting invasion function (*14*) and corrected distribution coefficient (*14*) are plotted in Fig. 5, using activity data from Harned and Owen (*15*) and

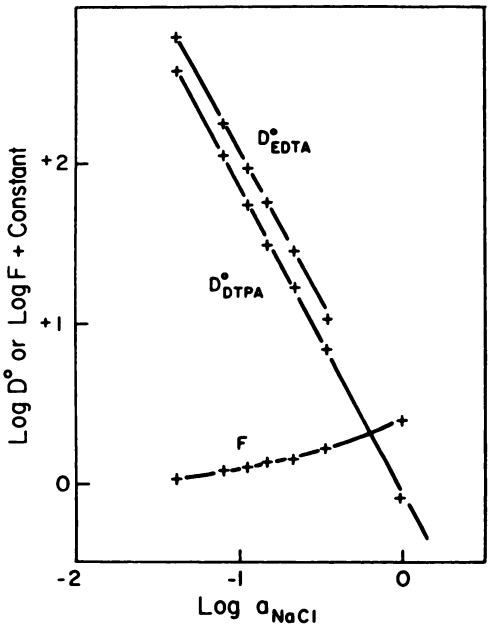

FIG. 5. Calculation of charge by method of Marcus and Eliezer (*14*). Corrected distribution coefficients, D°, for Tc-EDTA and for Tc-DTPA, and invasion function F, are plotted against effective chloride activity a_{NaCl}. Definitions (*14*): $a_{NaCl} = {}^{m}NaCl^{\gamma} \pm NaCl$; log F = log ($a_{NaCl}V'_{TcO_4^-}$) + constant; log D°_{DTPA} = log V'_{DTPA} − 2(log a_{NaCl} · $V_{TcO_4^-}$) + constant.

from Robinson and Stokes as tabulated by Meites (*16*). Slopes of -1.81 ± 0.11 and of -1.85 ± 0.06 were obtained for Tc-DTPA and Tc-EDTA, respectively, again indicating a net electrical charge of -2. This finding therefore agrees with Eq. 4. The two methods differ only in the handling of activity coefficients, and necessarily agree in the ideal case.

We have performed another series of measurements using perchlorate as eluent, buffered with acetate to pH 4.6, for both Tc-DTPA and nitroferricyanide. The measured charge for Tc-DTPA was 1.82 ± 0.03 and for nitroferricyanide 2.07 ± 0.12. This offers a further check on the procedure, since the charge on nitroferricyanide is known independently to be -2.

DISCUSSION

Although DEAE-cellulose is used mainly for biochemical separations, it also fractionates inorganic complexes efficiently (*17*). For example, Kaufman and Keyes used it to separate five thiocyanate complexes of Cr(III) (*18*), and it can also separate Tc-99m pyrophosphate complexes (*8*). The fractionation of a Tc-99m EDTA preparation is shown in Fig. 1. DEAE-cellulose has evident promise for the radiochemical analysis of technetium pharmaceuticals.

When ion-exchange chromatography is used for radiochemical analysis, the net charge on each stable fraction can be deduced from the effect of eluent electrolyte concentration on retention time. Previous measurements of this sort have used ion-exchange resins. The exchange capacity of swollen ion-exchange celluloses is lower than that of resins by nearly an order of magnitude (*17*), so that invasion by the electrolyte should occur at correspondingly lower concentrations (compare our Fig. 5 with Fig. 1 of Ref. *14*). Our method of data reduction takes invasion into account; it also allows for the presence of multiple counter-ions, so that pH buffers can be used unless they drastically affect the activity coefficients in the exchanger. (Preliminary experiments showed the effect of buffer in our system to be slight.) The final results show that DEAE-cellulose is a suitable ion-exchange medium for measurement of ionic charge.

Net ionic charge depends on several factors: the oxidation state of the metal, the inclusion of charged species such as chloride, and the relationship of pH to pK_a values for bound ligand and aquo groups. The principal stable oxidation states of technetium observed by polarography in aqueous complexing media are III, IV, and V (*19*). We assume, from the known chemistry of EDTA complexes (*20*), that all nitrogen atoms are coordinated with the metal; that all unbound carboxyl groups will be ionized at pH 7; and that chloride is absent from the complex, though present in the Tc-99m generator eluate. For oxidation states III, IV, and V, respectively, formulas compatible with the measured net charge are thus $TcOH(EDTA)^{2-}$, $TcO(EDTA)^{2-}$, and $TcO(OH)(EDTA)^{2-}$. (A number of water molecules may be included.) In the case of H_5DTPA, the possible formulas are $Tc(DTPA)^{2-}$, $Tc(OH)(DTPA)^{2-}$, and $TcO(DTPA)^{2-}$.

Only limited data are currently available on the redox chemistry of these chelates. Steigman, Meinken, and Richards have studied the reduction of TcO_4^- by Sn(II) at carrier concentrations in 0.4 *M* DTPA at pH 4 (*21*). Polarography and iodometric titration indicated the formation of Tc(III), whereas potentiometric titration indicated a mixture of Tc(III) and some higher state, presumably Tc(IV). Loberg studied a related complex (Tc-HIDA) at pH 6, and found Tc(III) by iodometric titration (*22*). Gorski and Koch prepared an EDTA complex of technetium by adding $Tc(ClO_4)_4$ to an EDTA solution (*23*), and judged the product to contain the pure species Tc(IV). We interpret these reports as indicating Tc(III) to be the most probable oxidation state, with Tc(IV) less likely. Probable formulas for the two species studied here are thus $Tc(OH)(EDTA)^{2-}$ and $Tc(DTPA)^{2-}$, analogous respectively to the known structures of Ni(II) EDTA (*24*) and Cu(II)DTPA (*25*). Reported metal-oxygen and metal-nitrogen bond distances for technetium (*26*) cause no conflict, and the Slater radii for Tc, Ni, and Cu are the same (*27*). Finding the same charge at both pH 7.0 and 4.6 supports the proposed structure for Tc-DTPA: because an aquo group bound to Tc(III) would probably have a pK_a around 7, as the EDTA complexes of other trivalent metals (*21*). In each case, a number of stereoisomers are possible (*24,25*), but whether this chromatographic procedure would separate stable isomers, or even nonisomeric species of the same charge, is uncertain.

The structure of Tc-DTPA has practical implications for radiopharmaceutical design. If the foregoing inferences are correct, the net charge of -2 can be attributed to two ionized acetate groups, one on each noncentral nitrogen atom. These are not bound to the metal, and can presumably be modified without affecting the stability of the chelate. Thus replacement of these free side arms with methyl, or conversion to ester or amide groups, should result in a stable complex with the zero net charge and lipophilic properties sought by McAfee (*28*) and by Oldendorf (*29*). Another approach would be the replacement of one free arm by a large lipophilic group, leaving the other unmodified, to obtain a hepatobiliary agent (*3*). Such derivatives, like DTPA itself, might bind technetium into stable complexes, radiochemically pure and resistant to oxidation. Thus basic studies of technetium chemistry can relate to tracer design. In this way it may be possible to develop a new class of technetium pharmaceuticals that combine the stability of Tc-DTPA with the specificity of other functional groups.

Technetium-DTPA is notable for its stability and radiochemical purity, compared with other technetium

pharmaceuticals. We chose this compound to validate both the theory and the technique of the column ion-exchange method before studying more complex cases in which chemical reactions may occur. One problem that may prove amenable to this approach is the identification of individual components present in Tc-pyrophosphate bone-scanning agents; these can be fractionated on DEAE-cellulose (*8*), but preliminary data suggest the existence of dissociation equilibria. Since data for Tc-DTPA and Tc-EDTA have been shown to fit Eq. 4, it seems likely that inclusion of appropriate mass-action equations will permit treatment of more complex cases.

Column ion-exchange methods may prove to be of general use in studying technetium pharmaceuticals because: (a) they are among the few methods that can be used at tracer concentrations; (b) the same measurements can be used both for elucidation of solution chemistry and for development of radioanalytical separations; and (c) the preparations used need not be radiochemically pure.

FOOTNOTE

* Baker, chromatographic grade.

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