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MASTER

DEVELOPMENT OF MORE EFFICACIOUS Tc-99m ORGAN IMAGING
AGENTS FOR USE IN NUCLEAR MEDICINE BY ANALYTICAL
CHARACTERIZATION OF RADIOPHARMACEUTICAL MIXTURES

Progress Report
for Period May 1, 1981-April 30, 1982

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ABSTRACT

The objectives of this year's research have been to develop a method for rapidly determining TcO_4^- in $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator eluates, to improve our ability to chromatographically determine individual Tc-HEDP complexes in radiopharmaceuticals, and to investigate the effects of TcO_4^- concentration and electrochemical reduction (instead of chemical reduction) on the types and relative amounts of Tc-HEDP complexes present in a radiopharmaceutical formulation.

A rapid and sensitive high performance liquid chromatographic (HPLC) method for the quantitative determination of pertechnetate (TcO_4^-) has been developed. The lower detection limit under ideal conditions (0.10 M acetate buffer) is 6.00×10^{-8} M TcO_4^- (12.1 ng/mL) while a lower limit of 1.30×10^{-7} M TcO_4^- (26.4 mg/mL) is obtained under conditions (0.03 M acetate buffer) useful for analyzing the eluates of $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators. Tracking of a 7,450 mCi generator through its useful clinical life leads to values of $[\text{TcO}_4^-]$ in the range 10^{-7} to 10^{-6} M. This HPLC-based analysis may be of considerable utility in assessing the history and function of $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators as well as in the routine analysis of reduced technetium radiopharmaceuticals for the presence of undesired TcO_4^- .

A number of chromatographic columns and mobile phases have been evaluated for improving the HPLC separation and determination of Tc-HEDP complexes in radiopharmaceuticals. Encouraging results have been obtained on a dimethyl amine column using aqueous $(\text{NH}_4)_2\text{SO}_4$ as the mobile phase. The capability for gradient elution will be necessary to realize the desired resolution of the individual complexes.

The preparation of $\text{Tc}(\text{NaBH}_4)\text{HEDP}$ radiopharmaceutical analogues using varying concentrations of total TcO_4^- shows a dramatic effect in the number and distribution of Tc-HEDP complexes over a TcO_4^- concentration range of 10^{-2} to 10^{-8} M. These results suggest that total TcO_4^- concentration is an important parameter to be considered in the preparation of a specific Tc-HEDP complex to improve skeletal imaging.

The preparation of $\text{Tc}(\text{electro})\text{HEDP}$ radiopharmaceutical analogues by using electrochemical reduction rather than chemical reduction by Sn^{2+} or NaBH_4 has been explored. The resulting solutions contain Tc-HEDP complexes that are tentatively identified as being the same complexes formed by NaBH_4 reduction, although the relative concentrations of these complexes are quite different with the two modes of reduction. Thus, electrochemical reduction shows promise as a viable route to the preparation of specific Tc-HEDP complexes for improved skeletal imaging.

OBJECTIVES

Objectives for the period May 1, 1981 through April 30, 1982 are as follows:

- (1) Develop an analytical technique capable of detecting total TcO_4^- concentration in eluates from $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators and track the levels of TcO_4^- during the lifetime of a generator operated under typical clinical conditions.
- (2) Evaluation of various column materials and mobile phases in search of a more efficient HPLC determination of Tc-HEDP complexes in Tc-HEDP radiopharmaceutical preparations.
- (3) Evaluate the effect of varying total TcO_4^- concentration on the distribution of Tc-HEDP complexes formed in the $\text{Tc}(\text{NaBH}_4)\text{HEDP}$ radiopharmaceutical analogue to determine the importance of this parameter in the radiopharmaceutical formulation procedure.
- (4) Evaluate the use of electrochemical modes of reduction for preparing Tc-HEDP radiopharmaceuticals to determine if electrochemical reduction generates different Tc-HEDP complexes than chemical reduction.

Determination of Total TcO_4^- in $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ Generator Eluates

Although the concentration of $^{99\text{m}}\text{Tc}$ in $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator eluates is of prime importance in nuclear medicine applications since it is the gamma ray emission from this isotope which provides the diagnostic image, the eluate also contains significant amounts of ^{99}Tc due to the characteristics of the decay scheme.

This total amount of technetium ($^{99\text{m}}\text{Tc}$ and ^{99}Tc) in generator eluates varies with both the age and history of the generator as well as the time since its last elution.

The amount of $^{99\text{m}}\text{Tc}$ can be readily determined by means of monitoring its gamma emission, however, the determination of ^{99}Tc by radiochemical means is very difficult due to the low concentration of this isotope in the eluate of generators, its low β emission (0.29 MeV) and the presence of other long-lived radioactive contaminants in the eluate. While theoretical calculations are available to estimate the total amount of technetium in generator eluates, the results of these calculations have never been confirmed experimentally.

The total chemical concentration of pertechnetate in the eluate is potentially significant since it may determine the chemistry and kinetics of the conversion of TcO_4^- into the radiopharmaceutical containing Tc in a reduced oxidation state. Therefore, this study was undertaken to determine the total amount of pertechnetate ($^{99\text{m}}\text{TcO}_4^-$ and $^{99}\text{TcO}_4^-$) in generator eluates by a chemical means.

We have developed a rapid and sensitive high performance liquid chromatographic method for the quantitative determination of pertechnetate in aqueous solution. This analytical technique has been utilized to track the concentration of pertechnetate in the eluates of commercially available 7.45 Ci $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators as they are used in a clinical setting over a two week period.

This non-radiochemical procedure employs HPLC to separate the TcO_4^- from other generator constituents and impurities, and UV detection at 254 nm to monitor the concentration. Thus, the method is not affected by the presence of chromatographically separated radiochemical impurities. The method utilizes a Spherisorb 5 μm amino bonded analytical column which is operated in an ion exchange mode by using acetate buffer, pH 4.5 as the mobile phase.

Calibration curves constructed from standard KTcO_4 solutions were shown to be linear over the range 10^{-8} M to 10^{-4} M, at least two orders of magnitude greater

than expected in a generator. A lower detection limit of $6.43 \times 10^{-8} \text{ M}$ (13.0 ng/mL) has been obtained under conditions useful for analyzing generator eluates. The unknown pertechnetate concentration of the generator eluates is calculated from the average peak height obtained from its chromatogram and the least squares parameters defining the calibration plot.

The results of the 7.45 Ci generator analyses reveal TcO_4^- concentrations in the range 10^{-7} M to 10^{-6} M with the total amount of chemical technetium recovered per unit of $^{99\text{m}}\text{Tc}$ activity slightly larger than expected. While this average difference between experimental and theory is only about 35% it is clear that either the experiment or the application of the theory to working generators contains a systematic error. The explication of this discrepancy will have to await further detailed calculations and analyses.

In addition to the quantitative evaluation of $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators, qualitative HPLC studies indicate that 1) while the predominant ($100 \pm 7\%$) technetium species in generator eluates is pertechnetate, some generator eluates also contain trace amounts (0.01%) of $^{99\text{m}}\text{Tc}$ impurities, and 2) generator eluates from different manufacturers contain varying amounts of UV absorbing impurities.

Thus, from these studies it is clear that HPLC with UV detection has the sensitivity and selectivity necessary to monitor total chemical pertechnetate in $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator eluates. This HPLC based determination may be of considerable utility for both environmental applications and the routine analysis of reduced Tc-radiopharmaceuticals.

A detailed description of this HPLC method for the determination of total TcO_4^- has been accepted for publication (1) and is attached as Appendix I. Our application of the method to monitoring TcO_4^- in generator eluates has also been accepted for publication (2,3) and is attached as Appendices II and III.

Development of Chromatographic Procedures for Determining Tc-HEDP Complexes in Radiopharmaceuticals

Radiopharmaceutical analogues prepared by the NaBH_4 reduction of millimolar concentrations of TcO_4^- in the presence of HEDP are complex mixtures. This reduced Tc preparation has been separated by HPLC into numerous Tc-containing components employing Aminex A-27, a moderately cross-linked polystyrene based quaternary ammonium strong base anion-exchange resin (4).

Although this stationary phase affords a moderate separation and is usable over a wide pH range, it does possess certain drawbacks. Its tendency to swell and shrink with changes in the mobile phase ionic strength limits its use to the buffering system in which it was packed. This lack of versatility as well as the length of time (~60 min) required for the separation of the Tc components, has led to the examination of other packings for improved performance. Thus, alternative HPLC methods are presently being evaluated which, while circumventing the problems associated with the polystyrene resins, will aid in improving the chromatographic resolution and decreasing the time of analysis.

The emphasis of this study has been directed towards the utilization of polar bonded phases which have advantages over the resins in that they equilibrate very rapidly to changes in mobile phase composition and are dimensionally stable. The two stationary phases examined thus far include: Spherisorb amino bonded phase (Phase Separations, Ltd.) and Nucleosil dimethyl amine (Machery-Nagel) both of which are totally porous silica particles of 5 μ m diameter.

Each column was examined by evaluating various parameters such as mobile phase, ionic strength and pH in order to achieve optimal chromatographic conditions. These mobile phases include: $(\text{NH}_4)_2\text{SO}_3$, $(\text{NH}_4)_2\text{SO}_4/\text{HEDP}$, $\text{Na}_2\text{SO}_4/\text{HEDP}$, NaClO_4 , $\text{NaClO}_4/\text{HEDP}$ for the amino bonded phase and $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{SO}_4/\text{methanol citrate}$, acetate, phosphate, and phosphate/methanol for the dimethyl amine column.

Our experience with the use of these columns indicates that the Tc preparation contains components which possess a wide range of selectivity for these packings. That is, chromatographic conditions necessary to improve the resolution of the early eluting components leads to loss of recovery of the later eluting components.

Presently, our most promising results have come from our preliminary studies on the dimethyl amine column using $(\text{NH}_4)_2\text{SO}_4$ as the mobile phase. We have been successful in partially resolving up to 5 Tc components. However, it appears that complete resolution of this preparation may be difficult to achieve with isocratic conditions. This necessitates the use of gradient elution. Gradient elution chromatography is easily implemented with the addition of a second pump and a gradient mixer to our existing chromatograph. We have requested this equipment in our 1982-83 Budget.

Effect of Formulation Parameters on Tc-HEDP Complexes in Tc(NaBH₄)HEDP Radiopharmaceutical Analogues

The use of HPLC to separate Tc(NaBH₄)HEDP radiopharmaceutical analogues reveals that such preparations consist of at least 7 Tc-containing components (4,5). These chromatographically separated Tc-components have been shown to exhibit distinctively different biodistributions in rats (5). The formation of such a radiopharmaceutical mixture containing primarily the component with optimal imaging properties would greatly enhance its effectiveness.

Thus, this study seeks to increase the yield of specific Tc-HEDP components by varying the preparation and handling conditions of the Tc(HEDP) radiopharmaceutical.

The first of several variables to be investigated is total TcO₄⁻ concentration used in the formulation. The preparation of Tc(NaBH₄)HEDP radiopharmaceutical analogues using concentrations of TcO₄⁻ varying over a range of 10⁻² to 10⁻⁸ moles/L shows a dramatic effect on the number and relative amounts of Tc-HEDP complexes formed. As many as 7 major component Tc-HEDP complexes are found in varying amounts when TcO₄⁻ concentrations of 10⁻⁴ moles/L and above are used in the preparation. At TcO₄⁻ concentrations lower than 10⁻⁴ moles/L, a single major Tc-HEDP component together with one or two minor components are obtained. These results suggest that total TcO₄⁻ concentration is an important parameter to be considered in the preparation of a specific Tc-HEDP complex to improve skeletal imaging.

Preliminary results indicate a strong influence of pH during formulation on which Tc-HEDP complexes are formed. We are systematically evaluating this effect of pH, which shows considerable promise as a simple means of controlling a radiopharmaceutical preparation to generate a high yield of a specific Tc-HEDP complex.

Electrochemical Reduction as a Means of Preparing Tc-HEDP Radiopharmaceuticals

The preparation of Tc-HEDP radiopharmaceuticals with electrochemical reduction, rather than chemical reduction with Sn²⁺, NaBH₄, etc., is being explored. Electrochemistry offers a convenient means of varying the reduction potential of an innocuous reductant (the electrode) to determine the effect of reducing strength on the resultant Tc-HEDP complexes.

Work has been initiated on the ligand HEDP in a sodium acetate buffer (pH 8.4) with the ratio of technetium to ligand at 1:80. Samples have been electrolysed under a nitrogen atmosphere at a mercury pool electrode with potentials of -0.700 V,

-0.800 V, and -0.900 V vs SCE. Cyclic voltammetry on the initial and final solutions verify that complete reduction of TcO_4^- is achieved electrochemically. The resulting preparation is analyzed by HPLC to determine which Tc-HEDP complexes are formed. Results thus far indicate that the same complexes are obtained as the ones formed with NaBH_4 reductant. However, the relative concentrations of these complexes are different than obtained with NaBH_4 reduction. The relative concentrations of the Tc-HEDP are influenced significantly by the potential used for electrolysis. We are systematically evaluating parameters such as TcO_4^- concentration, solution pH, HEDP concentration, electrode potential, and electrode material (Hg, Pt, Au, glassy carbon) to determine if electrochemical reduction is a viable route to the preparation of specific Tc-HEDP complexes for improved skeletal imaging.

Compliance to Contract Requirements

Contract requirements have been complied with.

Principal Investigator Effort

William R. Heineman

100% of time for 2 months

10% of time for 9 months during academic year

Edward Deutsch

100% of time for 2 months

10% of time for 9 months during academic year

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APPENDICES

Appendix I: Quantitative Determination of Pertechnetate by High Performance Liquid Chromatography with UV Detection

Appendix II: Preparation of "No Carrier Added" ^{99m}Tc Complexes

Appendix III: Preparation of "No-Carrier-Added" Technetium-99m Complexes:
Determination of the Total Technetium Content of Generator Eluents

APPENDIX I

QUANTITATIVE DETERMINATION OF PERTECHNETATE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV DETECTION

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(J. Chromatog. Biomed. Appl., in press)

SUMMARY

A rapid and sensitive high performance liquid chromatographic method for the quantitative determination of pertechnetate (TcO_4^-) has been developed. The method utilizes a Spherisorb 5 μm amino bonded analytical column and UV detection of pertechnetate at 254 nm. The column is operated in an ion exchange mode, the mobile phase being pH 4.5 acetate buffer at either 0.10 or 0.03 M. The lower detection limit under ideal conditions (0.10 M acetate buffer) is 6.00×10^{-8} M TcO_4^- (12.1 ng/mL) while a lower limit of 1.30×10^{-7} M TcO_4^- (26.4 ng/mL) is obtained under conditions (0.03 M acetate buffer) useful for analyzing the eluates of $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators. Application of this technique to the direct analysis of the first eluate of a commercial $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator revealed the presence of $(8.0 \pm 1.6) \times 10^{-7}$ M TcO_4^- (as both $^{99\text{m}}\text{TcO}_4^-$ and $^{99}\text{TcO}_4^-$) with $100 \pm 7\%$ of the initial gamma activity being recovered. This HPLC-based analysis may be of considerable utility in assessing the history and function of $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators as well as in the routine analysis of reduced technetium radiopharmaceuticals for the presence of undesired TcO_4^- .

INTRODUCTION

In the practice of diagnostic nuclear medicine, some chemical form of a gamma ray emitting isotope is administered to a patient with the goal of having the isotope localize in a specific organ. Subsequent scanning of the organ with a gamma ray camera provides valuable diagnostic and prognostic data by an essentially noninvasive technique (1,2).

Technetium-99m is the isotope of choice for diagnostic nuclear medicine because of its optimal nuclear properties, its diverse chemistry, and its ready availability by means of the $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator (3-5). This generator is based upon the nuclear decay scheme

and the chemical fact that the highest oxidation states of Mo and Tc have different ionic charges (MoO_4^{2-} and TcO_4^- , molybdate and pertechnetate, respectively in neutral, aqueous solution). The manufacturer loads $^{99}\text{MoO}_4^{2-}$ onto a shielded alumina column and then ships this generator to the point of use. In the hospital the column is eluted with normal saline (0.15 M NaCl) once every working day; upon elution, the -1 charged pertechnetate (both $^{99\text{m}}\text{TcO}_4^-$ and $^{99}\text{TcO}_4^-$) is eluted whereas the -2 charged molybdate is retained. This eluate can be used directly to obtain a " $^{99\text{m}}\text{Tc}$ pertechnetate" scan. But more often the eluate is subjected to one of a variety of chemical reactions in which the technetium is reduced to a lower oxidation state and simultaneously chelated by a ligand to generate a "reduced $^{99\text{m}}\text{Tc}$ radio-pharmaceutical" with specific biological properties (1-6).

The concentration of $^{99\text{m}}\text{Tc}$ in the generator eluate is of prime importance in nuclear medicine applications since it is the gamma ray

emission from this isotope which provides the diagnostic image. However, the eluate also contains significant amounts of ^{99}Tc because of the characteristics of the $^{99}\text{Mo} - ^{99\text{m}}\text{Tc} - ^{99}\text{Tc}$ decay scheme, and this concentration is important for two reasons. First, it is the total chemical concentration of pertechnetate (both $^{99\text{m}}\text{TcO}_4^-$ and $^{99}\text{TcO}_4^-$) in the eluate that determines the chemistry and kinetics of the conversion of pertechnetate into radiopharmaceuticals containing technetium in a reduced oxidation state (3). Secondly, the amount of ^{99}Tc , which is a long-lived ($t_{1/2} = 2 \times 10^5$ yr) beta emitter, injected into a patient must be taken into account when calculating the total radiation dose received by the patient.

The total amount of technetium, as both ^{99}Tc and $^{99\text{m}}\text{Tc}$, in generator eluates varies with the age and history of the generator, and, of course, with the time elapsed since the last elution of the generator. The amounts of $^{99\text{m}}\text{Tc}$ and ^{99}Tc in any given generator eluate can be calculated from the $^{99}\text{Mo} - ^{99\text{m}}\text{Tc} - ^{99}\text{Tc}$ decay scheme, given certain assumptions about the history of the generator (7,8). However, these assumptions are difficult to verify and, to date, the calculations have not been experimentally validated. Determination of $^{99\text{m}}\text{Tc}$ in the eluate presents no problems since this isotope can be accurately monitored by means of its gamma emission. Determination of ^{99}Tc by radiochemical means is very difficult because of its low energy beta emission (0.29 MeV), the low concentration of this isotope in the eluate, and the presence of other long-lived radioactive impurities in most generator eluates (9). We have therefore undertaken this study to determine the total amount of chemical pertechnetate (as both $^{99\text{m}}\text{TcO}_4^-$ and $^{99}\text{TcO}_4^-$) in generator eluates by chemical means. This non-radiochemical procedure employs HPLC to separate pertechnetate

from other generator constituents and impurities, and UV detection to quantitatively monitor the concentration of pertechnetate.

MATERIALS AND METHODS

Chemicals

Technetium in the form of TcO_4^- in 0.9% NaCl was obtained from the Radioisotope Laboratory at Cincinnati General Hospital as the first elution of a $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator (7.5 Ci, Union Carbide, Tuxedo, NY).

Crystalline $\text{NH}_4^{99}\text{TcO}_4$ (Oak Ridge National Laboratories, Oak Ridge, TN) was converted to $\text{K}^{99}\text{TcO}_4$ by metathesis with KOH. This material was recrystallized and dried before being used for the preparation of standard solutions.

Standard Solutions

Stock solutions of $6.00 \times 10^{-4} \text{ M}$ and $4.35 \times 10^{-4} \text{ M}$ $\text{K}^{99}\text{TcO}_4$ were prepared by weight in 0.10 M and 0.03 M acetate, pH 4.5, respectively. Appropriate dilutions of these with the corresponding elution buffers yielded a series of standard solutions.

Reagents

Elution buffers, 0.10 M and 0.03 M in total acetate concentration, pH 4.5, were freshly prepared from glacial acetic acid, A.R., sodium hydroxide, A.R., and triply distilled, charcoal filtered water. Prior to use, these were filtered through 0.22 μm GS membranes (Millipore, Bedford, MA) and deaerated by sonication under vacuum.

Chromatographic Apparatus

The chromatographic equipment included a Waters Associates Model M-45 solvent delivery system, a Rheodyne Model 7125 injection valve fitted with a 100 μL sample loop and a PD-2 12 inch pulse damper, all obtained from Bioanalytical Systems Inc., West Lafayette, IN.

A Lichrosorb 10 μm amino bonded phase 5 cm cartridge guard column (Brownlee Labs Inc., Santa Clara, CA) was installed between the

injection valve and the analytical column.

The analytical column was a 4.6 x 250 mm stainless steel Knauer column (Unimetrics Corp., Anaheim, CA) slurry packed with Spherisorb Amino Bonded Phase, 5 μ m (Phase Separations, Ltd., Hauppauge, NY). Isocratic elution of these columns with the aqueous acetate buffers at 1.5 mL/min resulted in typical operating pressures around 12.4 MPa (1800 p.s.i.) at ambient temperature.

UV Detection

The absorbance of the eluent was monitored at 254 nm with a Beckman Model 153 UV Detector equipped with an 8 μ L flow cell of 1 cm pathlength. The detector output was recorded with an Omniscribe B-5000 dual channel strip chart recorder (Houston Instruments, Austin, TX).

Radiometric Detection

Chromatographic detection of the gamma activity of eluting $^{99m}\text{TcO}_4^-$ was accomplished with a scintillation spectrometry system (Harshaw Chemical Co., Solon, OH). This consisted of a NA-23 stabilized amplifier/single channel analyzer, a NR-22 linear ratemeter, and a NV-32A high voltage power supply all mounted in an AP-2H nuclear instrument module (Berkeley Nucleonics Corp., Berkeley, CA). The detector was a shielded 5.1x5.1 cm cylindrical NaI(Tl) crystal with a 1.4 cm thru-side hole optically coupled to a 5.1 cm Amperex PM 2202 photomultiplier tube. The ratemeter signal was fed to the second channel of the recorder permitting simultaneous monitoring of gamma activity and optical absorbance. Finally, absolute amounts of ^{99m}Tc activity in collected fractions were determined with a CRC-6A Radioisotope Calibrator (E.R. Squibb and Sons, Inc., Princeton, NJ).

Quantitative Analysis

A standard curve (Figure 1, A) under conditions of minimum retention and maximum detectability was generated by sampling 18 different concentrations of KTcO_4 , ranging from $6.00 \times 10^{-8} \text{ M}$ to $6.00 \times 10^{-4} \text{ M}$ in 0.10 M acetate, pH 4.5, buffer. A second standard curve (Figure 1, B) under conditions more suited to the analysis of $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators was obtained by sampling 17 different concentrations of KTcO_4 , ranging from $1.30 \times 10^{-7} \text{ M}$ to $4.35 \times 10^{-4} \text{ M}$ in 0.03 M acetate, pH 4.5, buffer. For each concentration of $\text{K}^{99}\text{TcO}_4$, six replicate chromatograms were obtained; the peak heights were corrected for noise and measured manually. The six peak heights were then averaged, and the standard deviation of the mean (σ_m) was calculated. Logarithmic calibration curves were constructed by plotting average peak height (in absorbance units, weighted as $1/(\sigma_m)^2$) vs. $\text{K}^{99}\text{TcO}_4$ concentration, and were then analyzed by a linear least squares treatment (Table I).

RESULTS AND DISCUSSION

Initial experiments designed to optimize conditions for pertechnetate analysis resulted in a detection limit of $6.00 \times 10^{-8} \text{ M TcO}_4^-$ in 0.10 M acetate, pH 4.5, the retention time of pertechnetate being 4.3 min. However, it was found that analysis of pertechnetate eluted from a $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator was not possible under these conditions due to refractive index changes and other unknown chromatographic interferences occurring at or near this retention time. The mobile phase was therefore adjusted to increase the retention time of pertechnetate. With 0.03 M acetate, pH 4.5, the retention time of pertechnetate is 7.3 min and the detection limit for $\text{K}^{99}\text{TcO}_4$ is $1.30 \times 10^{-7} \text{ M}$. A typical chromatogram obtained under these conditions is shown in Figure 2A.

Figures
1 & 2

Figure 1 shows that the calibration curves under both sets of conditions are linear over four orders of magnitude of pertechnetate concentration. This linearity extends at least two orders of magnitude beyond the pertechnetate concentrations expected in $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator eluates. Statistical data describing each calibration curve are summarized in Table I.

Table
I

Analysis of the first eluate from a 7.5 Ci Union Carbide $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator (detection at 254 nm) yields the chromatogram shown in Figure 2B (—), with a single component eluting at $t_R = 7.3 \text{ min}$. Gamma detection (Figure 2B, ---) also shows a single major component with $t_R = 7.3 \text{ min}$, confirming that the component is indeed pertechnetate. From the slope and intercept parameters for standard curve B (Table I) the concentration of pertechnetate in the $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator eluate is calculated to be $(8.0 \pm 1.6) \times 10^{-7} \text{ M}$, five times greater than the detection limit. In this experiment, $100 \pm 7\%$ of

the initially injected gamma activity is recovered from the HPLC column within the peak at $t_R = 7.3$ min. Therefore, this particular generator eluate does not contain significant amounts of reduced forms of ^{99m}Tc , and the HPLC procedure itself does not induce significant reduction of pertechnetate. Upon increasing the gamma sensitivity, two additional radioactive components eluting prior to TcO_4^- are observed (Figure 3). These extremely minor components each comprise approximately 0.01% of the total activity applied and have been verified by multichannel pulse height analysis to contain only ^{99m}Tc (possibly as reduced hydrolyzed species).

Attempts at detecting MoO_4^{-2} (a breakthrough product of $^{99}\text{Mo}/^{99m}\text{Tc}$ generators) were performed by chromatographing standard Na_2MoO_4 solutions under similar conditions of generator analysis. Since no peaks were observed after 5.5 hrs of elution (1.5 mL/min), it is concluded that MoO_4^{-2} is either totally retained on the column or has been sufficiently diluted so as to be undetectable, and it thus poses no serious interference in the analysis of TcO_4^- .

From these results it is clear that HPLC with UV detection has the sensitivity and selectivity necessary to monitor total chemical pertechnetate in $^{99}\text{Mo}/^{99m}\text{Tc}$ generators eluates. The applicability of this technique has been substantiated by our current investigation involving the measurement of total TcO_4^- in the eluates of several $^{99}\text{Mo}/^{99m}\text{Tc}$ generators over their entire useful clinical lifetime (10). We have noticed that the absolute sensitivity varies slightly, however, due to both the age of the column and variability in column to column efficiencies.

Although some column deterioration (loss of retention) was observed over the course of the study, this analysis should be of considerable

utility in monitoring the function of clinical generators, as well as in the routine analysis of reduced ^{99m}Tc radiopharmaceuticals for the presence of undesired pertechnetate.

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TABLE I. Weighted Linear Least Squares Analysis for $K^{99}\text{TcO}_4$
Standard Curves (Log vs Log)

	<u>$t_R(A)$</u> 4.3 min	<u>$t_R(B)$</u> 7.3 min
Slope	1.011 ± 0.004	1.043 ± 0.006
Y-Intercept	-2.926 ± 0.015	-2.80 ± 0.03
Correlation Coefficient	0.9998	0.9997

Figure 1. Calibration graphs of $K^{99}TcO_4$ with mobile phases of A) 0.10 M acetate, pH 4.5 ($t_R = 4.3$ min) and B) 0.03 M acetate, pH 4.5 ($t_R = 7.3$ min). Conditions: Flow rate 1.5 mL/min; detection 254 nm; temperature ambient.

Figure 2. Typical chromatograms of (a) 1.30×10^{-7} M $K^{99}TcO_4$ and (b) $^{99}Mo/^{99m}Tc$ generator first eluate (37 $\mu Ci/mL$). Conditions: eluent 0.03 M acetate, pH 4.5; flow rate 1.5 mL/min; detection 254 nm (—), γ (---); temperature ambient.

Figure 3. Chromatogram of $^{99}Mo/^{99m}Tc$ generator eluate (≈ 60 mCi/mL) with γ detection. Conditions: eluent 0.1 M acetate, pH 4.5; flow rate 1.5 mL/min; temperature ambient.

Fig 1

Fig 2

Fig 3

elution yields may occur due to the reduction to the Tc(IV) form, which is strongly adsorbed on alumina.

The current biomedical applications of the ^{99m}Tc radioisotope are the basis of a \$100 million nuclear medicine business. The ^{99m}Tc generator is a large part of that nuclear medicine market. The growth of nuclear pharmacies has led to the development of large multicurie-size generators. The importance of the ^{99m}Tc generator makes it imperative that we understand and solve the problems associated with their manufacture.

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2. Preparation of "No Carrier Added" ^{99m}Tc Complexes, Edward Deutsch, William R. Heine-man, Julius P. Zodda (Univ of Cincinnati)

It is the purpose of this work to determine the chemical concentration of technetium (both ^{99}Tc and ^{99m}Tc) in "no carrier added" $^{99}\text{Mo}/^{99m}\text{Tc}$ generator eluants, and to assess the effects that this chemical parameter may have on the preparation of ^{99m}Tc radiopharmaceuticals.

Over the past few years, both inorganic and analytical chemists have played increasingly important roles in the preparation, characterization, and development of new ^{99m}Tc radiopharmaceuticals. Inherent in this interdisciplinary approach is the view that ^{99m}Tc radiopharmaceuticals are nothing more or less than coordination complexes of technetium prepared under the very dilute conditions dictated by the use of "no carrier added," generator-produced ^{99m}Tc . This, a currently popular route for radiopharmaceutical development involves first the preparation and chemical characterization of macroscopic quantities of a ^{99}Tc complex; preparative conditions are then varied to accommodate more and more dilute solutions of technetium until finally the reaction is conducted using "no carrier added" ^{99m}Tc as obtained from a ^{99}Mo generator. Quality control procedures (usually based on thin layer chromatography or high-performance liquid chromatography) are conducted to ensure that the technetium complex produced from the macroscopic ^{99}Tc preparation is the same as that produced from the "no carrier added" ^{99m}Tc preparation. However, there are two fundamental difficulties with this approach. First, the technetium chemistry observed at millimolar concentration levels

very often does not readily translate to the very dilute conditions of "no carrier added" ^{99m}Tc . Second, the chemical concentration of technetium (i.e., the sum of ^{99}Tc and ^{99m}Tc) in "no carrier added," generator-produced ^{99m}Tc is both variable (depending on the age and history of the generator) and unknown. While theoretical calculations are available to estimate the total amount of chemical technetium in a given generator eluant, the results of these calculations have never been confirmed experimentally. We have undertaken three experimental programs related to these points.

1. A rapid and sensitive high-performance liquid chromatographic method for the quantitative determination of pertechnetate in aqueous solution has been developed. This method utilizes a Spherisorb 5 micron amino-bonded analytical column, and an acetate buffer (pH 4.5) eluant. Detection of pertechnetate is accomplished at 254 nm using a commercially available, fixed-wavelength detector. The lower detection limit under ideal conditions is 60 nM TcO_4^- (independent of technetium isotope), while under conditions approximating those found in generator eluants, the lower detection limit is 130 nM TcO_4^- . Analysis of a typical "Monday morning" generator eluant showed that the total chemical concentration of pertechnetate was 700 nM. This analytical method promises to be of considerable utility in assessing the performance of $^{99}\text{Mo}/^{99m}\text{Tc}$ generators, and could also form the basis for the routine quality control of pertechnetate impurity in reduced ^{99m}Tc radiopharmaceuticals.

2. A model bone-seeking radiopharmaceutical, $\text{Tc}(\text{NaBH}_4)\text{-HEDP}$, can be prepared by the NaBH_4 reduction of pertechnetate in the presence of aqueous HEDP (1,1-hydroxyethylidene diphosphonate). When this preparation is conducted with 3 mM $^{99}\text{TcO}_4^-$, HPLC analysis of the reaction mixture shows the presence of at least seven different product complexes in roughly equivalent concentrations. When this preparation is conducted with "no carrier added" $^{99m}\text{TcO}_4^-$, however, only one dominant (>90%) and two minor products are observed. It is clear that in this system, there is a very dramatic concentration effect and that macroscopic ^{99}Tc chemistry does not readily translate into "no carrier added" ^{99m}Tc chemistry. This implies that at least some of the reactions controlling the product distribution are second-order in technetium concentration.

3. The ^{99}Tc complex $[\text{^{99}Tc}(\text{diars})_2\text{Cl}_2]\text{Cl}$ [diars = *o*-phenylene-bis(dimethylarsine)] may be prepared by refluxing excess diars with $^{99}\text{TcO}_4^-$ in aqueous alcoholic hydrochloric acid. This preparation utilizes the reducing power of the diars ligand to convert Tc(VII) to Tc(III) . With only minor modifications in the synthetic conditions, this preparative chemistry also succeeds with "no carrier added" $^{99m}\text{TcO}_4^-$ to yield the myocardial imaging agent $[\text{^{99m}Tc}(\text{diars})_2\text{Cl}_2]^+$. This implies that all of the reactions in this synthesis are first-order in technetium concentration.

The chemical concentration of technetium in "no carrier added" ^{99m}Tc generator eluants can be experimentally determined. This concentration is very important in determining whether or not chemistry developed with macroscopic concentrations of ^{99}Tc can be translated to the "no carrier added" ^{99m}Tc level. Success or failure of this translation could depend on whether the reaction controlling product formation is first- or second-order in technetium concentration.

3. The Chemistry of the Technetium Generator, Joseph Steigman (Downstate Med Center, Brooklyn)

This paper is a review of the chemistry of the alumina-based technetium generator and an interpretation of some of the published observations of generator performance.

APPENDIX III

PREPARATION OF "NO-CARRIER-ADDED" TECHNETIUM-99m COMPLEXES: DETERMINATION OF THE TOTAL TECHNETIUM CONTENT OF GENERATOR ELUENTS

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ABSTRACT

The total concentration of pertechnetate in $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator eluents can be readily determined by ion exchange HPLC with UV detection. Tracking of a 7,450 mCi generator through its useful clinical life leads to values of $[\text{TcO}_4^-]$ in the range 10^{-7} to 10^{-6} M, and values of the ratio (moles total technetium)/(mCi Tc-99m) in the range $(3 \text{ to } 35) \times 10^{-12}$ mol/mCi. Trace amounts (ca. 0.01%) of non-pertechnetate, $^{99\text{m}}\text{Tc}$ -containing impurities are detected, and generators of one manufacturer yield eluents with a large number of UV absorbing impurities. The total concentration of pertechnetate in generator eluents is important in determining whether or not chemistry developed with macroscopic concentrations of ^{99}Tc "carrier" can be translated to the "no-carrier-added" $^{99\text{m}}\text{Tc}$ level. Success or failure of this translation could depend on whether the reaction controlling product formation is first- or second-order in technetium concentration.

I. INTRODUCTION

Over the past few years both inorganic and analytical chemists have played increasingly important roles in the preparation, characterization and development of new ^{99m}Tc radiopharmaceuticals. Inherent in this interdisciplinary approach is the view that ^{99m}Tc radiopharmaceuticals are nothing more or less than coordination complexes of technetium prepared under the very dilute conditions dictated by the use of "no-carrier-added," generator-produced, ^{99m}Tc .^(1,2) Thus, a currently popular route for radiopharmaceutical development involves first the preparation and chemical characterization of macroscopic quantities of a ^{99}Tc complex; preparative conditions are then varied to accommodate more and more dilute solutions of technetium until finally the reaction is conducted using "no carrier added" ^{99m}Tc as obtained from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator. Quality-control procedures, usually based on thin-layer chromatography or high performance liquid chromatography, are conducted to insure that the technetium complex produced from the macroscopic ^{99}Tc preparation is the same as that produced from the "no carrier added" ^{99m}Tc preparation. However, there are two difficulties with this approach. First, the technetium chemistry observed at millimolar concentration levels very often is not readily translated to the very dilute conditions of "no-carrier-added" ^{99m}Tc . Second, the chemical concentration of technetium (i.e. the sum of ^{99}Tc and ^{99m}Tc in "no carrier added," generator produced, ^{99m}Tc is both variable (depending on the age and history of the generator) and unknown. While theoretical calculations are available⁽³⁻⁵⁾ to estimate the total amount of chemical technetium in a given generator eluent, the results of these calculations have never been confirmed experimentally. One determination of the amount of ^{99}Tc in liquid-liquid extraction generator extracts by liquid scintillation counting⁽⁶⁾ showed that these extracts contain high, and very variable, amounts of ^{99}Tc .

We have recently⁽⁷⁾ developed a rapid and sensitive high performance liquid

chromatographic (HPLC) method for the quantitative determination of pertechnetate in aqueous solution. This method is based on the UV absorption of the pertechnetate anion, not on the nuclear decay properties of ^{99}Tc , and is therefore not affected by the presence of radiochemical impurities. In this paper we utilize this analytical technique to track the concentration of total pertechnetate ($^{99\text{m}}\text{TcO}_4^- + ^{99}\text{TcO}_4^-$) in the eluents of commercially available $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators as they are used in a clinical setting. These experimental results are compared to theoretically predicted values, and are discussed in terms of their implications for the development and preparation of "no-carrier-added" $^{99\text{m}}\text{Tc}$ radiopharmaceuticals.

II. MATERIALS AND METHODS

Unless otherwise noted, all chemicals were of reagent grade. Water was triply distilled and passed through a charcoal filter. Sodium acetate eluent solutions were prepared by neutralization of acetic acid with sodium hydroxide, and before use they were filtered through 0.22- μm membranes and deaerated by sonication under vacuum. $\text{K}^{99}\text{TcO}_4$ was prepared from $\text{NH}_4^{99}\text{TcO}_4$ (ORNL, Oak Ridge, TN) by metathesis with KOH, and was purified by several crystallizations from warm water. Standard solutions of $\text{K}^{99}\text{TcO}_4$ were prepared by weight. Solutions of "no carrier added" $^{99\text{m}}\text{TcO}_4^-$ were obtained by elution of commercially available $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators with 0.9% aqueous NaCl solutions provided by the generator manufacturer. The $^{99\text{m}}\text{Tc}$ activity of the entire eluent volume was assayed with a Capintec CRC-6A dose calibrator that is regularly checked for accuracy, linearity of response, and daily constancy of response. ^{99}Mo breakthrough measurements were obtained using the calibrator with its "moly breakthrough" shield.

Generators from Manufacturer A were loaded with 7450 mCi of ^{99}Mo and eluted once by the manufacturer at noon $\pm$ one hour the Thursday before receipt. The first elution in our laboratories occurred 21 to 23 hours after the manufacturer's elution; data for this, and subsequent elutions (date and time of elution, volume

of eluent, total ^{99m}Tc activity per volume of eluent, and ^{99}Mo breakthrough) were recorded throughout the two-week clinical life of the generator. In general, 20 mL elutions were used for the first several days; 10 mL elutions were used when the expected ^{99m}Tc yields decreased due to the age of the generator or because of short ingrowth periods. On occasion, 5 mL elutions from generator 1 were used when minimal quantities of ^{99m}Tc were anticipated; this practice was discontinued when it was found that this small volume led to incomplete elutions (Table I), and the data from these elutions were not incorporated in the study. Generators from Manufacturer B were loaded with 880 mCi of ^{99}Mo and eluted once by the manufacturer at noon the Monday before receipt. The first elution in our laboratories occurred on Wednesday; 5 mL elutions were used at all times.

The details of the HPLC apparatus, with accompanying UV and radiometric detectors, used in this work have been described previously.^(7,8) The analytical column (4.6 mm/250 mm) was slurry packed with Spherisorb Amino Bonded Phase, 5 mic. on (Phase Separations, Hauppauge, NY) substrate. Isocratic elution with aqueous acetate buffer (pH 4.5) at 1.5 mL/min resulted in operating pressures around 1800 psi. The chromatographic UV detector (8 μL flow cell, 1.00 cm path-length) was operated at 254 nm to generate chromatograms in units of A_{254} vs. time. The concentration of pertechnetate was related to the height of the appropriate peak in this chromatogram. Calibration plots of peak heights (PH, in units of A_{254}) vs. the concentration of injected TcO_4^- (constant injection volume = 100 μL) were obtained using standard solutions of $\text{K}^{99}\text{TcO}_4$. For each concentration of injected $\text{K}^{99}\text{TcO}_4$, six replicate chromatograms were obtained, and the peak heights were corrected for noise and measured manually; the average peak height (weighted as $1/(\sigma_m)^2$ where σ_m is the standard deviation of the six replicate measurements) was plotted vs the concentration of injected pertechnetate, and the resulting calibration plot analyzed by a linear least-squares treatment. For solutions of unknown pertechnetate concentration, at least four replicate

chromatograms were obtained and the average resultant peak height was calculated; the concentration of pertechnetate in the injected solution (constant injection volume = 100 μ L) was then calculated from this average peak height and the least-squares parameters defining the calibration plot.

Experiments were conducted to ensure that the gamma-ray activity of the samples did not affect the output of the UV detector. First, it was shown that the total pertechnetate concentration determined in generator eluents was the same (within experimental error) both before and after decay of the ^{99m}Tc in the eluent. Second, it was shown that a 50 mCi ^{99m}Tc source held adjacent to the UV detector elicited no detectable response on the recorder output.

III. RESULTS

Figure 1 shows the calibration plot of peak height (measured as A_{254}) vs. $[\text{}^{99}\text{TcO}_4^-]$ for one of the two chromatographic columns used in this study. The slope and intercepts resulting from linear least-squares analysis of the two calibration plots are as follows: $942.3 \pm 1.1 \text{ M}^{-1}$ and $(-2.6 \pm 0.3) \times 10^{-5}$; $820.6 \pm 2.5 \text{ M}^{-1}$ and $(-9.7 \pm 2.3) \times 10^{-6}$. These parameters and sensitivity may vary from one column to the next due to the differing separation efficiencies of each individual column.

Two typical chromatograms are shown in Figure 2. Chromatogram 2A shows the A_{254} tracing that results from injection of 100 μ L of a $6.43 \times 10^{-8} \text{ M}$ solution of $\text{K}^{99}\text{TcO}_4$; this amount of $^{99}\text{TcO}_4^-$ represents the detection limit under the given conditions. The first two peaks in chromatogram 2A are artifacts resulting from refractive index changes, while the peak at 9.3 min results from $^{99}\text{TcO}_4^-$. Chromatogram 2B shows both the A_{254} tracing and the gamma-ray activity tracing that result from injection of 100 μ L of a typical "no carrier added" generator eluent. The gamma activity tracing has only one peak at 9.3 min, corresponding to $^{99m}\text{TcO}_4^-$;

the A_{254} tracing has several early peaks caused by refractive index changes, and a peak at 9.3 min resulting from $^{99}\text{TcO}_4^-$ and $^{99\text{m}}\text{TcO}_4^-$. The height of this peak in absorbance units corresponds (from the calibration plot) to a total pertechnetate concentration of $(9.21 \pm 0.14) \times 10^{-7} \text{ M}$; similar analyses lead to the data of Table I. The pertechnetate peak eluting at 9.3 min contains $100 \pm 7\%$ of the $^{99\text{m}}\text{Tc}$ activity originally injected. Increasing the sensitivity of the gamma activity tracing shows the presence of two minor radioactive components that elute prior to $^{99\text{m}}\text{TcO}_4^-$ (Figure 3); these two components comprise ca. 0.01% of the injected $^{99\text{m}}\text{Tc}$ activity, and, by gamma-ray spectroscopic analysis, contain only $^{99\text{m}}\text{Tc}$. Figures 4 and 5 respectively show typical tracings (both A_{254} and gamma-ray activity) for eluents obtained within the early lifetime of generators from Manufacturer A and Manufacturer B. While both generators yield essentially only one gamma-ray activity peak (i.e. $^{99\text{m}}\text{TcO}_4^-$), it is readily apparent that the eluent from generator B contains many more UV absorbing impurities than does the eluent from generator A.

IV. DISCUSSION

The total concentration of pertechnetate (as both $^{99\text{m}}\text{TcO}_4^-$ and $^{99}\text{TcO}_4^-$) in $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator eluents can be readily determined by ion exchange HPLC with UV detection. For a 7,450 mCi generator this concentration is in the range 10^{-7} to 10^{-6} M (Table I), considerably greater than is generally appreciated. While high, these concentrations are lower than those determined in earlier work⁽⁶⁾ by liquid-scintillation counting of liquid-liquid extraction generator extracts. In these extracts the concentration of ^{99}Tc ranges from $(2 \text{ to } 178) \times 10^{-7} \text{ M}$ and the ratio (moles total technetium)/(mCi $^{99\text{m}}\text{Tc}$) ranges from $(20 \text{ to } 717) \times 10^{-12} \text{ mol/mCi}$; ⁽⁶⁾ the comparable ranges from the data of Table I are $(1 \text{ to } 47) \times 10^{-7} \text{ M}$ and $(3 \text{ to } 35) \times 10^{-12} \text{ mol/mCi}$. Because of the variable history of commercially-provided liquid-liquid extracts, it is not unreasonable that they should contain

higher concentrations of ^{99}Tc than do $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator eluents.

Figure 6 compares experimentally determined values of the ratio (moles total technetium)/(mCi $^{99\text{m}}\text{Tc}$) for Generator #2 (Table I) with values predicted from theory.^(3,5) It is seen that the experimentally determined values are consistently higher than those predicted, i.e. the total amount of chemical technetium recovered per unit of $^{99\text{m}}\text{Tc}$ activity is larger than expected. The reproducibility of this phenomenon is readily seen in the $\Delta t = 24$ hr data, e.g. the points at 5, 6, 7 and 8 days in Figure 6. While the average difference between experiment and theory is only about 35%, it is clear that either the experiment or the application of the theory to working generators contains a systematic error. Explication of this discrepancy will have to await further detailed calculations and analyses.

While HPLC analysis clearly shows that the predominant ($100 \pm 7\%$) $^{99\text{m}}\text{Tc}$ species in generator eluents is $^{99\text{m}}\text{TcO}_4^-$, some generator eluents also contain trace amounts (ca. 0.01%) of $^{99\text{m}}\text{Tc}$ impurities. The chromatogram in Figure 3 shows two such minor impurities that elute prior to pertechnetate; component "a" elutes with the void volume, while component "b" has significant affinity for the column material. These components are possibly reduced, hydrolyzed forms of technetium that have not yet polymerized to TcO_2 . No evidence was obtained that could confirm a previous observation of large amounts (5 - 80%) of non-pertechnetate $^{99\text{m}}\text{Tc}$ impurities in generator eluents.⁽⁶⁾ However, we have examined only a limited number of generators, and the occurrence of non-pertechnetate impurities may be an infrequent phenomenon.

The eluents from generators of Manufacturer A contain very few UV absorbing impurities (Figure 4), whereas those from generators of Manufacturer B contain a large number of such impurities (Figure 5). We presume that the larger number and amounts of impurities in Generator B eluents result from the fact that Generator B contains ^{99}Mo in a plastic column while Generator A contains ^{99}Mo in a glass

column; the UV absorbing impurities are presumably leached from the plastic, a process that is possibly promoted by radiation damage to the plastic.

Detailed knowledge of the concentration of pertechnetate, that is, the total amount of pertechnetate available for chemical reaction, in generator eluents has important implications for the development and synthesis of "no-carrier-added" ^{99m}Tc radiopharmaceuticals. Since $^{99m}\text{TcO}_4^-$ and $^{99}\text{TcO}_4^-$ are chemically equivalent species, it is the sum of their concentrations that is important in determining the rates of reactions involved in the synthesis of ^{99m}Tc radiopharmaceuticals from $^{99}\text{Mo}/^{99m}\text{Tc}$ generator eluents. The effect of total pertechnetate concentration on the synthesis of reduced technetium radiopharmaceuticals will depend on whether the mechanistic step controlling product formation is first-order or second-order in total technetium concentration. (Only bimolecular reactions are considered since termolecular reactions are essentially never observed in solution.)

First-order. In radiopharmaceutical preparations most reagents (stannous ion, ligand, etc.) are present in much greater concentrations than is technetium. If the controlling mechanistic step involves reaction of technetium with a reagent present in large excess, the technetium reactant will disappear by first-order kinetics:

$$\frac{-d(\text{Tc})}{dt} = k(\text{Tc})(X) = k'(\text{Tc}) \quad (1a)$$

$$t_{99} = \frac{4.6}{k'} \quad (1b)$$

where X is the reagent present in large excess. In this situation the time required for the reaction to proceed to 99% completion (t_{99}) is not a function of technetium concentration and thus the synthesis should not depend on the generator eluent. Also, in this case "carrier-added" and "no-carrier-added" chemistries should be similar. This situation is apparently encountered in the synthesis of the potential myocardial imaging agent $[^{99m}\text{Tc}(\text{diars})_2\text{Br}_2]^+$ (9) (diars =

o-phenylene-bis(dimethylarsine)) from pertechnetate.

HPLC analysis shows that this reaction yields the identical product in >85% yield under "no-carrier-added" conditions where the total pertechnetate concentration is less than 10^{-7} M, and under "carrier added" conditions where the total pertechnetate concentration is greater than 10^{-2} M. Thus, as long as the diars ligand is present in great excess, a $>10^5$ variation in pertechnetate concentration does not affect the rate or yield of the synthetic reaction.

Second-order. If the mechanistic step controlling the synthesis involves the reaction of two technetium-containing species, then the technetium reactant will disappear by second-order kinetics:

$$\frac{-d(\text{Tc})}{dt} = k(\text{Tc})^2 \quad (2a)$$

$$t_{99} = \frac{99}{k(\text{Tc})} \quad (2b)$$

In this situation the time required for the reaction to proceed to 99% completion is a function of technetium concentration. Consequently the success of a synthesis may be dependent on the history of the generator used and its resultant total technetium concentration. For example, by pulse radiolytic techniques we have measured the second-order rate constant governing the bimolecular decay of Tc(VI) in neutral aqueous solution to be $10^8 \text{ M}^{-1}\text{s}^{-1}$; ⁽¹⁰⁾ from eq. 2b it can be calculated that while t_{99} is only 1 second when $(\text{Tc}) = 10^{-6}$ M, it is 100 seconds when $(\text{Tc}) = 10^{-8}$ M. Since the concentration of total technetium in radiopharmaceutical preparations can easily range from 10^{-8} to 10^{-6} M, it is readily seen that the fate of Tc(VI) in such a preparation (e.g. second-order decay vs. pseudo first-order

reaction with a reagent in excess) could depend on the history of the generator eluent. Also, in this second-order case, "carrier-added" and "no-carrier-added" chemistries could very likely be dissimilar.

This situation is apparently encountered in the synthesis of the model bone imaging agent $^{99m}\text{Tc}(\text{NaBH}_4)\text{-HEDP}$ via NaBH_4 reduction of pertechnetate in the presence of excess HEDP (1-hydroxyethylidene diphosphonate).⁽⁸⁾ When this reaction is conducted in the presence of 3 mM $^{99}\text{TcO}_4^-$ "carrier," at least seven different product complexes can be isolated by HPLC.⁽⁸⁾ Since there are only two or three oxidation states of technetium accessible under these conditions, it is possible that some of the product complexes are oligomeric or polymeric species which have been formed via bimolecular reactions of technetium-containing species. Consistent with this view, when the synthesis is conducted under "no-carrier-added" conditions, considerably fewer product complexes are formed.⁽¹¹⁾ However, even under "no-carrier-added" conditions, the distribution of product complexes is affected by the history of the generator eluent. A related phenomenon has recently been observed during clinical trials of the bone imaging agent $^{99m}\text{Tc}(\text{Sn})\text{-HMDP}$ (HMDP = hydroxymethylene diphosphonate).⁽¹²⁾ When standard "kits" containing stannous chloride and HMDP are formulated with 300 mCi $^{99m}\text{TcO}_4^-$ the resulting agent is inferior to agents prepared with 50 mCi $^{99m}\text{TcO}_4^-$. This effect has been traced to the varying amounts of total chemical pertechnetate being added to the kits. In controlled experiments it was shown that a 16-fold variation in the total amount of chemical, "no-carrier-added," pertechnetate added to the kit dramatically affects biodistribution in rats and blood clearance in dogs.⁽¹²⁾

In summary, radiopharmaceutical preparations developed with "carrier" ^{99}Tc will more likely be appropriate under "no-carrier-added" conditions if the mechanistic step controlling product formation is first-order in technetium chemistry. Concomitantly the resulting "no-carrier-added" formulation will be less susceptible to the variations in pertechnetate concentrations inherent in

$^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator eluents.

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Table I. Elution Profiles for Three 7450 mCi $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ Generators Used Clinically Over a Two Week Period

GENERATOR 1			GENERATOR 2			GENERATOR 3		
Δt (hr)	$^{99\text{m}}\text{Tc}$ (mCi/ml)	Total Tc (Molarity $\times 10^7$)	Δt (hr)	$^{99\text{m}}\text{Tc}$ (mCi/ml)	Total Tc (Molarity $\times 10^7$)	Δt (hr)	$^{99\text{m}}\text{Tc}$ (mCi/ml)	Total Tc (Molarity $\times 10^7$)
21	293	14.9	22.8	257	24.8	21.1	242	44.4
69.50	136.5	31.4	67.83	136.5	47.4	70.42	131.5	60.5
24.08	95.9	5.90	24.08	97.9	9.21	23.83	93.85	9.14
24.00	143.2	8.67	23.83	76.9	6.94	24.00	72.5	6.31
7.17	82.4	3.63	24.00	116.2	9.75	5.43	66.2	2.39
16.75	104.1	5.24	4.75	47.5	1.77	18.29	103.1	5.78
3.17	32.78	1.11**	19.25	84.4	5.93	3.00	32.3	0.582
20.83	87.3	5.80	2.58	24.7	0.865	6.32	45.4	1.48**
27.25	*	*	3.00	25.0	3.38	14.68	78.4	5.07
44.92	10.78	8.25**	66.42	45.1	14.2	2.92	25.8	0.845
23.83	34.7	9.80	24.00	33.1	3.51	28.5	*	*
24.08	15.8	2.02**	24.00	25.4	4.71	40.67	42.6	6.84
9.00	15.0	2.57**	7.50	14.2	1.63	23.83	30.9	2.59
15.08	14.8	2.02**	16.58	17.6	1.37	24.08	23.9	2.53
8.33	*	*	24.00	15.4	1.23	24.42	17.4	0.896**
15.50	9.76	1.23**				23.58	15.29	1.19

Δt = time from previous elution.

*No sample reserved for HPLC analysis.

**Previous elution volume was 5 mL, resulting in incomplete elution of the generator. This volume is thus erroneously high.

Table II. Comparison of Total Technetium Determinations by
Liquid Scintillation Counting and by HPLC

	<u>Beta*</u>	<u>HPLC**</u>
Size Generator, mCi	440 & 2200	7450
$10^7 \times [^{99}\text{TcO}_4^-], \text{ M}$	2 - 178	1 - 47
$10^{12} \times \frac{\text{moles Tc-99}}{\text{mCi Tc-99m}}$	20 - 717	3 - 35

*Data from reference 6.

**Data from Table I.

FIGURE CAPTIONS

- Figure 1. Calibration plot of peak height vs. $[K^{99}TcO_4]$ over the range 6.43×10^{-8} M to 6.43×10^{-6} M. Injected volume - 100 μ L.
- Figure 2. Typical chromatograms, with solid line representing UV detection and dashed line representing gamma-ray activity detection: (A) 6.43×10^{-8} M $K^{99}TcO_4$; (B) $^{99}Mo/^{99m}Tc$ generator eluent.
- Figure 3. Chromatogram of $^{99}Mo/^{99m}Tc$ generator eluent from Manufacturer A (ca. 60 mCi/mL) using gamma-ray activity detection with expanded sensitivity.
- Figure 4. Chromatogram of $^{99}Mo/^{99m}Tc$ generator eluent from Manufacturer A. Solid line represents UV detection and dashed line represents gamma-ray activity detection. Time elapsed since previous generator elution = 72 hr; UV peak height corresponds to 4.74×10^{-6} M TcO_4^- .
- Figure 5. Chromatogram of $^{99}Mo/^{99m}Tc$ generator eluent from Manufacturer B. Solid line represents UV detection and dashed line represents gamma-ray activity detection. Time elapsed since previous generator elution = 48 hr; UV peak height corresponds to 5.93×10^{-6} M TcO_4^- .
- Figure 6. Comparison of experimental and theoretical values of the ratio (moles total technetium)/(mCi ^{99m}Tc) in the eluent of a 7,450 mCi $^{99}Mo/^{99m}Tc$ generator as a function of time. Data taken from Table I (Generator #2).

GENERATOR "A"

GENERATOR "B"