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Separation Science and Technology

Publication details, including instructions for authors and subscription information:
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To cite this Article Rogers, Robin D. , Bond, Andrew H. , Zhang, Jianhua and Horwitz, E. Philip(1997) 'New Technetium-99M Generator Technologies Utilizing Polyethylene Glycol-Based Aqueous Biphasic Systems', Separation Science and Technology, 32: 1, 867 — 882

To link to this Article: DOI: 10.1080/01496399708003234

URL: http://dx.doi.org/10.1080/01496399708003234

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SEPARATION SCIENCE AND TECHNOLOGY, 32(1-4), pp. 867-882, 1997

NEW TECHNETIUM-99m GENERATOR TECHNOLOGIES UTILIZING POLYETHYLENE GLYCOL-BASED AQUEOUS BIPHASIC SYSTEMS

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ABSTRACT

Two new schemes for TcO_4^-/MoO_4^{2-} separations from OH^- and MoO_4^{2-} media using polyethylene glycol (PEG)-based aqueous biphasic systems (ABS) have been developed. The two most important salt solutions in current ^{99m}Tc-generator technologies, OH^- and MoO_4^{2-}, also salt out PEG to form ABS. In liquid/liquid PEG-ABS, pertechnetate can be separated from molybdate with separation factors as high as 10,000. Stripping is accomplished by reduction of the TcO_4^- and back extraction into a salt solution. The strip solution can be the salt of an imaging agent (e.g., Na_4HEDPA) and thus may, under the appropriate conditions, be injected directly into the human body. $^{99m}TcO_4^-$ can also be concentrated from a dilute load solution of $^{99}MoO_4^{2-}$ in NaOH using an aqueous biphasic extraction chromatographic technique (ABEC). A rinse with K_2CO_3 assures that all $^{99}MoO_4^{2-}$ is removed from the column, and this is confirmed by a rapid drop in ^{99}Mo activity by the fourth free column volume (fcv) of rinse. The $^{99m}TcO_4^-$ is then eluted with water. This chromatographic separation affords 94% of the $^{99m}TcO_4^-$ activity in 5 fcv, with the γ spectrum showing less than 2×10^{-4} of the original ^{99}Mo activity.

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INTRODUCTION

Although technetium-99m is used in the vast majority of all diagnostic nuclear medicine, its continued supply is at best uncertain (1). ^{99m}Tc is obtained as the daughter of ^{99}Mo, which is either isolated as a ^{235}U fission product or from neutron-irradiated ^{98}Mo (2-6). Technetium-99m from the former process is expensive, has few suppliers, requires extensive clean-up prior to use, and generates high levels of radioactive wastes. The latter process is cheaper and cleaner, but the supply of ^{98}Mo is dwindling due to the shutdown of some of the electromagnetic separators used to purify it. ^{99}Mo produced in this fashion has a low specific activity, and thus concentrating the ^{99m}Tc is problematic.

Currently, ^{99}Mo is converted to molybdate, MoO_4^{2-}, in base and adsorbed on alumina. The $^{99m}TcO_4^-$ is then eluted with physiological saline solution and either used in that form or reduced and complexed with an imaging agent. Contaminants include Al^{3+} and molybdate that also elute, and thus high-specific-activity fission-produced ^{99}Mo is required. A solvent extraction-based generator is known which uses methyl ethyl ketone solvent (5). This generator can utilize low-specific-activity neutron irradiation-produced ^{99}Mo, but it requires a flammable, toxic organic solvent and gives rise to a much more complicated process.

We discovered several aspects of the chemistry of polyethylene glycol (PEG)-based aqueous biphasic systems (ABS) which intrigued us enough to explore their potential as ^{99m}Tc generators (7-9). ABS consist of two immiscible phases formed when certain water-soluble polymers are combined with one another or with certain inorganic salts in specific concentrations. They are suitable, as two-phase systems, for carrying out liquid/liquid separations of various solutes such as biomolecules, metal ions, and particulates (10-14). In ABS *the major component in each of the two phases is water*, and because of this non-denaturing environment, these systems have been widely employed in biological separations for over 40 years. A number of reviews on the bioanalytical uses of and theory of bioseparations are available (10-16).

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We initially entered this field because of the potential for viable metal-ion separations (7-9, 17-22). PEG-ABS are virtually nontoxic and nonflammable, all components are commercially available in bulk quantities and are inexpensive, and the systems have reasonable phase separation characteristics (17, 18) and can even be used with traditional solvent extraction equipment (11, 16).

ABS have been criticized for metal-ion separations because of the necessity to strip into a salt solution so that a two-phase system is maintained. While this may actually be an advantage in some applications, we have recently obviated this need by adapting PEG-ABS to a solid-supported chromatographic mode (23, 24). Although the chromatographic techniques employed probably fit under the category of hydrophobic interaction chromatography (25, 26), we prefer to name this separation technique: Aqueous Biphasic Extraction Chromatography (ABEC). We believe this acronym is appropriate due to the behavior of our resins and the strong relationship with liquid/liquid PEG-ABS.

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ABS can thus be utilized in both liquid/liquid and solid chromatographic separations and can be fine tuned to meet a variety of demands. Given the current concerns and problems associated with using volatile organic compounds, ABS offer the possibility of clean separations technologies and should be fully explored to exploit this potential. In this contribution, we compare and contrast the utility of liquid/liquid PEG-ABS and ABEC resins for the separation and recovery of pertechnetate from molybdate.

EXPERIMENTAL

Reagents and Tracers

NaOH was purchased from Fisher Scientific and used without further purification. K_2CO_3, K_3PO_4, Na_2CO_3, Na_2MoO_4, Na_3(citrate)$\cdot 2H_2O$, Na_4(HEDPA) (HEDPA = 1-hydroxyethane-1,1-diphosphonic acid or its tetraanion), $SnCl_2 \cdot 2H_2O$, PEG-2000, Me-PEG-5000, and chloromethylated polystyrene-1% divinylbenzene beads (200-400 mesh) were obtained from Aldrich. (The salts were of reagent grade.) All water was deionized using a commercial deionization system.

The long-lived technetium-99 radioisotope ($t_{1/2} = 2.14 \times 10^5$ years) was used except as noted in the text. $NH_4{}^{99}TcO_4$(aq) was obtained from both Isotope Products Laboratories and Amersham. Ultima Gold scintillation cocktail was purchased from Packard Instrument Co., and the $Na_2{}^{99}MoO_4$ tracer was obtained from Medi-Physics. Technetium-99m was isolated as a daughter of the ^{99}Mo used.

PEGs are available from the suppliers (Aldrich, Union Carbide) as a blend with an average molecular weight. The molecular weights, which are determined by gel permeation chromatography, generally will fall within a fairly tight range. The PEG-2000 used in the majority of this work has a molecular weight range of 1900 to 2500 g/mol.

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Phase Characterizations

Phase diagrams for PEG-2000/salt systems were determined by turbidimetric titration (10). Systems with compositions in the two-phase region were weighed into tubes. Water was gradually added with vortexing until the turbid mixture just became clear. The final mass of the system was determined, and the compositions at the points of phase transition were calculated.

Partitioning Studies

The liquid/liquid partitioning studies were carried out from ABS formed by mixing 1 mL of salt stock solution and 1 mL of aqueous PEG stock solution. The systems were equilibrated by vortexing for 2 min followed by centrifugation for 2 min. A tracer quantity of $^{99}TcO_4^-$ or $^{99}MoO_4^{2-}$ was added to the equilibrated biphasic system followed by 2 min of centrifugation, 2 min of vortexing, and 2 min of centrifugation. The phases were then separated and placed into separate vials. 100-µL aliquots of each phase were removed and added to 3 mL of Ultima Gold liquid scintillation cocktail. The ^{99}Tc β^--decay was monitored using a Packard Tri-Carb 1900 TR Liquid Scintillation Analyzer, while the ^{99}Mo γ- decay was measured using a Packard Cobra

II gamma counter. Since equal volumes of each phase were counted, the distribution ratios (formally [tracer] (in the PEG-rich phase)/[tracer] (in the salt-rich phase)) were calculated as the ratio of the activity in counts per minute (cpm) in the upper PEG-rich phase compared to that in the lower salt-rich phase.

For studies involving $SnCl_2$, the reducing agent was dissolved to a given concentration in the salt stock solution used to form the ABS. 1 mL of each of these solutions and 1 mL of 40% (w/w) PEG-2000 were contacted. The systems were pre-equilibrated, spiked with tracer, equilibrated, separated, and sampled in the same way as described above.

All distribution ratios were performed in duplicate as a check on the experimental technique and precision. The distribution ratios generally agreed to within ±5%.

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Synthesis of ABEC-5000

Under an Ar atmosphere, NaH (0.38 g, 16 mmol) was weighed into a 1-L 3-neck flask which was capped and brought out to the benchtop. Under a positive flow of Ar, the system was equipped with a 400-mL slow addition funnel and a water-jacketed reflux condenser. THF (THF = tetrahydrofuran, ≈ 400 mL) was then transferred to the addition funnel via standard cannula techniques. 100 mL of THF was allowed to drain into the round-bottom flask in order to suspend the NaH with stirring. Me-PEG-5000 (15.0 g, 3.0 mmol) was then added to the addition funnel under a positive Ar flow. (Me-PEG-5000 is a solid and was melted in the THF in the addition funnel with the aid of a hot-air gun.) This solution was added dropwise over approximately 0.5-1 h to the NaH suspension at 0°C. Once addition was complete, the solution was allowed to stir at 0°C for 1 h. The mixture was then warmed to room temperature with stirring followed by the addition of chloromethylated polystyrene-1% divinylbenzene (3.0 g, 3.0 mmol reactive sites) under a positive Ar flow. The reaction was stirred at 25°C for 1-2 h followed by 36-72 h of refluxing with stirring. The remaining murky solutions were then filtered using a large Büchner funnel and the resin exhaustively extracted with THF in a Soxhlet extraction apparatus for 72 h to remove unbound Me-PEG. The resin was then dried *in vacuo* and small aliquots hydrated for the weight distribution ratio experiments as needed.

Metal-Ion Uptake Experiments

The dry weight conversion factors for ABEC-5000 were determined in the following manner. A quantity of the dry resin was gently stirred in an excess of water for 30 min at room temperature followed by 15 min of air drying on a Büchner funnel. A portion of the air-dried resin was then transferred to a tared crucible and dried in an oven at 110°C until a constant mass was achieved. Each gravimetric analysis was performed in duplicate and was repeated each time a new batch of hydrated resin was prepared. All resins were stored in tightly capped containers and were not exposed to air for any extensive period of time so as to avoid a change in water content.

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All weight distribution ratios were radiometrically determined by batch contacts of the resin with the desired solution. The dry weight distribution ratio is defined as

$$D_W = \left(\frac{A_O - A_f}{A_f}\right)\left(\frac{V}{m_R \cdot wcf}\right)$$

where A_O = the activity of the solution prior to contact with the resin, A_f = the activity of the solution after contact with resin, V = volume (mL) of solution contacted with resin, m_R = mass (g) of resin, and wcf = the dry weight conversion factor relating the mass of the hydrated resin to its dry weight.

The D_W experiments were carried out in the following manner. The radiotracer was added to 1.2 mL of the solution of interest, gently mixed, and a 100-µL aliquot removed for counting (A_O). One mL of the remaining solution (V) was added to a known mass of hydrated resin (m_R), and the mixture was centrifuged for 1 min. The solution was then stirred gently (so that the resin was just suspended in the solution) for 30 min, followed by 1 min of centrifugation, and another 30 min of stirring. After 1 min of centrifugation, the solution was pipetted away from the resin and filtered through a 45-µm pipette-tip filter so that any suspended resin would be removed. A 100-µL aliquot was then removed for counting (A_f).

Resin Irradiation Experiments

Small samples (ca. 0.5 g) of ABEC-5000 were placed in glass test tubes and covered with ca. 0.8 mL of either water or 5 M NaOH. The test tubes were covered with parafilm and placed together with a test tube containing a Co glass radiation monitor in the cave containing the 70,000-Ci ^{60}Co source in the Chemistry Division of Argonne National Laboratory. The samples were irradiated at a dose rate between 3-8 x 10^5 rads/h. The cumulative dose was measured using the Co glass plates. After irradiation, the samples were thoroughly washed with water and air dried. Metal-ion uptake studies were carried out as noted above.

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RESULTS AND DISCUSSION

Phase diagrams for the five PEG-2000/salt ABS investigated in this report are presented in Figure 1. The curves shown are the binodals: system compositions above the binodal for a given salt are biphasic, while systems compositions below the binodal are monophasic. The relative ability of the five salts to salt out PEG increases in the order NaOH < Na_2MoO_4 < K_2CO_3 < Na_3(citrate) < Na_4(HEDPA). This order follows from the water-structuring ability of each salt. The more water-structuring a salt is, the more it is capable of salting out PEG. We have previously correlated this ability to salt out PEG with the anion's Gibbs free energy of hydration (27).

We have also previously shown that the pertechnetate anion quantitatively partitions to the PEG-rich phase of PEG-ABS due to its low charge-to-size ratio and thus small ΔG_{hyd} (8, 9, 27). We also discovered that molybdate actually salts out PEG (8) as does NaOH (Figure 1). Since most technetium-99m generators rely on forming molybdate in base (1-6), we reasoned that it should be possible to effect a separation of TcO_4^- even from low-specific-activity molybdate solutions. This is substantiated by the data shown in Figure 2. Distribution ratios for TcO_4^- are plotted

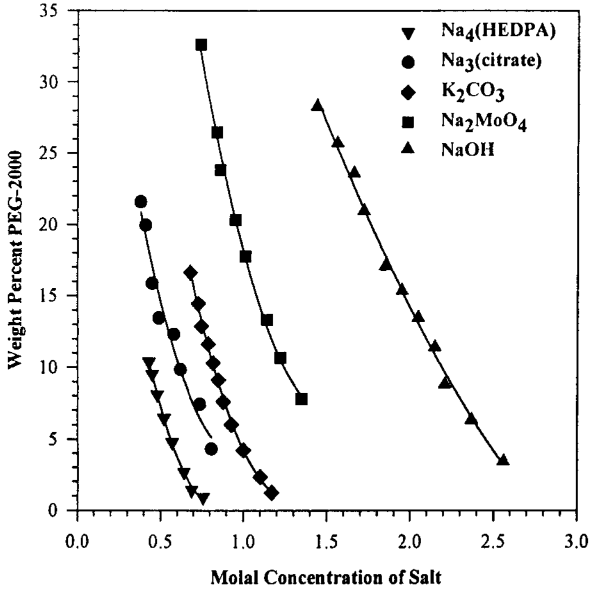

FIGURE 1. Phase diagrams for five PEG-2000/salt systems.

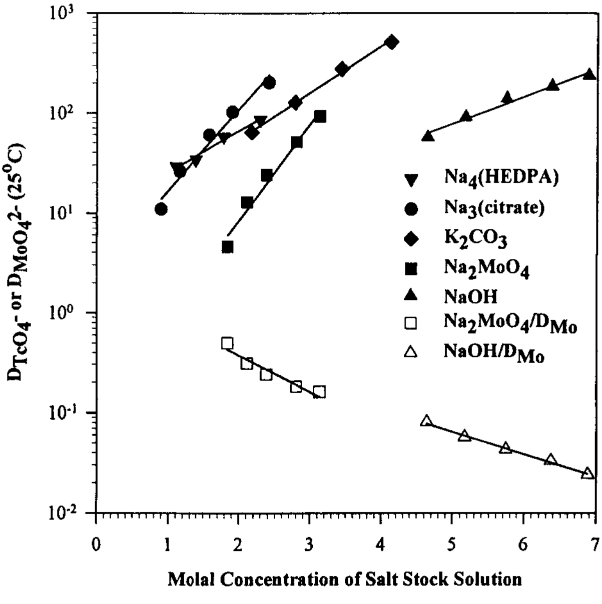

FIGURE 2. Distribution ratios for TcO_4^- or MoO_4^{2-} in 40% PEG-2000/salt ABS.

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versus the concentration of salt stock solution used to prepare the biphase. The trends we have associated with pertechnetate partitioning in PEG-ABS are apparent. As the salt or PEG concentrations increase, the phase incompatibility increases. The net result is that as phase incompatibility increases, the partitioning of species that prefer one phase or the other is enhanced to the phase of preference (15, 28-30). Thus, as salt concentrations are increased, TcO_4^- distribution ratios increase, while those for MoO_4^{2-} decrease. At 3.13 m Na_2MoO_4 stock solution and 40% PEG-2000, the separation factor for TcO_4^-/MoO_4^{2-} is 580. An even higher separation factor of 10,000 is achieved at 6.88 m NaOH.

Stripping can be achieved by reducing the pertechnetate to an oxy-chloro cationic species, which dramatically changes the interaction of the ion with water and thus the partitioning behavior. Figure 3 illustrates stripping into Na_3(citrate) and Na_4(HEDPA) using $SnCl_2$ as the reducing agent. (HEDPA is a widely used imaging agent in diagnostic nuclear medicine (6).) Since the reduced technetium species prefers the salt-rich phase, an increase in the phase incompatibility resulting from increasing the salt stock solution concentration results in a decrease in the technetium distribution ratios.

Based on the above results, a process for a ^{99m}Tc generator can be envisioned involving conversion of low-specific-activity ^{99}Mo to a molybdate salt in a highly alkaline solution (1-6). Contacting this solution with aqueous PEG will remove any $^{99m}TcO_4^-$ which can be stripped by contacting the loaded PEG-rich phase with a fresh solution of a phase forming salt containing $SnCl_2$. If the salt used is also an imaging agent, the resulting strip may be amenable to direct injection into the body following appropriate sterilization and formulation.

Because the back extraction of technetium into a salt solution in the liquid/liquid mode may be undesirable, we have also adapted aqueous biphasic separations to solid-supported chromatographic techniques (23, 24). Using our knowledge obtained from the liquid/liquid studies, we were able to design and test batch and column chromatographic separations of pertechnetate from molybdate.

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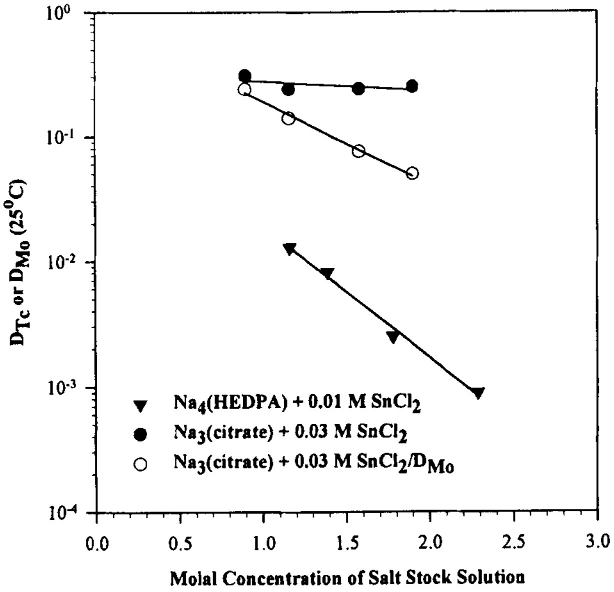

FIGURE 3. Distribution ratios for reduced technetium and molybdenum species in 40% PEG-2000/salt ABS containing $SnCl_2$.

The resin utilized in this study (ABEC-5000) consists of Me-PEG-5000 grafted to chloromethylated polystyrene-1% divinylbenzene beads. We prefer the acronym ABEC (for Aqueous Biphasic Extraction Chromatography) because we have applied the principles of extraction in ABS to solid chromatographic methods.

Dry weight distribution ratios for TcO_4^- from two representative salts (Figure 4) illustrate the relationship between liquid/liquid ABS and ABEC. ABEC-5000 extracts greater than 99.9% of the pertechnetate from NaOH and K_2CO_3. The D_w values from K_2CO_3 are higher than from NaOH as expected from the relative salting-out ability of carbonate vs. hydroxide (Figure 1). In addition, as observed in the liquid/liquid studies, distribution ratios increase as the salt concentration increases. Without a salt solution known to salt out PEG and form an ABS, ABEC-5000 does not retain TcO_4^-, **stripping can thus be achieved simply by washing with water!**

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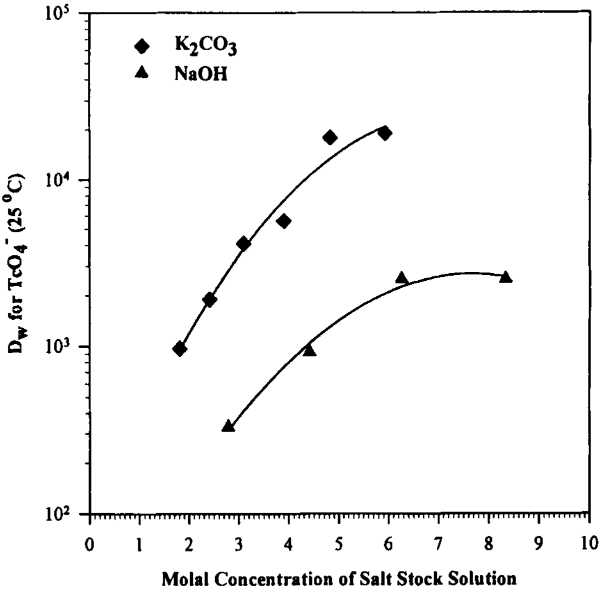

FIGURE 4. Dry weight distribution ratios for TcO_4^- on ABEC-5000 versus molality of salt stock solution.

A chromatogram of a $^{99m}TcO_4^-/^{99}MoO_4^{2-}$ separation using ABEC-5000 is presented in Figure 5. $^{99m}TcO_4^-$ was concentrated from a dilute load solution of $^{99}MoO_4^{2-}$ in 5 M NaOH using ABEC-5000. The rinse with K_2CO_3 assured that all $^{99}MoO_4^{2-}$ was removed from the column,and this was confirmed by the rapid drop in ^{99}Mo activity by the fourth free column volume (fcv) of rinse. **The pure $^{99m}TcO_4^-$ was then eluted with water.** This chromatographic separation afforded 94% of the $^{99m}TcO_4^-$ activity in 5 fcv, with the γ spectrum showing no trace of ^{99}Mo activity. This process is now the subject of two recent patent applications (23, 24) and represents a significant breakthrough in pertechnetate separation methods because: a) there is no load limitation for MoO_4^{2-}, b) separations can be carried out at high pH, c) no organic solvents are needed, d) there is no loss of PEG, and e) stripping can be achieved utilizing water.

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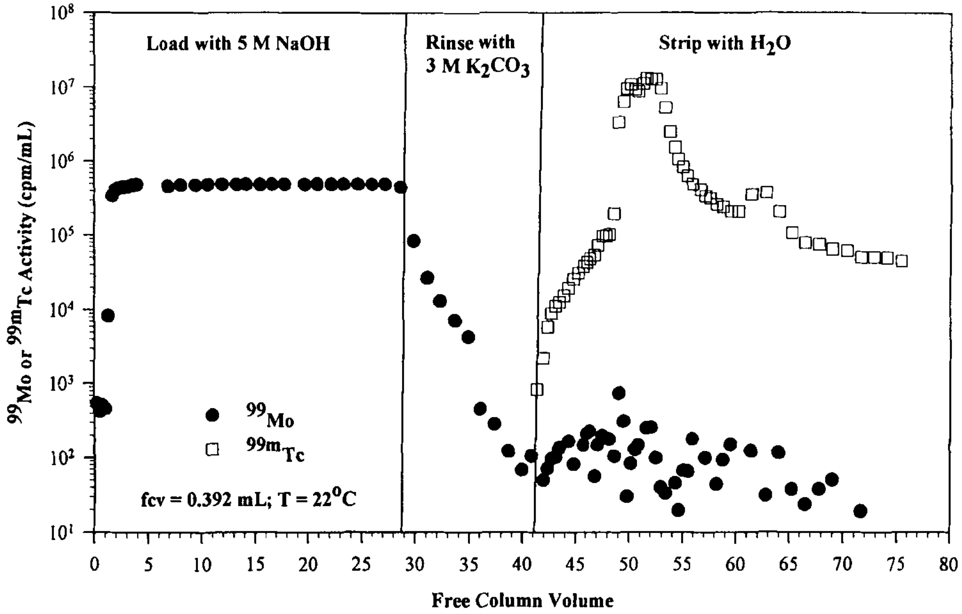

FIGURE 5. Chromatographic separation of $^{99m}TcO_4^-$ from $^{99}MoO_4^{2-}$ using ABEC-5000.

Utilization of ABEC-5000 in a ^{99m}Tc generator will require exposure to high levels of radioactivity. To test the radiation stability of ABEC-5000, several samples in either water or 5 M NaOH were exposed to the 70,000-Ci ^{60}Co irradiation source in the Chemistry Division at Argonne National Laboratory. Figure 6 presents D_w data for the uptake of TcO_4^- as a function of K_2CO_3 concentration for unexposed resin, resin with 1.2 and 12 Mrad exposure in water, and resin with 1.2 Mrad exposure in NaOH. There appears to be some drop-off in D_w at higher salt concentrations, but the uptake parameters are still well within acceptable limits. The small drop in D_w is consistent with some crosslinking of the PEG chains resulting in greater dehydration of the resin at high salt concentrations.

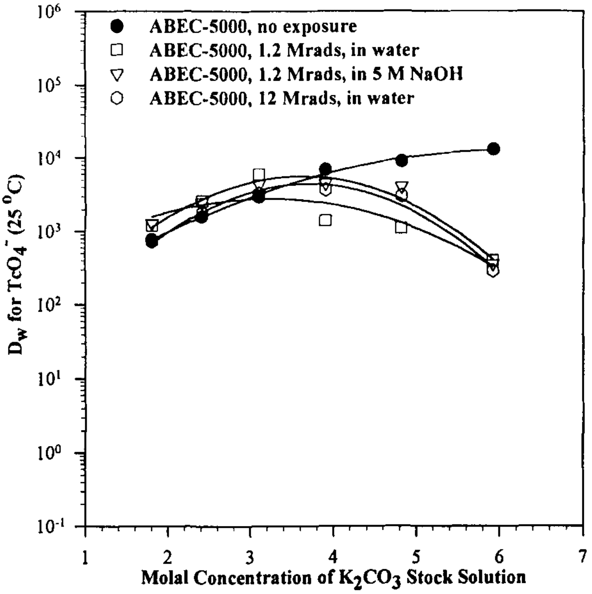

FIGURE 6. Dry weight distribution ratios for TcO_4^- versus molality of K_2CO_3 stock solution using unirradiated and irradiated ABEC-5000.

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CONCLUSIONS

Polyethylene glycol-based aqueous biphasic systems are suitable for the separation of pertechnetate from molybdate both in a solvent extraction and in a solid chromatographic process. The ABEC process has the advantages of giving higher D_w values at lower salt concentrations, being simpler and cleaner (there is no polymer loss), and allowing recovery of the pertechnetate in water. Radiation stability of the ABEC resins is good,and this material should be suitable for use in a ^{99m}Tc-generator. Further work in our research groups is progressing toward developing such a generator.

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ACKNOWLEDGEMENTS

Portions of this work were supported by U.S. National Science Foundation Grant CTS-9207264, and portions were performed under the auspices of the Office of Basic Energy Sciences, Divisions of Chemical Sciences and Advanced Energy Projects, U.S. Department of Energy. We thank Herbert Diamond for technical assistance and Michael W. and Christopher D. Drobnik, Medi-Physics, Inc., Arlington Heights, IL, for a gift of $Na_2{}^{99}MoO_4$.

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