Factors Influencing Paper Chromatographic Analysis of Technetium-99m Phosphorus Compounds: Concise Communication

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A single-paper chromatographic system has been developed, capable of resolving Tc-99m dioxide, [^{99m}Tc] pertechnetate and Tc-99m phosphorus compounds. The best separations are obtained with CM82 paper developed in 0.5 M NaCl, and 3MM or ashless No. 40 paper developed in 1 M sodium acetate buffer. In these systems, $^{99m}TcO_2$ remains at the origin, while $^{99m}TcO_4^-$ and Tc-99m phosphorus compounds move with R_f values of 0.56–0.75 and 0.80–1.0, respectively.

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Pertechnetate (TcO_4^-)* was initially considered to be the primary radiochemical impurity present in most Tc-tagged radiopharmaceuticals. Now it is well known that there is another Tc radiochemical impurity, reduced hydrolyzed technetium (*1,2*), which may include tin hydrolysis products (*3,4*). In this report, this insoluble material will be referred to as technetium dioxide, TcO_2.

Paper chromatography is one of the accepted tools for the analysis of radiochemical impurities in Tc radiopharmaceuticals. Among the current solvent systems in use, acetone, 85% methanol, and methylethyl ketone (MEK) are solvents of choice. Although these systems will separate TcO_4^- from the Tc-labeled compounds, they do not separate TcO_2. For example, these solvents do not separate Tc pyrophosphate (Tc-PPi) from TcO_2 (*1,2,5,6*). These solvent systems, therefore, are of limited value for the testing of the radiochemical purity of these radiopharmaceutical formulations.

Factors that govern compound migration on paper chromatographic strips include the solubility of the compound in the developing solution and the nature of the paper strip. We have investigated the influence of these two factors on radiopharmaceutical migration on different types of paper strips. The present account deals with the development of a single-paper chromatographic method that separates Tc hydroxyethylidene diphosphonate (TcHEDP), Tc methylene disphosphonate (TcMDP) and TcPPi from both TcO_4^- and TcO_2.

MATERIALS AND METHODS

The phosphorus compounds used were disodium hydroxyethylidene diphosphonate, methylene diphosphonate, and stannous pyrophosphate. Each was obtained in kit form; preparations were reconstituted by the addition of an appropriate volume of TcO_4^- according to the manufacturer's directions to form the corresponding Tc phosphorus compound. Technetium-99m dioxide was prepared by reducing TcO_4^- with stannous ion at pH > 5 in the absence of a complexing or chelating agent.

Whatman paper No. 3MM, ashless paper No. 40, and carboxymethyl cellulose ion-exchange paper No. CM82 were evaluated in this study. Whatman paper No. 3MM was washed in distilled water and dried for approximately 1 hr before use. Ashless No. 40 and CM82 papers were used as supplied.

The developing solvent systems used were 85% methanol, acetone, and methylethyl ketone (organic solutions); sodium chloride and sodium acetate buffer pH 4.75 (aqueous solutions). Sodium acetate buffer was made by mixing equal volumes and equimolar concentrations of sodium acetate and acetic acid. Preliminary studies included the use of various concentrations of the aqueous solutions.

A 1-μl aliquot of each Tc solution (TcO_4^-, col-

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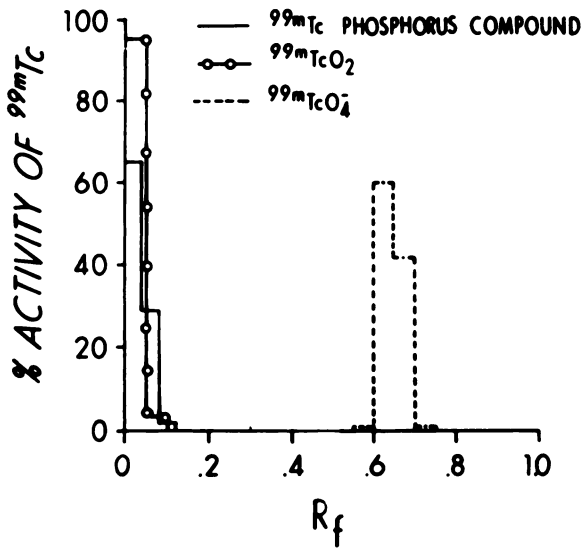

FIG. 1. Typical radiochromatogram of the tested Tc phosphorus compounds developed in 85% methanol, acetone, or methylethyl ketone using Whatman No. 3MM paper. Note that TcO_2 and the Tc phosphorus compound have about the same R_f value.

loidal suspension of TcO_2 or Tc phosphorus compound) was placed 2 cm from the end of the paper strip to be dipped in the developing solvent. The chromatographic papers were developed by the ascending method under atmospheric conditions. After drying, each strip was cut into 5-mm pieces and the Tc activity was assayed with a NaI(Tl) well scintillation crystal. The sum of the activities on each strip was compared with a 1-μl aliquot of the corresponding Tc solution to confirm that no activity was lost during analysis.

RESULTS

The probable radiochemical impurities present in the formulation of a Tc phosphorus compound include TcO_4^- and TcO_2. A Whatman No. 3MM paper strip developed in an organic solution such as methanol, acetone, or MEK can adequately separate TcO_4^- from Tc phosphorus compounds, but it cannot separate TcO_2 from the Tc phosphorus compounds. Both the TcO_2 and the Tc phosphorus compounds remain at the origin, whereas TcO_4^- migrates with an R_f value of 0.6–0.75 (Fig. 1).

Preliminary experiments using aqueous solutions as the developing solvent indicated that both TcO_4^- and Tc phosphorus compounds, in dilute solutions, migrate with, or close behind, the solvent front. The behavior of TcO_4^- migration on CM82, 3MM, and ashless No. 40 papers developed in sodium chloride and sodium acetate buffer was further studied. Figure 2 shows R_f values for TcO_4^- as a function of the concentration of the developing solutions, sodium chloride, and sodium acetate buffer. The results indicate that as the concentration of the solvent increases, the R_f value for TcO_4^- decreases.

Based on the extent of TcO_4^- migration in these systems, as in Fig. 2, further studies with Tc phosphorus compounds and TcO_2 colloidal solutions were done with CM82 paper developed in 0.5 *M* NaCl, and with 3MM ashless No. 40 papers developed in 1 *M* sodium acetate buffer. Figure 3 shows the radiochromatograms of TcHEDP, TcMDP, TcPPi, TcO_4^-, and TcO_2 using CM82 paper developed in 0.5 *M* sodium chloride solution. The TcHEDP (Fig. 3A) is well separated from TcO_2 ($R_f = 0.0$) and TcO_4^- ($R_f = 0.68$). Both TcMDP and TcPPi (Figs. 3B and C) show Tc activity along the entire paper strips. Figures 4 and 5 are the radiochromatograms of TcHEDP, TcMDP, TcPPi, TcO_4^-, and TcO_2 using 3MM and ashless No. 40 papers developed in 1 *M* acetate buffer. Both TcHEDP (Figs. 4A and 5A) and TcMDP (Figs. 4B and 5B) are well separated from TcO_2 ($R_f = 0.0$) and TcO_4^- ($R_f = 0.72$ (Fig. 4) and 0.68 (Fig. 5)). The Tc activity is spread along the entire length of the paper strips for TcPPi (Figs. 4C and 5C). The streaking of Tc activity is less, however, on ashless No. 40 paper than on the 3MM. The ashless No. 40 gives the best separation for TcPPi.

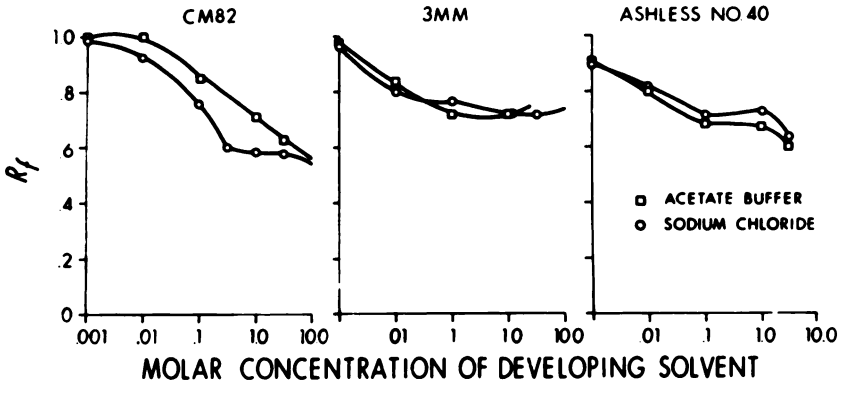

FIG. 2. R_f values of TcO_4^- as a function of concentration of developing solution.

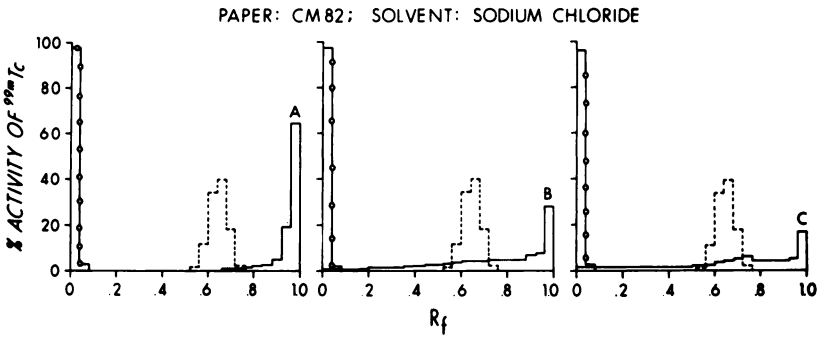

FIG. 3. Carboxymethyl cellulose ion-exchange paper (No. CM82) developed in 0.5 M NaCl, showing the relative positions of each Tc compound. (A) TcHEDP; (B) TcMDP; (C) Tc-PPi (- - - = TcO_4^-, -o-o- = TcO_2). Note that both TcMDP and TcPPi are smeared along the paper strips.

DISCUSSION

Pollard and McOmie (*7*) have shown that the solubility of an inorganic compound in an organic solvent will exert control over the migration of a compound along the paper strip. Very soluble compounds migrate with, or close behind, the solvent front, whereas less soluble compounds stay at the origin. Pertechnetate ion is fairly soluble in the organic solvents tested (acetone, MEK, or methanol) and therefore moves from the origin. Both Tc phosphorus compounds and TcO_2 are insoluble in these solutions, and therefore do not move from the origin. It is not uncommon for workers using these solvent systems (acetone, MEK, or methanol), to report over 95% labeling efficiency for these Tc phosphorus compounds (*6,8,9*). The basic error in these systems is that other radiochemical impurities such as TcO_2 may be included in the calculated labeling efficiency. This kind of error can be avoided if TcO_2 is properly separated from the Tc phosphorus compound.

Technetium-99m dioxide is not soluble in aqueous solutions and remains at the origin. Both TcO_4^- and Tc phosphorus compounds are soluble and migrate from the origin. In dilute solutions, both compounds migrate with, or close behind, the solvent front. The use of more concentrated solutions results in decreased TcO_4^- migration and enhances its separation from Tc phosphorus compounds. This decrease in R_f value of TcO_4^- is attributed to the increase in the ionic strength of the developing solvent. A similar observation has been made by Pluchet and Lederer (*10*). The R_f values of certain anions, VO_3^-, MoO_4^{2-}, and CrO_4^{2-}, were observed to decrease with increasing concentration of the developing solvent.

The spreading of Tc activity along the length of the paper strip is a definite problem with the TcPPi chromatogram. This may be due to a reaction between TcPPi and impurities on the papers. These impurities probably are cations such as alkali metals, calcium, magnesium, and iron (*11*). The percentage of Tc activity for TcPPi at an R_f range of 0.80–1.0 increased from 31.5% to 56.5% (Figs. 4C, 5C) when 3MM paper is replaced by either ashless No. 40 or CM82 paper. Ashless No. 40 paper is a refined form of 3MM paper in which some of the cations have been removed, whereas CM82 is an ion-exchange paper in which the main cation is H^+. Tetrasodium pyrophosphate is completely dissociated in solution, therefore, the TcPPi probably does not react with the H^+ ion on CM82 paper.

The procedure currently used for separating both TcO_4^- and TcO_2 from labeled compounds is to use 85% methanol and 0.9% sodium chloride solutions, and involves running two separate chromatographs (*1,2*). With 85% methanol, the presence of TcO_4^- can be detected and quantified; TcO_2 is detected and quantified with 0.9% sodium chloride solution. The basic problem with this approach is that the data used in the calculations are derived from experiments

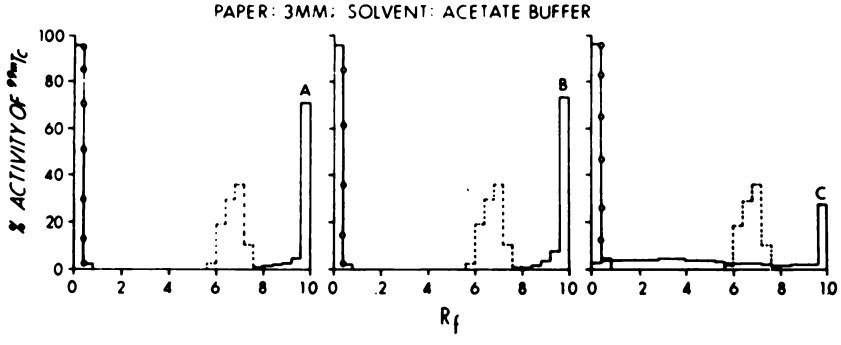

FIG. 4. Whatman No. 3MM paper developed in 1 M sodium acetate buffer showing the relative positions of each Tc compound. (A) TcHEDP; (B) TcMDP; (C) TcPPi (- - - = TcO_4^-, -o-o- = TcO_2). Note that both TcHEDP and TcMDP are well separated from TcO_2 and TcO_4^-; TcPPi is smeared along the paper strip.

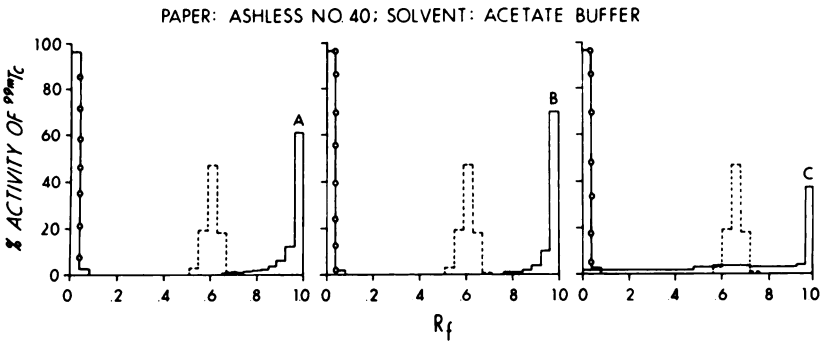

FIG. 5. Ashless No. 40 paper developed in 1 *M* sodium acetate buffer, showing the relative position of each Tc compound. (A) TcHEDP, (B) TcMDP, (C) TcPPi (- - - = TcO_4^-, -o-o- = TcO_2). Both TcHEDP and TcMDP are well separated from TcO_2 and TcO_4^-. Again TcPPi is smeared along the strip.

performed under different sets of conditions. The techniques developed in this study require a single chromatographic test that separates Tc phosphorus compounds from both TcO_4^- and TcO_2. With this method, it should now be possible to determine whether Tc phosphorus compounds stored at room temperature for long time periods will dissociate into TcO_2 or oxidize to TcO_4^-.

CONCLUSION

A single-paper chromatographic system for separating radiochemical impurities from Tc phosphorus compounds has been developed. This system requires the Tc phosphorus compound to be soluble in the developing solvent for migration. The factors that influence the extent of migration of each compound include both the nature of the paper and the concentration of the developing solvent. The following systems are recommended for routine testing: for TcHEDP—CM82 paper developed with 0.5 *M* sodium chloride and 3 MM or ashless No. 40 paper developed with 1 *M* sodium acetate buffer; for TcMDP—3MM or ashless No. 40 paper developed with 1 *M* sodium acetate buffer. The method developed here is not ideal for routine testing of TcPPi because of the streaking of Tc activity on the paper strip. As we have shown here, the paper-solvent characteristics greatly influence the resolution of the chromatograms. Our findings suggest that new efforts should be directed towards the development of discrete paper-solvent systems for specific Tc radiopharmaceuticals.

FOOTNOTE

* Throughout this paper the symbol "Tc" will be used to represent technetium-99m.

ACKNOWLEDGMENTS

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