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REDUCTION OF PERTECHNETATE-99m ION BY HYDROCHLORIC ACID

I. PREPARATION OF CHROMATOGRAPHICALLY PURE TECHNETIUM-99m(IV)

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SUMMARY

Chromatographically pure samples of pertechnetate-99m ion were reduced by concentrated hydrochloric acid at various temperatures. The reduction is more rapid than that of pertechnetate-99 ion. It was found to take place *via* technetium-99m(V) giving chromatographically pure technetium-99m(IV) as the end product. Technetium-99m(IV) is stable in dilute hydrochloric acid and in physiological saline solution. Loss of technetium-99m by volatilization can be minimized by carrying out the reduction at or below 70°.

INTRODUCTION

Technetium-99m and technetium-99 are the two most important isotopes of the element¹. Technetium-99, long since available in large amounts^{1,2}, has found use as a corrosion inhibitor, as a superconductor, and as a source of pure beta-radiation^{1,2}. It has also been the isotope used in almost all studies of the physical and chemical properties of the element³. Technetium-99m has been used for some time^{1,2} in the study of the effect of the chemical and physical state of the isotope on the radioactive decay constant. Technetium-99m generators, first introduced by the Brookhaven National Laboratory⁴, have been developed to take advantage of the long half-life (67 h) of the parent, molybdenum-99, and the short half-life (6 h) of the daughter isotope, technetium-99m, and of the difference between the adsorption of molybdate-99 (strongly held) and pertechnetate-99m (easily eluted) ions on alumina columns. These generators are now the main source of the short-lived radionuclide for laboratories remote from the production site of the parent radioisotope. Very favourable nuclear properties (half-life 6 h, no beta-emission and a monoenergetic gamma-emission of 140 keV) and the stability of the pertechnetate-99m ion on the generator column as well as in solution have made technetium-99m the radionuclide of choice in nuclear medicine^{5,6}. The ionic volume⁷, stability in solution and biological systems, quick uptake and elimination after a few hours⁸ of pertechnetate-99m ion have led to

its use in brain and thyroid gland scintigraphy⁵. As studies with technetium-99 have shown¹⁻² the element to be capable of existing in oxidation states ranging from 1- to 7+, the attention of researchers was drawn to the possibility of binding technetium-99m to carriers in order to prepare radiopharmaceuticals specific for the diagnostic imaging of other organs of the body. Chemical and electrolytic reduction of the pertechnetate-99m ion have been carried out in the presence of the compound to be labelled^{3,5,9}, and often the whole reaction mixture is injected into the patient for radionuclide imaging. In spite of the empiricism of the procedures used^{3,5,6} the products have almost always given satisfactory diagnostic results. However, the need is increasing not only for the development of new specific radiopharmaceuticals but also for improvements in the methods of production and purity control, and in the study of the properties of those compounds already in use in nuclear medicine. Hence, the need for an extensive study of the chemistry of technetium-99m has repeatedly been stressed^{3,5,6,10-14}. The extrapolation of the chemistry of technetium-99 studied at millimolar concentration to that of technetium-99m present at nanomolar level, without any experimental evidence, has been put in doubt by many authors^{3,5,6,10}. During our attempt to prepare tumour-specific radiopharmaceuticals labelled with technetium-99m, we were confronted with this problem because the pertechnetate-99m ion as such did not react with the compounds. A study of the reduction of pertechnetate-99m ion was, therefore, undertaken in order to prepare chromatographically pure lower oxidation states of the isotope and study their chemical and physiological properties. Hydrochloric acid, which is the simplest reducing agent and also the one first used in the reduction of pertechnetate-99 ion¹⁵ and in the preparation of chromatographically pure samples of technetium-99-(V) and -(IV)¹⁶⁻¹⁹, was chosen for the study reported here.

EXPERIMENTAL

The technetium-99m generators used for eluting sodium pertechnetate-99m were DRN Stercow TM99m of Philips-Duphar, B.V., Petten, Holland. The radio-nuclidic purity of the eluate was determined by gamma-spectrometry and half-life determinations. The radiochemical purity of the sodium pertechnetate-99m solutions was examined by paper chromatography and low-voltage electrophoresis. Ascending paper chromatograms on hydrochloric acid-washed Whatman 3MM paper strips (3 × 20 cm) with a chromatographic run of 15 cm were developed using physiological saline or 0.6 N hydrochloric acid as the mobile phase. The electrophoresis was carried out in Whatman 3MM paper strips (3 × 40 cm) impregnated with physiological saline and sandwiched between two thin glass plates allowing an electrophoretic run of 30 cm. The voltage applied was 250 V, and the current variation during the 1.5 h electrophoresis was 8-12 mA. After the run, the chromatograms or the electrophoregrams were dried in a current of warm air and were then cut into strips 5 mm in width. Each strip was counted in a well-type gamma-counter (Nuclear Chicago model 4230). When a single spot at R_f value of 0.68 and a single anionic spot at 6 cm from the point of application were observed, the solution under examination was taken to be chromatographically and electrophoretically pure sodium pertechnetate-99m solution. Only such solutions were used for the study.

An aliquot containing ca. 500 μ Ci of technetium-99m was evaporated on a

water bath and reconstituted with 2 ml of fuming hydrochloric acid (37%; Merck, Darmstadt, G.F.R.). The reduction was followed chromatographically as previously described¹⁵⁻²⁰. The effect of ageing on the solution of pertechnetate-99m ion was studied at -5, 21, 70 and 80°. For the reduction at 21° and at higher temperatures the radiochromatograms were developed in 0.6 N hydrochloric acid at room temperature (21°). The chromatograms of the solution aged at -5° were run at 4°.

RESULTS AND DISCUSSION

The first chromatographic evidence for the reduction of pertechnetate-99 ion by concentrated hydrochloric acid was given by Matsuura and Kojima²⁰. A systematic study of the reduction of technetium-99(VII) was undertaken in the Laboratorio di Cromatografia of the C.N.R., Rome, during 1964^{15-19,21-23}, when chromatographically pure samples of technetium-99-(V) and -(IV) were prepared^{16,17} and the solution chemistry of the three oxidation states of the isotope was studied. In addition to being more informative than the polarographic and spectrophotometric techniques²⁴⁻²⁸, the chromatographic method^{15,16} has also proved to be very suitable for the study of the solution chemistry of the oxidation states of radiotracers^{26,27,29-31}. The present study confirms the suitability of the method not only for the separation of the different oxidation states of technetium-99m but also for the isolation of these species for further study.

The reduction of pertechnetate-99m ion by concentrated hydrochloric acid

The reaction between the pertechnetate-99m ion and concentrated hydrochloric acid has been studied by Williams and Deegan²⁹, and by Münze and Noll^{26,27}, who reported that the pertechnetate-99m ion, unlike the pertechnetate-99 ion, is directly reduced to technetium(IV). No chromatographic spot of technetium-99m(V), always present during the reduction of the pertechnetate-99 ion, was observed by these authors. In a later paper³², Williams and Deegan claimed, without giving any experimental evidence, that concentrated hydrochloric acid reduces the pertechnetate-99m ion to technetium-99m(V), which binds to human serum albumin. This assumption has recently been criticized by Richards and Steigman¹⁴. There seems to be no disputing that the technetium-99m bound to the human serum albumin is technetium(IV). Cifka and Vesely^{30,31} have prepared technetium-99m(IV) by heating a solution of pertechnetate-99m ion in concentrated hydrochloric acid for ca. 5 min at 100°. They suggest that reduction of the pertechnetate 99m ion is stepwise, first to technetium-99m(V), and then to technetium-99m(IV) by disproportion. The experimental conditions used by these authors did not permit the observation of the technetium-99m(V) spot on the radiochromatogram.

Chromatograms of the reduction of chromatographically pure pertechnetate-99m ion by concentrated hydrochloric acid at 70 and -5° are shown in Figs. 1 and 2, respectively. Technetium-99m(V) is always observed to be present in fresh solutions of pertechnetate-99m ion in the concentrated acid. It remains there, although in relatively much smaller amounts than does technetium-99, until reduction of the pertechnetate ion to technetium-99m(IV) is complete. This would suggest that reduction of the pertechnetate-99m does take place *via* technetium-99m(V). The fresh solution of the pertechnetate-99m ion in concentrated hydrochloric acid at -5° contains

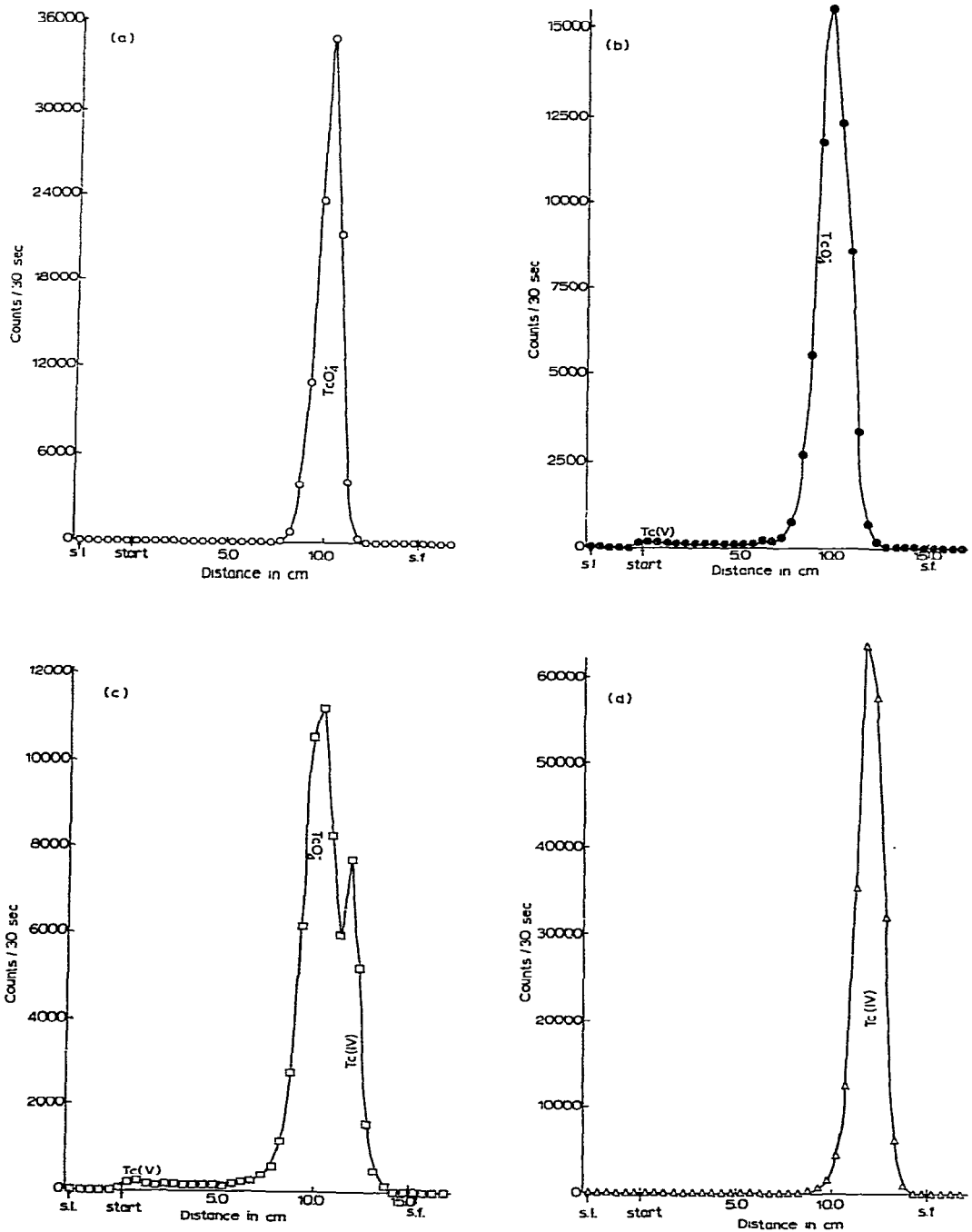


Fig. 1. Radiochromatograms of (a) the initial sodium pertechnetate- $99m$ solution; (b) a fresh solution of sodium pertechnetate- $99m$ in concentrated hydrochloric acid; (c) solution (b) aged for 2 h at 70° ; and (d) solution (b) aged for 3 h at 70° ; s.l., solvent level; s.f., solvent front. (Paper, HCl-washed Whatman 3MM; mobile phase, 0.6 N HCl; temperature, 21°).

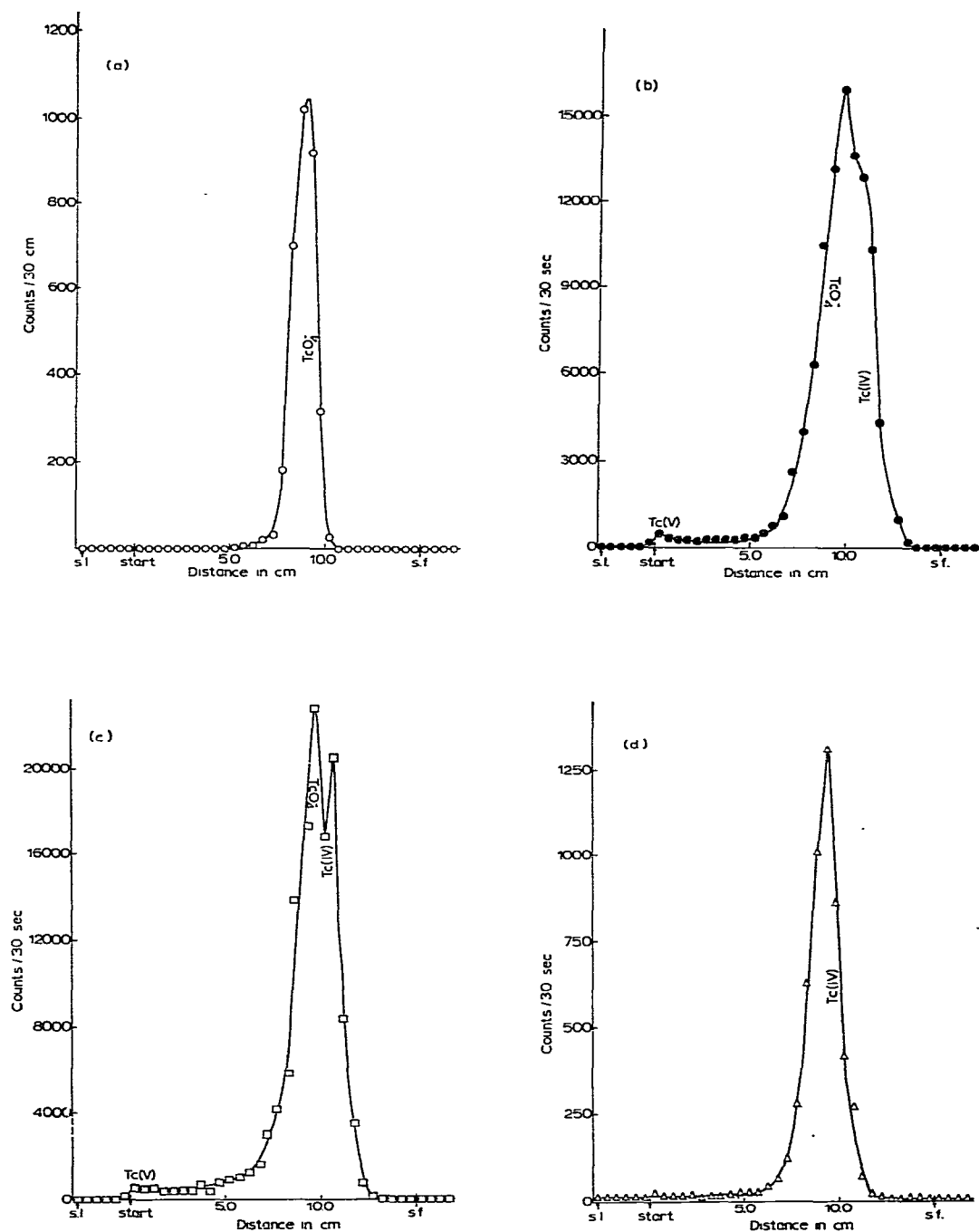


Fig. 2. Radiochromatograms of (a) the initial sodium pertechnetate-99m solution; (b) a fresh solution of sodium pertechnetate in concentrated hydrochloric acid at -5° ; (c) solution (b) aged for 1 h at -5° ; and (d) solution (b) aged for 48 h at -5° ; s.l., solvent level; s.f., solvent front. (Paper, HCl-washed Whatman 3MM; mobile phase, 0.6 N HCl; temperature, 4°).

all three technetium-99m-(VII), -(V), and -(IV) oxidation states (Fig. 2b). Per technetate-99 ion reduced at 0° gives¹⁷ chromatographically pure samples of technetium-99(V) stable at that temperature for *ca.* 4 days. Furthermore, reduction of pertechnetate-99 ion by concentrated hydrochloric acid requires ageing for *ca.* 2 years^{17,23} for chromatographically pure technetium-99(IV) to be obtained. The pertechnetate-99m ion, on the other hand, is reduced to chromatographically pure technetium-99m(IV) at -5° after ageing for only 48 h (Fig. 2d). These results show that reduction of technetium-99m-(VII) and -(V) to technetium-99m(IV) is very rapid. This difference between the reduction rates of the two isotopes may be attributed to the radiolytic products (H₂, H, OH, HO₂, *etc.*)^{33,34} present in the solution of the pertechnetate-99m ion.

Work is in progress to prepare chromatographically pure technetium-99m(V) and to study the physiological behaviour of the chromatographically pure technetium-99m(IV).

Effect of temperature on the reduction of pertechnetate-99m ion by concentrated hydrochloric acid

The reduction was carried out at -5, 21, 70 and 80°. Ageing times of 48, 10, 3 and 0.5 h, respectively, were required for chromatographically pure technetium-99m(IV) to be produced from the pertechnetate-99m ion. In no instance was pure technetium-99m(V) obtained. At 80°, technetium-99m is to some extent lost by volatilization. The reduction should therefore be carried out at or below 70°.

Table I gives the R_F values of the three oxidation states of technetium-99m and technetium-99. Similar R_F values are obtained.

TABLE I

R_F VALUES OF THE OXIDATION STATES VII, V, AND IV OF TECHNETIUM-99m AND -99

Oxidation state	Technetium-99m		Technetium-99*
	4°	21°	8°
Technetium (VII)	0.58	0.68	0.68
Technetium (V)	0.018	0.018	0.18
Technetium (IV)	0.62	0.78	0.78

* Taken from refs. 16 and 17.

Effect of pH on technetium-99m(IV)

Figs. 1d and 2d show chromatograms of the chromatographically pure technetium-99m(IV). The absence of trailing indicates the stability of the species in 0.6 *N* hydrochloric acid. Identical radiochromatograms were obtained both when technetium-99m(IV) in concentrated hydrochloric acid was chromatographed in physiological saline and when its solution in physiological saline was examined chromatographically. Technetium-99m(IV) is, therefore, stable in solution up to pH 7.

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