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THE EFFECT OF CONCENTRATION UPON THE CHROMATOGRAPHIC BEHAVIOUR OF TECHNETIUM IN CONCENTRATED HYDROCHLORIC ACID

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SUMMARY

The chromatographic behaviour of various concentrations of technetium in 11.4 *M* HCl was studied on Whatman No. 3MM and DE-81 papers with 0.6 *M* and 1.2 *M* HCl, respectively, as developing solvents. Within the concentration range, 0.5–2.0 mg per ml, results were similar to those already documented. In addition, two further species were detected, one appearing at concentrations above 2 mg per ml, and the other at concentrations below 0.1 mg per ml. The possible structures of these two species are discussed.

INTRODUCTION

Previous investigations[1,2] into the reaction between Tc(VII), as pertechnetate, and concentrated HCl have employed cellulose or ion-exchange paper chromatography to define the intermediates present. During a study involving reduction of trace quantities of [^{99m}Tc]pertechnetate in similar conditions, anomalous results were obtained with the same methods.

This paper reports the effect of concentration changes upon the reaction of Tc(VII) and concentrated HCl in terms of the chromatographic behaviour of the species involved.

EXPERIMENTAL

The technetium used in this study was a solution of ammonium [^{99}Tc]pertechnetate, which was adjusted with distilled water to a concentration of 10 mg ^{99}Tc per ml. In addition, [^{99m}Tc]pertechnetate, obtained by elution of a [^{99}Mo]molybdate generator with 0.15 *M* NaCl, was used to define chromatographic zones during analysis. (All isotopic materials were obtained from the Radiochemical Centre, Amersham, Bucks.)

Ascending chromatography was performed on 2.5 cm × 25 cm strips of both Whatman No. 3MM and DE-81 papers using 0.6 *M* and 1.2 *M* HCl, respectively, as developing solvents. Solutions for analysis were prepared by addition of approximately 30 μCi of [^{99m}Tc]pertechnetate to aliquots of the standard pertechnetate solution. After evaporation to dryness in vacuo, the residues were reconstituted in 11.4 *M* HCl (analytical grade) to give the concentration range investigated. The standard application to each strip was 5 μl. Development with No. 3MM papers was for 16–18 cm and with DE-81 papers, 12–14 cm. After drying, each strip was cut into 5 mm sections, the ^{99m}Tc-activities of which were determined in a well-type scintillation counter (Nuclear Enterprises Ltd., Beenham, Berks.). The activities of the individual sections were expressed as percentages of the total activity of the chromatogram.

RESULTS

The R_F values of the Tc(VII) and Tc(IV) states, as TcO_4^- and $TcCl_6^{2-}$, were determined for the two systems in preliminary experiments. The R_F value of the Tc(V) state was derived from the findings of the study. Comparison with the published data of SHUKLA[2] is presented in Table I.

TABLE I

R_F VALUES OF THE VARIOUS Tc SPECIES

	Present study	*Shukla (ref. 2)*
No. 3MM		
Tc (IV)	0.87	0.87
Tc (V)	0.18	0.18
Tc (VII)	0.70	0.68
DE-81		
Tc (IV)	0.10	0.10
Tc (V)	0.46	0.48
Tc (VII)	0.48	0.47

In general, the concentrations of the solutions were not defined in previous investigations of the reaction between Tc(VII) and concentrated HCl[1,2]. The range covered in the present study was from trace quantities ([^{99m}Tc]pertechnetate only) to 10 mg per ml. The results of the chromatographic analyses of the various solutions showed different effects dependent upon whether the concentration was greater or less than 0.5 mg per ml.

Concentration range: 0.5–10.0 mg per ml

The results of analyses of samples taken from solutions within 30 min of constitution are shown in Fig. 1.

On No. 3MM paper, the main species was the slow running Tc(V); some Tc(VII) was also detected in the R_F 0.7 region, and at higher concentrations a further species was apparent beyond R_F 0.8.

On DE-81 paper, most of the activity was detected in the area shared by Tc(V) and Tc(VII). Again, at higher concentrations a fast moving species with an R_F value greater than 0.6 was detected. The absence of appreciable activity in the low R_F region

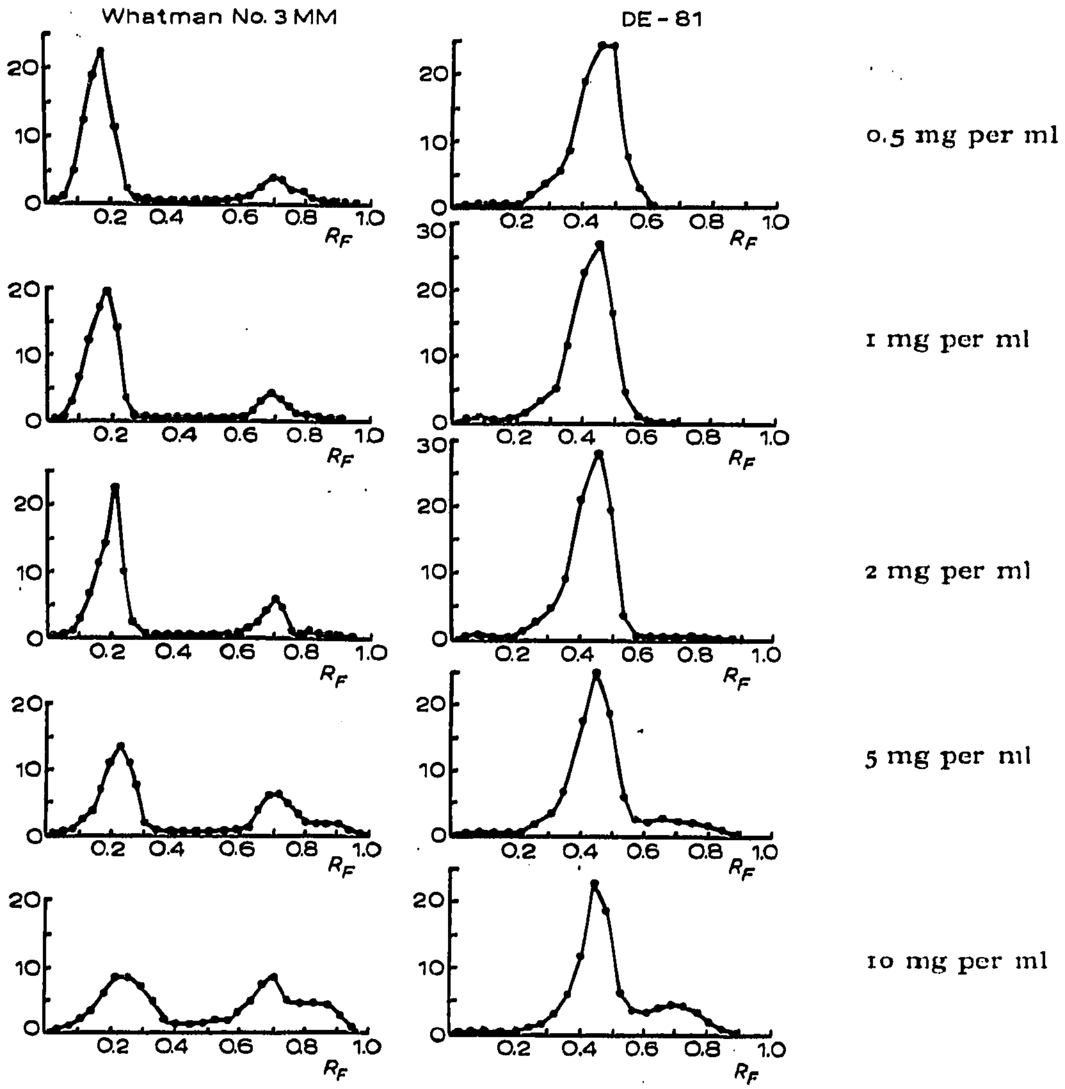

Fig. 1. Chromatograms of 0.5–10 mg per ml solutions, 30 min after constitution, on Whatman No. 3MM and DE-81 papers with 0.6 *M* and 1.2 *M* HCl, respectively, as developing solvents. Ordinates: percentage total chromatogram activity.

suggested that Tc(IV) was not present in quantity and could not have contributed much to the fast moving species on No. 3MM paper.

Repetition of the analyses with samples taken after 24 h reaction showed only small changes. On No. 3MM paper, a decrease in the amount of Tc(V) was apparent together with an increase in the activity in the R_F 0.7 region. As, judged by the amount of slow running material on DE-81 paper, the contribution of Tc(IV) was only increased minimally, it must be presumed that a greater quantity of Tc(VII) was present in the reaction mixture after 24 h.

By assessment of the areas under the peaks in the simultaneous chromatograms, coupled with the R_F values of the individual species in the two systems, it was possible to determine the proportions of each component in the reaction mixture at the various concentrations (Fig. 2). After 30 min the conversion of Tc(VII) to Tc(V) became less as the concentration of technetium increased and was accompanied by an

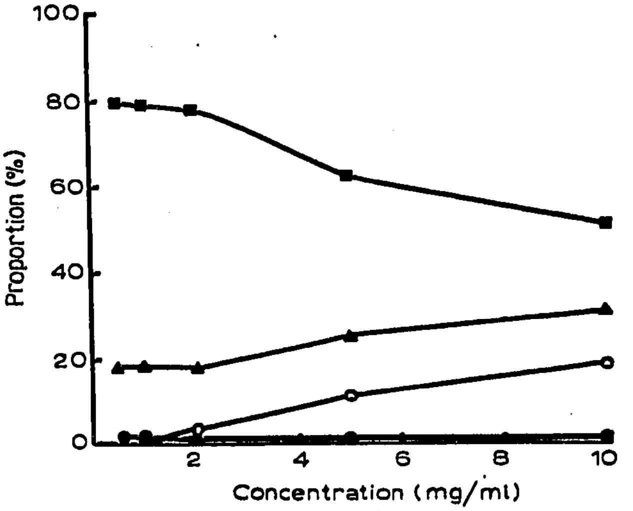

Fig. 2. Proportions of each component in the concentration range, 0.5–10 mg Tc per ml. Tc (IV) ●—●, Tc (V) ■—■, Tc (VII) ▲—▲, fast moving component (F) ○—○.

increasing amount of the fast moving component, F, at levels above 2 mg per ml. Throughout, the amount of Tc(IV) was very small.

Concentration range: less than 0.5 mg per ml

Samples taken within 30 min of constitution gave the results shown in Fig. 3

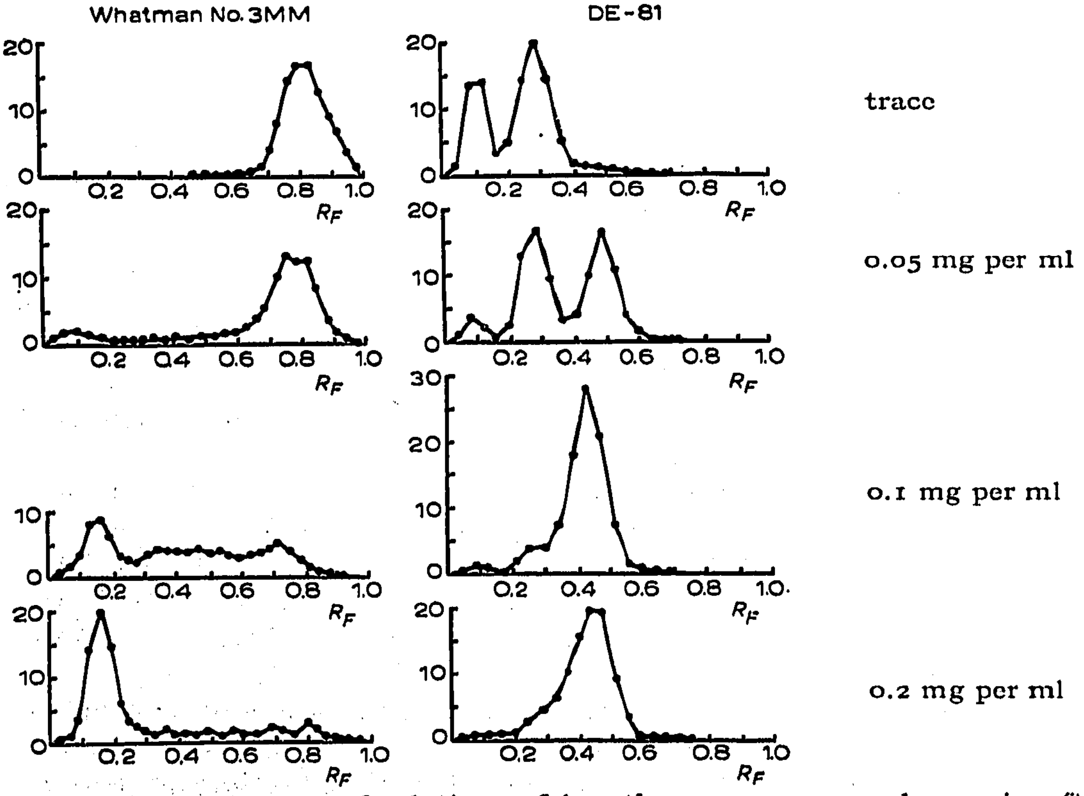

Fig. 3. Chromatograms of solutions of less than 0.5 mg per ml, 30 min after constitution, on Whatman No. 3MM and DE-81 papers with 0.6 M and 1.2 M HCl, respectively, as developing solvents. Ordinates: percentage total chromatogram activity.

for this range. In general, as the concentration decreased there was a movement of activity towards the solvent front on No. 3MM paper and towards the origin on the DE-81 paper.

At concentrations of 0.1 and 0.2 mg per ml. the discrete separations seen previously in analyses on No. 3MM paper became confused. Apart from the Tc(V) peak no other species was clearly defined. It is probable that the indeterminate area between R_F 0.3 and 0.8 resulted from secondary reactions on the paper since no parallel effects were observed in the DE-81 system. Here, as previously, the bulk of the activity was in the region of the common R_F of Tc(V) and Tc(VII) but the amount of material between R_F 0.2 and 0.3 produced a more definite shoulder to the peak at these concentrations. As previously, little change in distribution was apparent after 24 h.

When the concentration was reduced further to 0.05 mg per ml, marked changes appeared on both papers. On No. 3MM paper there was overall displacement of activity towards the solvent front with a peak at R_F 0.77. The Tc(V) peak was no longer a major feature and the trail between R_F 0.3 and 0.6 was much diminished. In the DE-81 system, the shoulder observed at higher concentrations emerged as a discrete peak at R_F 0.28 between the positions of Tc(IV) and Tc(V).

For the first time analyses after 24 h showed altered distributions (Fig. 4). On No. 3MM paper, the Tc(V) peak disappeared and all activity was concentrated in a double peak at R_F's 0.84 and 0.90. The Tc(V) peak was also absent on DE-81 paper

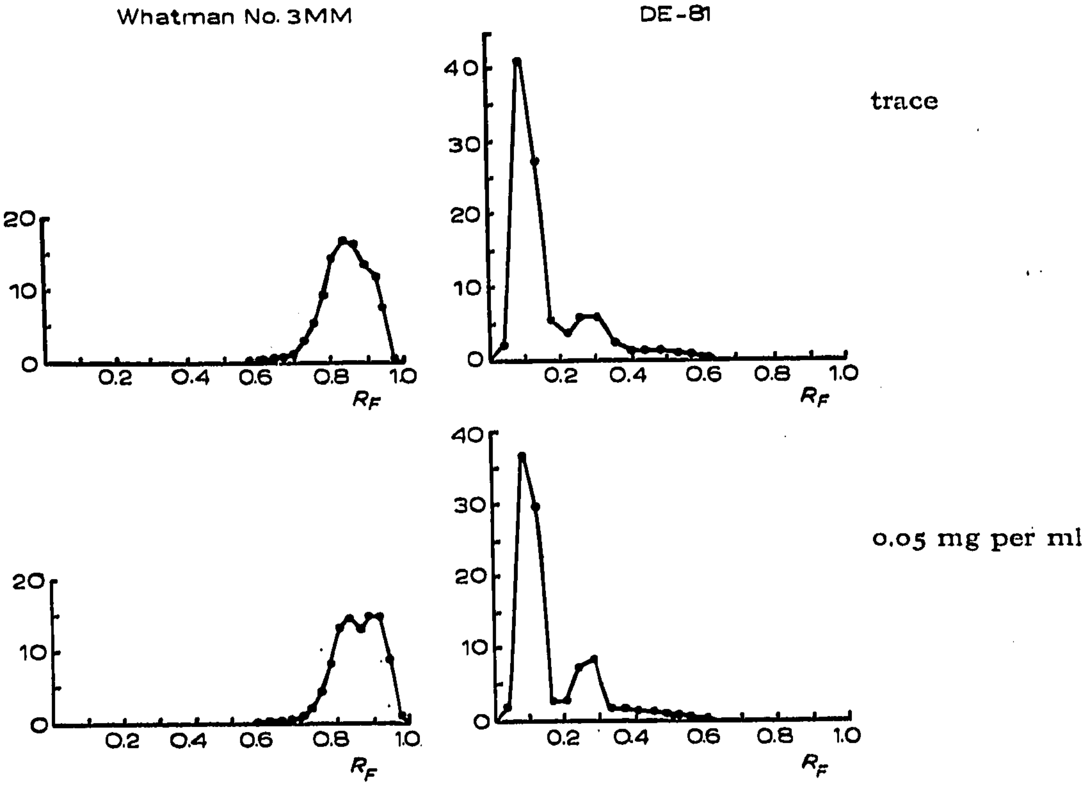

Fig. 4. Chromatograms of trace and 0.05 mg per ml solutions, 24 h after constitution, on Whatman No. 3MM and DE-81 papers with 0.6 M and 1.2 M HCl, respectively, as developing solvents. Ordinates: percentage total chromatogram activity.

and the activity at R_F 0.28 was reduced in quantity. The major component was the Tc(IV) peak.

The analyses of trace concentrations on No. 3MM paper after 30 min reaction showed a single wide peak with a maximum R_F value of 0.80. Two peaks were again evident on DE-81 paper at R_F's 0.10 and 0.28. No Tc(V) peak was detected in either system. After 24 h the results facsimilated those at 0.05 mg per ml; the peak maximum on No. 3MM paper had moved towards the solvent front whilst on DE-81 paper the Tc(IV) peak increased at the expense of the component at R_F 0.28.

DISCUSSION

Within the concentration range, 0.5–2.0 mg per ml, the distribution and type of species detected were similar to those designated previously as Tc(IV), Tc(V), and Tc(VII)[1,2]. Outside this range two further components were apparent, namely at higher concentrations one which was fast moving in both systems and at lower concentrations one which was fast moving on No. 3MM paper but rather slow moving on DE-81 paper.

From a spectroscopic investigation of the reaction between Tc(VII) and concentrated HCl, BUSEY[3] proposed that the most likely form of Tc(V) is $TcOCl_4^-$, but amongst alternatives possible are $TcOCl_3$ and $TcOCl_5^{2-}$. A further species detected when the reaction mixture was irradiated with sunlight was assigned the structure $TcCl_6^-$.

In the range above 2 mg per ml, no appreciable changes in the proportions in the reaction mixture were observed between analyses after 30 min and after 24 h, which suggested that stable equilibrium was quickly established. The differences in the relative distributions at the various concentrations involved only Tc(V), Tc(VII) and the fast moving component, F. The smallness of the contribution of Tc(IV) implied a slow rate of secondary reduction of Tc(V).

BUSEY[3] failed to detect any intermediate state between Tc(VII) and Tc(V) in the reaction which suggests that F is probably an alternative species to the usual $TcOCl_4^-$. In view of the chromatographic properties the most likely form is $TcOCl_3$, the neutral structure of which would imply a high R_F value on the anion-exchange paper. It is conjectural whether F occurs as an intermediate in concentrations less than 2 mg per ml, or arises at higher concentrations from the decreasing Cl^-/Tc ratio as the amount of nuclide increases.

In the low concentration range the main changes were the decrease in activity in the Tc(V) regions, culminating in the total absence at trace levels, and the changed distributions after 24 h. The latter, however, may have been relative rather than absolute since the same rate of secondary reduction would result in a more significant change in proportions in this range.

Another species was evident from the emergence of a peak at R_F 0.28 on DE-81 paper. Hydrolysis products with similar R_F values on anion-exchange papers occur in solutions of Tc(IV) in 1.2 M HCl[1]. The greater molarity of the present reaction conditions together with the increased amount of Tc(IV) after 24 h precludes this structure for the new species. HARPER *et al.*[4] has stated that Tc(V) is the valency necessary for ligand formation with such entities as albumin and WILLIAMS[5] has successfully combined trace quantities of ^{99m}Tc with this protein at efficiences of 90%

after reduction of Tc(VII) with concentrated HCl. This data suggests that the new component is a further alternative of Tc(V). The chromatographic properties point to two possibilities, $TcCl_6^-$ or $TcOCl_5^{2-}$, both of which would have relatively slow movement on DE-81 paper.

Summarising, it is considered that reduction of Tc(VII) to Tc(V) occurred throughout the range investigated but the nature of the pentavalent form present in the reaction mixture altered with concentration.

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