

Complexes of Technetium with Pyrophosphate, Etidronate, and Medronate

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The reduction of [^{99m}Tc]pertechnetate was studied as a function of pH in complexing media of pyrophosphate, methylene diphosphonate (MDP), and ethane-1, hydroxy-1, and 1-diphosphonate (HEDP). Tact (sampled d-c) and normal-pulse polarography were used to study the reduction of pertechnetate, and normal-pulse polarography (sweeping in the anodic direction) to study the reoxidation of the products. Below pH 6 TcO_4^- was reduced to Tc(III), which could be reoxidized to Tc(IV). Above pH 10, TcO_4^- was reduced in two steps to Tc(V) and Tc(IV), each of which could be reoxidized to TcO_4^- . Between pH 6 and 10 the results differed according to the ligand present. In pyrophosphate and MDP, TcO_4^- was reduced in two steps to Tc(IV) and Tc(III); Tc(III) could be reoxidized in two steps to Tc(IV) and TcO_4^- . In HEDP, on the other hand, TcO_4^- was reduced in two steps to Tc(V) and Tc(III), and could be reoxidized to Tc(IV) and TcO_4^- . Additional waves were observed; they apparently led to unstable products.

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The practical importance of the diphosphonate and pyrophosphate complexes of technetium lies in their medical use for radionuclide bone scanning. Their structures unknown, they are prepared by reducing tracer (nanomolar) amounts of [^{99m}Tc] pertechnetate with stannous ion in the presence of the appropriate ligand. Identification of the products remains an unsolved analytical problem despite their wide use as pharmaceuticals (1, 2). Although the preparations of interest are too dilute to be assayed except by radiochemical methods, such methods (principally chromatographic) are difficult to design and interpret without some prior knowledge of the system. In this respect, ignorance of the solution chemistry of technetium has been a great handicap. We have therefore undertaken to characterize by polarographic means the stable

complexes of technetium with all three ligands in clinical use. Stable oxidation states were positively identified for a wide range of pH, and unstable states were characterized by their polarographic half-wave potentials.

MATERIALS AND METHODS

The methods have been described in detail elsewhere (3) and representative polarograms shown (4). As previously described, the number of electrons transferred was determined for each wave by comparison with the diffusion current for the known 4-electron pertechnetate reduction in pH 3 citrate. If the ratio of tact (sampled d-c) to pulse-limiting current was that predicted by diffusion theory, the wave was regarded as diffusion-controlled. Methylenediphosphonic acid (MDP), $H_3PO_3 \cdot CH_2 \cdot PO_3H_3$, (medronate) and etidronate, the disodium salt of ethane-1, hydroxy-1,1-diphosphonic acid (HEDP), $CH_3 \cdot C(OH) \cdot (PO_3H_2)_2$, were used without further purification. The HEDP contained 0.7% NaH_2PO_3 , and the possible effect of

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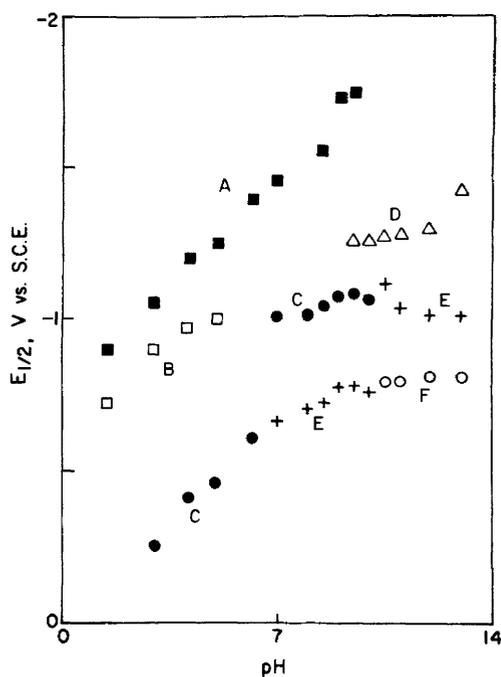


FIG. 1. Half-wave potentials for reduction of 0.14 mM TcO_4^- in 0.1 M MDP. A: $n = 6$ or 7; B: 5 or 6; C: 4; D: 4, 5, or 6; E: 3; F: 2.

TABLE 1. NUMBER OF ELECTRONS TRANSFERRED, CALCULATED FROM TAST (t) AND PULSE (p) CURRENTS IN MDP*

pH	Wave I		Wave II		Wave III		Calibration‡	
	t	p	t	p	t	p	t	p
1.5	4 [†]	4 [†]	5.9	¶	7.4	4.1		
3.0	4 [†]	4 [†]	6.8	6.0	8.0	7.0		
4.1	4 [†]	4 [†]	6.0	5.0	7.0	7.0		
5.1	4 [†]	4 [†]	5.4	4.7	6.5	6.6		
6.2	3.7	3.7			6.6	6.6	3.7	3.7
7.0	3.1	3.0	4 [†]	4 [†]	7.0	7.0		
8.0	2.9	2.7	4 [†]	4 [†]			4.0	3.9
8.5	3 [†]	3 [†]	3.9	4.0	§	7.7		
9.0	3 [†]	3 [†]	3.9	3.9		8.9		
9.5	3 [†]	3 [†]	3.9	4.0	§	4.9		
10.0	3 [†]	3 [†]	3.8	4.0	4.9	4.8	2.9	2.8
10.5	2.3	2 [†]	§	§	4.6	4.7		
11.0	2.4	2 [†]			3.0	5.6	5.8	
12.0	2.2	3.0	3 [†]	3 [†]	6.1	6.2	2.8	2.8
13.0	2.3	2.1	3 [†]	3 [†]		6.6		

* For successive cathodic waves I, II, and III in 0.1 M MDP, 0.14 mM NH_4TcO_4 , NaOH to stated pH.

† Reference wave, relative to which other n values are calculated.

‡ Measured value of n for reference wave relative to 4-electron pertechnetate reduction wave in pH 3 citrate.

|| not done

§ ill formed

¶ maximum

this impurity was not evaluated. Ammonium pertechnetate was used in 0.14 mM concentration for all measurements. The pH of the solution was adjusted by adding the required amount of sodium hydroxide or hydrochloric acid; acidic pyrophosphate solutions were prepared just before use to minimize hydrolysis. Measurements were made at 22–25°C, and all potentials relate to the saturated calomel electrode (S.C.E.).

RESULTS

Figure 1 shows the polarographic behavior of pertechnetate in 0.1 M MDP as a function of pH. The number of electrons transferred, n , is stated for each wave, basing the assignments on the diffusion current data displayed in Table 1. At each pH, the value of n was identified for one reference wave and the n values for the other waves were determined by proportion. Correct assignment of n for the reference wave was verified by comparison with the known 4-electron wave in citrate (3); the results appear in Table 1 under the heading "calibration." (At pH 6.2 no wave could reliably be assigned an integral value; there the n values are reported relative to the 4-electron wave in citrate.) The results are given both for tasc and pulse polarography; agreement between the two serves as a criterion for diffusion control. Figure 2 shows data for reoxida-

tion of the products, obtained by normal pulse polarography upon sweeping in the anodic direction from a resting (between-pulse) potential on the plateau of each cathodic wave. Table 2 shows the diffusion-current data used to determine the n values stated in Fig. 2. These relate to the same reference waves used in Table 1 and are adjusted for

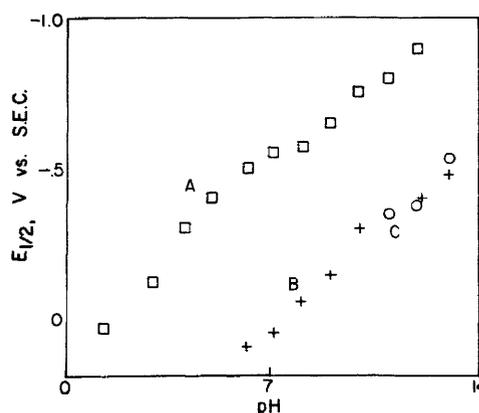


FIG. 2. Half-wave potentials of anodic waves for products of reduction of TcO_4^- in 0.1 M MDP. Products of cathodic TcO_4^- reduction are detected by anodic pulses, using normal pulse polarography and sweeping in the anodic direction from a resting point on the plateau of a cathodic wave. A: $Tc(II) \rightarrow Tc(IV)$; B: $Tc(IV) \rightarrow TcO_3^-$; C: $Tc(V) \rightarrow TcO_3^-$.

TABLE 2. REOXIDATION OF PRODUCTS IN MDP*

pH	Cathodic wave					
	I		II		III	
	$E_{1/2}(n)$	$E_{1/2}(n)$	$E_{1/2}(n)$	$E_{1/2}(n)$	$E_{1/2}(n)$	$E_{1/2}(n)$
1.5	+ .04(1.0)		+ .04(1.0)			
3.0	- .12(1.0)		- .20(.8)		- .3(.5) [†]	
4.1	- .30(.9)		- .35(.9)		- .5(.6) [‡]	
5.1	- .4(.9) [†]		- .4(.9) [†]		- .4(.7) [†]	
6.2	- .5(.6) [†]	+ .1(2) [‡]	- .5(.5) [†]	+ .1 [‡]		
7.0		+ .05 [‡]	- .55(.8) [†]	+ .05 [‡]	- .55(.8)	
8.0		- .05(3) [‡]	- .57(1.0)	0.0(4) [‡]		
9.0		- .13(2.8)	- .65(.8)	- .13(3.6)		
10.0		- .30(2.4)		- .26(3.1)	- .8(.8)	- .23(2.8)
11.0	- .34(2.2)				- .8(.8) [†]	- .26(2.4)
12.0	- .38(2.0)			- .39(2.6) [§]	- .9(.5) [†]	- .30(2.2)
13.0	- .53(1.8)			- .48(2.8) [§]		

* Half-wave potentials, $E_{1/2}$, and number of electrons transferred, n , for anodic-sweep pulse polarographic waves of products from cathodic waves I, II, and III; in 0.1 M MDP, 0.14 mM NH_4TcO_4 , NaOH to stated pH.
[†] merges with cathodic wave
[‡] ill-formed
^{||} not done
[§] slight maximum

the expected ratio of reverse pulse to forward fast diffusion currents [2.89, with the instrument parameters used (5, 6)]. Figures 3 through 6 portray similar measurements in pyrophosphate and HEDP solutions.

A curious phenomenon occurred in HEDP between pH 6 and 10. On the anodic-sweep pulse polarogram starting from the plateau of wave I, the

current, instead of becoming monotonically more anodic, turned cathodic at -0.4 V. This is illustrated in Fig. 7. Oscilloscopic examination of the current-time curve in this region of the anomalous cathodic wave showed that application of the anodic voltage pulse caused a large but very brief anodic current pulse, followed by abrupt reversal of the polarity of the current, then gradual decay of the cathodic current in a manner resembling qualitatively the $1/\sqrt{t}$ decay for a diffusion process. Triangular-wave voltammetry showed a similar anomalous wave at slow scan speeds (one V/sec) when the mercury drop was held initially at a potential corresponding to the plateau of the first cathodic polarographic wave; it was not seen, however, if the drop was held initially at a potential where pertechnetate was unreactive. To our knowledge this

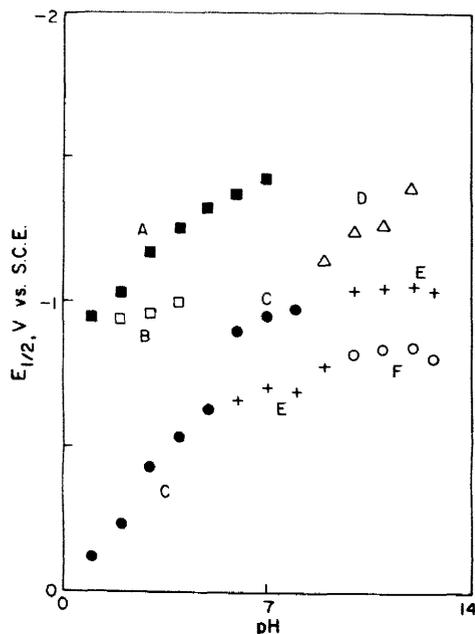


FIG. 3. Half-wave potentials for reduction of 0.14 mM TcO_4^- in 0.1 M pyrophosphate. A: $n = 6$ or 7; B: 5 or 6; C: 4; D: 4, 5, or 6; E: 3; F: 2.

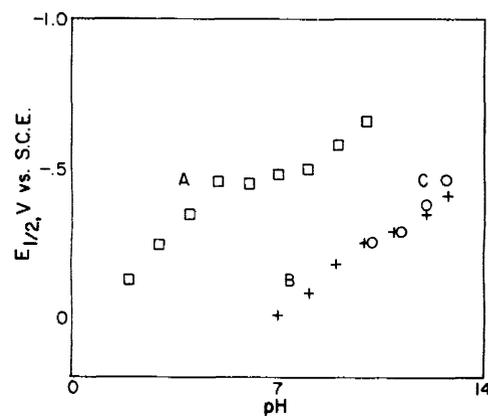


FIG. 4. Half-wave potentials or anodic waves for products of reduction of TcO_4^- in 0.1 M pyrophosphate. A: $\text{Tc(III)} \rightarrow \text{Tc(IV)}$; B: $\text{Tc(IV)} \rightarrow \text{TcO}_4^-$; C: $\text{Tc(V)} \rightarrow \text{TcO}_4^-$.

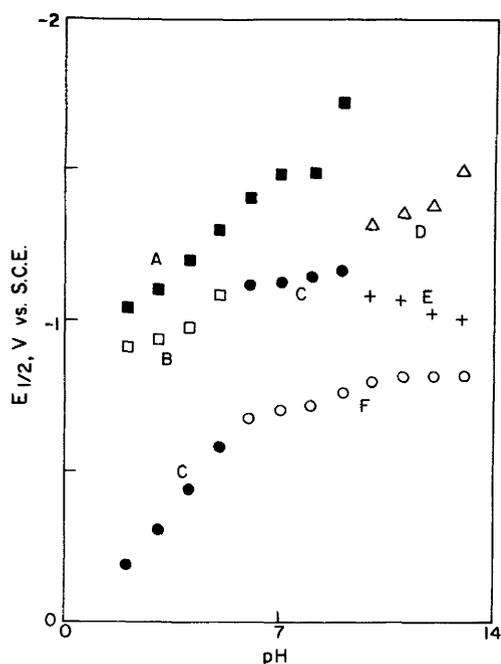


FIG. 5. Half-wave potentials for reduction of 0.14 mM TcO_4^- in 0.08 M HEDP. A: $n = 6$ or 7 ; B: 5 or 6 ; C: 4 ; D: $4, 5,$ or 6 ; E: 3 ; F: 2 .

phenomenon has not been described before in reverse-sweep pulse polarography. It apparently results from the inhibition of reduction of Tc(V) over a limited potential range, as described in classical polarography for persulfate and chloroplatinate (7), and is believed due to double-layer effects. To distinguish this mechanism from one involving formation of an insoluble film on the electrode surface, we prepared Tc(V) HEDP by controlled-potential electrolysis using methods described elsewhere (4). A polarogram of the product was quite similar to that in Fig. 7.

DISCUSSION AND CONCLUSION

The three media studied (pyrophosphate, MDP, and HEDP) all gave similar results (Figs. 1-6). In each case it is useful to discuss separately the behavior at acidic pH (below 6), near-neutral pH (6 to 10), and alkaline pH (above 10).

At acidic pH, initial reduction yields Tc(III) in all three media. This species can be reoxidized electrolytically at the dropping-mercury electrode to Tc(IV), as demonstrated by anodic-sweep pulse polarography. These findings agree with previous observations (3) that pertechnetate in acid media generally undergoes four-electron reduction to Tc(III), which can sometimes, depending on the medium, be reoxidized to Tc(IV) or Tc(V). Comparison of half-wave potentials for reduction of TcO_4^- to

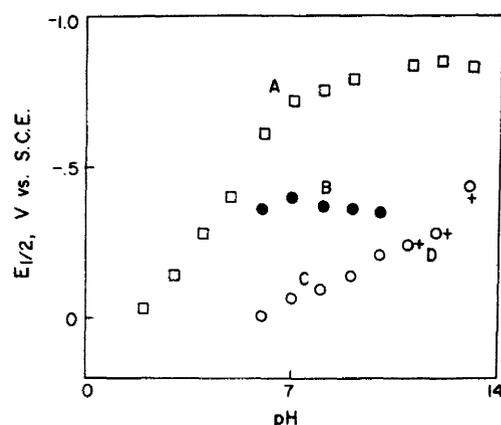


FIG. 6. Half-wave potentials of anodic waves for products of reduction of TcO_4^- in 0.08 M HEDP. A: $Tc(III) \rightarrow Tc(IV)$; B: $Tc(V) \rightarrow Tc(IV)$; C: $Tc(IV) \rightarrow TcO_4^-$; D: $Tc(V) \rightarrow TcO_4^-$.

Tc(III), and reoxidation of Tc(III) to Tc(IV) (Figs. 1-6), suggests that the Tc(III)/Tc(IV) couple approaches reversibility, as in triphosphates (8). If so, the variation of $E_{1/2}$ with pH indicates participation of two hydrogen ions in the reaction, as would also be expected in the absence of complexing agent ($Tc^{3+} + H_2O \rightarrow TcO^{2+} + 2H^+ + e^-$). The loss of two protons with one electron implies a higher net charge for the Tc(III) species than for Tc(IV) in acid solution: this is relevant to the correct interpretation of radioanalytical separations based on ion exchange or electrophoresis.

Two additional cathodic waves are found at more negative potentials. Each probably has a catalytic component, since the numbers of electrons transferred, n , estimated by fast polarography, are greater than those estimated by pulse polarography (Table 1). Salaria, Rulfs, and Elving also report a

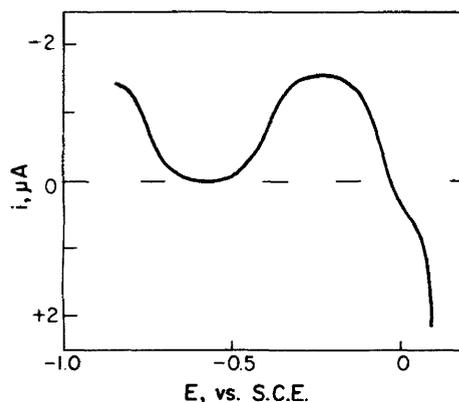


FIG. 7. Anodic-sweep pulse polarogram showing anomalous cathodic wave in 0.08 M HEDP, 0.14 mM NH_4TcO_4 , pH 7.0. Resting potential between pulses -0.85 V. vs. saturated calomel electrode. Cathodic currents are shown as positive.

catalytic component for the second pertechnetate wave in acidic media (9). The measured value of n can thus be regarded an upper limit for the true number of electrons transferred in the first reaction step. The n values for the preceding waves impose a lower limit. Therefore n must be 5 or 6 for the second wave, and 6 or 7 for the third. Some reduction to metallic Tc ($n = 7$) may best account for the deposition on the electrode of an insoluble product both at carrier (9) and at tracer (10) concentrations of pertechnetate. The products of the second and third waves probably reduce water with formation of Tc(III), since they give a reoxidation wave at the potential of the Tc(III)/Tc(IV) couple, but the yield of Tc(III) calculated from the height of the anodic wave is not always quantitative (Table 2). The oxidation states that are stable (on the polarographic time scale, see below) in acid media are thus Tc(III) and Tc(IV).

At alkaline pH all three media produce successive 2- and 3-electron cathodic waves. The Tc(V) and Tc(IV) formed are both reoxidizable to pertechnetate, the waves occurring at the same pH-dependent potential for reoxidation of both species (Figs. 1-6). Thus the wave for Tc(IV) \rightarrow Tc(V) appears to merge with that for Tc(V) \rightarrow TcO₄⁻. These polarographic properties are similar to those observed in strong base even in the absence of phosphates (3). A third cathodic wave occurs at more negative potentials. Reasoning as before, we believe this third wave contains a catalytic component with regeneration of Tc(III), which is reoxidizable in two steps of first one, then three electrons. The initial reduction step corresponds to $n = 4, 5, \text{ or } 6$. In alkaline media, the only stable oxidation states formed by the reduction of pertechnetate are thus Tc(IV) and Tc(V).

Only in the neutral pH range are differences apparent between the three compounds studied. In pyrophosphate and MDP, pertechnetate is reduced first to Tc(IV) and then to Tc(III). In HEDP, on the other hand, reduction yields first Tc(V) and then Tc(III). In all three the Tc(III) can be reoxidized, first to Tc(IV) and then further to pertechnetate. Tc(IV) and Tc(V) are reoxidized to pertechnetate at essentially the same pH-dependent potential. A curious phenomenon occurs in HEDP. On sweeping anodically from a potential where Tc(V) is formed, instead of a monotonically more anodic current we find a new cathodic wave of magnitude consistent with a one-electron diffusion current. This can be attributed to the reduction of Tc(V) to Tc(IV), inhibited at more cathodic potentials by double-layer effects (7). At very negative potentials, a third wave is seen for which n is 5, 6, or 7. The half-wave potentials for reoxidation of the

products of this third wave correspond to those identified for the successive oxidation of Tc(III), first to Tc(IV) and then to pertechnetate. We infer therefore that Tc(III) is regenerated by catalytic water reduction at the potential of the third wave, though the observed currents indicate less than quantitative yield of Tc(III). The stable oxidation states in neutral media are thus Tc(III), Tc(IV), and Tc(V).

Complexes identified as "stable" are stable on the polarographic time scale; this is, for a time at least on the order of the 2-sec drop time. Only in neutral pyrophosphate have we explored whether these are stable for a longer period. In that medium, the polarographic data have been used to design a controlled-potential electrolytic method for the preparation of polarographically pure Tc(III) and Tc(IV) complexes that are stable over the duration of a considerably longer experimental study (20 min) and may be stable indefinitely (4).

At certain pH values the solutions were poorly buffered, corresponding to endpoints in the titration curves of the acids studied. Since the electrode process can alter pH at the electrode surface, these results may not coincide with those measured in the presence of additional inert buffer (3). Because of the difficulty of proving that such added buffer is indeed inert, however, it was omitted.

The polarographic properties of pertechnetate in acidic media could be described simply (3), but we were previously perplexed by the nonintegral n values found for well-formed, apparently diffusion-controlled waves in several alkaline media. The present work, with its more detailed study of pH effects, showed such nonintegral values to occur only in the narrow pH range where transition occurs from one set of products to another. Except at these transition points, simple integral n values were observed. Just why the waves should simulate typical diffusion-controlled behavior at these points is unclear, but the explanation may lie partly in the fact that the ratio of rate constants for two totally irreversible processes is independent of potential if they have the same $n_a\alpha$. The available data, including some unpublished observations, suggest that the stable products of pertechnetate reduction in strongly alkaline media are usually Tc(IV) and Tc(V), whether or not complexing agents other than hydroxide are present. Thus a simple generalization also describes the usual polarographic behavior of pertechnetate in alkaline media.

How far these conclusions apply to tracer-level chemistry remains uncertain. In any event, carrier-level studies can serve as a fertile starting point for tracer-level investigations. For example, it was the demonstration of two stable technetium pyrophos-

phate complexes at carrier levels by means of polarography (3, 4) that led us to seek multiple component fractions in the corresponding clinical radiopharmaceutical. Whether the two fractions found by chromatography in the clinical agent (11) are identical to the two stable complexes identified by polarography is still unresolved. A number of studies both at tracer and at carrier levels may be necessary before the chemistry of technetium pharmaceuticals is well understood.

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