International Journal of Applied Radiation and Isotopes, Vol. 31, pp. 315 to 318
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0020-708X/80/0501-0315$02.00/0

The Valence State of Technetium-99 in its Complexes with Bleomycin, 1-Hydroxy-ethylidene-1,1-diphosphonate and Human Serum Albumin

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(*Received* 13 *October* 1978)

The valence state of technetium-99 in its complexes with bleomycin, 1-hydroxy-ethylidene-1,1-diphosphonate and human serum albumin was determined by titration of $^{99}TcO_4^-$ with Sn(II) in the presence of these complexing agents. Both direct titration, and addition of an excess of Sn(II) and back-titration with iodine was employed.

Introduction

MANY ^{99m}Tc radiopharmaceuticals are prepared via the reduction of pertechnetate by stannous chloride in the presence of the chelating agent. From the stoichiometry of the reduction reaction it is possible to elucidate the valence state of technetium in the chelate. However, meaningful analyses can be carried out only with millimolar amounts of technetium and not with the nanomolar quantities of ^{99m}Tc which are employed in medical practice. Therefore carrier technetium (^{99}Tc) was used in the experiments reported here. The applicability of the results to the nanomolar concentration level is not beyond doubt,[1, 2] but at least they will contribute to the knowledge of the chemistry of technetium-99*m* radiopharmaceuticals.

The oxidation state of technetium has already been investigated in a number of radiopharmaceuticals; among these are ^{99}Tc-gluconate and ^{99}Tc-1-hydroxy-ethylidene-1,1-diphosphonate (^{99}Tc-EHDP),[3] ^{99}Tc-diethylenetriamine-pentacetic acid,[1] and ^{99}Tc-*N*-(2,6-dimethylphenylcarbamoylmethyl)-iminodiacetic acid[4]. This paper describes the determination of the valence state of ^{99}Tc in its complexes with bleomycin (Bleo) and human serum albumin (HSA). As a check, the valence state of ^{99}Tc in the EHDP complex was also determined.

Two titration methods with potentiometric end point detection were employed, viz. direct titration of $^{99}TcO_4^-$ with $SnCl_2$ and iodimetric titration of unreacted $SnCl_2$ added in excess to pertechnetate.

It is generally known that the yield of technetium incorporated into a radiopharmaceutical is influenced by the order of combining the reagents, the concentration of the reagents and the pH.[2, 5–7] Therefore, after the reduction, the labeling percentage was determined using thin-layer and paper chromatography.

Experimental

Chemicals

^{99m}Tc-generator and NH_4 $^{99}TcO_4$: New England Nuclear. Bleomycin: Lundbeck, 67.4% A_2, 28.8% B_2 and 3.8% A_1. EHDP: synthesized according to CASTRONOVO.[8] Human serum albumin: Red Cross Laboratory, Amsterdam. Thin-layer plates: Merck, Kieselgel 60F254 Fertigplatten. Chromatography paper: Whatman No. 1. All other chemicals, from commercial sources, were of the highest obtainable purity.

Equipment

The titrations were performed with an automatic, potentiometric titration system with curve recording. The complete equipment comprised:

(1) A closed titration vessel containing a platinum indicator electrode, a saturated calomel reference electrode and a glass membrane combination electrode. The vessel and the solutions were deaerated with nitrogen.

(2) An automatic burette, Autoburette ABU11 (Radiometer, Copenhagen). In order to extend the titration time to about 3 h the input for remote control of the burette was shortcircuited periodically by means of a multivibrator.

(3) A Servogor RE 511 recorder connected to the output of a Knick Labor-pH-meter for the registration of the titration curve. The pH was measured with a Metrohm E 350 B pH meter.

Procedure

To prevent oxidation of Sn(II) all solutions were prepared oxygen-free and stored under nitrogen. A stock solution of 2×10^{-2} M $SnCl_2$ in HCl was pre-

pared every two weeks. A 2×10^{-3} M solution was made immediately before use and standardized against an iodine solution (5×10^{-3} M I_2 in 10^{-2} M KI). The pH of the diluted $SnCl_2$ solution was adjusted to 2.6 (for Bleo and EHDP) or 1.3 (for HSA).

The direct potentiometric titration of $^{99}TcO_4^-$ with $SnCl_2$ was performed as follows: first the chelating agent was put into the titration vessel (15 mg of Bleo or 5 mg of EHDP or 8 ml of 20% HSA). Next 1 ml of 1.89×10^{-3} M NH_4 $^{99}TcO_4$ was added. The pH was adjusted to the desired value with HCl. The volume was made up to 10 ml by adding a physiological saline solution of the appropriate pH. The titration was started by adding 0.1 ml of 2×10^{-3} M $SnCl_2$; then the mixture was stirred for about 20 min in order to achieve a constant potential reading. After this interval the titration was continued and at least an additional 100 min were spent to reach the end point. Titrations of $^{99}TcO_4^-$ in HCl solution (pH = 1.3 and 2.6) without complexing agent were performed in the same way with variable titration time.

The potentiometric titration of excess Sn(II) with iodine was performed as follows: first 15 mg of Bleo or 5 mg of EHDP were put into the titration vessel. Next 1 ml of 1.89×10^{-3} M NH_4 $^{99}TcO_4$ was added, the pH was adjusted and 5 ml of 2×10^{-3} M $SnCl_2$ was added. The reaction volume was made up to 10 ml with physiological saline of the appropriate pH. After the reaction mixture was stirred for 15 min the unreacted Sn(II) was titrated with iodine solution (5×10^{-3} M I_2 in 10^{-2} M KI). Also a number of titrations in HCl solution without complexing agent was performed.

The labeling percentage was determined using both thin-layer and paper chromatography. The procedure was as follows: the titrations were carried out as described above with the difference that an amount of $^{99m}TcO_4^-$ was added as a tracer for the chromatographic determinations. After the titration an aliquot of the solution was spotted on a thin-layer plate and/or paper strip. For Tc-Bleo a thin-layer chromatogram on silica gel was made, as described by BARTELS *et al.*[6] The labeling efficiency for Tc-EHDP and Tc-HSA was evaluated by means of a two-strip chromatographic procedure.[9] The activity distribution of ^{99m}Tc on the chromatograms was determined using a single channel analyser equipped with a well-type NaI crystal. In the case of Tc-HSA two paper chromatograms were made, using acetone and physiological saline as the developing solvents, respectively. In acetone the reduced unbound technetium, $Tc_{r,u}$, and Tc-HSA remain at the origin, whereas TcO_4^- migrates close to the solvent front. In saline $Tc_{r,u}$ remains at the origin, whereas TcO_4^- and Tc-HSA migrate rapidly (R_f = 0.65–0.80 and R_f = 0.85–0.95, respectively).[10]

As the iodine reagent might oxidize reduced technetium species,[1,2] labeling percentages were determined before and after the iodimetric titration. However, no significant difference was found.

Results and Discussion

Figure 1 shows the number of equivalents transferred to 1 mol of TcO_4^-, n, as a function of the titration time in the direct titration of pertechnetate in HCl solutions (pH = 2.6). Apparently 2 h should be spent on a titration to be sure that the reduction is complete. For this reason an automatic titration equipment was used, and at least 2 h were spent on the direct titrations in the presence of Bleo, EHDP or HSA.

Figure 2 shows a typical titration curve for reduction in the presence of Bleo; titration in the presence of EHDP gave a similar curve. In contrast with these results two end points were registrated for reduction in the presence of HSA, corresponding with Tc(V)-HSA and Tc(IV)-HSA (in the example of Fig. 2 the labeling percentage was 92%; see also Table 1).

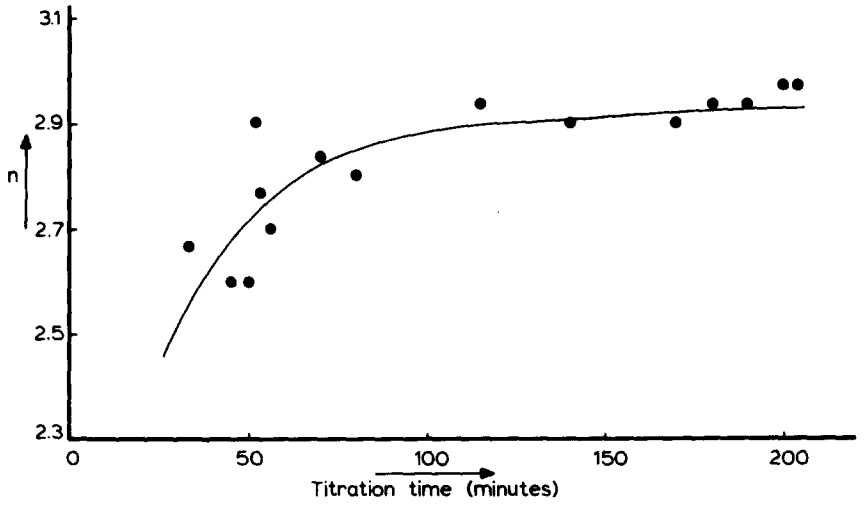

FIG. 1. Number of equivalents (n) transferred to 1 mol of $^{99}TcO_4^-$ as a function of the titration time.

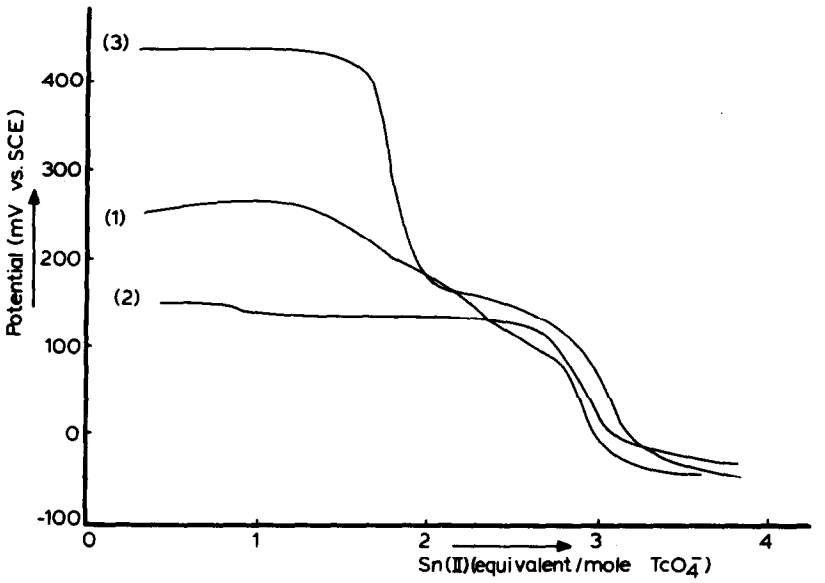

FIG. 2. Direct potentiometric titration of $^{99}TcO_4^-$ with stannous chloride in the presence of: (1) HCl, pH = 2.6; (2) Bleo, pH = 2.6; (3) HSA, pH = 1.3.

Apparently a Tc(V)-HSA complex is formed first, and this is further reduced to a Tc(IV)-HSA complex when more Sn(II) is added. It follows that in ^{99m}Tc-HSA chelates, prepared by reduction of $^{99m}TcO_4^-$ with (an excess of) Sn(II), ^{99m}Tc is in the (IV) state.

Table 1 shows the results of the direct and the iodimetric titrations and the observed labeling percentages of the investigated radiopharmaceuticals. (As iodine reacts with both Sn(II) and HSA a titration with iodine in the presence of HSA was meaningless). In HCl solutions both at pH = 1.3 and 2.6 the value of n is about 3; i.e. $^{99}TcO_4^-$ is reduced to the Tc(IV) state. Raising the pH above 3 gives a brown-black precipitate of $TcO_2.2H_2O$ as is reported by other authors.[11,12] This result is in agreement with data given by STEIGMAN *et al.*[1] and HAMBRIGHT *et al.*[3] on the reduction of $^{99}TcO_4^-$ by $SnCl_2$ in 0.1–2 M HCl and 5 M HCl respectively, and casts serious doubt on the statement of BRATU *et al.*[13] that $^{99}TcO_4^-$ is reduced to the Tc(V) state by $SnCl_2$ in 2 M HCl. The cause of this deviating opinion of the last mentioned authors is probably the inappropriate oxidant that they used to back-titrate the excess of Sn(II), i.e. Ce(IV). According to RULFS,[14] Ce(IV) oxidizes lower valence states of Tc to the Tc(V) state simultaneously with the oxidation of the excess of Sn(II). From Table 1 it appears that Tc(IV) is also found after reduction in the presence of Bleo or EHDP. The Tc(IV) oxidation state has also been reported by HAMBRIGHT *et al.*[3] for the EHDP system at pH = 5.5.

Previously we investigated the influence of the pH and of the Sn(II) and ligand concentrations on the efficiency of the labeling of HSA,[5] Bleo[6] or EHDP[7] with ^{99m}Tc, and established the optimum labeling conditions for these radiopharmaceuticals. It is interesting to investigate the applicability of these results to the higher concentration level of carrier ^{99}Tc. The estimated percentages, derived from the

TABLE 1. Results of titrations of pertechnetate in the presence of chelating agents and in HCl medium, and labeling percentages of the chelating agents

Chelating agent	Direct titration with Sn(II)			Iodimetric titrations		
	pH	n	Labeling percentage	pH	n	Labeling percentage
Bleo	2.6	2.9 ± 0.1	65 ± 2	3.1	2.7 ± 0.2	71 ± 3
EHDP	2.6	3.2 ± 0.1	73 ± 3	3.0	3.0 ± 0.2	92 ± 3
HSA	1.3	1.8 ± 0.1	86 ± 4	—	—	—
	—	3.0 ± 0.2[a]	—	—	—	—
HCl medium	1.3	2.9 ± 0.2	—	1.3	2.8 ± 0.1	—
HCl medium	2.6	3.1 ± 0.2	—	2.6	3.2 ± 0.2	—

n = number of equivalents transferred to 1 mol of TcO_4^-.
[a] In the presence of HSA two end points were registered.
On average 4 titrations were performed.

equations describing the labeling efficiencies with ^{99m}Tc as functions of the investigated variables are: for Bleo 64% at pH = 2.6 and 68% at pH = 3.1, for EHDP 97% at pH = 2.6 and 98% at pH = 3.0, and for HSA 93% at pH = 1.3. The labeling percentages with ^{99}Tc found in the present work (see Table 1) are in good agreement with the estimated values, with the exception of the low value of the labeling percentage of EHDP obtained at pH = 2.6.

Conclusions

$SnCl_2$ reduces $^{99}TcO_4^-$ to the Tc(IV) state in a HCl medium and in the presence of the chelating agent 1-hydroxy-ethylidene-1,1-diphosphonate and bleomycin. In the presence of human serum albumin a Tc(V)- or a Tc(IV)-albumin complex is formed, depending on the amount of reductant.

The labeling percentages of the chelating agents with millimolar amounts of ^{99}Tc are in good agreement with the values predicted on the basis of the results of investigations with nanomolar amounts of ^{99m}Tc.

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