RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Technetium Radiopharmaceuticals: Chemical Characterization and Tissue Distribution of Tc-Glucoheptonate Using Tc-99m and Carrier Tc-99

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The chemical structure of the kidney- and brain-imaging agent Tc-99m glucoheptonate has been established using the Tc-99 isotope. In a comparative study between Tc-99 and Tc-99m glucoheptonates, chromatographic, electrophoretic, and tissue distribution studies showed identical compounds in 0.9% NaCl. Optimal conditions for the formation of the Tc-99 glucoheptonate complex were investigated by uv and visible spectroscopy. The oxidation state of Tc-99 in the compound is V, measured by Sn^{2+} titration. The complex contains a Tc = O core and two glucoheptonate ligands (oxobis(glucoheptonato)technetate(V) anion (net charge: −1) in aqueous solution). NMR studies demonstrated two five-membered glucoheptonate rings, bidentate bound to Tc by the oxygens of the end carboxyl group and the adjacent hydroxyl group. The compound is stabilized by interaction between Tc-99 and one of the hydroxyloxygens of glucoheptonate at the vacant coordination site trans to the Tc = O core. Experiments with the reducing agent $NaBH_4$ demonstrated the absence of Sn (II or IV) in the complex and a biological behavior independent of the reducing agent used.

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Although Tc radiopharmaceuticals are widely used clinically, there is scanty knowledge of the chemical structures of the compounds and the relationship of their structures to their biological activity. This is due to two factors: (a) the Tc concentration in solutions of Tc radiopharmaceuticals (nanomolar scale) is too low to permit the use of normal chemical equipment; and (b) the lack of a stable Tc isotope (*1–7*). In recent years increasing fundamental research into the structure of Tc radiopharmaceuticals has been done using advanced chemical techniques and the long-lived Tc-99 isotope, which can be handled in the millimolar concentration range. When using Tc-99, one must bear in mind that differences can exist between the complex formations of Tc-99 and Tc-99m, resulting from the different concentration ranges used for the two isotopes (*8–11*). Detailed structural information concerning Tc radiopharmaceuticals is now available for Tc-dimethyl IDA (*12*), Tc-MDP (*13*), and oxotechnetium(V) compounds (*14–19*).

More detailed structural information regarding the radiopharmaceuticals in use today would greatly aid the prediction of in vivo stability and target-organ distribution of new radiopharmaceuticals. For this reason the study of the chemical structure of Tc glucoheptonate, a kidney- and brain-imaging agent, was undertaken (*20*). The net charge of Tc glucoheptonate (−1) has been determined by Owunwanne et al. (*21,22*) using an ion-exchange distribution method. A value of +4 for the oxidation state of technetium in Tc-99m glucoheptonate has been given by Eckelman and Levenson (*5*), but they have inferred this value from the corresponding Tc gluconate compound (*8*). In the case of Tc gluconate a value of +5 has been given for the oxidation state by Steigman et al. (*23,24*). Dewanjee and Brueggeman (*25*) determined the dissociation constant of Tc-99m glucoheptonate with human serum albumin ($K = 1.1 \times 10^{16}$).

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MATERIALS AND METHODS

Reagents. Solid $NH_4{}^{99}TcO_4$ was obtained from Oak Ridge National Laboratories. The concentration of a stock solution of 0.234 *M* $NH_4{}^{99}TcO_4$ (358 μCi/ml) in saline (0.9% NaCl) was measured spectrophotometrically using a molar extinction coefficient of 5,690 for the absorption bands at 244 and 248 nm (*26*). Glucoheptonate was >99% pure sodium α-glucoheptonate.* Technetium-99 glucoheptonate was prepared by the reduction of ^{99}Tc(VII) in $^{99}TcO_4^-$ with $SnCl_2$ or $NaBH_4$ in a saline solution of sodium α-glucoheptonate. The reaction conditions and the ratios Tc:Sn:glucoheptonate are described in the results and discussion section. Technetium-99m glucoheptonate was made by aseptic transfer of 3–7 ml of sodium pertechnetate (Tc-99m) into a vial of stannous glucoheptonate† containing 200 mg sodium glucoheptonate and 0.1 mg stannous chloride.

Analytical and physical measurements. Ultraviolet and visible electron spectra were recorded from 190–850 nm in saline using a spectrophotometer and a closed 10-mm Supracil quartz cuvette. Infrared spectra were recorded from 600–4000 cm^{-1}. Spectra were run on solid samples prepared as KBr pellets and in nujol oil using KBr plates. Raman spectra were recorded from 600–2000 cm^{-1} on a spectrophotometer‡ equipped with an argon-gas laser operating on the 488.0 and 458.0 argon lines. Spectra were run in liquid samples in sealed glass tubes. The C-13 NMR and proton NMR spectra were recorded on a 300-MHz spectrometer at 30°C in D_2O using sealed tubes. Carbon-13 and proton chemical shift values are reported as δ from (trimethylsilyl)-propane-sulfonic acid. Electrophoretic experiments were performed on cellulose acetate strips in a barbiturate buffer (pH = 8.6) at 100 V for 120 min (25°C) with an acetophor electrophoresis cell. Thin layer chromatography (TLC) was performed on silica-gel plates using saline or ethanol (70%) for the mobile phase. HPLC was performed by reverse-phase chromatography on a C18-column (mobile phase: 10% acetonitril in an aqueous buffer). Electrophoretic and TLC migrations were recorded with a radiochromatogram camera, on film by autoradiography, and in the case of Tc-99m on a radiochromatogram scanner, using a multichannel analyzer. pH measurements were performed with a digital pH meter and a standard glass- and calomel reference-electrode system in a closed cell previously flushed with dry nitrogen and containing a magnetic spin bar. Acid and base titrations were performed at 25°C, with 0.0454 *N* HCl and 0.0990 *N* NaOH.

In vivo distribution. This was determined in adult Wistar rats by injection into the tail vein of 0.1–0.5 ml Tc glucoheptonate (30–50 μCi/kg) in saline (0.9% NaCl). One hour later the kidneys, liver, spleen, stomach, bone and bone marrow, intestines, thyroid, and blood (obtained by heart puncture) were wet-weighted, solubilized and counted by liquid scintillation (Tc-99), or gamma counting (Tc-99m).

RESULTS AND DISCUSSION

Labeling with Tc-99m and Tc-99. Technetium-99m glucoheptonate was obtained in a commercial glucoheptonate labeling kit, with 95–100% of Tc-99m labeling. In this kit both glucoheptonate and Sn(II) are present in excess to the nanomolar amount of $^{99m}TcO_4^-$ added. The Sn(II) excess is necessary (see Fig. 4 of Ref. *27*), probably to overcome partial oxidation in the (O_2) solution buffered at pH 5.6 by the glucoheptonate excess. Partial hydrolysis of Sn(II) at pH 5.6 does not influence the reduction capacity, as will be shown in the section on oxidation state of technetium. TLC and HPLC experiments showed a single Tc-99m glucoheptonate peak at pH 7.0.

Technetium-99 glucoheptonate was formed within a few seconds of the addition of $SnCl_2$ and $^{99}TcO_4^-$ to a saline glucoheptonate solution. To obtain a high yield of Tc-99 glucoheptonate, a 25-fold excess of glucoheptonate, with respect to the amount Tc-99, is necessary in the saline solution. Under special conditions, described below, a twofold excess of glucoheptonate is sufficient for the formation of the complex. The chemical stability of Tc-99 glucoheptonate is high: a 0.01 *M* Tc-99 glucoheptonate solution in saline was chemically stable for more than 200 days at room temperature, as indicated by unchanged uv and visible (uv-vis.) spectra during that period. TLC experiments showed that there is one compound formed. This could not be confirmed by HPLC because of possible Tc-99 contamination of the HPLC system.

The electron (uv-vis.) spectrum of Tc-99 glucoheptonate shows two absorbance bands (λ_{max} = 502 nm, ϵ_0 = 65; λ_{max} = 270–280 nm, $\epsilon_0 \sim$ 2800; Fig. 1). The absorbance maximum at 502 nm can be assigned to an electron transition between the split d-electron orbitals of the Tc ion, caused by the ligand field of glucoheptonate and oxygen. The second band at 270–280 nm, observed in a region of increasing absorbance (maximum absorbance in the region of the self-absorbance of water,

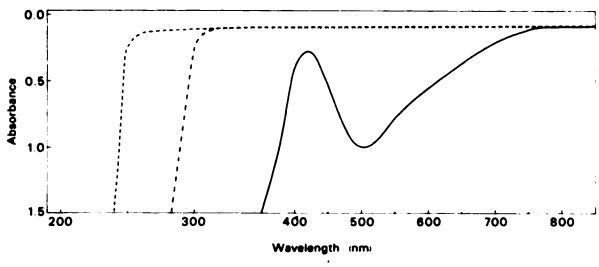

FIG. 1. Ultraviolet and visible spectra of various compounds in 0.9% NaCl at 25°C. Cuvette length: 10 mm. Reference: saline, — 1.1 × 10^{-2} *M* Tc-99 glucoheptonate, ····· 0.07 × 10^{-4} *M* Tc-99 glucoheptonate, -·- 2.0 × 10^{-2} *M* $SnCl_2$ and 1.0 *M* sodium glucoheptonate, - - - 1.0 *M* sodium glucoheptonate.

TABLE 1. ELECTROPHORETIC AND THIN-LAYER CHROMATOGRAPHIC BEHAVIOR OF Tc-99 GLUCOHEPTONATE AND Tc-99m GLUCOHEPTONATE IN 0.9% NaCl*

Compound	Reducing agent	Electrophoretic migration†	Thin layer chromatography R_f saline	Thin layer chromatography R_f ethanol (70%)
$^{99m}TcO_4^-$		1.0	0.92	0.85
$^{99}TcO_4^-$		1.0	0.90	0.84
^{99m}Tc-glucoheptonate	Sn(II)	0.30	0.89	0.65
^{99}Tc-glucoheptonate	Sn(II)	0.33	0.89	0.65
^{99}Tc-glucoheptonate	$NaBH_4$	0.32	0.89	0.60

* Electrophoresis on cellulose acetate strips in a barbiturate buffer (pH = 8.6) 2 hr, 100 V, 25°C. Thin layer chromatography on silica gel, mobile phases: saline and ethanol (70%), 25°C.

† Relative to the migration distance of $^{99m}TcO_4^-$ toward the positive pole.

<190 nm), can be assigned to a charge-transfer transition. The absorbance at 270–280 nm cannot be caused by a possibly existing Sn(II or IV)-glucoheptonate complex, since a 0.02 *M* Sn(II) with 1 *M* glucoheptonate solution in saline, in a nitrogen atmosphere, shows only an increasing absorbance at decreasing wavelength, without a band at 270–280 nm after dilution of the solution. After oxygen was bubbled through the solution, which causes the formation of Sn(IV), the spectrum did not change significantly. Glucoheptonate alone in saline solution gives no absorbance band at 270–280 nm in the uv-vis. spectrum (Fig. 1). Note that dissolving $SnCl_2$ (0.02 *M*) in water or in a glucoheptonate/saline solution did not give precipitation of hydrolysis products, probably due to the low Sn(II) concentration used.

(Dis)similarity of Tc-99 and Tc-99m glucoheptonate. Information concerning the chemical structures of Tc-99m radiopharmaceuticals can currently be obtained only by use of the long-lived Tc-99 isotope. The (dis)-similarity of the chemical structures of Tc-99 and Tc-99m glucoheptonates thus needs to be confirmed. The in vitro behavior of both complexes was investigated using electrophoretic and chromatographic methods. The relative electrophoretic migrations of both complexes were determined using cellulose acetate strips with a barbiturate buffer system (25°C) and are given in Table 1. No differences exist between the relative electrophoretic migrations of Tc-99 and Tc-99m glucoheptonates. If the complexes have sizes of the same order, then their overall charge will be the same. The TLC behavior of Tc-99 and Tc-99m glucoheptonates was investigated on silica-gel plates using two different mobile phases: saline and ethanol (70%). The R_f values of both complexes, determined by an autoradiographic method, are identical in the two mobile phases within the measuring error (Table 1). The phenomenon of adsorption on the silica gel at nanomolar concentrations can be excluded, because no differences exist between the R_f values of $^{99}TcO_4^-$ and $^{99m}TcO_4^-$ in either mobile phase. It can be concluded that the behavior of the two complexes in the TLC system is the same. Since no chemical differences in in vitro behavior were found between Tc-99 and Tc-99m glucoheptonates, identical chemical structures can be assumed.

TABLE 2. TISSUE DISTRIBUTION IN WISTAR RATS OF Tc-GLUCOHEPTONATE AT 1 HR AFTER INJECTION

	% dose/g*		
	Tc-99m glucoheptonate $SnCl_2$	Tc-99 glucoheptonate $SnCl_2$	Tc-99 glucoheptonate $NaBH_4$
Kidney	5.61 ± 0.87	5.14 ± 0.76	4.93 ± 0.92
Blood	0.09 + 0.01	0.14 ± 0.03	0.07 ± 0.01
Liver	0.06 ± 0.02	0.08 ± 0.04	0.11 ± 0.04
Spleen	0.06 ± 0.04	0.05 ± 0.03	0.09 ± 0.03
Stomach	0.09 ± 0.08	0.12 ± 0.10	0.12 ± 0.08
Bone and Marrow	0.04 ± 0.04	0.04 ± 0.02	0.09 ± 0.05
Intestines	0.10 ± 0.01	0.13 ± 0.04	0.11 ± 0.06
Thyroid	0.26 ± 0.14	0.24 ± 0.20	0.31 ± 0.11

* Mean of five animals ± s.d.

The in vivo tissue distribution in Wistar rats shows no significant differences between Tc-99 and Tc-99m glucoheptonates (Table 2). Although identical tissue distribution patterns for the two compounds is no proof of an identical chemical structure (see Eckelman, Ref. *5*), it can be regarded as an indication of chemical equivalence. The renal uptake (our figure 14 to 18% of injected dose; cf. 12.8–22.2%, Ref. *28*) is mainly in the cortex, with less in the medulla (*28*).

In both the in vitro and in vivo behavior of the Tc-99 and Tc-99m glucoheptonates no significant differences were found. We conclude that the two isotopes give the same complex with glucoheptonate and that there are no chemical structural differences in the nanomolar and the millimolar concentration ranges.

Chemical characterization of Tc-99 glucoheptonate. The net charges of Tc-99 and Tc-99m glucoheptonates are the same (−1), as indicated by the electrophoretic and TLC experiments (*21,22*). The electrophoretic migration of Tc-99 glucoheptonate is one third that of $^{99}TcO_4^-$. Decrease of migration velocity associated with increase of molecular radius (at equal net charge) is also found for oxobis(dithiolato)technetate(V) anions (*17*).

Oxidation state of technetium. Oxidation states of −1 to +7 are possible for technetium. The most frequently found values are +3, +4, and +5. In order to determine the oxidation state of technetium in the Tc-99 glucoheptonate complex, the reduction of Tc(VII) in $^{99}TcO_4^-$ is performed by titration of $^{99}TcO_4^-$ with $SnCl_2$ in the presence of a 100-fold excess of glucoheptonate (with regard to the amount $^{99}TcO_4^-$) in a nitrogen atmosphere (Fig. 2). At each point of the titration curve a uv-vis. spectrum delivered the Tc-99 glucoheptonate concentration (taken at λ_{max} = 502 nm; ϵ_0 = 65). No spectral changes were observed during titration, except the expected increase of absorbance. At $Sn^{2+}/^{99}TcO_4^- > 1.0$, no additional Tc-99 glucoheptonate complex is formed, so it can be concluded that $^{99}Tc(VII)$ is reduced in the complex formation to $^{99}Tc(V)$. A large excess of Sn(II) ions did not give any significant spectral change, except a decrease of ϵ_0 resulting from the increase of the pH by the formation of hydrolysis products of Sn(II) or Sn(IV), as will be described below. A two-step reaction mechanism in the formation of the complex—a reduction to Tc(IV) followed by a rapid O_2 oxidation—is not likely, because in the case of Tc(IV) gluconate, O_2 does not rapidly oxidize the Tc(IV) species (*8*). Following reduction, the oxidation state of technetium does not change under the influence of oxygen gas (*29*). A technetium oxidation state of +5 was also found for gluconate- (pH 12; *23*), oxocitrato- (*9,30*), oxodimethylglyoxime- (*14*), oxoborato- (*15*), and oxodithiolato- (*16–18,31*) technetium complexes. Tc(V) has a d^2- (outer) electron shell and is presumably paramagnetic in an octahedral configuration (*15,18,32*).

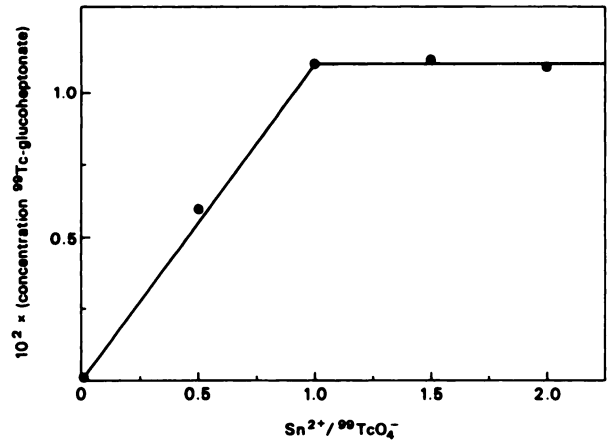

FIG. 2. Amount Tc-99 glucoheptonate formed at different molar ratios of Sn(II) and $^{99}TcO_4^-$, calculated from absorbance at 502 nm in the uv-vis. spectrum. Concentration $^{99}TcO_4^-$: 11 millimol in saline (25°C), N_2 atmosphere.

TABLE 3. MOLECULAR VIBRATIONS OF Tc-99 GLUCOHEPTONATE

	Infrared		Raman
	cm^{-1}	cm^{-1}	cm^{-1}
Tc-99 glucoheptonate	2080 w*	970 m	975 m
	1740 s	930 m	
	1180 s	860 m	
	1165 s	785 w	
		735 s	

* s = strong, m = medium, w = weak.

As can be observed in Fig. 2, a great excess of $SnCl_2$ (as described in Ref. *27*) is not necessary. The quantity of $SnCl_2$ is not crucial, because the same Tc-glucoheptonate compound is formed using Tc-99 and Tc-99m, whereas the ratios $Sn^{2+}/^{99}TcO_4^-$ and $Sn^{2+}/^{99m}TcO_4^-$ differ by a factor of 10^6–10^7.

Tc = O core. Tc(V) complexes frequently contain a Tc = O core (*5,14–18,30,31,33*). The molecular vibrations of Tc-99 glucoheptonate, obtained by ir and Raman spectroscopy, are given in Table 3. Vibration bands of the Tc = O core in oxodithiolatotechnetium(V) complexes have been described at frequencies of 928 cm^{-1} and 970 cm^{-1} (*15*); 930 cm^{-1} (Raman) and 925-945 cm^{-1} (ir) (*16,17*); 950 cm^{-1} (*18*); 800–1050 cm^{-1} (*31*), and 785 cm^{-1} (*33*). The molecular vibrations of Tc-99 glucoheptonate in the ir spectrum at 930 cm^{-1} and 970 cm^{-1} and possibly 785 cm^{-1}, and in the Raman spectrum at 975 cm^{-1}, indicate the presence of a Tc = O unit in the Tc-99 glucoheptonate molecule (bond length 1.656 Å (*15*) or 1.64 Å (*16,17*)).

Glucoheptonate/Tc ratio. The number of glucoheptonate ligands that coordinate with one Tc(V) ion was determined by a mol-ratio method. At different glucoheptonate concentrations (Tc:Sn:glucoheptonate = 1:

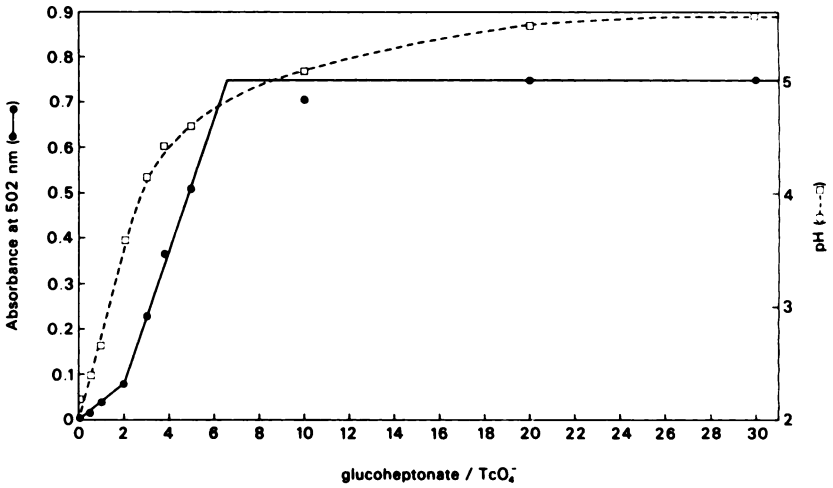

FIG. 3. Mol-ratio curve; absorbance at 502 nm (uv-vis. spectrum) and pH of Tc-99 glucoheptonate at different glucoheptonate to $^{99}TcO_4^-$ ratios (25°C).

1:x, where x = 0–30 and TcO_4^- is 0.115 *M*), uv-vis. spectra of the product were recorded. In all cases the same spectra were found. The absorbance at λ_{max} = 502 nm is given in Fig. 3 for the various glucoheptonate/TcO_4^- ratios. The pH of the solutions at the various glucoheptonate/TcO_4^- ratios is also plotted in Fig. 3. At low ratios the pH was not adjusted to pH 5.6 to avoid disturbance of the reaction mixture and the formation of other Tc complexes.

The mol-ratio curve has two bends, which indicate the existence of three different compounds at the different glucoheptonate/TcO_4^- ratios. The bend at the glucoheptonate/TcO_4^- ratio of 2 shows that two glucoheptonate ligands coordinate with one Tc-99 ion. The second bend of the mol-ratio curve (glucoheptonate/TcO_4^- = 6.5) is caused by the pH effect. The pH decreases as the glucoheptonate/TcO_4^- ratio decreases, as a result of the reduced buffering of glucoheptonate at lower glucoheptonate concentrations. The pH affects only the absorbance (see Fig. 4) and not the shape of the spectrum. The effect of pH on ϵ_0 is completely reversible and can be explained by protonation of the oxygen of the Tc = O core in the lower pH range. In this pH range an equilibrium exists between the protonated and the nonprotonated species.

Under the conditions of the mol-ratio curve [Sn(II) concentration 11 millimol; pH < 5.6] complex formation between Sn(II or IV) and glucoheptonate was not observed by Raman spectroscopy. Also no interaction was observed between Tc-99 glucoheptonate and Sn by comparing the uv-vis. spectra of the Tc-99 glucoheptonate compounds formed by $SnCl_2$ and $NaBH_4$ (see below).

The role of tin. Sn(II or IV) can be incorporated into the Tc complex or can stay as "free" Sn(II or IV) ions in the solution. These ions probably bind to glucoheptonate at high glucoheptonate concentrations. To investigate the role of tin, the nonmetal reducing agent $NaBH_4$ was used in place of $SnCl_2$. The Tc-99 glucoheptonate compound formed by $NaBH_4$ reduction gave the same uv-vis. spectrum (λ_{max} = 502 nm, ϵ_0 = 80; and λ_{max} = 270–280 nm, $\epsilon_0 \sim 2700$; pH 6.0) and no significant change in electrophoretic and TLC behavior compared with $SnCl_2$ (Table 1). The same compound is formed and Sn(II or IV) is not incorporated in Tc-99 glucoheptonate. This is in agreement with the absence of Sn(II or IV) in Tc-DTPA (*10,24*), Tc-HIDA (*12*), Tc-MDP (*13*), Tc-pyrophosphate (*24*), Tc-HEDP (*24,34*), Tc-gluconate (*24*), and Tc-dithiolato compounds (*16–19,31,33,35,36*). The only compound with Sn(IV) incorporation is Tc-dimethylglyoxime (*14*).

The tissue distribution in rats is identical, regardless of whether $NaBH_4$ or $SnCl_2$ is used as the reducing agent (Table 2). Sn(II or IV) plays no role in the in vivo localization. ^{113}Sn(II or IV) is not associated with Tc(V) (*37*) and localizes mainly in bone (*38*). The in vivo stability of Sn-glucoheptonate is very low (*38*). In human blood and serum a plasma/cell ratio for Tc-99 glucoheptonate of 1.5 has been found (*20*), and 85% in vitro

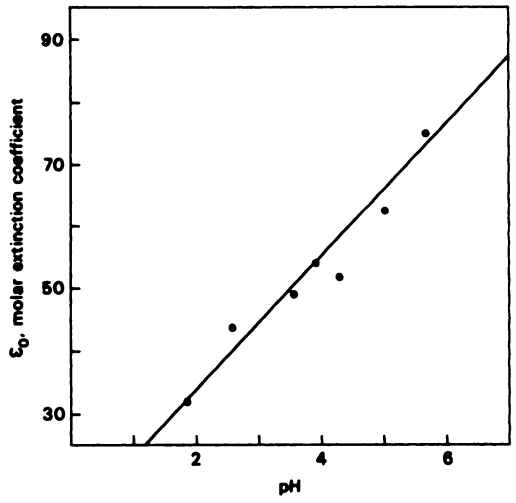

FIG. 4. pH dependence of the molar extinction coefficient at 502 nm of Tc-99 glucoheptonate (25°C).

binding between Tc-99 gluconate and HSA (*39*).

Structure. The complex can be described as an oxo-bis(glucoheptonato)technetate(V) anion. The compound does not crystallize from an aqueous solution after the addition of large cations (e.g., allyl-ammonium salts) and/or organic solvents. After extreme drying, a hard a-crystalline hydrated substance is obtained, so solid-phase characterization with x-ray crystallography is not possible. This phenomenon is normal in the coordination chemistry of carbohydrates. The structure in the liquid phase (not necessarily the same as in the solid phase) shows one oxo group and two glucoheptonate groups per molecule. A glucoheptonate molecule can coordinate to the Tc center with one or two oxygen atoms (mono- or bidentate).

The vibration absorbance of the carboxyl group in the ir spectrum of glucoheptonate (C–O stretch: 1620 cm^{-1}) is shifted in the Tc-99 glucoheptonate complex (C–O stretch: 1740 cm^{-1}). The glucoheptonate is bound to Tc by the carboxyl oxygen. pH titrations showed the release of one proton per glucoheptonate in the complex formation, so one of the hydroxyl oxygens must also be coordinated to Tc, with the release of a proton. The two glucoheptonate molecules form bidentate chelates to Tc. The overall stoichiometry is:

$$TcO_4^- + 2(C_7H_{13}O_8)^- + Sn^{2+} + H_2O \rightarrow [TcO(C_7H_{12}O_8)_2]^- + 2H^+ + [SnO_2(OH)_2]^{2-}$$

The Sn(IV) product can be a mixture of Sn(IV) hydrolysis products.

A glucoheptonate molecule can form 5- or 6-membered chelate rings with Tc-99. Information concerning the chelate size was obtained from NMR spectroscopic data. The C-13 NMR spectrum of "free" glucoheptonate showed seven lines corresponding with seven carbon atoms. The proton NMR data for the relevant protons of the glucoheptonate ligand are given in Table 4. The ligand itself is a linear molecule (Fig. 5) as given by these spectra, in particular indicated by the relatively small

TABLE 4. PROTON NMR SPECTROSCOPIC DATA

Compound	$\delta(H_2)$*	$\delta(H_3)$*	$\delta(H_4)$*
Glucoheptonate	4.123	3.927	3.995
	4.139	3.941	4.003
		3.956	4.009
			4.017
Tc-99 glucoheptonate	4.363	3.928	4.071
	4.377	3.942	4.076
		3.957	4.082
			4.092

* ppm relative to internal DDS standard; for indices of H see Fig. 5.

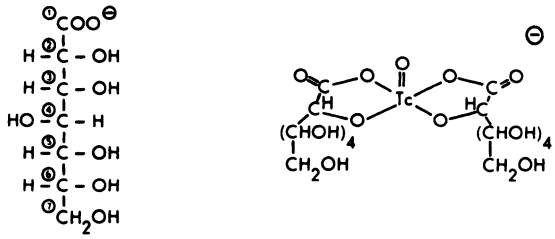

FIG. 5. Structures of glucoheptonate and Tc-99 glucoheptonate.

chemical shift value of the proton of the C_2 atom (see Fig. 5 for numbering) of glucoheptonate [$\delta(H_2)$] compared with the chemical shift values of anomeric protons of carbohydrate ring systems (*40*). In the proton NMR spectrum of Tc-99 glucoheptonate, the Tc ion acts as a shift reagent (*40*). Formation of a five-membered ring will increase the chemical shift of the proton on the C_2 atom, and for a six-membered ring a greater shift of the proton on the C_3 atom will occur.

After the complex formation, a low-field shift of the proton on the C_2 atom [$\delta(H_2)$] is observed (Table 4). Two five-membered ring systems are formed (Fig. 5).

It is known that the influence of an oxo group leads to a vacant trans coordination site. In solution, however, this vacant coordination site presumably contains a loosely bound solvent molecule. In the Tc-99 glucoheptonate molecule there is a small interaction between the vacant coordination site of Tc and the oxygen on the C_4 atom (a small additional, low-field shift is observed for the proton on the C_4 atom after complex formation). This additional interaction can explain the high stability of Tc-99 glucoheptonate compared with analogous compounds (*4*).

More study of Tc radiopharmaceuticals in solution is needed, since the structures of most routinely used imaging agents are currently unknown. Future research should lead to a more rational design of radiopharmaceuticals.

FOOTNOTES

* P-L Biochemicals, Inc.

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