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Cationic Technetium Complex with 1,4-Dithia-8,11-diazacyclotetradecane

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Abstract

The complex of ^{99}Tc with 1,4-dithia-8,11-diazacyclotetradecane was prepared via a ligand exchange reaction and was compared to the complex of ^{99}Tc with 1,4,8,11-tetraazacyclotetradecane. All the results are consistent with the formula $[TcO_2([14]\text{-}aneN_2S_2)]^+$. The reduction of $^{99m}TcO_4^-$ with Sn^{2+} in alkaline solution, in the presence of the 14-membered N_2S_2-macrocycle, led to the formation of a ^{99m}Tc–*cis*[14]aneN_2S_2 cationic complex. The results suggest the formation of the same complex with both ^{99}Tc and ^{99m}Tc radionuclides.

Introduction

During the last few years, the monocationic technetium complexes have been investigated with regard to their applications in nuclear medicine. For this purpose the complexes of ^{99m}Tc with 1,4,8,11-tetraazacyclotetradecane (cyclam) [1, 2], ethylenediamine (en) [3] and 1,4,8,11-tetraazaundecane (2,3,2-tet) [4] have been prepared and tested as radiopharmaceuticals. To understand the structures of these labelled compounds the ^{99}Tc analogs, with the above ligands, have been synthesized and characterized. The X-ray crystal structures of $[TcO_2\text{-}cyclam]ClO_4$ [5] and $[TcO_2(en)_2]X$ (X = Cl, I) [6] have been determined.

The chemistry of Tc complexes with macrocylic ligands has received little attention in comparison with that of complexes with open-chain ligands. To our knowledge there is no data on the complexes of Tc with macrocycles containing a N_2S_2-donor set. Recently, the preparation of the 1,4-dithia-8,11-diazacyclotetradecane has been reported [7], and its Cu^{2+} and Ni^{2+} complexes have been synthesized [7, 8].

The aim of this work was to prepare and characterize the complexes of ^{99}Tc and ^{99m}Tc with 1,4-dithia-8,11-diazacyclotetradecane (Fig. 1). A comparison was made between the complexes of ^{99}Tc with both 14-membered N_2S_2- and N_4-macrocycles. They were prepared via a ligand exchange reaction of $(nBu)_4N[TcOBr_4]$ with the appropriate macrocyclic ligand.

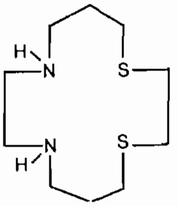

Fig. 1. 1,4-Dithia-8,11-diazacyclotetradecane (*cis*[14]aneN_2S_2).

Experimental

Materials

^{99}Tc was obtained from the Radiochemical Centre, Amersham as an ammonium pertechnetate solution. $(nBu)_4N[TcOBr_4]$ was prepared according to the method described in the literature [9]. The ligands *cis*[14]aneN_2S_2·2HCl and cyclam were kindly provided by the Institute of Inorganic Chemistry, Basel. All other reagents were p.a. quality. ^{99m}Tc was eluted from a Minitec Generateur (EIR).

Preparation of ^{99}Tc Complexes

^{99}Tc–cis[14]aneN_2S_2

13 mg (4.2×10^{-2} mmol) of *cis*[14]aneN_2S_2·2HCl was dissolved in 0.1 M NaOH and the free base was extracted three times with 2 ml CH_2Cl_2. To the organic phase, anhydrous Na_2SO_4 was added to dry the solution. The sodium sulfate was filtered off and the solvent was evaporated to dryness. The free base dissolved in 300 μl CH_2Cl_2 was added to a solution of $(nBu)_4N[TcOBr_4]$ (1.41×10^{-2} M) in THF. The resulting brown–yellow

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product was extracted three times with 0.5 ml H_2O. The aqueous solution was concentrated to 200 μl and was charged onto a Bio-Gel P2 column (h = 9 cm, diam. = 1.0 cm). The yellow coloured fractions eluted with water were combined and concentrated under a stream of nitrogen. Each of the eluted fractions and the final solution exhibited identical UV–Vis absorption spectra; the electrophoresis showed a single cationic species. The yellow solutions obtained from four identical preparations were combined and a few drops of a saturated KPF_6 solution were added. The yellow crystals formed upon slow evaporation were isolated and washed with isopropyl alcohol. The electrophoretic and spectrophotometric characteristics of the crystals, dissolved again in water, were the same as those of the initial yellow solution. The yield was 37% on the basis of the initial ^{99}Tc concentration. The cationic complex was isolated also with the $CF_3SO_3^-$ ion.

^{99}Tc–cyclam

Cyclam dissolved in CH_2Cl_2 was added to a solution of $(nBu)_4N[TcOBr_4]$ in THF. The pink–orange fractions eluted with water from a Bio-Gel P2 column were combined and concentrated; the complex yield in solution was 30%. With $LiCF_3SO_3$ solution an orange product was formed upon slow evaporation. The solution was allowed to evaporate to dryness and the residue was washed with isopropyl alcohol. A hygroscopic compound was obtained.

Preparation of ^{99m}Tc Complexes

^{99m}Tc–cis[14]aneN$_2$S$_2$

The following labelling method was found to be optimal. To a 10 ml penicillin vial containing 50 μl of ligand aqueous solution (10 mg/ml) were added successively: 500 μl 0.1 M phosphate buffer pH 11.5, 100 μl physiological saline with $^{99m}TcO_4^-$ (30 mCi/ml) and 20 μl of a solution of 0.25 mg/ml $SnCl_2 \cdot 2H_2O$ in 0.1 M HCl. The vial contents were mixed by inversion for one min. After 5 min 500 μl 0.1 M Na_2HPO_4 were added to set the pH value between 7 and 7.5 For 26 h the stability of the complex was regularly tested by electrophoresis; no degradation was detected.

^{99m}Tc–cyclam

50 μl of ligand solution (10 mg/ml), 250 μl 0.1 M Na_3PO_4, 10 μl physiological saline with $^{99m}TcO_4^-$ and 5 μl of 0.25 mg/ml $SnCl_2 \cdot 2H_2O$ in 0.1 M HCl were mixed in a 10 ml penicillin vial.

Analytical Methods and Activity Measurements

Electrophoresis

A Camag H.V.E. apparatus was used. The paper strips were Schleicher-Schull No. 2043B (1.5 X 40 cm^2). The electrophoretic conditions for the separation of the ^{99}Tc complexes are given in Table I. The separation of the ^{99m}Tc species was performed at pH 7 in a 0.1 M phosphate buffer at 2000 V for 30 min.

Paper chromatography

The separation was carried out on Schleicher-Schull 2043B paper in acetonitrile/water (65/35, *v/v*).

Activity measurements

The ^{99}Tc activity on the paper strips was measured with a TLC Linear Analyser LB 282 consisting of a position sensitive proportional counter tube connected to a Silena multichannel analyser.

The ^{99m}Tc activity was measured with a TLC linear analyser; the system consisted of a 3" X 3" NaI(Tl) detector arranged to scan the paper strip (velocity: 2–5 cm/min); the data were collected on a HP 86B computer for further processing.

TABLE I. R_f Values and Migrating Distances of Cationic ^{99}Tc Complexes

	Analysis					
	Paper chromatography (R_f)	Electrophoresis (cm)				
Solvent	CH_3CN/H_2O 65/35	0.05 M phosphate buffer pH = 8.5	0.05 M phosphate buffer pH = 7.0	0.1 M phosphate buffer pH = 7.0	0.1 M bicarbonate buffer pH = 8.6	0.1M[a] bicarbonate buffer pH = 8.6
Complex						
Tc–cyclam				9.5 ± 0.5		4.5 ± 0.5
Tc–*cis*[14]aneN$_2$S$_2$	0.6 ± 0.07	9.8 ± 0.8	9.4 ± 0.6	9.5 ± 0.5	9.5 ± 0.5	4.0 ± 1.0

[a] Conditions: 300 V for 1 h, all other analyses were performed at 2000 V for 30 min.

The Tc concentrations were determined by measuring the ^{99}Tc radioactivity with a Packard Tri-Carb 460 CD liquid scintillation system.

Spectrophotometry

Optical spectra in water were obtained with a HP 8450A Diode Array spectrophotometer connected to a HP 7475A Plotter.

Infared spectra in the 4000–400 cm^{-1} range were obtained with a Pye-Unicam SP 1100 spectrophotometer using KBr pellets. Those in the 1000–50 cm^{-1} range were recorded on a FTIR Brucker IFF 113C device, using polyethylene pellets.

Results

The electrophoresis of the brown–yellow solution extracted in water after the ligand exchange reaction with *cis*[14]aneN$_2$S$_2$ showed a single cationic product accompanied by 20% of TcO$_2$ and TcO$_4^-$ as impurities (Fig. 2a). After purification on the Bio-Gel P2 column the solution contained a pure cationic complex (Fig. 2b). The analysis of this solution by paper chromatography showed only one product.

When the ligand exchange was performed with cyclam the electrophoresis of the brown aqueous solution showed two species: a cationic complex (40%) and TcO$_2$ (60%). After the purification of the solution only the cationic Tc complex was found. In Table I, the electrophoretic and chromatographic characteristics of the complexes of ^{99}Tc with both *cis*[14]aneN$_2$S$_2$ and cyclam are given.

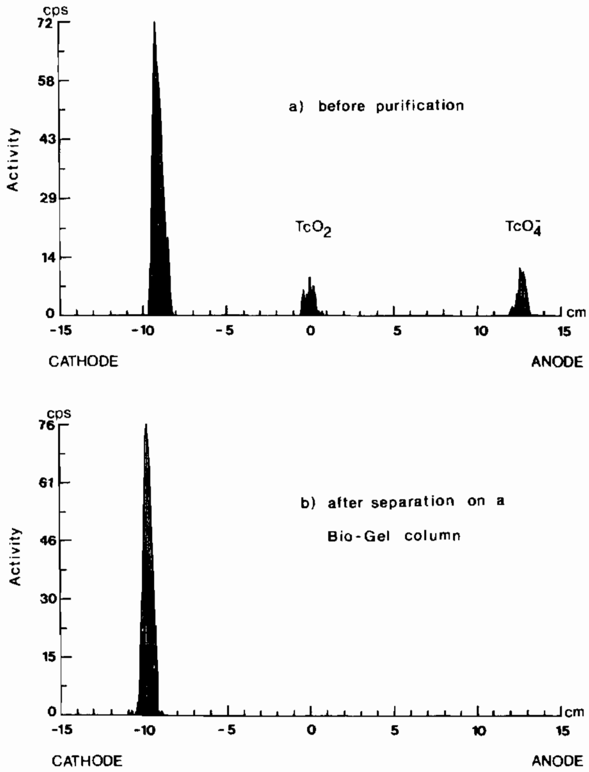

Fig. 2. Electrophoresis patterns in 0.05 M phosphate buffer at pH 8.5: (a) ^{99}Tc species in aqueous solution before purification; (b) pure cationic ^{99}Tc–*cis*[14]aneN$_2$S$_2$ complex.

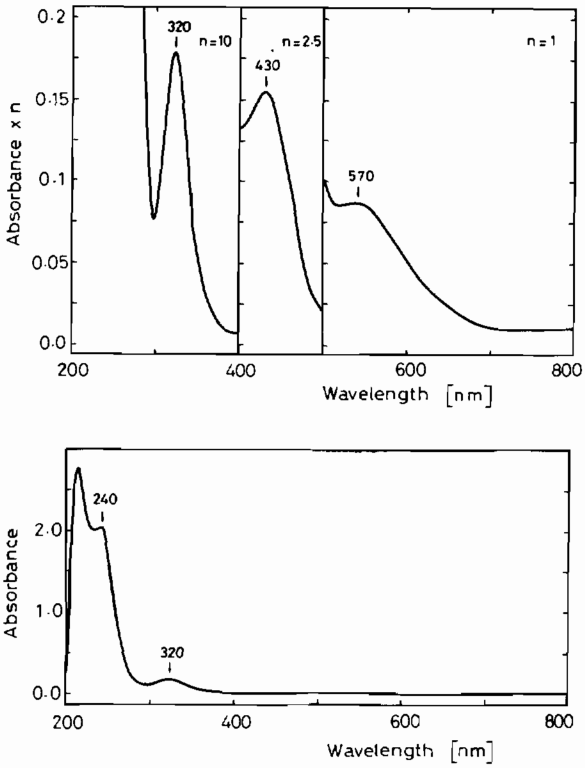

Fig. 3. Absorption spectrum of Tc–*cis*[14]aneN$_2$S$_2$ in water.

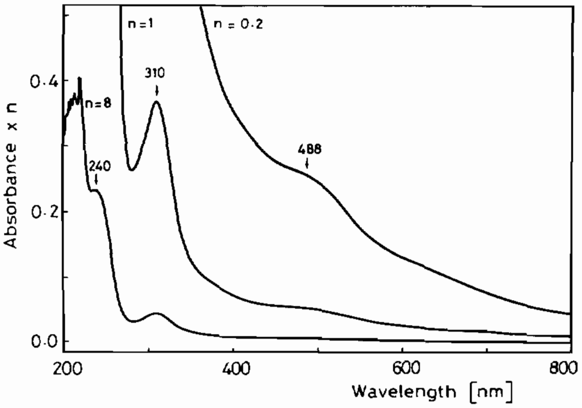

Fig. 4. Absorption spectrum of Tc–cyclam in water.

The UV–Vis spectrum of the complex of Tc with *cis*[14]aneN$_2$S$_2$ in water (Fig. 3) exhibits absorption maxima at 240, 320, 430 and a weak absorption at 570 nm ($\epsilon = 10\,200$, 858, 66 and 4 l M^{-1} cm^{-1} respectively). The electronic spectrum of Tc–cyclam in water (Fig. 4) shows absorption maxima at 240 ($\epsilon = 5076$) and 310 ($\epsilon = 1063$) nm and a shoulder at

TABLE II. Stability of ^{99}Tc-*cis*[14]aneN_2S_2 Complex

Solution	pH ± 0.5	[^{99}Tc] (M)	Time (h)	Complex (%)
H_2O	6.0	4.0×10^{-4}	240	100
H_2SO_4 0.1 M	1.0	1.0×10^{-4}	4	100
HCl 0.025 M	3.0	2.0×10^{-5}	20	99
Phosphate buffer 0.1 M	7.0	5.0×10^{-5}	24	100
Phosphate buffer 0.1 M	8.5	5.0×10^{-5}	20	98
			48	87
NaOH 0.1 M	12.0	2.2×10^{-4}	0.3	85
			18	0

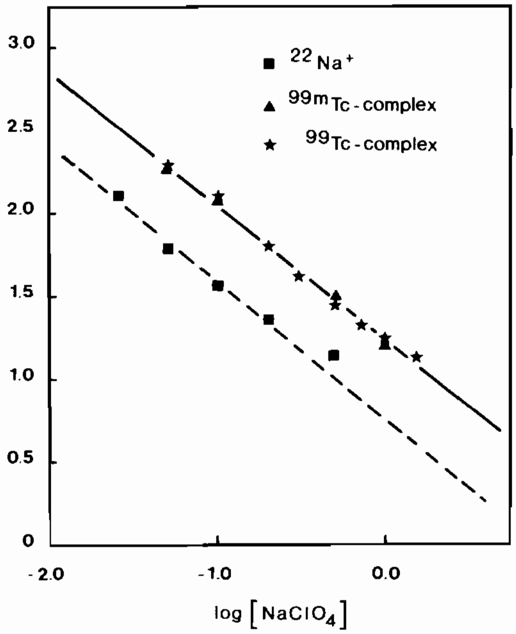

Fig. 5. Distribution, D_0, of ^{99}Tc– and ^{99m}Tc–*cis*[14]ane-N_2S_2 between resin and $NaClO_4$ solution plotted against $NaClO_4$ concentration.

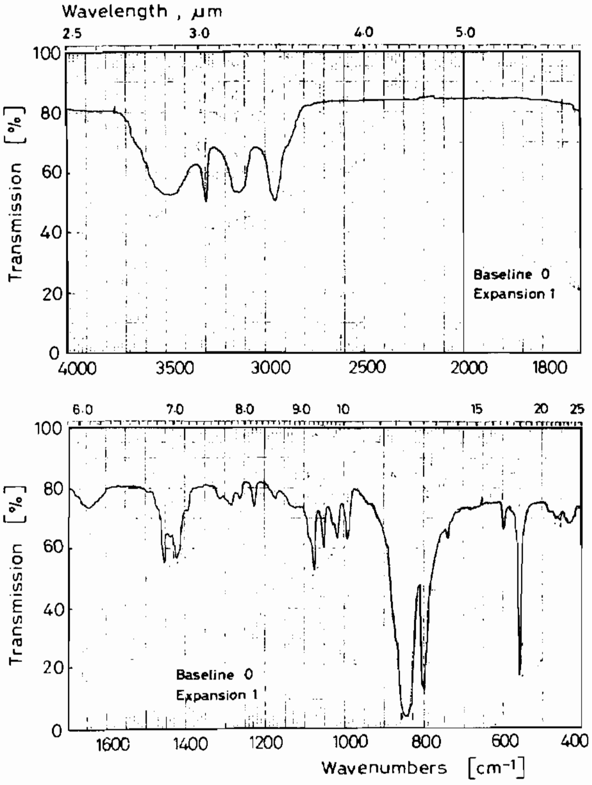

Fig. 6. Infrared spectrum of Tc–*cis*[14]aneN_2S_2 complex isolated with PF_6^-; spectrum obtained in a KBr pellet.

488 nm ($\epsilon = 159$). The complex was stable for several days in the spectrophotometric cell.

The stability of ^{99}Tc–*cis*[14]aneN_2S_2 in phosphate buffers, acid and basic solutions was studied by spectrophotometry and electrophoresis. The results obtained by the latter method are shown in Table II. The decomposition of the complex in strong basic solution led to formation of TcO_4^-. The hydrochloric acid induced the formation of a new species. Neither TcO_2 nor TcO_4^- resulted during this transformation. Further studies of this complex are now under way.

The charge of the ^{99}Tc-*cis*[14]aneN_2S_2 complex was determined by the ion exchange distribution technique [10, 11]. Let D_0 be the distribution of the Tc complex between the cationic resin and $NaClO_4$ solutions. The plot of log D_0 *versus* log[ClO_4^-] (Fig. 5) gives a straight line with a slope of 0.84. Complementary experiments performed with $^{22}Na^+$ gave the same results. It can be concluded that the net charge of the complex is +1. Attempts to determine the Tc oxidation state by titration with $Ce(SO_4)_2$ in 0.1 M H_2SO_4 were thwarted by the high stability of the complex.

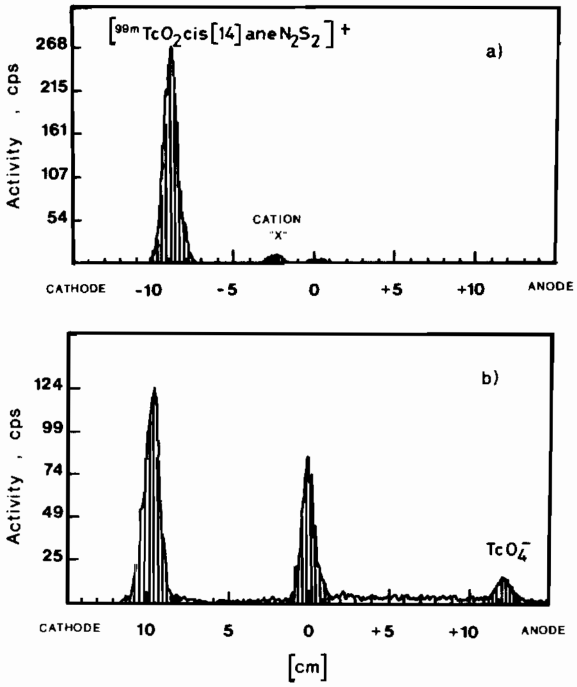

Fig. 7. Electrophoretic separation of ^{99m}Tc species coexisting in solution: (a) pH 11, (b) pH 7.

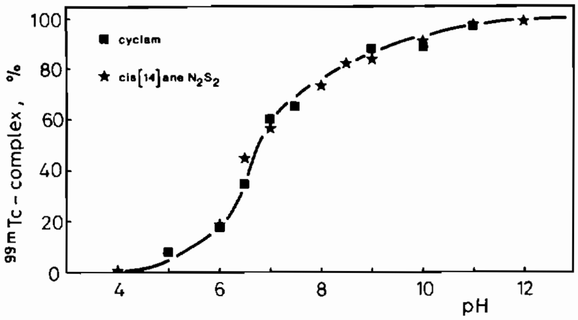

Fig. 8. Yield of ^{99m}Tc complex *vs.* pH: ★, Tc–*cis*[14]ane-N_2S_2; ■, Tc–cyclam.

Infrared spectra of the Tc–*cis*[14]aneN_2S_2 isolated with either PF_6^- (Fig. 6) or $CF_3SO_3^-$ exhibit a strong absorption at 800 cm^{-1} in addition to NH absorptions in the region of 3100–3300 cm^{-1}. The Tc–cyclam, isolated with $CF_3SO_3^-$, shows an absorption at 795 cm^{-1}. In none of the spectra, could absorptions assignable to the Tc–Br stretching be detected.

The chemical behaviour of ^{99}Tc–cyclam obtained by the ligand exchange reaction is similar to that of ^{99}Tc–cyclam prepared by $^{99}TcO_4^-$ reduction [2, 5]. Their electrophoretic patterns in bicarbonate buffer (Table I), UV–Vis spectra and infrared absorptions suggest that the two complexes are identical.

The reduction of $^{99m}TcO_4^-$ by Sn^{2+} in a *cis*-[14]aneN_2S_2 solution at pH 11.5 leads to the formation of the positively charged ^{99m}Tc–*cis*[14]aneN_2S_2 complex (96%), a cation 'X' (3%) and a neutral complex (1%). The yields of these species are dependent on the pH of the solution (Fig. 7a, b). At pH < 7, the major product is the neutral complex. The effect of pH on the yields of the complexes of ^{99m}Tc with both *cis*[14]aneN_2S_2 and cyclam is shown in Fig. 8. The *cis*[14]aneN_2S_2 complex is formed with a high yield at pH 11, but decomposes slowly in this basic solution leading to the formation of $^{99m}TcO_4^-$. To avoid this decomposition, the pH was adjusted to 7.5 immediately after the complex preparation.

Under similar electrophoretic conditions, the migration distances of both cationic complexes of ^{99m}Tc with *cis*[14]aneN_2S_2 and cyclam were the same indicating they have an identical positive charge. Indeed, the charge of +1 found for ^{99m}Tc–*cis*[14]-aneN_2S_2 (Fig. 4) is consistent with that of ^{99m}Tc–cyclam determined by Troutner *et al.* [2]. The electrophoretic pattern is the same for both ^{99m}Tc complexes which suggests that ^{99m}Tc–*cis*[14]ane-N_2S_2 is also structurally analogous to $[^{99m}TcO_2$-cyclam$]^+$ [2, 5].

Discussion

The results suggest that the technetium complex with a 14-membered N_2S_2-macrocycle belongs to the same class of $^{99}TcO_2^+$ complexes as $[TcO_2\text{-cyclam}]^+$, $[TcO_2(en)_2]^+$ and $[TcO_2(2,3,2\text{-tet})]^+$. As the UV–Vis spectrum of the complex with *cis*-[14]aneN_2S_2 shows two absorptions similar to those of complexes with TcO_2N_4 central coordination, we tentatively assign the absorption maxima at 240 nm ($\epsilon = 10\,230$) and at 320 nm ($\epsilon = 858$) to the N → Tc charge transfer transitions. Indeed, the spectra of en- and 2,3,2-tet-complexes given in the literature, show absorptions at 230 nm ($\epsilon = 8000$) and at 310 nm ($\epsilon = 540$ and 850 respectively). For the complex of cyclam the absorptions were found in the same region. Generally, the complexes containing metal–sulfur bonds have high-intensity visible absorption spectra. Though it is not the case here, the absorption at 430 nm ($\epsilon = 66$) in the ^{99}Tc complex of *cis*[14]aneN_2S_2 is temporarily attributed to the S → Tc charge transfer transitions. In the latter complex the weak absorption at 570 nm can be assigned to a d → d* transition.

The ^{99}Tc–*cis*[14]aneN_2S_2 complex exhibits electrophoretic, chromatographic and stability characteristics similar to those of $^{99}TcO_2$–tetraamine complexes. Independently of the type of ligand, the net charge of all complexes is +1. The IR absorption bands assigned to the O=Tc=O core in the complexes with en, 2,3,2-tet, cyclam and *cis*[14]-N_2S_2, are 833, 790, 790 and 800 cm^{-1} respectively.

The oxidation state of ^{99}Tc in $[TcO_2cyclam]^+$ has been reported to be +5 [2]; the complex was prepared by the reduction of $^{99}TcO_4^+$ with Sn^{2+}. The obtention of the same complex either by reduction of $^{99}TcO_4^-$ in the presence of cyclam or by a ligand exchange between $^{99}Tc(V)OBr_4^-$ and cyclam, indicates that the oxidation state +5 for technetium is conserved in the latter procedure. In the case of the *cis*[14]aneN_2S_2 complex, prepared by the ligand exchange reaction, the oxidation state of Tc should be +5 as the starting material is $(nBu)_4N[Tc(V)OBr_4]$. Thus, the above consideration and our results are consistent with the $[TcO_2([14]aneN_2S_2)]^+$ complex formula. By analogy with the hexa-coordinated dioxotechnetium–tetraamine complexes the configuration of the equatorial nitrogen and sulfur atoms should be planar with technetium in the ring and the oxygen atoms at the *trans* positions with respect to Tc. The X-ray structure analysis of this complex is under investigation. The electrophoretic, the stability characteristics and the charge of the ^{99m}Tc– and ^{99}Tc–*cis*[14]aneN_2S_2 complexes are identical; this proves the formation of $[TcO_2([14]aneN_2S_2)]^+$ with both radionuclides.

In $[^{99m}TcO_2([14]aneN_2S_2)]^+$ and $[^{99m}TcO_2cyclam]^+$ the central coordination spheres are $TcO_2N_2S_2$ and TcO_2N_4, respectively; this difference may influence the biodistribution patterns.

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