The synthesis and scintigraphic studies in rats of \(^{99m}\)Tc-amino acid complexes containing the \(\text{Tc} = \text{N}\) multiple bond are reported. Chromatographic analysis shows the formation of a multicomponent system affected by tertiary phosphines employed as reducing agents of pertechnetate-99m in the synthesis of the complexes with cysteine (CYS) and cysteine ethyl ester (CYS-OEt), while no influence of them is observed in \(^{99m}\)Tc-complexes having cysteamine (CSA) as ligand. Electrophoresis stresses a strong anionic character in all these compounds. It has not been possible to define their chemical identity by a comparison with the neutral technetium-99 nitrido complexes characterized by spectroscopic and X-ray crystallographic data due to the complexity of the \(^{99m}\)Tc-system. These complexes are stable at 80 °C over a period of 2 hours. Time-activity curves indicate a renal tissue retention only for the \(^{99m}\)Tc-(CSA) complexes.

The choice of technetium-99m in diagnostic nuclear medicine is due to its ideal photon energy (140 keV), half-life (6.02 h), and availability. In particular there is great interest in developing \(^{99m}\)Tc-radiopharmaceuticals suitable for monitoring the metabolic function of the brain and heart. The recent application in nuclear medicine of the lipophilic complex \(^{\text{[99mTcO(L)]}}\) (L=3,6,6,9-tetramethyl-4,8-diazaundecane-2,10-dione dioximate) as a cerebral blood flow imaging agent has stimulated the search for other lipophilic technetium complexes possessing a higher degree of localization in the cerebral region. The above complex as well as other neutral and lipid-soluble oxo-technetium(V) complexes with diaminodithiolates are characterized by a square-pyramidal geometry. They cross the blood-brain-barrier and it seems that the formation of a square-pyramidal structure with an apical Tc-X group (X = multiply bonded donor atom as O\(^{2-}\) or N\(^{3-}\)) is a favourable, although not sufficient, feature for obtaining \(^{99m}\)Tc-radiopharmaceuticals which may cross the blood-brain-barrier.

We reported the synthesis of a class of square-pyramidal nitrido technetium(V) complexes with bi- and tri-dentate Schiff bases derived from S-methyl dithiocarbazate \(\text{H}_2\text{N-N(R)}-\text{C(=S)}\text{SCH}_3\) (R= H, CH\(_3\)) and also the analogues oxo-technetium(V) complexes. An extension of our investigation on these reactions at the \(^{99m}\)Tc-level resulted in a new procedure for the preparation of \(^{\text{[99mTc]}}\)-radiopharmaceuticals containing the \(\text{Tc} = \text{N}\) multiple bond. Due to the great interest in the coordination chemistry of metal ions with amino acids and peptides with a view toward the understanding of the interaction of metal ions with proteins, antibodies, and biocatalytic processes, we recently described the synthesis and characterization of technetium-99 nitrido complexes with cysteine (CYS), cysteine ethyl ester (CYS-OEt) and cysteamine (CSA). These complexes are neutral and possess a square-pyramidal geometry.
with the TeN group in the apical position. A preliminary study of the formation of the corresponding $^{99m}$TeN compounds was also reported.

Here, we describe the detailed synthesis of some technetium-99m complexes, a comparison with those previously obtained at Tc-99 level and scintigraphic studies in rats.

**Experimental**

**Materials and Methods:** Reagents and solvents were used as received without further purification. Technetium-99, as [NH$_4$][TcO$_4$], was purchased from DuPont/Nen Products.

Technetium-99m, as Na[TcO$_4$], was obtained from a $^{99}$Mo/$^{99m}$Tc isotopic generator provided from Sorin Biomedical mod RF Dry-Gem. The nitrido nitrogen donor species H$_2$N-N(CH$_3$)-C(=S)-SCH$_3$ was prepared by literature method. Tertiary phosphines PR$_3$ (R=C$_6$I$_4$H$_5$ and CH$_2$CH$_2$CN) were obtained from Aldrich Chemical, and sodium tris-(m-sulfophenyl) phosphine Na[P(m-C$_6$I$_4$SO$_3$)$_3$] (TPPS) was obtained as a gift from the Laboratory of Dr. Dartiguenave, CNRS, Toulouse, France.

The amino acids cysteine (CYS), cysteine ethyl ester (CYS-OEt) and cysteamine (CSA) were Fluka Chemika reagents.

Technetium-99 nitrido complexes were synthesized as previous reported (vide infra). In order to characterize the compounds obtained at no carrier-added level ($^{99m}$Tc) with those prepared at carrier-added level ($^{99m}$Tc and $^{99}$Tc), a comparison of their chromatographic behaviour was performed. Yields and radiochemical purity, in terms of free $[^{99m}$TeO$_4$]$^-$ were determined by thin-layer chromatography (TLC) using non polar reverse phase Merck RP 18 F 254S and silica gel plates developed with gradient mixture of MeOH/H$_2$O ranged from 100% to 20% v/v in MeOH and with EtOH:CHCl$_3$:C$_6$H$_6$ in the ratio 1:5:2:1.5 v/v respectively.

After development of the chromatograms, the plates were dried and placed in contact with X-ray film (3M CRT 7) over night. A dark spot appeared on the film after development of the negative and it gave the location of the radioactivity. Labelling yield, stability, and charge of the final solutions were also determined by electrophoresis in a pH ranged from 2.0 to 8.5 using Whatman 3MM paper at 150-200 V for 60-120 min. A spot of $[^{99m}$TeO$_4$]$^-$ was applied on the same paper. It was dried and the direction which the radiopharmaceuticals migrated were determined by autoradiography. Chromatographic plates and electrophoresis paper were cut into 1.0 cm strips and counted with a gamma-counter (ACN mod. RAD-γ II).

**Preparation of $[^{99m}$TeN]$^-$-complexes:** Technetium-99m nitrido complexes were prepared through a two step reaction.

**STEP 1. Preparation of $[^{99m}$TeN]$^-$-intermediate:** Solid PR$_3$ (3 mg), S-methyl, N-methyl dithiocarbamate (nitrido-nitrogen donating agent) H$_2$N-N(CH$_3$)-C(=S)-SCH$_3$ (1.0 mg), 0.1 mL of aqueous HCl (1.0 mol dm$^{-3}$), 1 mL of physiological saline solution, and EtOH (0.1 mL if PPh$_3$ or P(CH$_2$CH$_2$CN)$_3$ were used) were mixed in a reaction vial and 0.5-1.0 mL of generator eluted $[^{99m}$TeO$_4$]$^-$ was added. The resulting mixture was heated at 100 °C for 15 min or at 80 °C for 30 min. The chromatographic analysis showed a tailed distribution of radioactivity but the presence of no unreacted pertechnetate.

**STEP 2. Preparation of $[^{99m}$TeN]$^-$-complexes:** The pH of the solution prepared as described in step 1 was raised to ca. 8.5 by adding 0.5 mL of a HCO$_3$^-/CO$_3^{2-}$ buffer (0.5 mol dm$^{-3}$), 0.5 mL of a water solution containing 10 mg mL$^{-1}$ of cysteine, cysteine ethyl ester, and cysteamine respectively was added, and the temperature was kept at 70 °C for 20 min.

**Scintigraphic Studies:** Scintigraphic studies were performed in Sprague Dawlay female rats (250-300 g weight). Anesthetized animals (i.p. xalasyle/ketamine mixture 1:3; 1.2 mL kg$^{-1}$) were injected in the jugular vein with a solution of the $[^{99m}$TeN]-amino acid complex (0.2 mL,