Synthesis And Characterization Of N-substituted Bis(2-mercaptoethyl)amino Ligands And Their Technetium Complexes.

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Lehigh University PH.D. 1982

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SYNTHESIS AND CHARACTERIZATION OF N-SUBSTITUTED BIS(2-MERCAPTOETHYL)AMINO LIGANDS AND THEIR TECHNETIUM COMPLEXES

by

Hayden T. Ravert

A Dissertation

Presented to the Graduate Committee

of Lehigh University

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CERTIFICATION OF PRESENTATION

This dissertation is respectfully submitted to the Graduate Faculty of Lehigh University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Hayden T. Ravert

A CERTIFICATE OF APPROVAL

Approved and recommended for acceptance as a dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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The author wishes to dedicate this thesis to his wife, Kathy and daughter, Heather for their patience and love... thank you.

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ABSTRACT

A present need in the field of technetium radiopharmacy is the isolation of a neutral mono-chelated technetium complex (one ligand to one technetium). If such a moiety can be attached to a receptor specific compound without hindering the specificity of the compound, a new class of "site specific" imaging agents may be formulated.

Recently a series of bidentate N-substituted 2-mercaptoethylamines were synthesized and complexed with technetium-99m and -99. The resulting complexes were found to be neutral. Their lipophilicity was dependent on the size and structure of group on the amine nitrogen. Also these complexes were discovered to contain two ligands per technetium.

To augment the search for neutral technetium radiopharmaceuticals, a series of N-substituted bis(2-mercaptoethyl)amines were synthesized and characterized along with the subsequent technetium-99m and -99 complexes. Analysis of a variety of synthetic pathways to the N-substituted bis(2-mercaptoethyl)amine ligands indicated that the isolation of pure ligand was impossible. Therefore a pure precusor- the N-substituted bis(2-isothioureylethyl)amine trihydrochloride salt- was obtained. After isolation, the isothiouronium salt was hydrolyzed and complexed with technetium-99m and -99. Each method developed for complexation proved to facile and reproducible.

Characterization of the technetium-99m complexes by electrophoresis and ion exchange methods indicated the complexes were neutral. The lipophilicity of the complexes was dependent on the length and type of substituent on the amine. The complex was found to be stable to ligand exchange. A mixed ligand procedure could not establish a simple

stoichiometry for the complex. Further analysis with technetium-99 complexes indicated a bridged technetium-oxo structure of the stoichiometry- $Tc_2O_2L_3$ (L = the N-substituted bis(2-mercaptoethyl)amine ligand) was present.

INTRODUCTION

Background

Although many milestones have occurred in nuclear medicine since Blumgart, Yeno and Weiss first injected radium into a human in 1926 (1), the most significant contribution to the field of imaging was the development of the cyclotron by E.O. Lawrence in 1934. The cyclotron while producing many artificial radioisotopes (2) also yielded the first clinically useful imaging radiopharmaceutical-iodine-131-applied with success in nuclear medicine today.

With the use of artificial radionuclides in medicine, the development of the tracer concept evolved. In essence the tracer concept is defined as "the ability to follow individual, labelled molecules as they move about the body" (3). Consequently for the tracer method to work the need arises for a radioisotope with qualities that enable it to be followed through the body. With good decay and detection characteristics many radiopharmaceuticals may be characterized as to their time course and localization in the body. What are the optimal imaging properties? Generally the radionuclide must emit a non-invasive particle or ray and must have a short half-life. Only a few nuclides are commercially available for imaging with these properties (4).

Unfortunately a large variety of diagnostic agents exist that are nonselective to disease states. The agents appear to detect only the "nonuniformities of diffusion" caused by a disease (5). Attempts to create selective uptake by radiolabelling drugs or natural substances known to "collect" in a disease state have been dismally unsuccessful. In many cases the main course of such radiolabelled substances is chemical decomposition and/or rapid excretion with no similarity to the

biodistribution of the nonlabelled substance observed. The reason for the change in biodistribution of the labelled substance is the drastic physical and chemical alteration of the biomolecule when the radiolabel is introduced. In most cases the desired radiolabel is a chelated metal ion radionuclide. For example, 1-aminocyclopentanecarboxylic acid is known to have pancreatic uptake as well as cytotoxic behavior in pancreatic tumors. A study of the technetium-99m-1-aminocyclopentanecarboxylic acid complex demonstrated that the complex was quickly collected and excreted by the kidney without any localization in the pancreas (6). Presumably the technetium complex is a charged species with no physical or chemical similarities to the original amino-carboxylic acid.

By contrast a few excellent imaging agents have been discovered by insightful or fortuitous modification of a biomolecule. One such example is the myocardial imaging agent γ-iodohaxadecenoic acid(7): cis-$ICH_2(CH_2)_6CH{=}CH(CH_2)_7CO_2H$. Fatty acids such as oleic acid are known to accumulate in the heart after intravenous injection. However a number of studies of radioiodinated natural fatty acids found them to be actively removed from the bloodstream as foreign substances. Conversely the unnatural iodinated hexadecenoic acid was found to have excellent myocardial uptake. Retrospectively the phenomenon of uptake of the unnatural fatty acid above may be explained. A comparison of a model of the iodohexadecenoic acid indicates it is a bioisoteric analog of oleic acid. Obviously a bioisostere of oleic acid should have similar selective uptake in the myocardium. With tracer biomolecules such as the one above, the future for the snythesis of radiolabelled analogs of biomolecules which have selective uptake or are receptor specific is positive.

Such logical approaches to the synthesis of imaging agents began with the development of structure-activity relationships of non-labelled drugs by Corwin Hansch. Hansch grasped the full meaning of Overton (8) and Meyers (9) efforts to study the relation between oil/water partition coefficients and the narcotic effect of certain drugs in 1899. This discovery of a relationship between the lipophilicity of compounds (the partition coefficient) and the biological effect produced was the first quantitative relation between structure and activity of molecules (10). Upon further edification through more experimental results, the relation between lipophilicity and activity was found to be parabolic in nature (10). The mathematical relation between the biological activity (log 1/C) and the partition coefficient (log P);

$$\log (1/C) = k_1(\log P^2) + k_2(\log P) + k_3;$$

indicates that some optimal lipophilic character must be attained for the drug to perform at its highest level (11). Since the compounds studied were nonspecific, the biological effect produced will likely depend on how the compound partitions through the organism. In these cases the effectiveness of a drug appears to depend entirely on its lipid to water interactions. Obviously other factors are involved in the compounds behavior besides lipophilicity. Steric, electronic and substituent effects along with active transport must be considered since they will affect the drugs final effectiveness at the site of action.

Since the primary objective of nuclear medicine is to make agents that will localize in specific tissues for accurate imaging, the use of structure-activity relations for labelled compounds is imperative. The use of trial and error methods of labelling drugs and natural substances will lead only to fortuitously obtained agents. However if isotopes may

be placed on known receptor specific or other agents in such a way as not to interfere with the function of the substance, excellent imaging agents may be obtained.

Technetium Radiopharmaceutical Design

A review of technetium-99m pharmaceutical chemistry reveals a number of agents used in nuclear medicine. The majority of these radiopharmaceuticals appear colloidal in nature.

Very few imaging agents have known receptor specificity and no technetium-99m [$E\gamma$=140 KeV, $t_{1/2}$ = 6.02 hr] labelled agents have any specificity or good partitioning qualities. Invariably "candidate" technetium moieties were observed to be excreted rapidly from test animals. However there are two technetium moieties known to have active transport in humans (13). The first, pertechnetate, is transported to the thyroid (14). The second, technetium HIDA, [HIDA= N-(2,6-dimethylphenylcarbamoylmethyliminodiacetate], has active uptake by hepatocytes (15). A primary reason for the lack of useful technetium-99m labelled radiopharmaceuticals is the poorly understood chemistry at carrier-free levels. Similar problems exist with the chemistry of isotopically stable technetium-99 [$E\beta$ = 292 KeV, $t_{1/2}$ = 2.1 x 10^5 yrs.]. By developing the chemistry on the "macroscale" of technetium-99, application to carrier-free levels of technetium-99m may ensue. However caution must be exercised when extrapolating properties to carrier-free levels. With the paucity of information that exists on both the technetium-99 and -99m levels, no real correlations have been made (16, 17). Equal precaution must be taken when attempting to extrapolate similarities between metals in the same group or neighboring groups to technetium. Examples of the impropriety of extrapolation exist (18,19). In general

enormous strides are being made on both the carrier-free and carrier-added levels. New complexes are being synthesized and standard radiopharmaceuticals reevaluated (20,21).

Established and new methods of production of known and candidate technetium-99m radiopharmaceuticals are constantly under scrutiny for complex reproducibility, purity and stability. Since spectroscopic measurements are impossible at the carrier free level (22), other physical methods such as electrophoresis and high pressure liquid chromatography (HPLC) have proven useful in delineating properties of the species.

Electrophoresis has shown utility for the determination of the charge on a species by measurement of the migration of the species in an electric field (23,24). Electrophoresis can not determine the exact charge on a complex but rather the charge to Stokes radius (25). However electrophoresis does aid in the determination of stoichiometry of a complex (if the complex is charged) by the correct use of the mixed ligand experiment. For example Burns, et al (23) using equivalent quantities of dimercaptopropane and dihydrothiotic acid complexed the ligands by reducing pertechnetate in their presence. The reaction mixture was electrophoretically separated at a pH>5. The negatively charged species showed significant differences in the migration with the complex containing the carboxyl anions moving the furtherest (toward the anode). In fact the reaction mixture resolved into three separate peaks with peak ratios of 1:2:1. Such results are indicative of a bis complex (2 ligands to 1 technetium).

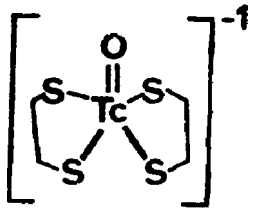
High pressure liquid chromatography besides being a quick, efficient and reliable for normal quality control in hospital radiopharmacies

is a necessity for basic research into reaction pathways and characterization of candidate radiopharmaceuticals (15,26,27,28). For instance Fritzberg and Lewis found, by use of LC, multiple peaks in standard commercial Tc-99m HIDA units (29). The peak sizes varied with pH, amount of reducing agent added and time. Collection of the various peaks and evaluation in mice indicated all species observed had the same distribution implying all the observed products were inherently the same.

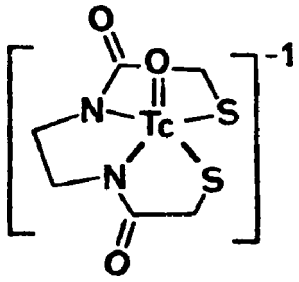
With the extreme interest in formulating technetium-99m radiopharmaceuticals there is imperative need to increase the knowledge of technetium chemistry. Since such knowledge can not be complete at the carrier-free level, work at the more macroscopic technetium-99 level would not only be practical but productive. New technetium-99 complexes appear regularly in journals. These complexes have been analyzed more completely by usual physical methods such as X-ray crystallography (30, 31,32), uv-visible spectrometry (33), infra-red spectrometry (34,35), proton and carbon-13 NMR (36), electrochemistry (37), and mass spectrometry (38,39) as well as methods used on the technetium-99m level-electrophoresis (33) and HPLC (15,40,41).

A number of review articles have been published on the chemistry of technetium-99 (18,19,21,42,43); and, literature searches for potential radiopharmaceuticals uncovered a number of interesting complexes ranging from porphyrins (44,45) to EDTA (46) species. However most recent publications contain reactions of technetium with sulfur and nitrogen type ligands. Perhaps one reason for the emphasis on such ligands is the fact that pertechnetate reduced in the presence of these ligands produces stable isolable products. A number of such complexes

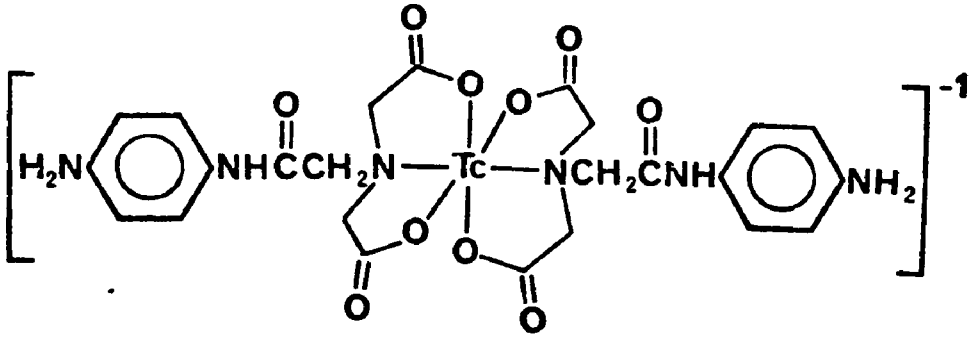
have been isolated and characterized (47-52). The chelating ligands vary from simple bidentates (ethanedithiol and ethylenediamine) to tetradentates - cyclam (1,4,8,11-tetraazacyclotetradecane) and ema [N,N'-ethylenebis(2-mercaptoacetamide)]. Four typical structures are drawn below. All the bidentate were found to form bis complexes with the technetium. The general formula is $[L_2TcO]^{1-}$ (where L = ligand) for the sulfur analogs; and, $[L_2TcO_2]^{1+}$ for the amine moieties. The



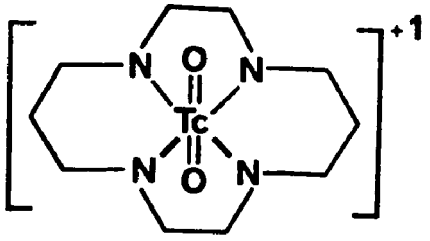
Ethanedithiol complex [32]



Tc-ema complex 39



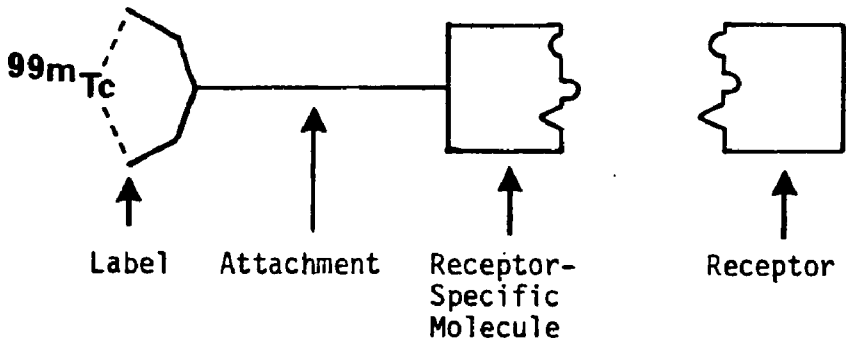
Tc-HIDA complex [47]



Tc-cyclam complex [51]

tetradentate ligands form a mono chelate with the formula $[LTcO_2]^{1+}$ and $[LTcO]^{1-}$ for the cyclam and ema derivatives respectively.

Except for the N-substituted aminoethanethiol ligands all the sulfur and nitrogen moieties form charged technetium complexes. The neutral bis chelate of the aminoethanethiols is a pharmacologically important event. A neutral species will enhance the probability of the complex moving to a site of action in the human system rather than excretion as a charged foreign substance. A stylized example of a hypothetical receptor-specific radiopharmaceutical is drawn below:



While the formation of a neutral complex is an important milestone, to be usable the complex must be inherently small and easily attached to carrier (receptor specific) molecules. Therefore the creation of a technetium complex which is neutral and forms a mono chelate that can be attached (functionalized) at only one position is a more attractive alternative.

OBJECTIVE

Due to its nuclear properties technetium-99m in radiopharmaceuticals will yield excellent imaging agents if compounds can be formulated that will localize as receptor, tumor or organ specific markers. A priority of creating good agents is to form a small, stable, neutral technetium chelate which can be chemically added to (or is a) receptor specific compound(s). In order to add to the present knowledge of technetium chemistry and radiopharmacy, the objective of this research is to synthesize N-substituted bis(2-mercaptoethyl)amines and their technetium-99m and -99 complexes. To characterize the complexes experiments are performed to: 1) describe the charge; 2) analyze the stability; 3) determine the effect of N-alkyl or aryl substituents; 4) determine the stoichiometry; and 5) explain the structure.

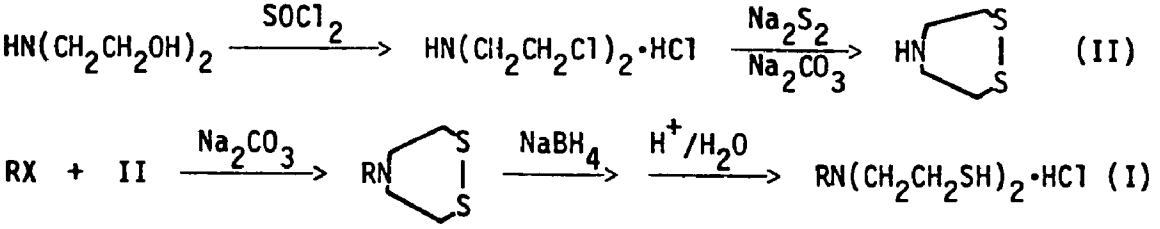
RESULTS AND DISCUSSION

I. BIS-MERCAPTOETHYLAMINE LIGANDS

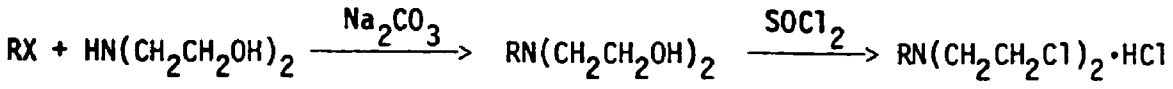
A. Bis-mercaptoethylamine precusors

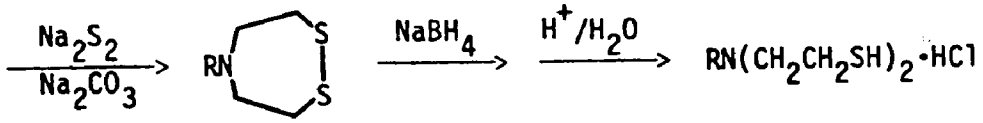
Since the bis-mercaptoethylamine compounds (I) were reported in the literature, a variety of synthetic schemes was devised to create these ligands. Two simple methods (scheme 1 and 2) detailed below were initially proposed. Scheme 1 involved the synthesis and reaction of 1,2-dithia-5-azapane (dithiazapane-II) with alkyl and aryl halides. Scheme 2 utilized the direct synthesis of the disulfide from the N-substituted nitrogen mustards.

In the first scheme dithiazapane was synthesized according to the procedure of Gunther and Mautner (53). HPLC and TLC analyses indicated only one product was isolated. The melting point agreed with that reported. However, several consistent combustion analyses at variance with the expected values indicated the desired product had probably not been obtained. Therefore, the synthesis was discontinued.



Scheme 1: Initial bis-mercaptoethylamino synthesis. R = benzyl, p-chlorobenzyl, p-cyanobenzyl, and p-nitrobenzyl (Ia-d respectively).

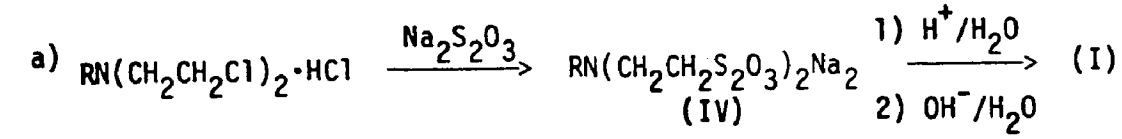




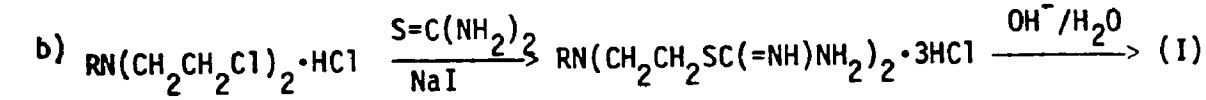
Scheme 2: Second bis-mercaptoethylamino synthesis. R = Ia-d.

In scheme 2 the disulfide was formed as in scheme 1 except the N-substituted nitrogen mustard was formed first. Analysis by TLC and HPLC of the disulfide indicated that impurities existed in the product. Figure 1 shows a typical elution profile (the cyano derivative) for a reaction mixture. For several of the dithiazapane derivatives the major peak was collected, the physical properties noted, and a sample sent for analysis. In all cases the analysis indicated the major peak in the HPLC elution was not the disulfide. In fact, no evidence for the presence of any of the desired product was obtained.

Since suspicion may be cast on the stability of the free base or hydrochloride salt of dithiazapane, two different synthetic routes (schemes 3 a and b) were devised in an attempt to create stable precusors to the dithiol ligands. Scheme 3a involved the direct reaction of sodium thiosulfate with a nitrogen mustard to yield a bis-Bunte salt (IV). After reflux and fractional recrystallization, the isolated



Scheme 3: a) Bunte salt route to the bismercaptoethylamines.
b) Final synthetic route to the bismercaptoethylamines.



product was discovered to be very impure by HPLC analysis. Liquid chromatograms displayed at least 6 major components with the peak

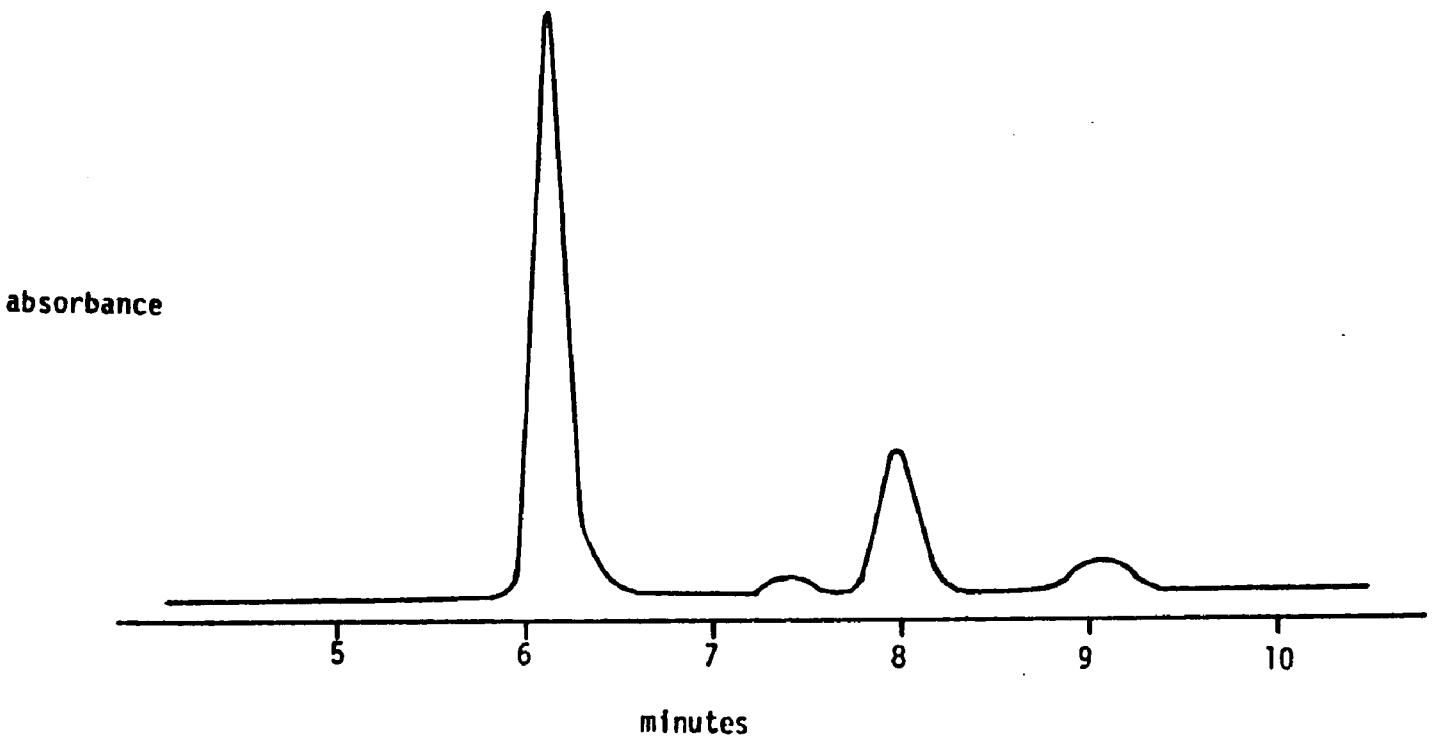


Figure 1: Elution Profile of crude p-cyanobenzyldithiazapane in 90% methanol/10% 0.01 N ammonium acetate.

nearest the void volume constituting unreacted thiosulfate. The chromatographic analysis of the unsubstituted bis-Bunte salt [$HN(CH_2CH_2SSO_3)_2Na_2$] is reproduced in Figure 2. Other authors have noted similar purification problems with amino-type Bunte salts including principally the formation of thiosulfuric acids (54). In brief no good published routes to the bismercaptoethylamines exist.

However scheme 3b, the reaction of thiourea with the nitrogen mustard, by comparison to the other methods above gave excellent yields of crystalline, easily purified isothiouronium salts. These salts were easily hydrolyzed to the desired aminomercaptan ligands. Typical liquid chromatograms for the benzyl and p-chlorobenzyl isothiouronium salts illustrated in Figure 3. Also, Table I lists the retention time, t_r, and the capacity factor, k', [$k'=(t_r - t_o)/t_o$ where t_o is the void volume time] for the isothiouronium salts and the nitrogen mustard precusors. Note the k' for the isothiouronium salt and starting material are different and distinguishable. The observation of one peak in the chromatograms of the isothiouronium salts under a variety of instrumental conditions; and the excellent elemental analysis confirm the isolation of pure product. Because of the exceptional purity of the isothiouronium salts, isolation of the bis-mercaptoethylamino ligand was attempted from the base hydrolysis of these salts.

B. Isothiouronium hydrolysis product

The crystalline isothiouronium salts appeared to be stable to long storage (greater than 12 months). However, the hydrolysis product of these salts (namely the bis-mercaptoethylamines), although easy to prepare in situ, proved difficult to isolate and characterize. All hydrolysis reactions were performed by simple addition of excess base

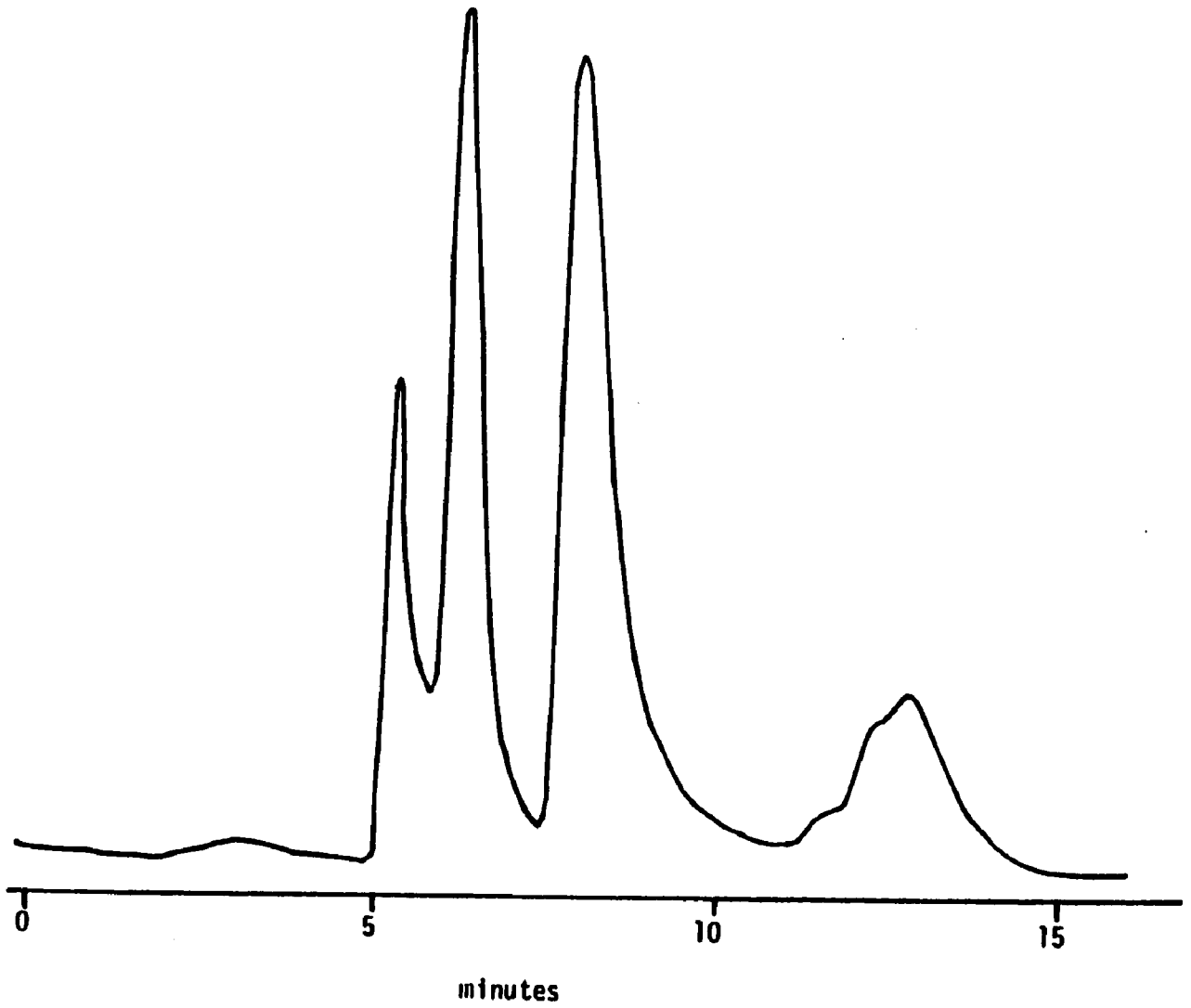


Figure 2: Elution profile of the crude unsubstituted bis-Bunte salt $[HN(CH_2CH_2SSO_3)_2Na_2]$ with 90% methanol/10% 0.01 N ammonium acetate.

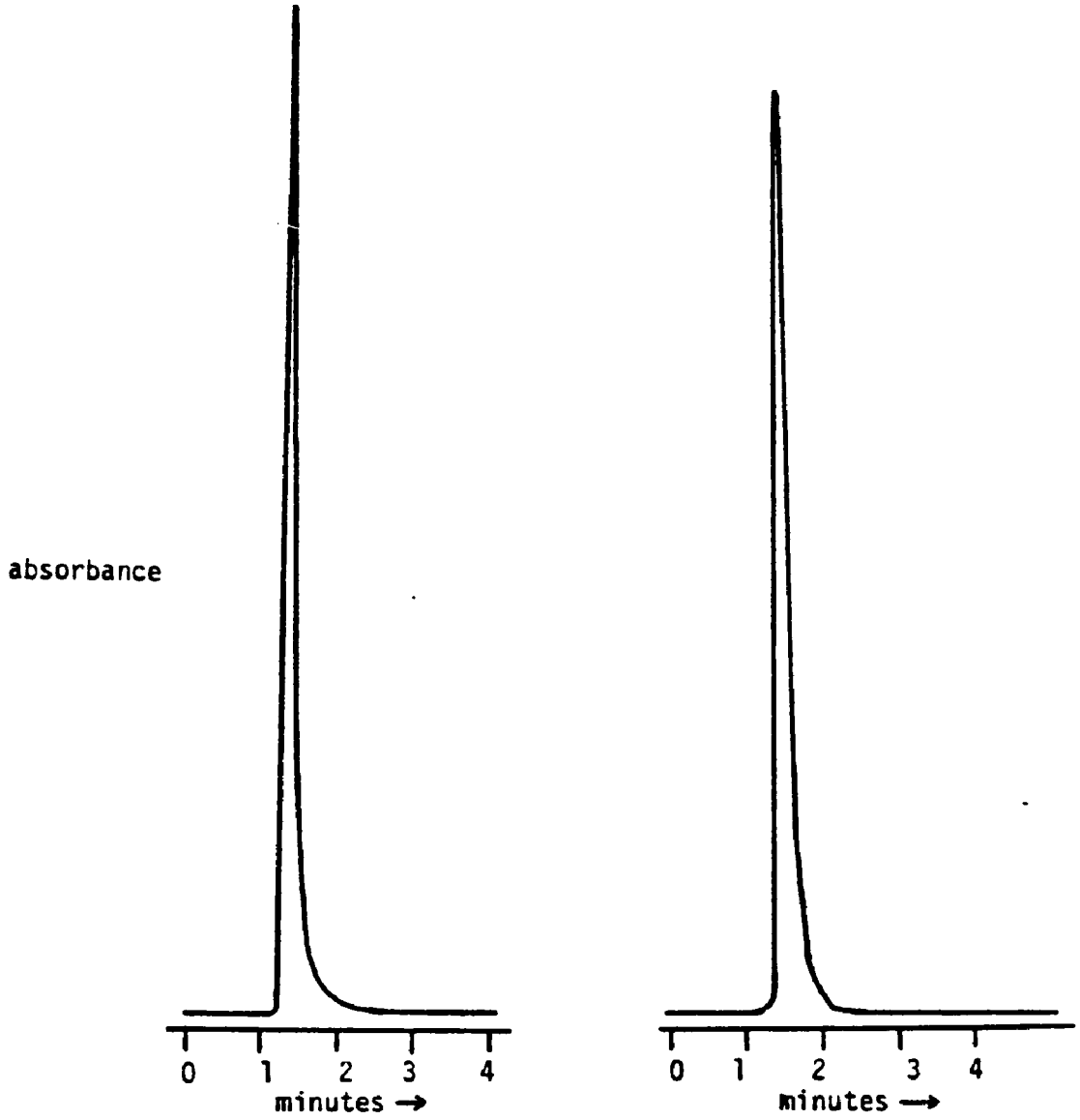


Figure 3: a) Elution profile of N-benzyl-N,N-bis(isothioureylethyl)amine.
b) Elution profile of N-p-chlorobenzyl-N,N-bis(isothioureylethyl)amine.

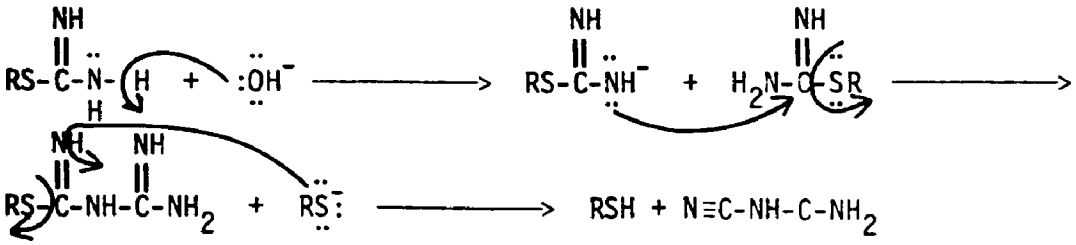
Table 1. The t_r and k' obtained for the isothiouronium salts, nitrogen mustards and thiourea

Compound[a]	t_r (minutes)	k'
Thiourea	1.70	1.13
$C_6H_5CH_2N(CH_2CH_2Cl)_2 \cdot HCl$	3.75	3.69
$p\text{-}ClC_6H_5CH_2(CH_2CH_2Cl)_2 \cdot HCl$	2.55	2.19
$CH_3(CH_2)_3N(CH_2CH_2Cl)_2 \cdot HCl$	1.95	1.44
$CH_3(CH_2)_{11}N(CH_2CH_2Cl)_2 \cdot HCl$	3.65	5.36
$1\text{-naphthyl-}CH_2N(CH_2CH_2Cl)_2 \cdot HCl$	2.90	1.63
$C_6H_5N[CH_2CH_2SC(=NH)NH_2]_2 \cdot 3HCl$	1.35	0.69
$p\text{-}ClC_6H_5CH_2N[CH_2CH_2SC(=NH)NH_2]_2 \cdot 3HCl$	1.40	0.75
$CH_3CH_2N[CH_2CH_2SC(=NH)NH_2]_2 \cdot 3HCl$	1.20	0.50
$CH_3(CH_2)_3N[CH_2CH_2SC(=NH)NH_2]_2 \cdot 3HCl$	1.60	1.00
$CH_3(CH_2)_{11}N[CH_2CH_2SC(=NH)NH_2]_2 \cdot 3HCl$	1.70	1.13
$1\text{-naphthyl-}CH_2N[CH_2CH_2SC(=NH)NH_2]_2 \cdot 3HCl$	1.60	1.00

a) All compounds were chromatogrphed using a Waters RCM-C18 column with a 90% methanol/10% 0.01 N ammonium acetate mobile phase at 2 ml/min; chart speed-1 cm/min; 254 nm.

to a solution of the isothiouronium salt (5:1 molar ratio). After reaction the solution was extracted with chloroform, and the thiol isolated from the organic layer. Chromatographic analysis of the isolated oils (Figure 4 the benzyl derivative) indicated a large number of products. Even the NMR spectra was very complex.

Major contaminents in the organic solution are hydrolysis by-products. The hydrolysis mechanism for isothiouronium salts might be a simple amide-like cleavage yielding ammonia and carbon dioxide. However, Reid (55) has indicated that the isothiouronium hydrolysis follows the mechanism outlined in scheme 4. This proposed mechanism involves an initial hydrogen ion abstraction with the eventual formation of the thiol and cyanoguanidine (dicyandiamide):



Scheme 4: Mechanism of hydrolysis of isothiouronium salts.

Since cyanoguanidine is not very soluble in chloroform, it does not appear to be a major contaminent in the extractable substances. LC analysis of commercially available cyanoguanidine indicates two major components (Figure 5). The two major peaks correlate with the first two peaks in Figure 4. However, these two minor assignments do not greatly clarify the chromatograms, for the other peaks can not be assigned to any other known contaminents (i.e. solvent, base).

What then may be the problem in obtaining pure isolable bis-mercaptoethylamines? Mason reported alkaline-induced polymerization of

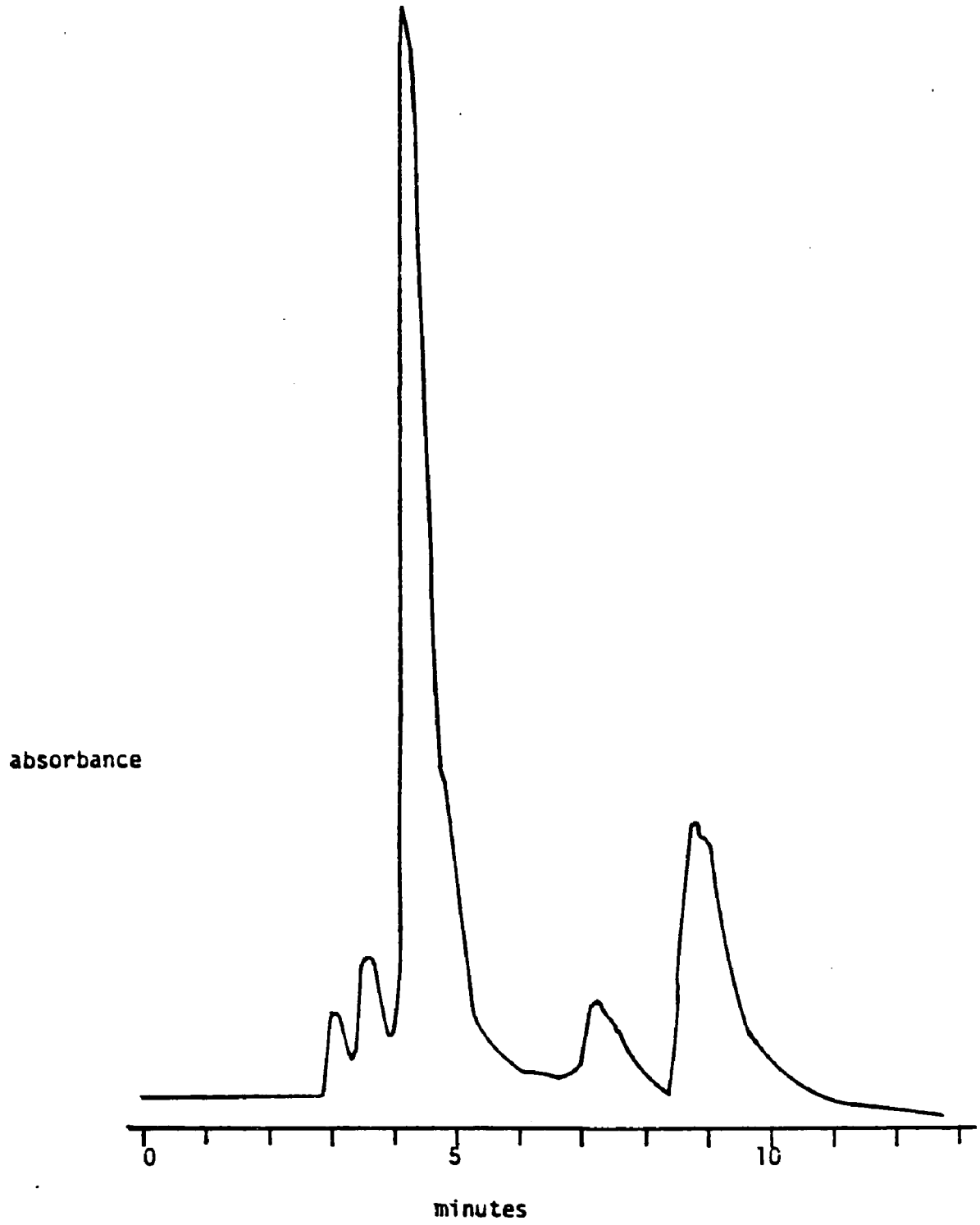


Figure 4: Elution profile of the hydrolysis mixture of $C_6H_5CH_2N(CH_2CH_2SH)_2$ with 90% methanol/10% 0.01 N ammonium acetate.

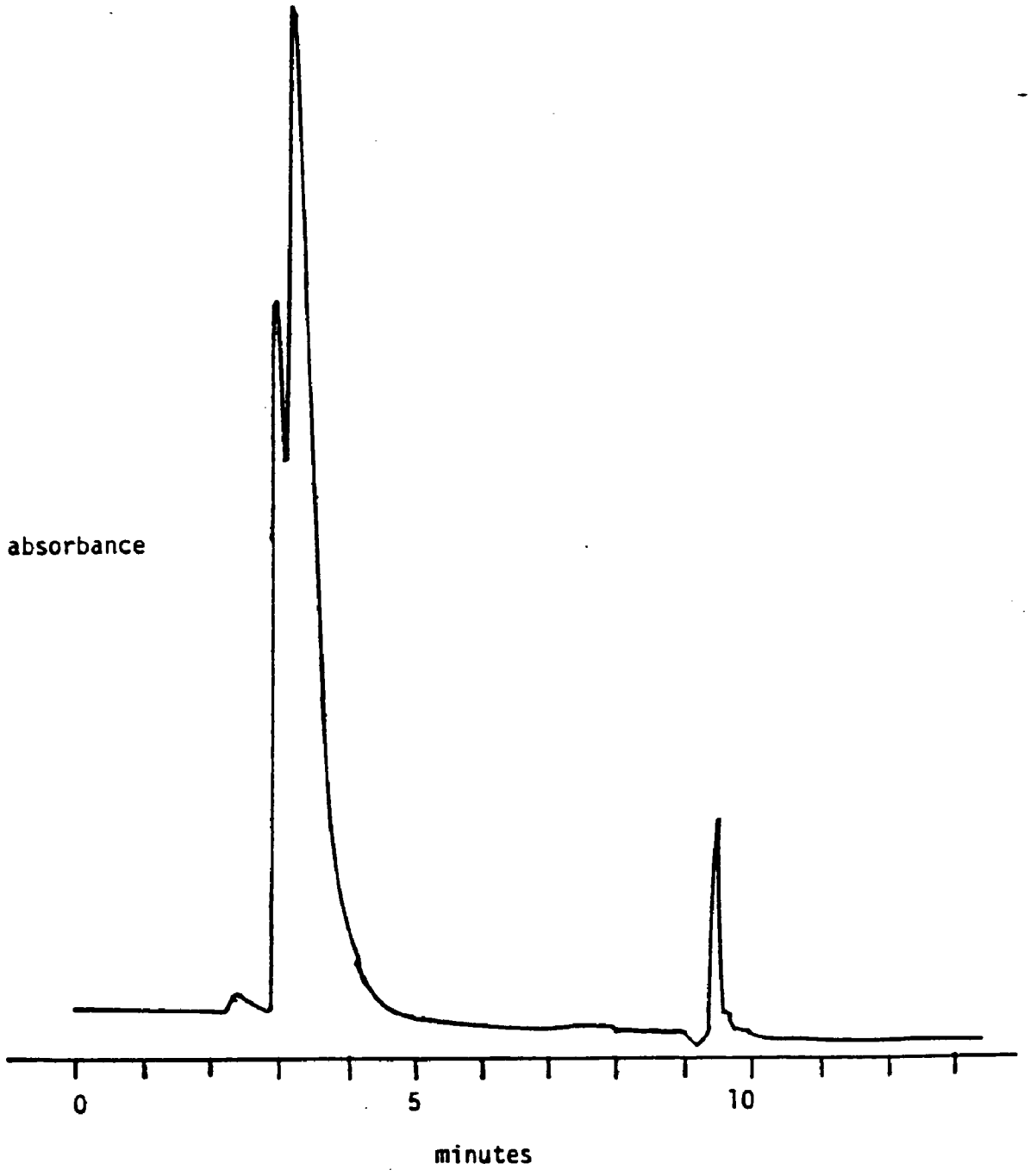


Figure 5: Elution profile of commercially available dicyandiamide (90% methanol/10% 0.01 N ammonium acetate).

similar mercaptoamines when exposed to air (56). In addition Wilson found that on standing in air in a caustic solution many bis-mercaptans yielded cyclic disulfides (57). In general only low yields were reported for the actual isolated cyclic disulfides obtained from bis-mercaptans (58,59), leading to the conclusion that polymerization may be more likely than disulfide formation (realizing that both reactions depend markedly on steric and entropic factors). In the bis-mercaptoethylamines of this study the liability seems more related to polymer formation than to cyclization. The initially isolated mercaptoamines were mobile colorless oils, highly soluble in chloroform and acetone. On standing these oils darkened, became viscous and lost solubility in chloroform, dimethyl sulfoxide and trifluoroacetic acid. These results are supported by the strong literature precedent for the linear polymerization of related mercaptoamines (60). Efforts to isolate and identify these presumed purified cyclic structures have been inconclusive (53,58,59). Rigorous exclusion of air did allow for a minor increase in stability. Infrared and NMR Spectra clearly demonstrate the presence of the thiol moiety (i.e. benzyl bismercaptoethylamine-ir: -SH stretch at 2545 cm^{-1} and NMR: see Figure 6). A second possible problem may arise from the incomplete hydrolysis of the isothiouronium salt. However, comparisons of LC tracings and NMR's of the isothiouronium salts with the dithiols suggest no residual isothiouronium amine hydrogens present.

Since the isolation of the dithiol from the crude mixture was difficult, derivatives were synthesized to characterize the labile ligands. The first derivatization attempted involved the reaction of benzoyl chloride with the dithiols:

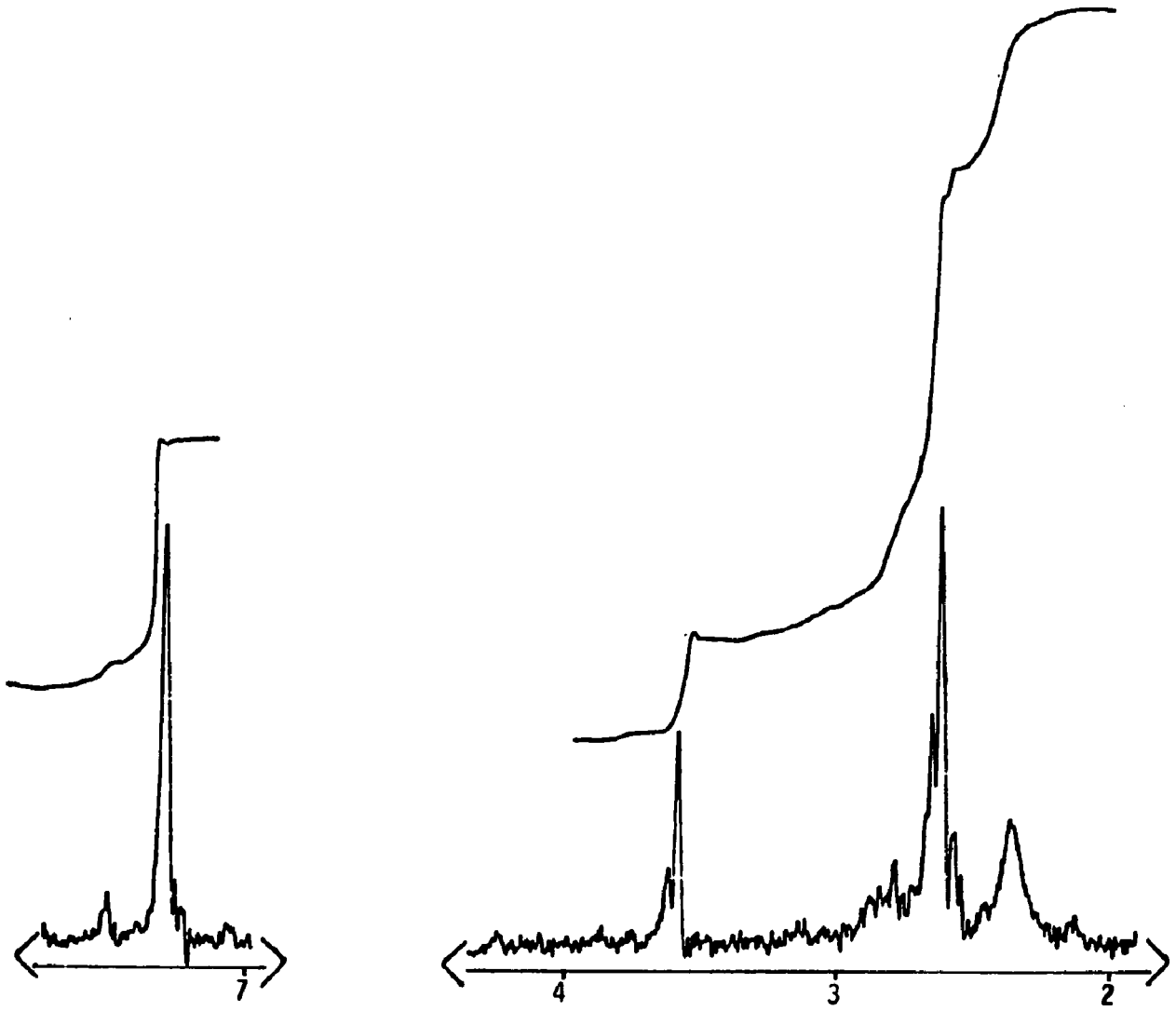


Figure 6: NMR of $C_6H_5CH_2N(CH_2CH_2SH)_2 \cdot HCl$.

$$RN(CH_2CH_2SH)_2 + 2\ C_6H_5COCl \xrightarrow{OH^-} RN(CH_2CH_2SCOC_6H_5)$$

After reaction an oil was isolated which was vacuum distilled to a clear liquid. However, the liquid darkened quickly. LC analysis indicated a number of substances were contained in the distillate. Combustion analysis was not in accord with the bis-thioester and NMR did not confirm the expected structure.

Second and third derivative attempts with heavy metal ions (Hg^{2+} and Pb^{2+}) were undertaken:

$$RN(CH_2CH_2SH)_2 + MX_2 \longrightarrow RN(CH_2CH_2S)_2M + 2\ HX$$

(assumed stoichiometry)

Following the procedure of Herbrandson and Wood (58), the mercuric mercaptide was prepared from the crude benzyl and dodecyl derivatives. Both mercaptides formed white percipitates from the aqueous solutions. Also both easily decomposed upon heating in the organic solvent used for recrystallization. Elemental analysis of the non-recrystallized solids did not approach theoretical values. The third derivative, a lead mercaptide, was synthesized by a procedure described by Reid (61). Addition of lead(II)acetate to the bis-mercaptoethylamines solution yielded a yellow precipitate. Upon isolation of the solid, rapid decomposition to a green vile-smelling solid occurred.

The conclusion of the above studies was that isolation, purification and characterization of discrete bis-mercaptoamines was not to be an easy task. Therefore, the preparation and analysis of the actual complexes of technetium-99m became the perferable objective.

II. TECHNETIUM-99m CHEMISTRY

A. Introduction

Initial efforts to determine the properties of the hydrolyzed ligands when complexed with a reduced species of technetium were developed with technetium-99m. The chelated species were subjected to a series of experiments to determine the: a) optimal reducing agent, b) charge on the complex, c) lipophilicity, d) chromatographic characteristics, e) relative stability, and f) ligand to technetium stoichiometry.

B. Synthesis

Introduction

The general reaction procedure in scheme 5 indicates the simplistic nature of the synthetic route pursued. The reaction mixtures are kept under nitrogen during the approximately three hour reaction sequence. The rigorous exclusion of oxygen minimizes polymer and disulfide formation of the ligand and possible reoxidation of the lower oxidation state(s) of technetium.

$$RN[CH_2CH_2SC(=NH)NH_2]_2 \cdot 3HCl \xrightarrow{NaOH} RN[CH_2CH_2SH]_2 \xrightarrow[[H]]{^{99m}TcO_4^-} {}^{99m}Tc\text{-Complex}$$

Scheme 5: General Synthetic Procedure for the Formation of ^{99m}Tc Complexes.

Reducing Agents

Three reducing agents, stannous chloride ($SnCl_2$), sodium borohydride ($NaBH_4$), and sodium dithionite ($Na_2S_2O_4$) were employed in the reduction of sodium 99m-pertechnetate ($NaTcO_4$) to a lower oxidation state for complex formation. Initially $SnCl_2$ reductions were performed. However, in all cases large quantities of solid formed

upon addition of the stannous solution to the basic medium. Since the technetium-99m is in very low quantity, the solid which forms is in all probability tin (II) insolubles. Experimentally little radioactivity was found in the isolated solids compared to the filtrate. Liquid chromatographic and thin layer chromatographic (TLC) analyses of the supernatant indicate large quantities of pertechnetate with little or no other substances eluted. These analyses verify that no reduction is taking place possibly due to precipitation of the tin salts.

In contrast $NaBH_4$ worked effectively as a reducing agent. Although solids formed in the reaction vial after a few hours, they readily dissolve upon the addition of water. Radioanalysis of these solids indicated insignificant activity. Also LC and TLC confirmed the complete reduction of the pertechnetate, the formation of a complex, and little indication of non-elutable substances. Finally, care must be taken in adding the correct amount of $NaBH_4$ to the reaction solution. Too little will not induce complete reduction of the pertechnetate and create no measureable complexed product. However, too much reductant, while reducing the pertechnetate, evolves a significant quantity of hydrogen gas. Although the hydrogen does not interfere with the TLC, a significant quantity is produced to create baseline instability, spiking of the tracing and dispersion of the HPLC peaks.

Preliminary work was also performed with sodium dithionite. From qualitative tests the dithionite appears to perform as well as $NaBH_4$. No precipitates form and no large quantities of TcO_2 are isolated. Chromatograms indicate the same major product seen in the $NaBH_4$ reduction is produced (although there appears to be an increase in minor impurities). Also no pertechnetate was observed. Apparently

$Na_2S_2O_4$ may prove more useful than $NaBH_4$ for reductions when the mixture is injected onto an HPLC, for no hydrogen evolves to create baseline instability, spiking or dispersion.

C. Complex Characterization

Thin Layer Chromatography

To establish optimum separations on TLC of the radioactive species in the reaction medium, a number of developing solvents were tested. The best separations were achieved with a 4:1 mixture of chloroform and methanol or methylene chloride and methanol. Samples of pertechnetate reduced with no ligands present yielded a TLC with all the radioactivity found at the origin. A non-mobile species is created by the reduction, namely, technetium dioxide ($TcO_2 \cdot xH_2O$). By contrast sodium pertechnetate elutes with a low R_f value as shown in Table II. Lastly reductions of the pertechnetate in the presence of ligand yielded results as illustrated by the chromatograms of butyl, ethyl, and benzyl complexed species depicted in Figures 7, 8, and 9. The R_f values for the complexes formed for all ligands reacted are given in Table II.

Interestingly a two peak phenomenon is noted on the TLC plates of most of the reacting species (Note: the second peak is not listed in Table II). In all cases the radioactive peak of lower R_f decreases with time. In the ethyl and butyl reactions the lower R_f species disappears in a matter of hours. However, the benzyl and p-chlorobenzyl species contain the lower R_f peak as much as 24 hours later. Conversely the naphthyl and dodecyl TLC contained one peak of high R_f.

Such multiple peak phenomenon are not unusual for technetium-99m species (29,62). In the cases above the low R_f species appears labile and converts to the high R_f species with time. In all likeli-

Table II: R_f values measured for the bismercaptoethylamine complexes of technetium-99m. All values are the averages of at least three trials.

Compound[a]	R_f	
	4:1 $CHCl_3/CH_3OH$	4:1 CH_2Cl_2/CH_3OH
Pertechnetate	0.13	0.07
Ethyl	0.76	b
Butyl	0.82	0.90
Dodecyl	0.91	0.87
Benzyl	0.83	0.80
p-Chlorobenzyl	0.99	c
Naphthyl	0.99	0.80

a) All compounds except the pertechnetate are the technetium-99m complexes of the N-substituted bismercaptoethylamine, b) not determined, c) indistinguishable from the solvent front.

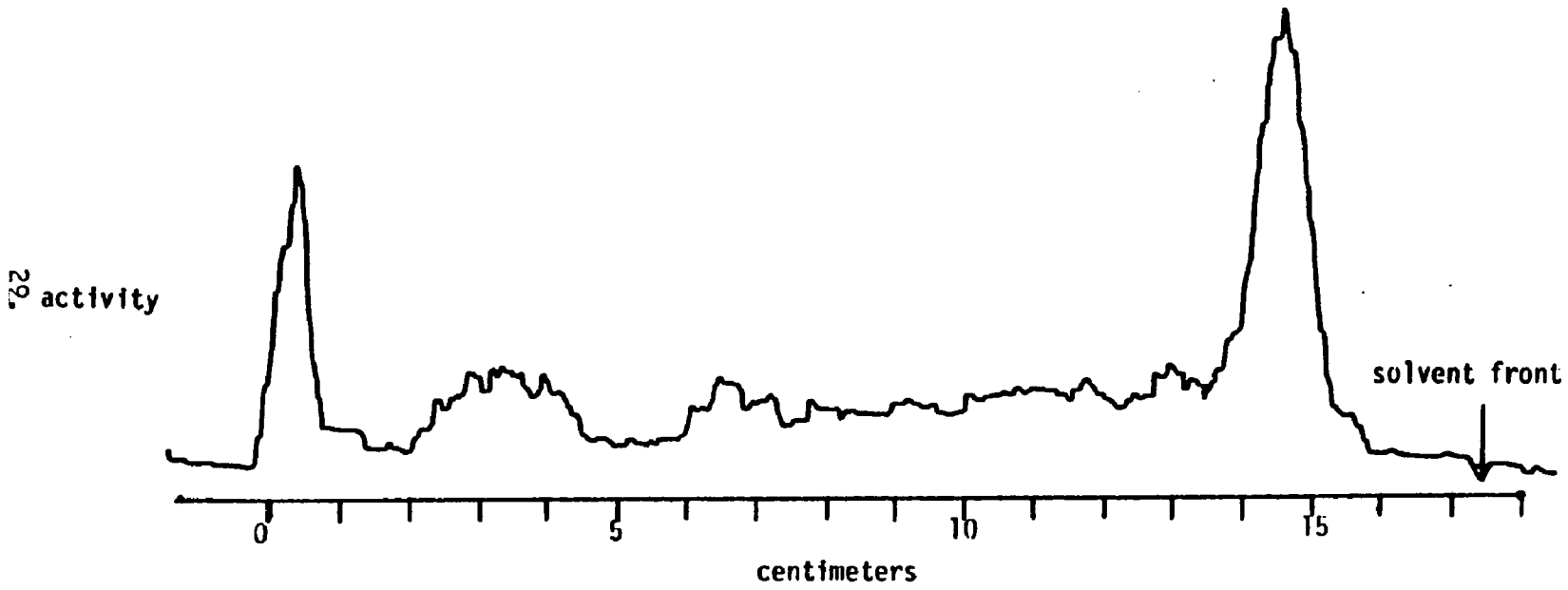


Figure 7: Radiochromatogram of the butyl- Tc-99m complex TLC with 4:1 chloroform/ methanol developer.

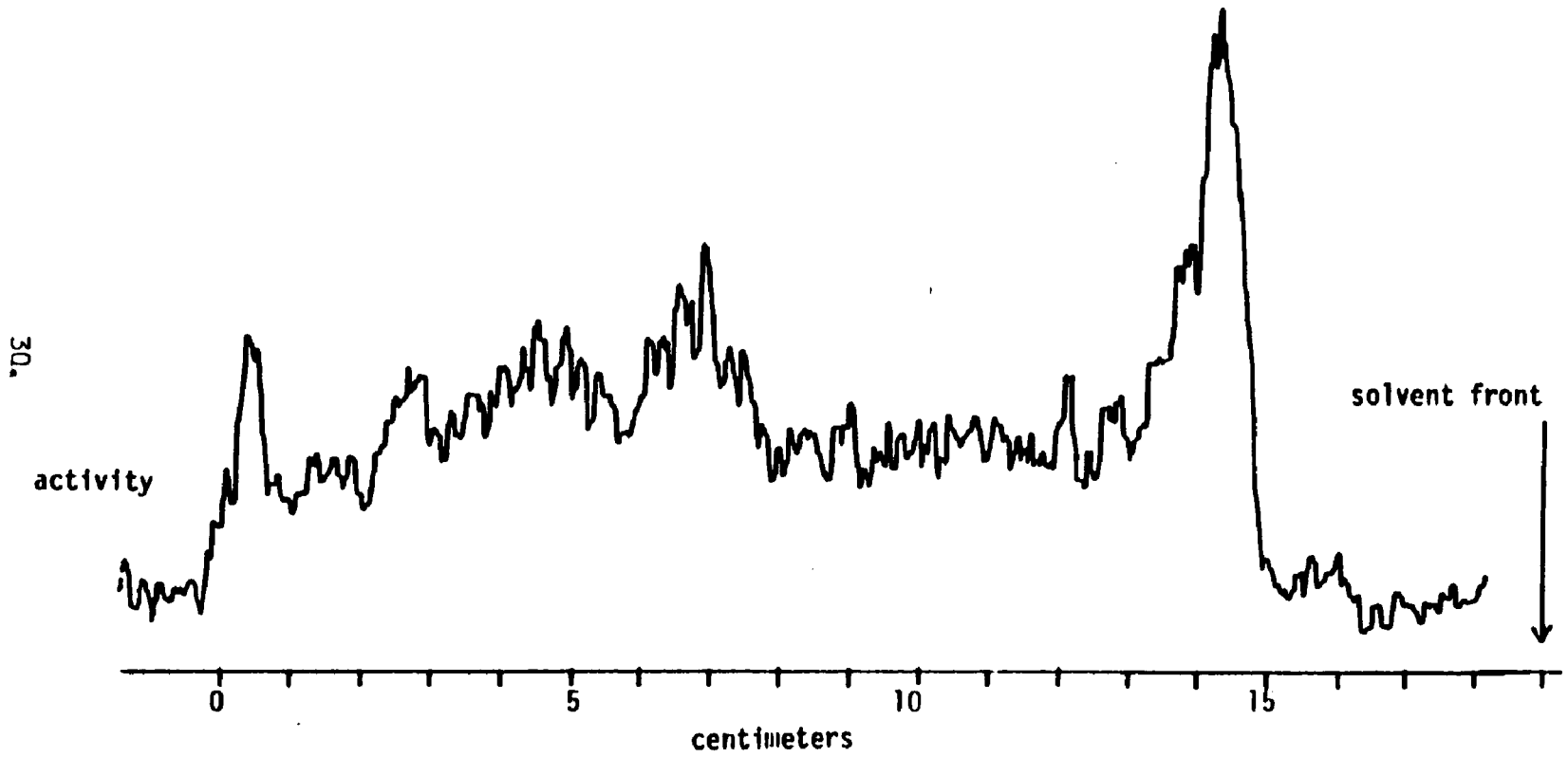


Figure 8: Radiochromatogram of the ethyl- Tc-99m complex in 4:1 chloroform/methanol developer.

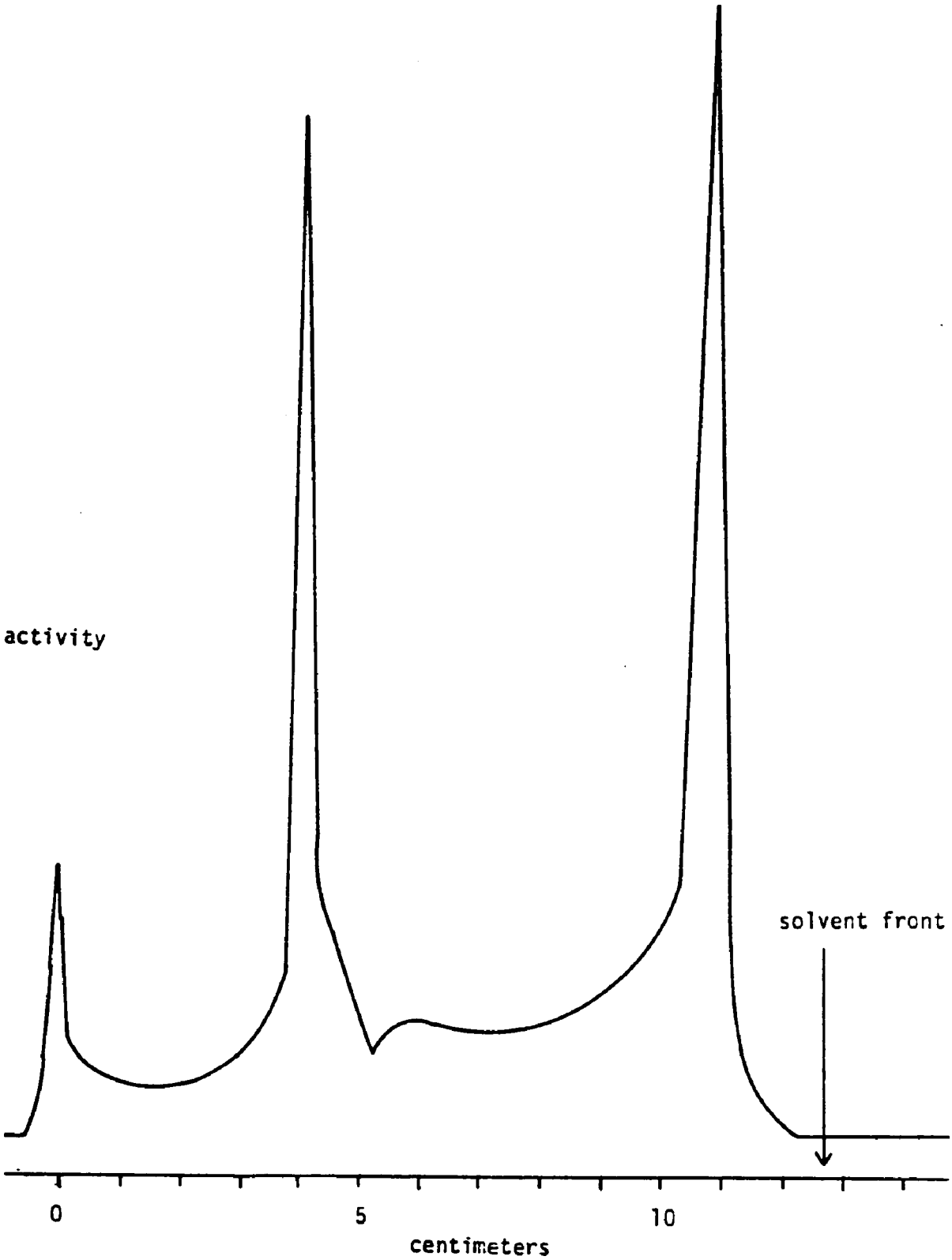


Figure9: Radiochromatogram of the benzyl-Tc-99m complex TLC in 4:1 chloroform/methanol.

hood, the conversion may simply be a change to a more stable complex. The one peak observation for the naphthyl and dodecyl species may indicate the labile species does not form in these cases or that the two species develop and exhibit the same R_f.

High Pressure Liquid Chromatography (HPLC)

To test the technetium-99m complexes with HPLC, a reverse phase column was chosen. Initially various concentrations of methanol or acetonitrile with water were tested for elution characteristics of the complexes. In general all the complexes eluted either within 1.0 minute of the void volume or did not elute. Table III lists the results obtained for a 90% methanol and 10% water mobile phase. Lower methanol to water ratios caused most of the complexes to elute at or near the void volume. Higher ratios created broad tailing peaks. Also the dodecyl and naphthyl complexes did not elute from the column with any mobile phase except a dimethylforamide wash. As with TLC, two peaks are also observed in the HPLC scan. Again the least retained peak disappears with time.

In general elution profiles of the complexes were poor with simple methanol-water mixtures. The few references that exist describing elution of technetium complexes by HPLC indicate that efficient fractionations occur on C-18 columns with the use of a buffered mobile phase (29,62,63). Therefore a series of experiments were performed to determine the optimum buffered mobile phase. Buffers from pH 4.8 to 8.9 were evaluated. All phosphate and bicarbonate buffers used with methanol or acetonitrile yielded radiochromatograms with no peaks or broad flat peaks. The use of ammonium acetate buffers provided improved chromatograms. But 0.01 N ammonium acetate solution (pH 7.0) with

Table III: Retention times and capacity factor for technetium-99m complexes eluted with 90% methanol/10% water.[a]

Complex	t_r (minutes)	k'[b]
Pertechnetate	2.1	0.31
Benzyl	(2.5)[c], 2.7	(0.56), 0.69
p-Chlorobenzyl	d	----------
Butyl	d	----------

a) RCM-C18 column; each complex was tested three or more times; b) t_o = 1.6 minutes, flow rate 2 ml/min; c) all numbers in parenthesis indicate the peak which decreases with time; d) not tested with this mobile phase.

methanol or acetonitrile provided the best results.

By variation of the percent ammonium acetate buffer and organic solvent, the best elution profiles were found with a 10% to 30% buffer to organic mobile phase (Tables IV and V). Higher percentages of buffer yielded elutions near the void volume. Lower percentages created some band broadening and tailing. Again the naphthyl and dodecyl complexes did not elute well and flat broad bands with long irreproducible retention times resulted. Figures 10 and 11 are representative elution profiles for the butyl and ethyl complexes after approximately two hour reaction time.

Lastly the two peak phenomenon observed on TLC and LC can be addressed more directly by HPLC analysis. With injections at regular intervals the conversion of one peak to the other may be quantitatively described. Figure 12 reveals the relative percent change obtained upon the study of the butyl and ethyl complexes over a three hour period. When the data is plotted as the logarithm of the disappearance of the labile peak [log L/(L+S) where L = labile peak and S = stable peak] with respect to time, a straight line is obtained. The correlation to a straight line is 0.998 and 0.938 for the butyl and ethyl complexes respectively. Accordingly the conversion from the labile to the stable species is first order or pseudo-first order. Values for the intercept, slope and k are indicated on the graph with the k value calculated from the relation: k = slope x 2.303. The half-life for the reaction may be calculated from the equation: $t_{1/2} = 0.693/k$. The half-life is 39 and 47 minutes for the butyl and ethyl species respectively In both cases the conversion from the labile to stable form is greater than 90% in three hours.

Table IV: Retention times and capacity factor for technetium-99m complexes eluted with 90% methanol/10% 0.01 N ammonium acetate.[a]

Complex	t_r (minutes)	k'[b]
Pertechnetate	2.1	0.31
Benzyl	(2.8)[c], 3.5	(0.75), 1.19
p-Chlorobenzyl	(3.1), 3.2	(0.94), 1.00
Ethyl	2.8	0.75
Butyl	4.1	1.56

a) RCM-C18 column; each complex was tested at least three times; b) t_0 = 1.6 minutes, flow rate = 2 ml/min; c) peak noted is the labile peak described in text.

Table V: Retention time and capacity factor for technetium-99m complexes eluted with 70% methanol/30% 0.01 N ammonium acetate.[a]

Complex	t_r (minutes)	k'[b]
Pertechnetate	2.0	0.25
Benzyl	5.6	2.50
p-Chlorobenzyl	5.1	2.19
Ethyl	6.2	2.88
Butyl	9.6	5.00

a) RCM-C18 column; each complex was tested at least three times; b) t_0 = 1.6 minutes, flow rate = 2 ml/min.

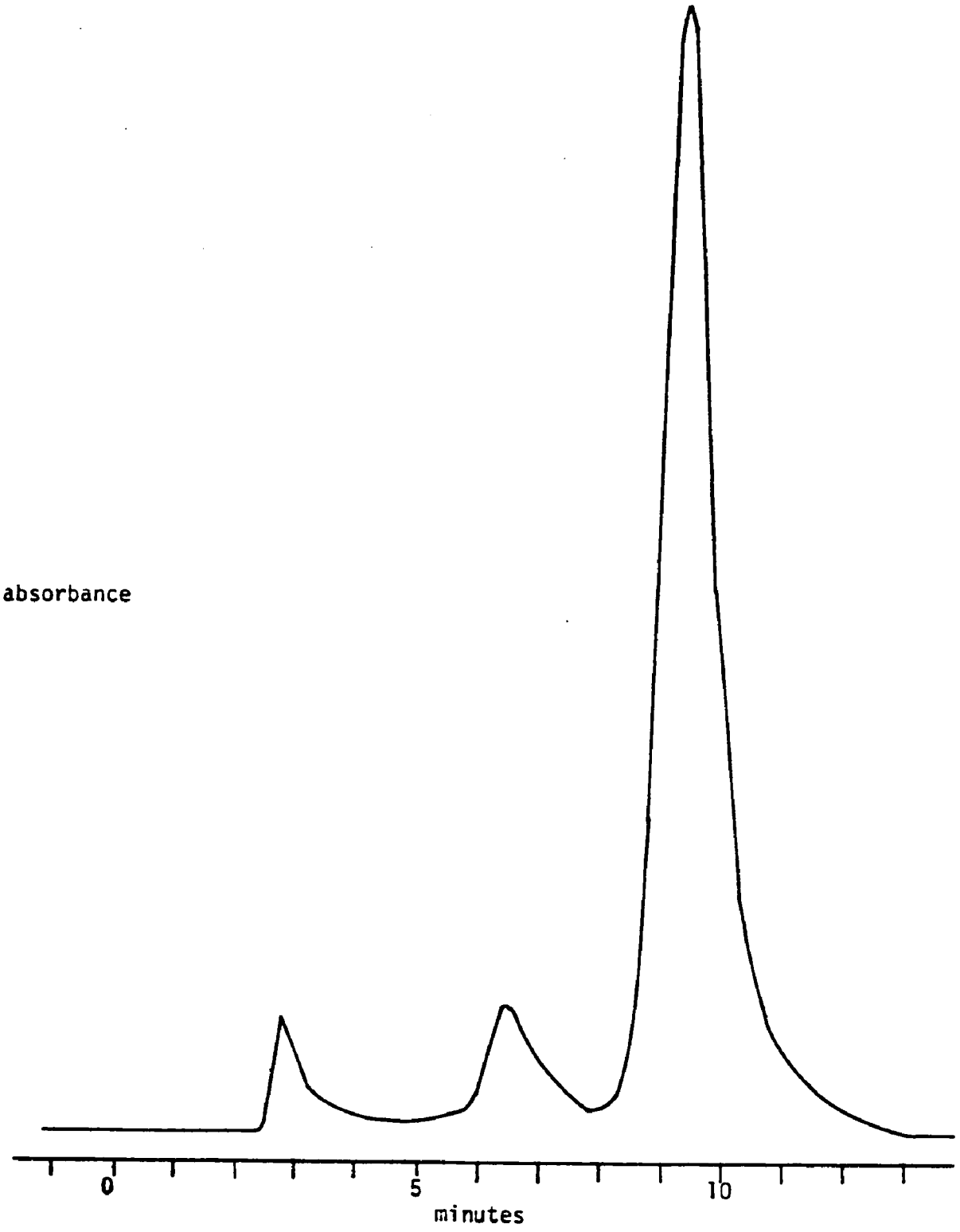


Figure 10: Elution profile of the butyl-Tc-99m complex mixture after a three hour reaction time; 70% methanol/30% 0.01 M ammonium acetate.

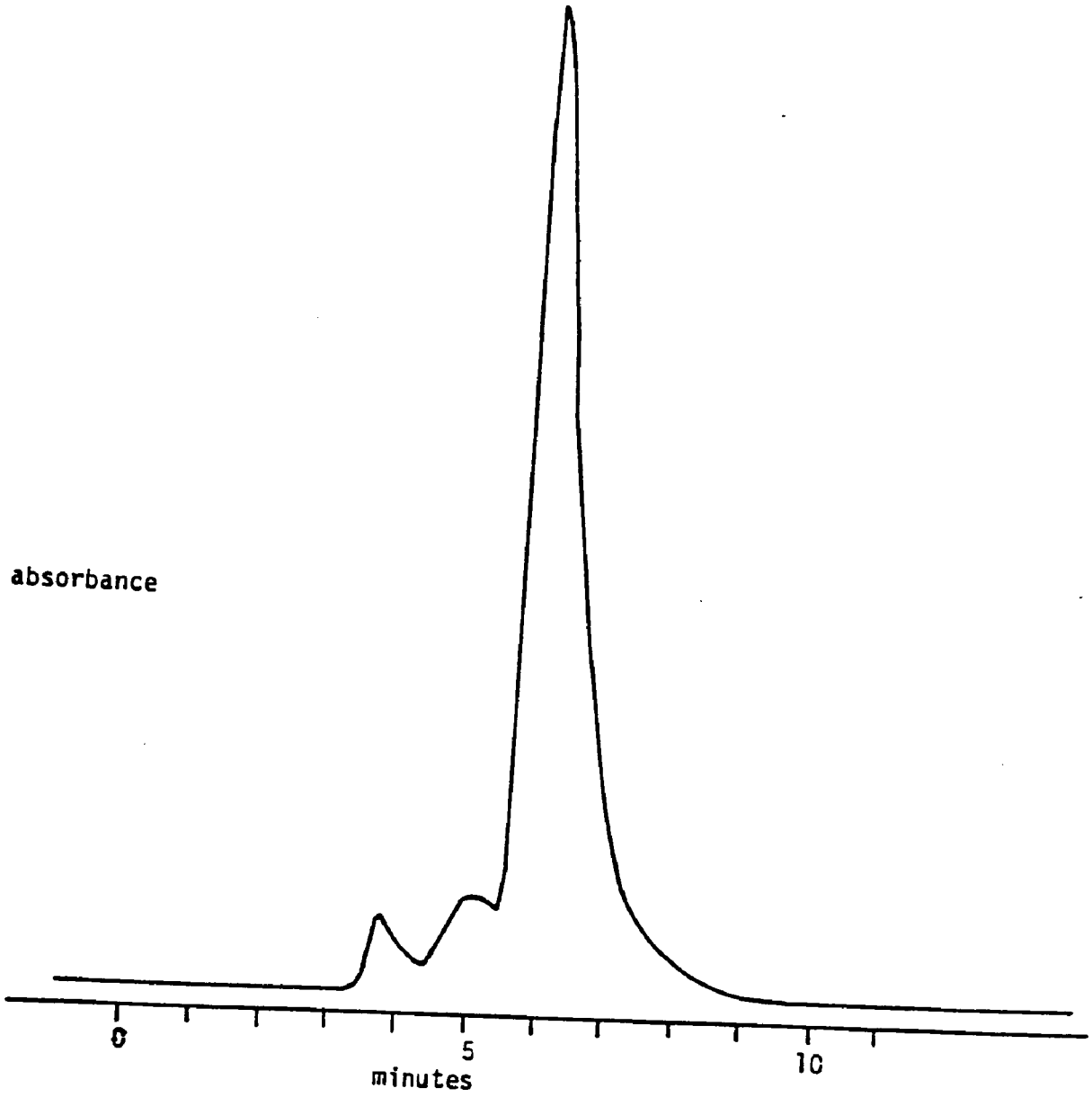


Figure 11: Elution profile of the ethyl-Tc-99m complex mixture after a three hour reaction time, 70% methanol/10% 0.01 M ammonium acetate.

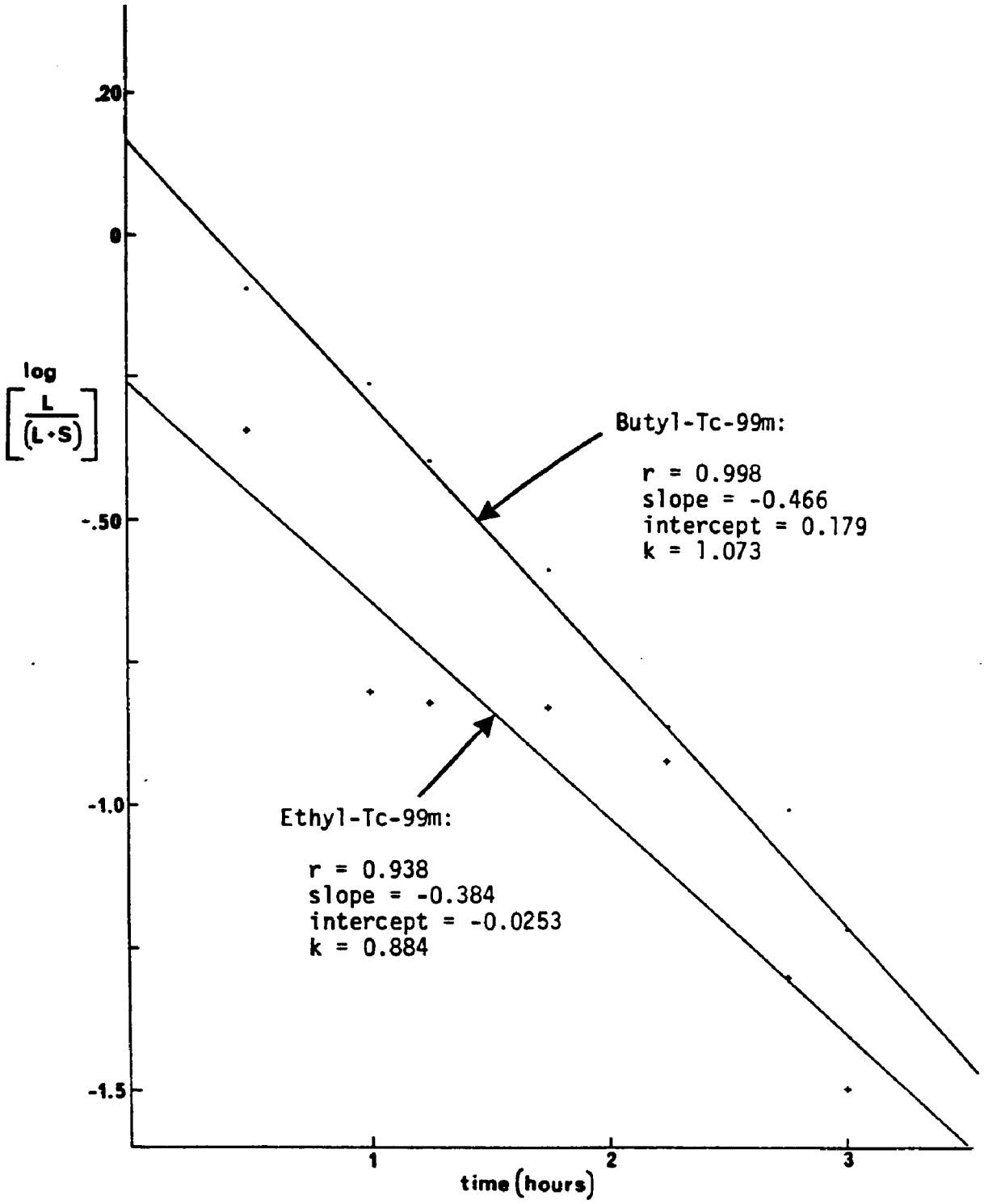


Figure 12: Semi-logarithmic plot of the disappearance of the labile peak with respect to time for the ethyl and butyl-technetium-99m complexes.

Charge on the Complex

To determine the charge on the technetium-99m complex, an electrophoretic experiment similar to that developed by Burns (23) was performed. Figure 13, the electrophorogram of sodium pertechnetate, demonstrates that the negatively charged species migrates toward the anode. Figures 14, 15 and 16 are representative electrophorograms for the dodecyl, benzyl and butyl chelates. These complexed species remain at the origin, indicating the complexes are neutral.

Two questions arise: 1) is the neutral species observed hydrolyzed reduced technetium ($TcO_2 \cdot xH_2O$) or a neutral bismercaptoethylamino complex; and 2) are there sites on the electrophoresis paper that might bind the technetium complex and prevent migration? The first question is addressed in detail in the chromatography section (vide ut supra). Briefly, TLC proves the species is not TcO_2 since no activity remains at the origin.

The question of the chromatography paper containing sites for chelation of technetium in the lower valence states has arisen in the past (64). Although migration of technetium complexes in several different oxidation states is well established (65,66) electrophoresis was performed on cellulose polyacetate paper to eliminate the possible interference from the cellulose hydroxyl groups. The results with the polyacetate paper were the same-no migration of the complexes from the origin. Apparently a technetium complexed to the bis-mercaptoethylamino ligand forms a neutral complex. Structure and stoichiometry, however, can not be predicted from these electrophoresis experiments.

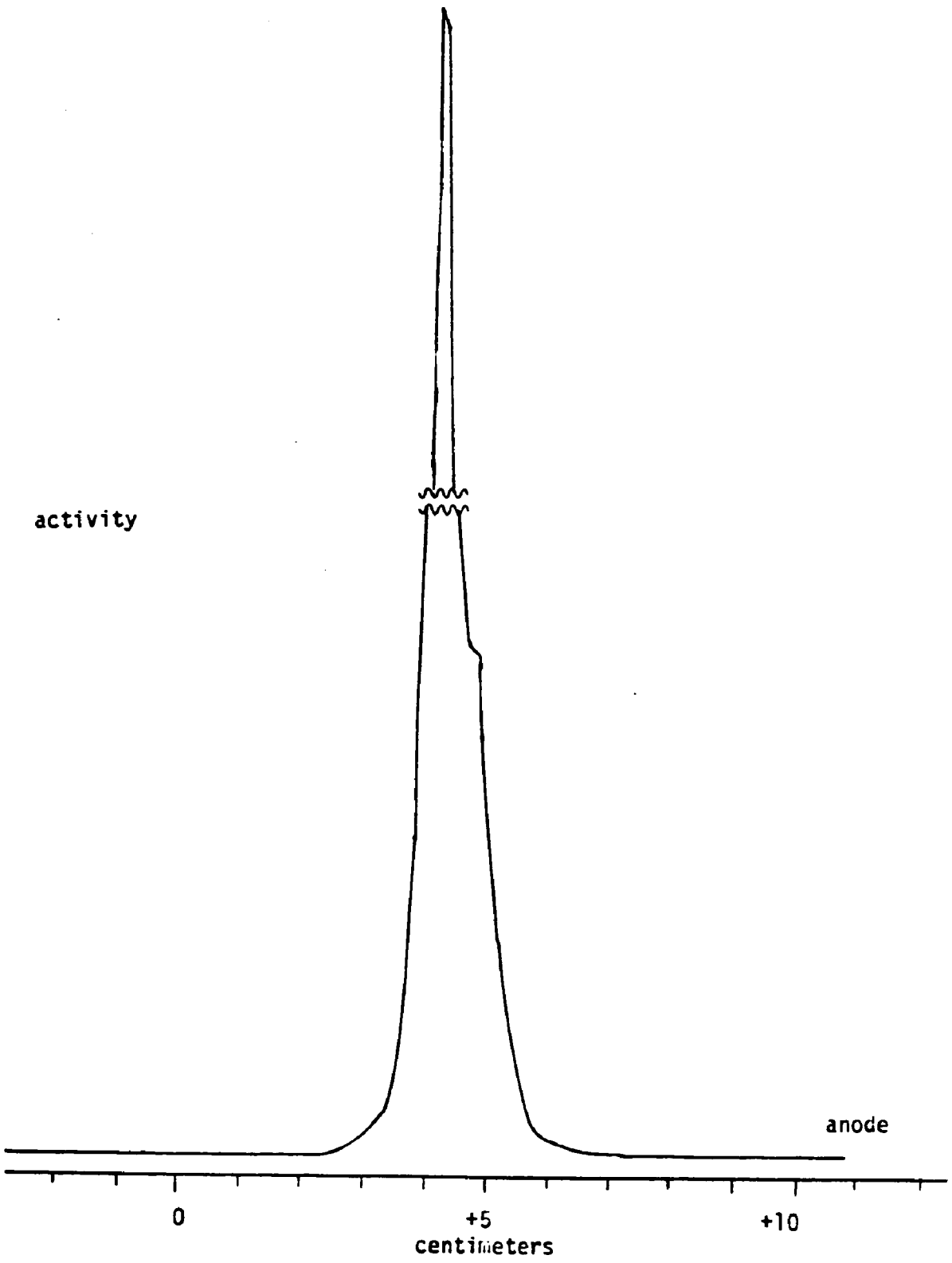


Figure 13: Electrophorogram of sodium pertechnetate on cellulose polyacetate in 0.01 N $NaHCO_3$ at 600 volts for 15 minutes.

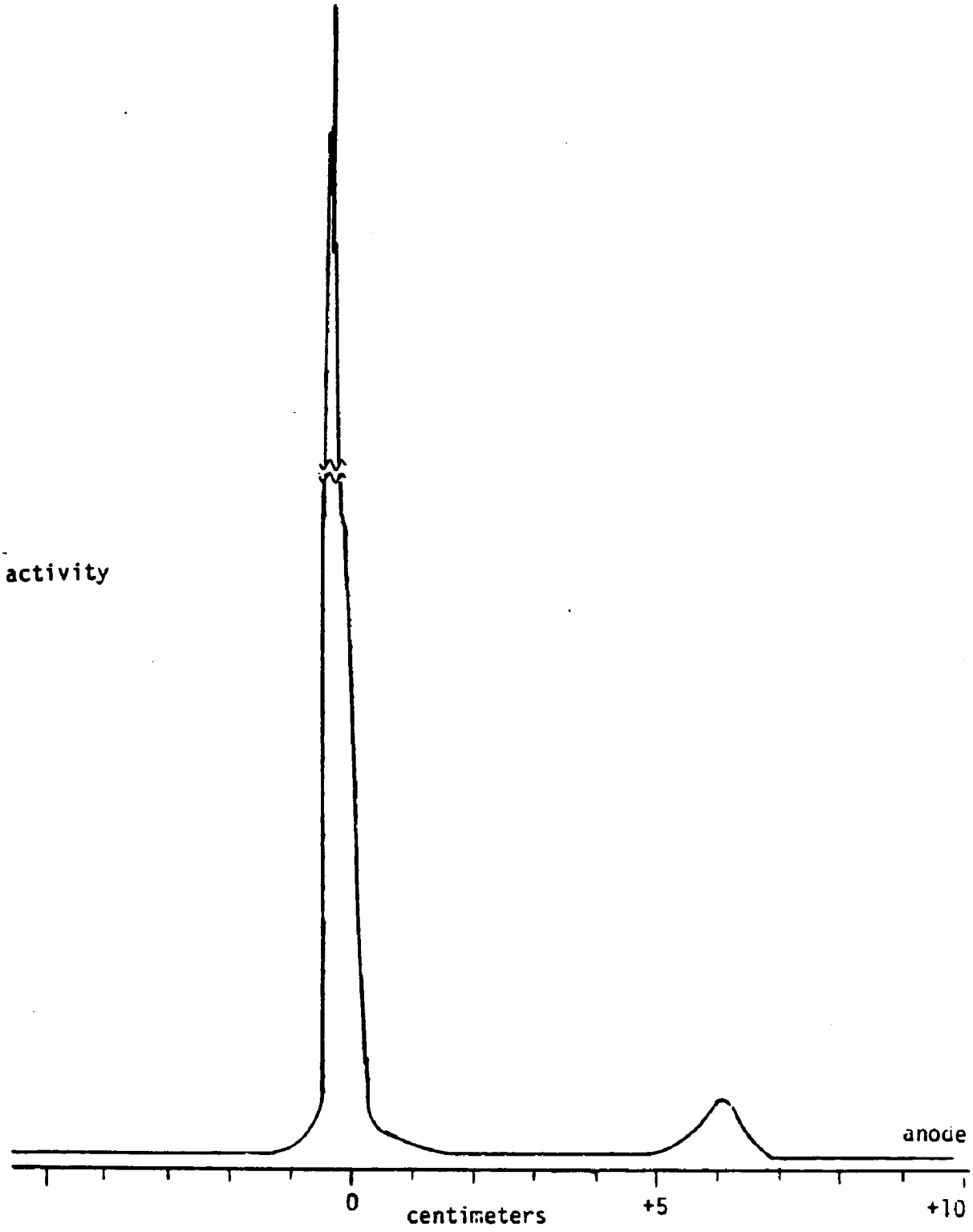


Figure 14: Electrophorogram of $CH_3(CH_2)_{11}N(CH_2CH_2S)_2$-Tc-99m complex on cellulose polyacetate in 0.01 N $NaHCO_3$ for fifteen minutes at 600 volts.

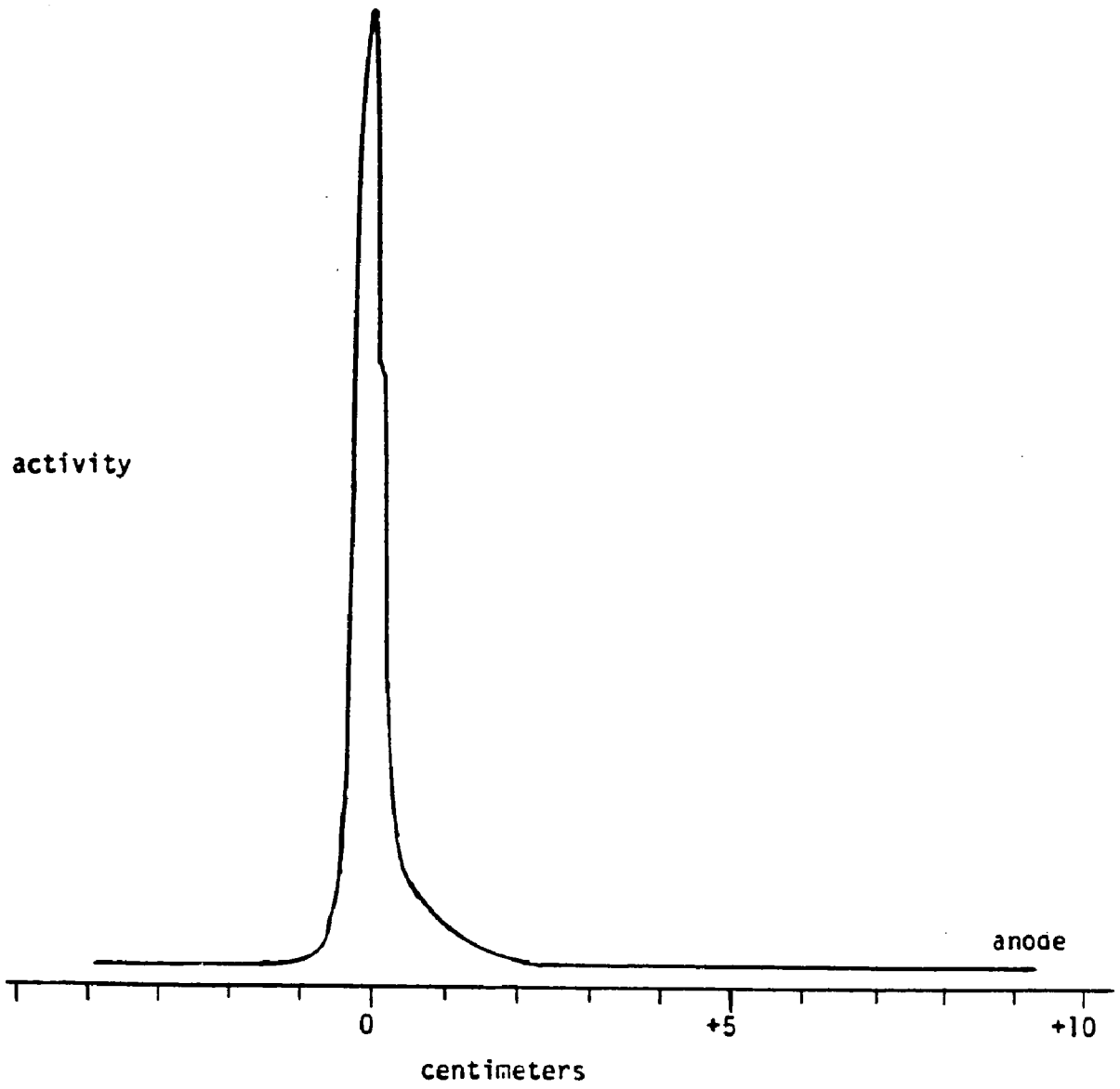


Figure 15: Electrophorogram of the benzyl-Tc-99m complex on cellulose polyacetate in 0.01 N $NaHCO_3$ at 600 volts for 15 minutes.

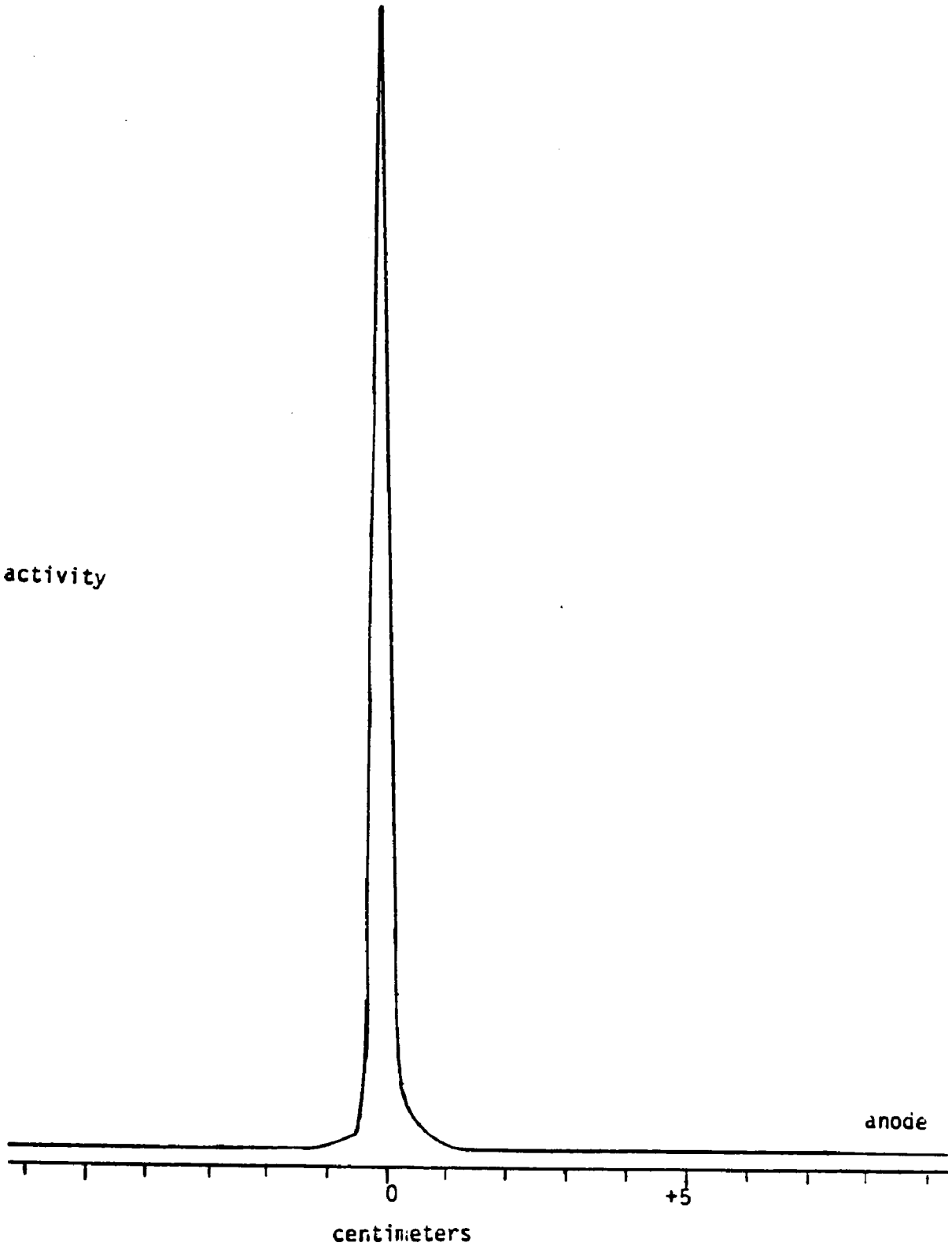


Figure 16: Electrophorogram of the butyl-Tc-99m complex on cellulose polyacetate in 0.01 N $NaHCO_3$ at 600 volts for 15 minutes.

Partition Coefficient

As noted in the introduction, the Hansch equation relates the effectiveness of a pharmaceutical to a number of parameters. The largest contributor in the equation is the partition coefficient of the pharmaceutical between n-octanol and saline. As another description of the complexes, the octanol/saline partition coefficients were obtained. Table VI lists these coefficients. A notable increase in the coefficient as the size of the alkyl chain increases is observed. Note also that the aryl functionalities have comparatively low coefficients.

Mixed Ligand Experiment

To aid in determining the stoichiometry of the technetium complex, a mixed ligand experiment was performed. Simplistically a mixed ligand experiment entails the combining of equivalent amounts of two different ligands, the complexing of these ligands to technetium-99m, and determining the number and ratio of products formed. Such experiments were performed with charged technetium-99m complexes with the products analyzed by electrophoresis (23). With neutral complexes the analysis may be performed by HPLC if the complexes are sufficiently different (k').

Experimentally the ethyl and butyl ligands were chosen for the mixed ligand experiment due to the relatively short reaction time to the formation of one major radioactive species and the HPLC retention time differences. After a three hour reaction time, the mixed ligand solution was injected into the liquid chromatograph. Figure 17 depicts the unexpected results-at least four peaks. As noted from Figures 10 and 11, the first and last radioactive peaks eluted represent the known

Table VI: Partition coefficients of the bismercaptoethylamino technetium-99m complexes

Complex	Octanol/saline
Pertechnetate	0.04
Ethyl	0.97
Butyl	2.89
Dodecyl	10.43
Benzyl	0.55
p-chlorobenzyl	0.28
1-Naphthylmethyl	0.52

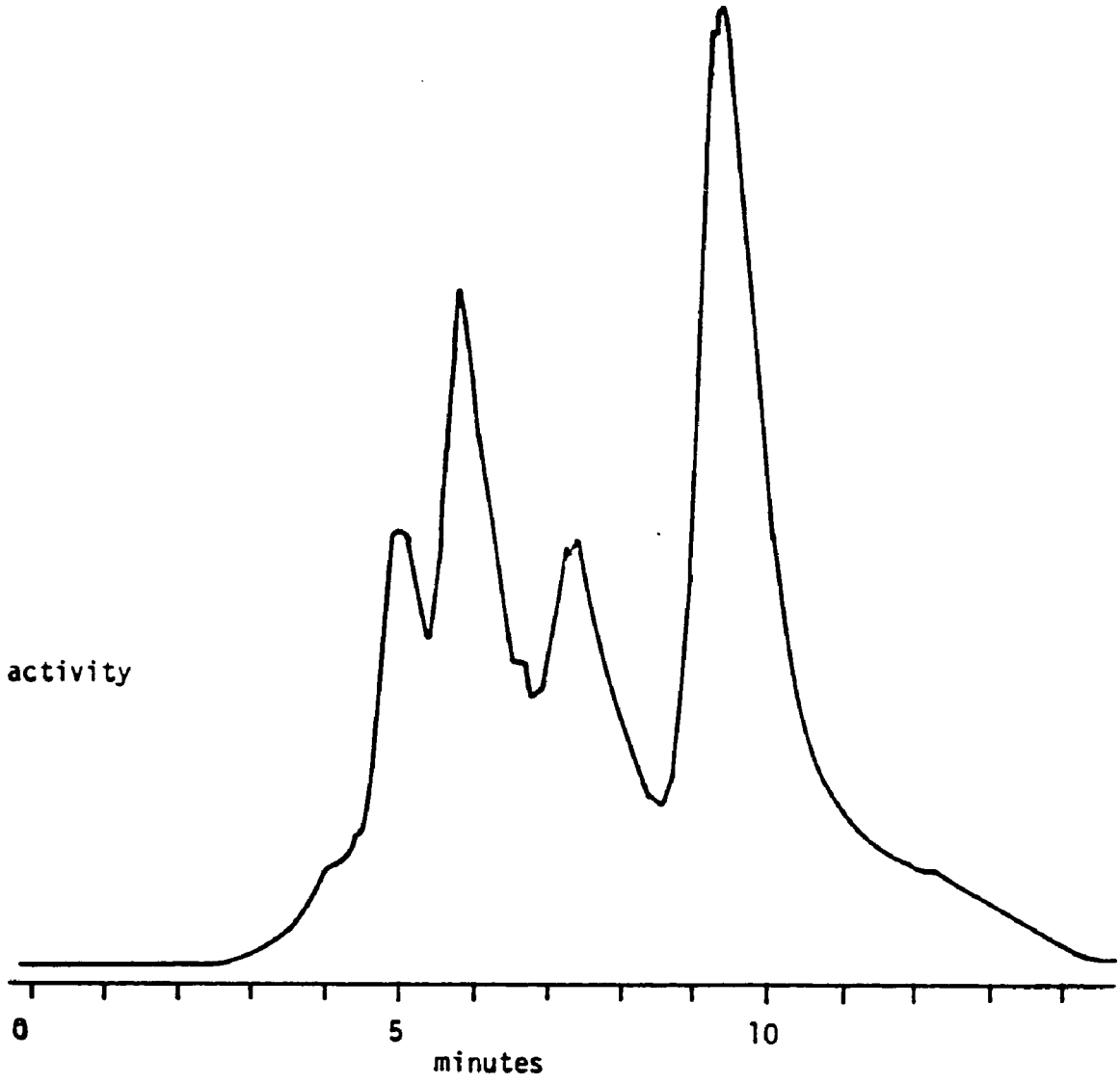


Figure 17: Elution profile of the mixed ligand reaction mixture using the butyl and ethyl ligand..

butyl and ethyl technetium complexes. The approximate relative peak ratios obtained by the peak height and half width method (67) are 1:1.4:1.4:2.7 for peaks 1 to 4 (as labelled in Figure 17) respectively. Modification of the LC conditions or reaction time did not reveal any differences in the number of peaks or relative peak intensities.

These mixed ligand experiments do not reveal any evidence of a simple stoichiometry. For example, a complex containing one ligand per technetium would yield a mixed ligand chromatogram containing only two peaks one for each complex. A complex containing two ligands per technetium would in all likelihood yield a chromatogram containing three peaks in a ratio of 1:2:1 if equivalent amounts of ligand with the similar kinetics are used. Apparently the multiple peak result above may be indicative of some higher order of stoichiometric complexation.

Ligand Exchange Experiment

In order to determine the relative chemical or kinetic inertness of the complex formed, a ligand exchange experiment was performed. Ethanedithiol, a known strong chelator of technetium (36), was added in large excess to a reaction vial containing the technetium-99m-butyl complex which was reacted under nitrogen for three hours. The chromatogram of the butyl complex appeared as in Figure 10. Also, the ethanedithiol-Tc-99m complex elution profile is represented in Figure 18. Aliquots of the exchange reaction were taken at approximately 15 minute intervals for the first three injections and at 30 minute intervals after the third injection. The result is indicated in the graph of Figure 19. As noted the butyl complex is converted to the ethanedithiol complex with a half-time of 25.5 minutes.

Using the definition of kinetic inertness as a half-time for

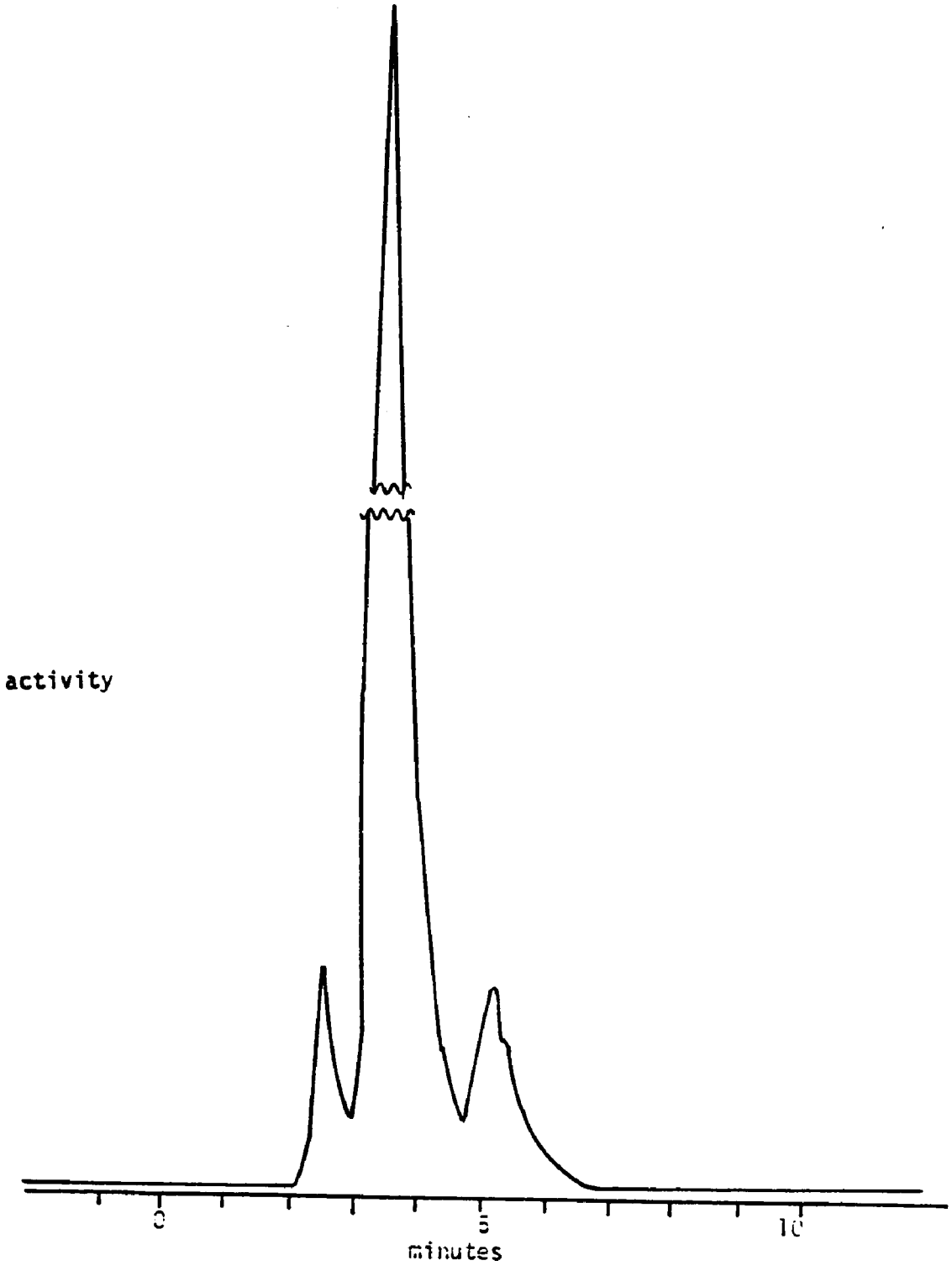


Figure 18: Elution profile of the ethanedithiol-Tc-99m complex.

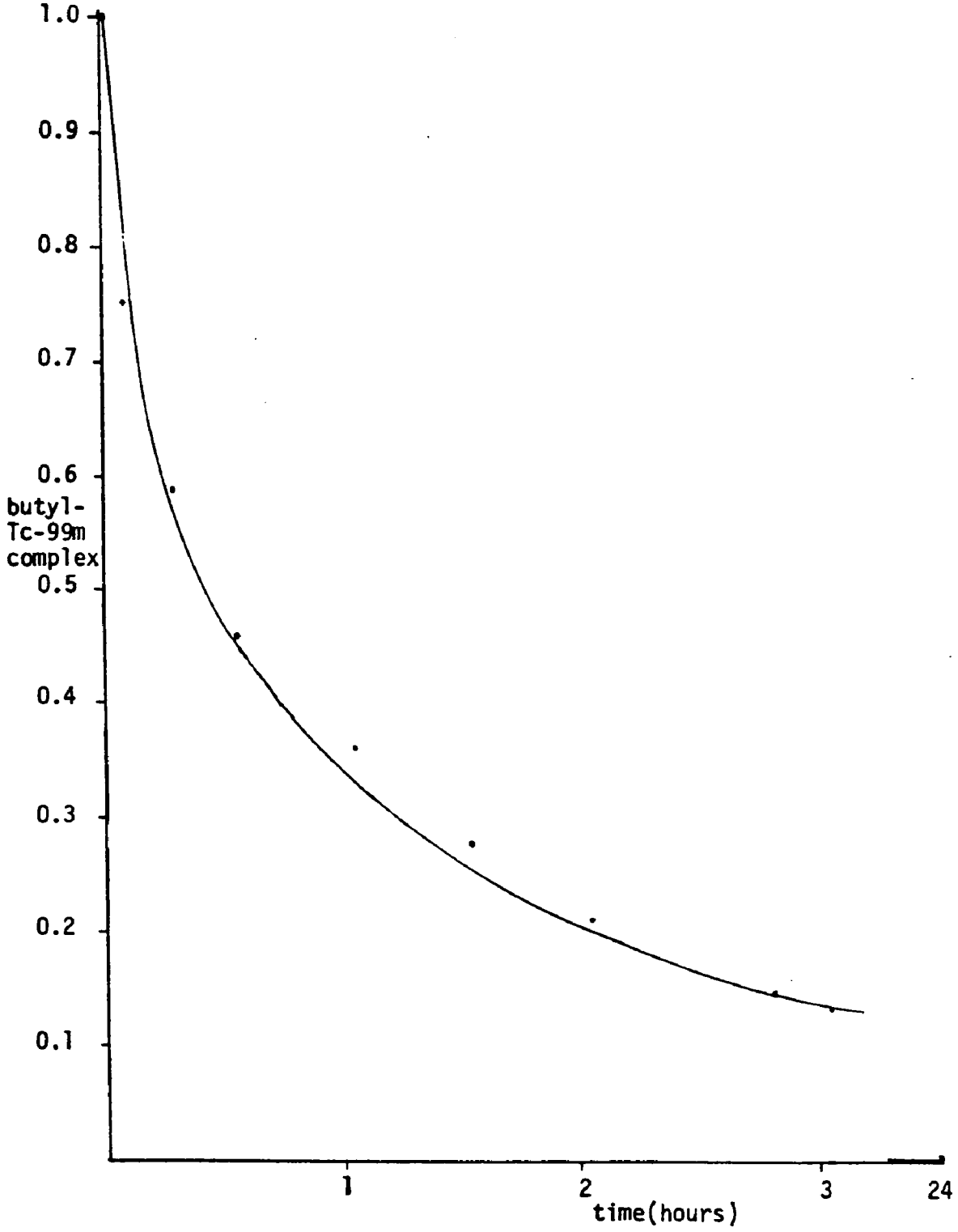


Figure 19: Ligand exchange rate between the complexed butyl and free excess ethanedithiol.

a reaction of greater than one minute (68), one may state that the butyl and therefore, possibly all such complexes are kinetically inert and chemically robust. In addition the ligand exchange reaction as performed experimentally does not appear to follow first, psuedo-first or second order reaction rates with respect to technetium. Attempts to plot the data under simple mathematical constraints yielded non-linear results.

Summary

In the study of the technetium-99m complexes of the N-substituted bisethanethiolamines, neutral complexes were easily synthesized by mild reduction of pertechnetate in the presence of ligand. In general the reaction pathway appears to involve the formation of a labile intermediate prior to the formation of product. The polar intermediate (as evidenced by TLC and HPLC) dissappears in a matter of 2-3 hours to yield a stable product. The stable product was found to have a variable lipophilicity depending on the size of the alkyl chain. The variable lipophilicity is indicative of a polar, non-ionic species. Lastly the complex has a complex stoichiometry which makes interpretation of the mixed ligand experiment difficult. Therefore in order to further characterize the complex stoichiometry and structure, technetium-99 chemistry was performed.

III. TECHNETIUM-99 CHEMISTRY

A. Introduction

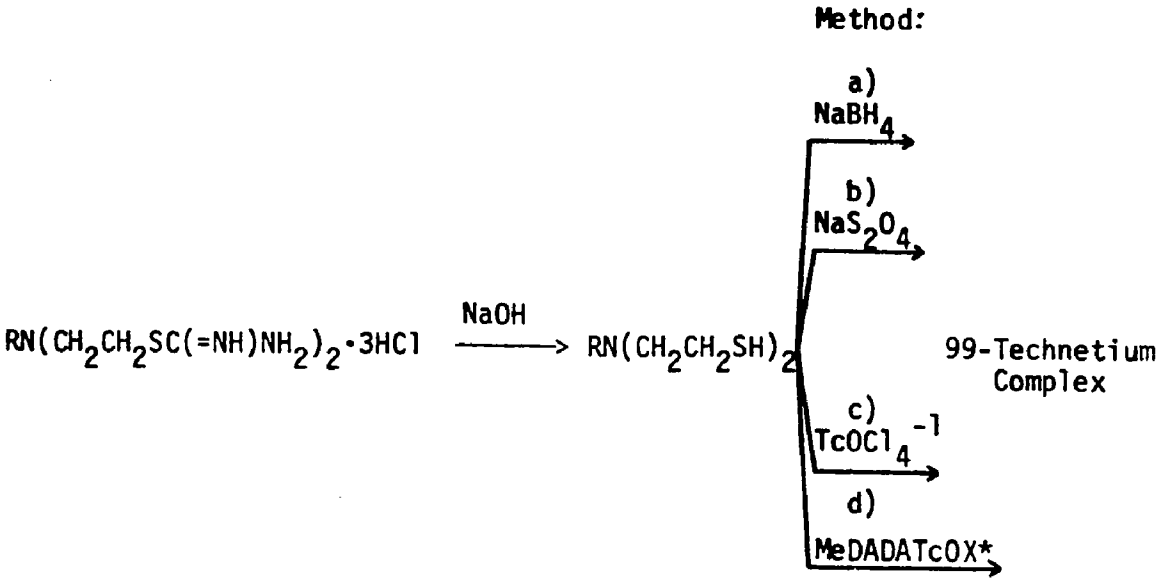
Although the technetium-99m results indicated one major neutral complex formed, the mixed ligand experiment made postulation of the exact nature of the stoichiometry of the complex impossible. To better evaluate the complex technetium-99 syntheses were developed (vide

infra). Actual isolation of complex would ultimately aid in the determination of stoichiometry and possibly elucidate the structure of the complex. The long half-life, low specific activity and emission of a weak beta particle allows technetium-99 complexes to be isolated on a milligram scale using non extra-ordinary safety precautions.

B. Complex Synthesis

Introduction

Technetium-99 complexes of the ligands were synthesized by the four routes described in scheme 6. After hydrolysis of the isothiouronium salt, reaction methods (a) and (b) included the reduction of NH_4TcO_4 in the presence of ligand. Methods (c) and (d) utilized the addition of ligand to a reduced state of technetium (+5 oxidation state). All four methods allowed the isolation of milligram quantities of crude complex. Description of synthetic, purification and characterization procedures follows.



Scheme 6: Technetium-99 Complex Synthetic Schemes. R = ethyl, butyl, dodecyl, benzyl, (1-naphthyl)methyl.

Complexation by Reduction

As stated in the introduction two methods of reduction were employed-sodium borohydride and sodium dithionite. The sodium borohydride ($NaBH_4$) reduction was initially used due the knowledge gained from the technetium-99m syntheses. Reduction of the pertechnetate to a lower oxidation state and consequent complexation was facile for each ligand listed in Scheme 6. In all cases a dark precipitate that readily dissolves in chloroform and methylene chloride is produced. Little to no technetium dioxide ($TcO_2 \cdot xH_2O$) precipitate was noted. The initial precipitates were in general gummy but became more crystalline upon washing with a dilute acid solution. TLC (vide infra) results indicate a number of spots with one major radioactive and colored spot predominating. Also HPLC results indicate one major uv active product as

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* Description of MeDADATcOX to follow later.

well as a large quantity of unreacted ligand and its polymeric products. Purification of the crude precipitate involved washes with dilute acid and water to remove excess ligand and salts, passage through a silica column, and recrystallization from chloroform/ether or chloroform/ethanol. Moisture stable solids or crystals were isolated.

The second reducing agent, sodium dithionite ($Na_2S_2O_4$), was found to create the complexes quickly and easily. In general the crude solids isolated were less gummy. Similar purification and recrystallization techniques to the $NaBH_4$ method appeared to yield more crystalline product with less difficulty. Also these products have the analytical characteristics of the borohydride reduced complexes.

Complexation by Metathetical Exchange

i. The tetrachlorooxotechnetium (V) anion ($TcOCl_4^-$):

Tetrachlorooxotechnetium (V) is a square pyramidal anion with easily displaced halogens (34). Therefore $TcOCl_4^-$ appears to be a viable intermediate for the complexation of ligands to technetium in a lower valence state. $TcOCl_4^-$ disproportionates in water but not in alcohols or other organics. In order to react the hydrolyzed mercaptoamine ligands with $TcOCl_4^-$ the ligand must be extracted from the basic aqueous reaction medium and dried before addition to an alcoholic solution of $TcOCl_4^-$. Upon addition of ligand to the $TcOCl_4^-$ solution an immediate color change is noted (green to yellow-brown). HPLC and uv-visible analysis indicates a complete conversion of the $TcOCl_4^-$ to the major product observed in the reduction methods. No further characterization was attempted with this method since less than a milligram of product may be expected at the stoichiometric levels utilized.

ii. The N-methyl-N,N'-diethoxyethylenediamineoxotechnetium(V)X

molecule (MeDADATcOX):

MeDADATcOX is a previously unknown complex of technetium in the +5 oxidation state. Although the structure is yet to be confirmed by X-ray crystallography, the stoichiometry, as noted in the formula, has been determined by other methods (ie- mass spectrometry) (69). The MeDADATcOX moiety has been found to be stable in solvents including water at a variety of pH conditions. Thus the complex may have unusually good potential as a precusor to the synthesis of other substances containing a low valence state of technetium (70). In fact crude hydrolysis solutions of the mercaptoamine ligand may be added directly to alcoholic solutions of the MeDADATcOX. An instantaneous conversion to product is observed colormetrically (light green to brown). Analyses indicate a quantitative conversion to the major isolated product observed in the reduction methods above.

C. Complex Characterization

Introduction

After extensive purification steps, the technetium-99 complexes were initially characterized for purity by TLC and HPLC. After purity was confirmed, structure and stoichiometric analyses were performed by uv-visible, infra-red, NMR and mass spectrometry. Final characterization was provided by a elemental analysis.

Chromatography

Thin Layer Chromatography

Preliminary investigation of isolated solids from the reactions above was performed on silica plates. Table VII contains the collective results for the isolated solids or solutions. TLC was not performed on the MeDADATcOX product due to the minimal quantities of

Table VII: R_f Values of the Technetium complexes[a]

R	Borohydride	Dithionite	$TcOCl_4^-$
Ethyl	0.76	0.74	0.75
Butyl	0.81	0.82	0.81
Dodecyl	0.91	----	----
Benzyl	0.36, 0.85	----	----
(1-Naphthyl)methyl	sf[b]	----	----

a) Eastman-Kodak silica plates, 4:1 chloroform/methanol; TcO_4^-: R_f = 0.13.

b) Solvent front.

Table VIII: Retention times and k' for the ethyl and butyl ligands complexed to technetium by the four methods in Scheme

Tc-99 complex/reaction method	Cyano[a] V_r	Cyano[a] k'	Silica[b] V_r	Silica[b] k'	Reverse Phase[c] V_r	Reverse Phase[c] k'
Ethyl/borohydride	5.4	2.0	4.7	1.6	6.2	2.4
Ethyl/dithionite	5.4	2.0	4.5	1.5	6.2	2.4
Ethyl/$TcOCl_4^-$	5.3	1.9	4.6	1.6	6.3	2.5
Ethyl/MeDADA	---	---	---	---	6.4	2.6
Butyl/borohydride	3.9	1.2	3.4	0.9	9.6	4.3
Butyl/dithionite	3.8	1.1	3.4	0.9	9.6	4.3
Butyl/$TcOCl_4^-$	4.0	1.2	3.3	0.8	9.4	4.2
Butyl/MeDADA	---	---	---	---	9.5	4.3
MeDADATcOX	---	---	---	---	4.9	1.7
TcO_4^-	2.1	0.2	---	---	1.8	0.0

a) Waters RCM-CN column, 90% methanol/10% water, flow rate 2 ml/min, chart speed 2 cm/min, 254 nm; b) Waters RCM-silica column, 50% chloroform/methanol, flow rate 2 ml/min, chart speed 2 cm/min, 254 nm; c) Waters RCM-C18 column, 70% methanol/30% 0.01N ammonium acetate, flow rate 2 ml/min, chart speed 2 cm/min, 254 nm.

product formed. The R_f values were confirmed visually and radiometrically. Figures 20 and 21 contain the radiometric TLC traces for the ethyl and butyl complexes reduced by $NaBH_4$. The R_f values are exceedingly reproducible. The minor impurities noted in the crude solid disappear with purification.

High Pressure Liquid Chromatography

HPLC analyses of the complexes listed in Scheme 6 were performed. Table VIII lists the various HPLC columns and conditions used for the ethyl and butyl complexes. Note that the benzyl, dodecyl and (1-naphthyl)methyl results are not reported due to irreproducibility or excessively long retention times. As indicated in Table VIII the four synthetic methods yield similar if not equivalent retention times for the major product of the reaction. Typical elution profiles for the crude butyl complexes verify one major component with the same retention time as the technetium-99m derivative along with a large quantity of unreacted ligand and its decomposition products.

Technetium Complexes of Hydrolysis Byproducts

In the early stages of the technetium-99m characterization the question of the chelating properties of the hydrolysis (or incompletely hydrolyzed) byproducts. During the course of characterization of the technetium-99 complexes, experiments were performed to determine if the complex formed was actually the dithiol moiety or some other ligand such as the neutralized (but not hydrolyzed) isothiouronium salt or a hydrolysis byproduct. The first test was to neutralize the bisisothiouronium salt without creating hydrolysis and attempting chelation of this species with technetium. Upon neutralization of the ethyl bisisothiouronium salt and addition of the pertechnetate with $NaBH_4$, a black precip-

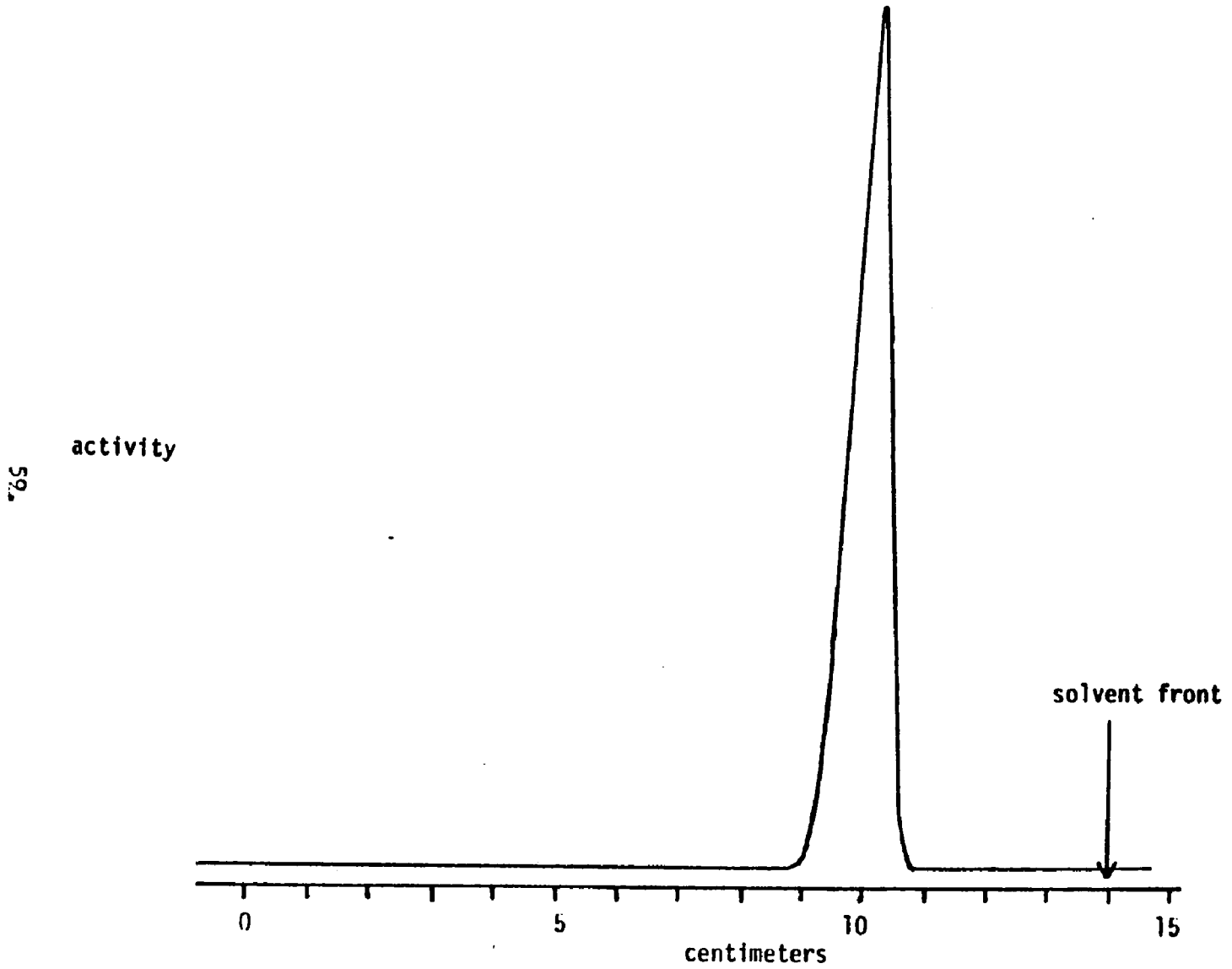


Figure 20: Radiochromatogram of the ethyl-Tc-99 complex in 4:1 chloroform/methanol.

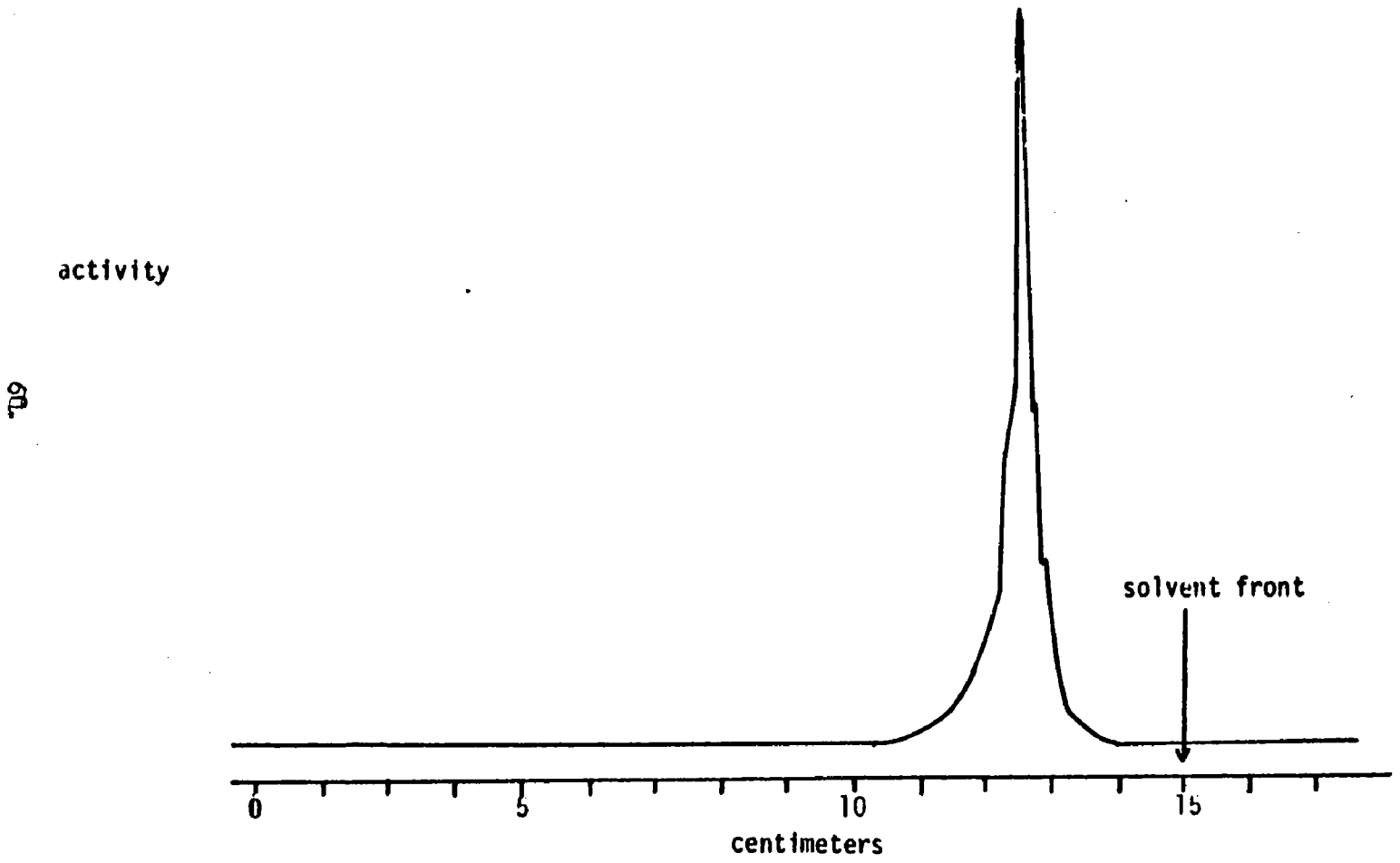


Figure 21: Radiochromatogram of the butyl-Tc-99 complex in 4:1 chloroform/methanol.

itate was found to be insoluble in organic solvents and appeared to be TcO_2.

A second test was to attempt to chelate technetium to the major hydrolysis byproduct dicyandiamide. Upon addition of pertechnetate and $NaBH_4$ to a dicyandiamide solution, a black precipitate formed-TcO_2. These two experiments confirmed the contention that the product obtained by the reduction methods was actually an organo-technetium complex containing a bis(thioethyl)amino moeity and not any of the expected hydrolyzed byproduct.

Charge on the Complex

To determine if the technetium-99 complexes formed by the four synthetic methods were neutral, electrophoresis and ion exchange chromatography experiments were pursued. The electrophoresis was performed on cellulose polyacetate strips saturated with 0.01 N $NaHCO_3$. A minimal amount of the technetium-99 complex was pipetted at the origin. 600 volts was applied for 15 minutes. Each complex tested did not move from the origin as noted by visual inspection and radiometric analysis.

To confirm the neutrality of the complexes a mixed ion exchange column was prepared. Elution of a methanolic solution of the complexes indicated almost complete non-retention of the radioactive species compared with complete retention of pertechnetate.

Partition Coefficients

The octanol/saline partition coefficients of the complexes listed in Table IX were determined in order to decide if the complex formed had the same inherent lipophilicity as the technetium-99m complexes. A comparison of the technetium-99m results (Table VI) with that of the technetium-99 indicates the lipophilic nature of the com-

Table IX: Partition coefficients of the bismercaptoethylamino technetium-99 complexes

Complex	Octanol/saline
Pertechnetate	0.04
Ethyl	0.99
Butyl	2.97
Dodecyl	11.36
Benzyl	0.69
p-Chlorobenzyl	0.54

plexes is the same.

Infrared Spectroscopy

Infrared spectrometry is a powerful tool for the identification of certain moieties bound to technetium. One such moiety is the Tc=O bond. As noted by some authors, the Tc=O stretching frequency is a strong absorption between 900 and 1000 cm^{-1}. As shown in Table X a number of technetium(V) complexes contain this absorption.

The stretching frequency for Tc=O of the charged species listed in Table X is found between 940 and 1050 cm^{-1}. Analogously the infrared spectra of the R-substituted bis(2-mercaptoethyl)amino complexes show two extremely strong absorptions at 920 and 895 cm^{-1}. A comparison of the ethyl and butyl technetium complexes (Figures 22 and 23) with the free ligand (Figures 24 and 25) and the isothiouronium salts (Figures 26 and 27) clearly indicate that no such band is evident in either the ligand or its precursor.

The two strong bands denote two different Tc=O stretches. Two conjectures to explain the double absorption phenomena are- 1) two different complexes are present or 2) a dimeric complex is formed containing two different technetium environments. Rigorous TLC and HPLC analyses demonstrate no perceptable separation of the isolated product. Unless very similar complexes are formed the suggestion of two different complexes may be rejected.

Ultraviolet-visible Spectroscopy

The uv-visible spectra of the technetium complexes while not yielding direct information on structure or stoichiometry does give a qualitative idea of the similarity between the R-substituted complexes. Figures 28 and 29 contain the uv-vis. spectra for the ethyl and butyl

Table X: Tc=O stretching frequencies for technetium-99 complexes

Complex	[Tc=O(cm^{-1})]	Reference
Tetraphenylarsonium oxo[N,N'-ethylenebis-(2-mercaptoacetamido)]technetate(5+)	945	39
Tetraphenylarsonium oxo[N,N'-propylene-bis(2-mercaptoacetamido)]technetate(5+)	960	39
Tetraphenylarsonium oxo[N,N'ethylenebis-(3-mercaptopropionimide)]technetate(5+)	940	39
Tetraphenylarsonium oxo[N,N'-o-phenylene-bis(2-mercaptoacetamido)]technetate(5+)	945	39
Tetrabutylammonium oxotetrabromo-technetate(5+)	1011	49
Tetrabutylammonium oxotetrachloro-technetate(5+)	1020	49
Dibromo[hydrotris(1-pyrazolyl)borato]-oxotechnetate(5+)	970	34
Dichloro[hydrotris(1-pyrazolyl)borato]-oxotechnetate(5+)	970	34
Dioxo bis(1,2-dimercaptoethane)-μ-1,2-dimercaptoethane ditechnetate(5+)	953 946	71

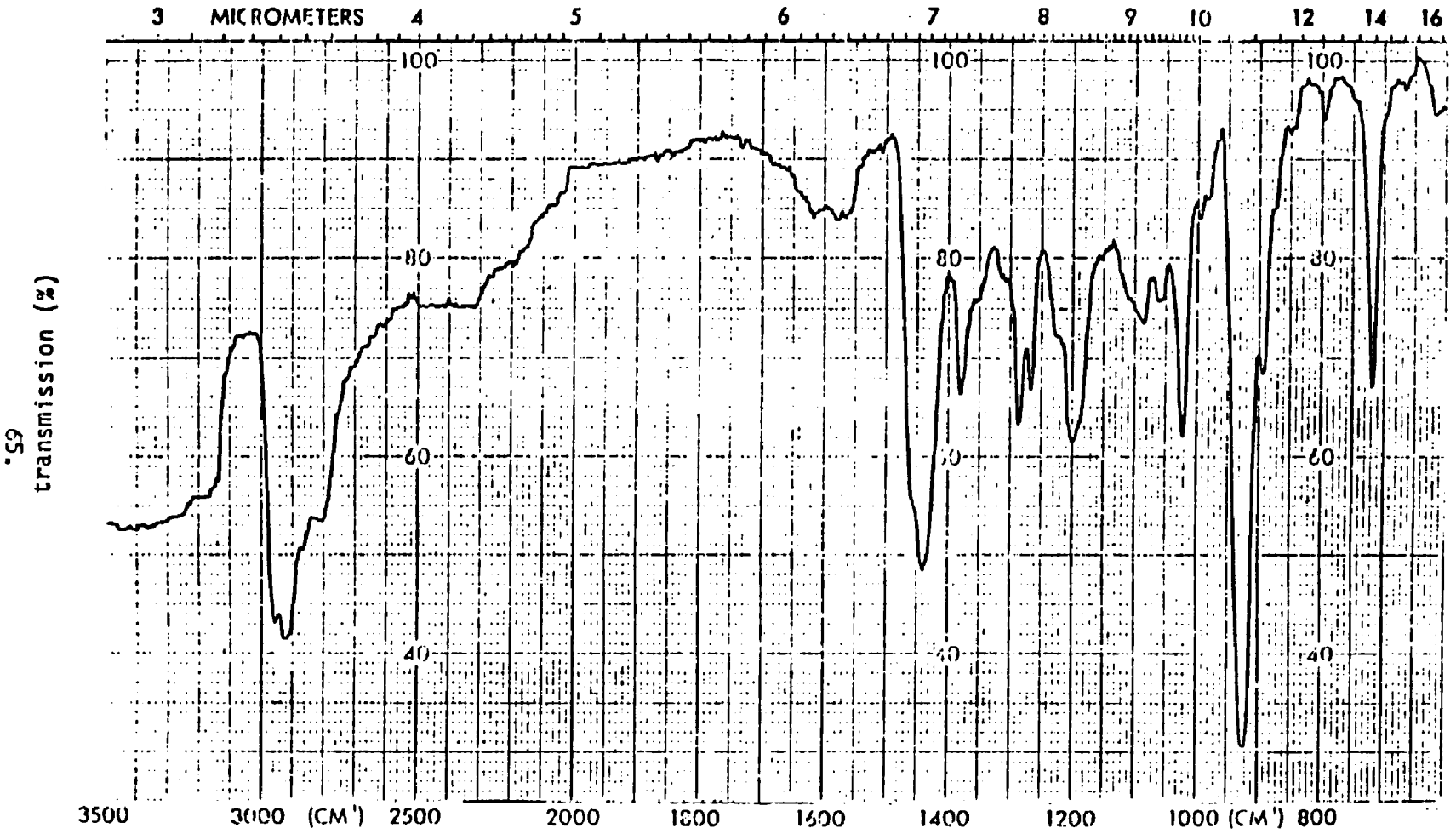


Figure 22: Infrared spectrum of the ethyl-Tc-99 complex.

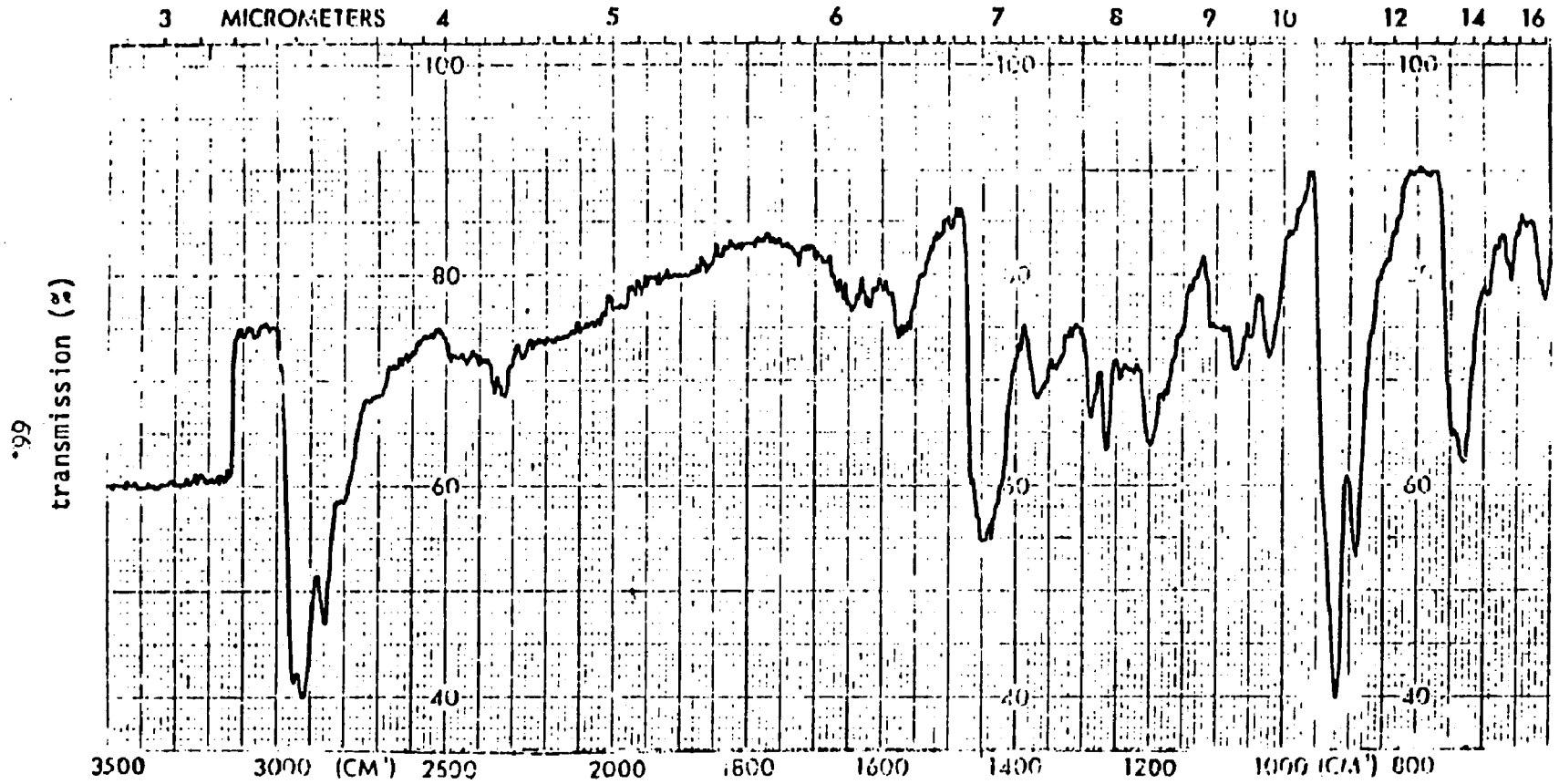


Figure 23: Infrared spectrum of the butyl-Tc-99 complex.

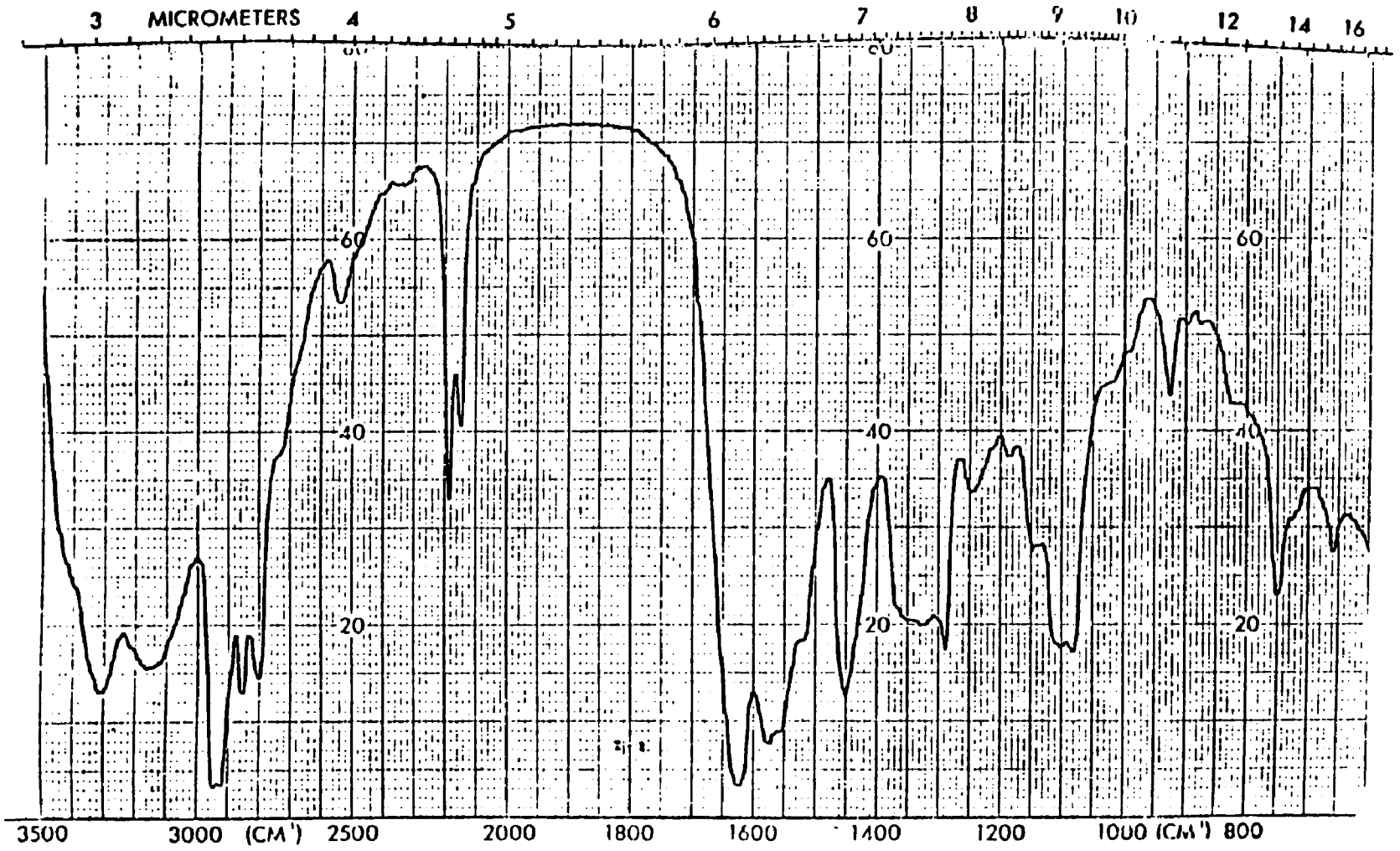


Figure 24: Infrared spectrum of the hydrolyzed butyl bisisothiouronium product.

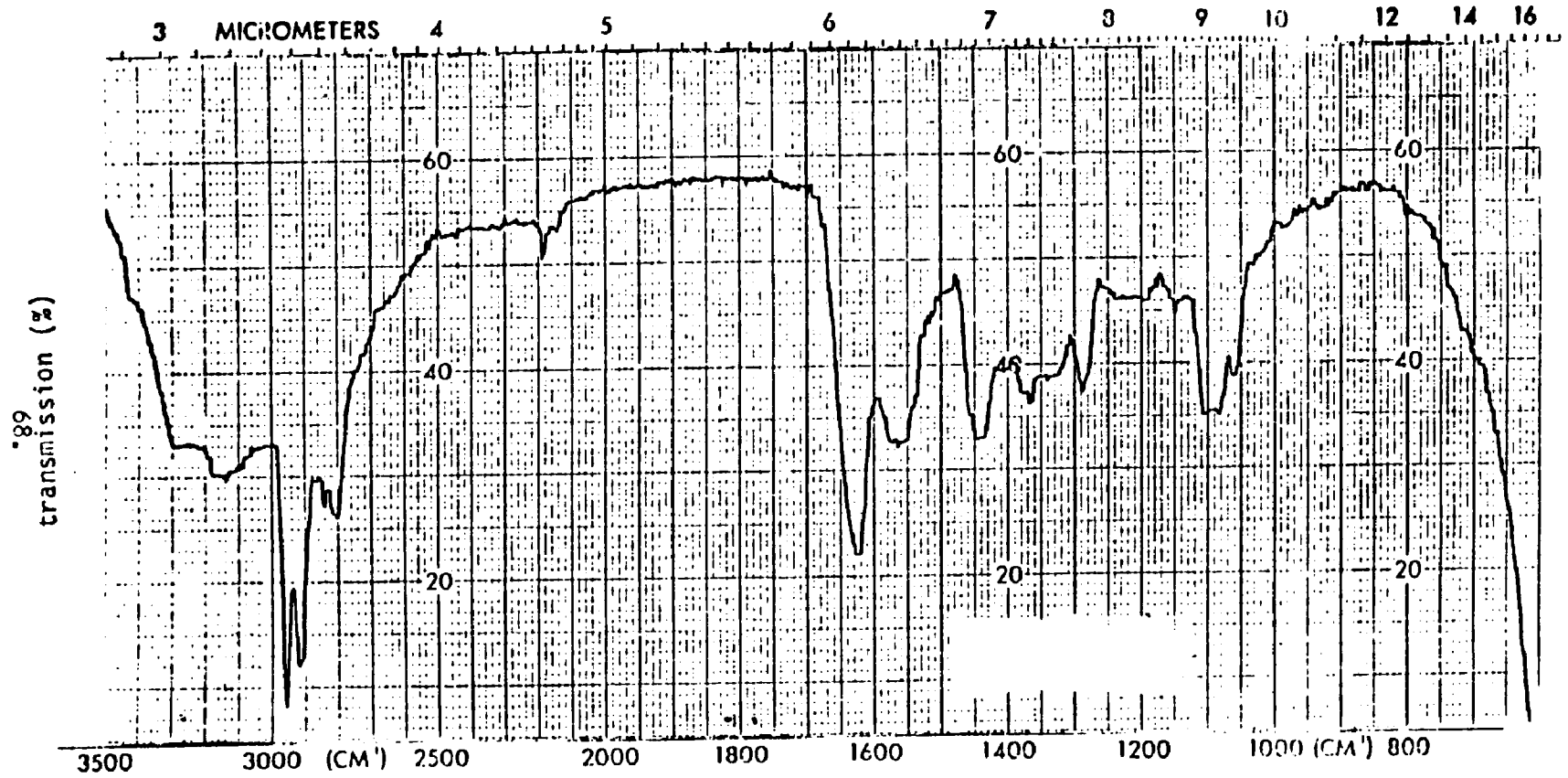


Figure 25: Infrared spectrum of the hydrolyzed ethyl bisisothiouronium product.

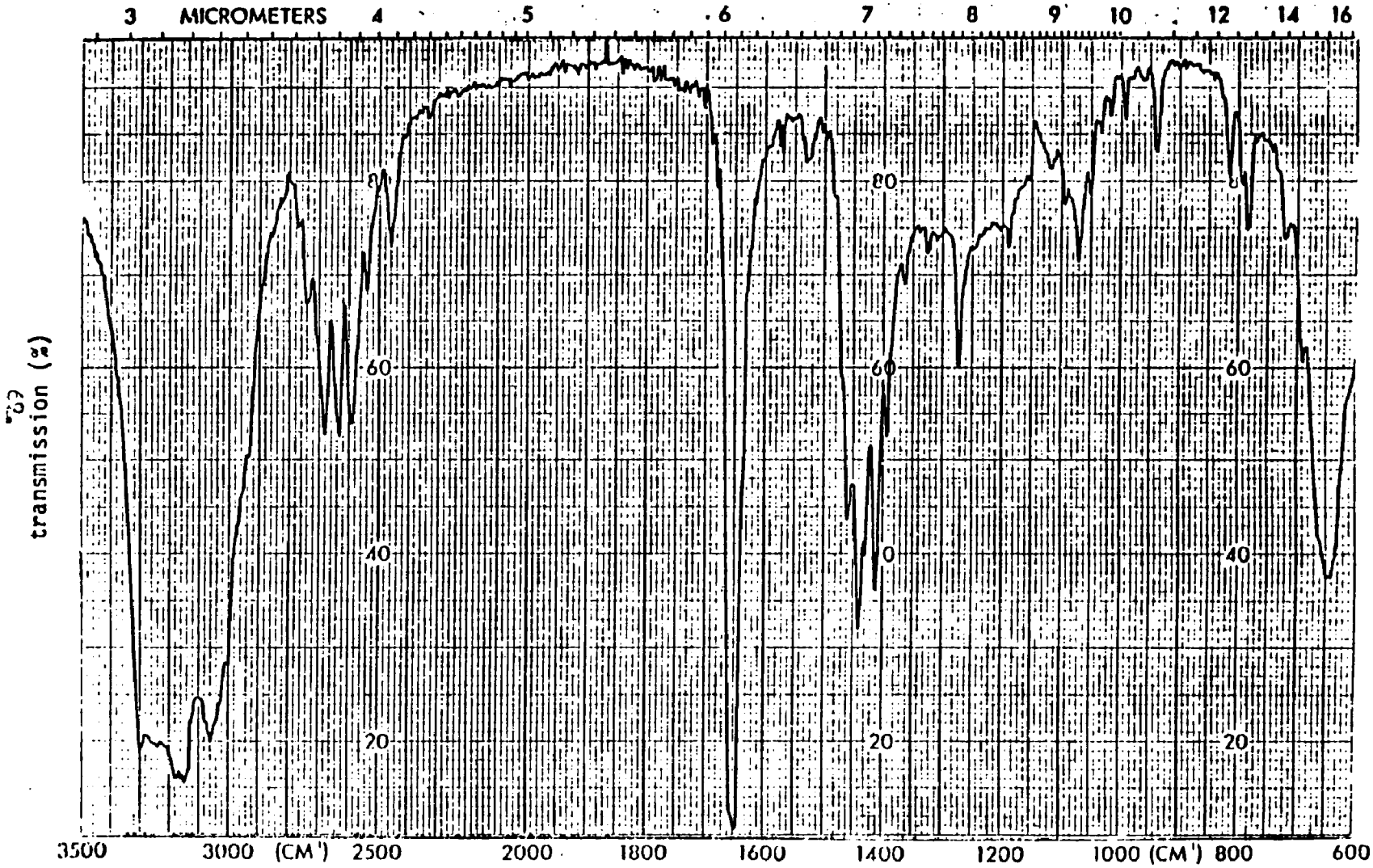


Figure 26: Infrared spectrum of the ethyl bisisothiouronium salt.

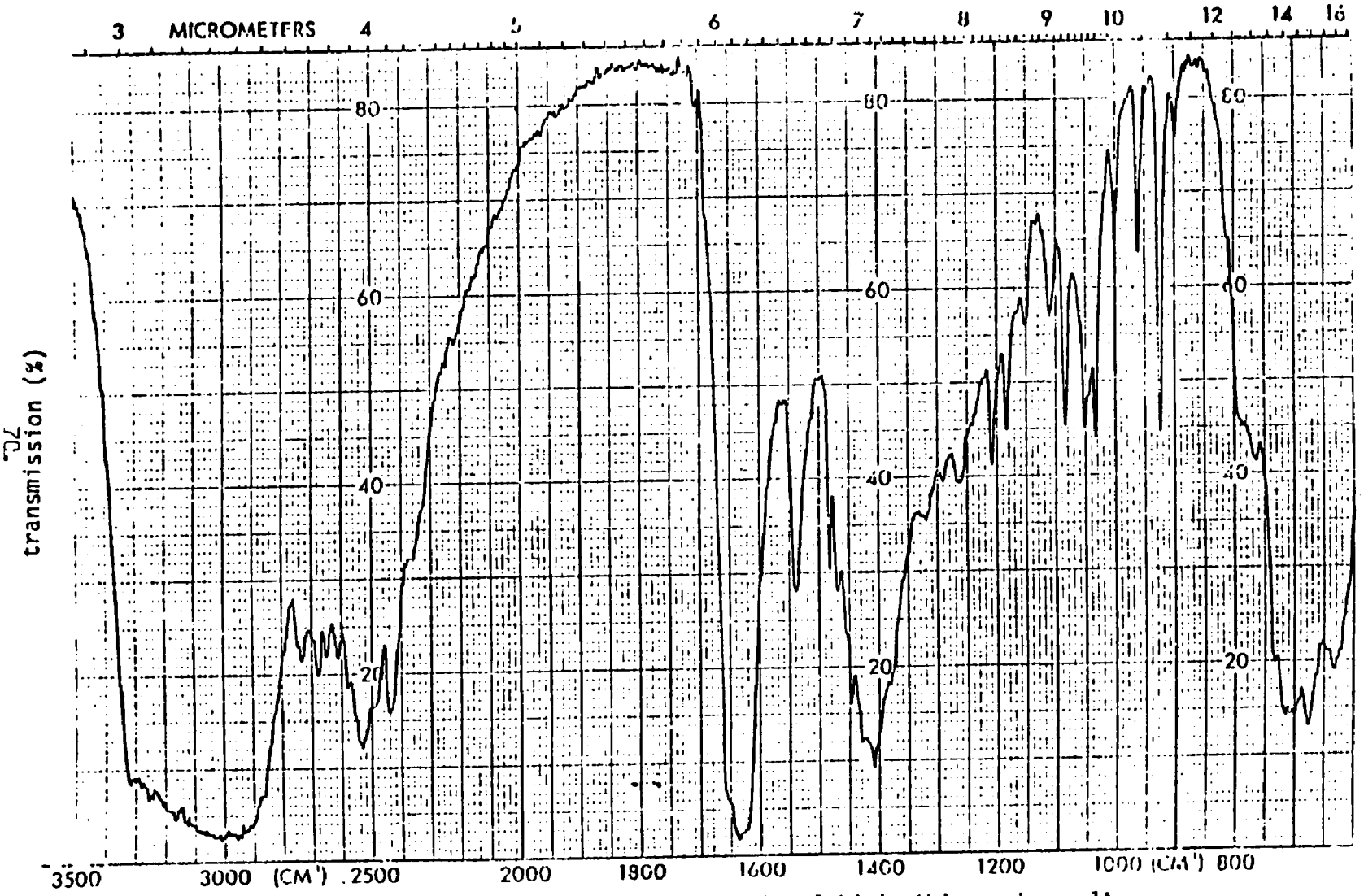


Figure 27: Infrared spectrum of the butyl bisisothiouronium salt.

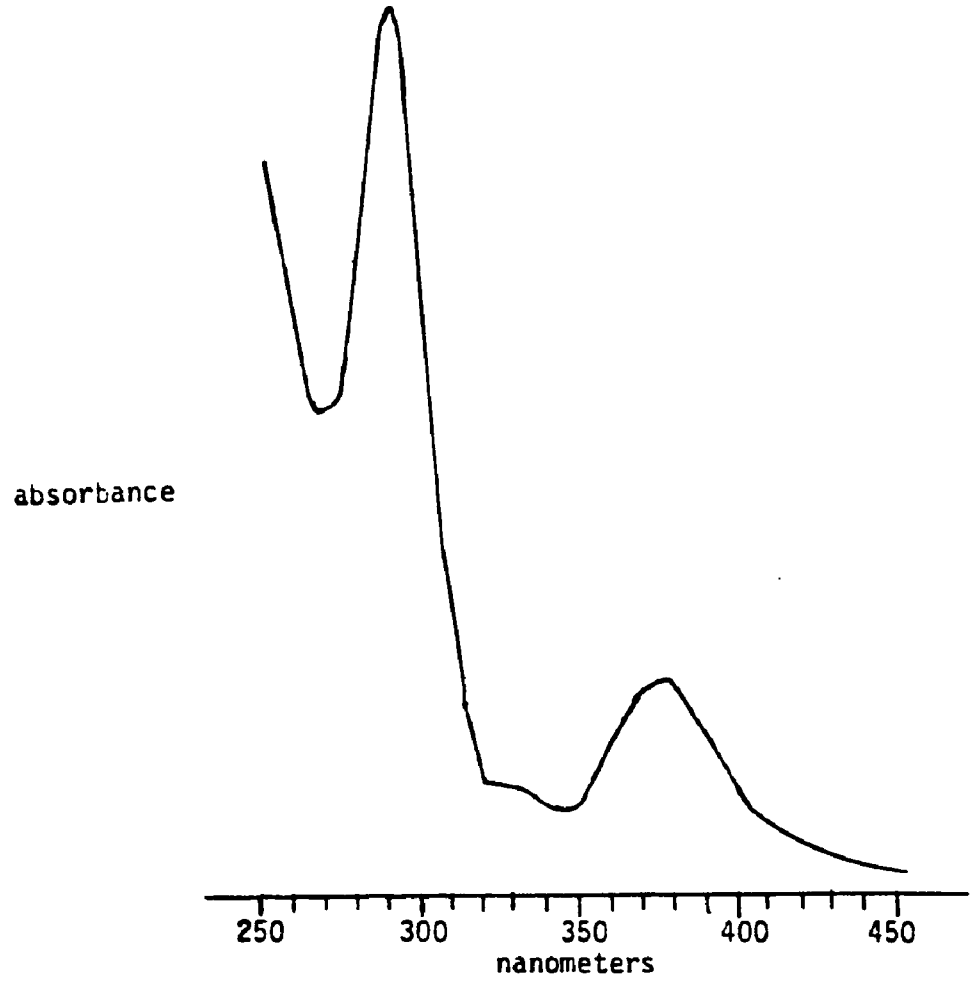


Figure 28: Ultraviolet-visible spectrum of the ethyl-Tc-99 complex.

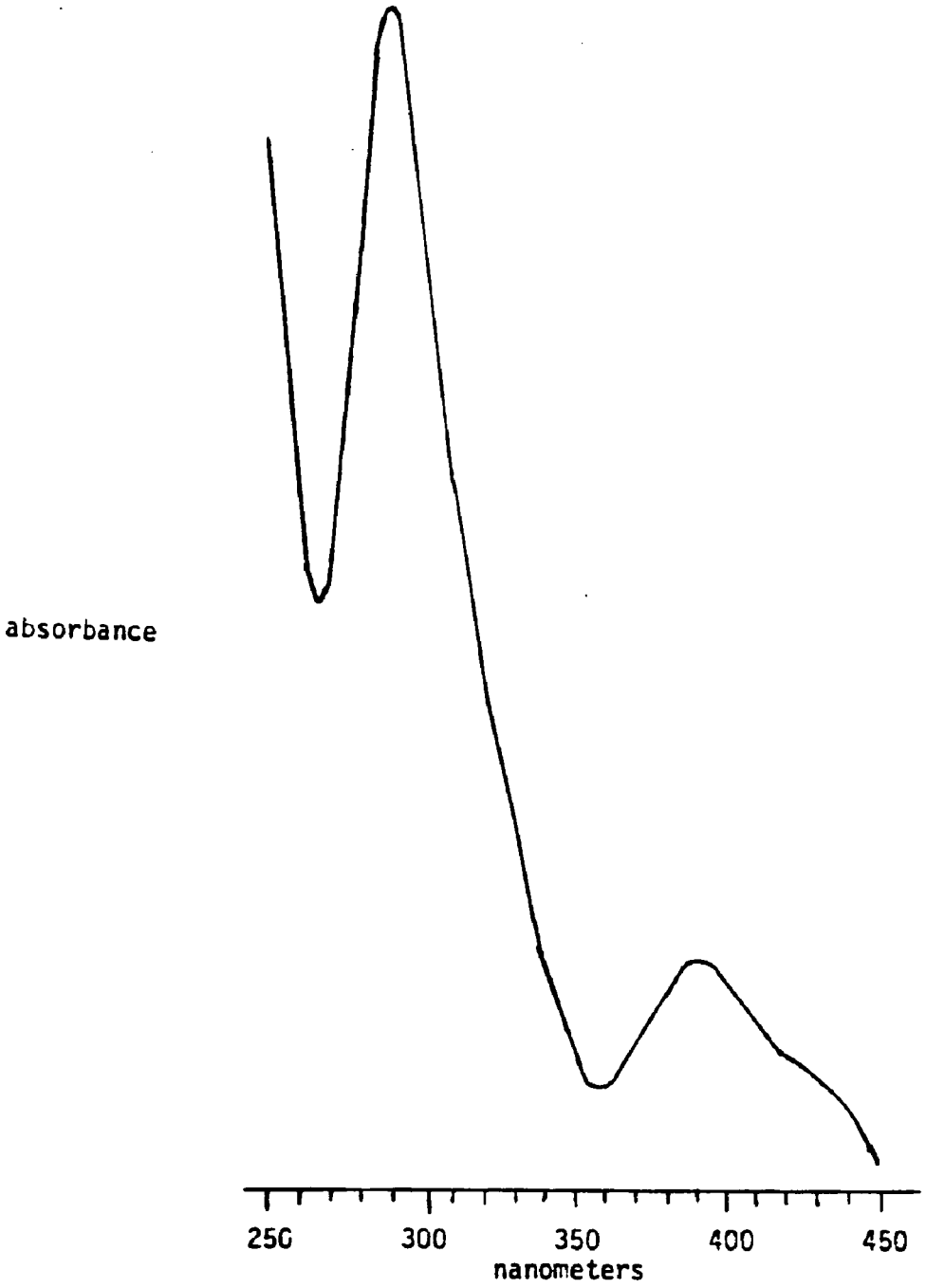


Figure 29: Ultraviolet-visible spectrum of the butyl-Tc-99 complex.

complexes obtained from the borohydride reduction method. As noted two maxima (λ_{max}) are seen at 285 and 390 nm(kk). A shoulder on the 390 nm maxima at approximately 420 nm exists in all spectra. Comparatively pertechnetate has λ_{max} at 244 and 287 nm (18). Again these spectra simply confirm that these complexes have the same or very similar complex coordination spheres. Also, the spectra of complexes isolated by the four synthetic methods were found to be exactly the same, suggesting the same type of complex is formed by each method.

Nuclear Magnetic Resonance

In the literature, proton and carbon-13 NMR spectra of technetium complexes or models there of are a rarity. The rarity is in part due to certain oxidation states of technetium being paramagnetic. As is known, paramagnetism may create unintelligible spectra or spectra with greatly shifted resonance frequencies (72). Conversely, diamagnetic technetium complexes yield relatively normal spectra. The technetium complexes isolated above were found to have normal decipherable spectra thereby indicating diamagnetic technetium is contained in the complex. This fact alone is a major aid on structural assignments.

^{1}H NMR

The proton spectra of the ethyl and butyl complexes are shown in Figure 30 and 31. The spectra are readable and not shifted from normal spectral parameters. The butyl and ethyl NMR indicates unusual peak features. Purusal of the ethyl spectrum shows two distinct methyl triplets in a ratio of 2:1. Two triplets may be indicative of two different complexes in the crystalline product; but, HPLC and TLC analyses negates this hypothesis. Another plausible explanation is that the complex contains ligands incorporated in two different environments

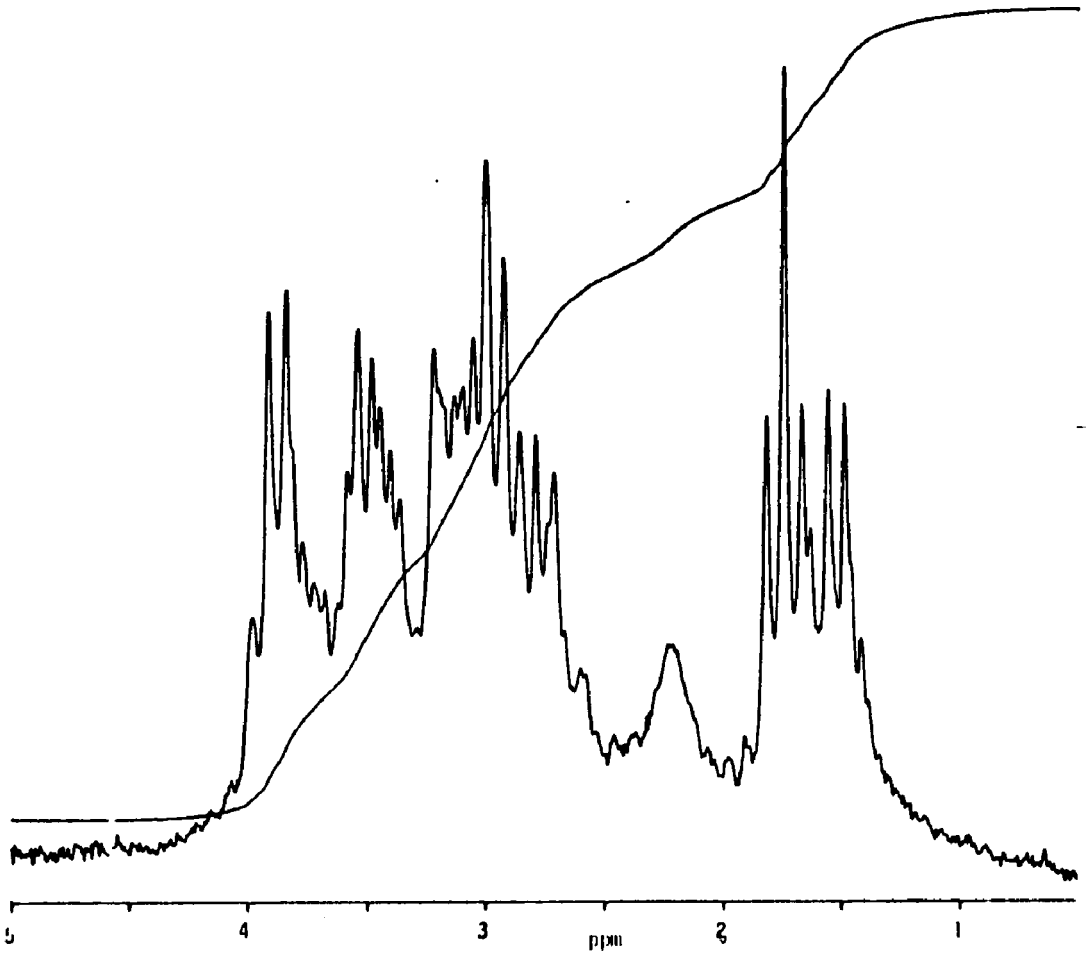


Figure 30: NMR spectrum of the ethyl-Tc-99 complex.

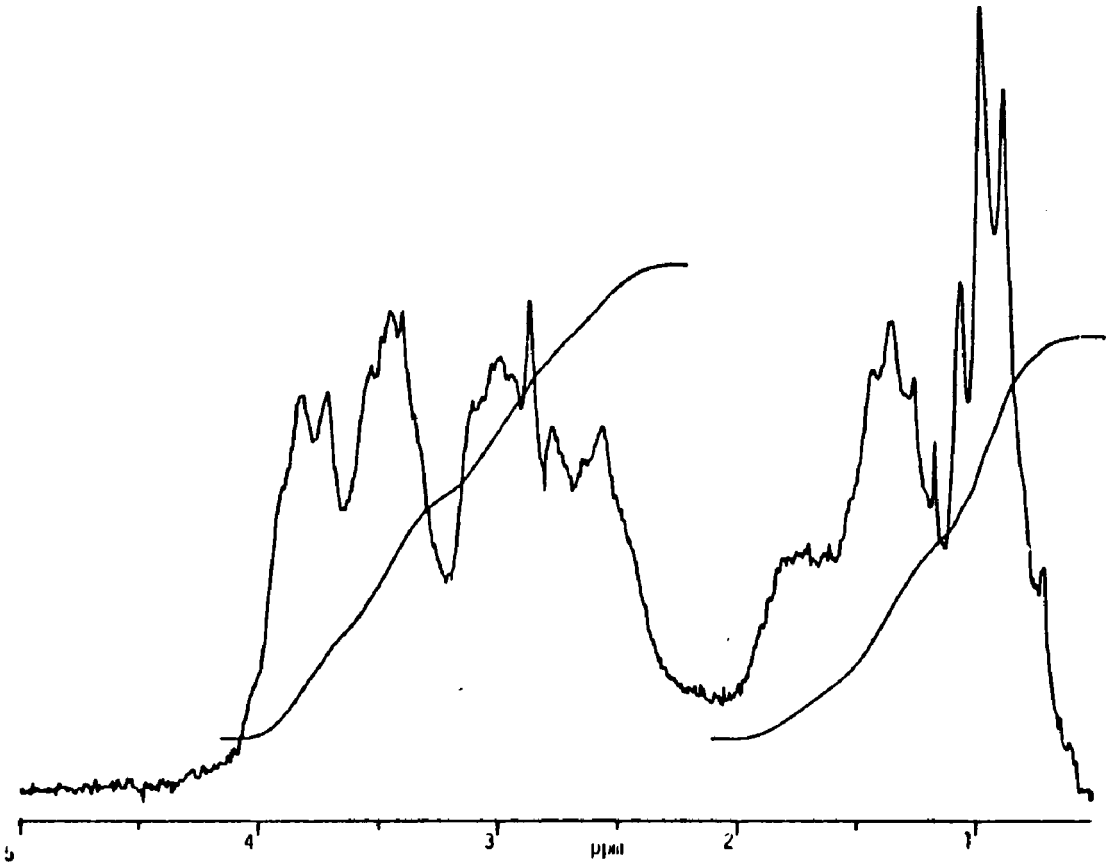


Figure 31: NMR spectrum of the butyl-Tc-99 complex.

in a 2:1 ratio.

^{13}C NMR

Figures 32 to 35 represent the carbon-13 spectra of the ethyl and butyl bisisothiouronium salts and technetium complexes respectively. (Note: The carbon-13 spectra of the hydrolyzed product of the bisisothiouronium salts can not be obtained due to the rapid formation of a precipitate in the NMR tube during scanning.) The resonances may be assigned by information from other authors (36,39,73,74,75):

1) Ethyl bisisothiouronium salt: 8.33, $\underline{C}H_3$; 24.3, $\underline{C}H_2S$; 47.8, $CH_3\underline{C}H_2N$; 50.4, $N\underline{C}H_2CH_2S$.
2) Butyl bisisothiouronium salt: 13.5, $\underline{C}H_3$; 19.5, $CH_3\underline{C}H_2$; 24.8, $\underline{C}H_2S$; 25.5, $NCH_2\underline{C}H_2CH_2$; 51.1, $N\underline{C}H_2CH_2S$, 52.5, $CH_2CH_2\underline{C}H_2N$.
3) Ethyl-technetium complex: 8.70, $\underline{C}H_3$; 36.6, $\underline{C}H_2S$; 48.3, 55.7, 56.1, 60.5, $\underline{C}H_2N$.
4) Butyl-technetium complex: 13.9, $\underline{C}H_3$; 20.7, $CH_3\underline{C}H_2$; 25.5, $CH_3CH_2\underline{C}H_2$; 36.8, $\underline{C}H_2S$; 54.3, 56.2, 61.3, 61.9, $\underline{C}H_2N$.

A comparison of the technetium complex NMR data indicate two magnetically non-equivalent ligands. Although the carbons next to the sulfur appear to be essentially equivalent, the carbons alpha to the nitrogen are non-equivalent as evidenced by two sets of ligand resonances. Also the carbons in the alkyl chain in the beta position (to the nitrogen) to the terminus are in an environment sufficiently far away from the technetium core as to have coincident peaks for all "different" ligands. Since the NMR spectra could not elucidate the stoichiometry, the ethyl and butyl complexes were subjected to mass spectrometry to help predict the structure of the complex (along with all other data obtained).

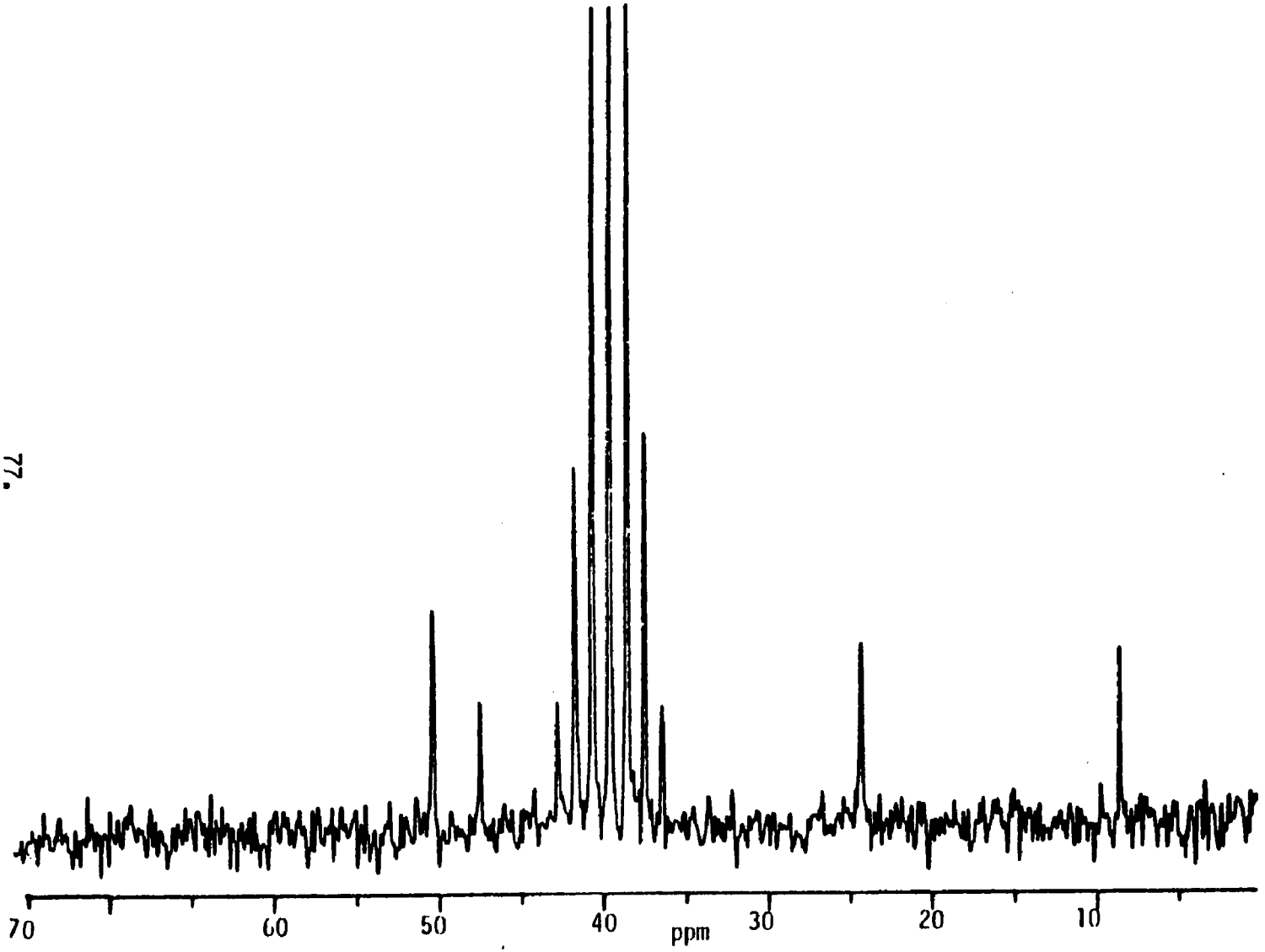


Figure 32: Carbon-13 nmr of the ethyl isothiouronium salt.

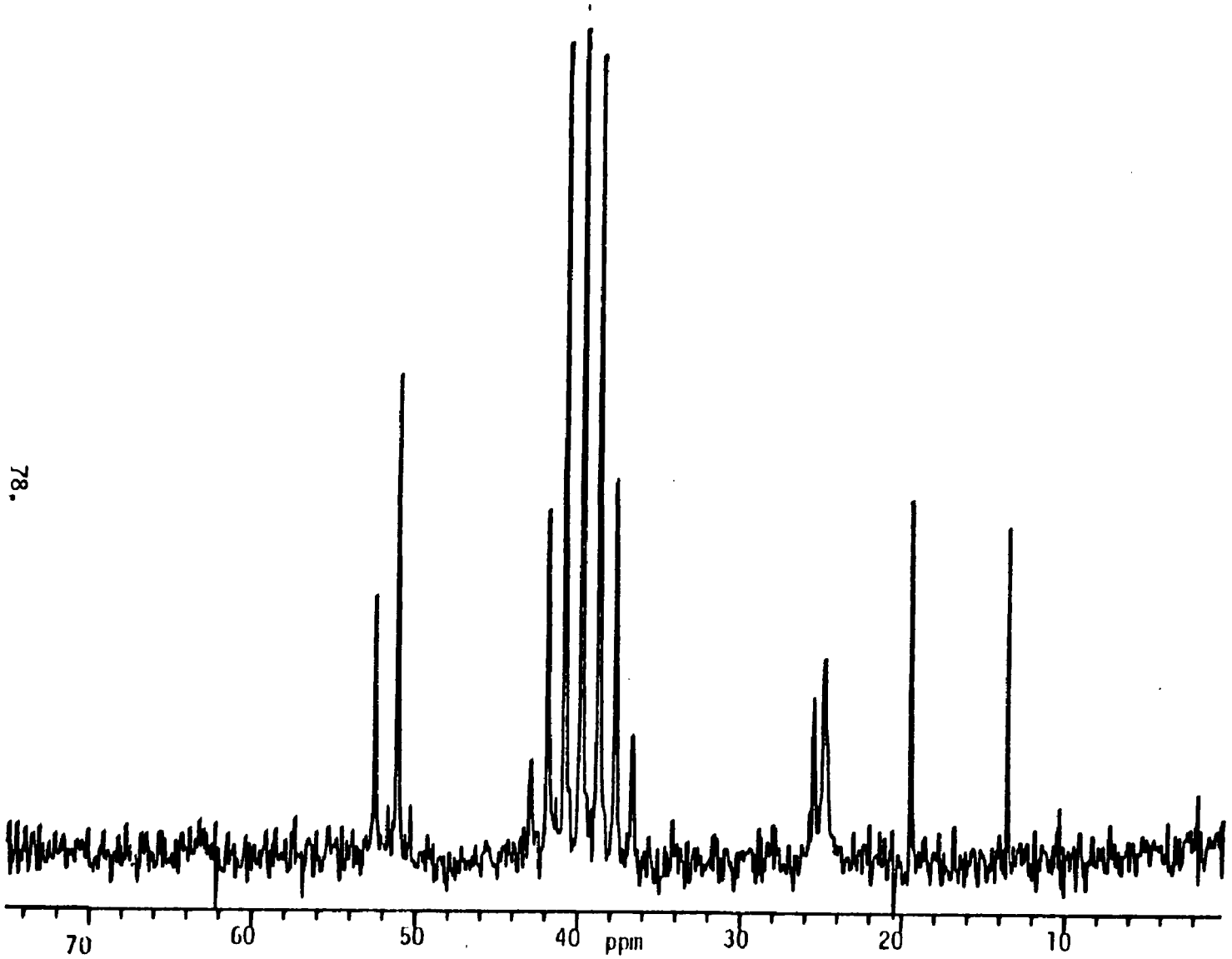


Figure 33: Carbon-13 nmr of the butyl isothiouronium salt.

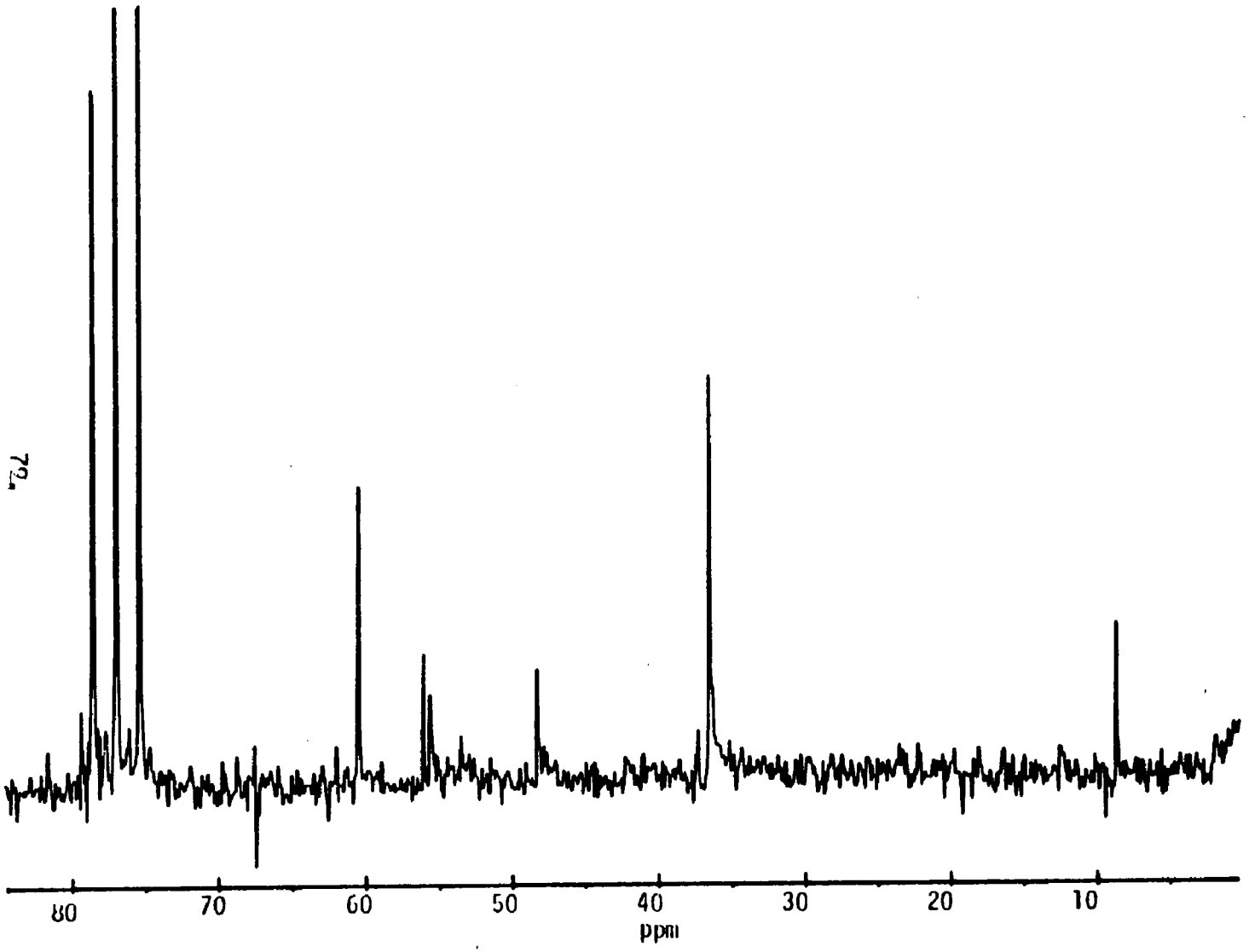


Figure 34: Carbon-13 spectra of the ethyl-Tc-99 complex.

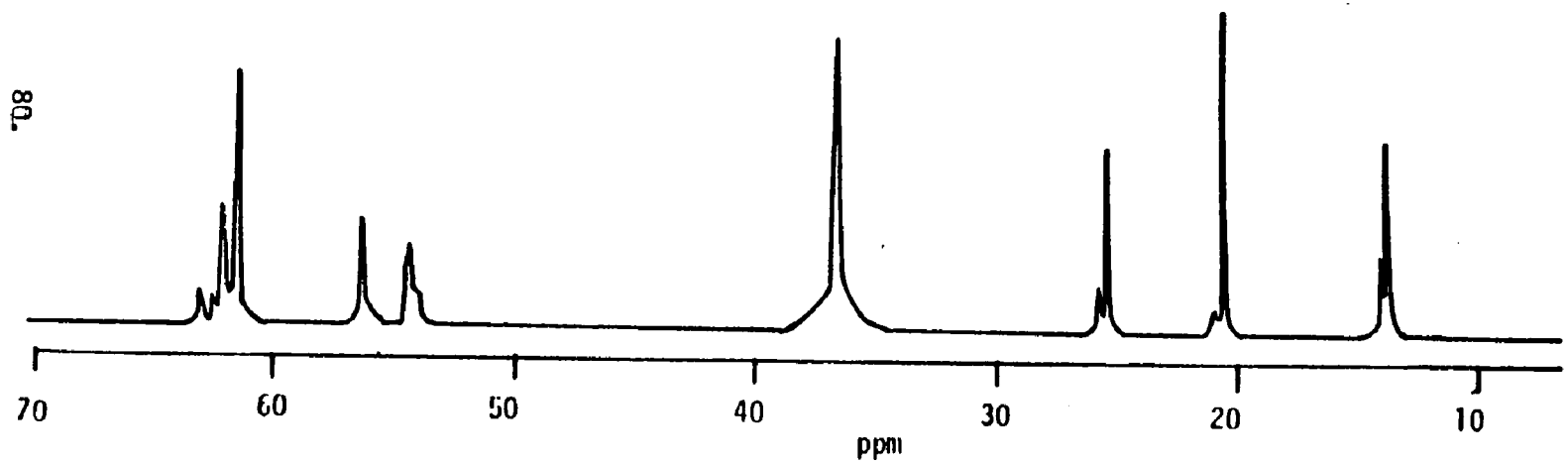


Figure 35: Carbon-13 spectra of the butyl-Tc-99 complex.

Mass Spectrometry

Analysis of the solid technetium-99 complexes was attempted by fast atom bombardment (FAB) and field desorption (FD) mass spectrometry. The positive ion FAB results were poor. The spectra contained large noise peaks and numerous spurious peaks that could not be correlated to any molecular formula for the complexes. Indirectly these results were helpful. The FAB technique is useful for the analysis of ionic species; therefore, the poor results suggest that the complexes are nonionic in character.

FDMS, in comparison, is useful for the analysis of nonionic polar molecules. As drawn in Figures 36 and 37 the butyl and ethyl complexes yield uncomplicated positive ion FD spectra. Due to the limited mass range of the FDMS system the figures are a composite diagram. The ion of highest mass to charge ratio (the parent ion = M^+) has a mass equivalent to the stoichiometry- $L_3Tc_2O_2$ where L = $RN(CH_2CH_2S)_2^{2-}$. Note also that the isotopic peaks of higher mass than the parent have approximately the same peak height ratios for a complex containing six sulfurs. Finally, the fragmentation ions (in order of decreasing mass) are identified as: L_2TcO^+, L_2TcOCl^+, and $LTcO^+$. The first and last fragment ions are typical cleavage products. The second fragment ion is due to sodium chloride. Being ubiquitous in nature, sodium chloride has been known to interact with some substances during mass spectrometry experiments.

Elemental Analysis

To confirm the stoichiometry described in the mass spectrometry section, elemental analysis of the butyl and ethyl complexes were obtained. As noted in Table XI, the analyses corroborate the mass

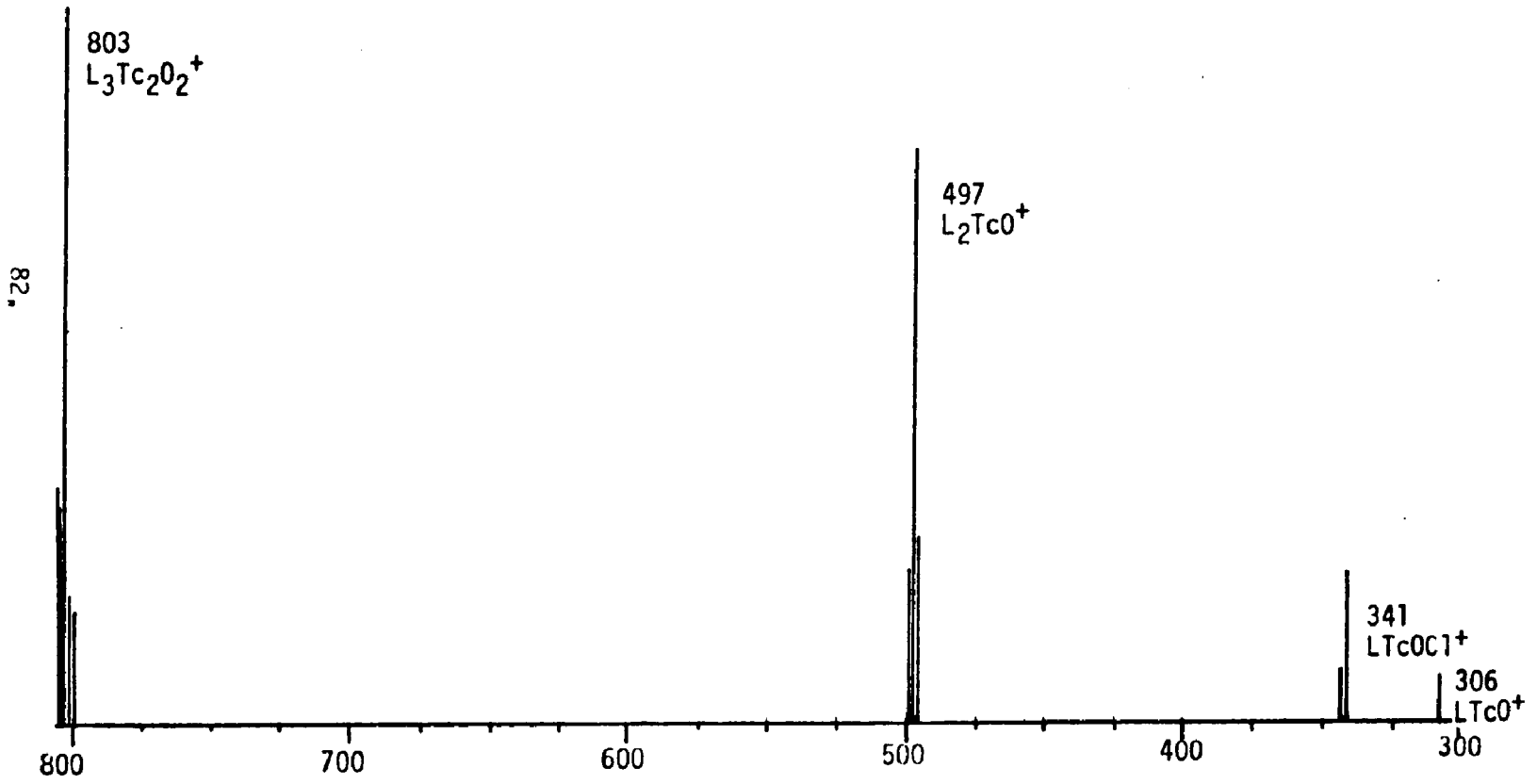


Figure 36: Composite mass spectrum of the butyl-Tc-99 complex.

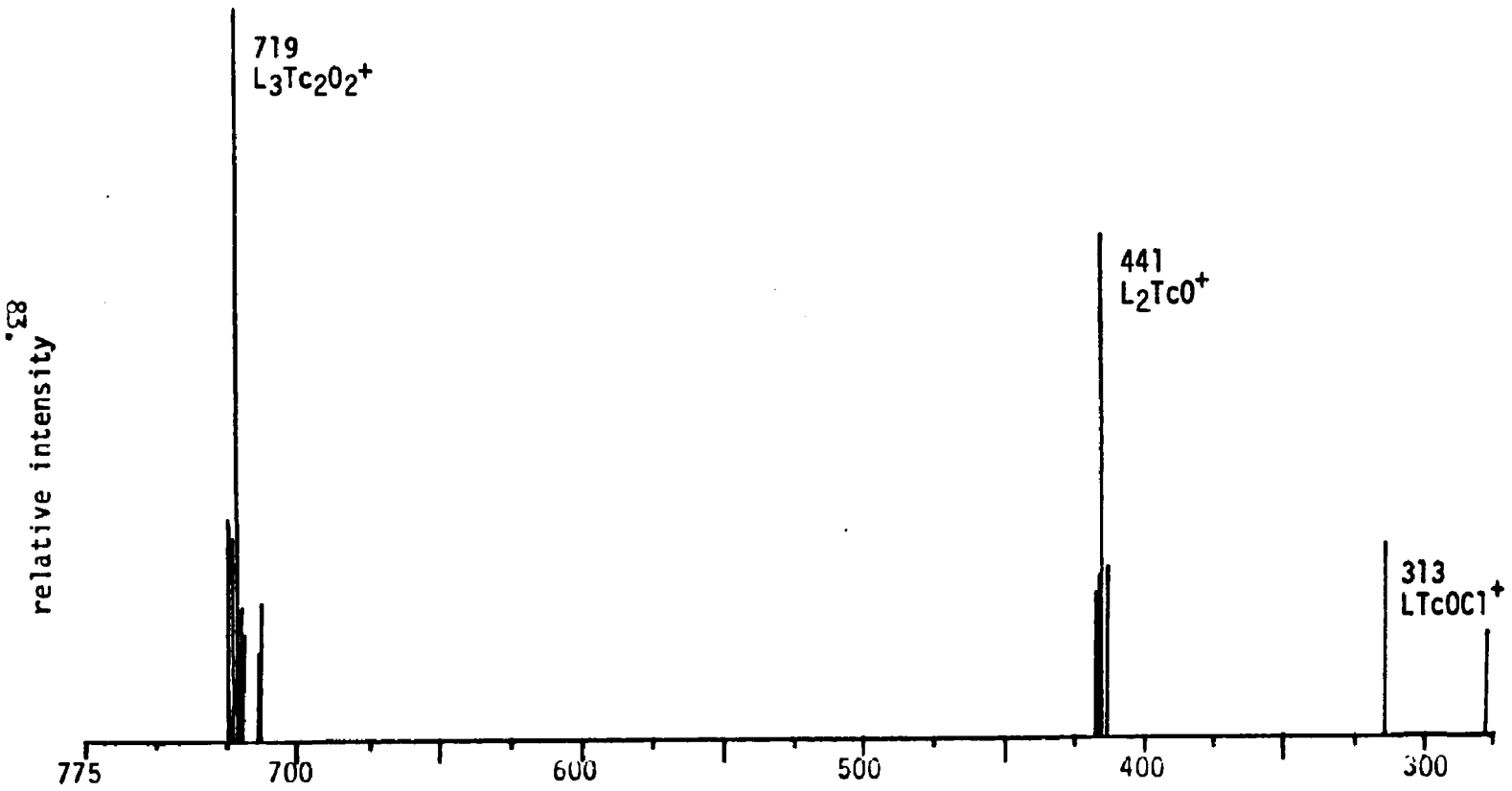


Figure 37: Composite mass spectrum of the ethyl-Tc-99 complex.

Table XI: Elemental analysis of the ethyl and butyl technetium-99 complexes

Complex		C	H	N
Ethyl	Found :	29.20	5.78	5.11
	*Calculated:	29.39	5.31	5.71
Butyl	Found :	34.85	6.01	5.14
	*Calculated:	35.84	6.39	5.22

*Assuming $Tc_2O_2L_3$ stoichiometry

spectral data. The stoichiometry of the complex is $L_3Tc_2O_2$.

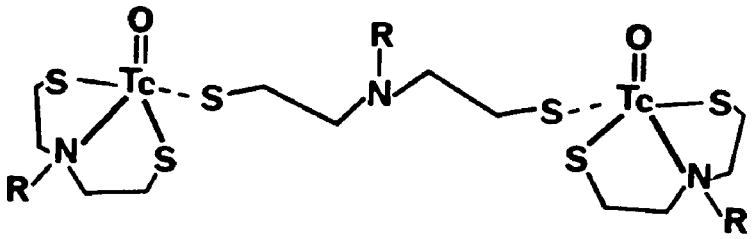
Summary and Conclusion

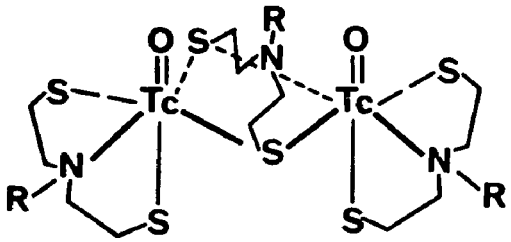
Interpretation of the collective data on the technetium-99 complexes leads to the conclusion that a neutral diamagnetic compound is created via four distinct synthetic methods. While the exact structure of the complex can not be ascertained without x-ray crystallography, a proposed structure and certain bonding details may be postulated. The specific data suggests the presence of:

1. Two Tc=O moieties,
2. N-substituted bis(2-mercaptoethyl)amino ligands,
3. Diamagnetic technetium,
4. Ligands in two different environments in the complex in a ratio of 2:1,
5. A neutral compound, and
6. A stoichiometry of $Tc_2O_2L_3$.

Of course the inferences from the data indicate a single complex of no overall charge containing two technetium centers with an oxo moiety on each center. Also, an interpretation of the two peak phenomenon observed in the technetium-99m experiments may be formulated. Since the final product actually formed in the technetium-99m and -99 studies is a two technetium center moiety, it is highly probable that in very dilute solutions used in the technetium-99m reactions the reaction kinetics are such that the formation of a mono-technetium intermediate is observed. Except for the aryl moieties, the mono-technetium species then proceeds rapidly to the di-technetium product. In order to place the three ligands around the Tc=O centers in a way that yields two different environments, a bridged species must be proposed. The

bridged species would contain one ligand bound to each Tc=O core and a third ligand bridging the two centers as shown below.





Again, these structures were drawn assuming that the diamagnetic technetium is in a +5 oxidation state with each technetium containing an oxo ligand in either a five or six coordinate site. The first structure drawn is less likely due to each technetium center containing only fourteen electrons. The second structure is more favorable due to each center having a sixteen electron count as well as a six coordinate structure. Also, the second structure has a similar literature precedent (71). Of course other structures can not be ruled out until x-ray structure determination is completed.

EXPERIMENTAL SECTION

EQUIPMENT AND ANALYSES

Melting points were determined on a Thomas-Hoover or Fisher-Johns melting point apparatus and are reported uncorrected. Combustion analyses of the ligand precusors were performed by Dr. G.I. Robertson (Florham Park, New Jersey) with radioactive samples analyzed by Schwartzkopf Microanalytical Laboratory (Woodside, New York). NMR spectra were obtained on a Hitachi Perkin-Elmer High Resolution Spectrometer or IBM NR/80 Spectrometer with tetramethylsilane as an internal standard. Infrared spectra were recorded on a Perkin-Elmer 283 or 399B spectrophotometer with samples as Nujol mulls, KBr pellets or in the neat state.

Thin layer chromatography was performed on J.T. Baker Bakerflex, Analtech 250 micron or Eastman-Kodak Silica plates.

Electrophoresis of the radioactive compounds was performed on Whatman 3MM chromatography paper and Gelman Sepraphore III cellulose polyacetate strips with a Gelman Flat bed electrophoretic chamber containing 0.01 N $NaHCO_3$ buffer at a constant 600 volts (direct current) for 15 minutes.

The TLC and electrophoretic radiochromatograms were examined under a 1" x 1" NaI crystal or thin window Geiger-Mueller tube encased in lead with a 1 mm slit.

High Pressure Liquid Chromatography analyses were performed on a Perkin-Elmer Series 212 instrument with a Perkin-Elmer LC-75 ultraviolet detector (254 nm) and/or a 1" x 1" NaI detector. The signals were recorded on a Linear Model 800 dual pen recorder.

SYNTHESIS

I. Synthesis of N-alkyl and N-aryl bis-(2-mercaptoethyl)amine precusors.

2,2'-dichlorodiethylamine hydrochloride (I).

Thionyl chloride (268 g/162 ml, 2.08 mol) dissolved in 100 ml of dry chloroform was added dropwise over a period of 3 hours to a solution of diethanolamine (110 g/100 ml, 1.04 mol) in 100 ml dry chloroform, protected from atmospheric moisture with a $CaCl_2$ drying tube. After a mild exothermic reaction, the solution was stirred overnight with a mechanical stirrer. Upon refluxing the mixture for 15 minutes, a large crystalline mass precipitated from solution. After cooling, the solid was filtered, washed with chloroform and air dried. Recrystallization from acetonitrile yielded 158 g of colorless plates (85% yield) with a melting point of 216- 218°C (lit- 217°C): ir (nujol) 2720, 2425 cm^{-1} (NH salt); NMR (D^6-dmso) δ3.38 (t- 2H, N-CH_2), 4.01 (t, 2H, Cl-CH_2), 9.90 (b, 2H, NH_2 disappears with the addition of D_2O).

1,2-dithia-5-azapane (II) (dithiazapane).

$Na_2S_2 \cdot 9H_2O$ (48.0 g, 0.12 mol) and 6.40 g (0.20 mol) of S were heated to a red melt, cooled and added to a solution containing 30.0 g (0.28 mol) Na_2CO_3. The mixture was diluted to 800 ml with H_2O and ice. To the resulting orange slurry was added 30.0 g (0.17 mol) of 2,2'-dichlorodiethylamine hydrochloride in 50 ml water. The solution was placed in the refrigerator for 48 hours and consequently steam distilled. After the pH of the distillate reached 8, 50 ml of 10% NaOH containing 0.5 g KCN was added to the distillation flask. Five liters of distillate was collected and neutralized with 6 N HCl. Flash evaporation of the distillate yielded a white solid. Recrystallization from absolute ethanol yielded 15.15 g (52%) of white cubic-like crystals:

mp 175-178°C (lit- 178°C); ir (nujol) 2700, 2650, 2550, 2500, 2450, 2385 (NH salt); NMR (D^6-dmso) δ3.22 (m, 8H, -CH_2-CH_2-), 9.85 (b, 2H, NH_2).

Anal. Calcd for $C_4H_{10}ClNS_2$: C, 26.91; H, 5.65

Found: C, 31.16, H, 6.62

N-benzyl-N,N-bis(hydroxyethyl)amine.

To a slurry of 53.0 g (0.50 mol) Na_2CO_3 in 500 ml absolute ethanol containing 52.7 g (0.50 mol, 48.0 ml) of dissolved diethanolamine was added 70.5 g (0.50 mol) benzyl chloride in 250 ml absolute ethanol. The mixture, protected from atmospheric moisture with a $CaCl_2$ tube, was refluxed overnight. After cooling the mixture was filtered and the unwanted salts washed with absolute ethanol. Flash evaporation of the solution yielded a light yellow viscous oil. Vacuum distillation produced 76.8 g (79%) fraction of colorless oil: bp 155- 158°C at 0.9 mm; ir (neat) 3340 cm^{-1} (OH); NMR ($CDCl_3$) δ2.64 (t, 4H, CH_2N), 3.56 (t, 6H, $ArCH_2$, CH_2O), 3.87 (s, 2H, OH), 7.28 (s, 5H, Ar-H).

N-benzyl-N'N-bis(2-chloroethyl)amine hydrochloride.

To 39.1 g (0.20 mol) of N-benzyl-2,2'-bis(hydroxyethyl)amine dissolved in 100 ml dry benzene was added 35.0 ml (0.45 mol, 58.0 g) of thionyl chloride dissolved in 50 ml dry benzene over a 3 hour period. The reaction was mildly exothermic with a oil forming as the $SOCl_2$ was added. The mixture was refluxed until a tan precipitate formed. After cooling, the crystalline solid was filtered, washed with benzene and air dried. Recrystallization from acetone yielded 48.1 g (90%) of a white solid: mp 147-149°C, ir (KBr) 2670, 2545, 2425, 2355 cm^{-1} (NH salt); NMR (D^6-dmso) δ3.45 (t, 4H, CH_2N), 4.11 (t, 5H, CH_2Cl, NH), 5.01 (s, 2H, $ArCH_2$), 7.51 (m, 3H, Ar-H), 7.63 (m, 2H, Ar-H).

Anal. Calcd for $C_{11}H_{16}NCl_3$: C, 49.19; H, 6.00; N, 5.22

Found: C, 49.40; H, 5.93; N, 5.06

N-benzyl-N,N-bis(2-isothioureylethyl)amine trihydrochloride.

To 10.7 g (0.04 mol) of the benzylnitrogen mustard was added 6.24 g (0.08 mol) of thiourea and 0.5 g NaI. After refluxing in 200 ml of absolute ethanol for 24 hours, the resultant yellow solution was cooled, filtered and flash evaporated to yield an off-white gum. The gum was redissolved in a minimum of boiling absolute ethanol and cooled to 0°C. 13.79 g (82%) of white crystalline product formed: mp 199-201°C (effervesces); ir (KBr) 3240 cm^{-1} (broad, NH), 1690 cm^{-1} (C=N); NMR (D^6-dmso) δ3.36 (b, 4H, $C\underline{H}_2N$), 3.79 (b, 4H, $C\underline{H}_2S$), 4.43 (s, 2H, $ArC\underline{H}_2$), 7.51 (m, 5H, Ar-$\underline{H}$), 8.87 (b, 9H, $N\underline{H}$ disappears with the addition of D_2O).

Anal. Calcd for $C_{13}H_{24}Cl_3N_5S_2$: C, 37.10; H, 5.75; N, 16.14

Found: C, 37.09; H, 6.02; N, 16.43

N-(p-chlorobenzyl)-N,N-bis(2-hydroxyethyl)amine.

8.05 g (0.05 mol) p,α-dichlorotoluene, 4.8 ml (0.05 mol, 5.27 g) diethanolamine and 5.30 g (0.05 mol) Na_2CO_3 were slurried into 200 ml absolute ethanol and reflux overnight. The mixture was cooled, filtered and flash evaporated to yield a light yellow oil. An 8.10 g (71%) fraction was collected upon vacuum distillation: bp 177-179°C at 1.6 mm; ir (neat) 3200 cm^{-1} (OH); NMR ($CDCl_3$) δ2.57 (t, 4H, $C\underline{H}_2N$), 3.57 (t, 6H, $C\underline{H}_2O$, $O\underline{H}$), 4.33 (s, 2H, $ArC\underline{H}_2$), 7.19 (s, 4H, Ar-$\underline{H}$).

N-(p-chlorobenzyl)-N,N-bis(2-chloroethyl)amine hydrochloride.

6.0 ml (0.08 mol, 10 g) of $SOCl_2$ dissolved in 20 ml dry benzene was added dropwise to 8.10 g (0.04 mol) of the p-chlorobenzyldialcohol over 3 hours. The solution was brought to reflux for 45 minutes. After

cooling an oil formed. Upon stirring overnight a tan solid formed. The solid was filtered, washed with benzene and air dried. Recrystallization from acetone yielded 10.3 g (97%) of a tan solid: mp 138-141°C; ir (KBr) 2675, 2550 cm^{-1} (NH): NMR (D^6-dmso) δ3.40 (t, 4H, $C\underline{H}_2N$), 4.05 (t, 5H, $C\underline{H}_2O$, $N\underline{H}$), 4.43 (s, 2H, $ArC\underline{H}_2$), 7.48 (d, 2H, Ar-$\underline{H}$), 7.72 (d, 2H, Ar-H).

N-(p-chlorobenzyl)-N,N-bis(2-isothioureylethyl)amine trihydrochloride.

The p-chlorobenzyl nitrogen mustard (4.80 g, 0.02 mol), 2.44 g (0.03 mol) of thiourea and 0.20 g of NaI were added to 250 ml of absolute ethanol and refluxed for 12 hours. The solution was cooled to room temperature and allowed to sit overnight. A white precipitate formed, was collected and recrystallized from absolute ethanol yielding 6.37 g (88%) of white crystals: mp 198-200°C; ir (KBr) 3260 cm^{-1} (broad, NH), 1650 cm^{-1} (C=N); NMR (D^6-dmso) δ3.33 (b, 4H, $C\underline{H}_2N$), 3.73 (b, 4H, $C\underline{H}_2S$), 4.40 (s, 2H, $ArC\underline{H}_2$), 7.49 (d, 2H, Ar-$\underline{H}$), 7.71 (d, 2H, Ar-$\underline{H}$), 9.48 (b, 9H, $N\underline{H}$).

Anal. Calcd for $C_{13}H_{23}Cl_4N_5S_2$: C, 34.29; H, 5.09; N, 15.38

Found: C, 34.01; H, 5.15, N, 15.11

N,N-bis(2-hydroxyethyl)-N-(1-naphthyl)methylamine.

The chloromethylnaphthylene (49.8 g, 0.28 mol) in 25 ml absolute ethanol was added to a slurry of 29.9 g (0.28 mol) Na_2CO_3 and 27 ml (0.28 mol, 29.7 g) diethanolamine in 150 ml absolute ethanol. After an overnight reflux, the solution was filtered and flash evaporated to yield a yellow oil. Vacuum distillation gave a golden yellow fraction-59.1 g (86%): bp 203-204°C at 1.2 mm; ir (neat) 3360 cm^{-1} (OH); NMR ($CDCl_3$) δ2.63 (t, 4H, $C\underline{H}_2N$), 3.22 (s, 2H, $O\underline{H}$, disappears with the addition of D_2O), 3.44 (t, 4H, $C\underline{H}_2O$), 4.03 (s, 2H, $ArC\underline{H}_2$), 7.40 (m, 4H,

Ar-H), 7.71 (m, 2H, Ar-H), 8.22 (m, 1H, Ar-H).

N,N-bis(2-chloroethyl)-N-(1-naphthyl)methylamine hydrochloride.

Thionyl chloride (7.80 ml, 0.10 mol) in 50 ml of dry chloroform was added dropwise to 12.3 g (0.05 mol) of the hydroxyethyl naphthalene in 100 ml chloroform over 3 hours. The solution was refluxed for 4.5 hours yielding a white precipitate upon cooling. The precipitate was elutriated with acetone after filtration and washing with chloroform. 14.4 g (87%) of solid was collected: mp 149-152°C; ir (KBr) 2680, 2550 cm^{-1} (NH); NMR (D^6-dmso) δ3.49 (t, 4H, CH_2N), 4.07 (t, 5H, CH_2Cl, NH), 4.91 (s, 2H, $ArCH_2$), 7.70 (m, 3H, Ar-H), 7.79 (m, 3H, Ar-H), 8.40 (m, 1H, Ar-H).

Anal. Calcd for $C_{15}H_{18}Cl_3N$: C, 56.53; H, 5.70; N, 4.24

Found: C, 56.61; H, 5.74; N. 4.01

N,N-bis(2-isothioureylethyl)-N-(1-naphthyl)methylamine trihydrochloride.

The naphthylnitrogen mustard (8.27 g, 0.03 mol), 3.81 g (0.05 mol) thiourea and 0.2 g NaI dissolved in 80 ml absolute ethanol were refluxed overnight. After filtering and flash evaporating the solvent, a light yellow-green gum was isolated and elutriated with benzene to yield 10.5 g (89%) of a hygroscopic solid: mp 142-145°C (effervesces at 111°C); ir (KBr) 3300 cm^{-1} (very broad, NH), 1680 cm^{-1} (C=N); NMR (D^6-dmso) δ3.35 (b, 4H, CH_2N), 3.74 (b, 4H, CH_2S), 4.45 (s, 2H, $ArCH_2$), 7.32 (m, 4H, Ar-H), 7.59 (m, 2H, Ar-H), 7.81 (m, 1H, Ar-H), 9.43 (b, 9H, NH).

Anal. Calcd for $C_{17}H_{28}Cl_3N_5OS_2$: C, 41.76; H, 5.77; N, 14.32

Found: C, 42.12; H, 5.95; N, 14.11

N-ethyl-N,N-bis(2-hydroxyethyl)amine.

Ethyl iodide, (7.80 g, 0.05 mol), 4.80 ml (0.05 mol, 5.27 g) diethanolamine and 5.30 g (0.05 mol) Na_2CO_3 were added to 100 ml absolute ethanol and stirred at 50°C for 24 hours. After filtering, the solution was flash evaporated to yield a light yellow oil. Vacuum distillation yielded 5.71 g (87%) of colorless oil: bp 149-151°C (2.5 mm); ir (neat) 3370 cm^{-1} (OH); NMR ($CDCl_3$) δ1.10 (t, 3H, CH_3), 2.58 (t, 6H, CH_2N), 3.59 (t, 4H, CH_2O), 4.55 (s, 2H, OH).

N-ethyl-N,N-bis(2-chloroethyl)amine hydrochloride.

To 10.5 g (0.08 mol) of the ethyldiethanolamine in 75 ml benzene was added dropwise over 3 hours 13.0 ml (0.17 mol, 21.6 g) $SOCl_2$ in 50 ml benzene. A 15 minute reflux yielded a brown oil which upon flash evaporation became a tan solid. Recrystallization from methylethylketone yields a white solid with a mass of 25.3 g (88%): mp 76-79°C; ir (KBr) 2605 cm^{-1} (broad, NH); NMR (D^6-dmso) 1.16 (t, 3H, CH_3), 3.61 (t, 7H, CH_2NH), 4.10 (t, 4H, CH_2Cl).

Anal. for $C_6H_{14}Cl_3N$: C, 34.86; H, 6.85; N, 6.78

Found: C, 34.75; H, 6.83; N, 6.74

N-ethyl-N,N-bis(2-isothioureylethyl)amine trihydrochloride.

The ethyl nitrogen mustard (6.46 g, 0.02 mol) dissolved in absolute ethanol, 3.05 g (0.04 mol) thiourea and 0.2 g NaI were added to 50 ml of absolute ethanol and reflux overnight. After filtration and vacuum evaporation, a white solid was isolated. After recrystallization from absolute ethanol, 5.81 g (90%) of a white crystalline solid was isolated: mp 106-108°C; ir (KBr) 3150, 2650 cm^{-1} (broad, NH), 1650 cm^{-1} (C=N); NMR (D^6-dmso) δ1.26 (t, 3H, CH_3-CH_2-N), 3.32 (b, 6H, CH_2N), 3.64 (b, 4H, CH_2S), 9.48 (b, 9H, NH).

Anal. for $C_8H_{12}Cl_3N_5S_2$: C, 26.79; H, 6.19; N, 9.88

Found: C, 26.83; H, 6.25; N, 9.69

N-butyl-N,N-bis(2-hydroxyethyl)amine.

Butyl bromide (4.50 ml, 0.05 mol, 5.74 g), 4.80 ml (0.05 mol, 5.27 g) of diethanolamine and 5.30 g (0.05 mol) of Na_2CO_3 were added to 100 ml of absolute ethanol and refluxed overnight. After filtration and vacuum evaporation of the solvent a yellow oil was isolated. The oil was vacuum distilled to yield 6.85 g (85%) of a colorless oil: bp 148-150°C at 1.2 mm; ir (neat) 3375 cm^{-1} (OH); NMR ($CDCl_3$) δ0.89 (t, 3H, CH_3), 1.38 (m, 4H, CH_3-(CH_2)$_2$); 2.60 (t, 6H, CH_2N), 3.56 (t, 4H, CH_2O), 4.39 (s, 2H, OH).

N-butyl-N,N-bis(2-chloroethyl)amine hydrochloride.

To 6.45 g (0.04 mol) of butyl diethanolamine in 30 ml $CHCl_3$ was added 7.0 ml (0.09 mol, 11.6 g) $SOCl_2$ over 3 hours. The solution was refluxed 30 minutes to yield a dark brown solution. Vacuum evaporation of the solvent left a brown waxy solid. The solid was recrystallized from methylethylketone and washed with dry diethylether to yield 7.75 g (83%) of an off-white solid; mp 89-91°C; ir (KBr) 3425, 2605 (NH); NMR (D^6-dmso) δ0.91 (t, 3H, CH_3), 1.49 (m, 4H, CH_3-(CH_2)$_2$), 3.19 (t, 7H, CH_2-NH), 4.08 (t, 4H, CH_2Cl).

Anal. for $C_8H_{18}Cl_3N$: C, 40.96; H, 7.73; N, 5.97

Found: C, 40.79; H, 7.69; N, 5.91

N-butyl-N,N-bis(2-isothioureylethyl)amine trihydrochloride.

The butyl nitrogen mustard (3.92 g, 0.02 mol), 2.59 g (0.03 mol) thiourea and 0.2 g NaI were refluxed overnight in 40 ml absolute ethanol. After filtration and vacuum evaporation, the resulting off-white gum was triturated with isopropyl alcohol to yield 4.70 g (72%) of an off-white

solid: mp 197-199°C, ir (KBr) 3000 cm^{-1} (broad, NH), 1640 cm^{-1} (C=N); NMR (D^6-dmso) δ0.90 (t, 3H, $C\underline{H}_3$), 1.52 (m, 4H, CH_3-($C\underline{H}_2$)$_2$), 3.48 (t, 6H, $C\underline{H}_2N$), 3.67 (t, 4H, $C\underline{H}_2S$), 9.51 (s, broad, 9H, N$\underline{H}$).

Anal. for $C_{10}H_{26}Cl_3N_5S_2$: C, 31.05; H, 6.72; N, 18.11

Found: C, 30.98; H, 6.84; N, 17.94

N-dodecyl-N,N-bis(2-hydroxyethyl)amine.

1-chlorododecane (10.2 g, 0.05 mol), 4.80 ml (0.05, 5.27 g) of diethanolamine and 5.30 g (0.05 mol) of Na_2CO_3 in 100 ml of absolute ethanol were refluxed overnight. After filtration and vacuum evaporation of the solvent, a yellow oil was isolated. Vacuum distillation yielded 9.30 g (68%) of a light yellow oil: bp 183-184°C at 1.2 mm; ir (neat) 3380 cm^{-1} (OH); NMR ($CDCl_3$) δ0.89 (t, 3H, $C\underline{H}_3$), 1.29 (m, 20H, CH_3-($C\underline{H}_2$)$_{10}$), 2.62 (t, 6H, $C\underline{H}_2N$), 3.60 (t, 1H, $C\underline{H}_2O$), 4.20 (s, 2H, O$\underline{H}$).

N-dodecyl-N,N-bis(2-chlorethyl)amine hydrochloride.

To 21.9 g (0.08 mol) of dodecyl diethanolamine in 75 ml of petroleum ether (50-110°C) was added 13.0 ml (0.17 mol, 21.58 g) of $SOCl_2$ over 3 hours. A vigorous reaction occurred with the formation of a brown oil. The mixture was refluxed 30 minutes; and, after evaporation of the solvent a brown oil was isolated which solidified upon sitting. The brown wax was dissolved in boiling absolute ethanol, 0.5 g decolorizing carbon added and the resultant mixture stirred for 30 minutes. After a hot filtration, a light brown wax was isolated from the vacuum evaporation of the solvent. Elutriation of the solid yielded 13.79 g (50%) of a white waxy solid: mp 61.5-63.5°C; ir (KBr) 2590 cm^{-1} (broad, NH); NMR (D^6-dmso) δ1.21 (bm, 23H, $C\underline{H}_3$-$C\underline{H}_2$)$_{10}$), 3.70 (m, 6H, $C\underline{H}_2N$), 4.10 (t, 4H, $C\underline{H}_2Cl$).

Anal. for $C_{16}H_{34}Cl_3N$: C, 55.41; H, 9.88; N, 4.03

Found: C, 55.42; H, 10.02; N, 3.53

N-dodecyl-N,N-bis(2-isothioureylethyl)amine trihydrochloride.

The dodecyl nitrogen mustard (6.34 g, 0.02 mol), 3.05 g (0.04 mol) thiourea and 0.2 g NaI were added to 50 ml absolute ethanol and refluxed overnight. The solution was filtered and flash evaporated to yield a gummy tan solid. The solid was elutriated with dry benzene followed by chloroform and placed on a high vacuum line for 8 hours to yield 6.47 g (72%) of a hygroscopic tan solid: mp 75-77°C, ir (KBr) 3250 cm^{-1} (broad, NH), 1670 cm^{-1} (C=N); NMR (D^6-dmso) δ0.82 (t, 3H, CH_3), 1.22 (m, 16H, CH_3-$(CH_2)_8$), 3.46 (m, 6H, CH_2N), 3.65 (m, 4H, CH_2S), 9.45 (b, 9H, NH).

II. General Experimental Procedure for Technetium-99m Complex Formation

To 0.01 mmols of an aqueous solution of the bisisothiouronium salt under nitrogen was added 0.06 mmol of aqueous NaOH. After 30 minutes, the reaction mixture was extracted with 2-4 ml chloroform. The chloroform layer was separated and vacuum evaporated to an oil. The oil was dissolved in absolute ethanol. To this thiol solution (under nitrogen) was added 0.1 ml of 99m-pertechnetate ($NaTcO_4$) in saline solution and 0.1 ml of 5 M $NaBH_4$ (.05 mmol) in sequence. After 2 to 3 hours, the reaction mixture was analyzed.

III. Synthesis of Technetium-99 Complexes

All technetium-99 complexes described in the results and discussion were synthesized by one or all the methods described below. In cases where solids were isolated, purification proceeded by liquid chromatography or serial recrystallizations.

A. Reduction with sodium borohydride.

The bisisothiouronium salt (2.4 mmol) was dissolved in water and placed under nitrogen. 12 mmol (4.8 ml of a 10% solution) of sodium hydroxide was added. The solution was allowed to react for 20 to 30 minutes with absolute ethanol added to those solutions where the ligand was less soluble. The aqueous ammonium 99-pertechnetate (0.6 mmol), sodium chloride (12 mmol, 0.7 g) and of sodium borohydride (0.6 mmol, 0.2 g) (dissolved in ethanol) were added to the solution in sequential order. Precipitation of the complex was noted upon addition of the $NaBH_4$. After 30 minutes, the precipitate was either collected by filtration and washed with water (or extracted with chloroform or methylene chloride), washed with 0.1 N HCl and water (dried with $MgSO_4$ when dissolved in solvent) and prepared for purification.

B. Reduction with sodium dithionite.

The bisisothiouronium salt (2.4 mmol) was hydrolyzed with 12 mmol of NaOH as described in part A. After the addition of 0.6 mmol of NH_4TcO_4 and 12 mmol NaCl, a freshly prepared solution of sodium dithionite (1.75 mmol, 0.23 g) in 10 ml of 0.20N NaOH was added dropwise. After 20 minutes the precipitate was prepared for purification by the methods listed in part A.

C. Metathetical exchange with tetrabutylammonium oxotetrachlorotechnetate(V) $[nBu_4N^+TcOCl_4^-]$.

Sodium hydroxide (0.25 mmol, 0.10 ml of a 10% solution) was added to an aqueous solution of 0.05 mmol of the bisisothiouronium salt (under nitrogen). After 20 to 30 minutes, the solution was extracted with 3 x 3 ml of chloroform. After separation, the combined organic layers were dried with $MgSO_4$ and filtered. This mercaptan solution was

added to an ethanolic solution of (.01 mmol) $nBu_4NTcOCl_4$ containing 0.10 mmol (13.6 mg) of sodium acetate. The final solution was filtered and analyzed without further isolation.

D. Metathetical exchange with N-methyl-N,N'diethanolethylenediamine-99 technetium complex [MeDADATcOX].

The bisisothiouronium salt (0.30 mmol) was hydrolyzed with 1.80 mmol (0.70 ml of a 10% solution) NaOH under nitrogen. After 20 minutes, the solution was added directly to 0.10 mmol MeDADATcOX in methanol. This solution was analyzed as is.

IV. Electrophoresis of the Technetium Complexes.

A 25 mm x 30 cm strip of Whatman 3MM chromatography paper and/or 25 mm x 30.5 cm strip of Gelman Sepraphore III polyacetate electrophoresis strips with the origin marked at the center of the strip was saturated with 0.01 N $NaHCO_3$ (pH7). After placing the strip on the electrophoretic flat bed containing 0.01 N $NaHCO_3$, approximately 5 µl of filtered technetium complex was spotted at the origin. 600 volts (DC) was applied to the strips for 15 or 30 minutes and the resultant strips air dried. These strips were scanned using a NaI scintillation detector (0.635 cm lead shield containing a 2.5 cm x 3 mm collimating slit) for the technetium-99m samples or a Geiger-Muller detector for the technetium-99 complexes.

V. Ion Exchange Procedure.

Equivalent amounts of Amberlite-IRA-400 (quaternary ammonium chloride) anion exchange resin and Amberlite IR-120 (sodium sulfonate) cation exchange resin were mixed in a 0.9% saline solution. The resins were placed in a disposable Pasteur pipette. Each filled pipette was rinsed with at least 5 ml of 0.9% saline and 5 ml of methanol. Each

column was eluted with 2 to 3 portions of methanol (1.5 ml each) after addition of approximately 0.1 ml of concentrated technetium solution. The effluent was collected, concentrated, placed in a stainless steel planchet and counted with a shielded Geiger-Mueller detector for a fixed time period.

VI. Partition Coefficient Measurement.

The aqueous borohydride reduced technetium-99m complexes (0.10 ml) or 0.10 ml of a 1.0 mg solution of the technetium-99 complexes were placed in glass stoppered vials. After evaporation of the solvent, 1.50 ml of n-octanol saturated 0.9% saline and 0.50 ml of saline saturated n-octanol were added. The layers were mixed by inversion of the vial 100 times over 5 minutes. After sitting 5 to 10 minutes, the technetium-99m layers were separated and counted under a NaI crystal. The separated technetium-99 layers were evaporated to dryness on stainless steel planchets and counted with a Geiger-Mueller detector.

APPENDIX: ABBREVIATIONS AND DEFINITION OF TERMS

Activity	- Counts per minute.
Bunte salt	- Compounds formed by the reaction of thiosulfate with alkyl halides.
Benzyl-Tc-99m complex	- N-Benzyl bis(2-mercaptoethyl)amine complexed to technetium-99m.
Butyl-Tc-99m complex	- N-Butyl bis(2-mercaptoethyl)amine complexed to technetium-99m.
Cyclam	- 1,4,8,11-Tetra-azacyclotetiadecane.
Dithiazapane	- 1,2-Dithia-5-azapane.
EDTA	- N,N,N',N'-Tetrakis(aceticacid)ethylene diamine.
Ema	- N,N'-Ethylene bis(2-mercaptoacetimide).
Ethyl-Tc-99m complex	- N-Ethyl bis(2-mercaptoethyl)amine complexed to technetium-99m.
FAB	- Fast Atom Bombardment Mass Spectrometry.
FDMS	- Field Desorption Mass Spectrometry.
HIDA	- N-(2,6-dimethyl phenyl carbamoyl methyl)-iminodiacetate.
HPLC	- High pressure liquid chromatography.
k'	- Capacity Factor; $k' = (t_r - t_o)/t_o$ where t_o = void volume time.
L	- Ligand; N-substituted-bis(2-mercaptoethyl) amines.
Labile peak	- The peak in the time dependent phenomenon that decreases with time.
LC	- High performance liquid chromatography.

MeDADATcOX	- N-methyl-N,N'-diethoxyethylenediamine-oxotechnetium(V).
R_f	- R_f = distance from origin to center of spot/ distance from origin to solvent front.
Stable peak	- The peak in the time dependent phenomenon observed by HPLC that remains with time.
TLC	- Thin Layer Chromatography.
T_r	- Retention time as observed by HPLC.
Void volume	- Volume of solvent needed to fill the voids in a chromatographic column.

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