Volume 28
Number 15
July 26, 1989

Inorganic Chemistry

© *Copyright 1989 by the American Chemical Society*

Communications

Technetium–Diphosphonate Skeletal Imaging Agents: EXAFS Structural Studies in Aqueous Solution

Technetium-99m-labeled diphosphonates are among the most widely used radiopharmaceuticals in diagnostic nuclear medicine.[1,2] These bone-seeking agents provide an effective means of diagnosing primary bone cancer, metastatic cancer to bone, bone trauma, Paget's disease, etc. However, despite the importance and widespread clinical use of these agents, almost no definitive information is available as to their chemical composition or structure.[3,4] This lack of fundamental chemical information restricts the development of improved ^{99m}Tc–diphosphonate formulations, and severely hinders the development of analogous diphosphonate formulations containing β-emitting radionuclides such as ^{186}Re, which could be used for the treatment of metastatic bone cancer.[5,6]

Over the past several years, we have devoted considerable effort to understanding the fundamental chemistry of ^{99m}Tc–diphosphonate radiopharmaceuticals. As a result of these efforts, we have formulated the working hypothesis that ^{99m}Tc–diphosphonate radiopharmaceuticals consist of a complicated, time-dependent mixture of oligomeric and polymeric technetium complexes.[7,8] The facts and observations upon which this "oligomer/polymer" hypothesis is based have been presented elsewhere.[9-18] The hypothesis has received some support from others,[19] but it has also been criticized,[20] although no experimental evidence has yet been presented with which it is inconsistent. Most criticisms of the "oligomer/polymer" hypothesis ultimately stem from the *indirect* nature of the evidence upon which it is based. No *direct* evidence for the existence of Tc–diphosphonate oligomers or polymers in solution has hitherto been available. In this paper, we present the first such evidence, which has been obtained by means of an EXAFS analysis[21] of an HPLC-purified Tc–MDP (MDP = methylenediphosphonate) complex in aqueous solution.

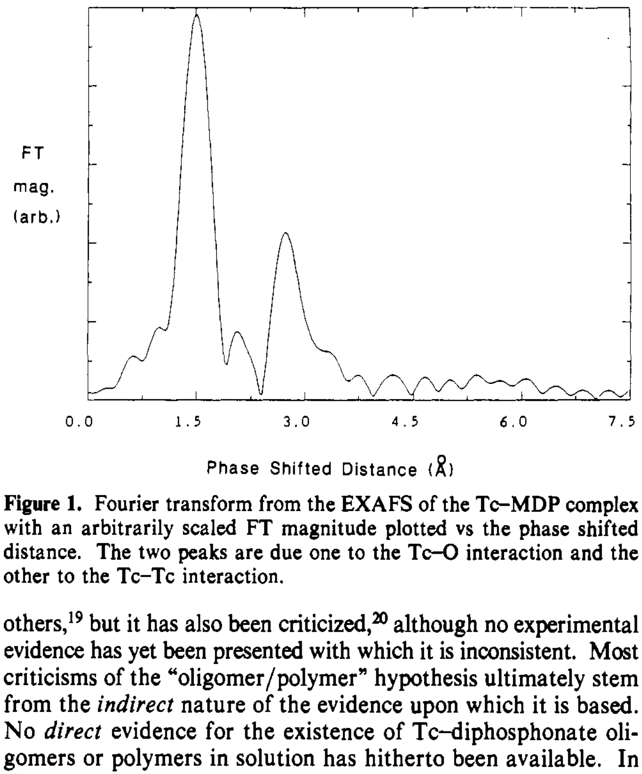


Figure 1. Fourier transform from the EXAFS of the Tc–MDP complex with an arbitrarily scaled FT magnitude plotted vs the phase shifted distance. The two peaks are due one to the Tc–O interaction and the other to the Tc–Tc interaction.

Technetium–diphosphonate radiopharmaceuticals are prepared by the reduction of pertechnetate, TcO_4^-, in the presence of excess diphosphonate ligand. HPLC has been used to investigate in detail the number and distribution of Tc–MDP components generated when pertechnetate is reduced by sodium borohydride in the presence of MDP.[14] These investigations demonstrated that the formulation pH is an effective variable for controlling the generation and ultimate interconversion of Tc–MDP components. In this present study, we have generated a remarkably stable Tc–MDP component by conducting the sodium borohydride reduction at pH 2 and then have isolated this component using minor modifications of previously described HPLC procedures.[14,22] This HPLC-isolated complex (i) is the principal (94%) component of the reaction mixture, (ii) exhibits the same retention time as does the principal component generated when "no-carrier-added"

---

(1) Clarke, M. J.; Podbielski, L. *Coord. Chem. Rev.* **1987**, *78*, 253–331.
(2) Lamki, L. M. *J. Nucl. Med.* **1985**, *26*, 312–314.
(3) Libson, K.; Deutsch, E. in *Technetium in Chemistry and Nuclear Medicine*; Deutsch, E., Nicolini, M., Wagner, H. N., Jr., Eds.; Cortina Internat.: Verona, Italy, 1983; pp 45–48.
(4) Deutsch, E.; Libson, L. *Comments Inorg. Chem.* **1984**, *3*, 83–103.
(5) Deutsch, E.; Libson, K.; Vanderheyden, J.-L.; Ketring, A. R.; Maxon, H. R. *Nucl. Med. Biol.* **1986**, *13*, 465–477.
(6) Maxon, H. R.; Deutsch, E. A.; Thomas, S. R.; Libson, K.; Lukes, S.J.; Williams, C. C.; Ali, S. *Radiology* **1988**, *166*, 501–507.
(7) Deutsch, E. In *Radiopharmaceuticals II*; Society of Nuclear Medicine: New York, 1979; pp 129–146.
(8) Pinkerton, T. C.; Heineman, W. R.; Deutsch, E. *Anal. Chem.* **1980**, *52*, 1106–1110.
(9) Uchtman, V. A. *J. Phys. Chem.* **1972**, *76*, 1304–1310.
(10) Jurisson, S. S.; Benedict, J. J.; Elder, R. C.; Whittle, R.; Deutsch, E. *Inorg. Chem.* **1983**, *22*, 1332–1338.
(11) Deutsch, E.; Libson, K.; Becker, C. B.; Francis, M. D.; Tofe, A. J.; Ferguson, D. L.; McCreary, L. D. *J. Nucl. Med.* **1980**, *21*, 859–866.
(12) Libson, K.; Barnett, B. L.; Deutsch, E. *J. Am. Chem. Soc.* **1980**, *102*, 2476–2478.
(13) Pinkerton, T. C.; Ferguson, D. L.; Deutsch, E.; Heineman, W. R.; Libson, K. *Int. J. Appl. Radiat. Isot.* **1982**, *33*, 907–915.
(14) Tanabe, S. Zodda, J. P.; Deutsch, E.; Heineman, W. R. *Int. J. Appl. Radiat. Isot.* **1983**, *34*, 1577–1584.
(15) Tanabe, S.; Zodda, J. P.; Libson, K.; Deutsch, E.; Heineman, W. R. *Int. J. Appl. Radiat. Isot.* **1983**, *34*, 1585–1592.
(16) Ferguson, D. L.; Libson, K.; Zodda, J. P.; Heineman, W. R.; Deutsch, E. *Appl. Radiat. Isot.* **1986**, *37*, 341–344.
(17) Zodda, J. P.; Tanabe, S.; Heineman, W. R.; Deutsch, E. *Appl. Radiat. Isot.* **1986**, *37*, 345–354.
(18) Martin, J. L. Ph.D. Thesis, University of Cincinnati, 1987, pp 121–127.
(19) Wilson, G. M.; Pinkerton, T. C. *Anal. Chem.* **1985**, *57*, 246–253.
(20) Gelsema, W. J.; DeLigny, C. L.; Tji, T. G. *Appl. Radiat. Isot.* **1987**, *38*, 251–254.
(21) Elder, R. C.; Eidsness, M. K. *Chem. Rev.* **1987**, *87*, 1027–1046.
(22) Martin, J. L. Ph.D. Thesis, University of Cincinnati, 1987, pp 153–154.

0020-1669/89/1328-2899$01.50/0 © 1989 American Chemical Society

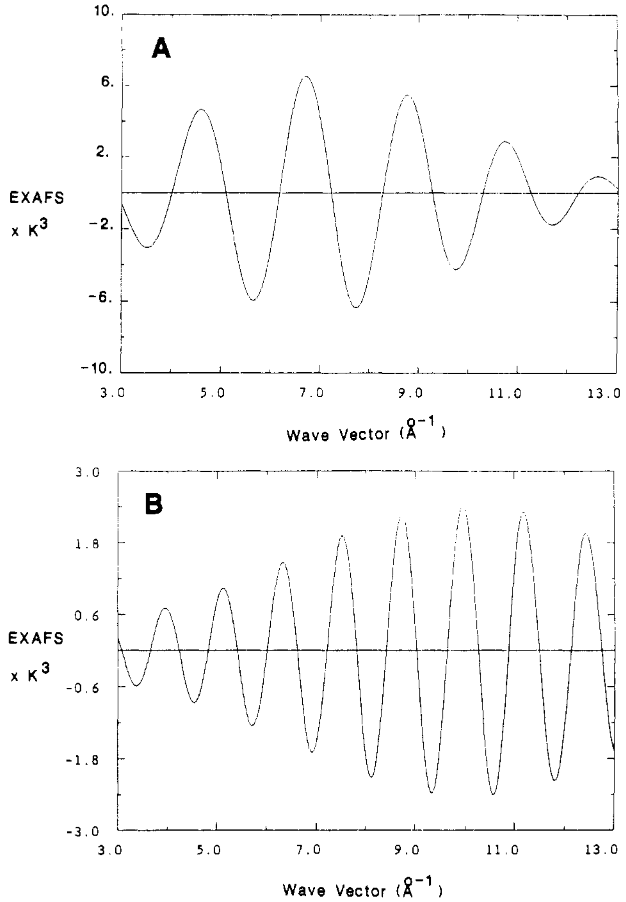


Figure 2. Back-transforms of the Fourier peaks at 1.5 Å (A) and that at 2.7 Å (B).

$^{99m}TcO_4^-$ is used in the reaction, (iii) has been shown in previous studies (peak D[15]) to be an effective bone-seeking agent in rats, and (iv) is stable (by HPLC analysis and visible–UV spectroscopy) for several months at technetium concentrations up to 35 mM. This component is thus the ^{99}Tc–MDP analogue of a ^{99m}Tc–MDP bone-seeking radiopharmaceutical; the fact that it can be prepared in relatively high concentrations and that it is stable at these concentrations provides the opportunity for its chemical characterization.

The EXAFS fluorescence spectrum of an 8 mM solution of the HPLC-isolated ^{99}Tc–MDP component was obtained at the technetium K edge (20.5–22.0 keV) by using a specially designed cell to guarantee containment of this radioactive solution (^{99}Tc decays by β emission with a half-life of 2.1 × 10^5 years). The Fourier transform of these data (Figure 1) shows two distinct peaks, one centered at 1.5 Å and one centered at 2.7 Å. Each of these peaks was isolated and separately back-transformed by using a Gaussian window. The filtered EXAFS times k^3 is shown in Figure 2A for the 1.5-Å peak and in Figure 2B for that at 2.7 Å. The difference in envelope shapes is readily apparent and suggests that the peak at the short distance arises from a Tc–O interaction while the peak at the longer distance results from a Tc–Tc interaction. Empirical phase and amplitude functions for Tc–O and Tc–P were developed by using EXAFS data measured from compounds of known crystal structure.[23] Those for Tc–Tc were taken from the theoretical values of Teo and Lee.[24] The results of single- and multiple-shell fits to the back-transformed data from the range 0.88–1.92 Å are as follows: (1) there are 5.9 ± 1.2 oxygen atoms at 1.99 ± 0.02 Å; (2) there is no detectable oxygen contribution at a distance ca. 1.72 Å; (3) no phosphorus atom contribution is detectable. The isolated peak centered at 2.7 Å was back-transformed over the range 2.38–3.10 Å. The resultant filtered EXAFS was fit by the single-shell contribution of 1.5 ± 0.5 Tc atoms at 2.99 ± 0.02 Å. No phosphorus component was detectable. All calculations were performed in the manner previously described[25] by using local modifications of the programs of Scott.[26]

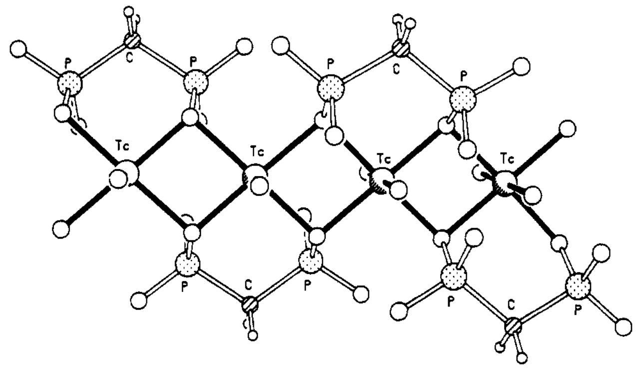


Figure 3. One of many possible tetrameric structures for Tc–MDP conforming with the EXAFS results. All unlabeled atoms are oxygen from either water or the diphosphonate ligand.

These EXAFS results provide the first direct evidence as to the structure of Tc–diphosphonate complexes in solution. Most importantly, the presence of a Tc–Tc interaction constitutes definitive evidence for the oligomeric–polymeric nature of these materials. While the chromatographic separation to isolate this material removes such known impurities as pertechnetate and TcO_2, we expect that the material obtained may be a mixture of several oligomers and the EXAFS results merely describe its average composition.

These results can be interpreted in terms of the following structural model. Given that each Tc has 1.5 ± 0.5 Tc neighbors, any value with the estimated error span from 1.0 to 2.0 requires at least the presence of a dimer (1.0 Tc) and at most a linear infinite polymer or a closed chain structure (2.0 Tc). The number 1.5 can be generated from a linear tetrameric material with two terminal Tc atoms (each having one Tc neighbor) and the two internal Tc atoms (each having two Tc neighbors).

The bridging unit appears likely to have two oxygen and two technetium atoms, which would form a nearly square array with a Tc–O distance of 1.99 Å and a Tc–Tc distance of 2.99 Å. This structure then gives an O–Tc–O angle of 83°. The six oxygen atoms surrounding the technetium are singly bonded. A typical Tc—O single bond length is 2.0 Å, whereas the Tc═O double bond is 1.7 Å.[27,28] The six oxygen atoms come either from the diphosphonate or water. We cannot say which, nor can we say which are involved as the bridging groups. However, since on average three of the six total oxygen atoms per technetium are involved in bridging, it is highly likely that at least some are from the disphosphonate. One of the many possible structures satisfying these constraints is shown as Figure 3.

Since all Tc(V) complexes prepared to date in aqueous media contain multiply bonded Tc═O linkages at 1.6–1.8 Å,[27] the lack of such a linkage within this Tc–MDP complex strongly implies that the Tc center is in an oxidation state less than +5. Considering that several dimeric Tc(IV,IV)– and Tc(IV,III)–polycarboxylate complexes have recently been characterized,[29–31] it

---

(23) For Tc–O the pertechnetate ion was used: Krebs, B.; Hasse, K.-D. *Acta Crystallogr., Sect. B* **1976**, *32*, 1334–1337. For Tc–P the complex cation tris(1,2-bis(dimethylphosphino)ethane)technetium(I) was used: Subramanyam, V.; Linder, K. E.; Sammartano, M. C.; Pendleton, D. B.; Delano, M.; Liteplo, M.; Camin, L. L. *J. Nucl. Med.* **1983**, *24*, P9.

(24) Teo, B. K.; Lee, P. A. *J. Am. Chem. Soc.* **1979**, *101*, 2815–2832.

(25) Watkins, J. W.; Elder, R. C.; Greene, B.; Darnall, D. W. *Inorg. Chem.* **1987**, *26*, 1147–1151.

(26) Scott, R. A. *Methods Enzymol.* **1985**, *117*, 414.

(27) Vanderheyden, J.-L.; Ketring, A. R.; Libson, K.; Heeg, M. J.; Roecker, L.; Motz, P.; Whittle, R.; Elder, R. C.; Deutsch, E. *Inorg. Chem.* **1984**, *23*, 3184–3191.

(28) Melnik, M.; Van Lier, J. E. *Coord. Chem. Rev.* **1987**, *77*, 275–324.

(29) Bürgi, H. B.; Anderegg, G.; Blauenstein, P. *Inorg. Chem.* **1981**, *20*, 3829–3824.

(30) Anderegg, G.; Muller, E.; Zollinger, K.; Burghi, H. B. *Helv. Chim. Acta* **1983**, *66*, 1593–1598.

is reasonable to assign this air-stable Tc–MDP complex as containing Tc(IV) centers. Further studies are now under way to verify this hypothesized structure and to probe the fundamental chemistry of this Tc–MDP complex.

Acknowledgment. Financial support by the National Institutes of Health (NIH) through Grants GM35404 (R.C.E.) and CA32863 (E.D.) and the Department of Energy (DOE) through Grant DE-FG02-86ER60487 (W.R.H.) is gratefully acknowledged. EXAFS measurements were performed at the Stanford Synchrotron Radiation Laboratories, which are operated by DOE and also supported by the NIH.

Supplementary Material Available: Figures A and B, including plots of the raw X-ray fluorescence data and the extracted EXAFS times $\mathbf{k}^3$, and Table A, listing the parameter values found in fitting the filtered EXAFS (3 pages). Ordering information is given on any current masthead page.

---

(31) Linder, K. E.; Davison, A.; Jones, A. G. In *Technetium Chemistry and Nuclear Medicine 2*; Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina Internat.: Verona, Italy, 1986, pp 129–130.

(32) To whom correspondence should be addressed at the Department of Chemistry.

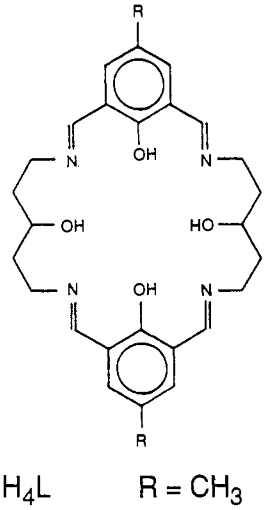
Biomedical Chemistry Research Center
Departments of Chemistry and Radiology
University of Cincinnati
Cincinnati, Ohio 45221-0172

John L. Martin, Jr.
J. Yuan
Craig E. Lunte
R. C. Elder*,[32]
William R. Heineman*,[32]
Edward Deutsch*,[32]

Received December 20, 1988

An Octacopper(II) Complex with μ_5-Oxo and Tripodlike Perchlorate Ligands: Formation and X-ray Structure of the $[Cu_4(L)O(ClO_4)]_2(ClO_4)_2 \cdot 2H_2O$ Dimer

Polynuclear complexes are of interest because of their bonding and magnetic interactions and also because of their potential value in catalysis. Recently[1] we reported the preparation of a planar tetracopper(II) complex, $[Cu_4(L')(OH)](NO_3)_3 \cdot 3H_2O$ (**1**), derived from the macrocyclic ligand H_4L'. We now record the dimerization of a copper complex of the related ligand H_4L, resulting in the formation of an octacopper(II) complex (**2**). The halves of the dimer are linked via a μ_5-oxo ligand in the center of each macrocyclic unit. Perchlorate anions are coordinated, in a new tripod bonding mode, to both open faces of the complex.



H_4L $R = CH_3$
H_4L' $R = C(CH_3)_3$

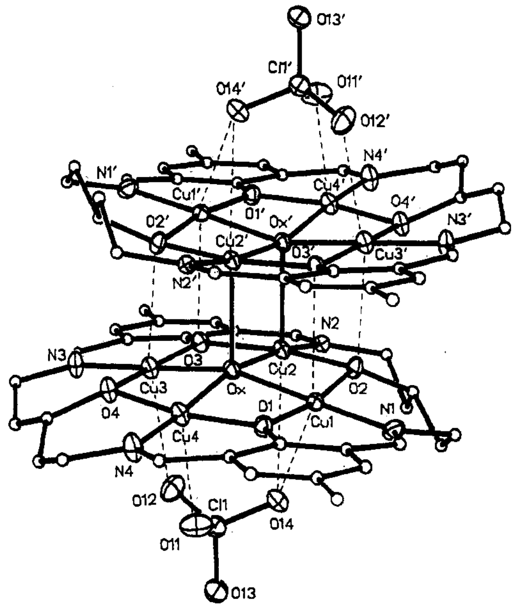


Figure 1. Perspective view of the $[Cu_4(L)O(ClO_4)]_2^{2+}$ dimer showing 30% probability thermal ellipsoids and atom labels for manganese, oxygen, and nitrogen atoms. Carbon atoms are shown as small spheres for clarity. Selected interatomic distances (Å): Cu(1)–Cu(2) = 2.852 (2), Cu(1)–Cu(4) = 2.876 (1), Cu(2)–Cu(3) = 2.918 (1), Cu(3)–Cu(4) = 2.844 (2), Cu(1)–Ox = 2.032 (6), Cu(2)–Ox = 2.066 (5), Cu(3)–Ox = 2.053 (6), Cu(4)–Ox = 2.054 (6), Cu(2)–Ox′ = 2.372 (7), Cu(1)–O(3′) = 2.84 (1), Cu(3)–O(2′) = 2.72 (1), Cu(1)–O(14) = 2.87 (1), Cu(2)–O(14) = 3.22 (1), Cu(3)–O(12) = 2.624 (8), Cu(4)–O(11) = 2.519 (8).

Complex **2** was prepared in 55% yield by refluxing 2,6-diformyl-4-methylphenol, 1,5-diamino-3-hydroxypentane, and copper(II) perchlorate hexahydrate in ethanol for ca. 24 h. A green product, **3**, was filtered from the hot solution, and **2** was obtained by recrystallization from 1:1 dimethylformamide (dmf)–methanol by diffusion of diethyl ether.[2] Although we have experienced no difficulties with the perchlorate salts described here, *these should be regarded as potentially explosive and handled accordingly*; i.e., make only small quantities and handle behind an explosion shield.

Figure 1 shows the X-ray crystal structure[3,4] of $[Cu_4(L)O(ClO_4)]_2^{2+}$, the dimeric cation of **2**. Within each macrocycle the arrangement is similar to that reported for **1**; the four copper atoms are coplanar, and each is coordinated to an imine nitrogen atom, to bridging phenoxide and alkoxide donors, and to a central oxygen donor. In **1** the central species is a hydroxo group located in the plane of the four copper atoms. In the present complex the central

---

(1) McKee, V.; Tandon, S. S. *J. Chem. Soc., Chem. Commun.* **1988**, 385.

(2) Copper(II) perchlorate hexahydrate (1.48 g, 4 mmol) and 2,6-diformyl-4-methylphenol (0.33 g, 2 mmol) were heated to reflux in dry ethanol (40 mL) on a steam bath. 1,5-Diamino-3-hydroxypentane dihydrochloride (0.38 g, 2 mmol), neutralized with KOH and dissolved in a 1:1 mixture of dry methanol and ethanol (20 mL), was added dropwise over 10 min. The resulting dark green solution was refluxed for 24 h, after which the dark green solid that separated was filtered from the hot solution, washed with dry ethanol (3 × 5 mL), and dried in vacuo at room temperature; yield of **3** 55%. Anal. Calcd for $C_{28}H_{33}Cl_3Cu_4N_4O_{17}$: C, 31.8; H, 3.1; N, 5.3; Cu, 24.0. Found: C, 31.8; H, 3.6; N, 5.1; Cu, 23.6. Recrystallization by slow diffusion of diethyl ether into a solution of this complex in 1:1 dimethylformamide–methanol gave crystals of the octanuclear complex **2**.

(3) Crystal Data: $C_{56}H_{62}Cl_4Cu_8O_{28}N_8$, green, dimensions 0.31 × 0.25 × 0.15 mm^3, triclinic, a = 11.497 (4) Å, b = 12.714 (3) Å, c = 13.546 (4) Å, α = 102.92 (2)°, β = 113.15 (2)°, γ = 100.36 (3)°, V = 1693 (1) Å^3, space group $P\bar{1}$, Z = 1, $F(000)$ = 980. Data were collected at 170 K on a Nicolet R3m four-circle diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.71069 Å). With use of 2° ω scans at 5.86° min^{-1}, 4049 unique reflections were collected in the range $4 < 2\theta < 45°$ and 2681 with $I > 3\sigma(I)$ were used in the structural analysis. The structure was solved by using Patterson methods, and refinement converged with R = 0.0497 and R_w = 0.0642. All programs used in the data reduction and structure solution are contained in the SHELXTL (Version 4.1) package. Full details will be published elsewhere.

(4) Sheldrick, G. M. *SHELXTL User Manual, Revision 4.1*; Nicolet XRD Corp.: Madison, WI, 1984.