STOICHIOMETRIC COMPLEXES TIN-METHYLENEDIPHOSPHONATE : COMPOSITION, BIODISTRIBUTION AND ELEMENTS OF STRUCTURE

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SUMMARY

Stoichiometric methylenediphosphonate (MDP) complexes of tin were prepared and their composition was determined as Sn (II)-$(MDP)_2$ and Sn (IV)-MDP. Biodistribution studies in mice have shown differences in organ uptake related to the valence of the metal ion and the nature of the injection solvent. N.M.R. spectra and gel filtration experiments indicated a simple, homogeneous structure for the stannous complex and the heterogeneous nature (hexa- and tetra-coordinated) as well as a high apparent molecular weight for stannic species.

Key words : methylenebisphosphonic acid; tin complexes; NMR spectra; bone scintigraphy.

INTRODUCTION

In most commercial kits for ^{99m}Tc bone scintigraphy, stannous tin is used as reductant of pertechnetate (1) and methylene diphosphonate (MDP) as a bone seeking ligand (2) (3). With very few exceptions (4) (5) (6) most of the recent publications on

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this subject relate to the composition and charge of Tc-diphosphonate complexes. The reductant is simply indicated as (Sn) or ($NaBH_4$), etc. There is, however, evidence that tin compounds have effects on the chemistry and metabolic fate of Tc-labelled agents, much more important than those of a simple reductant and its oxidation product (7) (8). Even the existence of mixed metal complexes (Tc-Sn) is still controversial (9) (10) (11).

Rather incredibly, many fundamental aspects of these apparently simple and widely used radiodiagnostic reagents still remain obscure.

All published experiments have taken place in a large excess of ligand and in the presence of other ions such as chloride. We have shown recently (12) that the ultraviolet spectra of Sn (II)-MDP complexes are sensitive to changes in pH as well as to the MDP/Sn ratio and Cl^- concentration. Similar effects were also apparent in ion exchange HPLC separations (unpublished data).

Since little meaningful data could be acquired from the investigation of complex and variable mixtures, we have endeavoured to prepare and study single chemical species according to the following criteria :

a) Minimum MDP/Sn ratio; b) constant MDP/Sn ratios from preparation to preparation and for each valence of tin; c) single tin valence per preparation within the limits of experimental possibilities.

Hydrous oxides, $SnO.xH_2O$ and $SnO_2.xH_2O$, precipitate upon the addition of aqueous ammonia to the solutions of stannous and stannic salts. The precipitates are washed free of foreign ions and redissolve easily in the solutions of methylene diphosphonic acid trisodium salt (Na_3MDP).

This procedure yielded reproducible, stoichiometrically well

defined complexes, free of contaminants. In the present paper we report results on the composition, the biodistribution and some data on the structure in solution of the Sn(II) and Sn(IV)-MDP complexes.

MATERIAL AND METHODS

I. APPARATUS

Radioactivity counter : Auto-gamma scintillation spectrometer, Model 5230, Packard Instruments Co. Samples containing ^{113}Sn were counted after a delay of 18-24 hrs to allow equilibration with the daughter isotope ^{113m}In.

Infra red spectrometer : Perkin-Elmer, Model 1700, FTIR. Solid samples (1-2 mg) in 300 mg KBr pellet.

NMR spectrometer : Bruker, Model AC-P 300 MHz.

Fraction collector : Redirac 2112, L.K.B.

Centrifuges : Ecco, Model E 2/12 and Christ, Model UJ 1-5000, angle-heads.

Glassware : crimp top glass vials 1-10 ml, Chrompack, with crimp caps and teflon coated silicone rubber septa. Hamilton gas-tight syringes.

II. REAGENTS

Methylenediphosphonic acid 98 % (H_4MDP), methylenediphosphonic acid trisodium salt, tetrahydrate 98 % (Na_3MDP), sodium cyanoborohydride ($NaBH_3CN$), trimethyl phosphite p.a. and deuterium oxide 99,8 atom % D(D_2O) were from Janssen Chimica Belgium.

^{113}Sn: $SnCl_2$ in 4M HCl, 20 mCi (740 MBq)/mg; Dupont de Nemours-N.E.N. Products.

^{119}Sn : metal powder, 86,7 % enriched, obtained through C.E.A. Saclay.

Bio-gel P-4, 240-400 mesh, Bio-Rad Laboratories

Nitrogen : dry nitrogen E1 (Air Liquide), containing less than 5 p.p.m. of O_2, passed trough a Chrompak Gas-Clean oxygen trap.

All other reagents were of analytical grade.

III. EXPERIMENTAL

1) To ensure uniform results, the following rules were applied to all preparation : the operations were conducted in glass vials of the appropriate volume; liquids were transferred by syringe, through the vial's septa; precipitates were separated from supernatants by centrifugation for at least 2 minutes at 4-6000 r.p.m. Oxygen was rigourously excluded from all Sn(II) preparations by purging reagents and maintaining the samples under a N_2 atmosphere. Care was taken to prepare all complexes in the presence of an excess of the tin hydrous oxides to achieve the constant, minimal ratios MDP/Sn.

2) Preparation of Sn(II)-MDP complexes.

A desired volume of 0,2 M $SnCl_2$ in 2M HCl was injected into a vial. The calculated volume of 2M ammonium acetate solution was slowly added with constant mixing, in order to neutralize most of the HCl, without precipitation of tin. A fivefold exces of 1M $NaBH_3CN$ solution in dry dimethylformamide was then added, again slowly and with mixing. After 60-90 minutes an excess of 2M aqueous ammonia was added and the resulting precipitate was left to settle for 10-20 minutes. The vial was then centrifuged, the supernatant removed with a syringe and discarded. The precipi- tate was washed 2-3 times by vigourous stirring in distilled water, followed by centrifugation and the disposal of the supernatant. Finally the precipitate was resuspended in the pre-calculated volume of 0,2M solution of Na_3-MDP, equilibrated for a suitable time and the supernatant

solution of the complex was removed for further use after spinning down the remaining excess of hydrous stannous oxide.

3) Preparation of Sn(IV)-MDP complexes.

Exactly the same procedure was followed, starting with 0,2M $SnCl_4$ in 2M HCl. However, the reductant was replaced with a slight excess of 30 % H_2O_2 and no particular steps were taken to exclude oxygen.

4) a) Stoichiometric composition

The hydrous oxides were prepared as described, starting with Sn(II) and Sn(IV) chlorides solutions spiked with ^{113}Sn. Small increments of Na_3MDP solution were added to the suspensions of the oxides in water. After suitable equilibration time aliquots of the supernatant were removed for counting. The results were corrected for the resulting dilution.

b) Biodistribution

Hydrous oxides containing ^{113}Sn were prepared as described and were dissolved in a pre-calculated amount of Na_3MDP. The complexes were withdrawn, and diluted in a suitable injection solvent.

c) Infrared spectra

Complexes, with no radioactive isotope added were prepared and freeze-dried. Weighted amounts of dry solid were mixed with 300 mg of solid KBr and compressed into pellets of 10 mm diameter.

d) NMR spectra

Hydrous Sn(II) and Sn(IV) oxides were prepared as described starting with a solution of 86,7 % enriched, stable ^{119}Sn. The last wash was performed in D_2O. Precipitates were then dissolved in a solution of H_4MDP in D_2O, previously neutralized with 3 equivalents of NaOH in D_2O/mole of acid.

e) Gel filtration studies

A chromatography column of 55 x 1 cm was loaded with Bio-Gel-P4, 200-400 mesh, equilibrated with 10^{-2} M Tris-acetic acid buffer,

pH 7,5. The V_o and the V_t of the column was calibrated at the flow rate used subsequently. The Sn(II) and Sn(IV)-MDP complexes spiked with ^{113}Sn were prepared and aliquots (10^{-5} M/0,2 ml) were loaded onto the column and eluted at the flow rate of 4,2-4,4 ml/h. Fractions of 30 min. were collected in a fraction collector and their radioactivity was counted.

RESULTS AND DISCUSSION

1) Stoichiometric composition

The results shown on Fig. 1 indicate the stoichiometric composition Sn(II)-$(MDP)_2$ and Sn(IV)-MDP for, respectively, stannous and stannic-methylene diphosphonate complexes. This composition has been also found consistently in complexes formed in the presence of an excess of oxide : no change in the ratios of MDP/Sn was observed after several days of equilibration.

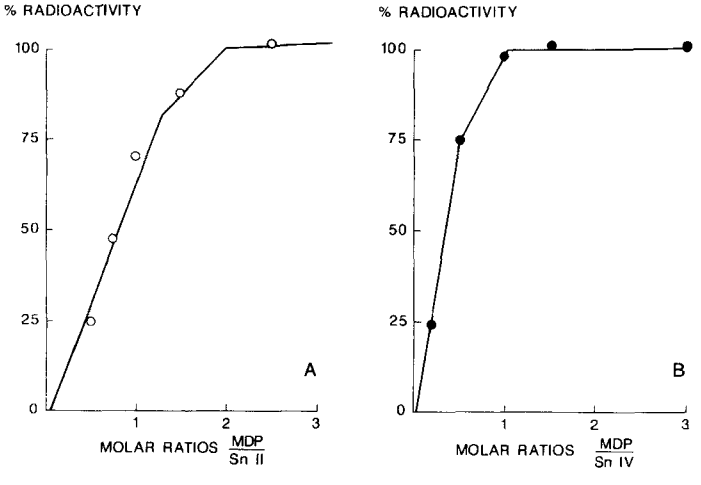

Fig. 1

Stoichiometric composition of tin-methylenediphosphonate complexes.
Results expressed as the minimum of mols MDP per At-g Sn necessary to dissolve completely the precipitate of hydrous tin oxide.
A : stannous complex. B : stannic complex.

2) General comments on preparations

a) A reduction step appears essential because Sn (II) is easily oxidized. Furthermore, solutions of ^{113}Sn and ^{119}Sn are often mixtures of Sn (II) and Sn (IV) compounds. Boron hydrides offer a rapid and quantitative route to pure stannous compounds. Reaction with $NaBH_4$ is rather violent in acid media, often leading to precipitates of metallic tin and to the loss of the element as a volatile stannane SnH_4 (13). $NaBH_3CN$, a much weaker reductant, reacts smoothly at acidic pH evolving almost no hydrogen gas (14).

b) In several experiments using preparations spiked with ^{113}Sn we have checked the yield of hydrous tin oxides obtained by ammonia precipitation and subsequent washings : it was consistently over 90 %. Since the second wash supernatant is neutral, contains no Cl^- and only faint traces of ammonium and acetate ions, we conclude that dissolving the precipitated oxides in the solutions of Na_3MDP yields pure Sn-MDP complexes.

c) There is an important kinetic difference in the dissolution of the two hydrous oxides in Na_3MDP. While the equilibrium SnO/MDP is achieved within minutes, with SnO_2 it is attained only after several hours. There is a difference in appearance between the two oxides : stannic oxide is a very fine amorphous gel, while the stannous compound seems a much coarser precipitate, settling rapidly. We would expect a fine precipitate with a larger surface area to react faster; therefore the observed difference reflects either a different reaction mechanism or a much lower stability constant of the stannic complex.

3) Biodistribution studies in mice

The solutions of complexes were made isotonic with sodium chloride or with glucose. Each mouse was injected into the caudal artery with 0,1 ml of solution, containing $0,5 \times 10^{-6}$

TABLE I

ORGAN	TIME hrs	Sn(II)-MDP (NaCl)	Sn(II)-MDP (Glu)	Sn(IV)-MDP (NaCl)	Sn(IV)-MDP (Glu)
BLOOD	2	4,94 ± 0,23	4,89 ± 0,23	0,45 ± 0,07	0,86 ± 0,29
	24	2,75 ± 0,06	2,64 ± 0,26	0,07 ± 0,01	0,09 ± 0,01
BONE	2	28,86 ± 2,44	31,74 ± 3,35	42,01 ± 5,44	47,14 ± 1,75
	24	33,22 ± 4,54	35,94 ± 8,15	43,21 ± 9,35	42,84 ± 4,52
MUSCLE	2	1,64 ± 0,19	4,50 ± 0,58	1,37 ± 0,34	2,42 ± 0,22
	24	1,06 ± 0,34	1,15 ± 0,33	0,75 ± 0,15	0,74 ± 0,13
STOMACH	2	0,28 ± 0,02	5,07 ± 3,62	0,45 ± 0,18	11,98 ± 1,87
	24	0,16 ± 0,04	1,76 ± 0,35	0,06 ± 0,01	0,06 ± 0,02
LIVER	2	8,53 ± 0,21	9,45 ± 1,19	22,17 ± 0,50	18,45 ± 0,70
	24	4,65 ± 0,39	4,47 ± 0,86	24,31 ± 2,10	22,09 ± 0,58
KIDNEY	2	5,99 ± 0,34	7,21 ± 0,90	0,71 ± 0,10	0,67 ± 0,09
	24	1,49 ± 0,32	1,71 ± 0,43	0,32 ± 0,03	0,37 ± 0,02
SPLEEN	2	0,29 ± 0,02	0,49 ± 0,06	0,40 ± 0,05	0,49 ± 0,02
	24	0,45 ± 0,31	0,49 ± 0,45	0,69 ± 0,13	0,56 ± 0,11

Biodistribution in the mouse

The results are expressed as percent dose per gram of a normalized 25 g animal : % dose/g x $\frac{\text{weight (g)}}{25}$ ± 1σ (n=3).

Percent dose/organ was calculated, assuming that blood, bone and muscle make up, respectively, 7 %, 10 % and 43 % of the body weight of a male, NMRI white mouse of 20-25 g.

(NaCl) : made isotonic with sodium chloride.

(Glu) : made isotonic with glucose (contains no foreign ions).

At-g of Sn(II) or Sn(IV) as MDP complexes and 4-6 x 10^5 cpm of ^{113}Sn. Animals were killed at 2 and 24 hrs post injection. All radioactivity counting was done after at least 18 hrs to allow for equilibration of $^{113}Sn/^{113m}In$.

Table I shows the overall results. There is a general qualitative agreement between our data and those published by Srivastava et al. (1985) : high bone uptake and slow clearance, lower uptake and faster clearance of Sn(IV)-complexes in blood, as compared to stannous compounds.

There are, however, some noteworthy differences. The injection medium exerts a significant influence on the level of the initial uptake and/or the subsequent clearance rate, especially in the muscle and stomach as shown in Tables II and III. The mechanism of this effect is at present unknown, although it confirms other data related to the effects of chloride ion concentration.

Another surprising finding is a high and persistant uptake, particularily of Sn(IV) compound by the liver and to a lesser degree by the spleen, indicating capture by the reticuloendothelial system. The significant capture of Sn(II) by the kidneys is also noteworthy.

Finally, in Table IV are reported the ratios of Sn(II)/Sn(IV) uptake. By comparison with Sn (IV), it appears that Sn(II) is taken up at a higher level by the blood, the kidneys and rather unexpectedly by the stomach, at least at 24 hrs.

The preference for Sn(IV) is shown by the liver and less strikingly by the bone, in disagreement with the results of Srivastava et al. (1985). We will therefore conclude that the influence of the excess ligand is quite significant. The results of experiments in vivo conducted with stoichiometric complexes of tin-MDP cannot be directly compared with those usually reported.

TABLE II

ORGAN	TIME hrs	Sn(II)-MDP (NaCl)	Sn(II)-MDP (Glu)	Sn(IV)-MDP (NaCl)	Sn(IV)-MDP (Glu)
BLOOD	2	-	-	0,45 ± 0,07	0,86 ± 0,29
MUSCLE	2	1,64 ± 0,19	4,50 ± 0,58	1,37 ± 0,34	2,42 ± 0,22
STOMACH	2	0,28 ± 0,02	5,07 ± 3,62	0,45 ± 0,18	11,98 ± 1,87
	24	0,16 ± 0,04	1,76 ± 0,35	-	-
LIVER	2	-	-	22,17 ± 0,50	18,45 ± 0,70
KIDNEY	2	5,99 ± 0,34	7,21 ± 0,90	-	-
	24	-	-	0,32 ± 0,03	0,37 ± 0,02
SPLEEN	2	0,29 ± 0,02	0,49 ± 0,06	0,40 ± 0,05	0,49 ± 0,02

Effects of diluent on the uptake of Sn

The results are expressed as in Table I.

Data reported only when $t_{obs.} > t_{calc.}$ at 95 % confidence level.

(NaCl) : made isotonic with sodium chloride.

(Glu) : made isotonic with glucose.

TABLE III

ORGAN	Sn(II)-MDP (NaCl)	Sn(II)-MDP (Glu)	Sn(IV)-MDP (NaCl)	Sn(IV)-MDP (Glu)
BLOOD	55	54,3	15,5	10,5
MUSCLE	67	28	54,7	30,6
STOMACH	57	24,2	13,3	0,5

Effects of diluent on the clearance rate

The results are reported as percent of radioactivity at 2 hrs remaining at 24 hrs.

(NaCl) : made isotonic with sodium chloride.

(Glu) : made isotonic with glucose.

TABLE IV

ORGAN	TIME	(NaCl)	(Glu)
		Sn(II)/Sn(IV)	Sn(II)/Sn(IV)
BLOOD	2	11,07	5,71
	24	41,25	28,28
BONE	2	0,69	0,67
	24	N.S.	N.S.
MUSCLE	2	N.S.	1,85
	24	N.S.	N.S.
STOMACH	2	N.S.	0,42
	24	2,72	27,84
LIVER	2	0,38	0,51
	24	0,19	0,20
KIDNEY	2	8,40	10,77
	24	4,64	4,58
SPLEEN	2	0,72	N.S.
	24	N.S.	N.S.

Effects of tin valence on the uptake

The results are expressed as Sn(II)/Sn(IV) ratios.
Data reported only when $t_{obs.} > t_{calc.}$ at 95 % confidence level.
N.S. : not significant.
(NaCl) : made isotonic whith sodium chloride.
(Glu) : made isotonic with glucose.

4) Elements of structure

a) Infrared spectra

No meaningful interpretation of the structure could be obtained from the infrared spectra of the ligand (free acid and its tri-sodium salt) and of its complexes with Sn (II) and Sn (IV). The superposition of absorption bands of ionic phosphonates, of free and hydrogen-bonded P=O stretching vibrations and, finally of P-OH bonds, did not show any significant differences between the spectra of various species (15) (16).

b) NMR spectra

In an effort to further characterize the structure in solution

of Sn (II)-and Sn (IV)-MDP complexes, a multi-nuclei (^{1}H, ^{13}C, ^{31}P and ^{119}Sn) nuclear magnetic resonance (NMR) study was undertaken (17). Free MDP in solution in D_2O shows a triplet centered at δ 2.10 ppm in ^{1}H-NMR and at δ-125.54 ppm ($^{2}J\ ^{31}P-^{1}H$= 19.0 Hz) in ^{31}P-NMR. As expected, a triplet centered at δ 29.38 ppm ($^{1}J^{13}C-^{31}P$ = 112.8 Hz) was equally observed. No significant modifications of these features was detected upon complexation of the ligand with Sn (II). However, when the Sn (IV)-MDP complex was examined, complex spectra were obtained in ^{1}H and ^{31}P-NMR. A signal of 19 lines with chemical shifts ranging from δ-128.0 to 129,1 ppm was observed in ^{31}P; a 14 components multiplet (δ 2.0-2.4) was produced in ^{1}H. Fortunately, a rather simpler situation was found in ^{13}C-NMR; a set of three triplets centered at δ 30.42 ($^{1}J^{13}C-^{31}P$ = 116.25 Hz), 30.03 (^{1}J = 115.46 Hz) and 29.93 (^{1}J = 114.5 Hz) was indeed observed. Very interestingly, a ^{119}Sn-NMR obtained with ^{119}Sn-enriched sample showed two signals, a pentuplet centered at δ 120.71 ppm (J = 27 Hz) and a heptuplet centered at δ 114.71 ppm (J = 18 Hz) (Fig. 2 & 3).

These data can be rationalized in terms of the existence of two complexes for Sn (IV) MDP : one is tetracoordinated and the other hexacoordinated. In the latter complex, coordinating nuclei will occupy diastereotopic positions and this gives rise to complex spectra, especially in ^{1}H and ^{31}P-NMR. The ^{13}C-NMR of the Sn (IV) species can be interpreted within this picture : the tetracoordinated species will produce one single triplet while the hexacoordinated species display two types of ^{13}C-nuclei occupying distinct diastereotopic loci. For the Sn (II)-species, no alteration of the spectra was observed compared to free MDP in ^{1}H, ^{13}C and ^{31}P-NMR. An interesting information however was provided by the ^{119}Sn spectrum : a broad signal (width at half-height : ± 2000 Hz) was obtained and this

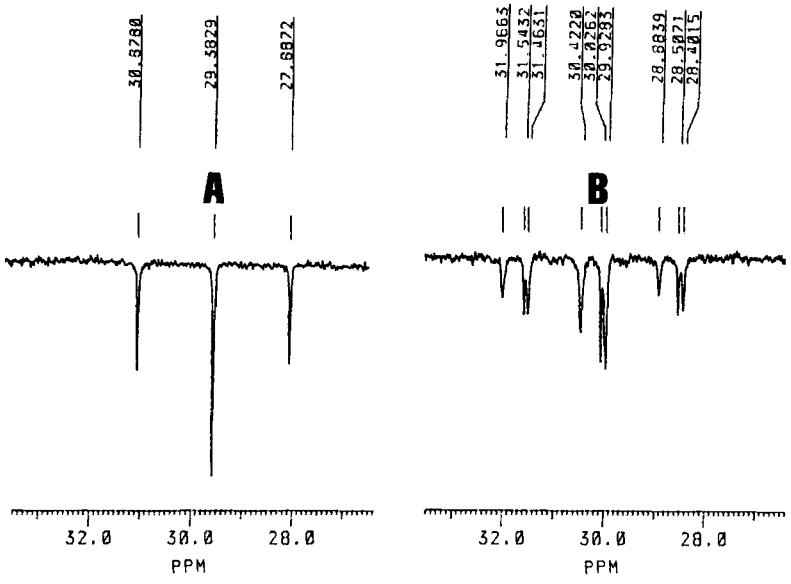

Fig. 2

^{13}C-NMR spectra (DEPT) of free MDP (A) and of Sn (IV)-MDP complexes.
Samples : A : 4 x 10^{-3} mol MDP-trisodium salt/4 ml D_2O.
B : 2 x 10^{-3} At-g of 87 % enriched ^{119}Sn as Sn (IV)-MDP.
N.B : Sn (II)-$(MDP)_2$ complex protected from oxidation yields NMR spectrum identical to A.

indicates the existence of a "loose" complex in a situation of intermediate rate chemical exchange process. By contrast, the tetracoordinated and hexacoordinated Sn (IV)-species experience a situation of fast dynamic exchange as indicated by the sharpness of narrow signals.

c) Gel filtration studies

Fig.4 shows the gel filtration results for (A), Sn (IV)-MDP complexes and (B), Sn(II)-MDP compounds. The main population of Sn (IV) species is excluded from the gel, although there is a smaller (less than 20 %) fraction of lower apparent molecular

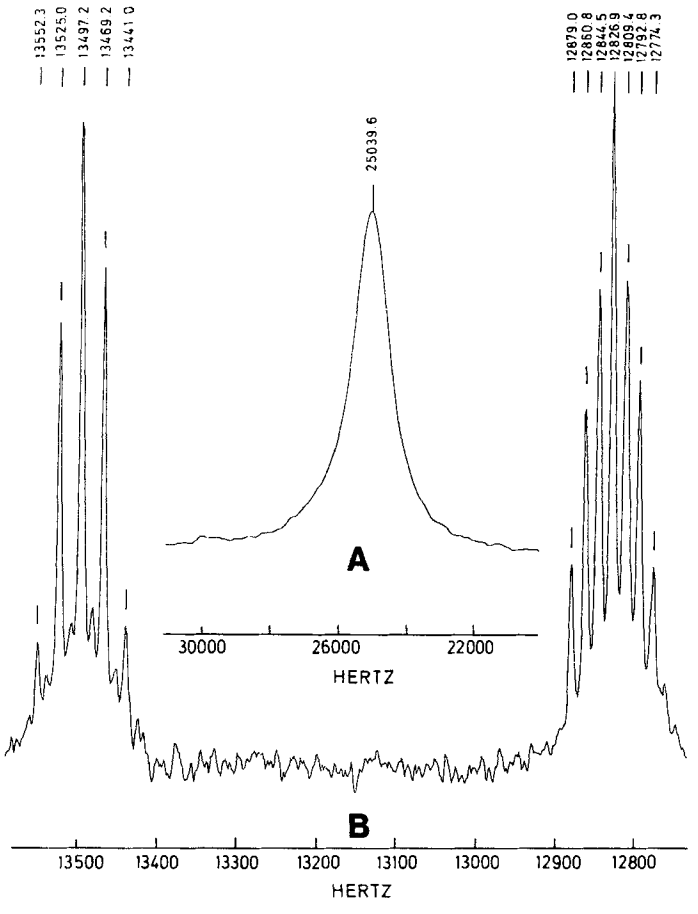

Fig. 3

^{119}Sn-NMR of tin-methylenediphosphonate complexes.
A : Sn (II)-$(MDP)_2$. B : Sn (IV)-MDP.
Samples : 2 x 10^{-3} At-g of 87 % enriched ^{119} Sn/4 ml D_2O.

weight. The Sn (II) samples run under non-oxidizing conditions are eluted much later, at a volume corresponding to the apparent molecular weight of a trimer. No structural conclusion concerning stannous complexes would be valid, however, as the elution order of highly charged ionic species depends on their Stokes radius.

It is also noteworthy, that neither the presence of 10^{-3} molar MDP in the eluent, nor the supra-stoichiometric amounts of the

ligand in the sample (MDP/Sn = 6) do not modify the gel filtration profile of the Sn (II & IV)-MDP complexes.

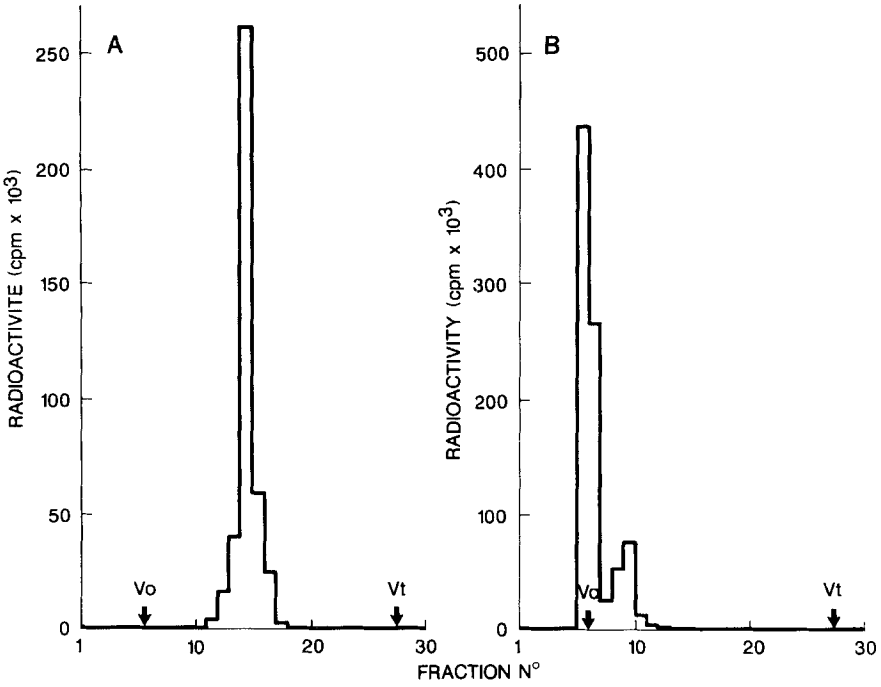

Fig. 4

Gel filtration of Sn-MDP complexes.
Bio-gel P-4, 55 x 1 cm, 0,01 M TRIS-Acetate, pH 7,5.
Flow rate : 4,2 ml/hour; fractions of 30 min.
Column/load : 5 $x10^{-6}$ At-g Sn spiked with ^{113}Sn/0,2 ml.
A : Sn (II)-$(MDP)_2$ B : Sn (IV)-MDP
Radioactivity yield for both A & B : 97 %

5) Conclusions

The correlation of the various sets of data allows us to draw several tentative conclusions.

a) The oxidation state of Sn exerts a profound influence on the structure and behaviour of its MDP complexes. Upon oxidation of tin in the Sn (II)-$(MDP)_2$ complex of low apparent molecular weight, the polymeric species $\left[\text{Sn (IV)-MDP}\right]_n$ are formed with the

concomitant release of 1 mole of MDP per atom-gram of Sn.

b) The polymeric nature and inhomogeneity of stannic complexes is indicated by NMR and gel filtration results. It also provides the most likely explanation of its strong uptake by the reticulo-endothelial system shown in the biodistribution studies.

c) As to the much debated question of the existence of mixed metal complexes Tc-Sn-MDP in the clinical preparation for bone scintigraphy, the stannic species also appear as likely candidate for the formation of inclusion compounds.

d) The influence of the isotonizing agent on the biodistribution has not been shown previously, as the 0,15 M NaCl is the usual eluent of pertechnetate produced from the ^{99} Mo generator.

e) Tentatively applied to the field of formulation of future bone scintigraphy radiopharmaceuticals, these conclusions indicate : that tin has a very similar tissue affinity to technetium and its excess might saturate the same sites; that the presence of large amounts of Sn (IV) species might exert a negative influence on the scintigraphic results; and that therefore tin should be replaced by some other reductant.

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