The Nucleus **49**, No. 3 (2012) 209-220

The Nucleus
A Quarterly Scientific Journal of Pakistan Atomic Energy Commission
NCLEAM, ISSN 0029-5698

LABELLING OF M-TRIMETHYL SILYLPHENYL)-ETHYLIDENE-1, 1-BISPHOSPHONATE WITH ^{99m}Tc AND ITS EVALUATION AS AN IMAGING AGENT

*K.M. SAJID and R. MAHMOOD

Multan Institute of Radiotherapy and Nuclear Medicine (MINAR), P.O. Box 377, Multan, Pakistan

(Received June 15, 2012 and accepted in revised form September 17, 2012)

Technetium-99m labeled phosphates and phosphonates have since long been in use for bone imaging to diagnose bone infection, bone metastasis and bone fracture. ^{131}I -labeled bisphosphonates have also been prepared for targeted radiotherapy of bone metastasis. Although animal experiments show good accumulation of bisphosphonates in bone. The agent has never been tried in humans because of high gamma and beta energy. The agent must first be tested in humans using a relatively safe radioisotope. Technitium-99m (^{99m}Tc) a radioisotope with relatively low gamma energy and short half-life can serve as a good label. Whether ^{99m}Tc-labeled bisphosphonates can be used as good imaging agents is another aspect that needs further investigation. A study was therefore, conducted to label m-trimethyl silylphenyl)-ethylidene-1, 1-bisphosphonate with ^{99m}Tc and standardize the labeling procedure. The labeling procedure involved reduction of technetium (TcO_4^-) with stannous chloride followed by chelation of technetium with bisphosphonates. Radiochemical purity was checked by paper chromatography. Pyrogenicity was checked by administration of the labeled compound into rabbits. The stability of the compound was determined by noting the radiochemical binding at several intervals of half an hour after preparation. Biodistribution of the agent was studied by injecting the labeled compound into rabbits. The results showed that the compound could be labeled with ^{99m}Tc without any difficulty. The ease of binding was excellent. There was more than 95% binding of technetium with the compound and the labelled compound was reasonably stable for 5 hours after labeling. The rectal temperature remained stable during this period, which showed that the animal accepted the compound and there were no pyrogenic reactions. Biodistribution studies on rabbit showed that accumulation of agent was poor in bones and the labeled compound remains in blood even after 4 hours. Comparison of bone scans with those obtained with ^{99m}Tc-MDP and ^{99m}Tc-PYP showed that the compound can not compete with these bone agents as for as the quality of bone scans is concerned. Tc^{99m}-MDP still remains the superior bone scanning agent. However, the retention of relatively higher quantities of radiopharmaceutical in blood for long period showed that the compound could serve as a good blood pool-imaging agent. Purification of HPLC (high pressure liquid chromatography) could also improve the distribution properties of the compound. This needs further studies.

Keywords : Technitium-99m, Radiopharmaceutical, Chromatography, Radiochemical purity

1. Introduction

Technetium-99m labeled phosphates and technetium-99m labeled phosphonates and di(bis) phosphonates have since long been used as bone imaging agents and are still most frequently used [1]. Since the early 1970s a number of such compounds have been introduced for bone imaging. Initially ^{99m}Tc-tripolyphosphate was introduced, which was soon replaced with ^{99m}Tc-polyphosphate. 1-hydroxyethylidene diphosphonate (HEDP), methylene diphosphonate (MDP), hydroxymethylene diphosphonate (HDP) and pyrophosphate labeled with ^{99m}Tc were then introduced with relatively better results in bone imaging. Study of Subramanian G et al. (1975) [2] showed that ^{99m}Tc -methylene diphosphonate is a superior agent for skeletal imaging due to its better concentration and retention in bone tissue, negligible fecal excretion, fast clearance relative to pyrophosphates and polyphosphates, 1-hydroxyethylidene diphosphonate (HEDP), hydroxymethylene diphosphonate (HDP) and better image quality as early as 2 hr after administration and so far ^{99m}Tc-MDP is accepted worldwide. However, there is still a need of a radiopharmaceutical where we could complete bone scan earlier than the time required for ^{99m}Tc -MDP scan. To achieve this objective there is a need do research on phosphonates other than ^{99m}Tc-MDP. For this we will require a radiopharma-

* Corresponding author : kmsajid@yahoo.com

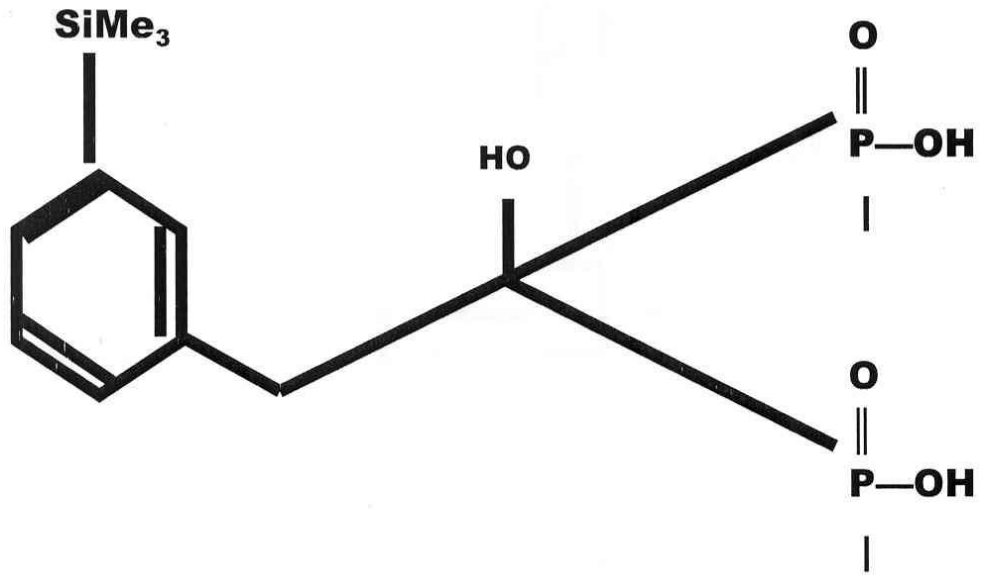


Figure 1. Structure of 1-Hydroxy (M-Trimethylsilylphenyl)-Ethylidene-1, 1-Bisphosphonate.

ceutical that is quickly adsorbed onto the monolayer of bone, has fast clearance from blood with negligible excretion through feces. Our recent efforts were therefore, an effort to label 1-hydroxy (m-trimethyl silylphenyl)-ethylidene-1, 1-bisphosphonate with technetium to see if it gives better results than ^{99m}Tc-MDP.

Bisphosphonates have also been labelled with ^{131}I. Animal experiments with ^{131}I -bisphosphonates purified by HPLC show good accumulation in bone but the labelled product has not been tried in humans [3]. Their potential for targeted radiotherapy needs to be evaluated. The labeling of bisphosphonates with ^{99m}Tc has not been done yet to see their superiority or inferiority relative to ^{99m}Tc -MDP.

A study was therefore, conducted with following objectives; (i) to label m-trimethyl silylphenyl)-ethylidene-1, 1-bisphosphonate with ^{99m}Tc and standardize the labeling procedure, (ii) to check radiochemical purity, pyrogenicity, stability and biodistribution of the compound, (iii) to compare the quality of ^{99m}Tc-bisphosphonate with ^{99m}Tc-MDP as a bone scanning agent, (iv) to predict on the basis of above findings the efficacy of compound (if labelled with ^{131}I) for targeted radiotherapy.

2. Materials and Methods

2.1. Labeling of 1-hydroxy (m-trimethyl Silylphenyl)-ethylidene-1,1-bisphosphonic acid with ^{99m}Tc and Standardization of the Labeling Procedure.

The principle of the technique was based on chemical behavior of technetium in solutions. Reduced technetium (Tc^{+4}) can accept lone pairs of electrons from oxygen atoms of bisphosphonate anions forming coordinate covalent bonds. The structure of 1-hydroxy (m-trimethyl Silylphenyl)-ethylidene-1,1-bisphosphonic acid is illustrated in Fig. 1. Tc^{+4} will form coordinate covalent bonds with oxygen atoms of bisphosphonate ions resulting in the formation of ^{99m}Tc-bisphosphonate coordinate complex (Fig. 2).

In experimental work on standardization of labeling procedure, three radiopharmaceutical preparations were tried. In first one (1 mg $SnCl_2.2H_2O$ and 1 mg bisphosphonate), we prepared a solution of 1-hydroxy (m-trimethyl Silylphenyl)-ethylidene-1, 1-bisphosphonic acid in normal saline at a concentration of 1mg/ml. To 1 ml of this solution (1 mg bisphosphonate) 50 µl (1 mg) of $SnCl_2.2H_2O$ in 3N HCl was added. Precipitates appeared in this mixture, which were filtered using disposable filter (pore size: 0.2 µm).

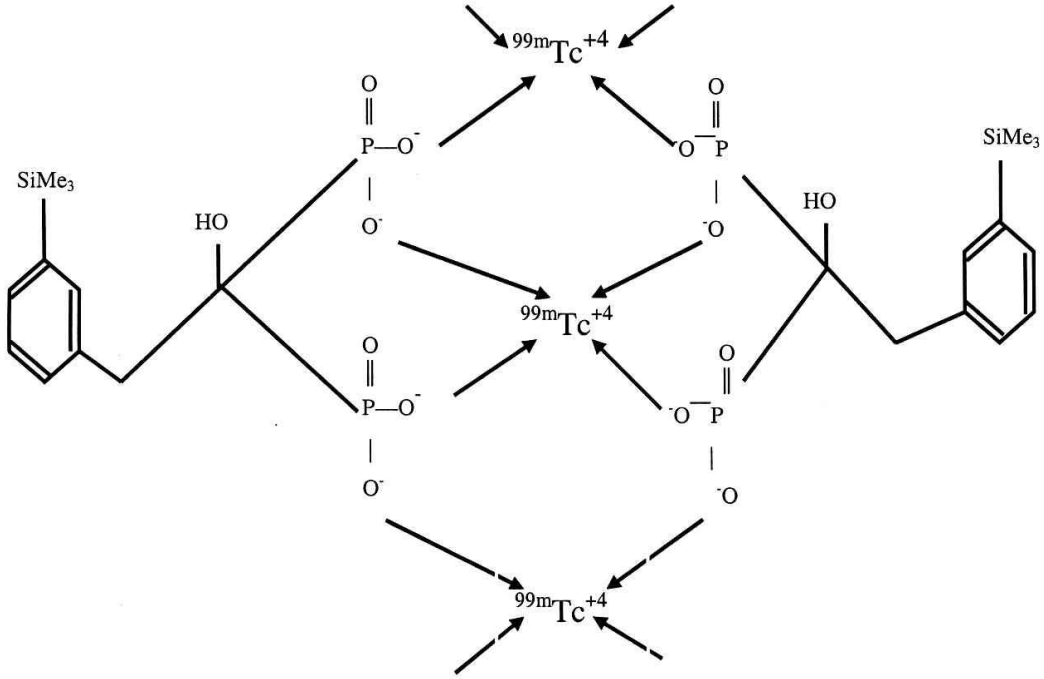


Figure 2. ^{99m}Tc-bisphosphonate complex.

The residue was discarded. To the supernatant about 25 mCi $^{99m}TcO_4^-$(in 0.06 ml saline) was added and paper chromatography was performed using filter paper (Whatman-1) as immobile phase. The chromatography data showed no binding of radioactivity (free 99.9%). In second formulation (0.5 mg bisphosphonate and 0.01 mg $SnCl_2.2H_2O$), dissolved 1 mg of bisphosphonate in 1 ml saline and added to it 100 µl (0.01mg) of $SnCl_2.2H_2O$ in 3N HCl. The mixture was filtered using disposable filter (pore size: 0.2 µm. 25 mCi $^{99m}TcO_4^-$ (in 0.5 ml saline) was then added to the supernatant. Adjusted pH of the supernatant to 6 and performed paper chromatography (in Acetone/Water system) to check the quality of labelling. Here about 50% binding of ^{99m}Tc with bisphosphonate was observed, remaining being free pertechnetate ($^{99m}TcO_4^-$). In the third preparation (0.5 mg bisphosphonate and 0.005 mg $SnCl_2$), we dissolved 1 mg of bisphosphonate in 2 ml saline and heated slightly. To 1 ml of this solution 100 µl (0.005mg) of $SnCl_2$ in 3N HCl. Clear solution was obtained. Again the mixture was filtered through disposable filter (pore size: 0.2 µm) and to the supernatant 25 mCi of $^{99m}TcO_4^-$ (in 0.5 ml saline) was added. Adjusted pH to 6 and performed paper chromatography to find the radiochemical yield. This preparation showed highest binding (more than 99.4%), the remaining being free pertechnetate).

For stability data on third preparation, serial paper chromatography experiments were performed upto 3 hours and % bound was calculated. To check pyrogenicity of the product 0.5 mCi of ^{99m}Tc-bisphosphonate was injected into a rabbit and rectal temperatures were noted for upto 5 hours. For biodistribution study of labelled product, 0.5 mCi of ^{99m}Tc-bisphosphonate was injected into a rabbit. The animal was sacrificed after 3 hours. Activity was measured in various organs. Activity per gram of tissue of different organs was calculated and compared to assess bone affinity of the compound.

2. Labeling of Methylene Diphosphonate (MDP) with ^{99m}Tc and Standardization of the Labeling Procedure

The principle of labeling was the same as described for labeling bisphosphonates. The structure of is methylene diphosphonate (MDP) is illustrated in Fig. 3. Tc^{+4} will form coordinate covalent bonds with oxygen atoms of bisphosphonate ions resulting in the formation of ^{99m}Tc-MDP coordinate complex (Fig. 4).

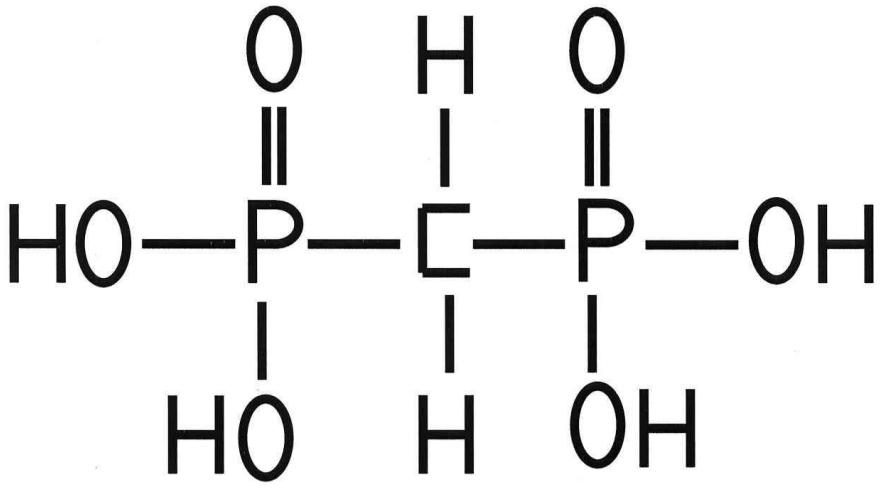


Figure 3. Methylene diphosphonate.

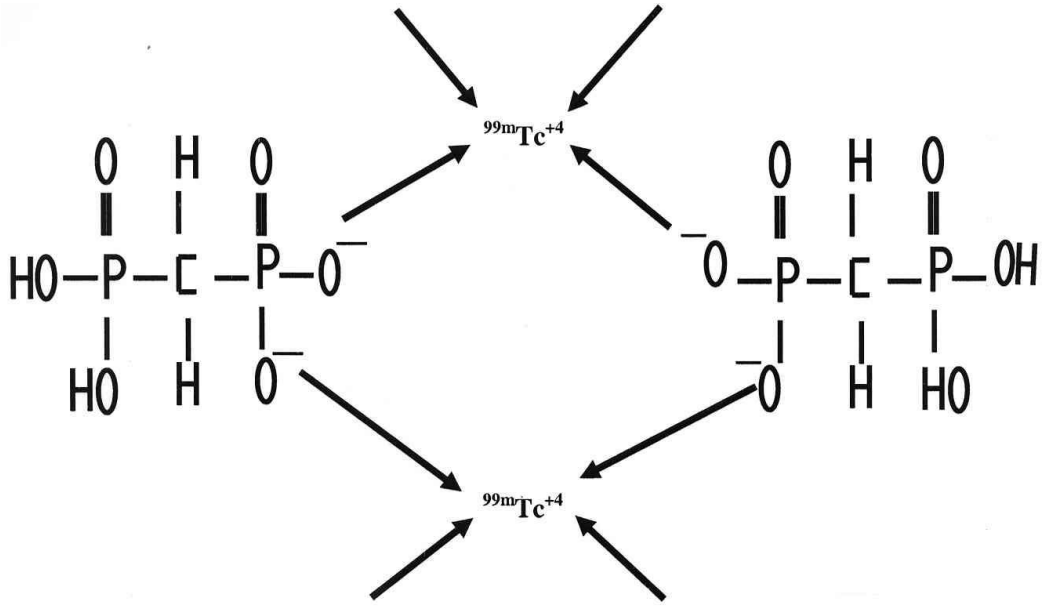


Figure 4. MDP – ^{99m}Tc complex .

For labeling we dissolved 5 mg of MDP in 1 ml saline followed by the addition of 250 µl (0.125 mg) of $SnCl_2.2H_2O$ in 3N HCl. Filtered the mixture through disposable filter (pore size: 0.2 µm) to obtain a clear solution. To the filtrate added 25 mCi of $^{99m}TcO_4^-$ (in 0.06 ml saline). Adjusted pH to 6 and performed paper chromatography (using whatman-1 paper) to check radiochemical yield. To check the stability serial paper chromatography (PC) experiments were performed upto 5 hours and % binding was calculated. For testing of pyrogenicity 0.5 mCi of ^{99m}Tc-MDP was injected into the rabbit and rectal temperatures were noted for upto 5 hours. For study of biodistribution of labelled product 0.5 mCi of activity was injected into a rabbit. The animal was sacrificed after 3 hours and activity appeared in various organs was measured. Activity per gram of tissue of different organs was compared to assess the bone affinity of the compound.

In order to study the effect of concentration of MDP on radiochemical yield, serial concentrations of MDP in normal saline, 10 mg/ml, 5 mg/ml, 2.5, mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.313 mg/ml, 0.156 mg/ml, 0.078 mg/ml were studied. Each concentration was mixed with o.125 mgs of $SnCl_2.2H_2O$ in 3N HCl followed by the addition of radioactivity. The rest of the procedures were the same as described above. To study the effect of concentration of $SnCl_2$ on radiochemical yield 20 mg of MDP was dissolved in 4 ml saline. Divided the solution in 4 parts (in four vials) each of 1 ml. Added 50 µl (0.2mg), 100 µl(0.4 mg), 150µl (0.6 mg) and 200 µl (0.8mg) of $SnCl_2$ in 3N HCL into each vial followed by addition of radioactivity and paper chromatography. Radiochemical data obtained on these experiments showed following optimum concentrations, MDP: 5-10 mg/ml, $SnCl_2$: 0.08—0.16.

Biodistributions of ^{99m}Tc-MDP and ^{99m}Tc-Bisphosphonate were compared to see which one is relatively a better bone imaging agent.

3. Labeling of Sodium Pyrophosphate with ^{99m}Tc and Standardization of Labeling Procedure

The procedure of labeling of sodium pyrophosphate with ^{99m}Tc and its biodistribution was similar to that described for MDP. It is also published in our previous work. [4]. Here more than 99.3% binding was observed. A comparison of biodistribution of ^{99m}Tc-PYP with ^{99m}Tc-MDP and ^{99m}Tc-Bisphosphonat is shown in Table 5 and displayed in Fig. 9.

3. Results and Discussion

3.1. Labeling of 1-hydroxy (m-trimethyl Silylphenyl)-ethylidene-1, 1-bisphosphonic acid with ^{99m}Tc and Standardization of the Labeling Procedure

Chromatographic data obtained with ^{99m}Tc labelled bisphosphonates (3rd preparation) is shown in Table1. More than 99% activity was tagged to bisphosphonate. The results on stability experiment are given in Table 2. The complex was fairly stable upto 3 hours after preparation. Data on biological distribution study is displayed in Fig. 5. There was very little activity in bone after 3 hours. High levels of activity remained in heart, liver and lungs. This shows poor chemisorption of radiopharmaceutical into the bone with sustained high activity in blood circulatory system. Radionuclide scans obtained with a rabbit (3 hours after injection) are shown in Fig. 6. The scans show poor bone uptake. There was very poor contrast between bone and other tissues.

Table 1. Labeling of Bisphosphonate with ^{99m}Tc.

Formulation-3: ~ 99.4% bound.

Strip number	Counts per 30 sec	%activity
1	2	0
2	105840	99.4
3	422	0.39
4	85	0.08
5	36	0.04
6	173	0.2
7	124	0.11
8	21	0.02
9	01	0
10	0	0
Total	106789	

Table 2. Labeling of Bisphosphonate with ^{99m}Tc.

Time (hr)	%binding
0	99
1	98
2	98.8
3	98.7

- The compound can be used for imaging until at least three hours after preparation.
- Fairly stable upto 3 hours after preparation.

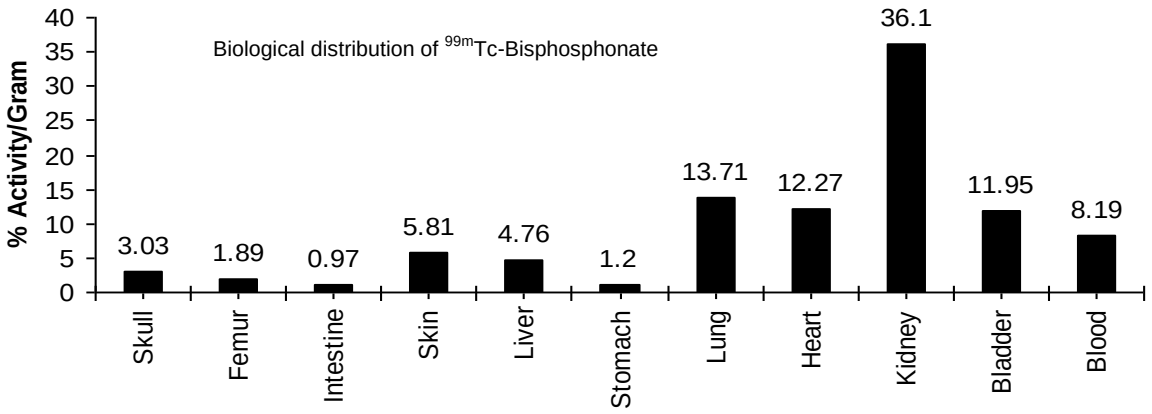
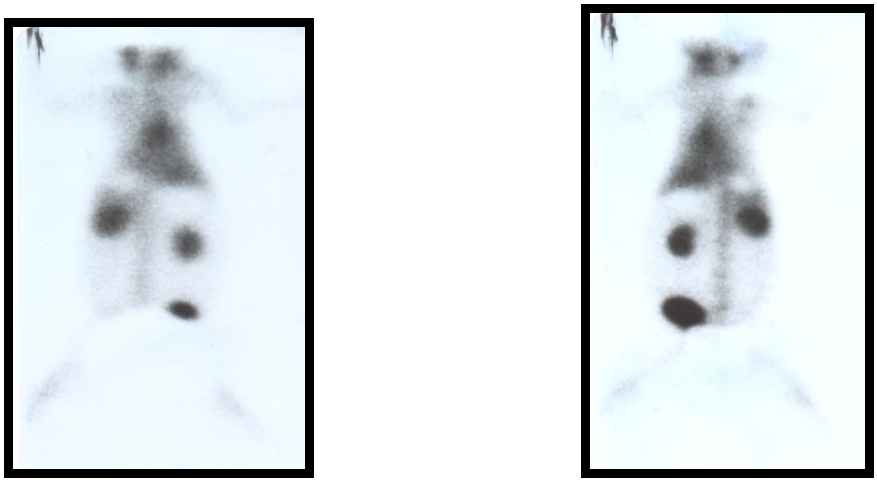


Figure 5. Results (Biodistribution In Rabbit) Labeling of Bisphosphonate with ^{99m}Tc.



Anterior View Posterior View

There was very poor contrast between bone and other tissues.

Figure 6. Biological Distribution Study (3 hr; RABBIT) Labeling of Bisphosphonate with ^{99m}Tc.

The scans taken 4 hours after injection are shown in Fig. 7. Here again high content of activity in blood was seen. Analysis of blood sample showed 81.2% activity bound to plasma protein whereas 18.9 % of activity was bound to RBCs.

3.2. Labeling of Methylene Diphosphonate (MDP) with ^{99m}Tc and Standardization of the Labeling Procedure

Data obtained in experiment on labeling of MDP with ^{99m}Tc is shown in Table 3. The compound was labeled very efficiently with technetium. The labeling efficiency was more than 98%. Chromatographic data obtained in stability experiment showed that more than 97 % activity remained tagged to the bone for more than 5 hours. Biological distribution data for MDP Labelled with ^{99m}Tc is shown in Table 4 and displayed in Fig. 8. Highest activity (28%) was seen in bone. Comparison of Biodistributions of MDP and bisphosphonates data (rabbit) is shown in Figure 9. ^{99m}Tc-MDP accumulates more in bone tissues. Bisphosphonate remains in circulation or excreted through kidneys. Bone Scans obtained with ^{99m}Tc-MDP (Fig. 10) show superior bone scans with MDP than bisphosphonate.

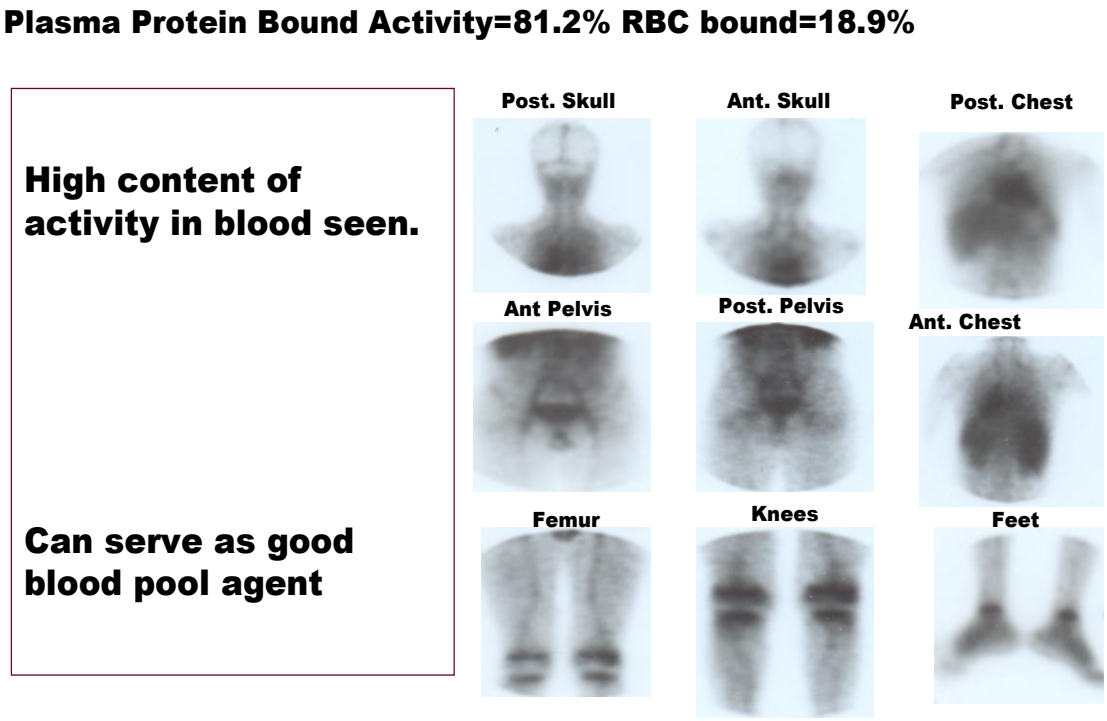


Figure 7. Results (study on patient) Labeling of Bisphosphonate with ^{99m}Tc Scan taken 4 hour after injection.

Table 3. Labeling of MDP with ^{99m}Tc (chromatography).

(More than 95 % activity remained tagged to the bone for more than 5 hours).

O hr		1 hr		2 hr		3 hr		4 hr		5 hr	
CPM	%Act	CPM	%Act.	CPM	%Act	CPM	%Act	CPM	%Act	CPM	%Act
3500	34.6	2378	39.13	8352	65.5	5153	51.24	3659	66.41	3751	48.46
6480	64.14	3593	59.12	4184	32.8	4720	46.93	1700	30.85	3662	47.31
5	0.05	4	0	13	0.10	20	0.19	24	0.43	35	0.45
13	0.13	22	0.36	30	0.23	29	0.28	15	0.27	7	0.09
10	0.10	10	0.16	23	0.18	0	0	6	0.10	11	0.14
18	0.17	15	0.24	5	0.03	24	0.23	20	0.36	106	1.36
28	0.28	6	0.09	4	0.03	13	0.12	24	0.43	48	0.62
21	0.20	20	0.32	52	0.4	13	0.12	21	0.38	47	0.60
18	0.18	15	0.24	35	0.27	63	0.62	20	0.36	37	0.47
09	0.17	14	0.23	14	0.10	11	0.10	20	0.36	35	0.45
9922		6077		12749		10056		5509		7739	

Table 4. Biological distribution of ^{99m}Tc-MDP .

No.	Organ or tissue	Weight (g)	Activity (µCi)	Activity(µCi/gram)	%Activity/gram
1	Femur	13.042	20.4	1.565	28.2
2	Skull	81.743	67.5	1.076	19.4
3	Bladder	1.939	2.6	1.342	24.3
4	Kidney	4.917	1.5	0.305	5.5
5	Liver	23.751	10.5	0.442	7.9
6	Lung	5.650	0.4	0.071	1.28
7	Heart	3.417	0.6	0.175	3.16
8	Stomach	47.275	3.4	0.072	1.30
9	Small intestine	49.723	2.4	0.048	0.87
10	Large intestine	87.032	1.8	0.021	0.37
11	Blood	8.176	1.0	0.122	2.2
12	Skin		13.5	0.237	4.2

Highest activity (28%) seen in bone

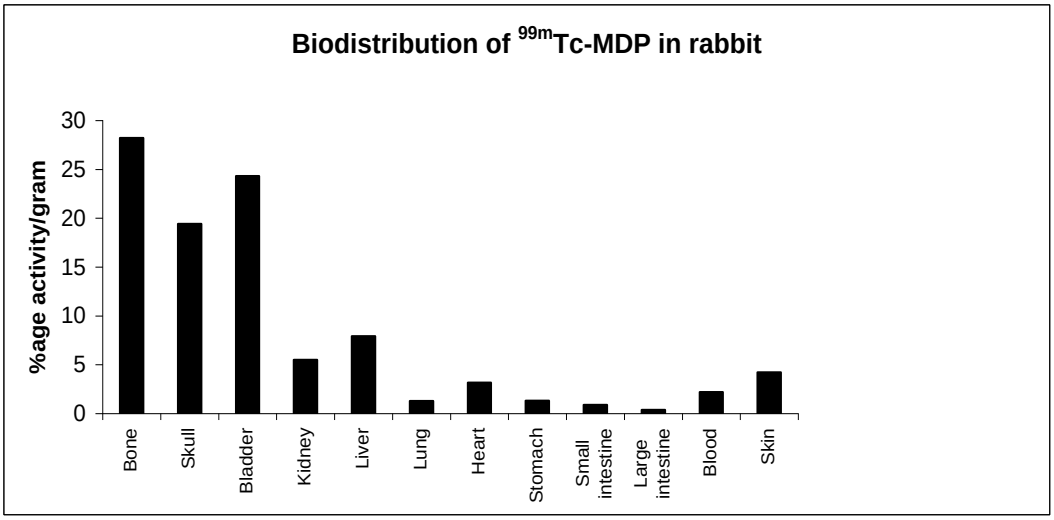


Figure 8. Labeling of MDP with ^{99m}Tc. Biodistribution data (rabbit) .

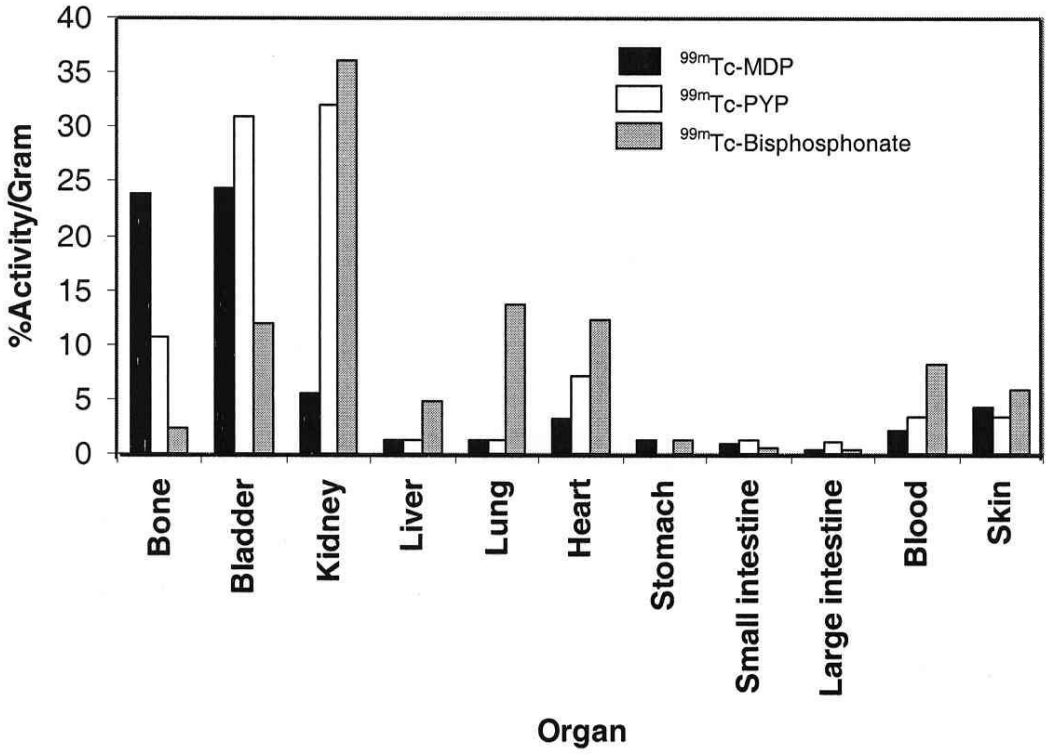
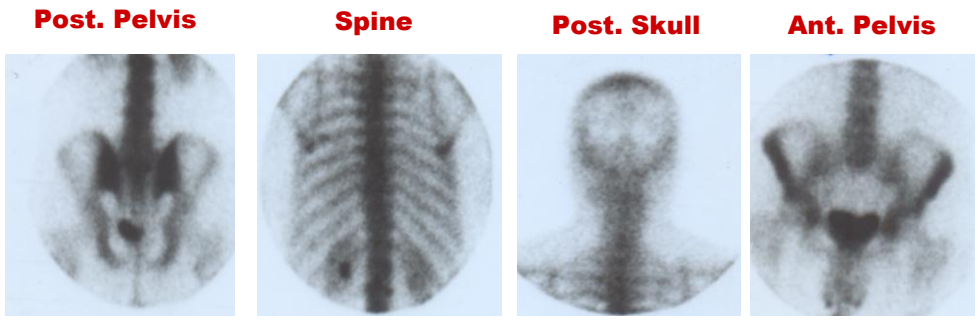


Figure 9. Comparison of Biodistributions of ^{99m}Tc-MDP, ^{99m}Tc-PYP and ^{99m}Tc-Bisphosphonate (^{99m}Tc-MDP accumulates more in bones compared to ^{99m}Tc-PYP and ^{99m}Tc-Bisphosphonate). ^{99m}Tc-Bisphosphonate remains in the circulation or excreted through kidneys.



Superior bone scans with MDP obtained compared to bisphosphonate scans

Figure 10. Bone Scans obtained with Tc-MDP .

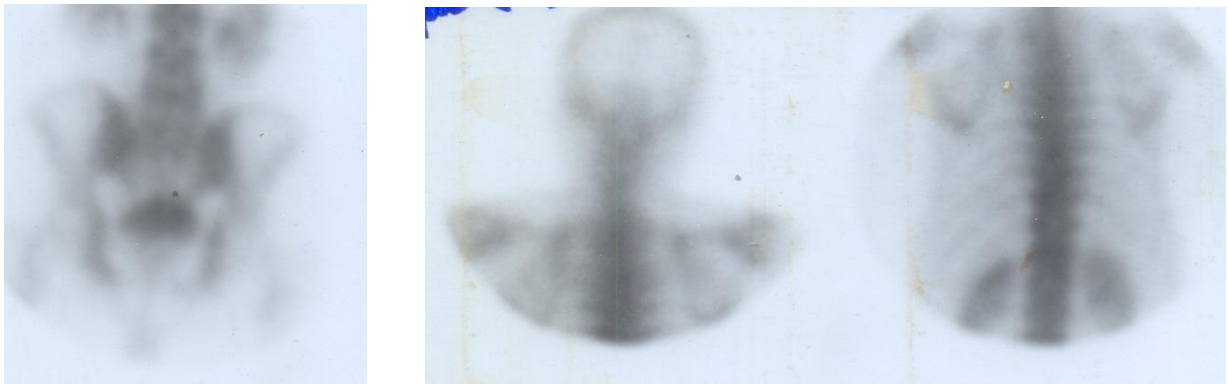


Figure 11. Bone Scans obtained with ^{99m}Tc-PYP

Table 5. Comparison between biological distribution of ^{99m}Tc -MDP and ^{99m}Tc –PYP and ^{99m}Tc-Bisphosphonate.

No	Organ or tissue	^{99m}Tc –MDP	^{99m}Tc –PYP* %activity per gram	^{99m}Tc–Bisphosphonate
1	Bone	23.8	10.60	2.3
2	Bladder	24.3	30.99	11.95
3	Kidney	5.5	32.08	36.10
4	Liver	1.31	1.31	4.76
5	Lung	1.28	1.24	13.71
6	Heart	3.16	7.07	12.27
7	Stomach	1.30	0.07	1.2
8	Small intestine	0.87	1.20	o.57
9	Large intestine	0.37	1.13	0.40
10	Blood	2.2	3.42	8.19
11	Skin	4.2	3.29	5.81

*K.M. Sajid, R.Mahmud. The Nucleus, 44 (3-4) 20071 43-153.

3.3. Labeling of Sodium Pyrophosphate with ^{99m}Tc and Standardization of Labeling Procedure

Bone scans obtained with ^{99m}Tc-PYP are shown in Fig. 11. The images reveal a better uptake in bone compared to ^{99m}Tc-bidphosphonates. However, 99mTc-Bisphosphate remains in the blood stream for long for very long period.

Bone scintigraphy with ^{99m}Tc–labeled bis(di) phosphonates is one of the most frequently performed radionuclide procedures because it has excellent sensitivity for many pathologic conditions like bone trauma, and osteomyelitis and will likely remain a popular and important imaging modality for years to come [1]. These compounds bind to the surface of bone by a process called chemisorption. Chemisorption or chemical adsorption results from chemical bond formation (strong interaction between the adsorbent and the adsorbate) in a monolayer on the surface [5]. The exact mechanism is not known although many mechanisms have been proposed [6-8]. Bis (di)phosphonates labelled with radioisotopes have also pain killing properties due to their radioactive nature [9]. Such radiopharmaceuticals have also found useful applications in the treatment of cancer. For example internally administered ^{186}Re-Iso(Sn) HEDP has also been used in some studies to treat multiple foci in bones [10]. Sodium phosphate (^{32}P), strontium chloride (^{89}Sr), and samarium (^{153}Sm) lexidronam. ^{117m}Sn-DTPA, ^{153}Sm phosphonate, ^{186}Re phosphonate have also been used because of their radiation properties that relieve pain [11].

Recently interest has grown in ^{131}I-labelled bisphosphonates [12]. However, the agents have never been tried in humans although the distribution studies have been carried out in mice [3]. As such their efficacy in the treatment of bone cancer remains to be evaluated. However, trials of the labelled compound on humans could result unnecessary exposure to radiation if favorable results are not obtained. We therefore tried to label the compound first with technetium to study its biodistribution in rabbits. The satisfactory accumulation of the technetium labelled compound could predict its safe and effective use in targeted radiotherapy. Our specific comments on the results obtained are summarized below:

The labeling efforts to prepare ^{99m}Tc-bisphosphonate were successful in achieving more than 99% radiochemical yield. The compound was radiochemically stable for more than 3 hours. Injection to rabbits showed no pyrogenic reactions. The compound was therefore safely accepted by the animal and no pyrogenic reactions were observed. Data displayed in Fig. 5 suggests high activities in blood, heart, lungs and kidney after 3 hours indicating slow clearance of the radiopharmaceutical from blood. The activity accumulated in bone was very low. Thus these observations showed poor efficacy of the compound as a bone agent. The radionuclide scan (Fig. 6) of rabbit obtained 3 hours after injecting the radiopharmaceutical showed very poor contrast between bone and other tissues. When tried on patient with bone disease it showed diffused pictures (Fig. 7). The labeling efforts to prepare ^{99m}Tc-Methylene diphosphonate were also successful and as high as 98% radiochemical yield was obtained. Injected dose was accepted by the animal without any pyrogenic reactions. The compound was fairly stable for upto 5 hours. The percentage binding remained between 95.6 to 98.3%. Biodistribution Studies on the labelled product showed about 20-28% activity in femur and skull.

Comparison of biodistribution of ^{99m}Tc-bisphosphonates with ^{99m}Tc-MDP and ^{99m}Tc-PYP (Fig.9) showed more accumulation of ^{99m}Tc-MDP (~23.8%) in bones relative to ^{99m}Tc-PYP(~10.8%) and ^{99m}Tc-Bisphosphonate (~2.3%). This suggests that ^{99m}Tc-Bisphosphonate cannot compete with ^{99m}Tc-MDP and ^{99m}Tc-PYP as bone agent. The comparison of images also shows that bone images obtained with ^{99m}Tc-Bisphosphonate are also relatively inferior in quality. The retention of high proportion of labeled activity in blood suggests that ^{99m}Tc-Bisphosphonate may be more suitable as blood pool imaging agent than a bone agent.

4. Conclusion

^{99m}Tc-Bisphosphate cannot compete with ^{99m}Tc-MDP and ^{99m}Tc-PYP for bone scanning. The retention of the radiopharmaceutical in circulation for very long periods shows that the compound can serve as a good blood-pool agent and may not be very suitable for bone scanning and targeted radiotherapy because of delayed accumulation in bones and poor target to non-target contrast. Purification of the radiopharmaceutical by HPLC

could probably improve its distribution properties (affinity for bones).

Acknowledgement

The authors acknowledge and appreciate the support of Dr. Durr-e-Sabih and Dr. Erik Arstad in arranging the supply of Compound (bisphosphonate) used in our study.

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