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《Original》

Studies on the Formation of Pyrophosphate—^{99m}Tc Complex

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Abstract

An instant labelling technique for lyophilized pyrophosphate with ^{99m}Tc is described. Labelling yield of about 90% is obtained at the pH range 3.5—5.5 on reconstitution with sodium pertechnetate-^{99m}Tc solution. The final product is controlled by a modified two dimensional paper chromatography using 85% methanol and 0.85% saline, and biodynamic investigations are performed on white mice. Generally, the less amount of stannous chloride is used, the higher labelling yield is obtained. The molar ratio of pyrophosphate to stannous chloride of 10 : 1~50 : 1 is sufficient. The more amount of reduced unbound ^{99m}Tc is injected, the more radioactivity is incorporated in the liver. Thus, the cause of the false bone-imaging is attributable to the presence of reduced unbound ^{99m}Tc which is known to be well adsorbed to oxidized tin colloidals.

The maximum uptake ratio of bone: liver in mice, in weight basis, 35 : 1 is achieved in time of 60 min. or so.

The preparation is suitable for clinical investigations on patients with bone diseases.

요 약

파이로포스페이트의 ^{99m}Tc 즉석표지 방법에 관하여 연구하였다. pH 3.5—5.5에서 냉동건조 상태의 파이로포스 페이트와 $SnCl_2$ 혼합물에 $Na^{99m}TcO_4$ 용액을 가하여 녹임으로써 대략 90%의 표지수율을 얻었다. 표지생성물의 순도와 수율은 85% 메탄올과 0.85% NaCl 용액을 전개용매로하는 2차원 종이 크로마토그래피로 검토하는 한편 생쥐를 실험동물로 하여 생성착물의 생체내 분포 실험도 실시하였다.

일반적으로 ^{99m}Tc (VII)의 환원제인 $SnCl_2$를 적은량 쓸수록 표지 수율이 좋았으며 파이로포스페이트와 $SnCl_2$의몰 비율은 10 : 1~50 : 1로 충분하였다. 환원된 미결합 ^{99m}Tc의 양이 많은 생성물을 생쥐체내에투여 할수록 간에 집적되는 방사능 양이 증가하는 것으로 보아 골격조영술에서 잘못된 영상을 얻게되는 원인은 환원된 미결합 ^{99m}Tc가 콜로이드 상태의 산화된 Sn 생성물에 흡착되어 일어 나는 것으로 생각되었다. 생성착물의 뼈와 간에 대한 분포비율은 투여후 60분 정도에서 최고 35 : 1이었으며, 파이로젠 시험결과도 양호함으로 생성착물은 골격질환의 연구나 진단목적에 적합함을 알수 있었다.

1. Introduction

The ^{99m}Tc bone radiopharmaceuticals pioneered by Subramanian and others[1)~6)] have revolutionalized skeletal imagings. A large number of these ^{99m}Tc bone scanning radiopharmaceuticals employ Sn (II) to reduce

^{99m}Tc (VII) (pertechnetate) to a lower valence state, and thereby making it more amenable to complex forming reactions. Thus, complexes of ^{99m}Tc with agents like diethylenetriaminepentaacetic acid (DTPA) [7),8)], human serum albumin (HSA)[9),19),11)], methylene diphosphonate (MDP)[12)], pyrophosphate (PYP)[13),14),15)] etc. are now routinely used in nuclear medicine laboratories. Reports about the unreliable performance of Sn (II) containing kits have appeared sporadically in the recent past, particularly with the use of PYP[7)].

Despite the inherent drawbacks with reference to troubles of the reducing agent in using instant labelling kits, Sn (II) has remained the most popular reducing agent for ^{99m}Tc radiopharmaceuticals. Srivastava et al.[7)] insisted that the unreduced ^{99m}Tc (VII) or reduced hydrolyzed colloidal ^{99m}Tc have very often detected in the preparations in quantities sufficient to produce faulty scans. Actually this kind of problems could be serious and warrants further studies.

In present paper, the authors have tried to establish a procedure of an efficient ^{99m}Tc labelling of PYP varying the amount and molar ratio of the reactants in acidic pH. This study also compares various analytical methods now in use to point out the advantage and disadvantage of each method. It is hoped that the data obtained throughout this study would also be helpful in elucidating the structure of the complex, and the labelling mechanisms further.

2. Experimental

2.1. Materials

a) Sodium pertechnetate ($Na^{99m}TcO_4$), radioactivity concentration; around 1mCi/ml, produced by neutron irradiations of ^{98}Mo target, radioisotope production group, KAERI

b) Tetrasodium diphosphate (pyrophosphate, PYP), a C.P. grade, E. Merck

c) Stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$), a G.R. grade, E. Merck

d) Argon gas, 99.998%, Matheson, U.S.A.

2.2. Procedures

2.2.1. Labelling of PYP

A procedure postulated by Huberty et al.[13)] was adopted varying the amount of the reactants; A 2 cm Teflon coated bar magnet was placed in a 50 ml Erlenmeyer flask. About 300 ml of redistilled water was heated to boil for about 10 min. Then Ar gas was bubbled into the boiled water while the water was kept cool in an ice-bath. The Ar gas purged water was used throughout the entire experiment. Five hundred mg of PYP and 15 ml of water were put into the 50 ml Erlenmeyer flask. By rotating the magnet stirring bar slowly the PYP was made dissolved. During which time Ar gas was also slowly flowed into the flask to minimize the possible contact with air. $SnCl_2$ solution containing 1.8 ml conc. HCl and 140 mg $SnCl_2 \cdot 2H_2O$ was prepared in a 10 ml volumetric flask with water added to a total volume of 10 ml. One ml of $SnCl_2$ solution was transfered into the PYP containing flask. The solution was well mixed, and the pH was checked with a narrow range pH paper. The pH was adjusted to about 5.5 with 1N-NaOH or 1N-HCl solution. One mCi (in some cases 10 mCi) of $Na^{99m}TcO_4$ solution was added to each 0.5 ml aliquot of the above $SnCl_2$-PYP solution, mixed, and kept for about 10 min. at room temperature with occational shaking. To change the molar ratio of PYP to $SnCl_2$, solutions of different concentra-

Table 1. Dependence of Labelling Yield on the Variation of Molar Ratios of PYP to $SnCl_2$*

$SnCl_2 \cdot 2H_2O$ in kit (ug)	Molar ratio (PYP:$SnCl_2 \cdot 2H_2O$)							Mean
	13.7 : 1	25 : 1	50 : 1	100 : 1	150 : 1	200 : 1	500 : 1	
31.25**	99.4		96.0	97.9		90.1	94.9	96
62.5	(99)***		(98)	(94)		96.7		
				96.3				
125	94.1		(100)	97.7	99.5			97
281.3	99.5	99.5	99.7	99.1				99
				(99)				
375	99.8	100	100					100
437.5	100	100	100					100
Mean	99	99	99	98		93		

*pH=5.5, yields were determined by a P.C. technique using 85% MeOH as a developing solvent.
**1.22×10^{-7} mole
***The values in parentheses indicate the yield determined for the the reconstituted samples which were millipore filtered prior to the freeze-drying.

Table 2. Dependence of Labelling Yield on the Variation of Molar Ratio of PYP to $SnCl_2$*

$SnCl_2 2H_2O$ in kit (ug)	Molar ratio (PYP:$SnCl_2 \cdot 2H_2O$)							Mean
	13.7 : 1	25 : 1	50 : 1	100 : 1	150 : 1	200 : 1	500 : 1	
31.25**	100		90.5	93.7		95.6	04.4	95
62.5	(56)***		(61)	(69)				
				94.2		95.7		95
125	84.4		(51)	90.5	91.2			90
281	94.2	94	100	94				96
			(88)					
375	64.6	49.8	52.6					56
437.5	64.3	54.3	16.8					45
Mean	94	84	65					

*pH=5.5, yields were determined by a P.C. technique using 0.85% NaCl as a developing solvent.
**1.22×10^{-7} mole
***The values in the parentheses indicate the yield determined for the reconstituted samples which were millipore filtered prior to the freeze-drying.

tions of $SnCl_2$ were prepared, and mixed as described above to eliminate the effects of volume change. (Table 1-3)

2.2.2. Determination of Labelling Yields

To determine labelling yields the following paper chromatography (PC) solvent systems were reviewed; 85% MeOH[16),17)] 15% H_3PO_4[18)], 30% acetone:HAc=7:3(v/v)[13)], 0.85% NaCl[1)7], 10% ammonium acetate: MeOH=1:1(v/v)[19)] etc. Using Whatman No. 1 paper, ascending PC were conducted and the radioactivities in each separated zone were counted. The labelling yields were expressed with radioactivities according to the following equation;

$$\% \text{ Labelling yield} = \frac{At}{Ap} \times 100$$

Ap; radioactivity in the zone of PYP-^{99m}Tc

At; radioactivity of other zones plus

Table 3. Dependence of Labelling Yield on the Variation of Molar Ratio of PYP to $SnCl_2$*

$SnCl_22H_2O$ in kit (ng)	Molar ratio (PYP:$SnCl_2 \cdot 2H_2O$)							Mean
	13.7 : 1	25. : 1	50 : 1	100 : 1	150 : 1	200 : 1	500 : 1	
31.25**	94.4	–	86.4	41.5	–	85.7	10.3	64
62.5	(55)***	–	(98)	(63)	–	92.4		
125	95.7*							
	88.6	–	(51)	88.2	91.2			90
281.25	93.5	93.5	99.7	93.1				95
			(88)					
375	64.4	49.8	52.6					56
437.5	64.3	94.3	16.8					45
Mean	81	66	64	78		88		

*pH=5.5, yields were determined by a modified two dimensional P.C. technique using 85% MeOH and 0.85% NaCl solution as developing solvents.

**1.22×10^{-7} mole

***The values in parentheses indicate the yields determined for reconstituted samples which were Millipore filtered prior to the freeze-drying.

#^{99}Tc:Sn (II) = 1.02×10^{-2} : 1[7]

Table 4. Comparison of Radiochemical Purity of PYP ^{99m}Tc Complex

Batch No.	^{99m}Tc(IV) (%)	^{99m}Tc(VII) (%)	PYP-^{99m}Tc (%)	Descriptions
523	26.0	0.6	73.4	Commercially available kits in Ref 28)
572	10.5	0.2	89.3	
572	9.5	0.1	90.4	
580	9.5	2.5	88.0	〃
582	19.4	0.1	80.5	〃
582	14.7	0.9	84.4	〃
601	25.2	0.4	74.4	〃
605	16.2	1.6	82.2	〃
Mean			83	〃
260979	14.4	0.6	85.5	kits prepared in present work, kept 2 day at -20°C
201079	11.9	0.5	87.6	
281179	10.0	0.2	89.3	
Mean			88	

*Determined by a modified two dimensional paper chromatography technique using 85% MeOH and 0.85% NaCl solution as developing solvents.

that of Ap.

2.2.3. Performance study

The radiochemical purity of the lyophilized kits prepared in lab-scale was compared with those of the kits of commercial source[28] applying the established PC systems. (Table 4).

To certify the correlation of the radiochemical purity of the complex with the bone distribution of the radioactivity, organ distribution studies were carried out using mice; About 0.1 ml of the labelled PYP having definite radiochemical purity was injected to the abdominal cavity of the mouse. After definite time intervals, the animals were sacrificed, and the radioactivities in liver, teeth, and bone were separately counted by using a well type γ counter (Aloka Model PC-10E) (Table 5, 6).

Pyrogen tests of PYP-^{99m}Tc were also performed by using rabbits (Table 7).

3. Results and Discussion

3.1. Labelling Efficiency and Chromatography

Table 1 shows a decreasing tendency of labelling yield as increasing the molar ratio of PYP to $SnCl_22H_2O$. An increasing tendency of labelling yield can also be observed as increasing the amount of $SnCl_2$. Thus,

Table 5. Distribution of PYP-^{99m}Tc Complex in Mouse (I)

Mouse No.	RCP*& amount injected	Injection time	Counting time	Weight of liver (g)	Weight of thigh bone (g)	Radioac'ty in liver (cps x 6)	Radioac'ty in thigh bone(cpsx6)	Radioac'ty per gm of liver	Radioac'ty per gm of thigh bone	Radioactivity ratio, thigh bone/liver	
1-1	23%0.1ml	13:35	15:00	2.3	0.4	430735	418434	187276	1046085	5.59	
1-2	〃	〃	〃	2.2	0.55	430658	429419	195754	780762	4.02	
1-3	〃	〃	15:50	1.2	0.45	431889	425731	359907	746069	2.63	Mean
1-4	〃	〃	〃	1.85	0.25	436859	431424	236140	1725696	7.31	value;
1-5	〃	〃	16:40	2.4	0.5	435001	437855	181250	875710	4.83	4.64
1-6	〃	〃	〃	1.9	0.5	448846	453283	236235	906566	3.84	
1-7	〃	〃	〃	1.85	0.4	357304	332875	193137	832188	4.31	
2-1	30%0.1ml	13:00	15:00	2.2	0.25	211745	356586	96248	1586344	16.48	
2-2	〃	〃	〃	3.2	0.25	363709	367931	113659	1471724	12.95	
2-3	〃	〃	15:50	1.55	0.45	209281	407479	135020	905509	6.71	
2-4	〃	〃	〃	3.15	0.4	246528	405428	78263	1013570	12.95	
2-5	〃	〃	〃	1.7	0.1	390091	183446	229465	1834460	7.99	11.88
2-6	〃	〃	16:40	2.3	0.2	214704	374704	93349	1873920	20.07	
2-7	〃	〃	〃	3.2	0.65	261960	360244	78738	554222	7.03	
2-8	〃	〃	〃	2.2	0.3	227795	338276	103543	1127587	10.89	
3-1	60%0.1ml	〃	15:00	2.3	0.45	94325	406009	41011	902244	22	
3-2	〃	〃	15:50	2.2	0.55	86099	559659	39136	1057560	26	25
3-3	〃	〃	16:40	1.9	0.32	9675	45620	5092	142565	28	
4-1	88%0.1ml	〃	15:00	3.2	0.53	66425	440070	20758	630321	40	
4-2	〃	〃	15:50	3.2	0.45	85337	372024	26668	826722	31	35
4-3	〃	〃	16:40	1.6	0.4	35507	1319572	22192	798930	36	

*Radiochemical purity of PYP-^{99m}Tc complex

when the amount of $SnCl_2 \cdot 2H_2O$ reached to 437.5 μg, the yield was 100 %. Generally, when the lyophilized samples were reconstituted, the yields were slightly decreased due probably to the exposure to air during membrane filtration and subsequent lyophilization. Anyway, the PC using 85 % MeOH indicated quite high labelling yield of more than 90 %. According to the data in Table 1, PYP can be labelled with ^{99m}Tc with high yield regardless the variation of conditions. The results indicate, however, the better yield is obtainable by increasing the amount of $SnCl_2$. The ^{99m}Tc(Ⅶ) would be well reduced with increasing the amount of $SnCl_2$. In contrast, when 0.85 % NaCl solution was used as a developing solvent[16], the yields were decreased sharply as increasing the amount of $SnCl_2$ and also there was an apparent tendency of yield decrease with increasing the molar ratio of PYP to $SnCl_2$ especially when the amount of $SnCl_2$ was increased to more than 375 ug. (Table 2). According to the literature[16], 0.85 % NaCl solution should be combined with 85 % MeOH to establish a two dimensional PC. The NaCl solution separates the reduced unbound ^{99m}Tc(Ⅳ) from the reaction mixture (composed mainly of the reduced unbound ^{99m}Tc(Ⅳ), the PYP-^{99m}Tc complex, and the unreduced ^{99m}Tc(Ⅶ) etc.) but not separates the sole PYP-^{99m}Tc complex. Since ^{99m}Tc(Ⅶ) can be separated by 85 % MeOH, a two dimensional PC combining these two solvents can well be adopted. (Fig. 1).

Table 6. Distributon of PYP-^{99m}Tc in Mouse(II)

Mouse No.	Radiochemical purity and amount injected	Time elapsed from injection	Radioactivity (cpm/mg organ)			Distribution ratio	
			Liver (A)	Thigh bone (B)	Teeth (C)	B/A	C/A
1	35%, 0.1 ml.	125 min	665	12169	5915	18.3	8.9
2	〃	140	467	4265	2102	9.1	4.5
3	〃	147	593	6223	3056	10.5	5.2
4	〃	152	402	5198	2079	12.9	5.2
5	〃	156	700	5268	2458	7.5	3.5
6	〃	169	247	1650	917	6.7	3.7
7	〃	175	346	4354	1840	12.6	5.3
8	〃	181	298	4429	1733	14.9	5.8
9	〃	185	660	2985	2061	4.1	3.1
10	〃	169	192	5228	1153	27.2	6.0
11	〃	194	443	648	1756	1.5	4.0
12	〃	200	151	411	579	2.7	3.8
13	〃	211	12	253	64	22.2	7.4
14	〃	217	379	1865	1556	4.6	4.1
15	〃	213	604	3177	6820	5.3	11.3
16	〃	222	1327	8219	2474	6.2	1.9
17	〃	228	677	2362	1570	3.5	2.3
18	〃	232	228	2836	31	12.4	—
19	〃	237	1251	2231	7252	1.8	5.8
20	〃	241	345	10968	4215	31.8	12.1
21	〃	245	180	6442	5363	35.8	29.8
Mean:						11.9	6.70

Table 7. Pyrogen Test of PYP-^{99m}Tc Complex

Rabbit No.	Body weight (g)	Amount injected* (ml)	Body temp. (1 hr intervals ·C)						Temp. rise (·C)	Description
			Before injection			After injection				
			1 st	2 nd	3 rd	1 st	2 nd	3 r9		
1	1700	0.5	39.2	39.3	39.1	39.3	39.2	39.4	0.3	negative
2	1750	0.5	39.5	39.5	39.4	39.3	39.5	39.6	0.2	〃
3	1800	0.5	39.8	39.7	39.6	39.5	39.6	39.6	0	〃

*K.P. regulates that the common pharmaceutical samples of 10 ml is injected. However, in present work about 1 mCi (=0.5 ml) was injected intravenously since the sample is a radiopharmaceuticals.

To simplify the PC manipulation, however, we have applied the two solvents separately; i.e., the percentage of the ^{99m}Tc (Ⅶ) measured on the chromatogram obtained by using 85% MeOH was subtracted from the percentage of PYP-^{99m}Tc complex plus ^{99m}Tc(Ⅶ) which was measured on the chromatogram obtained by using 0.85% NaCl solution. Table 3 shows the net labelling yields obtained by this method. Generally, the yields were increased with decreasing the molar ratio of PYP to $SnCl_2$, which is partly contradictory to the conclusion made by Srivastava et al.[7] They concluded that the yield increases with increasing the molar ratio of PYP to $SnCl_2$,

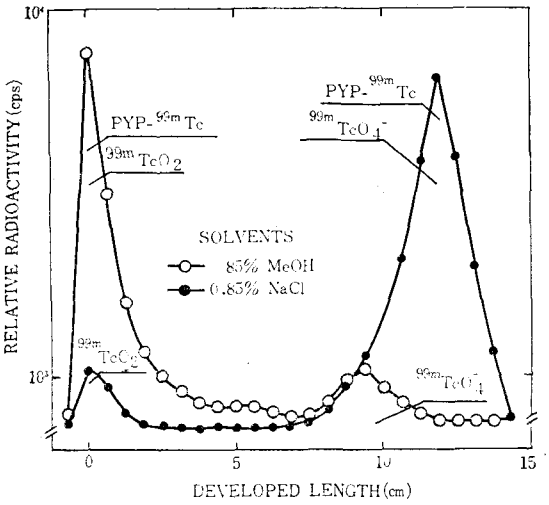
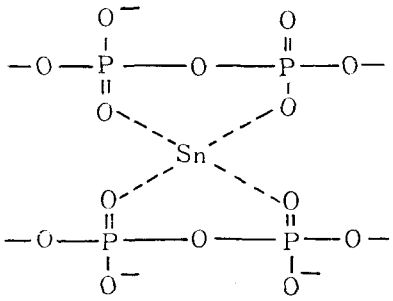


Fig. 1. Radiopaper Chromatogram Obtained by using 85% MeOH and 0.85% NaCl Solution (Sample: PYP Reaction Mixtures)

and with decreasing the amount of $SnCl_2$. When the amount of $SnCl_2$ is increased, the amount of the oxidized tin such as SnOHCl or $SnOCl_2$ etc. would also be increased, and since these compounds are hardly soluble in water, they remains as colloidal forms[7]. The reduced ^{99m}Tc(Ⅳ) (or ^{99}Tc(Ⅲ)) is apt to be adsorbed to the colloid and thus the labelling efficiency degenerates.[7].

Based on the postulated hypothetical structure of metal triphophate complex[20], the plausible structure of PYP-Sn complex is proposed as follows;



(I)

This structure also shows that the PYP-Sn complex has some possibilities of being a polynuclear one.

In the literature[7], the percentage of ^{99m}Tc labelling of PYP is inversely proportional to $^{99}Tc/Sn$ ratio. The data were discussed by S.C. Srivastava et al.[7] with that tin would be complexed with PYP and would be cut down on the competition between Sn and Tc for the ligand. However, in accepting their insist, following points are wondered; Firstly, Sn is complexed in advance with PYP in kits[7] (or in non kit form) since PYP is mixed with Sn prior to be mixed with ^{99m}Tc. Secondly, the number of ^{99m}Tc is far less than that of Sn in usual preparations for diagnostic use (15 mCi of ^{99m}Tc is about 2.5 ng while Sn in kit is in μg order). Thus, insofar as the complexing abilities of the two metals are equal, ^{99m}Tc can hardly, be labelled. Thirdly, the reduced form of ^{99m}Tc from pertechnetate ($^{99m}TcO^-_4$) is $^{99m}Tc^{2+7}$ which is apt to be hydrolyzed to a relatively stable $^{99m}TcO_2$[3,21] Therefore, the complexing ability of ^{99m}Tc is expected to be not stronger than that of Sn.

However, since the labelled complex is relatively well formed, it is considered that the complex would be stabilized by the two different metals (a mixed metal complex). We would like to explain the Srivastava's data not with a competition between Tc and Sn but with a stabilization by two metal-complex formations. The main reason of the contradictory argument is as follo-wings; When Tc/Sn ratio is large, the percentage of complex formation is low[7]. If there is a competition between Sn and Tc, the percentage of complex formation should rather be high. When Tc/Sn ratio is small, the percentage of complex form-

ation is high[7]. But if there is a competition between Sn and Tc, the percentage of complex formation should rather be low. Thus, the above described hypothetical structure of PYP-Sn should be modified similarly to the postulated hypothetical one for DTPA-Sn-^{99m}Tc[21,22,23] as (Fig. 2)

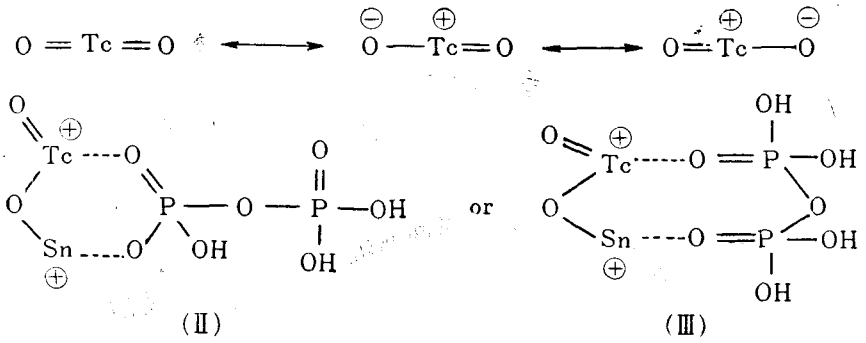
The latter structure is postulated only to emphasize the participation of the two metals regardless the ring member. Even though the participation of Sn in complex formation would be influenced more or less by the character of the complexing agent, the possibility of the formation of the two metal-complex cannot be excluded in the case of ^{99m}Tc labelling of PYP using $SnCl_2$. The mixed metal complex would therefore be expressed as PYP-Sn-Tc[15,24] while those of the electrolytically labelled complex (Sn-free preparations) is expressed simply as PYP-Tc.[25,26]

As Table 3 shows, when the amount of Sn(Ⅱ) is too small, the percentage of complex formation is decreased. The cause of such tendency may be attributable to the feasible oxidation of the small amount of Sn by air. When the amount of Sn is too large, the percentage of the complex formation is also decreased due probably to the increased amount of colloidal Sn[7,17,28] and consequent adsorption of ^{99m}Tc to the colloid. This tendency is consistent with that in the literature[25].

The cause of degenerating of labelling yield by increasing molar ratio of PYP to $SnCl_2$ is unclear. The similar tendency could be observed when some α-substituted carboxylic acids were used as ligands.[27]

Anyway, the conduction of ^{99m}Tc complex formation reaction using $SnCl_2$ was troublesome due to the rapid oxidation of Sn(Ⅱ) to Sn(Ⅳ) by oxygen in air and in water. Thus, the formation of $SnOCl_2$ colloid is inevitable. Since ^{99m}Tc(Ⅳ) or ^{99m}Tc(Ⅲ) is apt to be adsorbed to $SnOCl_2$, the amount of $SnCl_2$ should be minimized to the range of only reduction of ^{99m}Tc(Ⅶ) is possible. More than 200 μg $SnCl_2.2H_2O$ per labelling tube is too much. On the contrary, less than 30 μg would make a poor reproducibility. Also, the molar ratio of PYP to $SnCl_22H_2O$ of 10:1~50:1 would be better than 100:1~200:1.

The 15 % H_3PO_4[18] was not suitable solvent for PC of PYP-^{99m}Tc complex. since the PYP is easily hydrolyzed in acidic medium (at pH below 3.5).[13] phosphate-^{99m}Tc complex was made in similar mannar to that of PYP-^{99m}Tc complex and it was subjected to PC using 15 % H_3PO_4. Since the peak of H_3PO_4-^{99m}Tc complex was just superimposed upon that of PYP-^{99m}Tc complex it is wondered that the third peak of curve A is truly originated from the PYP-^{99m}Tc or not.



(Ⅱ) (Ⅲ)

Fig. 2. Hypothetical Structare of PYP-Sn-Tc Complex

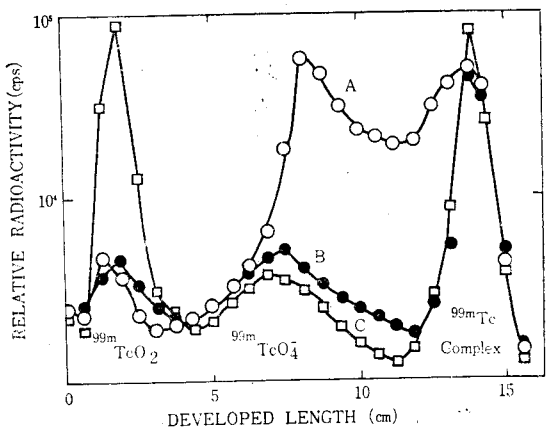


Fig. 3. Radiopaperchromatogram obtained by using 15% H_3PO_4
Sample: A: PYP—^{99m}Tc reaction mixture B: Mixture of $SnCl_2$+$^{99m}TcO^-_4$, C: Mixture of H_3PO_4+$SnCl_2$+$^{99m}TcO^-_4$.

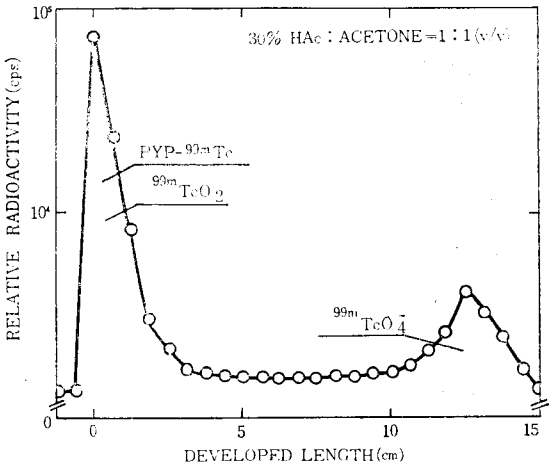


Fig. 4. Radiopaperchromatogram of PYP—^{99m}Tc ebtained by using 30% HAc: Acetone= 1:1(v/v)

(Fig. 3). When the mixture of $SnCl_2$ and $Na^{99m}TcO_4$ solution was subjected to PC, the third peak was also appeared. It indicates that the solvent(H_3PO_4) itself participates in a complex formation. Further, by H_3PO_4 solvent the resolution of PYP-^{99m}Tc against $^{99m}TcO_4^-$ is also poor.

On account of the above described drawbacks, the 15% H_3PO_4 is not suitable for the PC of PYP-^{99m}Tc.

The separation pattern by PC using the

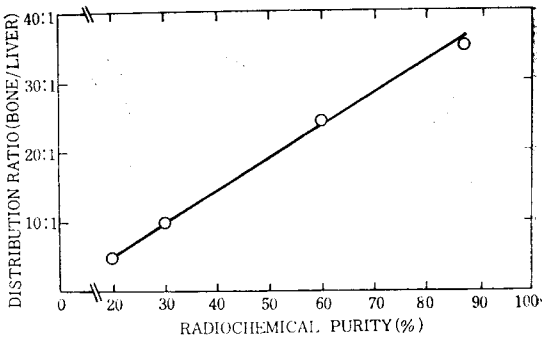


Fig. 5. Radiochemical purity v.s. radioactivity distribution (bone/liver) in mouse (Refer to Table 5)

acetone: HAC system[13] was simiar to that of 85% MeOH (Fig. 4). The acetone- HAc system separates only ^{99m}Tc(Ⅶ) and not the sole PYP-^{99m}Tc complex. Ammonium acetate:MeOH system which was originally proposed as a TLC solvent[19] also showed a poor resolution. Therefore, the modified two dimensional PC using 85% MeOH and 0.85% saline[17] is considered to be one of the best solvent for PC of PYP-^{99m}Tc.

3.2. Performance

The average radiochemical purity of our freeze-dried kits stored at 4°C for 3 days was about 88%. (Table 4). Such a purity is above of the average value (83%) for the commercial kits[28]. As Table 5 shows, the radioactivity ratio of bone/liver was lower when the complex of lower labelling yield (i.e, the product of low radiochemical purity of PYP-^{99m}Tc due to the presence of ^{99m}Tc(Ⅳ)) was injected comparing with that of higher labelling yield was injected. These results are consistent with that in the literature[24] althouh they are not directly comparable with ours due to the difference in data displays. (Fig. 5).

On the other hand, the complex having about 35% radiochemical purity was injected to check distributions in teeth (Table 6).

Even though the radioactivity distribution in teeth is less than that of bone, the complex has high affinity to teeth too. Thus, the PYP-^{99m}Tc complex is a potential biomedical tracer for the dentistry research. The prepared complex meets the requirements for intravenous injection as it is pyrogen-free as shown in Table 7.

4. Conclusions

For an efficient preparation of pyrophosphate-^{99m}Tc complex using stannous chloride, the amount of stannous chloride should be minimized as far as possible so as not to form excess colloidal tin compounds. The molar ratio of pyrophosphate to stannous chloride should be controlled to 10:1~50:1.

Using stannous chloride of highly pure grade and conducting the entire procedure in an inert atmospheric condition are both essential. Since the radiochemical purity determined by a modified two dimensional paper chromatography using 85 % MeOH and 0.85 % saline has close correlation with the biodynamic data, the method is considered to be a correct one for the determination of the radiochemical purity of pyrophosphate-^{99m}Tc complex.

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