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PHYSICOCHEMICAL STUDIES OF THE REACTION OF ^{99m}Tc WITH 2-THIOURACIL AND 5-NITROBARBITURIC ACID UNDER DIFFERENT CONDITIONS

Masoud M. S. [*], Soayed. A. A. [*] and Elkholany A. S. [**]

ABSTRACT

The reaction of ^{99m}Tc of different oxidation states (+7, +4) with 2-thiouracil and 5-nitrobarbituric acid have been studied at different temperatures, pH and concentrations. The reaction mixtures have been analyzed at different times using TLC and a radiodetctor to show the peaks at the plates.^{99m}Tc is obtained from the Mo generators with oxidation state (+7). The use of $SnCl_2$ as a reducing agent gave ^{99m}Tc with oxidation state (+4). It is very difficult to separate the complexes formed from the reactions in very small concentration. The percentage of ^{99m}Tc and its oxidation state involved the complexes can be determined.

* Faculty of Science, Alexandria University.
** International Medical Center.

INTRODUCTION

Technetium is present in the environment principally as a result of fallout from nuclear weapons testing, uranium enrichment, nuclear fuel processing, and disposal after pharmaceutical use [1].

During nuclear fuel processing, ^{99m}Tc is solubilized from spent fuels and is present in all waste streams principally as the pertechnetate anion [^{99m}Tc (VП) O_4]. ^{99m}Tc may exist in oxidation states VП.VI, V, or IV,but in the absence of strong complexing agents. Tc(VI) and Tc(V) may be expected to disproportionate to Tc(VП) and Tc(IV)[2]. The dominated oxidation state under oxic condition is ^{99m}Tc (V11), which is weakly, sorbed most soils and subsurface sediments at near neutral pH values [3,4] . Under anoxic conditions and in the absence of aqueous agents [2], ^{99m}Tc (IV) is largely immobile because it forms concentration-limiting solid phase and strong complexes with hydroxylated surface sites on Al and Fe oxide and clays [5,6].^{99m}Tc has become the mainstay of diagnostic nuclear medicine and in majority of the diagnostic scans performed each year in hospitals. This preferential use of ^{99m}Tc radiopharmaceuticals reflects the ideal nuclear properties of the isotope, as well as its convenient availability from commercial generator columns. ^{99m}Tc emits 140-KeV –γ-ray with 89% abundance, which is close to optimal for imaging with commercial gamma cameras. The availability of the relatively stable ^{99m}Tc isomer allows development of technetium coordination chemistry and modeling of technetium radiopharmaceutical [7].

Pyrimidine derivatives constitute an important class of compounds because they are components of the biologically nucleic acid. They have been shown to exert pronounced physiological effects [8]. Barbituric acid derivatives are well-known class of compounds many of which are widely used drugs having such disparate pharmacological activities as depressants, hypnotics and stimulants [9].

Aim of work.

Studying the reaction of ^{99m}TC of oxidation states (+7, +4) with 5-nitro-barbituric-acid at different temperatures, pHs, concentrations and different analytical times, and the reaction of ^{99m}Tc of oxidation states (+7, +4) with 2-thiouracil at different temperatures and analytical times by using thin layer chromatograph.

EXPERIMENTAL

Synthesis of ligands:-

a) ^{9m}TC-pertechnatate ($^{99m}TCO_4^-$) in isotonic saline was obtained from a commercial generators (Elutec^{TM} – Belgium, Syrtec-Syrian), being freshly eluted within 24 hours from previous elution.
b) 0.1N $SnCl_2$ stock solution was prepared and standardized. 0.1ml of this solution was added to $^{99m}TCO_4^-$ to reduce ^{99m}TC from oxidation state (+7) to (+4).
c) The silica gel TLC plates (Whatman-250µm layer) were used for thin layer chromatography, 5 µl of ^{99m}TC complexes solution was spotted on (0.8 x 10 cm) TlC plate, which was then placed into chamber and developed by a mixture of acitonitrile and water (95:5) to the top of the plate, finally the plate was dried.
d) Radio detector (Bioscan-AR-2000) was used to identify the position of the radioactive spots on the plate, to determine both the percentage of the activity and the R_f values of the spots on the plate.
e) Buffer solutions (4,7and 10),(Per-pH-ect^{TM}-Orion)were used.
5-nitro barbituric acid was dissolved in water, while 2-thiouracil ligand dissolved in warm dimethyl sulphoxide(DMSO).

The reaction of ^{99m}TC of oxidation states (+7, +4) with 5-nitro-barbituric-acid were studied at different temperatures, pHs, concentrations, and different analytical times.
For the reaction of 2-Thiouracil with ^{99m}TC of oxidation states (+7, +4) at different temperatures and different analytical times were studded. The reaction mixtures were prepared in a total volume 1ml containing 0.1ml- 30 % ammonia solution, 1-5 mCi of $^{99m}TCO_4^-$ and the ligand solution.

To prepare ^{99m}TC with oxidation state (+4), 0.1ml 0.1N solution of $SnCl_2$ was added to the reaction mixture.

RESULTS AND DISCUSSION

Characterization of the ^{99m}TC complexes as well as the determination of the extent of radiolabeling was done by thin layer chromatography using acitonitrile and water (95:5%) mixture as a solvent. The R_f-value [10] of $\mathbf{^{99m}TCO_4^-}$ (≈1),where that of ^{99m}TC (after reduction) is nearly zero[11].

The TLC data of $\mathbf{^{99m}TCO_4^-}$ -5-nitro-barbituric-acid complex at different pH-values, temperatures and analytical times are summarized in Table (1and 2) and represented ine Figur (1).

At the start of the reaction (0h) between $\mathbf{^{99m}TCO_4^-}$ and 5-nitro-barbituric-acid (at pH=10 and room temperature),no complexes were formed and only two bands of $\mathbf{^{99m}TCO_4^-}$ (+7)(R_f ~1) and $\mathbf{^{99m}TCO_2}$ (+4) (R_f ~0)were observed[11]Figure(1). After (1h) a band appeared at R_f ≈0.3 with 51% composition, indicating the formation of a complex. This

band disappeared between (3-6h), but after (20h) of the reaction start a new band appeared at R_f ≈0.7 with % composition of 46.36. The difference in position (from R_f ≈0.3 t R_f ≈0.7) indicates that there is an equilibrium between more than one complex.

If the above reaction was carried out at 40ºC, Table (1) a complex compound is formed at R_f ≈0.3-0.45 with 79.69% composition. Such complex was decomposed after 3h, and started to appear once more after (20h) of the reaction.
At 60ºC, two bands were given at R_f ≈0.3-0.45 an R_f ≈0.7 between (1-20h), the % composition of which changed by time indicates that there is an equilibrium between more than one complex.

At pH=7, no complexes were formed and most of the ^{99m}Tc ligand mixture moved to the top of the plate (R_f ≈1) indicating that ^{99m}Tc remains as **$^{99m}TCO_4^-$** and did not react with ligand at different temperatures. However, it is worth mentioning that only one complex was formed after 20h at 60ºC, and pH=7.

The TLC data of **$^{99m}TCO_4^-$** -5-nitro-barbituric-acid at pH=4 and room temperature, 40ºC and 60ºC are given in Table (2). This demonstrated the presence of two complexes at equilibrium at R_f =0.125 and R_f ≈0.8 but their % composition could not be detected due to its existence as noisy bands.

As for the reaction between the reduced form $^{99m}TCO_2$ ($^{99m}Tc^{+4}$) with 5-nitro-barbituric-acid, at different pH-values (4,7and 10), temperatures (RT, 40ºC and 60ºC) and different analytical times, the TLC (95:5) acetonitrile-water mixture, ,showed that $^{99m}Tc^{+4}$ is not reactive with the ligand except at pH=4 after (1h) at 60ºC, 20h at RT and pH=10 after 6h and 20h at 40ºC where a complex is indicated.

From Tables (1 and 2) and Figures (1,2and 3) we can conclude that the labeling efficiencies (% of complex) are maximum at pH=10 and that both oxidation states of ^{99m}Tc(+7, +4) appear at pHs 4 and10. But at pH=7 only the **$^{99m}TCO_4^-$** species was observed, where at pH=4, the reduced form **$^{99m}TCO_2$** is more pronounced.

Taking different concentrations of the ligand (5 m mol, 11.6 m mol, 21.4 m mol, 50 m mol and 105 m mol) in 0.1 ml of 30% ammonia solution and 4-5 m Ci of **$^{99m}TCO_4^-$** after 45 min of reaction, the TLC, Figure (4), depicts that increasing the ligand concentration results in increasing the labeling efficiencies of the complex.

The TLC data of ^{99m}Tc with 2-thiouracil at different temperatures and analytical times were studied and a representative example, Figure(5) is shown.

The reaction mixture consists of **$^{99m}TCO_4^-$** (1-5 m Ci), 0.1 ml (0.1N) $SnCl_2$ and 0.1 ml 30 % ammonia solution. From, Table (3),it could be noticed that at room temperature, the labeling efficiency of the complexes formed increases gradually by time, whereas that of **$^{99m}TCO_2$** decreases. Also by changing temperature to 40ºC, the % of complex

increased compared to those at room temperature. At 60ºC, the % of complex reached maximum value (81.11%) after 100 min. of the reaction, after which the complexes decomposed and only one complex appeared on TLC with different R_f values at different times.(Table (4)).

The reaction of $^{99m}TCO_4^-$ with 2-thiouracil at different temperatures and analytical times in the presence of 0.1ml. 30% ammonia solution was also studied. The results of TLC in (95:5) acetonitrile - water mixture are given in Table (5).

At roomtemperature, a complex was formed at $R_f \approx 0.6$ after (1h), then decomposed and reformed again after (4h). Another complex $R_f \approx 0.7$ was formed in very small amount and decreased by time. At 40ºC and 80ºC two complexes were formed with different % composition as shown in Tables. At 60ºC several complexes were formed together in the reaction mixture as shown in Table (6).

CONCLUSION

For the reaction $^{99m}TC^{+7}$ with 5-nitro-barbituric-acid we can conclude :

a) The labeling efficiencies (% of complex) increases mostly at pH=10 and that both oxidation states of ^{99m}Tc(+7, +4) appear at pHs 4 and10. But at pH=7 only the $^{99m}TCO_4^-$ species was observed, where at pH=4, the reduced form $^{99m}TCO_2$ is more pronounced.
b) At pH=7 no complexes were detected and most of ^{99m}Tc remains as $^{99m}TCO_4$.
c) By increasing the ligand concentration the labeling efficiencies of the complex increases.

$^{99m}TCO_2$ (+4) is not reactive with 5-nitro-barbituric-acid.

For the reaction of ^{99m}Tc of oxidation states (+4,+7) with 2-thiouracil at different temperatures and analytical times we can conclude that several complexes with different R_f values were observed in equilibrium and most of these complexes were unstable.

Table (1). Thin layer chromatography by (95:5) acetonitrile and water for $^{99m}TC^{+7}$ reaction with 5-nitro-barbituric-acid at (pH=10) at different times.

R_f range	speciation	Temp.	% at 0h	%after 1 h.	%after 3 h.	%after 6 h.	%after 20 h.
≈0	% Insoluble ($^{99m}TCO_2$)	Room Temp.	3.23	21	29.6	5.5	16.72
0.3 to 0.45	Complex(1)	Room Temp.	-	51	-	-	-
0.7	Complex(2)	Room Temp.	-	-	-	-	46.36
0.85 to1	%$^{99m}TCO_4^-$	Room Temp.	96.77	28	70.4	94.5	36.92
≈0	% Insoluble ($^{99m}TCO_2$)	40ºC	3.23	1.16	25.22	7.21	10.34
0.3 to 0.45	Complex(1)	40ºC	-	79.69	-	-	33.9
0.7	Complex(2)	40ºC	-	-	-	-	-
0.85 to1	%$^{99m}TCO_4^-$	40ºC	96.77		74.78	92.79	55.76
≈,0	% Insoluble ($^{99m}TCO_2$)	60ºC	3.23	54.61	10.35	8.46	1.94
0.3 to 0.45	Complex(1)	60ºC	-	-	-		
0.7	Complex(2)	60ºC	-	-	-		
0.85 to1	%$^{99m}TCO_4^-$	60ºC	96.77	45.39	89.65		

Table (2). Thin layer chromatography by (95:5) acetonitrile and water for $^{99m}TC^{+7}$ reaction with 5-nitro-barbituric-acid at (pH=4) at different times.

R_f range	speciation	Temp.	% at 0h	%after 1 h.	%after 3 h.	%after 6 h.	%after 20 h.
≈1	%$^{99m}TCO_4^-$	Room temperature	86.39	29.66	79.95	46.82	13.31
≈0	% Insoluble $^{99m}TCO_2$	Room temperature	13.6	41.32	13.92	2.75	8.61
≈1	%$^{99m}TCO_4^-$	40ºC	86.39	36.19	50.12		71.59
≈0	% Insoluble $^{99m}TCO_2$	40ºC	13.6	32.53	27.33	84.49	16.37
≈1	%$^{99m}TCO_4^-$	60ºC	86.39	55.31	52.72	45.26	45.88
≈0	% Insoluble $^{99m}TCO_2$	60ºC	13.6	18.22	20.38	54.42	9.11

Table (3). Thin layer chromatography by (95:5) acetonitrile and water for $^{99m}TC^{+4}$ reaction with 2-thiouracil at different temperatures and different analytical times.

Speciation	Temp.	% at 0.75 h	%after 1.66h.	%after 3 h.	%after 4 h.	% after 9 h.	%after 11.5h.	%after 21 h.
% Insoluble $^{99m}TCO_2$	Room temperature	75.15	71.8	70.73	51.8	53.75	52.26	
% complex	Room temperature	23.56	26.73	25.79	45.8	41.96		
% Insoluble $^{99m}TCO_2$	40ºC	61.41	60.86	36.33	44.37	36.12	44.37	
% complex	40ºC	35.36	36.27	62.62	54.88	63.24	55.18	
% Insoluble 40ºC	60ºC	67.79	9.81	40.31	62.77	23.84	45.16	43.73
% complex	60ºC	31.62	81.11	57.44	37.1	66.19	54.54	55.73

Table (4). Thin layer chromatography by (95:5) acetonitrile and water for $^{99m}TC^{+4}$ 2-thiouracil complexes at 60ºC and different analytical times.

Time	0.75	1.66	3	4	9	11.5	21
R_f	0.125	0.591	0.68	0.25	0.625	0.136	0.295

Table (5). Thin layer chromatography by (95:5) acetonitrile and water for $^{99m}TC^{+7}$ reaction with 2-thiouracil at different temperatures and different analytical times.

Speciation	Temp.	% after 0h	% after 1 h.	% after 2 h.	% after 4 h.	% after 5 h.
% $^{99m}TCO_4^-$	Room temperature	94.99	44.51	90.87	56.02	59.98
%complex	Room temperature	5.01	55.49	9.13	43.98	40.02
% $^{99m}TCO_4^-$	40ºC	94.99	≈100	54.96	50.34	65.46
%complex	40ºC	5.01	_	45.04	49.66	34.54
% $^{99m}TCO_4^-$	60ºC	94.99	25.47	34.44	34.09	50.46
%complex	60ºC	5.01	74.53	65.56	65.91	49.54
% $^{99m}TCO_4^-$	80ºC	94.99	49.7	99.42	65.81	36.58
%complex	80ºC	5.01	50.3	0.58	34.19	63.42

Table (6). Thin layer chromatography by (95:5) acetonitrile and water for $^{99m}TC^{+7}$ -2-thiouracil complexes at different temperatures and different analytical times.

Temp.	R_f	%After 0h	%After 1h	%After 2h	%After 4h	%After 5h
RT	≈0.6	0.498	38.02	"	34.06	40.02
RT	≈0.7	-	17.62	1.62	0.72	
40ºC	≈0.62	0.498	-	-	49.66	10.7
40ºC	≈0.73	-	-	45.04	-	23.74
60ºC	≈0.45		11.75	35.58	31.64	-
60ºC	≈0.55		39.09	15.92	25.96	49.02
60ºC	≈0.7		15.53	13.94	8.21	-
60ºC	≈0.8		8.17	-	-	_
80ºC	≈0.52		0.4	0.58	-	50.69
80ºC	≈0.72		49.39	_	33.83	10.97

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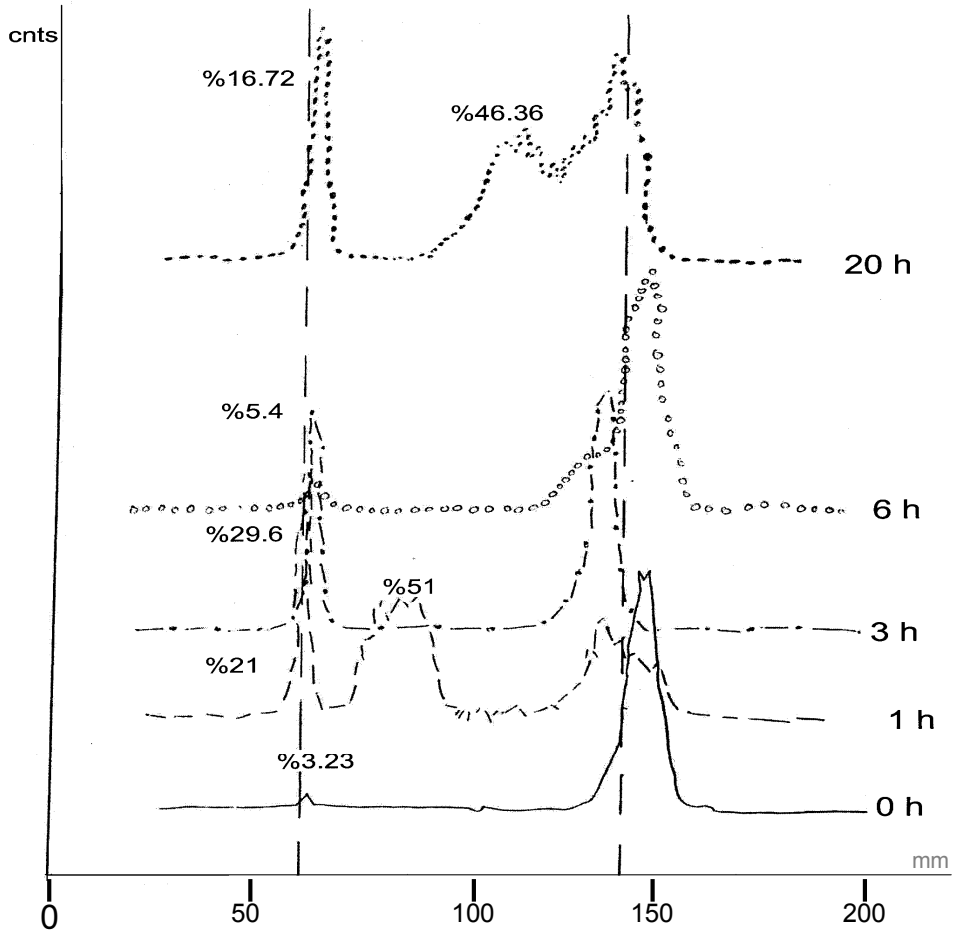
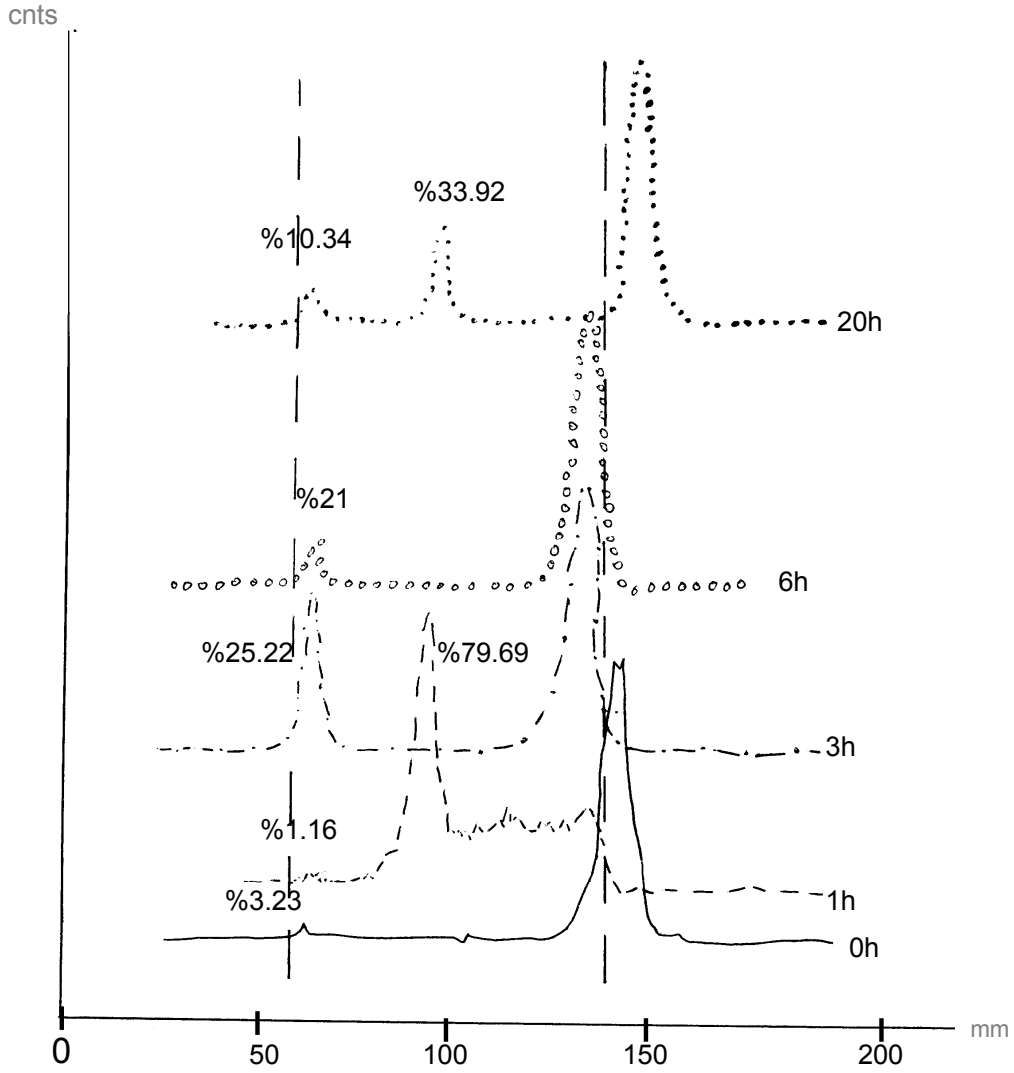
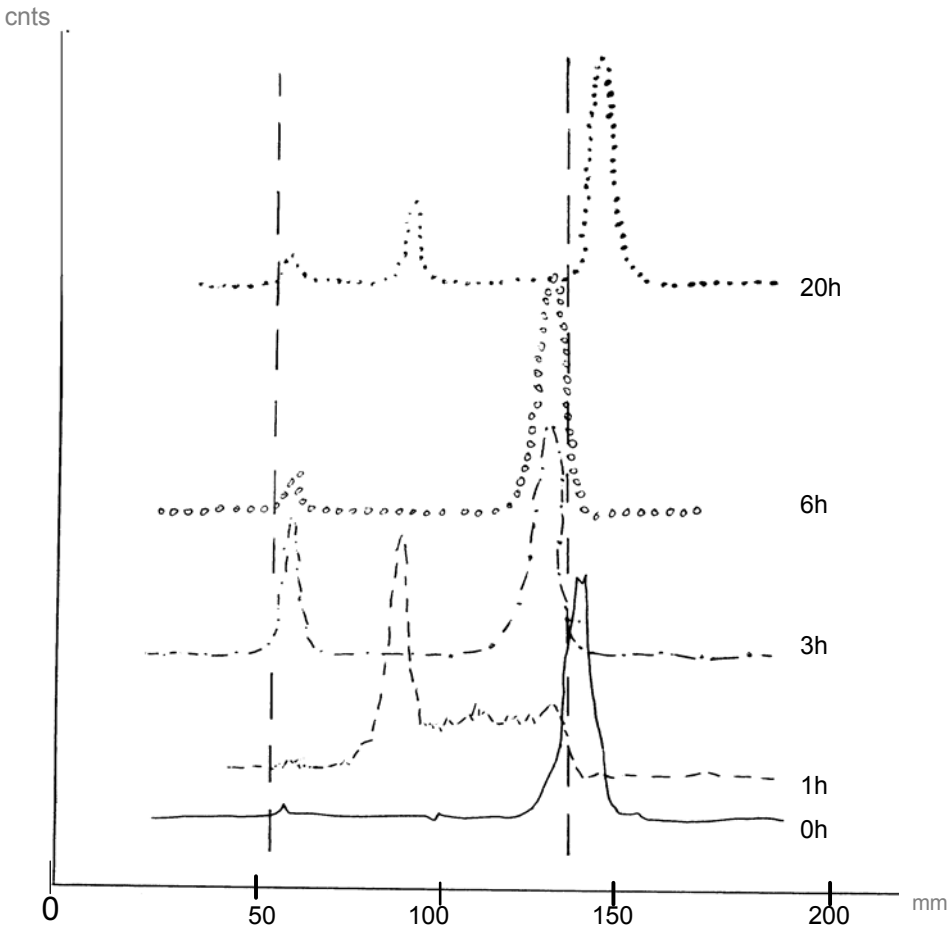


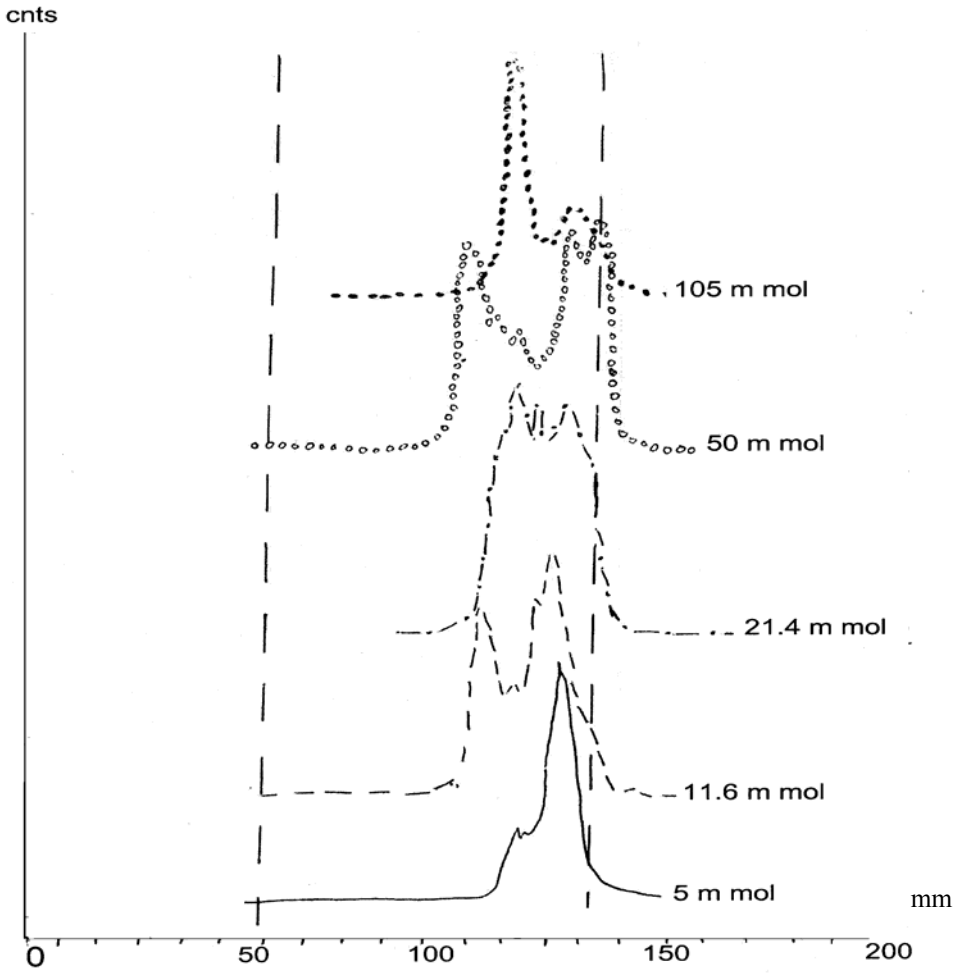
Figure(1),TLC diagram of $^{99m}Tc^{+7}$ with 5-nitro-barbituric-acid at pH=10 and R.T.



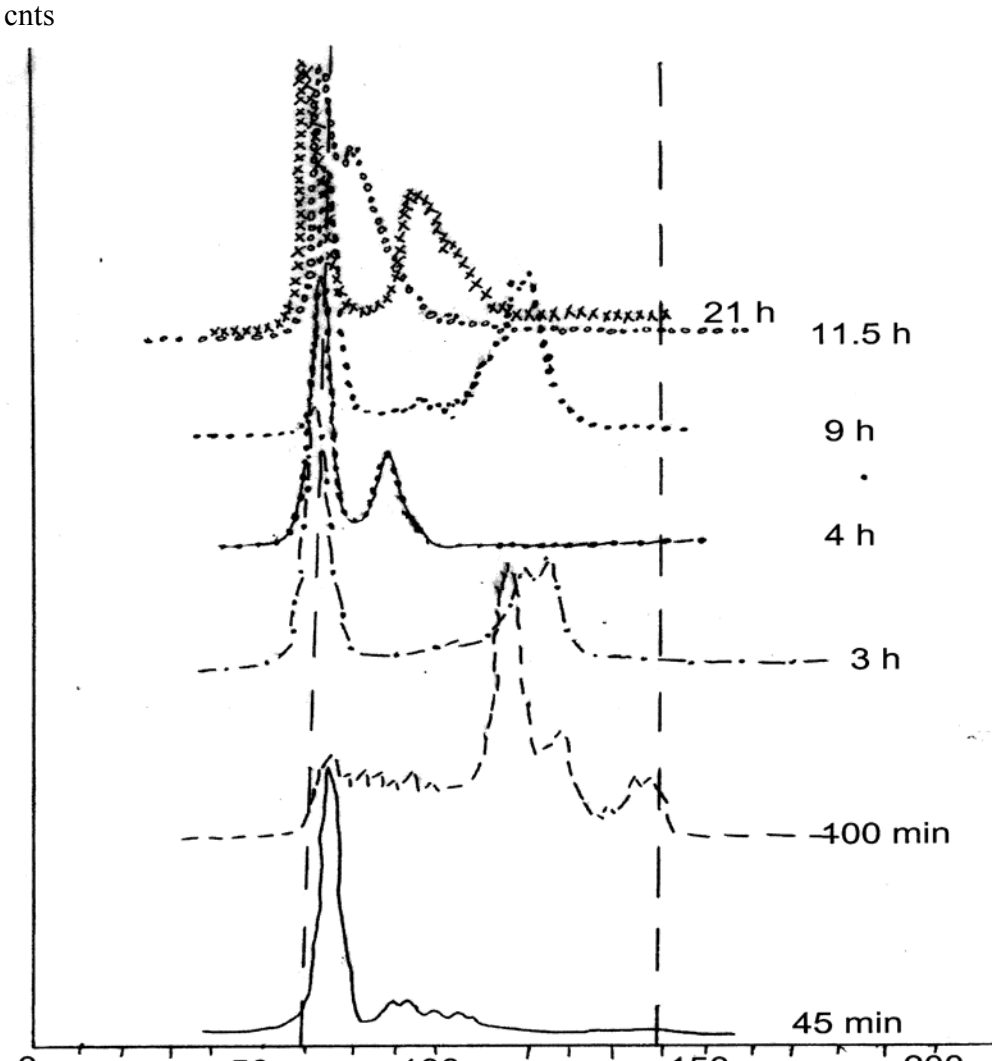
Figure(2),TLC diagram of $^{99m}Tc^{+7}$ with 5-nitro barbituruc acid at 40º C and pH=10



Figure(3),TLC diagram of $^{99m}Tc^{+7}$ with 5-nitro barbituruc acid at 60ºC and pH=10



Figure(4),TLC diagram of $^{99m}Tc^{+7}$ with different concentrations of 5-nitro-barbituric acid



Figure(5), TLC Diagram of $^{99m}Tc^{+4}$ WITH 2-thiouracil at 60 ° C