CHAPTER IV

RESULTS AND DISCUSSION

Calibration of assay systems.

The radiochromatograms of the standard ^{99m}Tc-DMSA, ^{99m}Tc(V)-DMSA and ^{99m}Tc-DMSA labeled with the excess amount of Sn(II) were visualized by autoradiography. The results from the **chromatographic system I** are shown in figure 5 and from the **chromatographic system II** are shown in figure 6. The Rf value of each standard was measured from the autoradiographs and calculated . The theoretical Rf values and the resulting calculations are given in table 1. The activity distributions, measuring by strip cut-and-count method, were plot versus Rf value as shown in figure 7 and 8 respectively. The Standard ^{99m}Tc-DMSA was used to represent the complex used as a renal scanning agent, the ^{99m}Tc(V)-DMSA was used to represent the complex occurred at alkaline pH. The ^{99m}Tc-DMSA labeled with excess of Sn(II) was used for confirmation of the complex for renal scanning agent as had been mention previously that an excessive amount of Sn(II) was necessary for the formation of this complex. The results from chromatographic system I show that this system is suitable for separation of labeled complex from unbound technetium but can not differentiate the complexes occur at different pH. The chromatographic patterns obtained from system II demonstrate that this system

can separate the complexes occur at different conditions. The elution time of the system II was approximately 2 hours , which was very long compared with 45 minutes of the system I . The system II is not official in the pharmacopoeia. The advantage of this system in this study is to characterize the changes of the labeled succimer.

Figure 5 The radiochromatograms of the pertechnetate (A), commercial standard ^{99m}Tc(III)-DMSA (B, C, D), ^{99m}Tc(V)-DMSA (E) and ^{99m}Tc(III)-DMSA labeled with excess of Sn(II) (F ,G) obtained from assay system I and visualized by autoradiography.

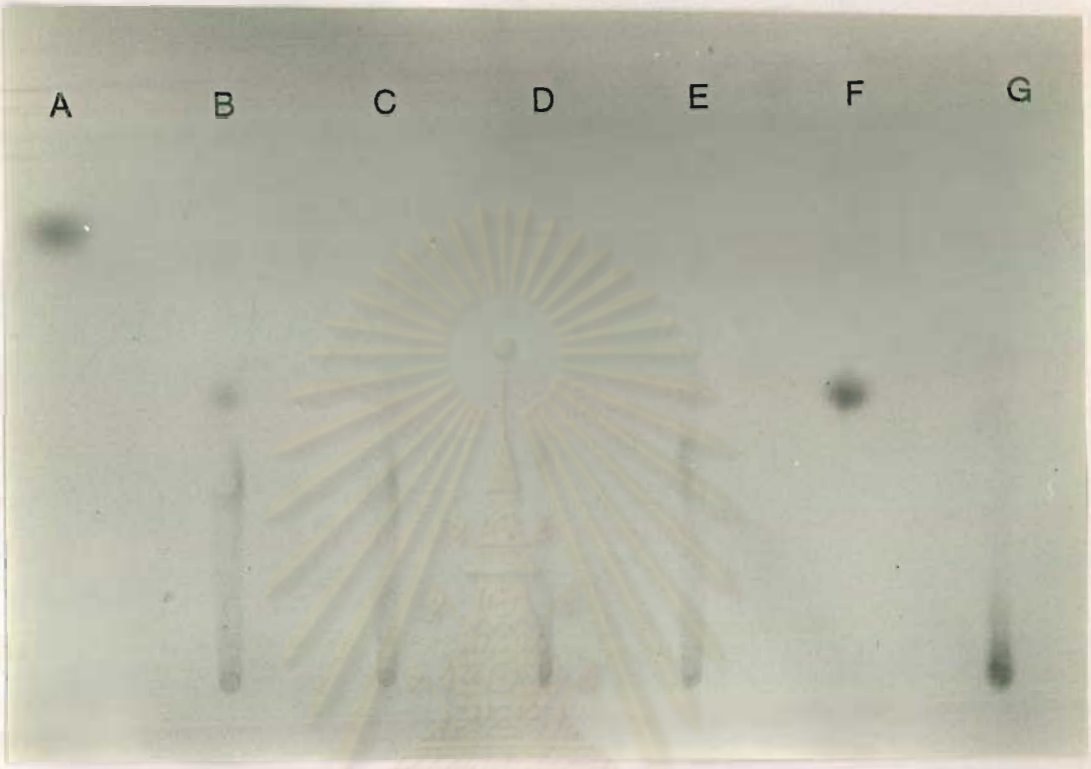

Figure 6 The radiochromatograms of the pertechnetate (A), commercial standard ^{99m}Tc(III)-DMSA (B, C, D, E), ^{99m}Tc(V)-DMSA (F) and ^{99m}Tc(III)-DMSA labeled with excess of Sn(II) (G) obtained from assay system II and visualized by autoradiography.

Table 1. The Rf value of the main radiochromatographic bands of pertechnetate and standard ^{99m}Tc-DMSA complexes.

Standard	Rf value (System I)		Rf value (System II)	
	theoretical	calculated	theoretical	calculated
TcO_4^-	1	0.95	0.7	0.7
^{99m}Tc(III)-DMSA	0.45-0.7	0.46	0	0-0.44
^{99m}Tc(V)-DMSA	-	0.46	0.5	0.49
excess Sn(II)	-	0.46-0.5	-	0-0.44
Hydrolyzed ^{99m}Tc	0-0.15	-	-	-

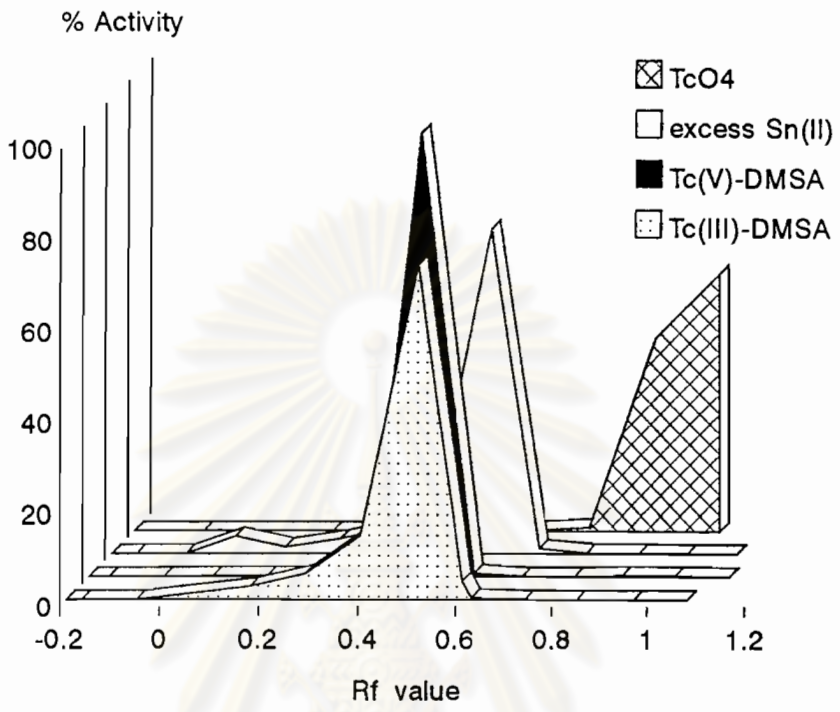

Figure 7 The plots of activity distributions versus Rf values of the pertechnetate standard, commercial ^{99m}Tc(III)-DMSA, ^{99m}Tc(V)-DMSA and ^{99m}Tc-DMSA labeled with excess of Sn(II) obtained from assay system I measured by strip cut-and-count method.

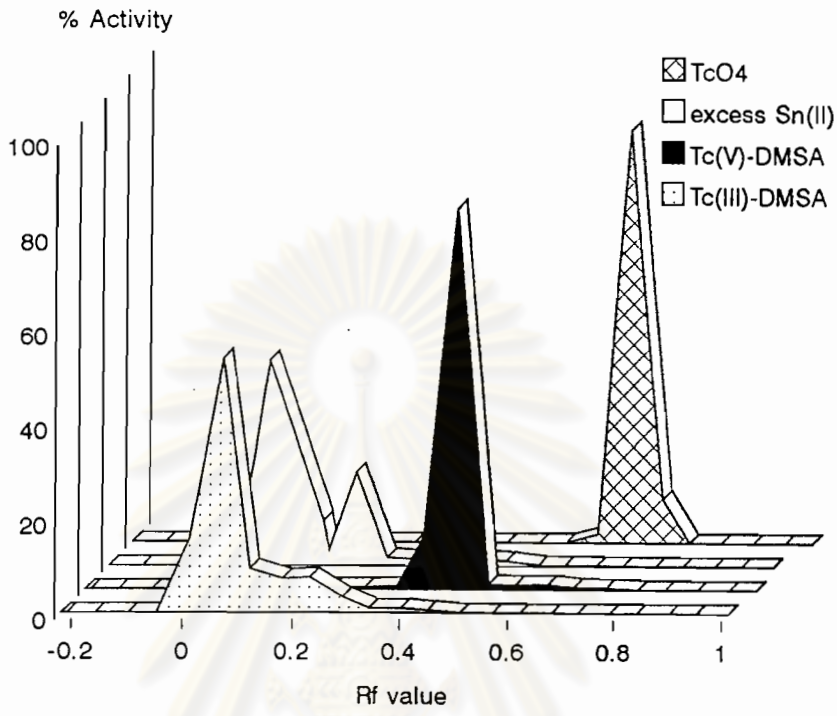

Figure 8 The plots of activity distributions versus Rf values of the pertechnetate standard, commercial ^{99m}Tc(III)-DMSA, ^{99m}Tc(V)-DMSA and ^{99m}Tc-DMSA labeled with excess of Sn(II) obtained from assay system II measured by strip cut-and-count method.

Formulation

Evalution of the formulations

1. Properties of succimer cold kit formulations.

The three succimer cold kit formulations were assayed for radiochemical purity by chromatographic system I, according to the schedule described in chapter III. The radioactivity distributions of the preparations of different storage times, after the labeling time of 15, 30, 60, 90, 120 minutes and 24 hours, measured from strip cut-and-count method were plot versus relative Rf values as shown in figure 9.1, 9.2, 9.3 and 9.4. The plots were devided into four segments along the Rf values as shown by the dash lines in the figures, the Rf segments were referred to as segment I, II, III and IV respectively. The radioactivity distributions in the chromatographic segments were calculated. The results are shown in table 2.1, 2.2 , 2.3 and 2.4 and the means, standard deviations and coefficient of variations were summarized in table 3. To evaluate the properties of the cold kit, the results were analyzed as follow ;

1.1 Labeling property of succimer cold kit The labeling property of the three succimer formulations has been determined by USP limit for radiochemical purity, which stated that the formulations should contain not less than 85 % of the labeled amount of ^{99m}Tc-DMSA. The results in table 2.1 , 2.2, 2.3 and 2.4 show that the percentage labeled amounts of the 3 formulations at various

testing conditions, represented by the percentage radioactivity of the main bands, are not less 85 % except for the formulation 1 at month 0, 24 hours after labeling. It can be seen from figure 9.1 f) that there was peak tailing that should be included in the labeled parts because the free pertechnetate moved to the solvent front and hydrolyzed ^{99m}Tc stayed at the origin. The peak shifting effect was also observed due to the evaporation of the solvent from the TLC edges when using different sizes of chromatography tanks, as well as the cutting technique when the band was located at the cutting position. Considering the data from 15 to 120 minutes it can be assumed that the labeling properties of the 3 formulations are within the USP standard .

Further evaluations have been done by comparing the percentage labeled amount of the 3 formulations from the same storage time by ANOVA. The results summarized in table 4 show that there are significant differences among group samples tested at month 1 as well as the group samples tested at month 2. However, there are no significant differences among group samples tested at month 0 and 3 respectively. From theoretical point of view if the differences are found at month 1 and 2 it should be found at month 3. On the other hands, the change might occur during three months at different rates so that the three preparations had similar labeling properties at the third month. However, these data are not sufficient for evaluation. The bioequivalent have been tested by the

biodistribution method to see whether the difference among the formulation existed, as will be discussed in the later part.

1.2 Stability of succimer cold kit formulation Stabilitiy of the formulations has been evaluated from the difference among the % labeled amount of each formulation tested at different storage times. The comparisons have been done simultaneously with the test for stability of labeled products by ANOVA (two-factor without replication) at a significance level of 0.05 as summarized in table 5. From table 5 significant differences are found within each of the three groups. These results together with those discussed in the labeling property topic confirm that there is tendency of the change in quality of the 3 cold kit formulations eventhough the tested results are still within the USP standard. The biodistribution tests have been performed to determine the change that might affect the biological property of the formulations .

1.3 Stability of the labeled products Stability of the labeled products has been determined by the means and the coefficient of variations of the percentage labeled amount at various post labeling times. From the values in table 3 the variations affected by the time after labeling by means of the % CV of all groups are not exceed 5 %, which is acceptable. Further analysis has been done by comparing the % labeling at 6 time intervals after labeling using data from four storage times by ANOVA . The results summarized in table 5 show that there is

significant different affected by the time after labeling occurred with formulation 1 but it is not found with formulation 2 and 3 ($p<0.05$). These results indicate that the formulation 1, which does not contain ascorbic acid, has inferior quality to 2 and 3 by means of the post labeling stability.

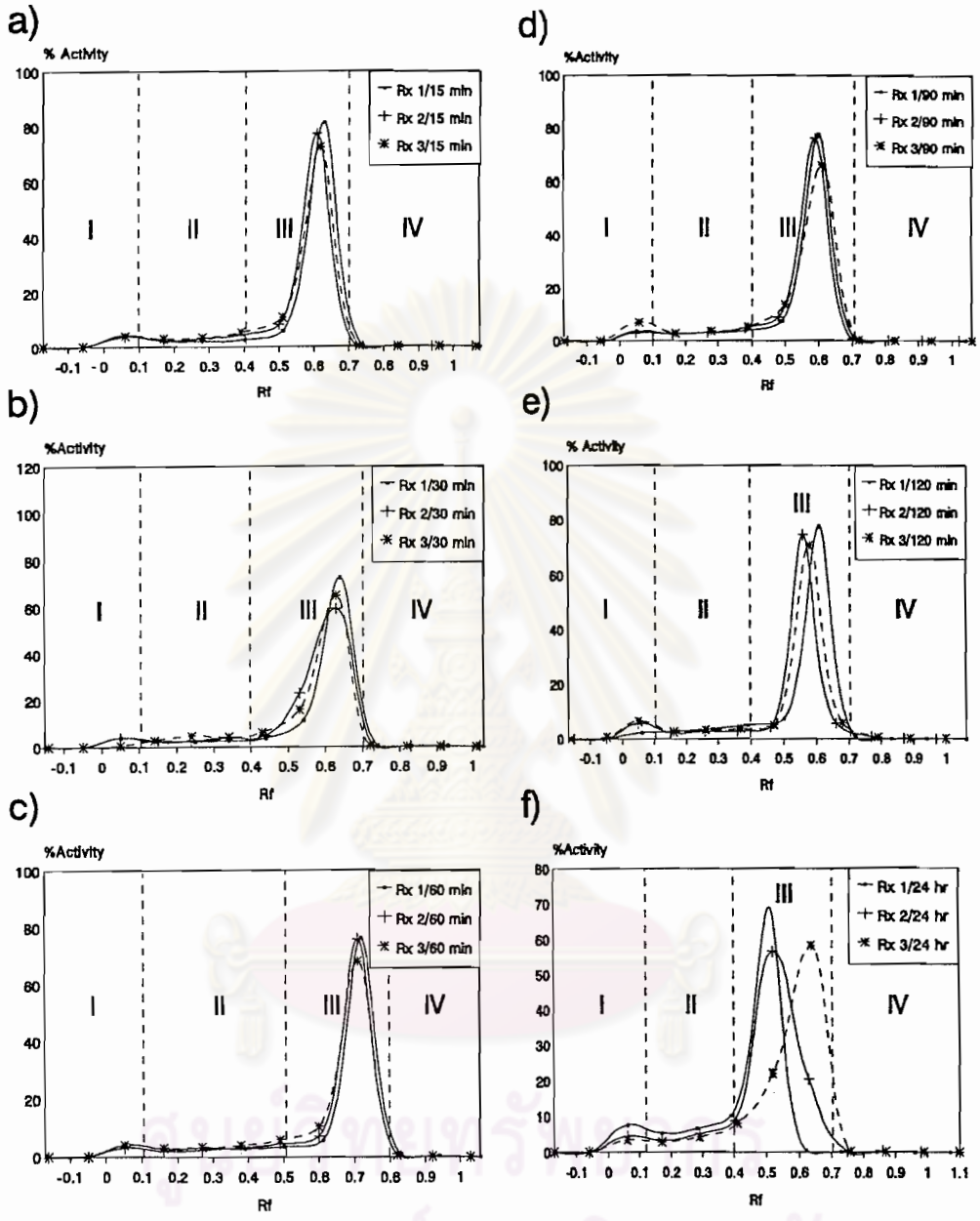

Figure 9.1 The radioactivity distribution plots of the radiochromatograms of three succimer formulations after labeling times of 15, 30, 60, 90, 120 minutes and 24 hours when assayed after freshly prepared. The assays were performed using chromatographic system I.

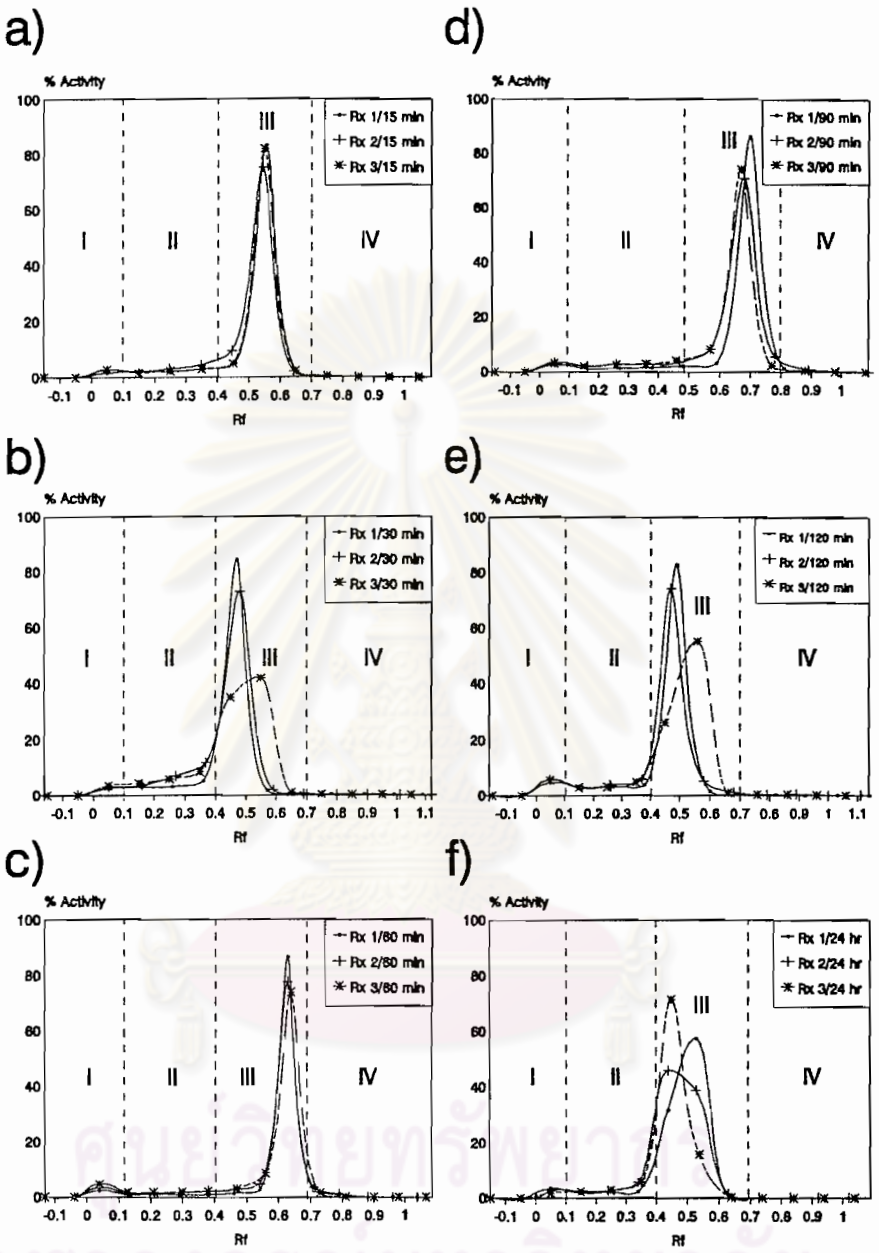

Figure 9.2 The radioactivity distribution plots of the radiochromatograms of three succimer formulations after labeling times of 15, 30, 60, 90, 120 minutes and 24 hours when assayed after one month storage. The assays were performed using chromatographic system I.

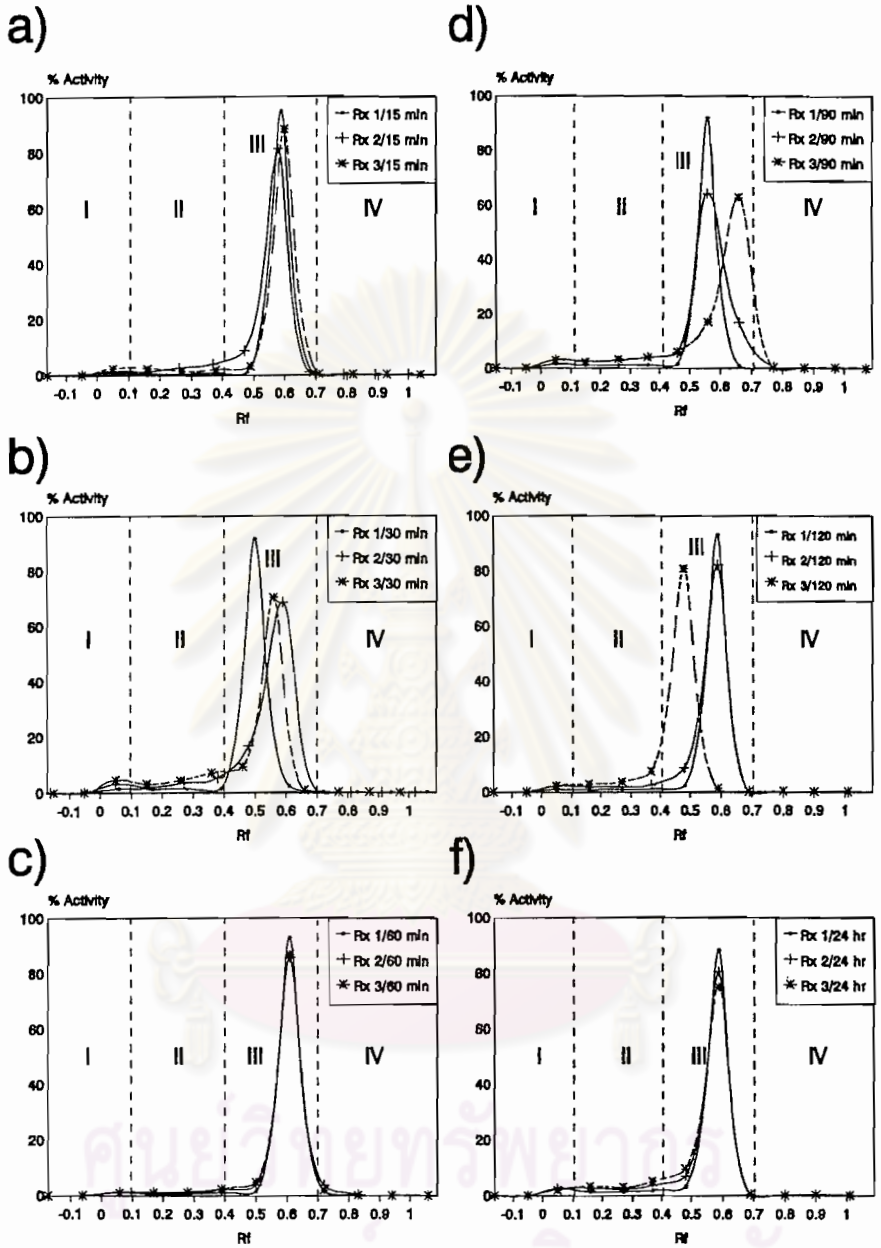

Figure 9.3 The radioactivity distribution plots of the radiochromatograms of three succimer formulations after labeling times of 15, 30, 60, 90, 120 minutes and 24 hours when assayed after two month storage. The assays were performed using chromatographic system I.

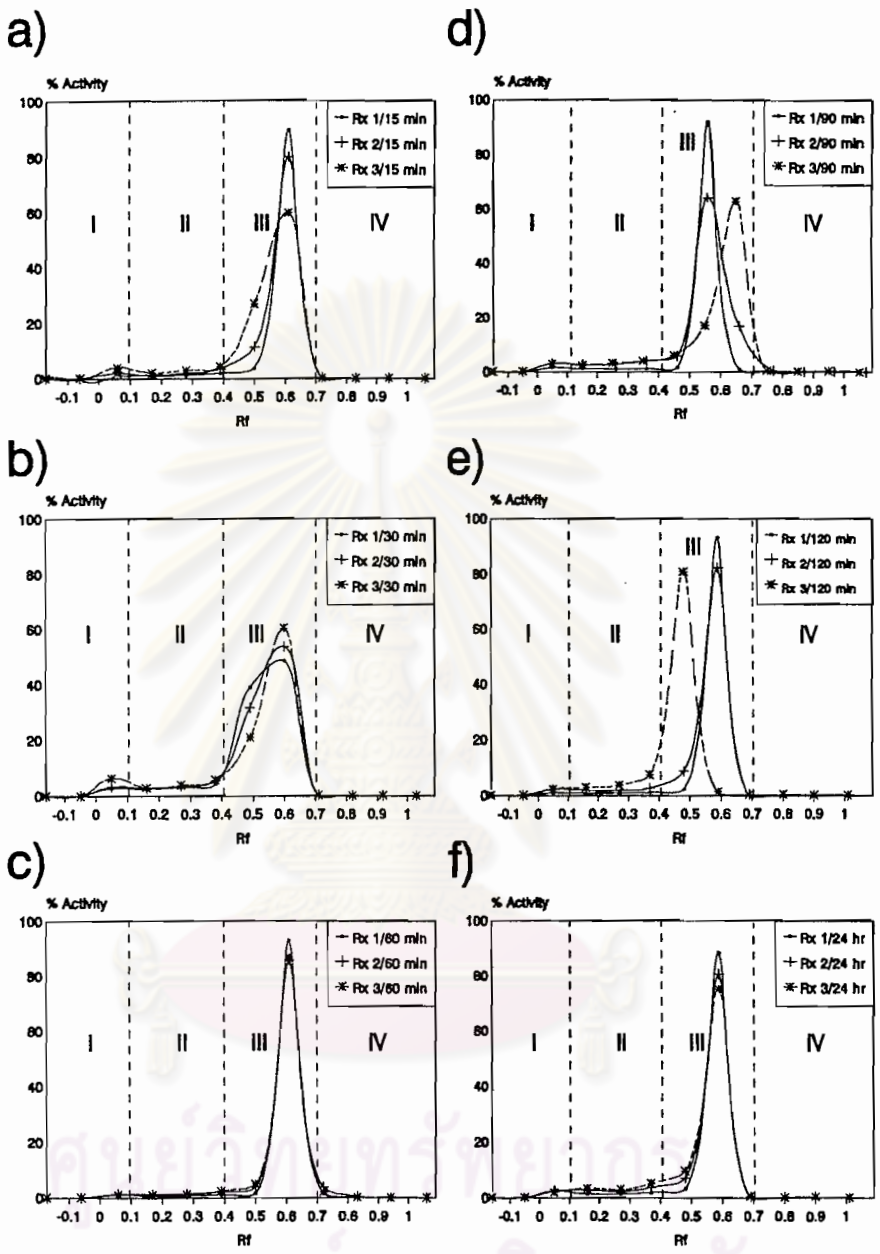

Figure 9.4 The radioactivity distribution plots of the radiochromatograms of three succimer formulations after labeling times of 15, 30, 60, 90, 120 minutes and 24 hours when assayed after three month storage. The assays were performed using chromatographic system I.

Table 2.1. Total % radioactivity of the chromatographic segments at different relative Rf ranges from 3 succimer cold kit formulations assayed after freshly prepared (month=0).

Month = 0	Post labeling time	% Activities at different relative Rf ranges			
Formulation		Rf segments			
		I	II	III	IV
1	15 min	4.34	6.68	88.73	0.25
	30 min	3.89	7.62	88.23	0.25
	60 min	4.18	8.65	85.81	1.36
	90 min	3.61	9.78	86.49	0.12
	120 min	2.27	11.69	85.86	0.18
	24 hr	7.58	11.91	79.51	0.18
2	15 min	3.78	9.82	86.28	0.12
	30 min	3.88	8.29	87.71	0.13
	60 min	3.07	7.58	89.03	0.31
	90 min	3.02	10.66	86.19	0.13
	120 min	5.84	7.91	85.30	0.95
	24 hr	4.50	8.41	86.25	0.84
3	15 min	4.04	6.51	89.41	0.04
	30 min	3.23	8.76	87.90	0.11
	60 min	4.15	10.39	85.33	0.13
	90 min	7.06	6.45	86.47	0.02
	120 min	7.22	9.36	86.12	0.73
	24 hr	3.44	7.22	89.06	0.28

Table 2.2 Total % radioactivity of the chromatographic segments at different relative Rf ranges from 3 succimer cold kit formulations assayed at one month storage (month=1).

Month =1 / Formulation	Post labeling time	% Activities at different relative Rf ranges			
		Rf segments			
		I	II	III	IV
1	15 min	1.59	7.46	89.92	1.03
	30 min	2.33	5.87	90.93	0.87
	60 min	2.44	5.06	91.49	1.01
	90 min	2.53	6.01	90.96	0.50
	120 min	4.74	6.11	88.55	0.60
	24 hr	3.44	6.38	89.85	0.33
2	15 min	2.68	9.64	86.95	0.73
	30 min	2.71	10.41	86.41	0.47
	60 min	3.47	9.38	85.94	1.21
	90 min	3.43	7.43	88.26	0.88
	120 min	5.95	6.57	85.63	1.85
	24 hr	2.56	10.84	86.47	0.13
3	15 min	2.85	6.93	89.39	0.83
	30 min	3.40	9.98	86.17	0.45
	60 min	4.72	7.67	87.43	0.18
	90 min	3.09	7.38	88.99	0.51
	120 min	5.66	5.97	87.71	0.66
	24 hr	1.79	10.75	87.40	0.06

Table 2.3 Total % radioactivity of the chromatographic segments at different relative Rf ranges from 3 succimer cold kit formulations assayed at two month storage (month=2).

Month = 2 Formulation	Post labeling time	% Activities at different relative Rf ranges			
		Rf segments			
		I	II	III	IV
1	15 min	1.09	1.50	97.08	0.33
	30 min	1.34	2.61	95.60	0.45
	60 min	1.58	1.83	96.20	0.39
	90 min	1.52	3.49	94.56	0.43
	120 min	1.01	2.83	95.53	0.63
	24 hr	2.38	4.63	92.22	0.77
2	15 min	1.38	7.70	90.81	0.11
	30 min	3.02	10.76	86.07	0.15
	60 min	1.13	6.15	93.30	0.55
	90 min	3.02	9.32	87.02	0.64
	120 min	2.09	6.49	91.26	0.16
	24 hr	2.48	8.78	88.20	0.59
3	15 min	2.31	5.50	92.07	0.12
	30 min	4.45	7.45	87.87	0.23
	60 min	1.08	4.69	94.07	0.13
	90 min	2.93	5.37	91.39	0.31
	120 min	2.26	6.38	91.10	0.26
	24 hr	1.90	6.09	91.72	0.29

Table 2.4 Total % radioactivity of the chromatographic segments at different relative Rf ranges from 3 succimer cold kit formulations assayed at three month storage (month=3).

Month = 3 Formulation	Post labeling time	% Activities at different relative Rf ranges			
		Rf segments			
		I	II	III	IV
1	15 min	2.11	3.96	93.79	0.14
	30 min	2.68	5.31	91.86	0.15
	60 min	7.14	6.42	85.57	0.87
	90 min	2.34	7.06	90.43	0.17
	120 min	3.27	7.45	88.79	0.49
	24 hr	6.79	7.67	84.43	1.11
2	15 min	1.80	6.74	91.40	0.06
	30 min	3.19	5.97	90.76	0.08
	60 min	6.30	5.55	87.86	0.29
	90 min	2.28	7.61	90.06	0.05
	120 min	3.38	7.32	88.88	0.42
	24 hr	4.63	6.88	88.17	0.32
3	15 min	3.83	5.60	91.14	0.03
	30 min	6.23	6.60	87.10	0.07
	60 min	8.43	5.46	85.84	0.27
	90 min	2.85	5.59	91.51	0.05
	120 min	2.86	9.25	87.82	0.07
	24 hr	3.57	8.63	87.59	0.21

Table 3. Means, standard deviations and % coefficient of variations of the % labeled amount of the formulations by using the data from six different sampling times.

Month	Rx 1		Rx 2		Rx 3	
	Mean±SD	% CV	Mean±SD	% CV	Mean±SD	% CV
0	85.77±3.30	3.84	86.79±1.34	1.54	87.38±1.66	2.07
1	90.28±1.06	1.17	86.61±.0.92	1.07	87.85±1.17	1.33
2	95.20±1.68	1.76	89.44±2.79	3.11	91.37±2.10	2.21
3	89.15±3.63	4.06	89.52±1.44	1.61	88.51±2.30	2.59

Table 4. Summarized results of the comparison of % labeled amounts of 3 formulations taken from different storage times.

	$F_{calculated}$	$F_{critical}$	P	Statistical significance ($p<0.05$)
Different formulations				
month 0	0.772	3.682	0.480	N
month 1	18.938	3.682	7.87×10^{-5}	S
month 2	10.557	3.682	0.001	S
month 3	0.234	3.682	0.794	N

(Calculation details : see table 22 of appendix B)

Table 5. Summarized results of the comparison of the % labeled amount of each of the three formulations at different storage time and different post labeling time. The comparison was performed by ANOVA.

	$F_{calculated}$	$F_{critical}$	P	Statistical significance ($p<0.05$)
Different storage time				
Rx 1	24.643	3.287	$4.77x10^{-6}$	S
Rx 2	4.404	3.287	0.021	S
Rx 3	7.107	3.287	0.003	S
Different post labeling time				
Rx 1	4.555	2.901	0.010	S
Rx 2	0.550	2.901	0.736	N
Rx 3	1.979	2.901	0.140	N

(Calculation details : see table 23 of appendix B)

2. Biodistribution studies

The biodistribution studies have been performed using group samples taken from freshly prepared products and three month storage ones. The results of the freshly prepared formulations were calculated as shown in table 6.1, 6.2 and 6.3. and the results of the three months old formulations are shown in table 7.1, 7.2 and 7.3. According to the USP specification, which stated that " not less than 40 % of the administered radioactive dose is found in the kidneys and a ratio of not less than 6:1 of the administered dose is found in the ratio kidney/(liver and spleen), in not fewer than two of the animals", all the freshly prepared formulations were within the limits while the three month old formulation 1 was out of limit. Further data analysis was done by determining the differences of organ distribution among 3 formulations for both groups by ANOVA at significant level of 0.05. The results were summarized in table 8. Differences within the same formulation at different storage times were also determined in the same manner as summarized in table 9. From table 8 there are significant differences among distribution of freshly prepared formulations in blood, which can be seen from table 6.1- 6.3 that the distribution of formulation 1 is higher than the others, but there are no significant different in other organs. The results of the three months old formulations show that significant differences are found with the distribution in bladder, head, kidney, legs and the remaining carcass. The high percentages of the radioactivity are accumulated in the bladder and the remaining carcass. These can produce the high-background scintigraphic images in vivo. It can be noticed from table 7.1-7.3 that the

radioactivity ratio in kidney of the formulation 1 is lower than the others while in other organs are higher. These results imply that any change occurred with the formulation 1 can cause the reduction of the distribution in the kidney, which is the target organ. Furthermore, these results also support the results previously discussed in the chemical assay part that formulation 1 has tendency to deteriorate more than the other 2 formulations. Ascorbic acid in formulation 2 and 3 should play an important role in stabilizing the succimer formulations. The statistical significant different in table 9 is to confirm that the change has occurred with formulation 1 is greater than with the other formulations even though some significant changes occur with distribution of formulation 2 in the heart, which is actually small ratio of radioactivity.

Further attempts had been made to characterize the change pattern of the three formulations and if possible, to improve preparation procedures. The three months old formulations were assayed for radiochemical purity by system II to see whether the change pattern could be identified or not .

Table 6.1 Biodistribution tests of ^{99m}Tc-DMSA Injections. The tests were performed using the freshly prepared formulation 1.

Organ	Activity (%)			
	Rat no.1	Rat no.2	Rat no.3	Mean±SD
Bladder	5.51	8.23	7.10	6.95±0.02
Blood	16.32	11.80	11.80	13.31±2.13
Head	4.54	3.68	4.79	4.34±0.48
Heart	0.71	0.44	0.57	0.57±0.11
Kidney	39.84	45.60	43.10	42.87±1.12
Legs	5.66	4.66	4.01	4.78±0.68
Liver	6.25	4.80	5.95	5.67±0.62
Remaining carcass	20.88	21.10	23.00	21.70±0.96
Spleen	0.28	0.32	0.32	0.31±0.02
Kidney/ (Liver and Spleen)	6.10	8.90	6.87	7.29±1.18

Table 6.2 Biodistribution tests of ^{99m}Tc-DMSA Injections. The tests were performed using freshly prepared formulation 2.

Organ	Activity (%)			
	Rat no.1	Rat no.2	Rat no.3	Mean±SD
Bladder	11.09	3.91	14.90	10.00±4.59
Blood	3.87	6.27	4.18	4.77±1.07
Head	3.93	4.69	4.08	4.23±0.33
Heart	0.34	0.33	0.18	0.28±0.07
Kidney	51.42	49.40	52.20	51.04±1.15
Legs	6.09	7.19	4.20	5.83±1.24
Liver	3.55	4.72	3.33	3.87±0.61
Remaining carcass	19.50	23.1	16.60	19.76±2.68
Spleen	0.20	0.24	0.19	0.21±0.02
Kidney/ (Liver and Spleen)	13.71	9.96	14.83	12.83±2.08

Table 6.3 Biodistribution tests of ^{99m}Tc-DMSA Injections. The tests were performed using freshly prepared formulation 3.

Organ	Activity (%)			
	Rat no.1	Rat no.2	Rat no.3	Mean±SD
Bladder	10.07	5.87	8.72	8.22±1.75
Blood	5.69	5.51	4.82	5.34±0.37
Head	4.59	4.44	3.68	4.24±0.40
Heart	0.34	0.35	1.62	0.77±0.60
Kidney	50.41	50.68	40.81	47.30±4.59
Legs	5.61	4.49	5.45	5.18±0.49
Liver	4.23	4.68	12.29	7.07±3.70
Remaining carcass	18.80	23.69	21.46	21.32±2.00
Spleen	0.26	0.30	1.14	0.57±0.40
Kidney/ (Liver and Spleen)	11.23	10.18	3.04	8.15±3.64

Table 7.1 Biodistribution tests of ^{99m}Tc-DMSA Injections. The tests were performed using the three months old formulation 1.

Organ	Activity (%)			
	Rat no.1	Rat no.2	Rat no.3	Mean±SD
Bladder	20.10	15.15	18.21	17.82±2.04
Blood	6.68	6.63	8.72	7.24±1.05
Head	5.59	6.81	7.05	6.48±0.64
Heart	0.30	0.34	0.38	0.34±0.03
Kidney	29.46	30.14	29.11	29.57±0.43
Legs	6.87	8.93	8.72	8.17±0.93
Liver	4.06	6.88	3.72	4.89±1.42
Remaining carcass	26.80	25.06	23.91	25.26±1.19
Spleen	0.14	0.35	0.18	0.22±0.09
Kidney/ (Liver and Spleen)	7.01	4.17	7.46	6.21±1.46

Table 7.2 Biodistribution tests of ^{99m}Tc-DMSA Injections. The tests were performed using the three months old formulation 2.

Organ	Activity (%)			
	Rat no.1	Rat no.2	Rat no.3	Mean±SD
Bladder	12.09	9.81	8.87	10.26±1.35
Blood	5.99	5.60	8.21	6.60±1.15
Head	4.12	4.83	4.28	4.41±0.30
Heart	0.45	0.41	0.54	0.47±0.05
Kidney	48.68	46.68	49.18	48.18±1.08
Legs	5.55	6.51	5.76	5.94±0.41
Liver	4.86	4.11	3.60	4.19±0.52
Remaining carcass	18.07	21.87	19.26	19.73±1.59
Spleen	0.19	0.19	0.30	0.23±0.05
Kidney/ (Liver and Spleen)	9.64	10.86	12.61	11.04±1.22

Table 7.3 Biodistribution tests of ^{99m}Tc-DMSA Injections. The tests were performed using the three month olds formulation 3.

Organ	Activity (%)			
	Rat no.1	Rat no.2	Rat no.3	Mean±SD
Bladder	9.41	6.49	9.84	8.58±1.49
Blood	8.90	5.74	8.09	7.58±1.34
Head	4.91	5.03	4.93	4.96±0.05
Heart	0.39	0.39	0.73	0.50±0.16
Kidney	45.66	47.52	46.88	46.69±0.77
Legs	6.20	6.17	6.07	6.15±0.06
Liver	3.50	4.47	4.35	4.11±0.43
Remaining carcass	20.85	23.96	18.78	21.20±2.13
Spleen	0.17	0.25	0.34	0.25±0.07
Kidney/ (Liver and Spleen)	12.44	10.06	9.99	10.83±1.14

Table 8. Summarized statistical tests for differences of the organ distributions among the 3 succimer formulations. The tests were performed by ANOVA at significant level of 0.05.

Organ	P value		Significant difference (P<0.05)	
	0 month	3 months	0 month	3 months
Bladder	0.603	0.003	N	S
Blood	0.002	0.697	S	N
Head	0.959	0.005	N	S
Heart	0.438	0.299	N	N
Kidney	0.095	7.32×10^{-7}	N	S
Legs	0.827	0.016	N	S
Liver	0.401	0.659	N	N
Remaining carcass	0.614	0.039	N	S
Spleen	0.356	0.904	N	N

(Calculation details : see table 24 of appendix B)

Table 9. Summarized statistical tests for differences of the organ distributions between the freshly prepared and the three months old formulations.

Organ	Rx 1		Rx 2		Rx 3	
	P value	Sig. dif (P<0.05)	P value	Sig. dif (P<0.05)	P value	Sig. dif (P<0.05)
Kidney	0.001	S	0.067	N	0.861	N
Blood	0.006	S	0.066	N	0.085	N
Remaining carcass	0.029	S	1.001	N	0.956	N
Legs	0.014	S	0.908	N	0.052	N
Head	0.019	S	0.607	N	0.064	N
Bladder	0.003	S	0.935	N	0.835	N
Spleen	0.273	N	0.696	N	0.342	N
Heart	0.045	S	0.047	S	0.577	N
Liver	0.515	N	0.598	N	0.323	N

(Calculation details : see table 25 of appendix B)

Studies on the factors affecting the quality of the formulations

1. Study on the degradation patterns of the three Succimer formulations

The three months old formulations have been assayed for radiochemical purity by system II as described previously in page 33. The activity distribution determined by strip cut-and-count method are plotted versus Rf value as in figure 10. From figure 10 the chromatographic patterns have been divided into 6 segments as marked by the dash lines. The interesting segments are from the Rf 0-0.2, 0.2-0.4 and 0.4-0.5 , which will be referred to as segment no. 1, 2 and 3 respectively. The segment no.1 and 3 were previously identified as ^{99m}Tc(III)-DMSA for renal scintigraphy and ^{99m}Tc(V)-DMSA but segment no.2 was not identified. It can be noticed from figure 10 that the peak in segment no.3 of formulation 1 is highest among the 3 formulations. Difference can be seen between the proportion of segments no.2 to no.1 of formulation 2 and 3 while there is no significant difference in biological behaviors. From these results the different biological distribution of formulation 1 to the others might affect by the presence of peak in segment no.3. The different peak no.2 to peak no.1 ratios of formulation 2 and 3, which have the same amount of ascorbic acid demonstrate that there are also the slow deteriorations of both formulations occurred at different rate, however, the different in biological distribution can not be notified. The inositol, which is

intended to use as suspending medium, may have some effects on protection of deterioration.

From the experimental results it can be concluded that to formulate the succimer cold kit it is necessary to stabilize both cold kit and labeled product with ascorbic acid. The experiments were performed to determine the optimum amount of ascorbic acid in the formulation.

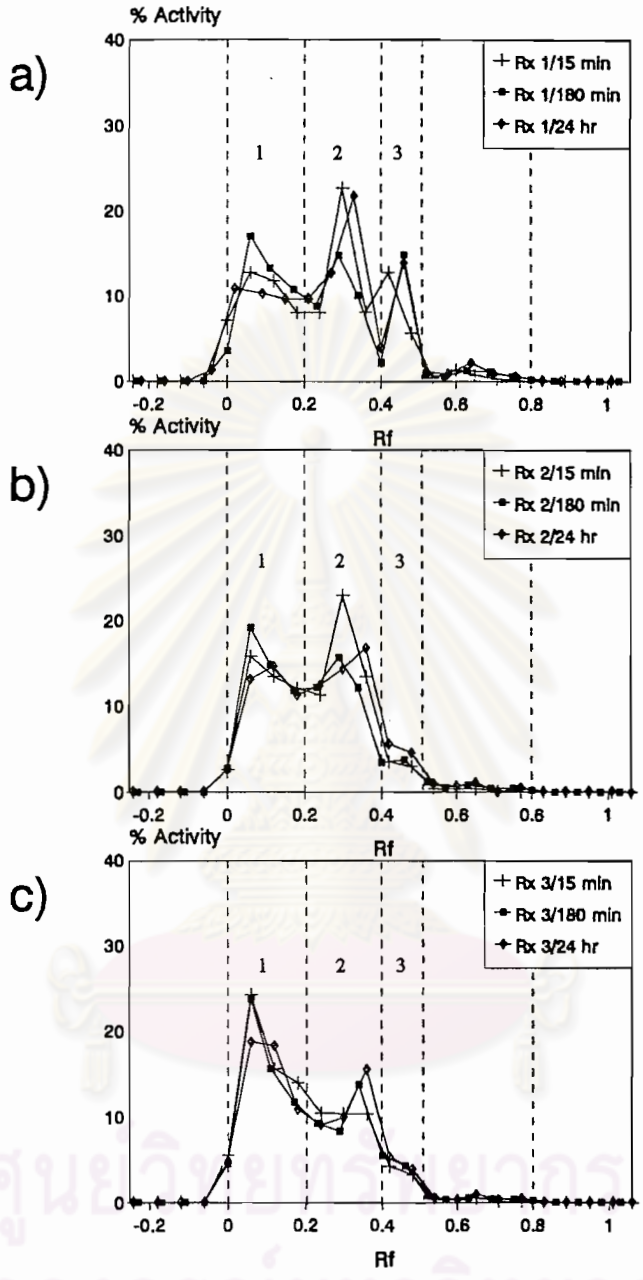

Figure 10 The radioactivity distribution plots of the radiochromatograms of the three succimer formulations assayed after three month storage. The assays were performed using chromatographic system II..

Table 10 Total radioactivity of each chromatographic segments at different Rf ranges from three succimer cold kit formulations after three month storage. The assays were performed using chromatographic system II.

Month = 3	Post labeling time	% Activities at different Rf ranges					
Formulation		Rf range					
		0	0--0.2	0.2--0.4	0.4--0.5	0.5--0.8	0.8--1
1	15 min	0.09	39.79	38.81	18.85	2.42	0.08
	180 min	0.13	44.59	35.77	15.56	3.76	0.19
	24 hr	0.14	32.11	47.95	14.92	4.71	0.17
2	15 min	0.13	43.93	51.37	3.41	1.04	0.14
	180 min	0.17	48.66	43.4	5.03	2.55	0.19
	24 hr	0.27	41.68	49.14	5.59	3.01	0.31
3	15 min	0.21	59.41	35.4	3.67	1.13	0.18
	180 min	0.18	55.66	36.75	5.11	2.09	0.22
	24 hr	0.31	52.71	39.85	4.51	2.32	0.31

2. Determination of the optimum amount of ascorbic acid

After strip cut-and-count process the percentage activity and relative Rf value of each strip were calculated. The percentage activity was plotted versus Rf value as shown in figure 11 and the total activity of peak in each chromatograpic segment was summarized in table 11. To determine the difference among the 5 preparations, the percentage activity in two main chromatographic segments at Rf ranges of 0-0.2 and 0.2-0.4, obtained at three post labeling times (15, 180 minutes and 24 hours) were analyzed by analysis of variance (two-way) at a significant level of 0.05 (see table 26 of appendix B) . The resulting calculations were summarized in table 12.

From the analysis of variances the results show that there were no significant differences among the percentage activity of the two main chromatographic segments at Rf 0-0.2 and 0.2-0.4 from the 5 preparations, but there were significant difference among different post labeling times. Further calculations (Duncan's new multiple range test) showed that the percentage activity of the 24 hour group were different from the 15 and 180 minute groups for both Rf ranges . These can be concluded that the amount of ascorbic acid range from 0.175 to 2.8 mg /vial is enough to prevent oxidation occur in the presence of oxygen in the closed 10 ml vial. These amounts can also maintain the change of the ^{99m}Tc-DMSA complexes within 180 minutes after labeling, which is enough for routine use as a radiopharmaceutical. From the figure 11 e), the change in chromatographic pattern at 24 hours after labeling is smaller than at others post

labeling times, however, it is not necessary to stabilize the formulation for too long time due to the limitation of the radioactive half-life of ^{99m}Tc. Thus, the amount of ascorbic acid ranges from 0.175 to 2.8 mg/vial (0.018 to 0.28 %) can be used to stabilize the formulation. The theoretical range of ascorbic acid for use as an antioxidant is 0.05-3%.

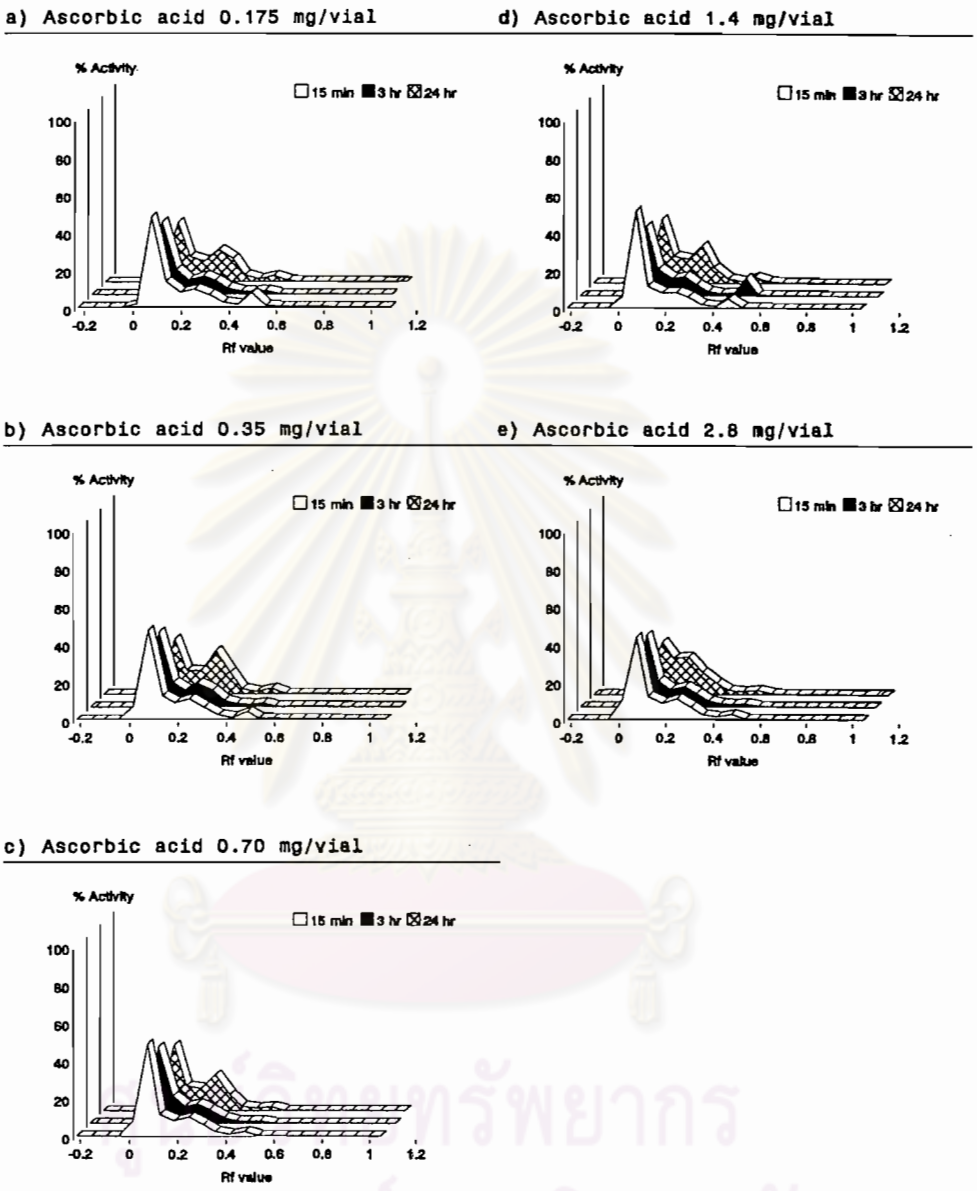

Figure 11 Effect of various amounts of ascorbic acid on the properties of ^{99m}Tc-DMSA at 15 minutes, 180 minutes (3 hr) and 24 hours after labeling.

Table 11. Total activity of each chromatographic band at different values obtained from the 5 succimer preparations with different concentration of ascorbic acid.

Ascorbic acid	post labeling time	% Activity at different Rf ranges			
		Rf range			
		0-0.2	0.2-0.4	0.4-0.6	0.6-1
0.175 mg/vial	15 min	73.94	19.84	5.29	0.93
	180 min	71.67	24.61	2.79	0.93
	24 hr	60.49	34.28	4.51	0.72
0.35 mg/vial	15 min	74.69	20.01	4.54	0.76
	180 min	69.85	27.32	2.26	0.57
	24 hr	57.63	38.26	3.69	0.42
0.7 mg/vial	15 min	76.88	19.45	3.11	0.56
	180 min	73.57	24.17	1.93	0.33
	24 hr	65.43	31.46	2.85	0.26
1.4 mg/vial	15 min	76.30	17.16	5.94	0.60
	180 min	75.23	18.70	5.44	0.63
	24 hr	63.45	31.64	4.60	0.31
2.8 mg/vial	15 min	74.84	21.01	3.66	0.49
	180 min	72.66	24.54	2.46	0.31
	24 hr	75.83	21.09	2.91	0.17

Table 12. Summarized results of the comparison of ^{99m}Tc-DMSA occurred in the presence of 5 concentrations of ascorbic acid at different post labeling times.

	$F_{calculated}$	$F_{critical}$	P	Statistical significance ($p<0.05$)
Rf 0-0.2				
Different time	10.328	4.459	0.006	S
Different conc.	1.592	3.838	0.266	N
Rf 0.2-0.4				
Different time	11.441	4.459	0.004	S
Different conc.	1.335	3.838	0.336	N

(Calculation details : see table 26 of appendix B)

3. Effect of efficiency of equipment on the quality of the formulations

Because degradation of succimer cold kits results from oxidation, prevention of the leakage of air into the vial is one of the important step to preserve the quality of the kits. After lyophilization the nitrogen gas was added into the vial at atmospheric pressure. There was a problem occurred with this step because of the stoppering mechanism of the machine. Lyph lock 12^{R} used air bag to stopper the vial closure by lowering pressure outside the bag, which was the pressure of the sample container. For this reason the pressure inside the vial was lower than outside and there might be a possible leakage of air and moisture into the vial during storage and labeling process. Fortunately, the new equipment with different stoppering mechanism was available in the later stage. This FTS^{R} machine used motor drive system for stoppering the vial closure that did not interfere with the pressure system. The new batches of the three formulations were prepared using the new equipment. The new batch formulations were radiolabeled and assayed by using system II. The plots of activity distributions versus Rf values are shown in figure 12 and the total radioactivity in the bands is shown in table 13. From the figure 12 the formulation 3 yields neglegible amount of the peak at Rf range 0.4-0.5, which corresponds to the ^{99m}Tc(V)-DMSA peak, whereas the formulation 1 and 2 have noticable peaks. The other interesting point is the difference of the proportion of peak no.1(Rf 0-0.2) and peak no. 2 (Rf 0.2-0.4) among the three formulations. Furthermore, it is clearly seen from the plots at time = 24 hours that

the reduction of peak no.1 of the formulation 2 is quicker than the formulation 3 . These two formulations contain similar ingredients except formulation 3 has inositol as suspending agent. These results support the postulate that inositol has some effects on stabilizing the formulation. This effect may be because the molecules of active ingredients are embedded in the inositol cake, this mechanism can lower the chance of exposure to oxygen. The stabilization effect also probably occurred after radiolabeling, resulting in the slower reduction of peak at Rf range 0-0.2. The dispersion of the labeled compound in an unlabeled diluent is one of the method that can protect self-decomposition results largely from the attack on the labeled compound by chemically reactive species produced by the effects of radiation on the solvent.

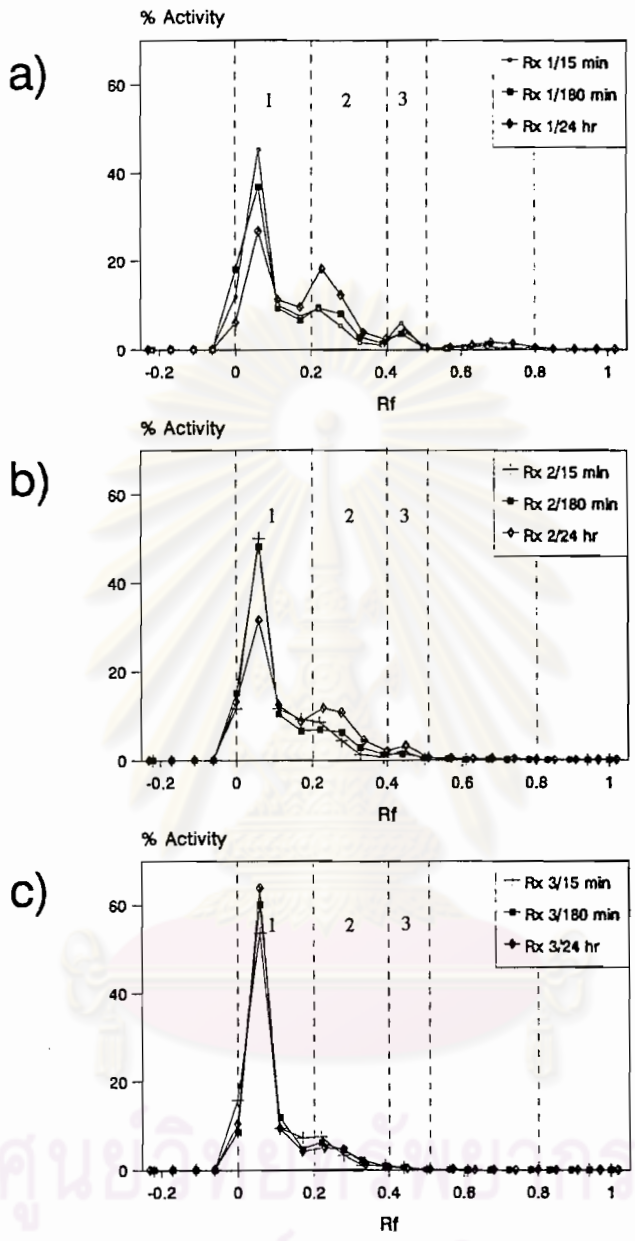

Figure 12 The radioactivity distribution plots of the radiochromatograms of the second batch of three succimer formulations assayed after freshly prepared. The assays were performed using chromatographic system II.

Table 13 Total radioactivity of each chromatographic segment at different Rf range from the second batch of 3 succimer cold kit formulations. The kits were assayed after preparations by using chromatographic system II.

Month = 0	post	% Activities at different Rf range					
Formulation	labeling time	Rf range					
		0	0--0.2	0.2--0.4	0.4--0.5	0.5--0.8	0.8--1
1	15 min	0	74.96	17.08	6.27	1.65	0.04
	180 min	0.01	70.81	21.76	3.95	2.88	0.62
	24 hr	--0.01	53.66	36.64	4.43	4.84	0.43
2	15 min	0.01	82.46	14.74	2.25	0.56	0
	180 min	0.01	80.18	17.27	1.82	0.63	0.08
	24 hr	0.02	65.75	29.01	3.69	1.45	0.11
3	15 min	0.01	83.12	15.36	1.15	0.35	0.02
	180 min	0.01	80.35	18.11	1.21	0.33	0
	24 hr	--0.01	84.13	15.01	0.63	0.25	0