Table (17) Effect of the reaction time on the %-labelling yield of ^{99m}Tc-DCAA-AP

Time, min.	$^{99m}TcO_4^-$ %	RH-^{99m}Tc %	^{99m}Tc- DCAA-AP %
005	38.68±0.37	13.82± 0.92	47.50±0.51
010	31.48± 0.77	16.92± 0.89	51.60±0.67
015	26.45±0.34	20.15 ±0.59	53.40±1.23
030	18.80± 0.26	22.78 ±0.51	58.42±0.22
060	23.75 ±0.61	12.13 ±0.63	64.12±0.91
120	25.41±0.32	08.42± 0.97	66.17±1.30
240	18.12± 0.91	09.88 ±0.91	72.00±1.10
360	05.12±0.89	09.54 ±0.77	85.34±2.30
420	11.78± 0.81	08.39± 0.88	79.83±1.22
480	16.30±0.79	08.40 ±0.36	75.30±0.98

(10 mg, DCAA-AP, 0.1mg tin, (II), pH 3 at room temp. and different reaction times.)

Table (18) Effect of the reaction time on the %-labelling yield of ^{99m}Tc-DCAA-AT.

Time, min.	$^{99m}TcO_4^-$ %	RH-^{99m}Tc %	^{99m}Tc- DCAA-AT %
005	42.08±0.77	17.62± 0.22	40.30±0.20
010	39.53± 0.27	15.94± 0.72	44.53±0.90
015	29.91±0.69	22.17 ±0.44	47.92±0.60
030	21.00± 0.29	26.37 ±0.82	52.63±0.32
060	31.68 ±0.37	13.15 ±0.76	55.17±0.79
120	25.59±0.62	14.60± 0.27	59.81±0.33
240	16.90± 0.92	19.18 ±0.83	63.92±0.72
360	07.79±0.31	16.60 ±0.35	75.61±0.50
420	14.52± 0.91	16.30± 0.66	69.18±0.60
480	13.76±0.38	20.61 ±0.27	65.63±1.20

(10 mg, DCAA-AT, 0.1mg tin, (II), pH 2.8, at room temp. and different reaction times.)

Table (19) Effect of reaction time on the % -labelling yield of ^{99m}Tc-Br-IDA

Time , min.	$^{99m}TcO_4^-$ %	RH-^{99m}Tc %	^{99m}Tc- Br-IDA %
005	1.50±0.70	1.37±0.19	97.13±0.22
010	1.40±0.90	1.15±0.63	97.45±0.34
015	2.10±0.82	0.37±0.82	97.53±0.17
030	1.30±0.63	0.89±0.79	97.81±0.52
060	3.00±0.27	0.69±0.93	96.31±0.42
120	3.10±0.65	0.49±0.34	96.41±0.42
240	3.60±0.42	0.78±0.57	95.62±0.51
360	3.20±0.73	0.63± 0.93	96.17±0.33
420	2.70± 0.61	1.58± 0.29	95.72±0.18
480	4.10 ±0.53	0.74 ±0.83	95.16±0.28

(40 mg Br-IDA, 0.3 mg tin (II), pH 7, at room temp. and different reaction times.

II.3.1.6. Effect of temperature

Tables (20) and (21) summarize the effect of raising the temperature on the yield of ^{99m}Tc–DCAA-AP and ^{99m}Tc – DCAA-AT.Increase in the temperature is one of the important factors affecting the percent labelling yield where elevation in the temperature from 25 – 50 °c increases the percent labelling yield from 58.42 % and 52.63 % to 65.23 % and 62.13 % for ^{99m}Tc–DCAA-AP and ^{99m}Tc–DCAA-AT respectively. By increasing the temperature up to 100 °c the percent labelling yield increased to a maximum value of 93.18 % and 91.20%. The increase in the temperature above 100 °c leads to the decomposition of ^{99m}Tc–DCAA-AP and ^{99m}Tc–DCAA-AT. But ^{99m}Tc-Br-IDA was not affected by increasing the temperature.

Table (20) Effect of temperature on the % labelling yield of ^{99m}Tc-DCAA-AP.

Temp. °c	$^{99m}TcO_4^-$ %	RH-^{99m}Tc %	^{99m}Tc- DCAA-AP %
025	18.80±0.26	22.78±0.51	58.42±0.22
050	24.97±0.73	09.80±0.49	65.23±0.29
075	20.20±0.29	00.37±0.56	79.43±0.92
100	05.67±0.83	01.15±0.97	93.18±0.21
120	10.67±0.68	07.63±0.89	81.70±0.42

(10 mg DCAA-AP, 0.1mg tin (II), pH 3.0, at 30 min. reaction time and different Temps.)

Table (21) Effect of temp. on the % labelling yield of ^{99m}Tc-DCAA-AT.

Temp. °c	$^{99m}TcO_4^-$ %	RH-^{99m}Tc %	^{99m}Tc- DCAA-AT %
025	21.00±0.29	26.37±0.82	52.63±0.32
050	20.67±0.22	17.20±0.51	62.13±1.30
075	13.13±0.67	09.26±0.82	77.61±0.70
100	06.16±1.70	02.64±0.37	91.20±1.30
120	09.16±0.25	04.47±0.91	86.37±0.76

(10 mg DCAA-AT, 0.1mg tin (II), pH 2.8, at 30 min. reaction time and at different temps.)

II.3.1.7. In-vitro stability of ^{99m}Tc–IDA complexes:

The reaction of ^{99m}Tc with DCAA-AP and DCAA-AT analogs is fast and high labelling yields of 93.18 % and 91.20 % were achieved within 30 min at 100 °c. The results in tables (22) and (23) also indicate that the ^{99m}Tc – complexes remain stable up to 8 h after labelling with ^{99m}Tc as determined by ITLC – SG chromatography, in complete agreement with the results reported by **Chervu et al (1984) and Zmbove et al (1990).**

Table (22): In-vitro stability of ^{99m}Tc–DCAA–AP

Type of Species	Radiochemical species, %				
	Time after labelling				
	5 min.	1 hr	2 hr	4 hr	8 hr
$^{99m}TcO_4^-$	05.67	06.64	06.99	07.15	08.62
RH-^{99m}Tc	01.15	00.86	00.11	00.15	00.15
^{99m}Tc-DCAA-AP	93.18	92.50	92.90	92.70	91.23

Table (23): In-vitro stability of ^{99m}Tc–DCAA–AT

Type of Species	Radiochemical species, %				
	Time after labelling				
	5 min.	1 hr	2 hr	4 hr	8 hr
$^{99m}TcO_4^-$	06.16	06.10	07.30	07.50	08.10
RH-^{99m}Tc	02.64	03.60	04.00	04.50	04.80
^{99m}Tc-DCAA-AT	91.20	90.30	88.70	88.00	87.10

II.4.Biodistribution study of ^{99m}Tc- HIDA derivatives in mice

The localization of ^{99m}Tc- complexes in particular target organs like liver, kidney, brain, bone, etc, is the basis for the use of these complexes as diagnostic agents in nuclear medicine. All ^{99m}Tc -IDA derivatives are bound to hepatocytes in the liver. The mechanism of their binding varies depending mainly on the strength and the amount of the binding to interhepatocyte proteins. An increase of the percentage of binding to proteins leads to a decrease of the glomerular filtration rate, but it can also slow down the preparations binding to hepatocytes.

The biodistribution properties of the IDA derivatives after labelling them with technetium –99m were evaluated in mice. The test is carried out by injecting the reconstituted IDA derivative kit in the tail vein of Albino – Swiss mice. The urine was collected in vessels containing filter paper at the bottom. The animals were killed by decapitation and their organs were collected and radioactivity assayed. The dose per organ was calculated by the following formula:

$$\%\text{Injection dose / organ} = \frac{\text{Sample cpm x organ weight}}{\text{Standard cpm x sample weight}} \times 100$$

Tables (24), (25) and (26) represent the biodistribution data of the complexes ^{99m}Tc-DCAA-AP, ^{99m}Tc-DCAA-AT and ^{99m}Tc-Br-IDA respectively. The data clearly indicate that all these complexes were cleared by both hepatobiliary pathway and the urinary pathway with a varying degree of renal excretion and liver retention. The data presented in Table (24) clearly shows high extraction index of the ^{99m}Tc- DCAA-AP.

The high liver uptake (26.1 %) was noticed after 5 min post injection .A fast biliary excretion for the ^{99m}Tc -DCAA-AP was observed as the intestine activity was increased markedly from 5.6 % at 5 min post injection up to 26.85 after 3h. This indicates a very short hepatobiliary transport time for the ^{99m}Tc-DCAA-AP. Also, the accumulation of the activity in both kidneys is negligible reaching 1.65 % at 3h-post injection. Although the complex was cleared from the body through hepatobiliary pathway, it is also cleared through the urinary pathway where the activity reaches 60.68 % after 3 h. Compared to ^{99m}Tc -DCAA-AP, ^{99m}Tc- DCAA-AT has a low urinary excretion rate of 50.47 % after 3h, as shown in Table (25). We must note that, in case of ^{99m}Tc- DCAA-AP the clearance of activity from the liver is high and decreased from 26.1 % at 5 min post injection to 10.36 % at 1h post injection and this washout through urinary pathway (60.68 % in the urine after 3h). In case of ^{99m}Tc- DCAA-AT the accumulation of the activity in the liver is high and increased from 16.2 % after 5-min post injection to become 26.69 % after 1 h. which is suitable for imagining with γ-camera. In the two prepared compounds, it was observed that no significant uptake of the chelates were observed in non –target organs and the accumulation of very small amounts of radioactivity in the stomach especially indicates that there was no appreciable decomposition of these chelates resulting in the formation of free pertechnetate as reported by **Challeryes M. and Bagerjee S. (1991).** But in case of ^{99m}Tc-Br-IDA as shown in table (26) approximately, 79 % of the injected dose is cleared by intestine during the first 15 min. and 90.20 % during 30 min. It is observed that the liver concentration is high (13.20 %). It could be concluded that ^{99m}Tc-Br-IDA has a high hepatic extraction, rapid clearance from liver, low urinary excretion and high bilirubin tolerance. This is in complete agreement with the data reported by **Abedin et al. (1995).**

Table (24) Biological distribution of ^{99m}Tc-DCAA-AP complex in mice organs

% Injected dose / organ and body fluid at different times post injection.					
Organs and body fluid	**5 min.**	**15 min.**	**30 min.**	**60 min.**	**3 hr**
Liver	26.10±0.10	16.00±0.34	14.90±0.33	10.36±0.10	04.26±0.10
Intestine	05.60±0.33	07.75±0.32	11.70±0.35	32.59±0.22	26.85±0.92
Urine	23.50±0.21	29.03±0.51	32.20±0.46	39.62±0.87	60.68±0.27
Kidneys	10.70±0.61	08.20±0.20	05.01±0.37	03.76±0.48	01.65±0.33
Stomach	01.10±0.29	01.00±0.74	01.00±0.38	00.83±0.27	03.90±0.41
Lung	01.20±0.28	0.90±0.61	00.70±0.50	00.26±0.37	00.10±0.28
Heart	01.40±0.67	0.92±0.23	00.60±0.61	00.16±0.48	00.06±0.78
Spleen	00.30±0.29	0.25±0.41	00.10±0.29	00.07±0.28	00.04±0.27
Bone	07.70±0.58	5.66±0.27	05.50±0.28	02.60±0.27	00.22±0.29
Muscles	07.50±0.12	16.00±0.29	17.59±0.42	06.75±0.81	00.88±0.63
Blood	14.90±0.23	14.29±0.61	10.70±0.62	03.00±0.72	01.36±0.15

Table (25) Biological distribution of ^{99m}Tc-DCAA-AT complex in mice organs

% Injected dose / organ and body fluid at different times post injection.					
Organs and body fluid	**5 min.**	**15 min.**	**30 min.**	**60 min.**	**3 hr**
Liver	16.20±0.33	17.80±0.10	21.30±0.10	26.69±0.71	09.59±0.52
Intestine	15.60±0.37	22.63±0.21	25.90±0.30	29.71±0.38	26.10±0.73
Urine	19.20±0.29	24.60±0.63	25.30±0.50	28.36±0.76	50.47±0.64
Kidneys	16.90±0.10	09.30±0.71	06.10±0.20	03.31±0.17	03.35±1.02
Stomach	01.30±0.27	01.32±0.26	00.93±0.60	00.41±0.20	00.52±0.43
Lung	00.63±0.40	00.45±0.20	00.71±0.52	01.11±0.33	00.25±0.66
Heart	00.25±0.20	00.22±0.11	00.13±0.67	00.10±0.83	00.08±0.92
Spleen	00.18±0.41	00.14±0.63	00.11±0.39	00.16±0.89	00.15±0.72
Bone	06.20±0.33	03.10±0.78	02.52±0.62	00.67±0.93	02.33±0.60
Muscles	07.80±0.29	11.40±0.86	10.10±0.67	05.75±0.34	04.90±0.21
Blood	15.74±0.30	9.04±0.21	6.90±0.25	3.73±0.61	2.26±0.10

Table (26) Biological distribution of ^{99m}Tc-Br-IDA complex in mice organs

% Injected dose / organ and body fluid at different times post injection.					
Organs and body fluid	**5 min.**	**15 min.**	**30 min.**	**60 min.**	**3 hr**
Liver	13.20±1.30	01.95±0.17	00.76±0.10	00.96±0.04	00.54±0.20
Intestine	59.21±0.23	79.00±3.80	90.20±0.25	91.89±1.03	89.60±0.54
Urine	00.39±0.20	01.97±0.20	01.76±0.50	01.10±1.40	01.77±0.83
Kidneys	02.24±0.53	00.80±0.40	00.45±0.31	00.36±0.40	00.30±0.17
Stomach	03.40±0.10	00.75±0.27	00.97±0.07	01.70±0.83	02.50±1.20
Lung	00.51±0.80	00.33±0.70	00.82±1.03	00.93±0.60	00.27±0.63
Heart	00.93±0.80	00.26±1.40	00.18±0.76	00.10±0.33	00.07±0.53
Spleen	00.19±0.82	00.15±0.07	00.12±0.21	00.19±0.18	00.11±0.27
Bone	08.20±0.76	04.20±0.23	03.50±0.61	01.20±0.55	02.70±0.03
Muscles	09.8±0.33	09.74±0.15	00.56±0.41	01.05±0.11	01.81±0.27
Blood	1.93±0.31	0.85±0.17	0.68±0.70	0.52±0.19	0.33±0.27