

# Radiosynthesis of $^{99m}\text{Tc}$ -nitrofurantoin a novel radiotracer for in vivo imaging of *Escherichia coli* infection

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Received: 3 July 2010 / Published online: 19 August 2010  
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**Abstract** Nitrofurantoin (NFN) radiolabeling with technetium-99m ( $^{99m}\text{Tc}$ ) was investigated using different concentration of the NFN, sodium pertechnetate ( $\text{Na}^{99m}\text{TcO}_4$ ), reducing agent ( $\text{SnCl}_2$ ) at different pH ranges (5.1–6.00). The suitability of the  $^{99m}\text{Tc}$ -NFN was evaluated in terms of the radiochemical purity (RCP) yield, in vitro stability in saline, serum, in vitro binding with *E. coli*, biodistribution in *E. coli* infected model rat (ERT), and scintigraphic accuracy in *E. coli* infected model rabbit (EBT). The superlative radiochemical succumb at 2.5 mg NFN, 125  $\mu\text{L}$  of  $\text{SnCl}_2$  (1  $\mu\text{g}/\mu\text{L}$  in 0.01 N HCl), 2.5 mCi of  $\text{Na}^{99m}\text{TcO}_4$ , at pH 5.2 at 30, 60, 90, and 120 min after reconstitution was  $64.50 \pm 0.11$ ,  $97.50 \pm 0.16$ ,  $94.25 \pm 0.10$ ,  $92.15 \pm 0.14$  and  $90.75 \pm 0.013\%$ . The complex was found stable in saline and serum for 90% up to 120 min and showed 50–65% in vitro binding with *E. coli*. The absorption of the  $^{99m}\text{Tc}$ -NFN, primarily at *E. Coli* infected (ECT) muscle of ERT was lower but after 60 min it went up to  $7.25 \pm 0.17\%$ . The absorption in the blood, liver, spleen, stomach, intestines, inflamed muscle (N.T1) and normal muscle (N.T2) went down while in the kidney and urine it went up with time. The ratio of the ECT/N.T1 was 7:1 and N.T2/N.T1 was 2:1. The Whole Body Static

(WBS) imaging of the ERB confirmed the suitability of the  $^{99m}\text{Tc}$ -NFN as radiotracer. The superlative radiochemical succumb, significant in vitro stability in saline and serum, in vitro binding with *E. coli*, ideal biodistribution and scintigraphic accuracy confirmed the viability of the  $^{99m}\text{Tc}$ -NFN as radiotracer for infection.

**Keywords** Nitrofurantoin · Technetium-99m · *E. coli* · Infection

## Introduction

A wide range of techniques are available for the early diagnosis of infection and its discrimination from inflammation. Amongst all the Radionuclide Scintigraphy (RS) showed promising results for in vivo localization of infectious foci at early stages [1, 2].

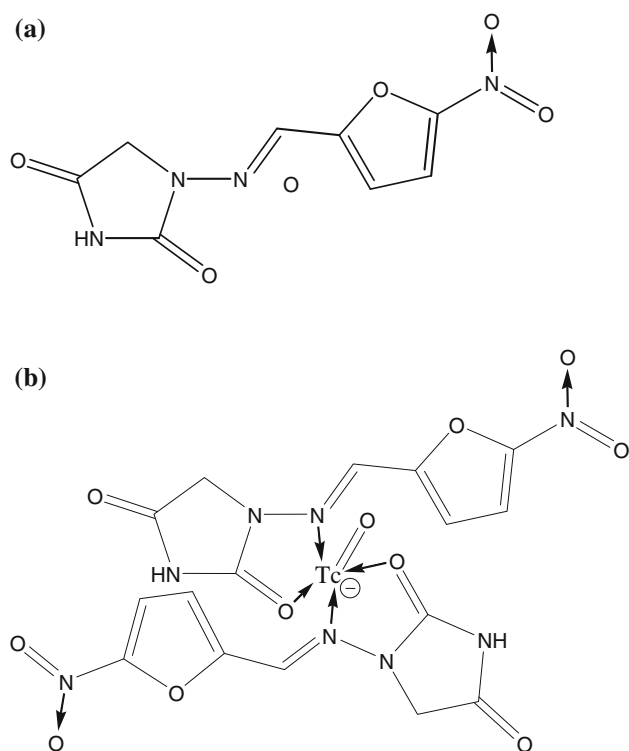
The existing radiotracers akin to  $^{67}\text{Ga}$ -citrate,  $^{111}\text{In}$ -WBC and the labeled peptides showed encouraging results but the factors that limit their suitability and usefulness include the intricate availability, high radiation dose and awkward labeling procedure [3, 4]. The technetium-99 m labeled ciprofloxacin [5], ciprofloxacin dithiocarbamate [6], lomefloxacin and ofloxacin [7], cefuroxime [8], pefloxacin [9], kanamycin [10], sparafloxacin [11], moxifloxacin [12], sitafloxacin [13], and rifampicin [14], after intensive evaluation have been anticipated for infection imaging that opening new ways for exploration of a new class of imaging agents.

Nitrofurantoin (NFN); (*E*)-1-[(5-nitro-2-furyl) methylideneamino] imidazolidine-2,4-dione (Fig. 1), is another antibiotic (bacteriocidal) oftenly intended for the Urinary Tract Infection (UTI) caused by *E. coli* showing resistance to other antibiotics. NFN is also safe in pregnancy

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**Fig. 1** **a** Chemical structure of nitrofurantoin. **b** Proposed structure of <sup>99m</sup>Tc-NFN complex

as and when it is used in combination with sulfisoxazole [15]. The mechanism of action of NFN is unique and complex as it damaged the pathogen Deoxy Ribonucleic Acid (DNA) in its reduced form. Flavoproteins (nitrofurantoin reductase) in the pathogen cell rapidly reduced the NFN into multiple reactive intermediate that attack ribosomal DNA, respiration, pyruvate metabolism and other macromolecules [16].

To exploit the bacteriocidal activity of the NFN, herein, we report the radiolabeling of NFN with technetium-99 m, its in vitro radiochemical stability in saline, serum, binding with *E. coli*, biodistribution in *E. coli* infected rat (ERT) and scintigraphic accuracy in *E. coli* infected rabbit (ERB).

## Experimental

### Materials

Nitrofurantoin (Macrochantin USA, TLC strips (Merck), and all the other chemicals and solvents of analytical grade (Sigma). RP-HPLC (Shimadzu, Japan), well counter, scalar count rate meter (Ludlum USA), Dose calibrator (Capintech USA), and the Gamma camera (GEADE Nuclearmedicine system, Germany).

### Method

#### Synthesis of <sup>99m</sup>Tc-nitrofurantoin complex

Stannous chloride (1 µg/µL in 0.01 N HCL), 25 µL (with 25 µL rise) to 250 µL were taken in 10 sterile vials. The pH of the vials was set between 5.1 (with 0.1 augmentation) to 6 and nitrofurantoin (NFN), 0.5 (with 0.5 mg tallying) to 5.0 mg was added to it. Thereafter, 0.5 (with 0.5 mCi rise) up to 5.0 mCi of sodium pertechnetate was aseptically poured and the preparations were incubated at room temperature followed by filtration prior to investigation.

#### HPLC analysis

Shimadzu SCL-10 AVP system, equipped with SDP-10 AVP UV detector operating at 254 nm, Packard 500 TR series flow scintillation analyzer, binary pump, online degasser, was used for determination of % yield of <sup>99m</sup>Tc-NFN, free <sup>99m</sup>Tc and radio-colloids applying the reported method [13]. Briefly, 5 µL of the preparation was injected into C-18 column (4.6 × 150 mm, 5 µM) followed by 1 mL/min water:acetonitrile (10:90) elution. The fraction collected (1 mL/min) was analyzed for of % yield of <sup>99m</sup>Tc-NFN, free <sup>99m</sup>Tc and radio-colloids at 30, 60, 90 and 120 min after reconstitution using well counter interface with scalar count rate meter (WCSR).

#### In vitro stability in saline

In vitro stability of the <sup>99m</sup>Tc-NFN complex was investigated using the reported HPLC method [14], Briefly, 10 µL of the <sup>99m</sup>Tc-NFN complex was injected to the C-18 column (4.6 × 150 mm, 5 µM) of the shimadzu SCL-10 AVP system, with SDP-10 AVP UV detector operating at 254 nm, Packard 500 TR series flow scintillation analyzer, binary pump, using online degasser, with a flow rate of 1 mL/min for 15 min using water:acetonitrile (W:A) (10:90) as the mobile phase. The labeled complex was examined for in vitro stability in serum at 30, 60, 90, and 120 min. The eluent collected at 1–15 min of separation was collected vials and counted for % in vitro stability of the complex using gamma well counter.

#### In vitro stability in serum

In serum the in vitro stability of the <sup>99m</sup>Tc-NFN was evaluated by incubating 0.2 mL of the complex in saline with 1.8 mL of serum at 37 °C for 120 min. Aliquots, 1 µL at 1–120 min (in incubation period) were applied to the TLC strip. The strip after drying was developed in acetone and ethanol:ammonia:water (2:1:5). The developed TLC

strips were divided at ( $R_f$  5) and counted for activity, using gamma rays well counter.

#### *In vitro* binding with *Escherichia (E.) coli*

Binding of  $^{99m}\text{Tc}$ -NFN with *E. coli* was determined by the reported method [17], briefly, 0.1 mL of sodium phosphate buffer (Na-PB) containing 10 MBq of the  $^{99m}\text{Tc}$ -NFN was shifted to a test tube and 0.8 mL of 50% (v/v) of 0.01 M acetic acid in a Na-PB containing approximately  $1 \times 10^8$  colony forming units (CFU) pathogen were added to it followed by incubation at 4 °C for 1 h with a final pH 5 and centrifugation at 2000 rpm for 5 min. The supernatant was removed and the bacterial pellet was resuspended in 1 mL of Na-PB and recentrifuged as above. The supernatant was removed and the *E. coli* pellet was counted for activity using gamma well counter. The activity related with bacteria was expressed as % of the added activity bound to bacteria in regard to the total activity. *In vitro* binding efficacy of  $^{99m}\text{Tc}$ -NFN was compared with  $^{99m}\text{Tc}$ -sitafloxacin ( $^{99m}\text{Tc}$ -STF),  $^{99m}\text{Tc}$ -rifampacin ( $^{99m}\text{Tc}$ -RMP).

#### *Biodistribution in rat*

Bacterial broth containing  $1 \times 10^8$  colony forming units (CFU) in 0.1 mL saline was injected intramuscularly (I.M) into the right thigh of the anaesthetized male Sprague–Dawley rat: weight range, 150–200 g. After 12 h sterile turpentine oil was injected I.M into the left thigh followed by intravenous administration of 0.1 mL (37 MBq) of  $^{99m}\text{Tc}$ -NFN. The rats were scarified in accordance with the guide lines of the Nuclear Medicine Research Laboratory University of Peshawar. Accurately, one gram each of the blood, infected (IFT), inflamed (IFM), normal (NL) muscle, liver, spleen and kidney were weighed and percent injected dose absorbed per gram (% I.D/g) was calculated using gamma well counter.

#### *Scintigraphy of rabbit*

*Escherichia coli*, 0.5 mL in saline containing  $1 \times 10^8$  CFU was intramuscularly injected to the right thigh of the anesthetized New Zealand white rabbits (male, weight: 3.0–4.0 kg) and after 10 h 0.5 mL autoclaved sterile turpentine oil to the left thighs. Afterward, 0.2 mL (2 mCi) of the  $^{99m}\text{Tc}$ -NFN complex was intravenously administered and the rabbit was placed prone on the bed of the Gamma camera equipped with low energy general purpose collimator (LEGPC). Whole body images (WBS) of the rabbits were obtained at 15, 30, 45, 60 and 90 min of injection.

## Results and discussion

### Yield of $^{99m}\text{Tc}$ -nitrofurantoin complex

The radio-chromatogram of the  $^{99m}\text{Tc}$ -NFN complex showed different peaks at retention time 5.1 and 14.8 min as shown in Fig. 2. The radio peak at 14.8 min represents the  $^{99m}\text{Tc}$ -NFN complex. The  $^{99m}\text{Tc}$ -NFN prepared by mixing 2.5 mg NFN, 125  $\mu\text{L}$  of  $\text{SnCl}_2$  (1  $\mu\text{g}/\mu\text{L}$  in 0.01 N HCl), and 2.5 mCi of  $\text{Na}^{99m}\text{TcO}_4$ , at pH 5.2, gave  $97.50 \pm 0.16\%$   $^{99m}\text{Tc}$ -NFN,  $0.55 \pm 0.16\%$  free  $^{99m}\text{Tc}$  and  $2.00 \pm 0.12\%$  radio-colloids after 30 min of reconstitution and was found stable in saline up to 120 min for more than 90% as shown in Fig. 3. The % yield of  $^{99m}\text{Tc}$ -NFN, free  $^{99m}\text{Tc}$  and radio-colloids are given in Table 1.

$^{99m}\text{Tc}$  can complex with the bidentate nitrofurantoin through one of the nitrogen and C=O and between two

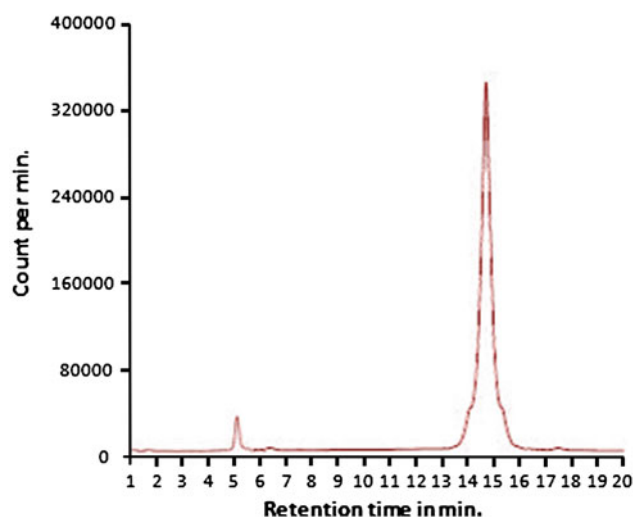


Fig. 2 HPLC radio-chromatogram of the  $^{99m}\text{Tc}$ -NFN complex

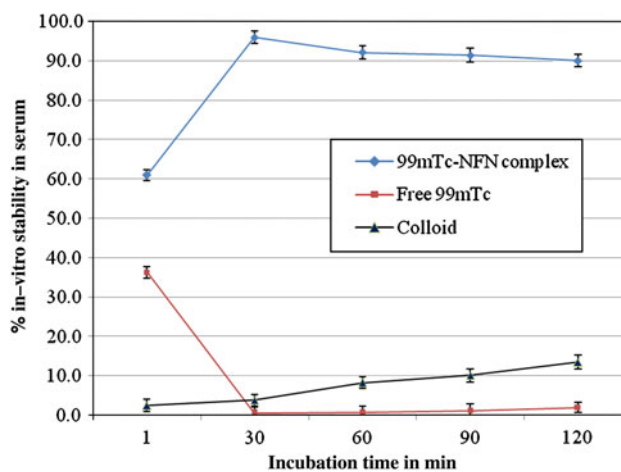


Fig. 3 In-vitro stability of  $^{99m}\text{Tc}$ -NFN complex in saline

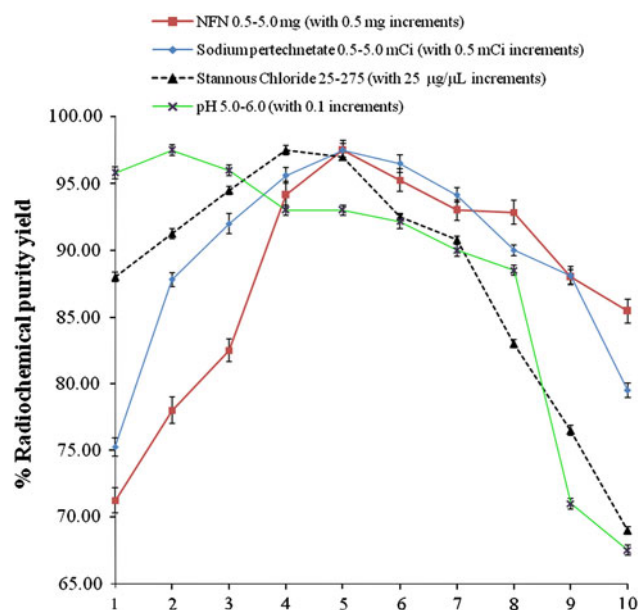
**Table 1** % Radiochemical yield of  $^{99m}\text{Tc}$ -NFN complex (Mean  $\pm$  SD) ( $n = 3$ )

Post labeling investigation time (min)	Radiochemical purity yield (%)		
	Free $^{99m}\text{Tc}$	Colloids	$^{99m}\text{Tc}$ -NFN complex
1	33.75 $\pm$ 0.16	1.50 $\pm$ 0.15	64.50 $\pm$ 0.11
30	0.55 $\pm$ 0.16	2.00 $\pm$ 0.12	97.50 $\pm$ 0.16
60	0.80 $\pm$ 0.14	5.85 $\pm$ 0.09	94.25 $\pm$ 0.10
90	1.20 $\pm$ 0.11	7.10 $\pm$ 0.11	92.15 $\pm$ 0.14
120	1.75 $\pm$ 0.14	8.75 $\pm$ 0.12	90.75 $\pm$ 0.13

molecules of the ligand (Fig. 1b). At pH 5–6  $\text{SnCl}_2$  reduces  $\text{Tc(VII)}$  to  $\text{Tc(V)}^{3+}$  which get stabilized by the ligand. By analogy to  $\text{Cu(II)}$  complex derived from 5-nitrofurantoin-2-carboxaldehyde [18], the complex will have a square pyramidal geometry, 1:2 stoichiometry of  $\text{Tc:Ligand.}$ , with the coordination number five, with the oxidant core  $\text{Tc(V)}^{3+}$  and a negative charge on the  $^{99m}\text{Tc}$ .

#### Effect of nitrofurantoin, stannous chloride, sodium pertechnetate and pH

The effect of nitrofurantoin (NFN), stannous chloride, sodium pertechnetate and pH on the radiochemical purity yield of the  $^{99m}\text{Tc}$ -NFN is shown in Fig. 4. The maximum RCP yield of the  $^{99m}\text{Tc}$ -NFN observed by mixing 2.5 mg NFN, 125  $\mu\text{L}$  of  $\text{SnCl}_2$  (1  $\mu\text{g}/\mu\text{L}$  in 0.01 N HCl), and 2.5 mCi of  $\text{Na}^{99m}\text{TcO}_4$ , at pH 5.2 was 97.50  $\pm$  0.16%.

**Fig. 4** Effect of nitrofurantoin, sodium pertechnetate, stannous chloride, and pH on the % radiochemical purity yield of the  $^{99m}\text{Tc}$ -NFN complex

#### In vitro stability in serum

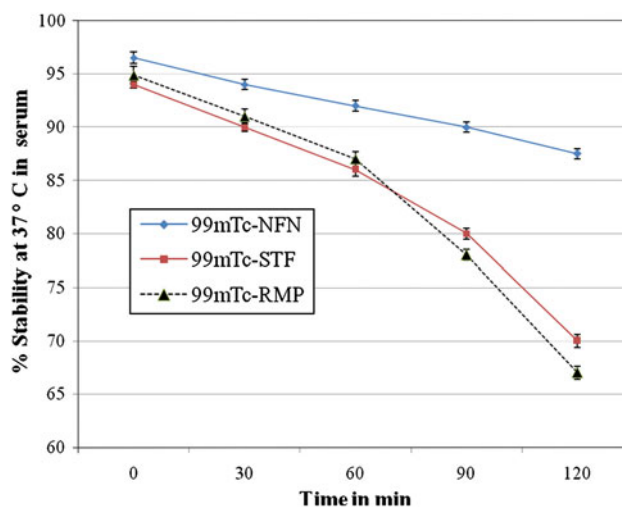
The undesirable activity observed in vitro in serum at 37  $^{\circ}\text{C}$  after 120 min of incubation for the  $^{99m}\text{Tc}$ -NFN complex was 9.15  $\pm$  0.14% ( $n = 10$ ). The comparative in vitro stability profile of  $^{99m}\text{Tc}$ -NFN,  $^{99m}\text{Tc}$ -STF and  $^{99m}\text{Tc}$ -RMP in serum at 37  $^{\circ}\text{C}$  is given in Fig. 5.

#### In-vitro binding with Escherichia (E.) coli

The  $^{99m}\text{Tc}$ -NFN prepared by mixing NFN (2.5 mg),  $\text{SnCl}_2$  (125  $\mu\text{L}$ ),  $\text{Na}^{99m}\text{TcO}_4$  (2.5 mCi) at pH 5.2 showed 50–65% ( $n = 15$ ) binding with *E. coli*, while that for  $^{99m}\text{Tc}$ -STF and  $^{99m}\text{Tc}$ -RMP was 45–55% ( $n = 15$ ) and 55–60% ( $n = 15$ ) respectively.

#### Biodistribution into the *E. coli* infected rats

The percent absorption of the  $^{99m}\text{Tc}$ -NFN in blood, *E. coli* infected (ECT) muscle, inflamed (T1) muscle, normal (T2) muscle, liver, spleen, stomach, intestines, kidney and urine of the ERT are given in Table 2. After 30 min of the administration 26.25  $\pm$  0.22, 3.60  $\pm$  0.018, 2.25  $\pm$  0.16, 1.50  $\pm$  0.018, 24.50  $\pm$  0.14, 6.80  $\pm$  0.015, 18.15  $\pm$  0.18, 2.25  $\pm$  0.17 and 4.15  $\pm$  0.14% of the injected dose was observed in blood, ECT muscle, T1 muscle, T2 muscles, liver, spleen, stomach & intestines, kidney and urine respectively. Within 90 min the absorption of the complex in the ECT muscle increased from 3.60  $\pm$  0.18 to 7.25  $\pm$  0.17% and decreased from 2.25  $\pm$  0.016 to 1.5  $\pm$  0.15% in the T1 muscle. No change in T2 (control) muscle was observed and the uptake was 1.50%, during the analysis. Decrease in the order of activity in all the organs of the

**Fig. 5** In-vitro binding of the  $^{99m}\text{Tc}$ -Nitrofurantoin (NFN),  $^{99m}\text{Tc}$ -Sitafoxacin (STF),  $^{99m}\text{Tc}$ -Rifampacin (RMP) complexes in serum at 37  $^{\circ}\text{C}$

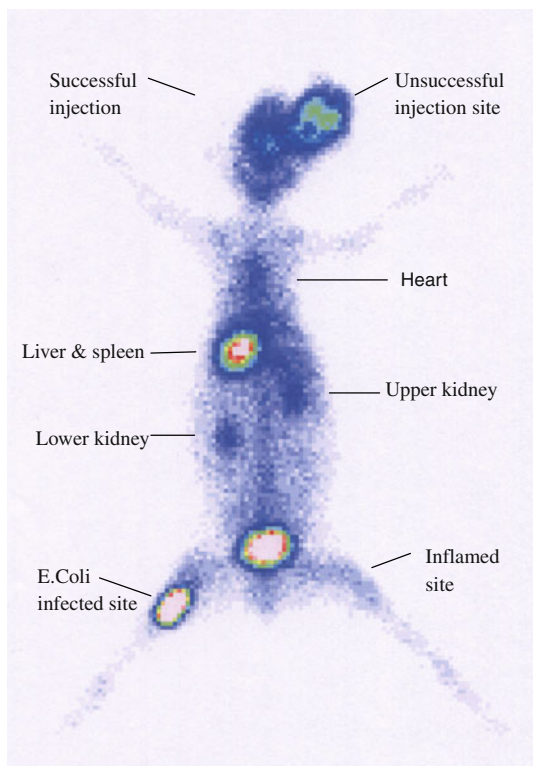
**Table 2** Biodistribution behavior of the <sup>99m</sup>Tc-NFN complex at 30, 60, 90 and 120 min in *Escherichia coli* infected rats (Mean ± S.D) (n = 3)

Organs of the <i>E. coli</i> infected rats	% Absorbed dose per gram in different organs of the <i>E. coli</i> infected rat at different intervals after I.V administration of the <sup>99m</sup> Tc-NFN complex (min)			
	30	60	90	120
<i>E. coli</i> infected (ECT) muscle	3.60 ± 0.18	6.80 ± 0.20	7.25 ± 0.17	5.50 ± 0.20
Inflamed (NT1) muscle	2.25 ± 0.16	2.00 ± 0.18	1.50 ± 0.18	1.50 ± 0.15
Normal (NT2) muscle	1.50 ± 0.18	1.50 ± 0.16	1.50 ± 0.15	1.50 ± 0.15
Blood	26.25 ± 0.22	14.50 ± 0.18	7.00 ± 0.17	3.30 ± 0.16
Liver	24.50 ± 0.14	10.25 ± 0.15	5.85 ± 0.18	2.00 ± 0.20
Spleen	6.80 ± 0.15	5.00 ± 0.15	3.15 ± 0.18	1.85 ± 0.22
Kidney	2.25 ± 0.17	14.50 ± 0.17	18.85 ± 0.22	26.25 ± 0.19
Stomach & intestine	18.15 ± 0.18	13.50 ± 0.14	8.20 ± 0.17	4.00 ± 0.22
Urine	4.15 ± 0.14	15.55 ± 0.22	19.85 ± 0.22	25.00 ± 0.17
ECT/NT2	2.40 ± 1.00	4.53 ± 1.25	4.83 ± 1.13	3.67 ± 1.33
NT1/NT1	1.50 ± 0.89	1.33 ± 1.13	1.00 ± 1.20	1.00 ± 1.00

ERT was observed with the exception of kidney, urine and ECT muscle, which inveterate the typical itinerary of excretion of the <sup>99m</sup>Tc-NFN complex as infection radiotracer from the body.

Scintigraphy of *E. coli* infected rabbits

After 15 min of the intravenous injection of the <sup>99m</sup>Tc-NFN normal appearance in the whole body of the EBT was



**Fig. 6** Whole Body Scan (WBS) of *E. coli* infected rabbit at 60 min of I.V injection of the <sup>99m</sup>Tc-NFN

observed. The appearance of activity in the ECT (target organ) muscle and T1 muscle was zilch after 15 min of scintigraphy. After 30 min of injection the activity slightly appeared in the ECT muscle and T1 muscle. After 45 min, simultaneously, the appearance of activity in ECT and disappearance from T1 muscle was observed. Maximum activity uptake was noted in ECT and almost similar negligible level of activity was observed in T1 and T2 (control) muscle after 60 min as shown in Fig. 6. After 90 min no change in the scan was observed with regard to the uptake in ECT, T1 and T2 muscle.

**Conclusion**

<sup>99m</sup>Tc-NFN complex, a new radiotracer for in vivo infection was synthesized that showed soaring in vitro stability in saline and serum, lofty binding with *Escherichia coli*, precise and suitable in vivo distribution in ERT and infection imaging accuracy in EBT. In view of the exceeding facts, we recommend the <sup>99m</sup>Tc-NFN as promising radiotracer for in vivo infection.

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