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# Synthesis and biological evaluation of technetium-sarafloxacin complex for infection imaging

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**Abstract** Technetium-99m-sarafloxacin was synthesized and formulated for the development of a potential diagnostic imaging agent for the bacterial infection and inflammation with higher efficiency than the commercially available $^{99m}$Tc-ciprofloxacin. Factors influencing the labeling yield such as sarafloxacin amount, pH of the labeling reaction, $SnCl_2$ amount and reaction time were optimized. The labeled drug was subjected to preclinical evaluations in mice. The biodistribution studies indicated that $^{99m}$Tc-sarafloxacin displayed relatively high uptake in the infectious lesion (T/NT $= 4.2 \pm 0.1$) at 2 h post-injection. The results revealed that $^{99m}$Tc-sarafloxacin cannot discriminate infection from sterile inflammation.

**Keywords** Sarafloxacin · Diagnosis · Infection · Labeling · Inflammation

## Introduction

Early discrimination and diagnosis of infection from inflammation is vital for therapists for its effective treatment with convenient cure to diminish the percentage of morbidity and mortality [1]. Elegant radiological imaging instruments such as computer tomography (CT), magnetic resonance imaging (MRI), ultrasonography (US) and X-ray are commercially available and have been used for the diagnosis of infection. These medical diagnostic imaging techniques are not the convenient methods for early detection of infection [2, 3]. The objective of nuclear medicine scintigraphy (NMS) techniques to recognize the sites of infections and its distinction from non-infectious inflammations is medically essential for the suitable care of patients with microbial infectious or inflammatory diseases [4, 5]. Some of infection imaging agents were stated including the radiolabeled leukocytes, $^{111}$In and $^{67}$Ga-citrate [6, 7]. As a result of the lack and limitations of the conventional infection imaging agents such as the lack of specificity, time-consuming preparation, sensitivity and high radiation burden, the development of novel and improved radiopharmaceuticals for infection imaging raised a major challenge for nuclear medicine practice for in vivo localization of the bacterial infection and inflammation [8–11].

Different promising antibacterial agents have been synthesized, labeled and formulated for the diagnostic imaging and observation of infective lesions [12–15]. The selected antibiotics used in the diagnostic process should be highly localized in the infectious foci, where they are considerably taken up, metabolized and demonstrated rapid clearance in vivo. Quinolone derivatives such as gatifloxacin [16], norfloxacin [17] difloxacin, pefloxacin [18], sparfloxacin [19], lomefloxacin [20] and enrofloxacin [21] representing the major antibiotics used to cure serious bacterial infections were successfully labeled with technetium-99m and compared to the commercially available $^{99m}$Tc-ciprofloxacin used for infection imaging procedures [22–24]. However, biodistribution investigations in experimental animals and in clinical trials revealed that the

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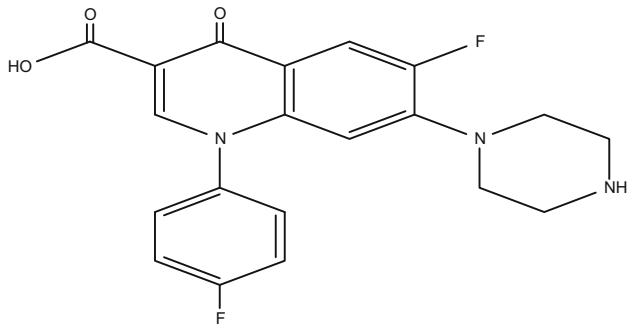


**Fig. 1** Chemical structure of sarafloxacin

specificity of $^{99m}$Tc-ciprofloxacin for infection is unfavorable [25–29]. $^{99m}$Tc-ciprofloxacin formulation has many disadvantages which are reported in the literatures [30–33].

Sarafloxacin is a fluoroquinolone derivative operating against a wide range of disease-causing bacteria including both aerobic and anaerobic pathogenic bacteria. The structure of sarafloxacin was illustrated in Fig. 1 [34]. The objective of the present study was to develop an easy and efficient method for synthesis and labeling of sarafloxacin with $^{99m}$Tc as a convenient infection imaging radiopharmaceutical to recognize the sites of bacterial infections in vivo. The formulation of the radiolabeled sarafloxacin will be developed depending on preclinical evaluation of different parameters affecting the labeling yield including in vitro and in vivo stability, radiochemical purity and pharmacokinetic studies in mice.

## Materials and methods

Sarafloxacin ($C_{20}H_{17}F_2N_3O_3$; Mwt: 385.36 g/mol) was obtained from Sigma-Aldrich Chemical Company, USA. Whatman No. 1 paper chromatography (PC) was purchased from Whatman International Ltd, Maidstone, UK. $^{99m}$Tc was eluted as $^{99m}TcO_4^-$ from $^{99}$Mo/$^{99m}$Tc generator, Gentech, Turkey. The radioactivity was determined in a well-type NaI(Tl) γ-ray scintillation counter coupled to SR-7 scaler ratemeter. All chemicals were used directly without further purification. Ultrapure water was used in all experiments for the preparation of solutions and dilutions.

### Labeling procedure

$^{99m}$Tc-sarafloxacin was synthesized by direct reaction of sarafloxacin with $^{99m}$Tc ($t_{1/2}$ = 6 h) under reducing conditions in the presence of $SnCl_2{\cdot}2H_2O$. One ml of $^{99m}$Tc eluate containing 400 MBq was added to the reaction vessel. Thereafter, the reaction mixture was allowed to react at room temperature (25 °C) for the recommended time before estimating the yield of $^{99m}$Tc-sarafloxacin complex. Factors affecting the radiolabeling efficiency like the concentration of reducing agent (25–200 mg/ml), amount of sarafloxacin (0.5–2.5 mg), pH of the reaction medium (5–12) and the reaction time (1–480 min) were adjusted in order to optimize the labeling yield. The radiochemical purity was assessed by PC and high performance liquid chromatography (HPLC).

### Analysis

Radiochemical yield and purity of $^{99m}$Tc-sarafloxacin is the proportion of the total radioactivity in the desired radiochemical form. It was carried out using strips of Whatman No. 1 PC by ascending paper chromatographic technique. An aliquot of 1–2 µl of the labeling reaction mixture was spotted 2 cm above the bottom edge of two PC strips (10 cm length, 1.5 cm width) and allowed to evaporate spontaneously. Then one strip was developed with acetone to determine the percentage of free $^{99m}TcO_4^-$ while the other strip was developed with a mixture of ethanol: water: ammonia (2:5:1) in order to determine the percentage of reduced hydrolyzed $^{99m}$Tc. After entire development, the strips were dried, cut into pieces (0.5 cm) and individually counted using NaI(Tl) scintillation detector to determine the percentage of $^{99m}$Tc-complex, free $^{99m}TcO_4^-$ and hydrolyzed $^{99m}$Tc. The radiochemical purity was further verified by HPLC (Hitachi model, Japan). The HPLC analysis was completed by injection of 10 µl purified $^{99m}$Tc-sarafloxacin complex into a reversed-phase column (Lichrosorb RP C-18, 4 × 250 mm; 5 µm) coupled to a UV detector (SPD-6A) operated at 319 nm and eluted with a mobile phase containing a mixture of 10 % ethanol in 0.2 M phosphate buffer at pH 7. The filtered mobile phase was degassed prior to use and a flow rate of 1 ml/min was applied [35]. Radioactivity measurements in the HPLC eluates was detected by NaI(Tl) scintillation detector coupled to a single channel analyzer.

### Stability of $^{99m}$Tc-sarafloxacin in serum

In vitro stability was performed by incubating 2 ml of the human serum and 0.2 ml of $^{99m}$Tc-sarafloxacin at 37 °C for 24 h. An aliquot of 0.2 ml was withdrawn during the incubation period at different time intervals for up to 24 h and analyzed using PC and HPLC to calculate the percentage of $^{99m}$Tc-complex, free pertechnetate and reduced hydrolyzed technetium.

### Induction of inflammation in mice

Induction of infectious foci was estimated using a single clinical isolation of *Staphylococcus aureus* (*S. aureus*) from biological samples to yield focal infection [36]. Single colonies were diluted in order to obtain a turbid suspension. Five mice were injected with 200 µl of the produced

suspension in the left lateral thigh muscle. Thereafter, the animals were abandoned for 24 h to obtain a gross swelling in the infected thigh. Then, creation of non-infected inflammation (sterile inflammation) was generated by injecting 200 µl of sterile turpentine oil intramuscularly in the left lateral thigh of the mice. Accordingly, swelling appeared 2 days later. In the same manner, generation of heat killed non-infected inflammation was induced by injecting 200 µl of heat killed *S. aureus* [37–40].

### Biodistribution studies

Biodistribution experiment was performed according to the guidelines outlined by the Egyptian Atomic Energy Authority and authorized by the ethics committee. In vivo experiments were carried out in normal Albino mice ($n = 5$). Animals were injected intravenously in the tail vein with 100 µl of $^{99m}$Tc-sarafloxacin (4 MBq). Mice were housed alive in groups of 5 and supplied with food and water for different intervals of time. Animals were sacrificed by cervical dislocation at 2, 4 and 24 h after intravenous injection of $^{99m}$Tc-sarafloxacin. A blood sample was obtained by heart puncture. Both target and non-target thighs were dissected and counted. After dissection, different organs and tissues were washed with saline, gathered in plastic containers and weighed. Each sample was counted and corrected for background and physical decay using a well-type NaI(Tl) gamma detector. Results were expressed as percent-injected dose per gram organ and reported as % ID/g organ ± SD in a population of five mice for each time point. Statistics were calculated with the Student *t* test and all results were presented as mean ± SEM.

## Results and discussion

Radiochemical purity and in vitro stability of $^{99m}$Tc-sarafloxacin complex were estimated by PC and HPLC. Acetone was effective as the developing solvent in PC where free $^{99m}TcO_4^-$ proceed with the solvent front ($R_f = 1$) while $^{99m}$Tc-sarafloxacin and reduced hydrolyzed technetium stayed at the origin. Reduced hydrolyzed technetium was evaluated in vitro using a mixture of ethanol: water: ammonium hydroxide (2:5:1 v/v) as the developing solvent, where reduced hydrolyzed technetium remained at the origin ($R_f = 0$) while other species migrated with the solvent front ($R_f = 1$). It was found that in the majority of the preparations the greater part of radioactivity was in the bound form. The free and hydrolyzed fractions are undesirable radiochemical species and must be eliminated or reduced to a minimum level in order not to interfere significantly with the diagnosis. The radiochemical purity was assessed by subtracting the sum of the percentage of reduced hydrolyzed technetium and free pertechnetate from 100 %. The results of the radiochemical yield were expressed as mean value of three experiments. It was further confirmed by HPLC analysis, where the retention time of free $^{99m}TcO_4^-$ and $^{99m}$Tc-sarafloxacin was 4.8 and 16.5 min, respectively as presented in the radiochromatogram (Fig. 2). The HPLC was capable of isolate $^{99m}$Tc-sarafloxacin and can be applied efficiently for the purification and quality control of the complex. Factors influencing the radiochemical yield will be discussed in details.

### Effect of sarafloxacin amount

Sarafloxacin was labeled with $^{99m}$Tc using the direct technique, in which the reduced $^{99m}$Tc reacts with sarafloxacin to form the labeled chelate. The influence of the labeling yield on the amount of sarafloxacin was shown in Fig. 3. The reaction was carried out at different sarafloxacin amount (0.5–2.5 mg). Exactly 1 mg was the ideal ligand amount necessary to obtain the largest radiochemical yield, 96 %. Below this value, the ligand amount was insufficient to react with all the reduced $^{99m}$Tc, consequently the reduced hydrolyzed technetium was 62 % at

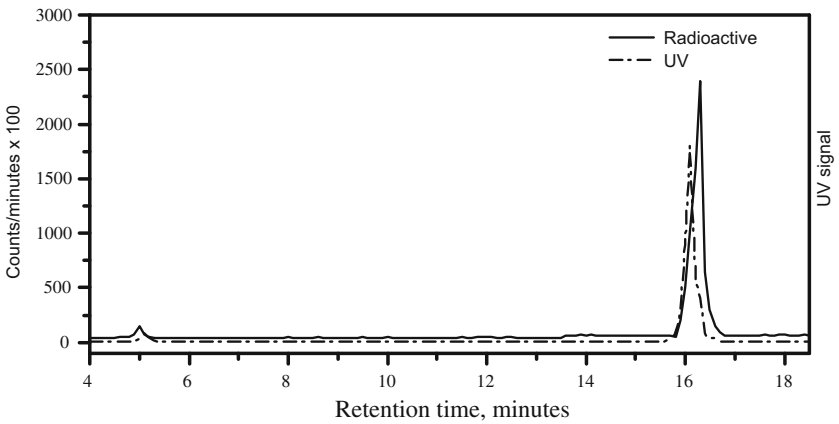


**Fig. 2** Chromatogram of $^{99m}$Tc-sarafloxacin

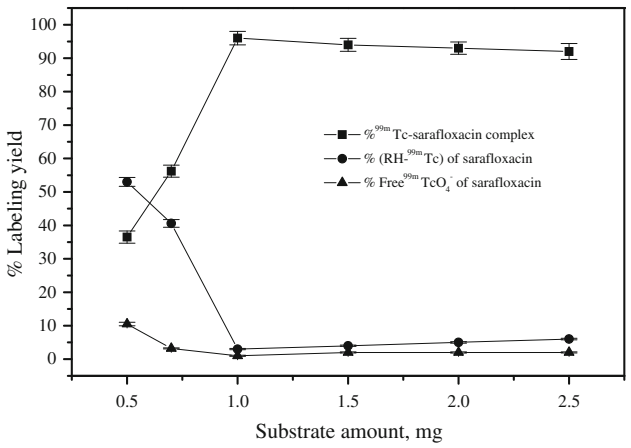


**Fig. 3** Effect of substrate amount on the labeling yield of $^{99m}$Tc-sarafloxacin complex

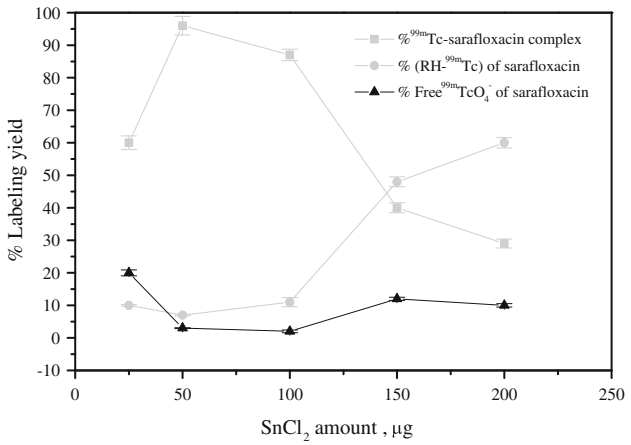


**Fig. 4** Effect of $SnCl_2{\cdot}2H_2O$ amount on the labeling yield of $^{99m}$Tc-sarafloxacin complex

0.5 mg of sarafloxacin. At ligand amount above 1 mg, the labeling yield was slightly reduced and stayed stable.

## Effect of $SnCl_2$ content

$SnCl_2{\cdot}2H_2O$ is the most common reducing agent used to reduce $^{99m}$Tc(VII) to lower valence state, which speed its chelation by sarafloxacin. The labeling yield was relied on the amount of stannous chloride present in the reaction mixture as displayed in Fig. 4. At 25 μg $SnCl_2{\cdot}2H_2O$, the labeling efficiency of $^{99m}$Tc-sarafloxacin was 60 %, which may be attributed to the incomplete reduction of $^{99m}TcO_4^-$ and hence unreliable yield of the complex due to the presence of free $^{99m}TcO_4^-$ (20 %). The labeling yield was maximized considerably to 96 % by increasing the amount of $SnCl_2{\cdot}2H_2O$ from 25 to 50 μg, Increasing the amount of $SnCl_2{\cdot}2H_2O$ to 200 μg minimized the

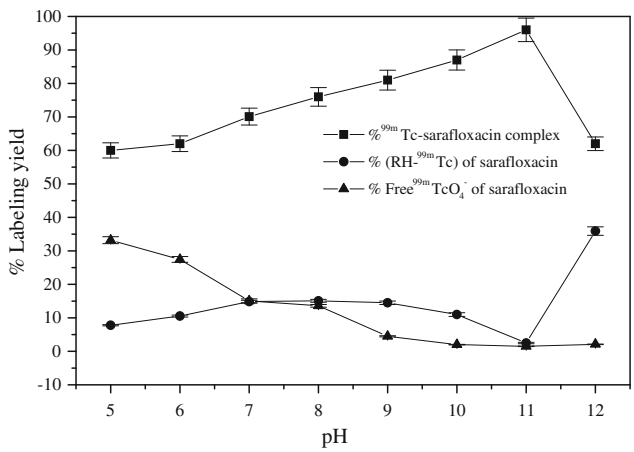


**Fig. 5** Effect of pH of the reaction on the labeling yield of $^{99m}$Tc-sarafloxacin complex

labeling yield to 29 % due to the formation of tin colloids (60 %), which may compete with sarafloxacin for the reduced $^{99m}$Tc. This may be referred to the consumption of most of the ligand molecules in the formation of complex, consequently the excess pertechnetate was further reduced to technetium(IV) in the absence of ligand or the unreacted stannous chloride lead to the production of the undesired stannous hydroxide colloid ($Sn(OH)_3^-$) in alkaline medium [5, 40].

## Effect of pH of the reaction mixture

The effect of the pH of the reaction on the radiochemical yield was shown in Fig. 5. The pH of the labeling reaction medium was evaluated at pH range from 5 to 12. At pH 11 the radiochemical purity was equal to 96 %, which may be referred to the deprotonation of the sarafloxacin that is certainly existing at high pH values and lead to great stability of TcO(V)-sarafloxacin complex. Elevated $OH^-$ concentration may lead to partial hydrolysis of the complex and oxidation of Tc(V) to pertechnetate.

## Effect of reaction time and stability test

The stability of $^{99m}$Tc-sarafloxacin was investigated to decide the convenient time for injection to prevent the formation of unfavorable products that may arise from the radiolysis of the labeled complex. The resultant undesired radioactive products may be concentrated in non-target organs. Figure 6 depicted the rate of formation of $^{99m}$Tc-complex, which indicated that the labeling yield increased from 89 to 96 % when increasing the reaction time from 1 to 30 min. Figure 7 revealed the in vitro stability of $^{99m}$Tc-sarafloxacin with respect to time. The labeled fluoroquinolone derivative was stable for up to 120 min after

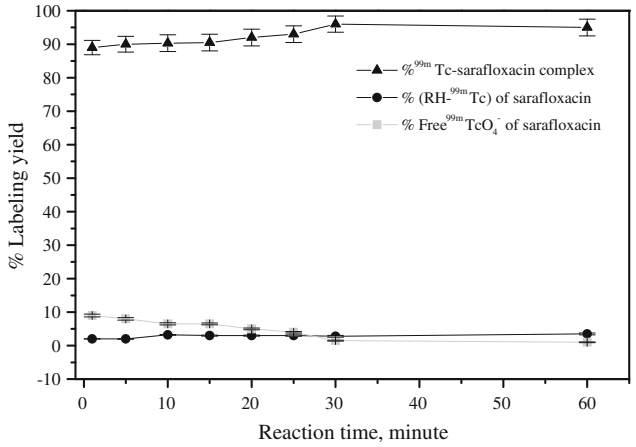


**Fig. 6** Effect of reaction time on $^{99m}$Tc-sarafloxacin complex

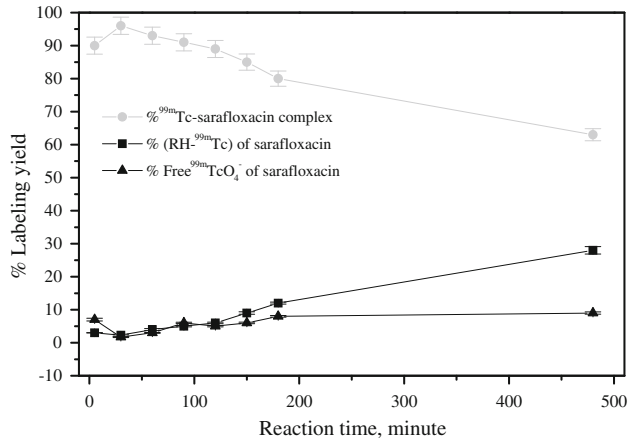


**Fig. 7** Stability test for $^{99m}$Tc-sarafloxacin

labeling. The stability was found to be time-dependent with regard to several parameters such as radiolysis, temperature and light, which may induce the degradation of the compound and restricted its availability before injection.

### Stability test

As shown in Fig. 8, incubation of $^{99m}$Tc-sarafloxacin in normal serum for 24 h at 37 °C lead to a limited release of radioactivity from $^{99m}$Tc-complex (10.6 ± 0.6 %, $n$ = 5) as confirmed by PC and HPLC.

### Biodistribution

Biodistribution results showing the uptake of the $^{99m}$Tc-sarafloxacin in different organs of the mice infected with

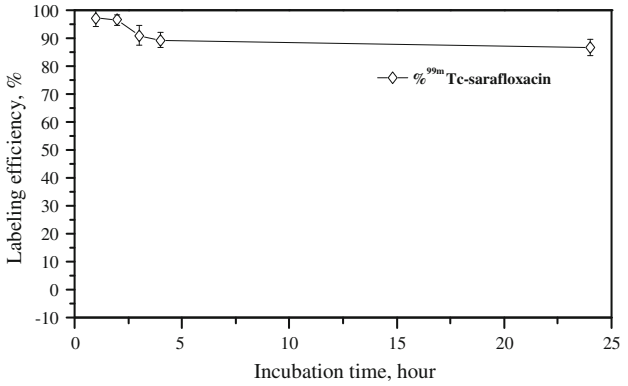


**Fig. 8** In vitro stability of $^{99m}$Tc-sarafloxacin in human serum

living, heat killed *S. aureus* and turpentine oil was presented in Table 1. The uptake of $^{99m}$Tc-sarafloxacin was considerably small in heat killed *S. aureus* and turpentine oil infected group of mice (aseptic inflammation) as compared to infected group with living bacteria (abscess). The results illustrated rapid distribution of the radioactivity throughout the body and uptake in the inflamed areas was noticed within 2 h after intravenous injection of the labeled complex.

The biodistribution data revealed that after 24 h of $^{99m}$Tc-sarafloxacin injection the great part of radioactivity was located in both kidneys (8.7 ± 0.6 % ID) and urine (31.9 ± 4.1 % ID). In contrast, a significant amount of $^{99m}$Tc-sarafloxacin radioactivity remained in the liver (7.4 ± 0.2 % ID). Clearance of $^{99m}$Tc-sarafloxacin appeared to proceed through both renal and hepatic routes. Mice with infectious lesions injected with $^{99m}$Tc-sarafloxacin depicted a mean abscess-to-muscle (target to non-target, T/NT) ratio equal to 4.2 ± 0.1 after 2 h post-injection where this $^{99m}$Tc-fluoroquinolone revealed greater uptake in infected tissue than the commercially available $^{99m}$Tc-ciprofloxacin (T/NT = 3.8 ± 0.8) [41] as shown in Fig. 9.

## Conclusions

This work depicted the in vitro and in vivo performance of $^{99m}$Tc-sarafloxacin complex crucial for designing a potentially useful radiopharmaceutical for diagnosing the bacterial infection. Sarafloxacin was efficiently labeled with $^{99m}$Tc by direct labeling method at room temperature with a labeling efficiency of 96 % in the presence of stannous chloride as a reducing agent. It demonstrated favorable radiochemical and metabolic stability in vivo. The acceptable localization of the tracer in the induced foci of

**Table 1** Biodistribution of $^{99m}$Tc-sarafloxacin in *S. aureus*, heat killed *S. aureus* and turpentine oil inflamed mice at different time periods

| Organs and body fluids | Percent Injected dose/organs at different time periods (h) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *S. aureus* | | | Heat killed *S. aureus* | | | Turpentine oil | | |
| | 2 | 4 | 24 | 2 | 4 | 24 | 2 | 4 | 24 |
| Inflamed muscle | 1.10 ± 0.1 | 0.75 ± 0.2 | 0.30 ± 0.0 | 1.0 ± 0.2 | 0.71 ± 0.1 | 0.28 ± 0.1 | 0.90 ± 0.0 | 0.69 ± 0.1 | 0.21 ± 0.2 |
| Control muscle | 0.28 ± 0.0 | 0.22 ± 0.1 | 0.16 ± 0.1 | 0.30 ± 0.0 | 0.22 ± 0.1 | 0.17 ± 0.0 | 0.29 ± 0.1 | 0.23 ± 0.1 | 0.14 ± 0.0 |
| Liver | 19.1 ± 2.1 | 15.1 ± 1.0 | 7.3 ± 0.2 | 20.6 ± 2.7 | 15.2 ± 3.2 | 7.9 ± 0.3 | 19.5 ± 2.0 | 15.2 ± 2.3 | 8.1 ± 0.5 |
| Urine | 20.5 ± 2.8 | 26.8 ± 3.2 | 31.9 ± 4.1 | 19.1 ± 1.5 | 27.3 ± 2.1 | 33.4 ± 3.4 | 19.0 ± 0.8 | 27.4 ± 2.0 | 31.8 ± 3.2 |
| Kidneys | 12.4 ± 1.4 | 17.9 ± 1.6 | 8.7 ± 0.6 | 13.7 ± 2.1 | 20.2 ± 2.3 | 8.3 ± 0.7 | 13.6 ± 1.8 | 18.6 ± 1.3 | 7.8 ± 0.2 |
| Blood | 6.9 ± 0.2 | 4.5 ± 0.3 | 1.0 ± 0.2 | 5.9 ± 0.5 | 4.8 ± 1.2 | 1.3 ± 0.4 | 6.0 ± 0.1 | 4.5 ± 0.3 | 1.0 ± 0.1 |
| Heart | 0.8 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.0 | 0.5 ± 0.2 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.6 ± 0.1 | 0.2 ± 0.0 | 0.1 ± 0.0 |
| Lung | 1.4 ± 0.3 | 0.4 ± 0.1 | 0.1 ± 0.0 | 1.2 ± 0.1 | 0.3 ± 0.0 | 0.1 ± 0.0 | 1.3 ± 0.1 | 0.5 ± 0.0 | 0.2 ± 0.0 |
| Intestine and stomach | 21.9 ± 3.1 | 5.9 ± 0.4 | 4.10 ± 0.6 | 22.1 ± 2.9 | 4.6 ± 1.3 | 4.6 ± 0.9 | 19.9 ± 2.1 | 6.7 ± 1.1 | 4.4 ± 1.4 |
| Spleen | 2.3 ± 0.3 | 1.2 ± 0.4 | 0.4 ± 0.0 | 2.5 ± 0.2 | 1.6 ± 0.4 | 0.7 ± 0.2 | 2.3 ± 0.2 | 1.4 ± 0.1 | 0.5 ± 0.0 |
| Bone | 1.0 ± 0.1 | 0.7 ± 0.1 | 0.4 ± 0.0 | 1.3 ± 0.1 | 0.6 ± 0.1 | 0.4 ± 0.0 | 1.3 ± 0.1 | 0.6 ± 0.2 | 0.2 ± 0.0 |

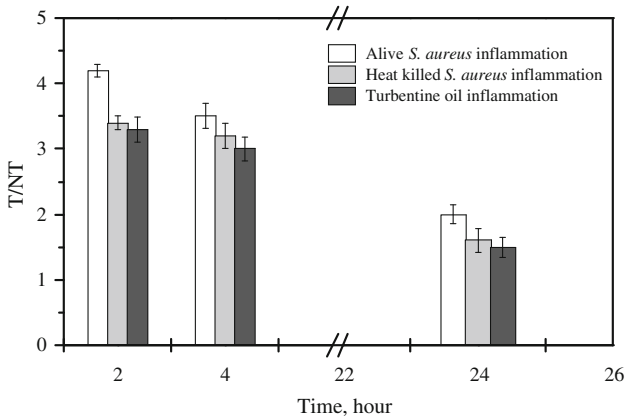


**Fig. 9** The ratio of target muscle (T) to non-target muscle (NT) of $^{99m}$Tc-sarafloxacin in different inflammation models at different post-injection times

inflammation expressed the effectiveness of this complex for targeting infectious lesions, which was greater than the commercially available $^{99m}$Tc-ciprofloxacin. $^{99m}$Tc-sarafloxacin was not able to distinguish between infection and sterile inflammation.

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