ISSN 1066-3622, Radiochemistry, 2015, Vol. 57, No. 3, pp. 307–311. © Pleiades Publishing, Inc., 2015.
Published in Russian in Radiokhimiya, 2015, Vol. 57, No. 3, pp. 260–263.

Radiolabeling, Quality Control, and Biodistribution of ^{99m}Tc-Sulfadiazine as an Infection Imaging Agent[1]

I. Essouissi*[a,b], F. Darghouth[a], N. M. Saied[b], Maroua Saidi[b], A. Kanoun[a,b], and Mouldi Saidi[b]

[a] *Ibn al-Baytar Society of Pharmaceutical Industries, Tunisia*
[b] *Radiopharmaceutical Unit, National Centre for Nuclear Sciences and Technologies, Tunisia*
** e-mail: imenessouissi@yahoo.fr*

Received November 11, 2014

Abstract—Sulfadiazine (antibiotic used for treating bacterial infections) was labeled with ^{99m}Tc with the aim of the development of a new radiopharmaceutical for infection imaging. The influence of the reaction parameters such as the substrate and $SnCl_2 \cdot 2H_2O$ concentrations, pH of the reaction mixture, and reaction time on the labeling yield was examined, and the labeling conditions were optimized. The maximum radiochemical yield of ^{99m}Tc-sulfadiazine (94.7%) was obtained by using 50 μg of $SnCl_2 \cdot 2H_2O$ and 1 mg of sulfadiazine at pH 5. The radiochemical purity of the labeled compound was evaluated by ITLC and HPLC. The biological distribution of ^{99m}Tc-sulfadiazine in mice with experimentally induced *Staphylococcus aureus* infection in the right thigh was studied, and the bacterial infected thigh/normal thigh (target-to-nontarget, T/NT) ratio was evaluated. The T/NT ratio for ^{99m}Tc-sulfadiazine was found to be 3.6, which is comparable to the commercially available ^{99m}Tc-ciprofloxacin (3.8), indicating that ^{99m}Tc-sulfadiazine can be used for infection imaging.

Key words: sulfadiazine, technetium-99m, radiolabeling, infection, diagnosis

DOI: 10.1134/S106636221503011X

The early and accurate localization of infectious foci is a major challenge in contemporary nuclear medicine. Early and accurate diagnosis and localization allow prompt and successful treatment and decrease the associated morbidity. Radiopharmaceuticals such as ^{67}Ga-citrate, in vivo and in vitro labeled leukocytes, and labeled human immunoglobulins are used for the diagnosis of inflammation. They are able to detect the physiological and biochemical changes that occur during the early phases of inflammation. However, none are capable of reliably differentiating sterile inflammation from septic infection and none are able to identify the presence of microorganisms causing the infection [1, 2]. The use of radiolabeled antibiotics is rapidly emerging as a promising diagnostic test for the detection of infective lesions. Antibiotics localize in the infectious focus, where they are frequently taken up and metabolized by microorganisms. One of the most important radiopharmaceuticals that are currently available for infection imaging is the antimicrobial agent ciprofloxacin labeled with ^{99m}Tc, which has probably shown the best results. However, previously reported data on the specificity of ^{99m}Tc-ciprofloxacin for infection are contradictory [3–9]. ^{99m}Tc-ciprofloxacin preparation has some disadvantages related to radiochemical purity (81%) [10] and stability, which are discussed in detail in the literature [3, 10–14]. Therefore, other antimicrobial agents such as levofloxacin [15], pefloxacin [16], lomefloxacin [17], cefoprazone [18], and cefuroxime [19] were labeled with ^{99m}Tc with the aim of using them for imaging infection sites of overcoming the drawback of ^{99m}Tc-ciprofloxacin.

Here we suggest sulfadiazine as the labeling substrate. It is an antibiotic of the sulfonamide family, used for medical treatment of infections [20]. Sulfonamide is a bacteriostatic, acting by inhibiting the synthesis of bacterial folic and dihydrofolic acids. We have optimized the conditions for radiolabeling of sulfadiazine with the most widely used imaging radionuclide, ^{99m}Tc. The radiolabeling was performed using $SnCl_2 \cdot 2H_2O$ as the reducing agent. In addition, the radiochemical purity, stability, and biodistribution in infected mice were studied.

EXPERIMENTAL

Materials. Sulfadiazine was obtained as active principle from Sigma. Techentium-99*m* was obtained

[1] The text was submitted by the authors in English.

as a saline eluent (Mon-Tek $^{99}Mo/^{99m}Tc$ generator). Tin chloride was purchased from Sigma. All other chemicals were of analytical grade (AR), obtained from reputed manufacturers. *Staphylococcus aureus* strain (American Type Culture Collection, ATCC 25923) was obtained from New England Biolabs.

Animals. Normal Swiss mice males, weighing 18–20 g, were obtained from the Pasteur Institute (Tunis). Animals were housed for 1 day before starting the experiments in housing facilities of our laboratory. The experiments were carried out in accordance with the principles of the guide to the care and use of experimental animals [21].

Radiolabeling procedure. An accurately weighed 1-mg portion of sulfadiazine was transferred to a penicillin vial, and the vial was evacuated. A solution containing exactly 50 μg of $SnCl_2 \cdot 2H_2O$ was added, pH of the mixture was adjusted to 5 with HCl or NaOH, and the volume of the mixture was brought to 1 mL with N_2-purged distilled water. One milliliter of freshly eluted $^{99m}TcO_4^-$ (about 75 MBq) was added to the above mixture. The reaction mixture was vigorously shaken and allowed to react at room temperature (22 ± 2°C) for a time sufficient to complete the reaction.

Radiochemical analysis of ^{99m}Tc-sulfadiazine. The radiochemical purity of ^{99m}Tc-sulfadiazine was determined by thin-layer chromatographic method using silica gel impregnated glass fiber sheets (ITLC-SG; Gelman). Acetone and 4 N HCl (this concentration was found to ensure the best separation) were used as developing solvents [22]. It was further confirmed by reversed-phase high-performance liquid chromatography (HPLC) on a 250 × 4.6 mm C18 column (Shimpack VP-ODS, Shimadzu) using a linear gradient with water supplemented with 0.1% (v/v) trifluoroacetic acid (TFA) as ion-pairing agent (solution A) and methanol (solution B), at a flow rate of 1 mL min^{-1}. The gradient was from 65 to 75% methanol for 20 min. The fractions were analyzed with a NaI γ-ray detector for monitoring the radioactivity.

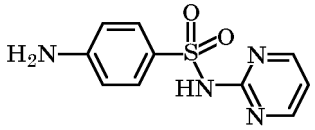
In vitro stability. The stability of ^{99m}Tc-sulfadiazine in saline solution after labeling and the impact of time on the complex were evaluated by measuring the relative content of the product at different time intervals (10, 30, 60, 120, 180, and 360 min) after labeling [23].

Biological evaluation. The biodistribution of ^{99m}Tc-sulfadiazine was evaluated in normal Swiss mice males. To induce the infection, a turbid suspension containing approximately 10^6–10^7colony-forming units (CFU) of *S. aureus* in 0.1 mL of saline was injected into the right thigh muscle of the mice. 24 h later, when visible swelling appeared in the infected thigh, 0.1 mL of ^{99m}Tc-sulfadiazine (~1.6 MBq) was injected via the tail vein. Four mice were used for one set of experiments. After a definite time, the mice were sacrificed under ether anesthesia, and the biodistribution was determined at 15, 30, and 60 min post injection. The blood sample was collected at the time of decapitation. Both thighs (right thigh muscle as target and left thigh muscle as control) and organs were dissected and weighed, and their radioactivity was measured using a well gamma counter.

Statistical analysis. Experiments were performed in triplicate unless otherwise indicated. The results are reported as mean ± standard deviation. Differences between the data were evaluated with the Student's *t*-test. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The chemical structure of sulfadiazine is shown below. Various complexes of ^{99m}Tc may be formed by the interaction between the electron-donor atoms and reduced technetium [24–26]. In our case, the complexation probably occurs via nitrogen atoms, but the structure of the complex is unknown.



Sulfadiazine

The labeling efficiency, radiochemical purity, and stability of the ^{99m}Tc-sulfadiazine complex were assessed by thin-layer chromatography. With acetone as the solvent, free pertechnetate moved with the solvent front ($R_f = 1$), whereas ^{99m}Tc-sulfadiazine and reduced hydrolyzed technetium remained at the origin. Reduced hydrolyzed technetium was determined with 4 N HCl as the mobile phase, where reduced hydrolyzed technetium remained at the origin ($R_f = 0$), whereas the other species migrated with the solvent front ($R_f = 1$). The radiochemical purity was determined by subtracting the sum of the percentage of reduced hydrolyzed technetium and free pertechnetate from 100%. The radiochemical yield is the mean value of three experi-

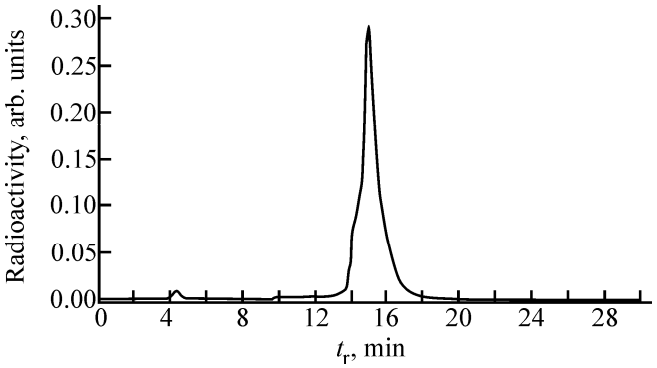


Fig. 1. HPLC chromatogram of ^{99m}Tc-sulfadiazine on reversed-phase C18 column.

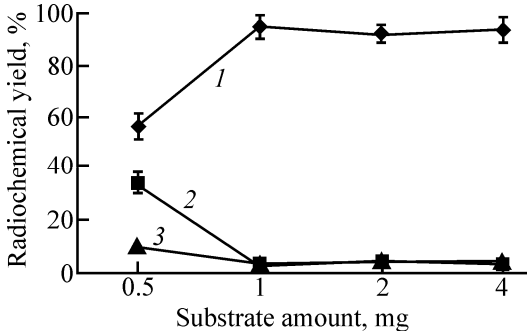


Fig. 2. Radiochemical yield of ^{99m}Tc-sulfadiazine as a function of substrate amount. Conditions: 50 μg of $SnCl_2 \cdot 2H_2O$, 1 mL (~75 MBq) of $^{99m}TcO_4^-$, pH 5, room temperature, 10 min. (*1*) ^{99m}Tc-sulfadiazine, (*2*) free $^{99m}TcO_4^-$, and (*3*) colloid; the same for Figs. 2–6.

ments. An HPLC radiochromatogram is shown in Fig. 1. It has two peaks: the minor peak at a retention time of 4.2 min, which corresponds to $^{99m}TcO_4^-$, and the major peak at 14.9 min for ^{99m}Tc-sulfadiazine.

Factors affecting the labeling yield. *Amount of sulfadiazine.* As the sulfadiazine amount was increased from 0.5 to 1 mg, the radiochemical yield of ^{99m}Tc-sulfadiazine increased from 56.1% to 94.7% (Fig. 2). The yield did not change noticeably as the sulfadiazine amount was increased further to 4 mg. Thus, the optimum amount of sulfadiazine was 1 mg.

Amount of $SnCl_2$. As shown in Fig. 3, the Sn(II) amount is the most important factor influencing the labeling yield of ^{99m}Tc-sulfadiazine. At low Sn(II) amount (25 μg of $SnCl_2 \cdot 2H_2O$), the labeling yield was low (75.1%), because this amount of Sn(II) was insufficient to reduce all the $^{99m}TcO_4^-$ (the amount of the remaining $^{99m}TcO_4^-$ was 22.1%). With increasing the amount of Sn(II), the labeling yield increased, reaching the maximum value of 94.7% at 50 μg of $SnCl_2 \cdot 2H_2O$. At the Sn(II) amount increased further, the yield decreased again, reaching 79.1% at 150 μg of $SnCl_2 \cdot 2H_2O$, because of colloid formation (19.7%).

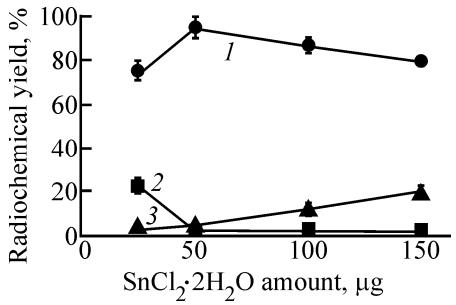


Fig. 3. Influence of the $SnCl_2 \cdot 2H_2O$ amount on the radiochemical yield of ^{99m}Tc-sulfadiazine. Conditions: 1 mg of sulfadiazine, 1 mL (~75 MBq) of $^{99m}TcO_4^-$, pH 5, room temperature, 10 min.

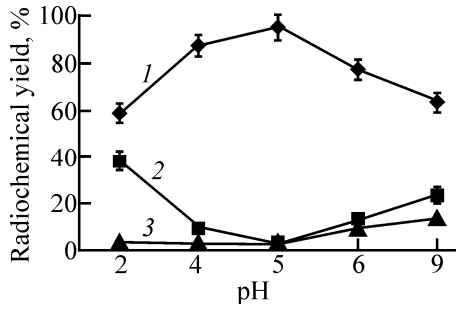


Fig. 4. Influence of pH on the radiochemical yield of ^{99m}Tc-sulfadiazine. Conditions: 1 mg of sulfadiazine, 50 μg of $SnCl_2 \cdot 2H_2O$, 1 mL (~75 MBq) of $^{99m}TcO_4^-$, room temperature, 10 min.

pH. The influence of pH (Fig. 4) on the radiolabeling yield was examined at pH in the range 2–9. The highest labeling yield was obtained at pH 5. In alkaline solutions, the yield decreases, probably because of increased formation of hydrolyzed technetium, ^{99m}Tc-stannous colloids, and free pertechnetate [27].

Reaction time. Figure 5 shows the relationship between the reaction time and the yield of ^{99m}Tc-sulfadiazine. The radiochemical yield significantly increased from 73.3 to 94.7% with an increase in the reaction time from 1 to 10 min. Extending the reaction time to 30 min produced no significant change in the radiochemical yield.

In vitro stability. Data on the in vitro stability of the ^{99m}Tc-sulfadiazine complex show that the relative

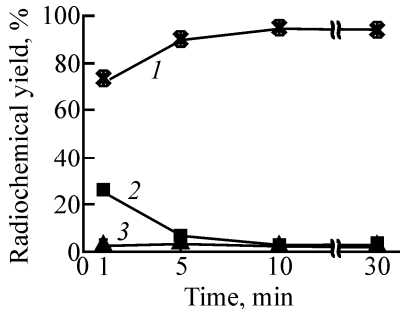


Fig. 5. ^{99m}Tc-sulfadiazine yield as a function of reaction time. Conditions: 1 mg of sulfadiazine, 50 μg of $SnCl_2 \cdot 2H_2O$, 1 mL (~75 MBq) of $^{99m}TcO_4^-$, pH 5, room temperature.

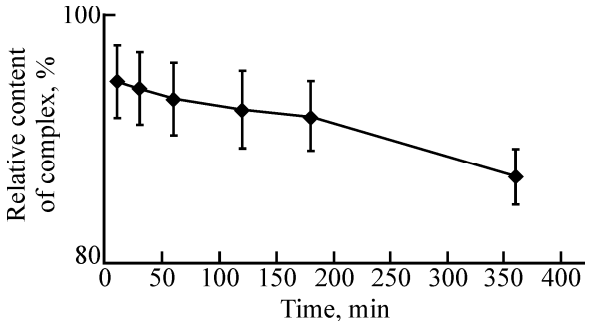


Fig. 6. Effect of time on the stability of ^{99m}Tc-sulfadiazine.

content of the labeled product slightly decreased (from 94.7 to 91.8%) in 3 h post labeling. In the subsequent 3 h, the relative content of the complex decreased further to 87.1% (Fig. 6).

Biodistribution. The table shows the biodistribution of ^{99m}Tc-sulfadiazine in mice and accumulation in infected and normal muscles. Values are given as the percent of the injected dose per gram of tissue (% ID/g) at 15, 30, and 60 min after administration. The accumulation of ^{99m}Tc-sulfadiazine complex at the site of infection was expressed as the target-to-non-target (T/NT) ratio. As indicated in the table, the accumulation of ^{99m}Tc-sulfadiazine at the site of infection was maximal at 30 min after intravenous injection. The T/NT ratio was equal to 3.6, which was close to that for ^{99m}Tc-ciprofloxacin [10]. On the other hand, ^{99m}Tc-sulfadiazine showed higher T/NT ratio than most of the labeled antibiotics such as streptomycin (T/NT = 2.4) [28], sulfadimidine (T/NT = 2.2) [29], alafosfalin (T/NT = 2.75) [30], and *N*-sulfanilamide (T/NT = 2.9) [23].

Biodistribution of ^{99m}Tc-sulfadiazine (uptake, % ID/g, at indicated time post injection[a]

Organ or body fluid	15 min	30 min	60 min
Blood	12.7 ± 2.2	11.0 ± 1.1	7.6 ± 0.8
Liver	2.49 ± 0.23	4.5 ± 1.0	5.2 ± 0.6
Heart	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Lungs	4.0 ± 0.8	6.3 ± 1.0	5.5 ± 0.9
Spleen	2.39 ± 0.09	3.01 ± 0.03	2.97 ± 0.12
Kidneys	4.1 ± 0.7	7.2 ± 1.0	9.5 ± 1.3
Stomach	5.1 ± 0.4	8.24 ± 0.08	11.3 ± 1.1
Intestine	2.22 ± 0.21	3.4 ± 0.7	4.4 ± 0.7
Normal muscles	0.07 ± 0.00	0.10 ± 0.01	0.13 ± 0.02
Infected muscles	0.16 ± 0.02	0.36 ± 0.04	0.31 ± 0.01

[a] Data are presented as mean ± standard deviation; $n = 4$.

The accumulation of activity at the site of infection slightly decreased with time, with the T/NT ratio reaching 2.38 at 1 h post injection. The early maximum tracer accumulation followed by gradual decline may be due to the subsequent bacterial killing caused by the antimicrobial activity, which is manifested in complexation with *p*-aminobenzoic acid in the course of bacterial DNA synthesis [31], followed by clearance from circulation.

Thus, sulfadiazine can be easily labeled with ^{99m}Tc by direct labeling method at room temperature using stannous chloride as a reducing agent. The labeling yield under optimum conditions is as high as 94.7%. The complex is selectively taken up by the infected muscle. ^{99m}Tc-sulfadiazine shows promise for clinical investigations as a new ^{99m}Tc agent for detecting sites of infection.

ACKNOWLEDGMENTS

This project was conducted within the framework of MOBIDOC-Post doc as specified in PASRI program, funded by the EU and managed by ANPR.

REFERENCES

1. Becker, W., *Eur. J. Nucl. Med.*, 1995, vol. 22, pp. 1195–1211.
2. Becker, W. and Meller, J., *Lancet. Infect. Dis.*, 2001, vol. 1, pp. 326–333.
3. Vinjamuri, S., Hall, A.V., Solanki, K.K., et al., *Lancet*, 1996, vol. 347, pp. 233–235.
4. Hall, A.V., Solanki, K.K., Vinjamuri, S., et al., *J. Clin. Pathol.*, 1998, vol. 51, pp. 215–219.
5. Sonmezoglu, K., Sonmezoglu, M., Halac, M., et al., *J. Nucl. Med.*, 2001, vol. 42, pp. 567–274.

6. Yapar, Z., Kibar, M., Yapar, A.F., et al., *Eur. J. Nucl. Med*., 2001, vol. 28, pp. 822–830.
7. Larikaa, M.J., Ahonen, A.K., Niemela, O., et al., *Nucl. Med. Commun*., 2002, vol. 23, pp. 167–170.
8. Dumarey, N., Blocklet, D., Appelboom, T., et al., *Eur. J. Nucl. Med*., 2002, vol. 29, pp. 530–535.
9. Sarda, L., Saleh-Mghir, A., Peker, C., et al., *J. Nucl. Med*., 2002, vol. 43, pp. 239–245.
10. Rien, H.S., Huub, J.R., Otto, C.B., et al., *J. Nucl. Med*., 2004, vol. 45, pp. 2088–2094.
11. Pirmettis, I., Limouris, G.S., Papadopoulos, M., et al., *Eur. J. Nucl. Med*., 1999, vol. 26, p. 1108.
12. Seung, J.O., Jin, S.R., Joong, W.S., et al., *Appl. Radiat. Isot*., 2002, vol. 57, p. 193.
13. Lima, J.E.T., Maliska, C., Goncalves, M.R.B., et al., *World J. Nucl. Med*., 2004, vol. 3, pp. 284–289.
14. Sarda, L., Cremieux, A.C., Lebellec, Y., et al., *J. Nucl. Med*., 2003, vol. 44, pp. 920–926.
15. El-Ghany, E.A., Amine, A.M., El-Kawy, O.A., et al., *J. Label. Compd. Radiopharm*., 2007, vol. 50, pp. 25–31.
16. El-Ghany, E.A., El-Kolaly, M.T., Amine, A.M., et al., *J. Radioanal. Nucl. Chem*., 2005, vol. 266, pp. 131–139.
17. Motaleb, M.A., *J. Radioanal. Nucl. Chem*., 2007, vol. 272, pp. 95–99.
18. Motaleb, M.A., *J. Radioanal. Nucl. Chem*., 2007, vol. 272, pp. 167–171.
19. Yurt, L.F., Durkan, K., and Unak, P., *J. Radioanal. Nucl. Chem*., 2008, vol. 275, pp. 161–164.
20. Jesús, M., García, G., Silvia, M., et al., *Trends Anal. Chem*., 2008, vol. 27, pp. 1008–1022.
21. *FELASA Euroguide*, British Library, 2006.
22. Theobald, A.E., *Textbook of Radiopharmacy: Theory and Practice*, New York: Gordon and Breach, 1990.
23. Essouissi, I., Ghali, W., Saidi, M., et al., *Nucl. Med. Biol*., 2010, vol. 37, pp. 821–829.
24. Barreto, V.G., Iglesias, F., Roca, M., et al., *Rev. Esp. Med. Nucl*., 2000, vol. 19, pp. 479–483.
25. Roohi, S., Mushtaq, A., Jehangir, M., et al., *J. Radioanal. Nucl. Chem*., 2006, vol. 267, pp. 561–566.
26. Vallee, F. and Lebel, M., *J. Antimicrob. Agents Chemother*., 1991, vol. 35, pp. 2057–2064.
27. El-Ghany, E.A., Amine, A.M., El-Sayed, A.S., et al., *J. Radioanal. Nucl. Chem*., 2005, vol. 266, pp. 125–130.
28. Meral, T., Erean, T., and Isil, S.U., *Nucl. Med. Biol*., 1992, vol. 19, pp. 802–806.
29. Amin, A.M., Ibrahim, I.T., Attallah, K.M., et al., *Radiochemistry*, 2014, vol. 56, no. 1, pp. 72–75.
30. Tsopelas, C., Penglis, S., Ruszkiewicz, A., et al., *Nucl. Med. Biol*., 2003, vol. 30, pp. 169–175.
31. Eshima, D., Taylor, A.J., and Fritzberg, A.R., *J. Nucl. Med*., 1987, vol. 28, pp. 1180–1186.