^{99m}Tc-radiolabeled Levofloxacin and micelles as infection and inflammation imaging agents

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A B S T R A C T

Easy and early detection of infection and inflammation is essential for early and effective treatment. In this study, PEGylated micelles were designed and both micelles and Levofloxacin were radiolabeled with ^{99m}TcO$_4^-$ to develop potential radiotracers for detection of infection/inflammation. Radiolabeling efficiency, *in vitro* stability and bacterial binding of ^{99m}Tc-Levofloxacin and ^{99m}Tc-micelles were compared. The aim of this study is to formulate and compare ^{99m}Tc-Levofloxacin and ^{99m}Tc-micelles as infection and inflammation agents having different mechanisms for the accumulation at infection and inflammation site. PEGylated micelles were designed with a particle size of 80 ± 0.7 nm and proper characterization properties. High radiolabeling efficiency was achieved for ^{99m}Tc-Levofloxacin (96%) and ^{99m}Tc-micelles (87%). The radiolabeling efficiency was remained stable with some insignificant alterations for both radiotracers at 25 °C for 24 h. Although *in vitro* bacterial binding of ^{99m}Tc-levofloxacine was higher than ^{99m}Tc-micelles, ^{99m}Tc-micelles may also be evaluated potential agent due to long circulation and passive accumulation mechanisms at infection/inflammation site. Both radiopharmaceutical agents exhibit potential results in design, characterization, radiolabeling efficiency and *in vitro* bacterial binding point of view.

1. Introduction

The diagnosis of infections with the help of radiological imaging modalities such computed tomography (CT), ultrasonography (US) and magnetic resonance imaging (MRI) provides both non-invasive diagnosis and accurate indication of the area of lesion. Depending on the basis of these anatomical imaging techniques, the infections can only be detected after formation of a morphological alteration. Therefore, early stage detections are not possible by the use of these routine medical imaging modalities especially in deeply seated infections, for example, osteomyelitis, intraabdominal infections and endocarditis. For this purpose, the use of scintigraphic imaging modalities such as gamma scintigraphy, single photon emission computed tomography (SPECT), positron emission tomography (PET) and hybrid imaging modalities comprising SPECT/CT, PET/CT, PET/MRI can provide physiologic and metabolic information about the lesion.

Specific radiopharmaceuticals have been searched for obtaining lesion imaging with physiologic and metabolic information at the early stage of microbial infections. ^{99m}Tc-labeled leukocytes was the first radiopharmaceutical in 1980s having the ability to image deeply seated infections to indicate regions of inflammation [1–3]. However, the cumbrous methods are one of the most commonly faced disadvantageous [4–6].

Detection of infection by non-specific tracers can be performed including ^{67}Ga-Citrate [7], radiolabeled nonspecific Immunoglobulins such as human polyclonal immunoglobulin (HIG) [8], liposomes [9], Avidin-Biotin system [10]. Apart from these non-specific tracers some specific tracers were also developed such as radiolabeled white blood cells including ^{111}In-Oxine-labeled leukocytes [11], ^{99m}Tc-HMPAO-labeled leukocytes [12], antigranulocyte antibodies and antibody fragments [13], chemotactic peptides [14], cytokines [15], Interleukin-1 [16], Interleukin-2 [17], Interleukin-8 [18], Platelet Factor-4 [19], ^{18}F-FDG [20], antibiotics and antimicrobial peptides [21].

Although many alternatives were exist and developed for specific and non-specific detection of infection, the development of radiolabeled antibiotics against bacteria progressed faster and gained popularity due to simple radiolabeling procedure of ^{99m}TcO$_4^-$ and higher availability [22]. Fluoroquinolone antibiotics specifically bind and inhibit bacterial DNA gyrase. ^{99m}Tc-Ciprofloxacin was one of the first radiolabeled antibiotics in 1990s to image microbial infections or aseptic inflammation [23]. Ciprofloxacin was labeled using formamidine sulfuric acid (FSA) as ^{99m}Tc reducing agent at 100 °C for 10 min. However, due to the instability of FSA, stannous ion has been used to increase labeling yield and reduce ^{99m}Tc to a lower oxidation state [23]. Afterwards, was

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[99m]Tc-Ciprofloxacin was approved for infection imaging and marketed as "Infecton®" [24,25]. Other fluoroquinolone family antibiotics were also labeled for this purpose [26]. By the improvements in the production of alternative severe acute respiratory syndrome (SARS) antibiotics and growing drug resistance, a variety of ^{99m}Tc-labeled flourquinolone and cephalosporin antibiotics were prepared including ^{99m}Tc-Pefloxacin [27], ^{99m}Tc-Lomefloxacin and ^{99m}Tc-Ofloxacin [28], ^{99m}Tc-Sparafloxacin [29], ^{99m}Tc-Difloxacin [30], ^{99m}Tc-Moxifloxacin [31], ^{99m}Tc-Gemifloxacin [32], ^{99m}Tc-Rufloxacin [33], ^{99m}Tc-Clinafloxacin [34], ^{99m}Tc-Sitafloxacin [35], ^{99m}Tc-Levofloxacin [36], ^{99m}Tc-Temafloxacin [37], ^{99m}Tc-Ceftizoxime [38], ^{99m}Tc-Cefuroxime [5], ^{99m}Tc-Ceftriaxone [39], ^{99m}Tc-Cefotaxime [40] and ^{99m}Tc-Cefoperazone [41,42].

Radiolabeled antibiotics have been used for the diagnosis of infection. The advantage of the utilization of radiolabeled antibiotics is its ability to differentiate between infection and aseptic inflammation [25,43]. Levofloxacin is a broad-spectrum antibiotic and belong to third-generation fluoroquinolones. It has an antibacterial activity of optically active L-isomer of ofloxacin. The antibacterial activity of Levofloxacin is due to blockage of bacterial cell growth by inhibiting DNA gyrase (bacterial topoisomerase II) which is an enzyme required for DNA replication, RNA transcription, and repair of bacterial DNA [44].

Some other agents for scintigraphic imaging were developed to perform quick and efficient imaging of inflammation and infection with high sensitivity and specificity [45,46]. Radiolabeled liposomes were designed as imaging agents for inflammation and infectious processes. It was previously demonstrated liposomes with small particle size and surface coating by a hydrophilic polymer (such as PEG) show enhanced blood circulation time providing an increased accumulation at the site of infection [47–51]. Liposomes which are formulated in a particle size of nanometer range and surface coated for passive targeting are called stealth liposomes. A variety of stealth liposomes were prepared and evaluated for this purpose [49,52–54]. Although many previous studies were performed for ^{99m}Tc-radiolabeling of antibiotics and liposomes, these techniques are still very valuable for their accumulation in the site of the infection and inflammation [25]. Infection specific radiopharmaceuticals can be successfully used for the diagnosis, imaging and therapy monitoring. There has been an intensive research for the development of specific infection and inflammation imaging probes because routinely used tracers in clinics still cannot evaluate infection and inflammation efficiently.

Although, micelles are another efficiently used delivery systems for imaging or therapy of many diseases, the research about the diagnosis and imaging of infection and inflammation with micelles is very limited. Micelles are formed of lipid monolayers with a fatty acid core and polar surface. Inverted micelles are defined as the vice-versa of micelles in which polar core is in the center with fatty acids on the surface. Therefore, research about the evaluation of infection and inflammation potential of micelles may be beneficial.

[99m]Tc-Levofloxacin was radiolabeled in two studies [36,55] by different radiolabeling methods including cysteine·HCl as co-ligand. *In vivo* efficacy of ^{99m}Tc-Levofloxacin in infection model small animals was found high for both studies. However, *in vitro* bacterial binding of ^{99m}Tc-Levofloxacin was never investigated and compared with ^{99m}Tc-labeled PEGylated, phosphatidylcholine (PC), sodium dodecyl cholate (SDC) and DTPA-PE containing nanosized micelles.

In this study, PEGylated, PC, SDC and DTPA-PE containing nanosized micelles were designed and both micelles and Levofloxacin were radiolabeled with [99m]TcO4^{-} by tin reduction method to develop potential radiotracers for detection of infection and inflammation. The aim of this study is to formulate and compare radiolabelled micelles and radiolabelled antibacterial agent Levofloxacin as infection and inflammation agents having different mechanisms for the accumulation at infection and inflammation site. Radiolabeling of ^{99m}Tc-Levofloxacin was evaluated with changing antibiotic concentration, reducing agent ($SnCl_2.2H_2O$) concentration, pH and incubation time. Among these

processes, radioactivity was kept constant and percent labeling of ^{99m}Tc-Levofloxacin was measured using ITLC plates. Characterization studies were performed for ^{99m}Tc-radiolabeled micelles and radiolabeling efficiency was also evaluated. Bacterial binding of ^{99m}Tc-labeled Levofloxacin and micelles were compared in *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) for evaluating and comparing their *in vitro* bacterial binding and specificity as potential infection and inflammation imaging agents.

2. Materials and methods

2.1. Materials

Levofloxacin hemihydrate was obtained from Drogsan, Turkey. Phosphatidylcholine from Soybean (98%) (PC) was a kind gift from Lipoid GmbH, Germany and sodium dodecyl cholate (SDC) was obtained from Sigma-Aldrich, USA. Tin(II) chloride was obtained (Sigma-Aldrich, USA) for radiolabeling procedure. 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (PEG2000-DSPE) (Avanti Polar Lipids, Inc., USA) was used for PEGylation. 1,2-Dioleoiyl-sn-glicero-3-phosphoethanolamine (DOPE) (Avanti Polar Lipids, Germany), Diethylenetriaminepentaacetic acid anhydride (DTPA) (Sigma-Aldrich, USA), and dimethyl sulfoxide (DMSO) (Merck, Germany) were used for DTPA-PE synthesis. Membrane filters (MS® Nylon Membrane Filters, USA) were used for filtration sterilization. ITLC-SG Plates were obtained from Gelman Sci, Germany.

2.2. Radiolabeling of Levofloxacin

This was performed to determine the best conditions for the labeling of Levofloxacin with [99m]TcO4^{-}. Labeling efficiency was calculated by changing $SnCl_2.2H_2O$ concentrations from 15 to 150 μg, Levofloxacin concentration from 0.5 to 3 mg, pH from 3 to 7 and incubation time from 15 to 120 min with the same amount of sodium pertechnetate (5 mCi) which was freshly eluted from [99m]Mo/^{99m}Tc generator. The pH was arranged by using 0.1 N HCl and NaOH solutions [36,55].

2.3. Radiochemical analysis

Radiochemical purity was evaluated by ITLC by using miniaturized ITLC-SG Plates to evaluate the percentage of unbound pertechnetate ([99m]TcO4^{-}) and hydrolyzed/reduced technetium ([99m]TcO2) by using acetone and saline as running solvents, respectively. ^{99m}Tc-Levofloxacin was spotted at ITLC plates. The radiochemical purity of ^{99m}Tc-Levofloxacin was measured by the using Equation (1) [36,55].

%Colloid = (Activity before filtration–Activity after filtration)/Activity before filtration × 100

%Free Pertechnetate ([99m]TcO4^{-}) = (Activity at Rf 0.75 to 1.0/Total activity) × 100

$$\%^{99m}\text{Tc-Levofloxacin} = 100 - (\%\text{Colloid} + \%\,^{99m}\text{TcO}_4^-) \qquad (1)$$

For the purpose of obtaining efficient and maximum radiolabeling, optimum amount of Levofloxacin, reducing agent, pH and incubation time were evaluated. The highest radiolabeling yield was calculated after passing ^{99m}Tc-Levofloxacin through a 0.22 μm filter and by ITLC analysis [36,42,44,55].

2.4. Synthesis of DTPA-PE

DTPA-PE was used as chelating agent for radiolabeling of micelles. It was synthetized by mixing 0.1 mM of DOPE in 4 mL of chloroform, supplemented with 30 μL of triethylamine. This was then added to 1 mM of DTPA anhydride in 20 mL of DMSO by stirring. This mixture

was incubated for 3 h at 25 °C under argon gas. Afterwards, the solution was dialyzed against 6 L of water at 4 °C for 48 h. Purified DTPA-PE was freeze-dried and stored frozen at −80 °C [56,57].

2.5. Preparation of micelles

Film forming method was used for the preparation of PEGylated DTPA-PE containing micelles containing PC:PEG2000-DSPE:SDC:DTPA-PE (55:0.9:44:0.1 in % molar ratios). Lipids (15 mg total lipids/mL) were dissolved in chloroform which was evaporated at 40 °C under reduced pressure. After removing of the solvent, lipid film was hydrated by HEPES (1 M, pH 7.4) buffer at 30 °C. Afterwards, the vesicles were dispersed for 15 min via ultrasonicator [58,59].

2.6. Characterization of micelles

The characterization of PEGylated, PC, SDC and DTPA-PE containing nanosized micelles was determined by measuring mean particle size and zeta potential.

2.6.1. Mean particle size and zeta potential

Mean particle size, polydispersity index (PDI) and zeta potential of micelles were measured using the Nano-ZS (Malvern Instruments, Malvern, UK) by dynamic light scattering method at 25 °C.

2.7. Radiolabeling of PEGylated micelles

PEGylated, PC and SDC containing micelles including amphiphilic chelating agent DTPA-PE were labeled with $^{99m}TcO_4^-$ by tin reduction method. In brief, 0.5 mL $SnCl_2.2H_2O$ (1 mg mL^{-1}) and (1.5 mCi) $^{99m}TcO_4^-$ were incubated with micelles for 30 min to perform trans-chelation of ^{99m}Tc with micelles. Afterwards, samples were dialyzed against HEPES Buffer (pH 7.4) for 5 h at 4 °C to remove un-chelated ^{99m}Tc by using cellulose ester dialysis tubes (3500 Da cut-off size). Quality control of binding was checked by ITLC-SG Plates in saline and acetone as running solvent system. After completion of the development procedure, strips were cut and measured in a well type gamma counter.

2.8. In vitro stability studies

In vitro stability of ^{99m}Tc-Levofloxacin and radiolabeled, PEGylated, PC and SDC containing micelles was determined at room temperature in the presence of saline (NaCl 0.9% (w/v)) or serum. For this purpose, 0.1 mL of samples were added to 0.9 mL of saline or equal volume of cell culture medium (PBS) supplemented with 10% FBS. Afterwards, they were analyzed after incubation with ITLC at 1, 2, 4, 6, 8, and 24 h to estimate the radiochemical stability of ^{99m}Tc-Levofloxacin and radiolabeled micelles [55].

2.9. In vitro binding assay with bacteria

In vitro binding efficiency of ^{99m}Tc-Levofloxacin and ^{99m}Tc radiolabeled PEGylated PC:SDC micelles were investigated against *S. aureus* and *E. coli*. For the evaluation of bacterial binding, 0.9 mL of a bacterial suspension containing approximately 10^8 CFU was taken, and exactly 0.1 mL of PBS containing approximately 1 mCi of radiolabeled Levofloxacin or micelles were added to the test tubes. The mixtures were then incubated for 1 h at 37 °C. Afterwards, they were centrifuged for 5 min at 2000 rpm at 4 °C and the pellets were then resuspended in 1 mL of PBS. The resuspended pellets were centrifuged for 5 min, the supernatants were separated and 1 mL of PBS was added. The supernatants were removed and the radioactivity in the bacterial pellets were determined by well-type gamma counter. The bacterial binding of radiopharmaceuticals was calculated according to Equation (2) [60–62].

Bacterial Binding(%) = Counts in Pellet/(Counts in Supernatant + Counts in Pellet)x100.   (2)

2.10. Statistical analysis

All values were expressed as the mean ± SD and n = 6. Non-parametric test methods were used for the evaluation of number of data less than 30. Depending on the group number, Student T test was used for the comparison of two groups and the Kruskal Wallis was used for the comparison of three or more groups. The significance level was set at $p < 0.05$.

3. Results and discussion

^{99m}Tc-Levofloxacin and ^{99m}Tc-radiolabeled micelles were prepared for the purpose of effective infection and inflammation imaging.

3.1. The characterization of ^{99m}Tc radiolabeled, PEGylated micelles

PEGylated, PC, SDC and DTPA-PE containing nanosized micelles were prepared and radiolabeled for imaging of infection and inflammation. The characterization of micelles was performed by measuring particle size and zeta potential. The average particle size of micelles was 80 ± 0.7 nm, polydispersity index was 0.14 and the zeta potential was 21.15 ± 1.2 mV.

3.2. Radiochemical purity of ^{99m}Tc radiolabeled, PEGylated micelles

Nanosized, PEGylated, PC, SDC and DTPA-PE containing micelles show a high labeling efficiency (87 ± 1.21%).

3.3. Radiolabeling process and quality control of ^{99m}Tc-Levofloxacin

The optimization of radiolabeling provides the best conditions to obtain maximum labeling efficiency. ^{99m}Tc radiolabeling of Levofloxacin was tried in varying conditions and the labeling yield was optimized.

3.3.1. Effect of Levofloxacin amount
The radiolabeling efficiency of Levofloxacin was performed in varying concentrations (0.5–3 mg) with the same amount of radioactivity. The best radiolabeling efficiency of Levofloxacin was 96 ± 2.12% which was achieved by addition of 1 mg Levofloxacin. It was observed that lesser amounts of Levofloxacin resulted in decreased labeling efficiency. The radiolabeling efficiency of 0.5 mg of Levofloxacin was 83%. Higher amounts than 1 mg resulted no significant alteration on the labeling efficiency. Our findings were in agreement with the literature [55].

3.3.2. Effect of reducing agent
Reducing agent ($SnCl_2.2H_2O$) was used in the range of 15–150 μg for the evaluation of optimum amount of $SnCl_2.2H_2O$ to achieve highest labeling efficiency. It was observed that at low amounts of reducing agent, the labeling efficiency was very low (~57%). The highest radiolabeling efficieny was obtained at 50 μg mL^{-1} of $SnCl_2.2H_2O$ (~96%). It may be concluded that lower amounts (lower than 50 μg mL^{-1}) of reducing agent can not effectively reduce whole $^{99m}TcO_4^-$ for the labeling process (Fig. 1). This observation was in agreement with previous studies [36].

3.3.3. Effect of pH
Different pH values (pH:3–7) were evaluated for determining the effect of pH in radiolabeling efficieny. Any significant difference in labeling yield at acidic conditions was observed. On the other hand, the labeling efficiency decreased at slight basic pH which may be due to
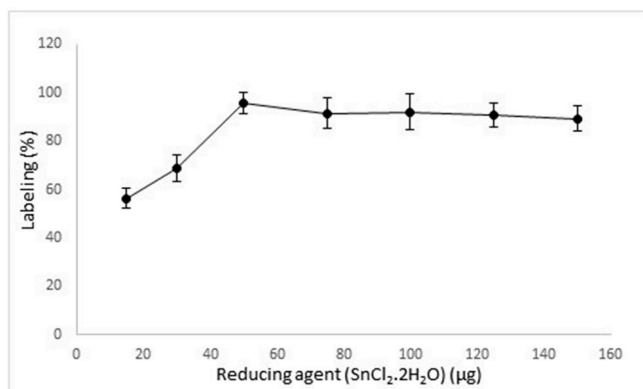
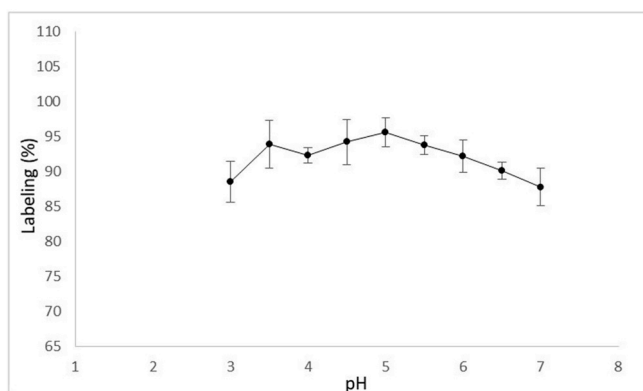**Fig. 1.** The effect of $SnCl_2.2H_2O$ on labeling efficiency.

Fig. 2. The effect of pH on labeling efficiency.

any possible change in the structure of the Levofloxacin related with carboxylic moiety at basic pH (Fig. 2). Its carboxylic moiety may be neutralized to prevent labeling with $^{99m}TcO_4^-$ during the metal exchange reaction. The maximum radiolabeling (96%) was achieved at pH:5. Higher and lower pH values cause decrease in labeling efficiency. This observation was in paralell with other studies [36,55].

3.3.4. Effect of incubation time

Incubation time is an essential parameter in radiolabeling efficiency of radiopharmaceuticals. The effect of incubation time on labeling efficiency of ^{99m}Tc-Levofloxacin was given in Fig. 3. Maximum radiolabeling efficiency (96%) was obtained after 15 min of incubation of Levofloxacin with sodium pertechnetate. It was observed that longer incubation time did not cause any significant difference in the radiolabeling efficiency which was in agreement with the literature [36].
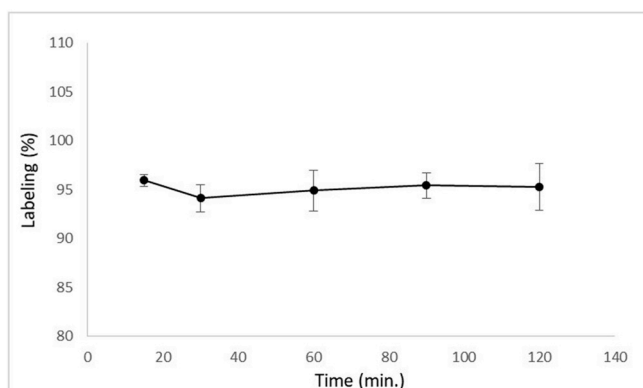

Fig. 3. The effect of incubation time on labeling efficiency.

After evaluation of the effects of changing antibiotic concentration, reducing agent concentration and pH, it was concluded that maximum labeling efficiency was achieved by using 1 mg Levofloxacin, 50 µg $SnCl_2.2H_2O$ were dissolved in 1 mL of saline at pH 5 and incubated with Sodium pertechnetate (5 mCi) for 15 min. Afterwards, radiolabeled Levofloxacin was filtered through 0.22 µm filter at room temperature. The highest radiolabeling yield was observed as 96 ± 2.13% which was in paralell with previous studies [36,55].

3.4. In vitro stability studies

According to the stability test performed at room temperature (25 °C), the radiolabeling efficiency was remained stable with some insignificant alterations and degradations carried out at 25 °C for 24 h (Table 1). Any significant difference was not observed in the serum and saline stability of ^{99m}Tc-Levofloxacin and ^{99m}Tc-micelles which were in agreement with the literature [36,55,63,64].

3.5. In vitro bacterial binding

In vitro bacterial binding of radiolabeled Levofloxacin and micelles after *S. aureus* incubation at 37 °C was observed as 75 ± 1.3% and 45 ± 2.1%, respectively. *In vitro E. coli* binding of radiolabeled Levofloxacin and micelles at 37 °C showed 63 ± 2.4% and 40 ± 1.9%, respectively (Fig. 4). Both radiolabeled Levofloxacin and micelles show high *in vitro* bacterial binding. Tc-99 m radiolabeled PC:SDC micelles exhibit lesser bacterial binding for both *S. aureus* and *E. coli* cultures (p < 0,05). This may be due to the fact that ^{99m}Tc-radiolabeled PC:SDC micelles are not specific to bacterial infections as much as ^{99m}Tc-Levofloxacin. However, Tc-99 m radiolabeled PC:SDC micelles can also be assumed as sensitive radiopharmaceutical agents due to the mechanism of imaging of infection and inflammation. ^{99m}Tc-radiolabeled, PEGylated, PC, SDC and DTPA-PE containing nanosized micelles can accumulate at infection and inflammation sites due to long vascular circulation of vesicles by small particle size and surface coating by an hydrophilic polymer (such as PEG) [47,52–54]. It was reported in some previous studies that the accumulation of radiolabeled liposomes in at infectious sites is performed by leakage of the vesicles through vessels. This leakage depends on the enhanced vascular permeability and following phagocythosis by macrophages of infected tissue. It was reported that the endothelial junctions existing in the blood vessel provides the penetration of the particles smaller than 200 nm from the vascularization [48,54]. Therefore, surface coated, nanosized drug delivery systems like liposomes can be accumulated at the site of infection and inflammation due to long circulation, enhanced vascular permeability and reduced removal by opsonisation [49,52,65].
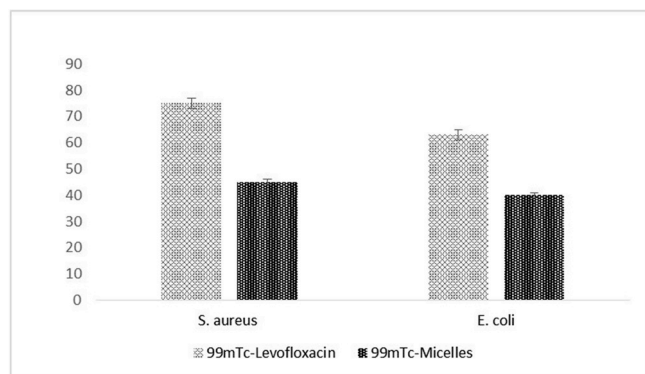
Therefore, ^{99m}Tc-Levofloxacin and ^{99m}Tc-radiolabeled, nanosized, PEGylated, PC, SDC and DTPA-PE containing micelles were observed as potential imaging agents for the diagnosis and imaging of inflammation and infectious sites [66]. Despite, radiolabelled antibiotics could represent a promising tool for specifically image infective processes, radiolabelled micelles do not show a similar specific mechanism of accumulation in infectious/inflammatory sites that is mainly due to increased permeability (that is present in both infective and inflammatory processes) and small particle size. Therefore, this "passive" and non-specific mechanism implies that they could be useful for monitoring the inflammatory burden but, since radiolabelled micelles are not able to discriminate between an infection from a sterile inflammation, their use for imaging of infections is limited in clinical practice.

4. Conclusion

Radiolabeled compounds and nanocarriers lead to direct researchers toward easy and quick diagnosis and imaging of infection and inflammation. Although, radiolabeled Levofloxacin is particularly an

Table 1

In vitro stability of labeling efficiency of ^{99m}Tc-Levofloxacin and ^{99m}Tc-PC:SDC micelles at room temperature.

Radiotracers	Medium	Labeling Efficiency (%)					
		Time (h)					
		1	2	4	6	8	24
^{99m}Tc-Levofloxacin	Serum	95.86 ± 1.32	95.01 ± 0.87	94.90 ± 2.43	94.51 ± 3.24	93.83 ± 1.92	93.13 ± 1.97
	Saline	96.00 ± 0.98	95.07 ± 2.12	95.04 ± 1.98	94.08 ± 2.89	94.42 ± 3.76	93.97 ± 2.23
^{99m}Tc-PC:SDC Micelles	Serum	85.01 ± 0.87	84.08 ± 1.36	84.07 ± 2.65	84.02 ± 3.79	83.60 ± 4.70	83.59 ± 3.75
	Saline	84.97 ± 1.12	84.68 ± 1.79	84.42 ± 3.12	83.87 ± 2.31	83.54 ± 2.86	83.12 ± 3.73

Fig. 4. *In vitro* bacterial binding of ^{99m}Tc-Levofloxacin and ^{99m}Tc-micelles after *S. aureus* and *E.coli* incubation at 37 °C.

excellent alternative for the detection of chronic infections caused by gram-positive and gram-negative bacteria, radiolabeled PEGylated, PC, SDC and DTPA-PE containing nanosized micelles were also evaluated as a potential candidate in the detection of infection and inflammation. As it was known, micelles similar to liposomes tend to accumulate at the site of infection based on the long vascular circulation of vesicles by small particle size and surface coating by a hydrophilic polymer (such as PEG). Both radiopharmaceutical agents exhibit potential results in design, characterization, radiolabeling efficiency and *in vitro* bacterial binding point of view. *In vivo* potential of radiolabeled Levofloxacin and micelles should also be evaluated in the infection and inflammation animal models in the future.

CRediT authorship contribution statement

Asuman Yekta Ozer: Writing - review & editing.

Declaration of competing interest

The authors state no decleration of interest.

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