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Preparation and biological evaluation of radio conjugated cephradine complex for infection imaging

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Abstract: Antimicrobial resistance is a major challenge in the field and threat to human life. Many patients are suffering from cancer, infection and other diseases simultaneously. Therefore, early detection of infection can lead to treatment of these patients with an appropriate antibiotic. Hence, the development of a specific imaging molecule can increase the speed of infection analysis and thereby application of proper antibiotic. The present work involves the optimization of labelling conditions for an antibiotic of cephalosporin family, cephradine with technetium-99m (^{99m}Tc) and establishment of quality control tests. Labelling of cephradine was also determined by applying MALDI-TOF mass spectrometry. Evaluation of *in vitro* binding with *S. aureus* bacteria was carried out. Animal model was used to conduct *in vivo* binding studies. For this, infected animals were injected with the radiolabelled ligand and images were captured by Gamma camera, to observe target to non-target uptake of radiolabelled complex. Furthermore, we optimized various parameters to achieve best labelling efficacy and stability of cephradine. Our results show that cephradine can be used as potential infection imaging agent for advanced clinical care.

Keywords: Antibiotics, bacterial infection, molecular imaging, radiolabelling, cephradine .

INTRODUCTION

Bacterial infections are posing one of the major challenges to humanity (Wareham D *et al*., 2005, Eggleston and Panizzi, 2014). World Health Organization has declared antimicrobial resistance as one of the top threats to humans (Mota *et al*., 2020). The currently available clinical and laboratory assessments usually do not provide a clear etiology of the disease. Therefore, an early detection of infection in patients simultaneously suffering from different diseases is of great importance. This can prevent a delay in proper treatment and overuse of antibiotic. Molecular imaging can help to differentiate between inflammations due to infections and other disease states such as cancer and sterile inflammation(Jain, 2017). Molecular imaging like magnetic resonance imaging and computed tomography are not much useful in infection imaging. As these techniques are specific for anatomical changes that occur during the progress of disease. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are sensitive techniques but they have limitations also. They show the physiological changes that arise as a result of host response to infection and are a part of inflammatory process (Ordonez *et al*., 2019). Nuclear medicine techniques are found to be an effective tool in the management of diseases caused by infection, when used in combination with bacteria specific radiolabelled molecules, which bind directly with live bacteria (Britton *et al*., 1997, Asikoglu *et al*., 2000, Becker and Meller, 2001, Oyen *et al*., 2001, Yigit *et al*., 2006, Petruzzi *et al*., 2009). Most of the nuclear medicine imaging procedures are based on technetium-99m labelled agents (Ilem-Ozdemir *et al*., 2016, Akbar *et al*., 2016, Salahinejad and Mirshojaei, 2016) but others are also available (Salmanoglu *et al*., 2018). Technetium- 99 m is a versatile imaging radionuclide eluted from commercially available Mo-99/Tc-99m generator system that is the primary tool for all nuclear medical centers. It has ideal characteristics such as half-life (6 h) and 140 keV gamma ray emissions that make it one of the best imaging agents. Several Technetium- 99 m labeled compounds have been established for imaging (Ercan *et al*., 1992, Bhatnagar *et al*., 1995, Britton *et al*., 1997, Sonmezoglu *et al*., 2001, Larikka *et al*., 2002, Lupetti *et al*., 2002, Oh *et al*., 2002, Appelboom *et al*., 2003, Sarda *et al*., 2003, Siaens *et al*., 2004, El-Ghany *et al*., 2005, Gomes Barreto *et al*., 2005, Roohi *et al*., 2005, Roohi *et al*., 2006, El- Ghany *et al*., 2007, Motaleb, 2007, Yurt Lambrecht *et al*., 2008). A recent *in vitro* study showed promising results of [^{99m}Tc] levofloxacin and [^{99m}Tc] micelles as radiopharmaceutical agents (Silindir-Gunay and Ozer, 2020). Informative Comprehensive reviews regarding advancements in tracer development for imaging bacterial infections have been published (Mick M. Welling *et al*., 2019, Northrup *et al*., 2019).

Keeping in view radiolabelling studies of cephalosporin family, research work was designed with *Staphylococcus aureus* (*S. aureus*) so a candidate with activity against gram positive bacteria was selected among first generation antibiotics of cephalosporins on the basis of high activity against gram positive bacteria because activity against gram positive bacteria decreases as we proceed to next

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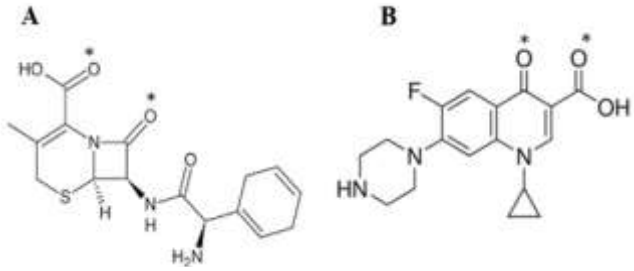
generations so a member from first generation cephalosporin-cephradine was selected. cephradine is a member of broad range antibiotics of cephalosporin group that works against bacteria in body (Breuer and Starke, 1978, Chester *et al*., 1983, Kang *et al*., 2019). Mode of action of cephradine starts with binding to penicillin-binding proteins (PBP) that are present on the bacterial cell wall and inactivates them. So, weakening of the bacterial cell wall results in cell lysis. Scheme 1a shows the structure of cephradine and probable binding site with technetium-99m. Scheme 1b shows the structure of ciprofloxacin. A previous mass spectrometric study of [^{99m}Tc] ciprofloxacin complex showed that a bis-chelate complex is formed between ciprofloxacin and technetium-99m, by coordinating through carbonyl and carboxylate oxygen atoms (Lecina *et al*., 2014). This study adequately reveals the possibility of a bis-chelate complex formation of cephradine and technetium-99m through carbonyl and carboxylate oxygen atoms. The objective of the current study was to regulate, improve and finalize a simple and efficient procedure for radiolabelling of cephradine at room temperature followed by evaluation of its bio distribution and uptake in infection by imaging animal model using Gamma camera.



Scheme 1: A) Chemical structure of cephradine and B) chemical structure of ciprofloxacin.
(*Indicating probable binding site with technetium-99m).

MATERIALS AND METHODS

Cephradine (Velosef) was purchased from local pharmacy in Lahore, Pakistan. Technetium-99m was eluted from PAKGEN Mo-99/^{99m}Tc generator that is locally produced in Pakistan Institute of Science and Technology (PINSTECH) by fission-based products. Analytical grade (AR) grade chemicals were used in all experiments. Preclinical studies were based on rabbit animal models with approval from the "Institutional Ethical Review Board" of Institute of Nuclear Medicine and Oncology (INMOL), Lahore, Pakistan, in compliance with the ARRIVE guidelines. It is further to approve that all procedures were conducted according to relevant guidelines and protocols.

Characterization

Well shaped Gamma counter (SCALER TIMER ST7). Centrifuge machine, incubator, Dual-headed Infinia (GE) gamma camera, Matrix-Assisted Laser Desorption-Ionization-Time of Flight Mass Spectrometry (MALDI-TOF), UV-Visible Spectrophotometer HITACHI (U-2910).

Radiolabelling of cephradine with [^{99m}Tc]

Various parameters were adjusted to maximize the radiolabelling efficacy of cephradine with ^{99m}Tc. Cephradine was radiolabelled with [^{99m}Tc]. Technetium-99m was taken ~370 MBq in the form of $^{99m}TcO_4^-$ in saline after eluting from Mo-99/^{99m}Tc generator. Amount of ligand (cephradine) was varied between 0.1-1.0mg to monitor labelling efficacy at each amount of ligand. $SnCl_2.2H_2O$ was added as a reducing agent. The amount of stannous chloride was varied between 5-15µg to select the optimum concentration. The pH of mixture was changed in series of 4-7 by using either 0.1M NaOH or 0.1M HCl. The mixture was kept at room temperature 25±2°C for 30 min.

Quality control of [^{99m}Tc] cephradine radio-conjugate

The percentage of free $^{99m}TcO_4^-$ in the mixture was assessed by chromatographic procedure using Whatman chromatographic paper (3) and acetone as stationary and mobile phase respectively. The distribution of labelled and free $^{99m}TcO_4^-$ was measured by cutting the chromatographic strip into 1cm segments and checked the counts of radioactivity by a gamma-counter (SCALER TIMER ST7). Percentage of colloid content was estimated by TLC/SG paper as stationary phase while mixture of ethanol, water and ammonium hydroxide in ratio of (3:5:1) as mobile phase.

Stability of [^{99m}Tc] cephradine at room temperature

After selection of best labelling conditions, [^{99m}Tc] cephradine was kept at room temperature to monitor the stability of the complex till 24h. Chromatographic assessment after 2, 4 and 24h of incubation was performed that showed the stability of labelled product.

Protein binding and lipophilicity test of [^{99m}Tc] cephradine

In vitro protein binding study was performed by using human blood plasma from a healthy volunteer with his informed consent. For this, 3 ml of human blood plasma was added to 1ml of labelled complex [^{99m}Tc] cephradine and kept at 37°C for one hour. Afterwards, the mixture was centrifuged at 3000rpm for 10min to separate serum from blood cells. The supernatant was added to equal volume of trichloroacetic acid and centrifuged again at 3000 rpm for 10min. Two layers were formed that were separated and counted for radioactivity in well-shaped gamma counter. All experiments were performed in triplicate.

Partition coefficient (p)

Partition coefficient was planned to check the lipophilicity by measuring the counts of radioactivity between organic and aqueous layers. For this purpose, 100µl of labelled complex [^{99m}Tc] cephradine was mixed in separate vials having mixture of 200µl of phosphate buffer of pH 6.6, 7.0

and 7.6 and 200µl of n-octanol in each vial. Vials were shaken and kept intact for appearance of two layers after 15minutes. The two visible layers were separated carefully and counted in well-shaped gamma counter for radioactivity counts in them.

Stability in human serum

Stability in human serum was estimated by collecting blood from a healthy volunteer with his informed consent. To start each experiment, approximately, 5ml of blood from the same volunteer was collected and centrifuged at 3000 rpm. About 3ml of serum was separated from each blood sample. This extracted serum was added in 0.2ml of [^{99m}Tc] cephradine. The mixture was set aside at 37°C. Stability in serum was checked by drawing an aliquot from sample at different time intervals till 24 h and labelling efficacy was monitored by paper chromatography. Degradation of labelled complex was indicated by decrease in the labelling efficacy.

In vitro cell binding studies

S. aureus culture was developed using brain heart infusion (BHI) media. 10ml aliquots of suspensions of harvested bacteria containing almost 5×10^8 colony forming units (CFU) of viable stationary phase bacteria were snap frozen in liquid N_2 and stored at -10°C in freezer. *In-vitro* binding study of [^{99m}Tc] cephradine was done by taking 3 ml of frozen bacterial culture of S. aureus. It was centrifuged to obtain a pellet. The pellet was mixed with 1.5ml (0.2M) sodium phosphate buffer (Na-PB) having ~7MBq activity of Technetium-99m labelled cephradine and 0.2M acetic acid (1.5mL) was also added and incubated at 4ºC for 1h. The mixture was centrifuged at 4ºC temperature for 10min and after removing supernatant radioactivity in the pallet containing bacteria was checked by gamma-counter.

[^{99m}Tc] cephradine scintigraphy in rabbit

We used rabbit as animal model to evaluate the efficacy of infection imaging of novel radiolabelled conjugate [^{99m}Tc] cephradine. The study was performed after getting approval from ethical committee of INMOL. It is an ISO certified laboratory that restricts our activities and permits us to work according to international standards. Strict standard operating procedures and work instructions are followed in this regard. Record of each sacrificed animal is also kept. For this study, 3ml of frozen bacterial culture was kept at room temperature and centrifuged at 3000rpm. The pellet was mixed with 3ml of 0.9% saline. Approximately, 0.3-0.5ml of this saline culture was injected in thigh of rabbit. The rabbit was kept separate with proper food and water for 48 hours for appearance of infection in injected leg. For imaging we used a Dual-headed Infinia (GE) gamma camera connected to an online dedicated computer (Xeleris Functional Imaging Workstation).Animal was injected with (2mL) Diazepam injection into the left thigh muscle and positioned with hind legs spread out and all legs were fixed with surgical tape on a flat wood surface. [^{99m}Tc] cephradine (0.3ml) containing 130MBq of activity was then injected into the marginal ear vein of rabbit intravenously. To study the bio distribution of tracer in animal having swelling in its right leg due to S. aureus infection, images were captured at 10 min, 2 h, 3h and 24h of post injection time.

MALDI-TOF mass spectrometric analysis

Mass spectrometric analysis of technetium labelled and non-labelled cephradine was performed on MALDI-TOF system Auto-flex III, Smart beam 200 system, Germany). As an experimental procedure, 1-2µL of freshly-prepared sample solution (cephradine radio-conjugated with technetium-99m) was mixed with 10-15ul of α-cyano-4-hydroxycinnamic acid (HCCA) in its diluent comprising of 1/3 acetonitrile, 2/3 of 0.1%TFA in water and 1-2µL sample was loaded on MALDI anchor chip plate. The sample was dried for 10-15 min at room temperature and analysed by MALDI-TOF mass spectrometry in reflectron positive mode.

UV-Visible spectroscopic analysis

UV-Visible spectrometric analysis of technetium labelled and non-labelled cephradine was performed on UV-Visible Spectrophotometer HITACHI (U-2910). For this analysis cold and radiolabelled cephradine samples were filled in cells and measured in equipment to see the effect on absorption maximum of cephradine before and after labeling with Tc-99m.

RESULTS

Various parameters were studied to maximize radiolabelling of cephradine. It was observed that maximum radiolabelling was accomplished by using 0.5mg of ligand in 10µL of volume, 10µg of reducing agent and at pH=7. Process of radiolabelling of cephradine with technetium-99m may result in the labelled ligand (cephradine), free radionuclide in the form of pertechnetate ($^{99m}TcO_4^-$) and formation of colloid/ hydrolysed ($^{99m}TcO_2$). UV-VIS data confirmed the successful labelling of cephradine by [^{99m}Tc]. After labelling of cephradine with [^{99m}Tc] peak clearly shifted from 259nm to 275nm as shown in fig. (1)

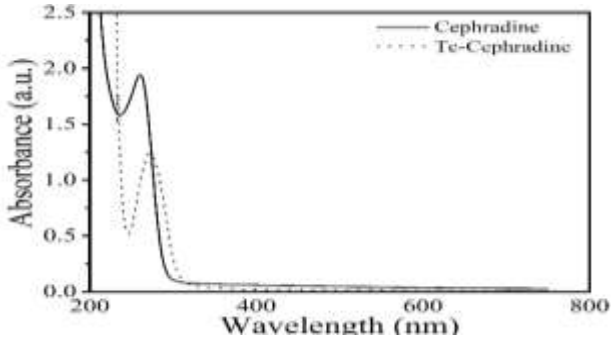


Fig. 1: UV data of cephradine and [^{99m}Tc] labelled cephradine.

Radiochemical purity that is actually percentage of labelled cephradine was determined by ascending paper chromatography. For this estimation, two different chromatographic systems were used. In first system Whatman paper No.3 and acetone were used as stationary phase and mobile phase respectively for the assessment of free radionuclide. In this case when strip was developed in acetone and then after drying and cutting all segments were measured in Gamma counter. Free pertechnetate was estimated by counts of radioactivity on upper side of strip that is having R_f value=1 while counts on base of strip having R_f value=0 of strip were due to colloid and labelled content. Second system was used for colloid determination with thin layer chromatographic paper coated with silica gel (TLS/SG) as stationary phase while mixture of ethanol, water and ammonium hydroxide in ratio of (3:5:1) as mobile phase. In this system, the free and labelled moved with solvent so counts were more on upper side of strip having R_f value=1, while colloid remained at origin (R_f=0). Radiochemical labelling efficacy was calculated by formula given below:

% Colloid content = (Activity counts at R_f = 0/ Total activity counts on TLC strip) x100
% Free pertechnetate = (Activity counts at R_f =1/ Total activity counts on Whatman 3 strip) x 100
% Labelled cephradine = 100 - (% colloid + % free pertechnetate)

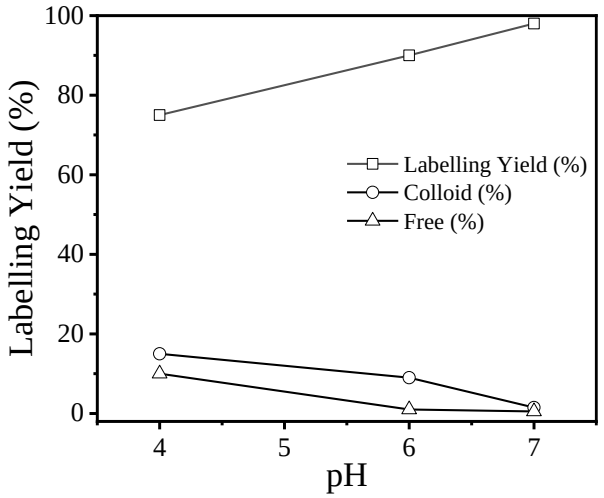


Fig. 2: Effect of pH on radiolabelling efficacy

Fig. (2) shows effect of pH on the labelling efficacy. To get maximum labelling yield i.e.,>95% radiolabelling of cephradine was checked at different pH values (4-7). 10μg of $SnCl_2$. $2H_2O$ was added as reducing agent. Results specify 75±1% labelling at pH 4 that increased to 90±1% at pH 6 and maximum labelling98±1%was attained at pH 7. Thus pH 7 was selected as optimum for radiolabelling and it was in good agreement with physiological pH value of blood ~ 7.4. So labelling was not checked at pH beyond 7.

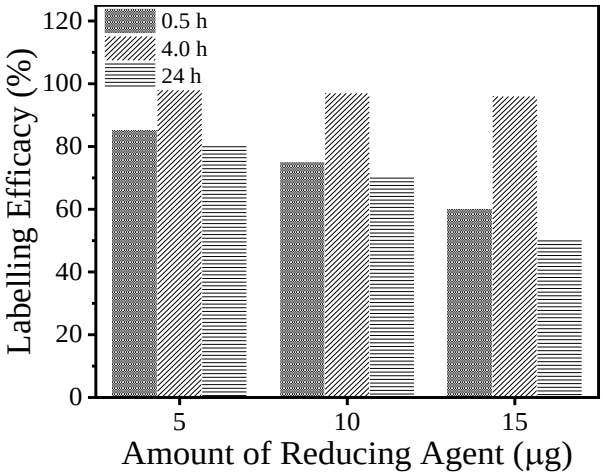


Fig. 3: Amount of reducing agent and radiolabelling efficacy

The effect of the amount of reducing agent on labelling efficacy was determined by labelling the ligand with various quantities of reducing agent and keeping other factors constant as shown in fig. (3). at first, amount of reducing agent was taken as 5μg and labelling was checked that was 85%. Its stability was checked after 4h and 24h but results were not appreciable due to fall in labelling efficiency to 75% and then 60% respectively. Next trial was done with 10µg of reducing agent. This gave good labelling efficacy and stability. Further trial experiment was carried out with 15µg of reducing agent. This concentration gave good initial labelling efficacy i.e., 80%. However, it was dropped to70% and 50% after 4h and 24h respectively.

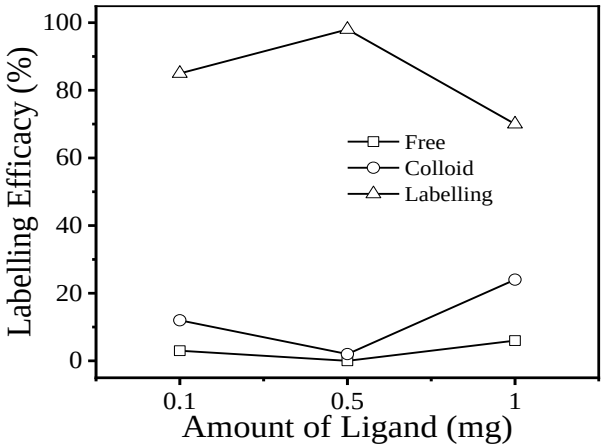


Fig. 4: Radiolabelling affected by of amount of ligand (cephradine)

The concentration of ligand also affects the labelling, as shown in fig. (4). It was observed that at 0.1mg of ligand, labelling was 80±2% after half hour of incubation time that increased to 97±1% at 0.5mg of ligand. Further increase in the concentration of cephradine leads to drop in labelling yield.

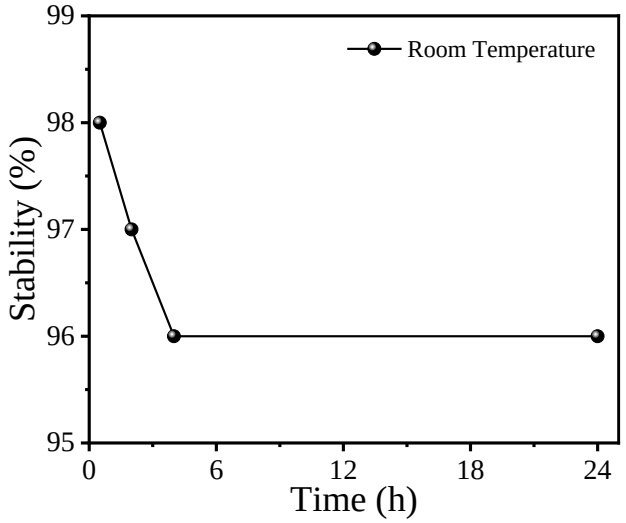


Fig. 5: Stability of radiolabelled complex at room temperature

After optimization of parameters for the preparation of, [^{99m}Tc] cephradine stability studies were performed. The radio conjugate was kept at room temperature to check its stability as shown in fig. (5). Results showed 98 ± 1% labelling after 30 min that was slightly decreased to 97 ± 1% after 2-4 h and decreased to 96±1% after 24h. Stability studies of radioconjugate in fresh human serum were also performed. It was mixed with fresh human serum and incubated for 24 h to determine its behaviour in serum. Radiolabelled cephradine was stable more than 95 ± 2 % up to 24h in serum. Results are shown in fig. (6).

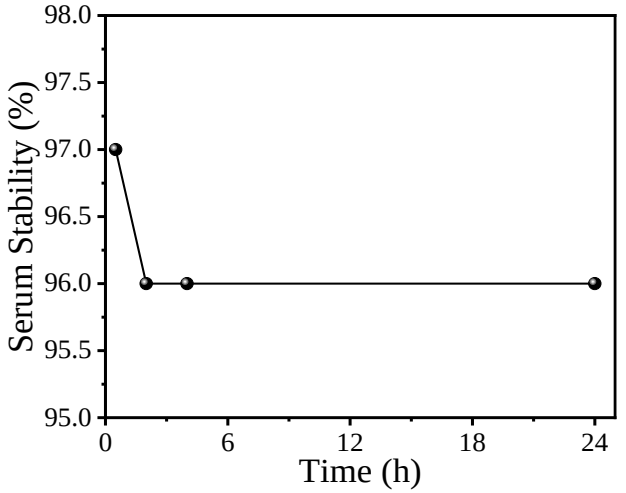


Fig. 6: Serum Stability of radiolabelled complex

The results of protein binding study showed 52% binding with serum protein and 48% was left unbound. Percentage of binding was calculated by following formulas.

% Binding in supernatant = (counts in supernatant/total counts) x 100

Total counts = counts in supernatant + counts in residue

% Binding in Residue = (counts in Residue/total counts) x 100

Partition co-efficient study reflected that the complex is hydrophilic in nature because of showing low Log p value that is -2.95.

Log p Value = Log (0.11/99.89) = -2.95

Table 1: *In vitro* binding (%) of the [^{99m}Tc] cephradine to viable S. aureus, n = 3

[^{99m}Tc] cephradine (mg)	*S. aureus*		
	1 h	4 h	24 h
0.1	98 ± 1.2	96 ± 1.1	95 ± 1.2
0.5	97 ± 1.2	97 ± 1.2	96 ± 1.3
1.0	95 ± 1.4	94 ± 1.2	94 ± 1.2

In vitro binding with gram-positive S. aureus bacteria was also checked. The results indicate strong binding potential of our radiolabelled conjugate with bacteria. Table 1 shows binding of [^{99m}Tc] cephradine for S. aureus. MALDI-TOF mass spectrometry was performed to determine the labelling of cephradine with technetium-99m. The $[M + H]^+$ ion peaks were obtained at 350.364 and 765.426 corresponding to the masses of unconjugated and conjugated cephradine with technetium-99m (theoretical masses 349.406 a.m.u. and 797.812 respectively). fig. (7a), shows $[M + 1H]^{+1}$ ionic peak at 765.426 corresponded to the ionic peak of cephradine conjugated with technetium-99m (with the abstraction of two water molecules). This peak is absent in mass spectrum of unconjugated cephradine fig. (7b), fig. (7c) shows zoomed in peak at 765.426. These spectra support the mass spectrometric identification of technetium-99m conjugated cephradine.

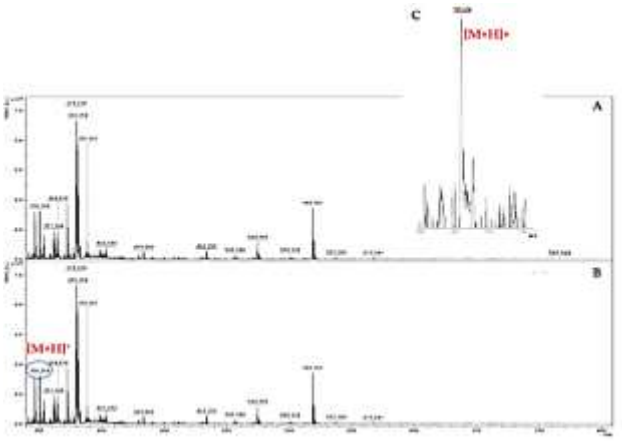


Fig. 7: MALDI mass spectra of technetium labelled cephradine. a) The spectra showing $[M + 1H]^{+1}$ ionic peak at 765.426 corresponded to the $[M + 1H]^{+1}$ value of cephradine conjugated with technetium (with the abstraction of two water molecules) which is absent in unconjugated cephradine (spectrum B). b) Mass spectrum of cephradine .c) Magnified peak of 765.426 from spectrum (A) is shown in spectrum (C).

Table 2: Biodistribution of [^{99m}Tc] cephradine

Time(h)	Liver n Heart	Right Kidney	Left Kidney	Bladder	Infected Leg	Normal Leg
0.1	145025(V) 147708(D)	21940(V) 28326(D)	26076(V) 35367(D)	101297(V) 77261(D)	6275(V) 8640(D)	2185(V) 2545(D)
2.0	62724(V) 59014(D)	27632(V) 33155(D)	26235(V) 32958(D)	200008(V) 144202(D)	6591(V) 6146(D)	2024(V) 2363(D)
3.0	30256(V) 32145(D)	29523(V) 15475(D)	90158(V) 42792(D)	502400(V) 236164(D)	6704(V) 4271(D)	1785(V) 1786(D)
24	2545(V) 2758(D)	8754(V) 8963(D)	10367(V) 10851(D)	107423(V) 109409(D)	406(V) 745(D)	239(V) 233(D)

In vivo study involved imaging in rabbit having inflammation in its right leg. After injection of [^{99m}Tc] cephradine Scanning was performed at 0.1h, 2h, 3h and 24h of post injection. The standard uptake value of labelled complex in different organs of animal was checked along dorsal (D) and ventral (V) side as shown in table 2 and fig. (8), show the dynamic study after 10 min and scans of static view after 2h for whole body while selective imaging of legs was performed to mark the infection site at 3h post injection and 24h post injection for rabbit. Dynamic study showed normal distribution of labelled complex through heart, liver, kidneys and finally excreted to bladder. Radiolabelled conjugate uptake in inflamed leg showed binding of radio conjugate with bacteria. Maximum activity was reached in target after 3 hours. T/NT value after 10minutes was 2.87 that increased to 3.25 after 2 h and it was 3.75 after 3 h that decreased to 1.69 after 24 hours of post injection.

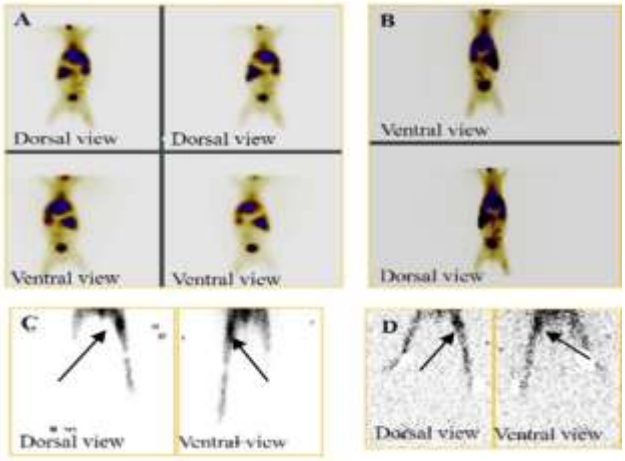


Fig. 8: Uptake and excretion pattern of ^{99m}Tc-cephradine in a rabbit model at various time intervals. a) Dynamic view over 10 min imaged immediately after intravenous injection, b) Static view at 2 h, c) and d) Static view of selective imaging of legs to mark the infection site at 3 h and 24 h of post injection respectively.

DISCUSSION

Nuclear imaging approaches have gained significant ranking in the diagnosis of various infective diseases (Kis *et al*., 2020). Multidrug resistant strains of bacteria are even able to resist third generation antibiotics. Methicillin-resistant *S. aureus* (MRSA) is resistant to cephalosporins. Some other strains have also established resistance of certain degree for cephalosporins (Sutaria *et al*., 2018). Antibiotics are sometimes co administered with avibactam, which acts an inhibitor of β-lactamase (Both *et al*., 2017). Infection imaging using radionuclide can be a promising method in clinics treating patients suffering from other diseases like cancer. The use of infection imaging allows a quick distinction between sterile and non-sterile inflammation and thereby administration of a specific antibiotic. Further, it can save the patient with sterile inflammation from unnecessary use of antibiotics.

Different radioactive labelled antibiotic compounds and antimicrobial peptides have been used for the diagnosis of infection (Brouwer *et al*., 2010). The compounds are either covalently attached or chelated to the radionuclide depending on the nature of the radioisotope (Northrup *et al*., 2019). Technetium-99m is one of the best radioisotopes in nuclear imaging (Northrup *et al*., 2019). In the present work, we have also used this radioisotope. It is challenging to find the right chemical conditions that allow the development of a radiotracer, which has properties identical to the patent medicine (Mota *et al*., 2020). Previous studies have reported different labelling conditions for different antibiotics. A study showed that [^{99m}Tc] levofloxacin gave maximal binding at a pH of 5 and the concentration of stannous chloride used as reducing agent for radiolabelling and ligand used was 50 micro gram and 1mg respectively (Silindir-Gunay and Ozer, 2020). In our case maximal binding of cephradine took place at pH 7 that is in good accordance of pH conditions of blood. However, in our work only 10µg of the reducing agent and 0.5mg of ligand were used. The variation in pH for labelling can be attributed to different structures of both ligands.

The biodistribution studies of labeled cephradine fig. 8, show very clear image similar to that of [^{99m}Tc] norfloxacin (Svetlana Ivanovna Sazonova *et al*., 2015). If we compare T/NT values after 2-3 hours of post injection with antibiotics that belong to cephalosporin family then our labeled cephradine that is having T/NT 3.75 after 3his almost second in position on basis of published value of ceftazidime that was having T/NT 1.4±0.2 (Mirshojaei *et al*., 2013) but later another research group reported a value of T/NT as 5.06±0.1 for ceftazidime (Bekheet *et al*., 2014) and cefotaxime is having T/NT value 3.77±2.38 (Akbar *et al*., 2016) that is in close proximity to cephradine while ceftriaxone is having 3.11 (Brouwer *et al*., 2010) that is lower than cephradine. Comparison with antibiotics of other families show that cephradine value for T/NT 3.25 after 2h is slightly lower than well-known ciprofloxacin 3.6±0.4 (Siaens *et al*., 2004) and levofloxacin 3.57 (El-Ghany *et al*., 2007). So, this showed that [^{99m}Tc] cephradine can be used as potential imaging agent for

bacterial infections. Results obtained from target-to-non target ratio are in good accordance with *in vitro* binding results also.

STATISTICAL ANALYSIS

One sample t-test was used for statistical analysis. Frequencies with percentages were used for categorical variables. Chi square test was used for calculating P-values in categorical variables. All P-values were calculated with significance considered at $P < 0.001$.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this manuscript. Also, there is no conflict of interest regarding funding among the authors. Since this study contains collection of blood sample from a healthy volunteer, so it is declared that all procedures performed were in accordance with the ethical standards of the institution with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. It is to further declare that informed consent was obtained from the individual participant included in the study. It is furthermore declared that the manuscript contains original research data which have not been previously published and not been submitted for publication elsewhere.

INFORMED CONSENT STATEMENT

It is declared that consent was obtained from the individual participant, before collecting their blood samples.

CONCLUSION

In this work cephradine was labelled successfully with [^{99m}Tc] and high radiochemical purity. *In vitro* binding study with *S. aureus* bacteria is in good agreement with uptake of labelled complex in swelling of leg of rabbit due to infection by S. aureus. This concludes that [^{99m}Tc] cephradine is a strong candidate for imaging of bacterial infection.

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