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Radiolabeling, quality control and kit formulation of a new ^{99m}Tc-labeled antibiotic: ^{99m}Tc-doxycycline hyclate

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Abstract Since radiolabeled antibiotics specifically bind to the bacterial components they are promising radiopharmaceuticals for the precise diagnosis and detection of infectious lesions. Doxycycline hyclate (DOX) was chosen to investigate as a new radiolabeled antibacterial agent since its bacteriostatic activity against a wide variety of microorganisms. The aim of the present study is to develop simple and easy formulation of DOX with ^{99m}Tc ready to use kit. ^{99m}Tc-DOX was developed and standardized under varying conditions of reducing and antioxidant agent concentration, pH, radioactivity dose and reducing agent type. Labeling studies were performed by changing the selected parameters one by one and optimum labeling conditions were determined. After observing the conditions for maximum labeling efficiency and stability, lyophilized freeze dry kits were prepared accordingly. Radiochemical purity was determined with RTLC and RHPLC which was found more than >95 %. Two different freeze dry kits were formulated with optimum labeling conditions. The improved kits were found stable up to 6 months.

Keywords Doxycycline hyclate · Radiopharmaceutical · Radiolabelling · 99m-Technetium · 99mTc-doxycycline hyclate

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Introduction

The evaluation of diagnostic nuclear medicine can be principally attributed to the existence and chemical versatility of ^{99m}Tc-sodium pertecnetate (^{99m}Tc) the ideal radiotracer. The nuclear properties of ^{99m}Tc consist of its 140 keV gamma photon emission, which is optimum for imaging with gamma cameras used in nuclear medicine. Half-life of 6 h is optimum for preparing the radiopharmaceutical, performing its quality control, and injecting into the patient for imaging studies, yet it is short enough to minimize the absorbed radiation dose [1, 2]. Scintigraphic detection of acute, subacute and chronic infection and inflammation is an important problem in clinical practice, because it may have important implications for the management of patients with infectious or inflammatory diseases. The ideal radiopharmaceutical for infection/inflammation imaging should accumulate efficiently to inflammatory foci, clear rapidly from background tissue, discriminate between bacterial infection and sterile inflammation, cost low, have kit to prepare easily [3]. Since radiolabeled antibiotics specifically bind to the bacterial components they are promising radiopharmaceuticals for the precise diagnosis and detection of infectious lesions [4]. So far various antibiotics studied for infection imaging in nuclear medicine. Some of those are the ciprofloxacin, enrofloxacin, moxifloxacin, kanamycin, lomefloxacin, ofloxacin, cefoperazone, doxorubicin [5–15] were also labeled with ^{99m}Tc-pertechnetate and evaluated as infection imaging agents. Also Örümlü [16] has described a radiolabeling procedure for Doxycycline hyclate (DOX) with ^{99m}Tc. DOX is a well-known broad-spectrum tetracycline antibiotic obtained by modification of the oxytetracycline molecule. It has bacteriostatic activity against a wide variety of microorganisms, including aerobic and anaerobic Gram-

positive and Gram-negative bacteria, chlamydiae, rickettsiae and mycoplasmas. It exerts bacteriostatic effect by inhibiting the bacterial protein synthesis due to the disruption of transfer RNA and messenger RNA at the ribosomal sites [17, 18]. Generator produced ^{99m}Tc is the most commonly used radionuclide in nuclear medicine. Availability and affordability of ^{99m}Tc and accessibility of single photon emission computed tomography cameras (SPECT) are important preconditions for clinical practice [19–21]. The aim of this study was to prepare ^{99m}Tc-DOX in a simple radiochemical method with good labeling efficiency and evaluate the ready to use cold kit formulation thus making it available to the other nuclear medicine centers.

Materials and methods

All chemicals and solvents were of either HPLC or analytical grade and were used without further purification. DOX was obtained from AppliChem (Germany). Stannous tartrate and stannous chloride dehydrate (Stannous chloride) were purchased from Sigma-Aldrich (USA) and ascorbic acid was purchased from Sigma-Aldrich (United Kingdom). ^{99m}Tc was eluted from the Molybdenum-99 (^{99}Mo)/^{99m}Tc-generator (Nuclear Medicine Department of Ege University). 13 mm Syringe filter with 0.22 μm pore size GH Polypro membrane was purchased from Pall Life Science. All solvents were obtained from Merck (Germany). Radioactive samples counted in a counting unit (Atomlab 100 Dose Calibrator Biodex Medical Systems). Experiments were performed in triplicate unless stated otherwise. Results are reported as mean ± standard error.

Radiolabeling studies

To investigate the optimum conditions, radiolabeling was tested with different types and concentrations of reducing and antioxidant agent. Radiochemical purity (RP) was determined with Radio Thin Layer Chromatography (RTLC) and Radio High Performance Liquid Chromatography (RHPLC) analysis. Two different freeze dry kits were formulated with optimum labeling conditions and stability of kits were performed.

Effect of reducing agent on labeling

^{99m}Tc pertecnetate was eluted from ^{99}Mo/^{99m}Tc-generator in +7 oxidation state which does not able to label with any compound on direct addition. So prior to labeling procedure, reduction of ^{99m}Tc is required for converting ^{99m}Tc from the +7 state to a desired lower oxidation state, which can make complexes with the ligand to form the radiopharmaceutical. So far, different types of reduction agents have been used for radiopharmaceuticals, stannous chloride and stannous tartrate is used extensively [22].

To examine the effect of reducing agent concentration on labeling; DOK (1 mg) was dissolved in 0.9 % sodium chloride solution (1 mL). To this stock solution, stannous chloride was added under an atmosphere of bubbling nitrogen. Reduction of ^{99m}Tc was performed at acid pH (1 mg reducing agent dissolved in 1 mL 0.1 N HCl) with different amount of stannous chloride (20, 30, 40, 50, 100, 200 and 400 μg). Radiolabeling was performed with ^{99m}Tc (37 MBq) in 0.9 % sodium chloride solution (0.1 mL) and solution was allowed to stand at room temperature for 15 min prior to radiochemical analyses.

Also stannous tartrate has been used as reducing agent for radiolabeling studies. DOK (1 mg) was dissolved in 0.9 % sodium chloride solution (1 mL) in adequate number of vials. 20, 30, 40, 50, 100, 200 and 400 μg stannous tartrate (1 mg reducing agent dissolved in 1 mL 0.1 N HCl) was added to each individual vial. Freshly eluted 37 mBq ^{99m}Tc was added to each vial. The vials were allowed to stand at room temperature for 15 min prior to radiochemical analyses.

Effect of antioxidant agent on labeling

^{99m}Tc radiopharmaceuticals may undergo auto radiolysis during preparation, release, and storage. Decomposition of the radiopharmaceutical prior to or during administration will decrease the targeting capability. Therefore, it is very important to use a stabilizer to minimize the auto radiolysis. Radiolytic stabilizers are often antioxidants, such as ascorbic acid, gentisic acid, and *p*-aminobenzoic acid [23].

To examine the effect of antioxidant agent on labeling efficiency and stability of the complex, labeling studies were performed in the absence and presence of an antioxidant agent. DOK (1 mg) was dissolved in 0.9 % sodium chloride solution (1 mL) in four groups of vials. 30, 40, 50 and 100 μg stannous chloride was added to each individual group. Each group has three vials and labeling was performed in the absence and presence (0.05 and 0.1 mg) of ascorbic acid. Freshly eluted 37 mBq ^{99m}Tc was added to each vial. The vials were shaken for 30 s, filtered through a 0.22 μm pore size membrane filter and incubated for 15 min at room temperature. The labeling efficiency was analyzed by RTLC.

Antioxidant agent effect on labeling efficiency was also performed with the formulations described above by using stannous tartrate instead of stannous chloride.

Effect of incubation time on labeling

After radiolabeling the RP of the complexes were investigated with RTLC studies which performed at different time intervals (5, 15, 30, 45 and 60 min postlabeling).

Effect of pH on labeling

The RP of a ^{99m}Tc radiopharmaceutical is highly dependent on the pH of the kit mixture. The effect of the pH on labeling efficiency of ^{99m}Tc-DOX was examined for pH 4.75–7.4.

In vitro stability

After labeling DOX with ^{99m}Tc, the preparation was left at room temperature for 6 h. The labeling stability of the complex was evaluated by RTLC studies for every hour.

RTLC procedure

Whatman No:1 papers and silica gel coated (SG) plates were used as stationary phases. Free ^{99m}Tc was determined by using SG plates as stationary phase and acetone as the mobile phase. Reduced/Hydrolyzed (R/H) ^{99m}Tc was determined by Whatman No:1 papers which developed in Acetonitrile/Water/Trifluoroacetic acid (ACN/W/TFA; 50/25/1.5) solvent system. The radioactivity on plates was measured using a TLC scanner (Bioscan AR 2000), and RP % of ^{99m}Tc was calculated from the following equation by subtracting from 100 the sum of measured impurities percentages.

$$RP\,\% = 100 - \left(\text{Free}\,^{99m}\text{Tc}\,\% + \text{R/H}\,^{99m}\text{Tc}\,\%\right)$$

Radio high performance liquid chromatography (RHPLC) analysis

The compounds were further analyzed by an Ultra-HPLC system equipped with a C_{18} column connected to a photodiode array detector (PDA) and additional NaI gamma detector for the ^{99m}Tc compounds. The flow rate was 1.0 mL/min for analytical runs. In all runs the eluent was 0.1 % TFA in H_2O (solvent A) and 0.1 % TFA in CH_3CN (solvent B). For the analytical control and semi preparative separation the method was as follows: 0–2.5 min, 95 % solvent A-5 % solvent B; 2.5–5 min, 50 % solvent A 50 %-solvent B; 5–7.5 min 20 % solvent A 80 %-solvent B; 7.5–10 min 5 % solvent A-95 % solvent B.

Freeze dry kit formulation and radiolabeling

After observing the effect of different parameters on labeling, subsequently two lots were prepared as follows: Lot-A was prepared by mixing 1 mg DOX, 30 µg stannous tartrate and 0.1 mg ascorbic acid. Lot-B was prepared by mixing 1 mg DOX, 30 µg stannous chloride and 0.1 mg ascorbic acid. Both kits solution were filtered through a 0.22 µm pore size filter to glass vials, frozen in a freezer at −80 °C and lyophilized at −20 °C for 24 h.

In vitro radiolabeling studies were performed with 37 mBq ^{99m}Tc for the regard to radiation safety of personnel and the environment. Since, human studies with radiopharmaceuticals are performed with higher radiation doses, labeling with freeze dry kits also examined with higher doses of ^{99m}Tc ranged between 37 and 370 mBq.

Stability of the freeze dry kits

The kits were stored at +5 ± 3 °C (in a refrigerator) and +25 ± 2 °C/60 % HR ± 5 % (in a stability cabin). The labeling efficiency of the ^{99m}Tc-DOX in each kit was checked at different time intervals up to 6 months. The kits were reconstituted with 37 MBq of ^{99m}Tc-pertechnetate and the percentage of the radiolabeled drug was determined by RTLC and RHPLC studies.

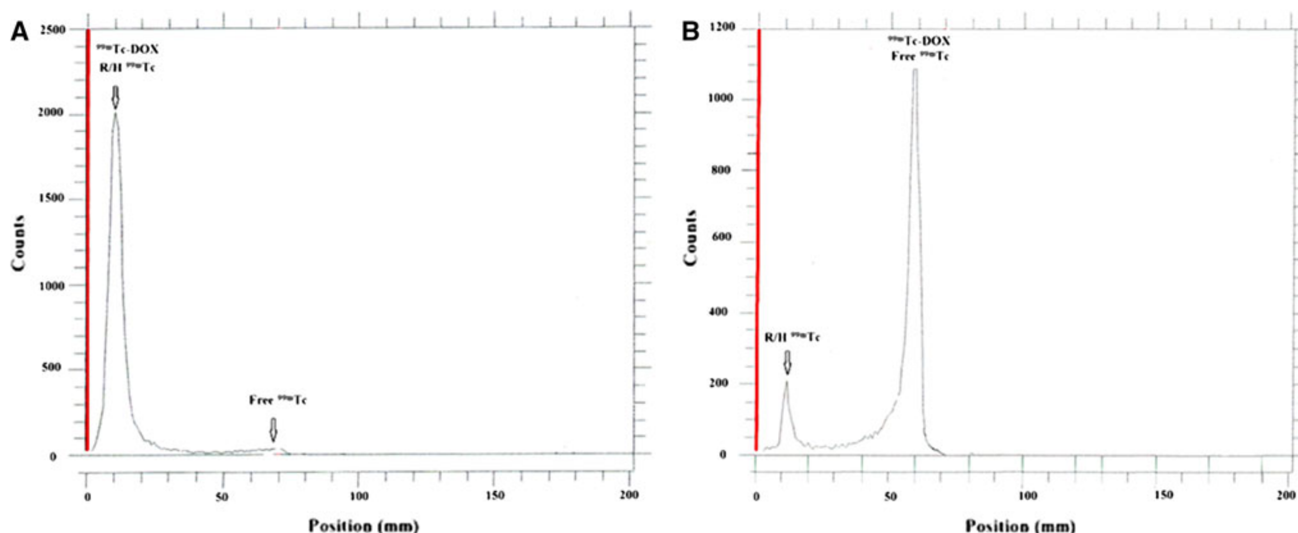

Fig. 1 RTLC chromatogram of ^{99m}Tc-DOX in different mobile phases: **a** Acetone, **b** ACN/W/TFA (50/25/0.15)

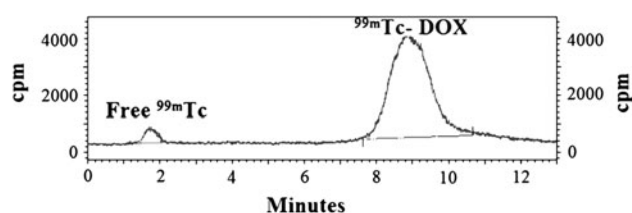

Fig. 2 RHPLC analysis of ^{99m}Tc-DOX

Statistical analysis

The calculation of means and standard deviations were made on Microsoft Excel. *t* test was used to determine statistical significance. Differences at the 95 % confidence level ($p < 0.05$) were considered significant.

Results and discussion

Radiolabeling studies

A new, simple, rapid and efficient direct method for labeling of DOX with ^{99m}Tc was developed. Labeling efficiency of the ^{99m}Tc-DOX was assessed by both RTLC and RHPLC studies. Two solvent systems were used to distinguish and quantify the amounts of radioactive contaminants (Free ^{99m}Tc, R/H ^{99m}Tc).

Radiochemical purity and stability of ^{99m}Tc-DOX were assessed by RTLC and RHPLC studies. In RTLC using acetone as the solvent, free ^{99m}Tc moved with the solvent front, while ^{99m}Tc-DOX and R/H ^{99m}Tc remained at the spotting point. R/H ^{99m}Tc was determined by using ACN/W/TFA (50/25/0.15) as the mobile phase where the R/H ^{99m}Tc remained at the point of spotting while free ^{99m}Tc and ^{99m}Tc-DOX moved with the solvent front. The RTLC chromatogram of ^{99m}Tc-DOX was presented in Fig. 1.

The RP of ^{99m}Tc-DOK was >95 %, acquired via RTLC and also RHPLC. The RHPLC chromatogram was presented in Fig. 2 and showed two peaks, first one was corresponds to free ^{99m}Tc, while the second peak for ^{99m}Tc-DOK. The elution times for ^{99m}Tc and ^{99m}Tc-DOX complex was established as 1.942, 8.873 min respectively.

Effect of reducing agent on labeling

To investigate the effect of reducing agent type on labeling yield, labeling experiments were performed with the same amounts of active ingredient, reducing agent and radionuclide including formulations. Two groups of formulation were prepared. While first group include stannous chloride as reducing agent, second group include stannous tartrate instead. Comparative results for both formulations were shown in Fig. 3.

By increasing the amount of stannous chloride, the labeling efficiency was decreased while the amounts of colloid increased (Fig. 3). By increasing the reducing agent concentration over the optimum values, the labeling yield was slightly decreased. According to radiolabeling studies, no significant differences between groups were assessed.

Effect of antioxidant agent on labeling

In the presence of ascorbic acid stability of the complex was increase slightly while labeling efficiency for early hours was not affected significantly. Increasing of the labeling efficiency takes place due to a decreasing of the R/H ^{99m}Tc percentage. The results obtained in these experiments revealed that, maximum RP was obtained with

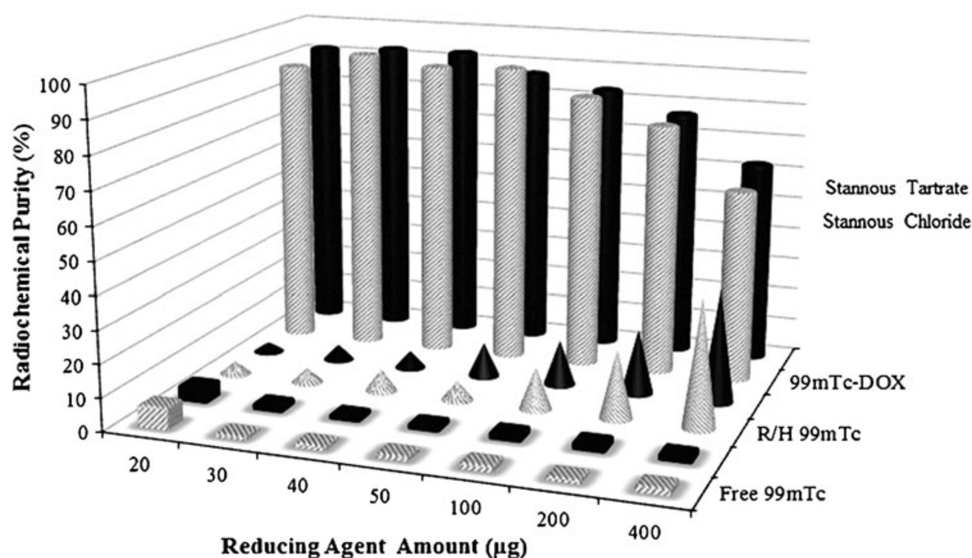

Fig. 3 Variation of the Free ^{99m}Tc, R/H ^{99m}Tc and ^{99m}Tc-DOX as a function of stannous chloride and stannous tartrate amount

Table 1 30 μg stannous chloride including formulations radiochemical purity in the absence and presence of ascorbic acid

Time (h)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		0.05	0.1
0.25	92.92 ± 0.99	94.71 ± 0.43	94.89 ± 0.13
1	93.52 ± 0.64	95.61 ± 1.07	95.93 ± 0.52
2	93.51 ± 1.90	95.58 ± 0.55	96.28 ± 0.17
3	96.21 ± 0.89	96.36 ± 0.62	97.51 ± 0.46
4	94.46 ± 1.65	94.06 ± 1.17	95.79 ± 0.82
5	94.12 ± 0.19	94.68 ± 0.52	96.17 ± 0.87
6	94.23 ± 0.33	96.33 ± 1.49	97.28 ± 0.57

Table 2 40 μg stannous chloride including formulations radiochemical purity in the absence and presence of ascorbic acid

Time (h)	Absence of ascorbic acid	Radiochemical purity (%)	
		Ascorbic acid (mg)	
		0.05	0.1
0.25	91.45 ± 0.84	93.17 ± 0.76	91.35 ± 2.33
1	90.30 ± 0.57	93.70 ± 0.37	91.98 ± 2.01
2	93.33 ± 0.61	94.43 ± 2.74	96.09 ± 0.34
3	93.04 ± 1.96	94.50 ± 1.12	94.72 ± 0.30
4	88.34 ± 2.59	93.58 ± 1.06	92.67 ± 1.01
5	89.96 ± 0.36	92.47 ± 0.16	93.39 ± 1.57
6	89.66 ± 2.06	88.46 ± 3.27	91.58 ± 0.72

Table 3 50 μg stannous chloride including formulations radiochemical purity in the absence and presence of ascorbic acid

Time (h)	Radiochemical purity		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		0.05	0.1
0.25	94.06 ± 1.66	93.13 ± 1.56	94.26 ± 0.35
1	91.96 ± 1.23	90.78 ± 2.42	93.88 ± 0.18
2	89.63 ± 2.38	90.50 ± 2.15	90.75 ± 0.15
3	89.54 ± 1.93	89.34 ± 0.91	91.47 ± 3.36
4	89.32 ± 1.87	90.58 ± 4.18	91.48 ± 3.17
5	89.69 ± 2.05	89.42 ± 2.75	94.07 ± 0.72
6	88.10 ± 2.35	92.59 ± 7.06	92.74 ± 2.12

30 μg stannous chloride, 0.1 mg ascorbic acid and 30 μg stannous tartrate, 0.1 mg ascorbic acid including formulations (Tables 1–8). Effect of incubation time, pH and stability of these formulations were investigated to evaluate the optimum labeling conditions for DOX.

Table 4 100 μg stannous chloride including formulations radiochemical purity in the absence and presence of ascorbic acid

Time (h)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		0.05	0.1
0.25	90.23 ± 1.21	86.95 ± 3.46	86.23 ± 2.10
1	92.53 ± 4.45	78.14 ± 8.78	80.44 ± 1.11
2	87.43 ± 1.46	82.14 ± 2.00	82.58 ± 2.41
3	83.48 ± 4.31	71.71 ± 9.02	72.41 ± 3.13
4	83.56 ± 4.65	81.11 ± 3.70	82.56 ± 1.58
5	81.82 ± 3.24	72.94 ± 3.31	83.22 ± 4.52
6	80.20 ± 3.70	78.58 ± 3.14	80.09 ± 2.29

Table 5 30 μg stannous tartrate including formulations radiochemical purity in the absence and presence of ascorbic acid

Time (h)	Absence of ascorbic acid	Radiochemical purity (%)	
		Ascorbic acid (mg)	
		0.05	0.1
0.25	93.42 ± 0.54	95.70 ± 0.21	96.57 ± 0.46
1	93.12 ± 0.23	95.41 ± 0.42	96.07 ± 0.24
2	93.75 ± 0.32	95.42 ± 0.72	96.63 ± 0.93
3	93.55 ± 0.15	95.62 ± 0.27	96.76 ± 0.34
4	93.92 ± 0.65	96.33 ± 0.32	97.02 ± 0.23
5	93.77 ± 0.14	96.18 ± 0.53	96.42 ± 0.14
6	93.93 ± 0.33	96.90 ± 0.11	96.71 ± 0.13

Table 6 40 μg stannous tartrate including formulations radiochemical purity in the absence and presence of ascorbic acid

Time (h)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		0.05	0.1
0.25	91.60 ± 0.22	93.31 ± 0.25	94.20 ± 0.33
1	91.37 ± 0.36	93.39 ± 0.23	94.50 ± 0.64
2	91.98 ± 0.75	93.48 ± 0.37	94.19 ± 0.72
3	91.80 ± 0.46	94.18 ± 0.61	94.26 ± 0.26
4	92.90 ± 0.28	94.30 ± 0.73	93.95 ± 0.54
5	92.91 ± 0.39	94.34 ± 0.52	94.76 ± 0.28
6	92.35 ± 0.16	94.22 ± 0.58	94.94 ± 0.35

Effect of incubation time on labeling

30 μg stannous chloride, 0.1 mg ascorbic acid and 30 μg stannous tartrate, 0.1 mg ascorbic acid including formulations were labeled with 37 mBq ^{99m}Tc. After radiolabeling the RP of the complexes were investigated with RTLC studies which performed at 5, 15, 30, 45 and 60 min

Table 7 50 μg stannous tartrate including formulations radiochemical purity in the absence and presence of ascorbic acid

Time (h)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		0.05	0.1
0.25	87.93 ± 0.23	90.92 ± 0.64	92.24 ± 0.11
1	88.46 ± 0.31	91.20 ± 0.41	91.70 ± 0.26
2	88.99 ± 0.44	91.71 ± 0.36	91.85 ± 0.92
3	89.19 ± 0.73	91.44 ± 0.27	91.90 ± 0.45
4	90.30 ± 0.73	92.50 ± 0.75	91.97 ± 0.32
5	90.11 ± 0.92	92.21 ± 0.19	91.93 ± 0.74
6	90.06 ± 0.23	92.33 ± 0.32	92.42 ± 0.82

Table 8 100 μg stannous tartrate including formulations radiochemical purity in the absence and presence of ascorbic acid

Time (h)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		0.05	0.1
0.25	85.04 ± 0.38	89.55 ± 0.32	90.28 ± 0.35
1	84.60 ± 0.26	90.27 ± 0.27	90.64 ± 0.21
2	84.81 ± 0.37	90.21 ± 0.18	90.18 ± 0.13
3	84.71 ± 0.18	90.31 ± 0.63	90.70 ± 0.27
4	86.32 ± 0.29	90.71 ± 0.47	90.54 ± 0.85
5	86.75 ± 0.84	91.31 ± 0.91	91.00 ± 0.29
6	86.84 ± 0.15	91.76 ± 0.58	90.46 ± 0.11

postlabeling (Table 9). According to the experiments the RP of the complex was reached over 90 % in 5 min after labeling. Optimal radiolabeling yield was obtained at 15 min incubation period (∼95 %) and incubation for longer time intervals did not show any remarkable change.

Effect of pH on labeling

The effect of pH on labeling efficiency was examined for pH 4.75–7.4. According to experiments results the pH of the reaction medium was not found to play an important role in the labeling process (Fig. 4). While keeping other reaction conditions constant and varied the pH of the reaction from 4.75 to 7.4, there is no significant differences on labeling efficiency was observed.

Table 9 Effect of incubation time on radiochemical purity

Time (min)	Reducing agent	
	Stannous chloride Radiochemical purity (%)	Stannous tartrate
5	92.37 ± 0.93	92.19 ± 1.28
15	94.82 ± 1.12	94.90 ± 0.73
30	94.97 ± 0.52	95.02 ± 0.25
45	95.02 ± 1.21	95.18 ± 1.26
60	95.72 ± 0.61	95.27 ± 0.59

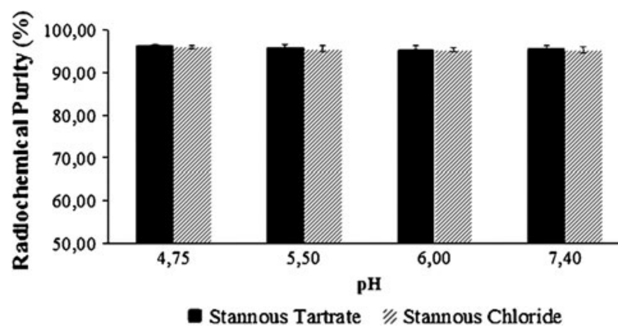

Fig. 4 Effect of the pH on labeling efficiency of ^{99m}Tc- DOX

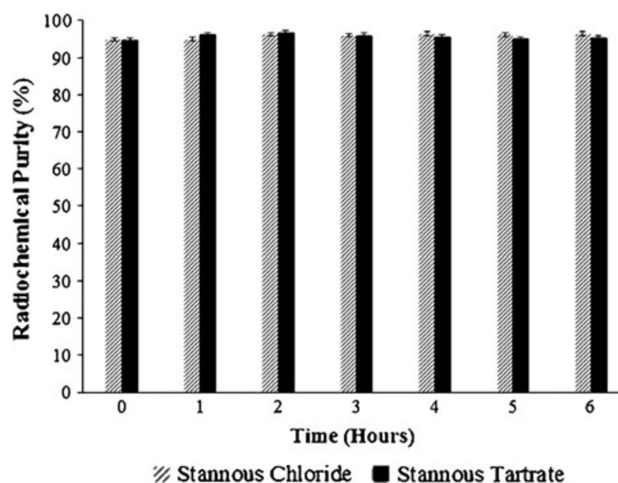

Fig. 5 ^{99m}Tc- DOX stability over 6 h

In vitro stability

The complex stability was checked up to 6 h at room temperature. During the incubation period the compound were found stable as determined by RTLC (Fig. 5).

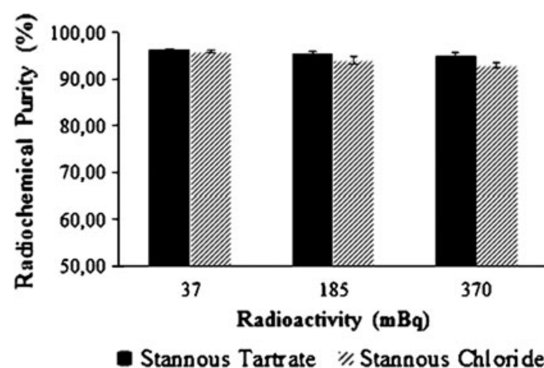

Fig. 6 Effect of radiation dose on radiochemical purity of ^{99m}Tc-DOX

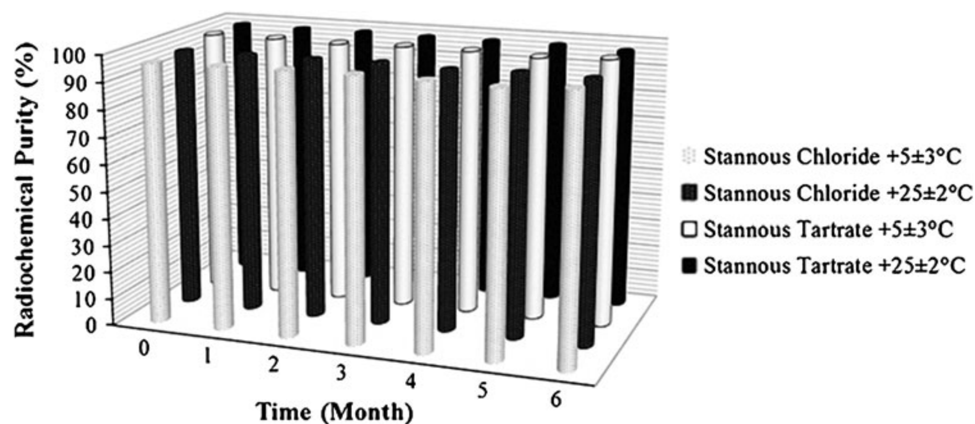

Fig. 7 Radiochemical purity of freeze dried kits at different incubation conditions and periods

Stability of the freeze dry kits

Kits were labeled with 37, 185 and 370 mBq ^{99m}Tc. Slightly decrease in RP was observed with increasing of radioactivity ($p < 0.05$) (Fig. 6).

The stability of the freeze dry kits were determined at 1, 2, 3, 4, 5 and 6 months after storage both at $+5 \pm 3$ °C (in a refrigerator) and $+25 \pm 2$ °C/60 % HR $\pm$ 5 % (in a stability cabin). According to experiments, both kits (Lot-A and Lot-B) were found stable up to 6 months without any significant decrease in labeling yield (Fig. 7) ($p > 0.05$).

The freeze dry kit preparation of a radiopharmaceutical leads to instant labeling by mixing with ^{99m}Tc at the time of use. The reconstituted kit solution was clear and did not contain any visible particle. The freeze dry kits developed in this study were found to be stable with a shelf –life of 6 months when preserved at both at $+5 \pm 3$ °C (in a refrigerator) and $+25 \pm 2$ °C/60 % HR $\pm$ 5 % (in a stability cabin). Based on the findings of this study, the complex easily formed by the reconstitution of these kits without any requirement for boiling and post-labeling purification [24].

Conclusion

DOX, is a broad spectrum of activity against a wide range of gram positive and gram negative pathogens [17, 18]. So far various antibiotics were labeled with ^{99m}Tc [5]. According to Örümlü's studies, DOX was labeled with ^{99m}Tc [16]. But radiochemical impurities weren't separated from the complex with RTLC studies. The aim of this study was to standardize and develop a new, simple and ready to use kit of DOX for radiolabeling with ^{99m}Tc. ^{99m}Tc-DOX was developed and standardized under varying conditions of reducing and antioxidant agent concentration, pH, radioactivity dose and reducing agent type. Labeling studies were performed by changing the selected parameters one by one and optimum labeling conditions were determined. After observing the conditions for maximum labeling efficiency and stability, lyophilized freeze dry kits were prepared accordingly.

Simple method for radiolabeling of DOX with ^{99m}Tc has been developed and standardized. Labeling efficiency of ^{99m}Tc-DOX was assessed by both RTLC and RHPLC and found higher than 95 %. The resulting complex was quite stable and labeling yield >95 % was maintained for up to 6 h. Two different freeze dry kits was developed and evaluated. Based on the data obtained from this study, both products was stable for 6 months with high labeling efficiency. To examine the role of ^{99m}Tc-DOX in imaging of infection at early stage, in vivo studies are in progress.

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