ISSN 1066-3622, Radiochemistry, 2021, Vol. 63, No. 4, pp. 512–519. © Pleiades Publishing, Inc., 2021.

A Comparative Study of Stannous Chloride and Sodium Borohydride as Reducing Agents for the Radiolabeling of 2,3,7,8,12,13,17,18-Octaethyl-21*H*,23*H*-Porphine with Technetium-99*m* for Tumor Imaging

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Received December 10, 2018; revised June 1, 2021; accepted June 7, 2021

Abstract—2,3,7,8,12,13,17,18-Octaethyl-21*H*,23*H*-porphine (OEP) was successfully radiolabeled with technetium-99*m* by direct technique using two different reducing agents: stannous chloride and sodium borohydride. High radiochemical yield (97%) and in vitro stability for up to 8 h were obtained using sodium borohydride. Paper chromatography (PC), paper electrophoresis, and high-performance liquid chromatography (HPLC) were used to evaluate the radiochemical yield and identify the purity of the final product. Biodistribution studies of the ^{99m}Tc-OEP complex (prepared using sodium borohydride) in tumor-bearing mice showed high target/nontarget (T, right thigh/NT, left thigh) ratio of 4.40 ± 0.12 at 60 min post injection, suggesting that this complex can be used as a solid tumor imaging agent.

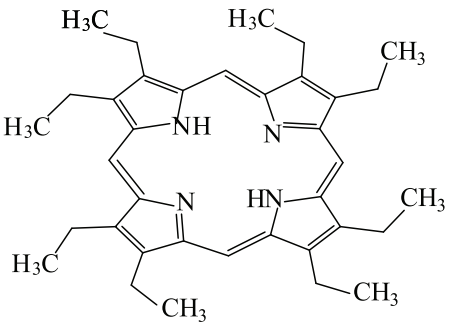
Keywords: porphyrin, technetium-99*m*, stannous chloride, sodium borohydride, tumor, imaging

DOI: 10.1134/S1066362221040159

INTRODUCTION

Technetium-99m complexes are routinely used in the diagnosis of many cancer and non-cancer diseases involving various organs such as heart, bones, kidneys, liver, lungs, thyroid, etc., because ^{99m}Tc has favorable nuclear properties (γ-ray energy 140 keV, $t_{1/2}$ = 6 h) and is cheap and readily available. These good imaging characteristics and economic reasons make ^{99m}Tc an ideal radionuclide for drug radiolabeling [1–5]. ^{99m}Tc is available as pertechnetate ion from ^{99}Mo/^{99m}Tc generators, but the pertechnetate ion cannot be attached to any organic moiety. Therefore, reduction of pertechnetate ion to a lower oxidation state is essential for ^{99m}Tc complex formation in high yield. Several reducing agents were used for this purpose such as Sn(II), Fe(II) ascorbate, and sodium borohydride. It is important here to mention that stannous chloride is the most commonly used reducing agent in most preparations of ^{99m}Tc-labeled compounds. Porphyrins have been extensively studied as potential photosensitizers in photodynamic therapy (PDT), because they are able to accumulate in many kinds of cancer cells and exhibit favorable magnetic and optical properties [6–8]. PDT technique has many disadvantages such as low efficacy in treating large tumors and low sensitivity of detection [9–11]. A wide variety of porphyrin derivatives with various types of peripheral moieties have been radiolabeled with several medically important radionuclides for developing an ideal porphyrin-based tumor-specific agent [12–23]. In this paper, we have reported the use of both stannous chloride (to give complex I) and sodium borohydride (to give complex II) as reducing agents for reduction of ^{99m}Tc(VII) ions and subsequent complexation with OEP (see structure below). The radiotracer, ^{99m}Tc-OEP complex II prepared by thy sodium borohydride labe-

ling procedure, was purified chromatographically, and its biodistribution in tumor-bearing mice was studied.



EXPERIMENTAL

Chemicals. OEP was purchased from Sigma–Aldrich. All other chemicals were of analytical grade and were used without additional purification. Deionized water was used in all experiments for preparing the solutions. Technetium-99m was eluted as $^{99m}TcO_4^-$ from a $^{99}Mo/^{99m}Tc$ generator (radionuclidic purity 99.99%, radiochemical purity 99.99%, activity 1 Ci, Elutec, Brussels, Belgium).

Labeling procedure. The reaction mixture volume was fixed at ~2000 μL. An accurately weighed portion (1 mg) of OEP was dissolved in a mixture of equal volumes of DMSO and ethanol [1 mg : 0.5 mL : 0.5 mL, in total 1 mg/ mL)] and was transferred into an evacuated penicillin vial. Then, a portion of this solution was taken with a sterilized syringe. The required amount of the $SnCl_2$ (20–100 μg of $SnCl_2$; optimum: 60 μg/mL] (to obtain complex I) or $NaBH_4$ (10–50 mg; optimum: 20 mg/mL) solution was added, and the required pH (2–12; optimum: pH 6 in the case of Sn(II) and pH 10 in the case of $NaBH_4$) of the mixture was adjusted with 0.1 N NaOH and phosphate buffer, after which the volume of the mixture was adjusted to 1 mL with nitrogen-purged distilled water. 1 mL of freshly eluted $^{99m}TcO_4^-$ (~200–400 MBq) was added. The reaction mixture was vigorously shaken and allowed to react at room temperature for 30 min without filtration. When studying the effect of various factors on the reaction, all the variables were kept constant except the factor under study until the optimum conditions are achieved. The radiolabeling yields were determined and checked by paper electrophoresis, paper chromatography (PC), and high-performance liquid chromatography (HPLC).

Quality control. The radiochemical yield of the ^{99m}Tc-OEP complex was determined by ascending paper chromatographic technique using two strips of Whatman no. 1 paper; one strip was developed with acetone, and the other was developed with saline. A spot of the ^{99m}Tc-OEP solution was applied using a hypodermic syringe onto the spotting point, and then the strip was developed in an ascending manner in a closed jar filled with N_2 gas to prevent oxidation of the labeled complex spot. After complete development, the strip was dried, cut into 1 cm pieces, and separately counted using the NaI(Tl) γ-ray scintillation counter to determine the ratio of the hydrolyzed ^{99m}Tc as colloid, free $^{99m}TcO_4^-$, and ^{99m}Tc-OEP complex. The Shimadzu HPLC device consisted of LC-9A pumps, a Rheodyne injector, a UV spectrophotometer (SPD-6A) operated at a wavelength of 285 nm, a NaI(Tl) detector used for radioactivity measurements, and a Lichrosorb reversed-phase column (C-8, 250 × 4.6 mm, 5 μm), with methanol : water (50 : 50) used as a mobile phase at a flow rate of 0.25 mL min^{-1}. Fractions of 0.25 mL volume were collected separately using a fraction collector to a total volume of 20 mL and were counted in a well-type NaI(Tl) γ-counter (BLC-20, BUCK Scientific).

Biodistribution study. The study was approved by the animal ethics committee and was in accordance with the guidelines set out by the Egyptian Atomic Energy Authority (EAEA).

Induction of tumor in mice. The biodistribution study was done in tumor-bearing mice. The parent tumor line (Ehrlich ascites carcinoma) was withdrawn from 7-days-old donor female Swiss Albino mice and diluted with sterile physiological saline solution to give 12.5×10^6 cells/mL. Exactly 0.2 mL of the solution was then injected intramuscularly in the right thigh to produce a solid tumor. The animals were maintained for 4–6 days until the tumor development became apparent.

Biodistribution assay. Normal Swiss Albino mice (20–30 g) were intravenously injected with 100 μL (50–150 MBq) of sterile solutions of the purified complexes (I, II) via the tail vein and kept alive in metabolic cages for different time intervals under normal conditions (5, 15, 30, 60, and 120 min post injection (p.i.)). Samples of fresh blood, bones, and muscles were collected in preweighed vials and counted. For the quantitative

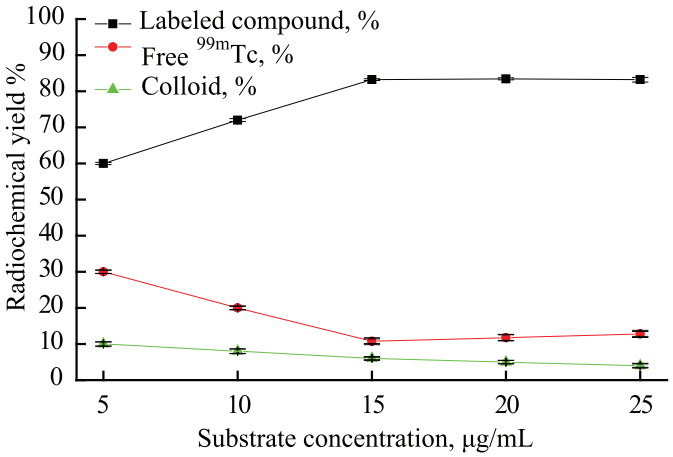


Fig. 1. Radiochemical yield of ^{99m}Tc-OEP complex I with $SnCl_2$ reducing agent as a function of OEP concentration [20 μL (~50 MBq) of $^{99m}TcO_4^-$ solution, 60 μg/mL Sn(II), pH 6, reaction time 15 min, 25 ± 2°C].

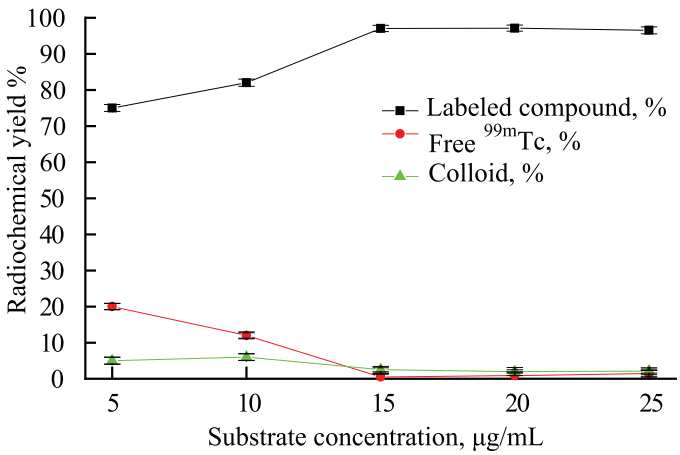


Fig. 2. Radiochemical yield of ^{99m}Tc-OEP complex II with $NaBH_4$ reducing agent as a function of OEP concentration [20 μL (~50 MBq) of $^{99m}TcO_4^-$ solution, 20 mg/mL $NaBH_4$, pH 10, reaction time 15 min, 25 ± 2°C].

determination of the organ distribution, five mice were used for each experiment. The mice were sacrificed at different times post injection. Various organs were removed and counted. The average percent values of the administrated dose per gram (% ID/g ± SD) were calculated. Blood, bones, and muscles were assumed to make up 7, 10, and 40%, respectively, of the total body weight. Corrections were made for the background radiation and decay during the experiments. The data were estimated using the one-way ANOVA test. All the results are given as mean ± SD. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Factors Affecting the Radiochemical Yield of ^{99m}Tc-OEP

The factors affecting the radiochemical yield of ^{99m}Tc–OEP complex were studied. These factors include the substrate concentration, reducing agent concentration, pH of reaction mixture, reaction time, and reaction temperature. The radiochemical yield in all the cases was determined using the paper chromatographic technique.

Effect of OEP concentration. Figures 1 and 2 summarize the effect of the OEP concentration on the radiochemical yield. At low OEP concentration, the radiochemical yield was low, because this concentration was insufficient to chelate all the reduced ^{99m}Tc. The radiochemical yield increased with increasing the OEP concentration until the maximum was reached at its 15 μg/mL concentration: 83.2 ± 0.5% in the case of Sn(II) and 97 ± 0.6% in the case of $NaBH_4$. Further increase in the OEP concentration over 15 μg/mL did not led to significant changes in the yields of both complexes I and II. Each experiment was repeated three times.

Effect of Sn(II) concentration. Figure 3 shows how the Sn(II) concentration influences the radiochemical yield of the ^{99m}Tc-OEP complex. At too small amounts of $SnCl_2$, the $^{99m}TcO_4^-$ reduction was incomplete. As the Sn(II) concentration was increased from 20 to 100 μg (optimum: 60 μg/mL Sn(II)), the radiochemical yield increased from 56.9 ± 0.4 to 83.2 ± 0.5%, which was the maximum. Further increase in the Sn(II) concentration over 60 μg/mL led to a decrease in the radiochemical yield, probably because of increasing hydrolysis of Sn(II), leading to the formation of Sn colloids which can compete with the ligand for the reduced technetium and form the ^{99m}Tc–Sn colloid [24–27].

Effect of $NaBH_4$ concentration. The optimum concentration of sodium borohydride, ensuring the maximum radiochemical yield of 97 ± 0.9%, was 20 mg/mL as shown in Fig. 4. At lower $NaBH_4$ concentrations, high fraction of free pertechnetate remained; i.e., these $NaBH_4$ concentrations were insufficient for complete reduction of pertechnetate to form ^{99m}Tc-OEP complex. No further increase in the radiochemical yield was observed at the $NaBH_4$ concentration increased further over 20 mg/mL. The results obtained show that

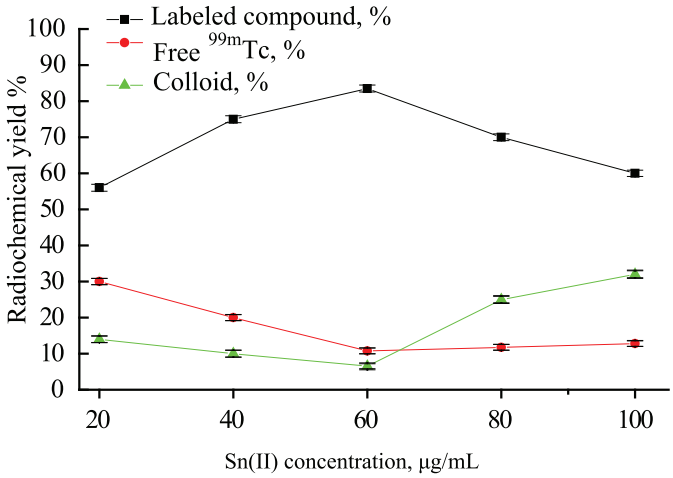


Fig. 3. Radiochemical yield of ^{99m}Tc-OEP complex I as a function of Sn(II) concentration [15 μg/mL OEP, 20 μL (~50 MBq) of $^{99m}TcO_4^-$ solution, pH 6, reaction time 15 min, 25 ± 2°C].

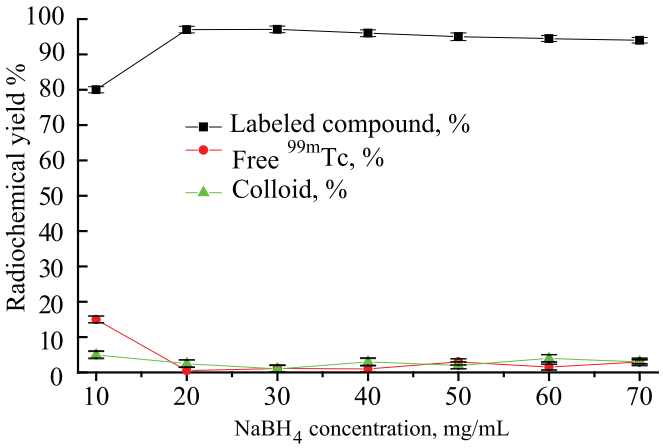


Fig. 4. Radiochemical yield of ^{99m}Tc-OEP complex II as a function of $NaBH_4$ concentration [15 μg/mL OEP, 20 μL (~50 MBq) of $^{99m}TcO_4^-$ solution, pH 10, reaction time 15 min, 25 ± 2°C].

sodium borohydride as a reducing agent ensures higher radiochemical yield than stannous chloride does.

Effect of pH of the reaction mixture. The dependence of the radiochemical yield on pH of the reaction mixture is shown in Figs. 5 and 6. The radiochemical yield of ^{99m}Tc-OEP was low at acidic pH values. This can be attributed to the protonation of the OEP amino groups which coordinate with reduced technetium to form ^{99m}Tc-OEP [26]. The maximum radiochemical yield of 83.2 ± 0.8 and 97 ± 0.5% was obtained at pH 6 and 10 with Sn(II) and $NaBH_4$, respectively. With an increase in pH above the optimum value, the radiochemical yield significantly decreased, and this may be due to partial hydrolysis of the complex and oxidation of ^{99m}Tc(V) to pertechnetate $^{99m}TcO_4^-$ at high OH^- concentration. This is a major reason for the emergence of colloids.

Effect of reaction temperature. The maximum radiochemical yield of ^{99m}Tc-OEP, 83.23 ± 0.19 and 97 ± 0.9% with Sn(II) and $NaBH_4$, respectively, was obtained at 25 ± 2°C. Increasing the reaction temperature to 40, 60, 80, and 100°C caused a significant decrease in the radiochemical yield, which may be due to the thermal decomposition of ^{99m}Tc-OEP.

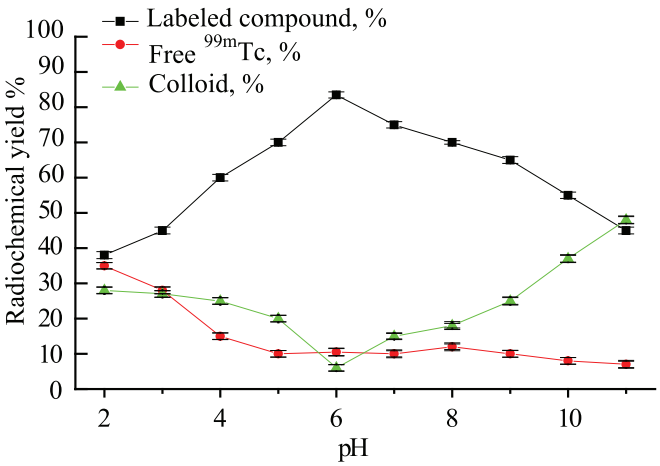


Fig. 5. Radiochemical yield of ^{99m}Tc-OEP complex I as a function of pH [15 μg/mL OEP, 20 μL (~50 MBq) of $^{99m}TcO_4^-$ solution, 60 μg/mL Sn(II), reaction time 15 min, 25 ± 2°C].

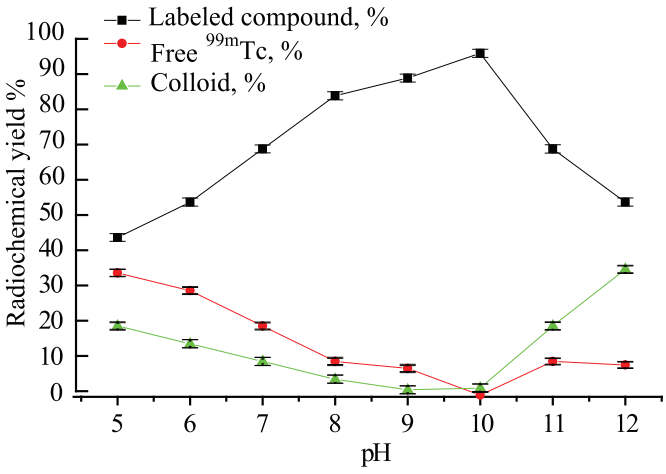


Fig. 6. Radiochemical yield of ^{99m}Tc-OEP complex II as a function of pH [15 μg/mL OEP, 20 μL (~50 MBq) of $^{99m}TcO_4^-$ solution, 20 mg/mL $NaBH_4$, reaction time 15 min, 25 ± 2°C].

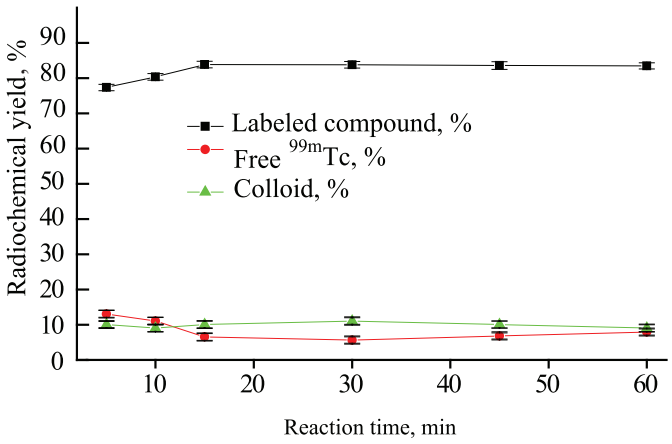


Fig. 7. Radiochemical yield of ^{99m}Tc-OEP complex I as a function of reaction time [15 μg/mL OEP, 20 μL (~50 MBq) of $^{99m}TcO_4^-$ solution, 60 μg/mL Sn(II), pH 6, 25 ± 2°C].

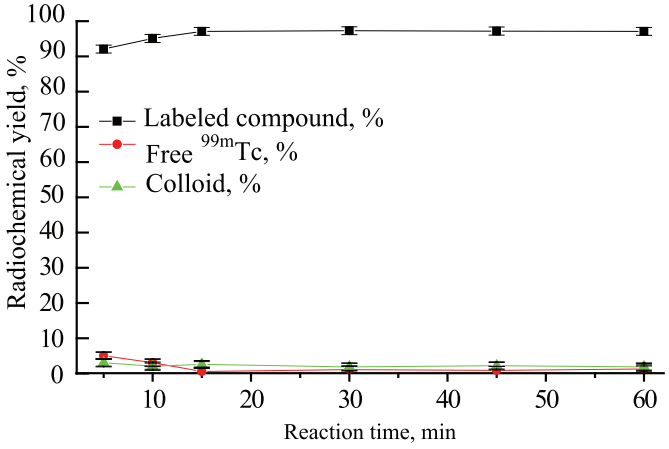


Fig. 8. Radiochemical yield of ^{99m}Tc-OEP complex II as a function of reaction time [15 μg/mL OEP, 20 μL (~50 MBq) of $^{99m}TcO_4^-$ solution, 20 mg/mL $NaBH_4$, pH 10, 25 ± 2°C].

In vitro stability of ^{99m}Tc-OEP. The stability of ^{99m}Tc-OEP was studied in order to determine the suitable time for injection to avoid the formation of the undesired products that result from the oxidation and γ-radiolysis of the labeled compound during storage post labeling with technetium. These undesired radioactive products may be accumulated in non-target organs. The results showed that ^{99m}Tc-OEP complex was stable (maximum yield 97 ± 0.5%) with no significant decrease up to 8 h when using $NaBH_4$, whereas with Sn(II) the stability of ^{99m}Tc-OEP complex decreased after 2 h post labeling. This may be due to the fact that the optimum Sn(II) concentration used (60 μg/mL) was very low and insufficient to prevent the reoxidation of ^{99m}Tc; on the other hand, sodium borohyride rapidly reduces $^{99m}TcO_4^-$ in dilute aqueous solution and excess $NaBH_4$ is rapidly hydrolyzed to boric acid, which shows a good antioxidant activity resulting in stabilization of the reaction mixture [28–30].

Effect of reaction time. The dependence of the radiochemical yield on the reaction time is shown in Figs. 7 and 8. At short reaction times, the radiochemical yield was low (cf. data from [31, 32]). Increasing the reaction time to 15 min caused an increase in the radiochemical yield to 83.2 ± 1.0 and 97 ± 0.9% for Sn(II) (complex I) and $NaBH_4$ (complex II), respectively. Further increase in the reaction time over 15 min had a slight effect on the radiochemical yield.

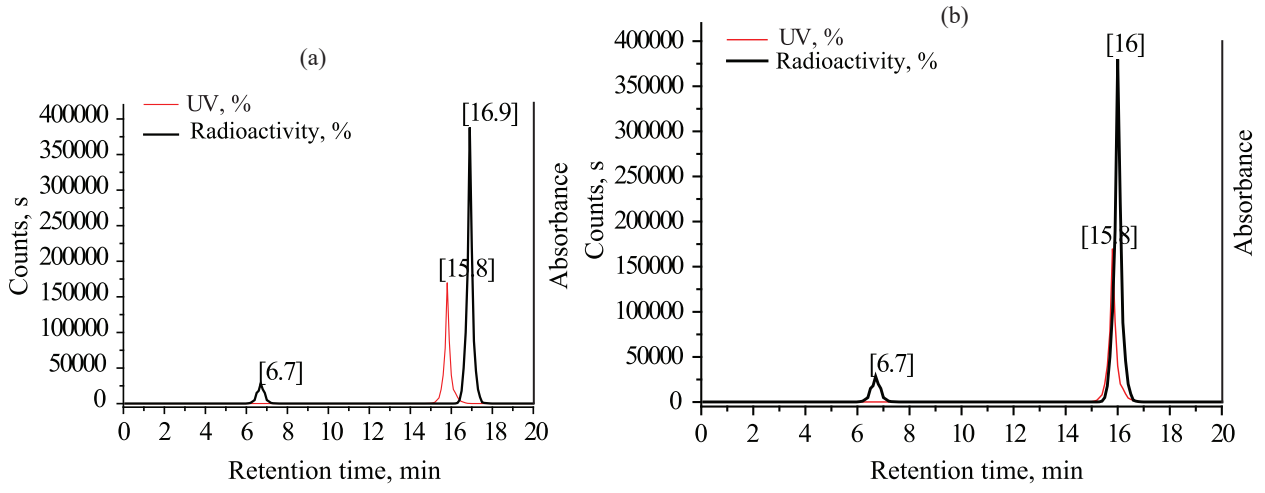


Fig. 9. HPLC radiochromatogram of ^{99m}Tc-OEP complexes (a) I and (b) II and UV profile of OEP.

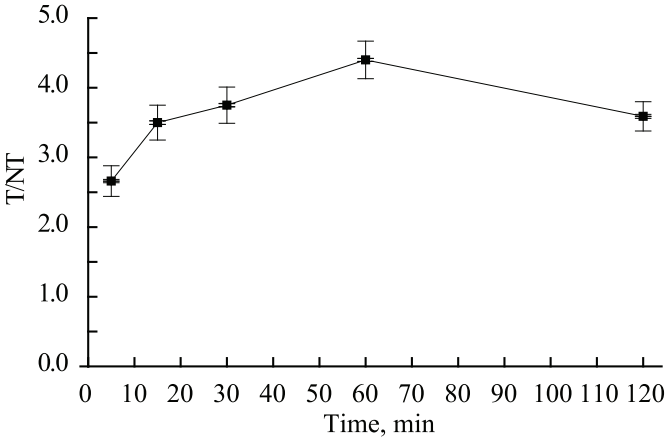


Fig. 10. Variation of T/NT for ^{99m}Tc(NaBH$_4$)-OEP complex II in solid tumor bearing mice with time post injection.

Radiochemical yield assay of ^{99m}Tc-OEP complex. In the above study, the radiochemical yield of ^{99m}Tc-OEP was determined using ascending paper chromatography [34–37]. The conditions were optimized, and the product obtained under optimum conditions was subjected to HPLC. A 10-μL aliquot of the reaction mixture containing complex I or II reaction mixture was injected into an RP-18 column. The results are shown in Fig. 9. The retention times of pertechnetate detected by NaI(Tl) detector (BLC-20, BUCK Scientific) used for radioactivity measurements, OEP (UV detection), and the complex are 6.7, 15.8, and 16.9 (complex I) or 16 min (complex II), respectively.

Biodistribution of ^{99m}Tc(NaBH$_4$)-OEP (complex II). The biodistribution of ^{99m}Tc-OEP in solid tumor bearing mice is shown in Table 1. The low stomach uptake during 2 h confirms in vivo stability of ^{99m}Tc(NaBH$_4$)-OEP [31–33]. The kidney uptake increased to 30.19 ± 0.15% (here and hereinafter, % of injected dose (ID) per gram of organ or tissue) at 30 min p.i. and decreased to 9.2 ± 0.9% at 2 h p.i. The liver uptake increased to 9.18 ± 0.22% at 30 min p.i. and decreased to 2.87 ± 0.07% at 2 h p.i. This fact demonstrates renal and hepatobiliary excretion of the radiotracer. The solid tumor uptake (right thigh) was high at 60 min p.i., which clearly indicates the ability of ^{99m}Tc(NaBH$_4$)-OEP to accumulate and localize selectively in solid tumor sites. The selectivity of ^{99m}Tc(NaBH$_4$)-OEP was evaluated by the T/NT ratio between tumor muscle (muscle of right thigh) and normal muscle (muscle of left thigh). Figure 10 shows the T/NT ratio of ^{99m}Tc-OEP in solid tumor bearing mice. As can be seen, ^{99m}Tc(NaBH$_4$)-OEP is highly selective to the tumor cells with an accumulation ratio (T/NT) of 3.75 ± 0.01 at 30 min p.i. To compare, the tumor-to-muscle (T/M) ratios obtained were 2.0 and 3.19 for ^{99m}Tc(V)-DMSA (DMSA = dimercaptosuccinic acid) and ^{177}Lu-labeled porphyrin-*p*-NH$_2$-benzyl-DOTA (*p*-aminobenzyl-1,4,7,10-tetraazacyclododecane-

Table 1. Biodistribution of ^{99m}Tc-OEP complex II in tumor bearing mice at different times (mean ± SEM, mean of five experiments)

Organs and body fluids	% ID/g at indicated time post injection				
	5 min	15 min	30 min	60 min	120 min
Blood	19.6 ± 0.80	9.1 ± 0.30	4.2 ± 1.00	3.1 ± 0.30	2.00 ± 0.11
Bones	1.12 ± 0.18	1.2 ± 0.30	1.11 ± 0.19	0.95 ± 0.11	0.90 ± 0.01
Brain	1.01 ± 0.01	0.95 ± 0.02	0.88 ± 0.01	0.80 ± 0.01	0.66 ± 0.00
Heart	1.1 ± 0.11	1.11 ± 0.01	1.0 ± 0.03	0.91 ± 0.01	0.90 ± 0.02
Liver	5.65 ± 0.12	7.7 ± 0.50	9.18 ± 0.22	4.55 ± 0.01	2.87 ± 0.07
Kidneys	16.6 ± 1.00	23.8 ± 1.00	30.19 ± 0.15	19.3 ± 0.90	9.2 ± 0.90
Spleen	1.1 ± 0.02	1.12 ± 0.01	1.24 ± 0.02	1.10 ± 0.01	0.95 ± 0.00
Intestine	3.19 ± 0.15	4.11 ± 0.17	4.77 ± 0.03	3.11 ± 0.01	2.12 ± 0.03
Stomach	1.12 ± 0.02	1.10 ± 0.01	1.00 ± 0.02	0.95 ± 0.01	0.91 ± 0.00
Tumor muscle (right thigh)	14.55 ± 0.19	15.3 ± 1.00	13.33 ± 0.09	12.77 ± 0.19	9.1 ± 0.90
Normal muscle (left thigh)	5.47 ± 0.11	4.38 ± 0.22	3.55 ± 0.01	2.90 ± 0.19	2.54 ± 0.01
T/NT ratio	2.66 ± 0.02	3.5 ± 0.30	3.75 ± 0.01	4.40 ± 0.12	3.59 ± 0.02

1,4,7,10-tetraacetic acid) at 30 min p.i., respectively. Thus, $^{99m}Tc(NaBH_4)$-OEP shows higher selectivity to the target organ (solid tumor) than the radiotracers mentioned above do.

CONCLUSIONS

An optimized protocol for the synthesis of $^{99m}Tc(NaBH_4)$-OEP has been developed. The complex is stable in vitro for up to 8 h. Biodistribution studies show high T/NT ratio (4.40 at 60 min p.i.). Thus, $^{99m}Tc(NaBH_4)$-OEP has high tumor affinity and retainable accumulation characteristics in tumor muscles; it could potentially be used for solid tumor imaging. As in [28], we have found that variation of the reducing agent can cause changes in the radiochemical yield and biological results.

ACKNOWLEDGMENTS

This work was supported by STDF – National Research Centre under project number 41535 – PI Ahmed Sayed Morsy Fouzy.

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