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Synthesis and biodistribution in mice of ^{99m}TcN–DBODC–DMSEt

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Nitrido technetium(V)-mixed ligand complex of ^{99m}TcN–DBODC–DMSEt [DMSEt: Monoethyl ester of (meso) 2,3-dimercaptosuccinic acid, DBODC: bis(2-ethoxyethyl)carbamodithioate] has been prepared in a two-step procedure by first reaction of $^{99m}TcO_4^-$ with succinic dihydrazede in the presence of stannous chloride as a reducing agent and propylenediamine tetraacetic acid as a complexant, followed by the addition of DMSEt and DBODC. The complex was stable over 6 h at room temperature. The partition coefficient indicated that it was a hydrophilic complex. Biodistribution in mice demonstrated that the complex accumulated mainly in liver, lungs and kidneys.

Keywords: nitrido technetium; ^{99m}TcN–DBODC–DMSEt; biodistribution

Introduction

Nitrido technetium(V) complexes have been widely studied since the introduction of an efficient method for the production of $[^{99m}Tc{\equiv}N]^{2+}$ core in the past years. Pioneering investigation on nitrido technetium(V) chemistry performed by Baldas and co-workers in the 1980s[1] has been sharply tuned into nuclear medicine applications with the synthesis of the neutral myocardial imaging agent [^{99m}TcN–NOET].[2] Another typical example about nitrido technetium(V) complexes are asymmetrical complexes in recent years. Complexes such as $[Tc(N)(PXP)(OS)]^{+}$,[3] $[Tc(N)(L^n)(PNP)]^{+}$,[4] $[Tc(N)(DTC)(PNP)]^{+}$,[5] $[^{99m}Tc(N)(PXP)(cysOEt)]^{+}$, $[^{99m}Tc(N)(PXP)(cysNAc)]$[6] and $[Tc(N)(R_2PS)(S\text{^}Y)]$[7] were synthesized and thoroughly characterized by Bolzati *et al*. Here we synthesized another asymmetrical nitrido technetium complex, which was characterized by the presence of two kinds of ligands bound to the same $[^{99m}TcN]^{2+}$ core.

Results and discussion

Preparation of $[^{99m}TcN]^{2+}$

The method for preparing $[^{99m}TcN]^{2+}$ was based on the reaction of $^{99m}TcO_4^-$ with succinic dihydrazide (SDH) in the presence of stannous chloride as a reducing agent to form a technetium-99m nitride intermediate. The radiochemical purity of $[^{99m}TcN]^{2+}$ was more than 98% by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The chromatography analyses were performed on a polyamide film with saline as the mobile phase. TLC experiments indicated that $^{99m}TcO_4^-$ and $^{99m}TcO_2 \times H_2O$ remained at the origin and the R_f value for $[^{99m}TcN]^{2+}$ was 0.7–1.0. HPLC chromatograms were shown in Figure 1.

Preparation of ^{99m}TcN–(DBODC)$_2$

^{99m}TcN–DBODC was prepared by adding DBODC to $[^{99m}TcN]^{2+}$ solution. The preferable labeling condition was at pH = 7–8 and room temperature. The radiochemical purity of ^{99m}TcN–(DBODC)$_2$ was more than 98% by TLC and HPLC. The chromatography analyses were performed on a polyamide film with a solution [CH_2Cl_2:CH_3OH = 9:1(V/V)] as the mobile phase. TLC experiments indicated that $^{99m}TcO_4^-$, $^{99m}TcO_2 \times H_2O$ and $[^{99m}TcN]^{2+}$ remained at the origin and ^{99m}TcN–(DBODC)$_2$ moved at the solvent front. HPLC experiments indicated that there was only one complex. The results were shown in Figure 2.

Preparation of ^{99m}TcN–(DMSEt)$_2$

^{99m}TcN–(DMSEt)$_2$ was prepared by adding DMSEt to the $[^{99m}TcN]^{2+}$ solution. The preferable labeling condition was at pH = 8–9 and room temperature. The RCP of the product is more than 98% by TLC and HPLC. The chromatography analysis was performed on the polyamide film with saline as mobile phase. TLC experiments indicated that $^{99m}TcO_4^-$ and $^{99m}TcO_2 \times H_2O$ remained at the origin and the R_f value for $[^{99m}TcN]^{2+}$ was 0.7–1.0, for ^{99m}TcN–(DMSEt)$_2$ was 0.1–0.3. HPLC experiments indicated that there was one complex. The results were shown in Figure 3.

Preparation of ^{99m}TcN–DBODC–DMSEt

^{99m}TcN–DBODC–DMSEt was prepared by the reaction of DMSEt and DBODC with $[^{99m}TcN]^{2+}$. Some experiments were carried

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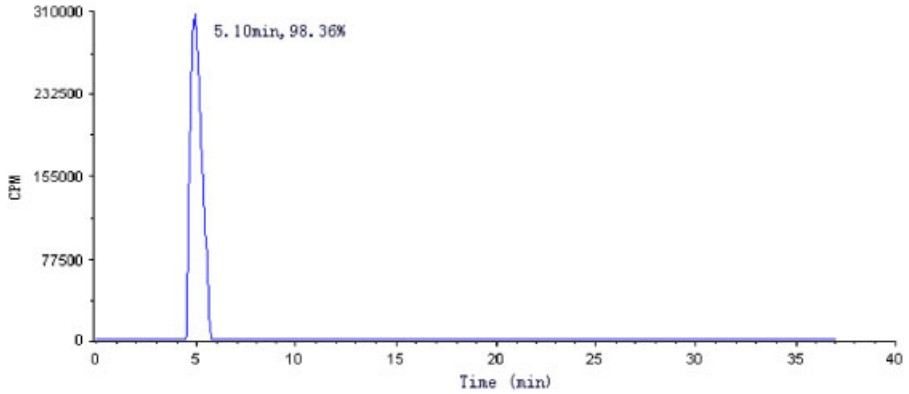


Figure 1. HPLC chromatograms of $[^{99m}TcN]^{2+}$. This figure is available in color online at www.interscience.wiley.com/journal/jlcr.

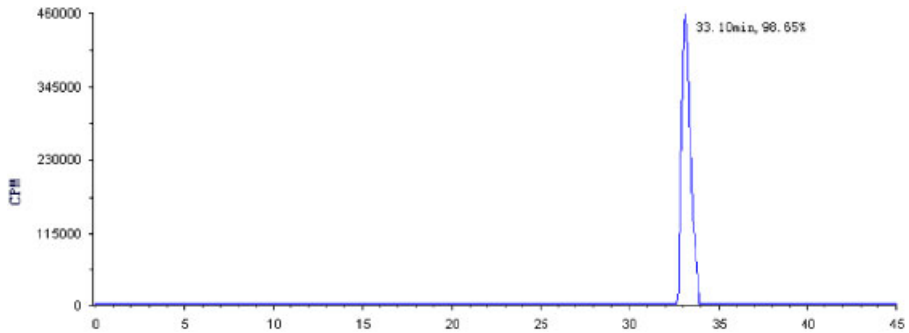


Figure 2. HPLC chromatograms of $^{99m}TcN–(DBODC)_2$. This figure is available in color online at www.interscience.wiley.com/journal/jlcr.

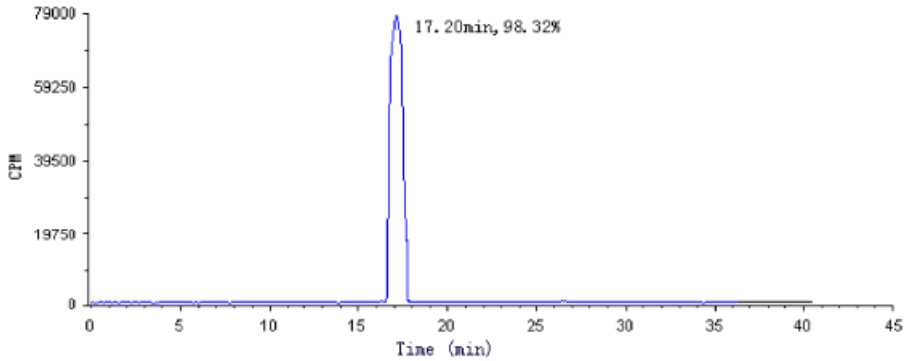
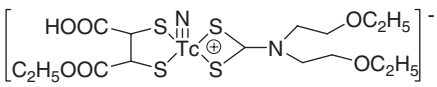


Figure 3. HPLC chromatograms of $^{99m}TcN–(DMSEt)_2$. This figure is available in color online at www.interscience.wiley.com/journal/jlcr.

out to optimize the condition for obtaining a maximum labeling yield. The preferable labeling condition is as follows: 0.5 mL of freshly prepared $[^{99m}TcN]^{2+}$ intermediate was added to a solution of 0.1 mL ethanol containing 5 mg of DMSEt and 0.1 mL water containing 5 mg sodium bis(2-ethoxyethyl)carbamodithioate. The pH of the mixture was controlled at 8.0 by adding sodium hydroxide solution (0.1 M) and kept in room temperature for 30 min. HPLC experiments indicated that there was one complex after separation. The Radiochemical Purity (RCP) of the product is more than 95% by HPLC. The results were shown in Figure 4. The retention time for $^{99m}TcN–DBODC–DMSEt$ (22.80 min) was between $^{99m}TcN–(DBODC)_2$ and $^{99m}TcN–(DMSEt)_2$. The reason for this was that the polarity of $^{99m}TcN–DBODC–DMSEt$ was between $^{99m}TcN–(DBODC)_2$ and $^{99m}TcN–(DMSEt)_2$. The ratio of DBODC/DMSEt (m/m) is between 0.5 and 1.0 resulting in a higher labeling yield of $^{99m}TcN–DBODC–DMSEt$ (Figures 5 and 6). The effect of ratios of DBODC/DMSEt (m/m) on the labeling yield was investigated and the results are shown in Figure 7. Further studies are in process to determine the structure of the complex. The presumed structure of $^{99m}Tc–DBODC–DMSEt$ is described in Scheme 1



Scheme 1. The presumed structure of $^{99m}Tc–DBODC–DMSEt$.

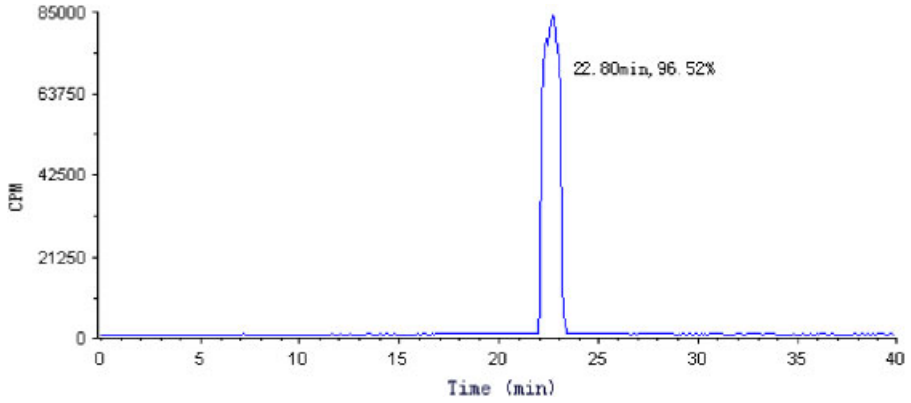


Figure 4. HPLC chromatograms of ^{99m}TcN–DBODC–DMSEt. This figure is available in color online at www.interscience.wiley.com/journal/jlcr.

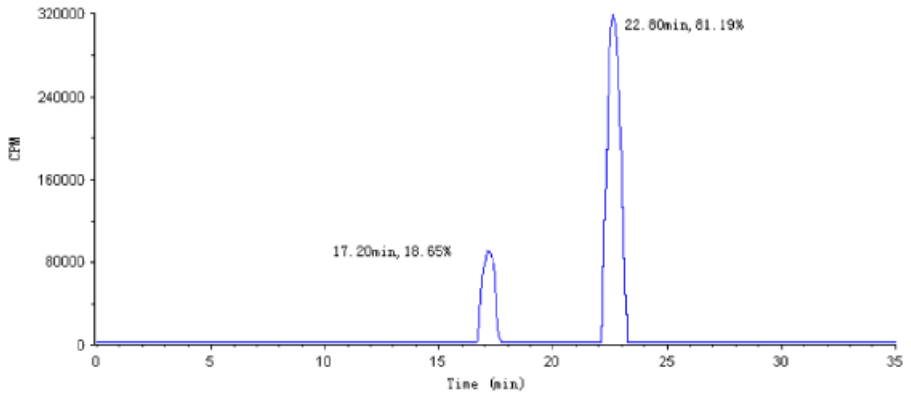


Figure 5. HPLC chromatograms of ^{99m}TcN–DBODC–DMSEt [DBODC:DMSEt = 1:2, (m/m)]. This figure is available in color online at www.interscience.wiley.com/journal/jlcr.

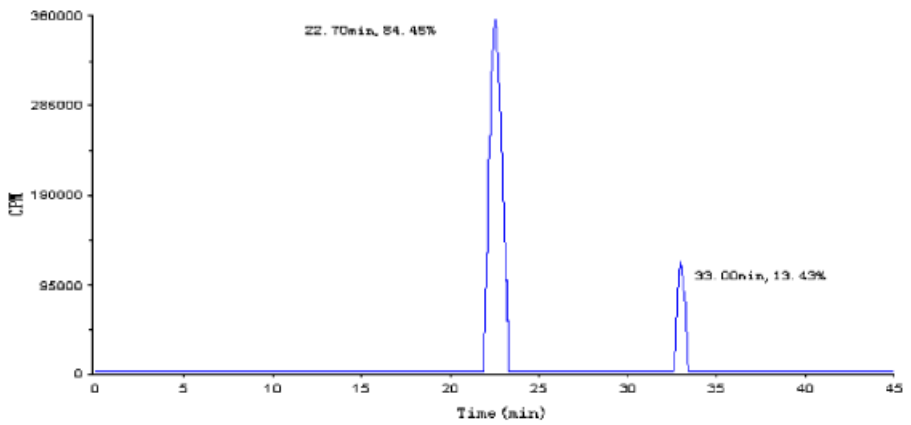


Figure 6. HPLC chromatograms of ^{99m}TcN–DBODC–DMSEt [DBODC:DMSEt = 1:1, (m/m)]. This figure is available in color online at www.interscience.wiley.com/journal/jlcr.

Partition coefficient (log P), electrophoresis and stability experiments

According to the result of the octanol/water partition coefficient experiment, the partition coefficient value for ^{99m}Tc–DBODC–DMSEt was -0.9 ± 0.1. The complex migrated to the anode in electrophoresis experiments, which indicated that the complex was negatively charged. The radiochemical purity at different times after separation showed that the complex had high stability and no decomposition was observed over a period of 6 h by HPLC. The RCP at different times after preparation was given in Table 1. The complex of ^{99m}Tc–DBODC–DMSEt was not very stable in the solution of the plasma. For example, the HPLC chromatograms at 120 min in the plasma showed that the complex was decomposed (Figure 8). The study about the unknown components was in progress.

Biodistribution studies

Biodistribution of ^{99m}TcN–DBODC–DMSEt in mice is given in Table 2. Biodistribution of the complex indicated that it accumulated mainly in the liver and the blood at 5 min post-injection. As compared with ^{99m}TcN–DBODC–DMSEt and ^{99m}TcN–DBODC, ^{99m}TcN–DMSEt accumulated mainly in the

kidneys. Liver uptake was higher for ^{99m}TcN–DBODC–DMSEt and ^{99m}TcN–DBODC at 60 min post-injection.

Experimental

Materials

^{99}Mo/^{99m}Tc generator was obtained from the China Institute of Atomic Energy. DMSEt was synthesized under the following procedure[8] and was obtained as white crystals with 13.5% yield. M.P.: 104–105°C, v/IR (cm^{-1}): 2557(SH), 2560(SH), 1730 (CO) and 1710 (CO), ^{1}H NMR (500 MHz, 0.09 M $NaHCO_3/D_2O$, TMS)δ1.21–1.24 (3 H, CH_3), 3.28–3.31 (1 H, CHCOOH), 3.51–3.53 (1H $CHCOOCH_2$), 4.12–4.17 (2H $CHCOOCH_2$). Anal. calculated for $C_6H_{10}O_4S_2$: C, 34.27; H, 4.79. Found: C, 34.06; H, 4.66. DBODC was donated by the Beijing SHIHONG Pharmaceutical Center. All the other chemicals were obtained from the Beijing Chemical Reagents Company.

Preparation of ^{99m}TcN–(DBODC)$_2$

One milliliter of saline containing $^{99m}TcO_4^-$ (activity ranging from 1.85 to 185 MBq) was added to a vial containing 0.05 mg of $SnCl_2 \cdot 2H_2O$, 0.5 mg of propylenediamine tetraacetic acid (PDTA) and 5.0 mg of SDH in a freeze-dried form.[9] The resulting solution was kept at room temperature for 30 min. Two milligrams of DBODC dissolved in 0.2 mL water was added to the solution. The reaction vial was maintained at room temperature for 30 min.

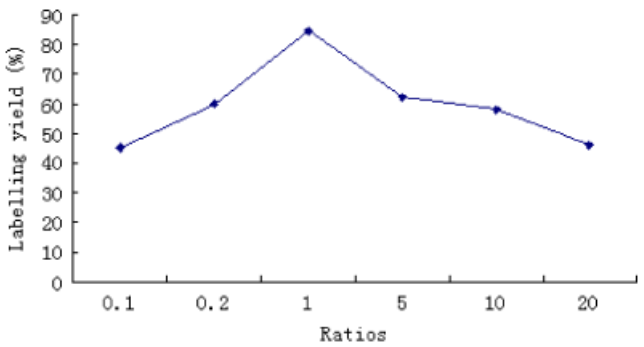


Figure 7. Effect of ratios of DBODC/DMSEt (m/m) on the labeling yield. This figure is available in color online at www.interscience.wiley.com/journal/jlcr.

Preparation of ^{99m}TcN–(DMSEt)$_2$

One milliliter of saline containing $^{99m}TcO_4^-$ (activity ranging from 1.85 to 185 MBq) was added to a vial containing 0.05 mg of $SnCl_2 \cdot 2H_2O$, 0.5 mg of PDTA and 5.0 mg of SDH in a freeze-dried form.[9] The resulting solution was kept at room temperature for 30 min. A total of 0.5 mg of DMSEt dissolved in 0.1 mL ethanol was added to the solution and then the pH was maintained at 8.0 by adding sodium hydroxide solution (0.1 M). The reaction vial was maintained at room temperature for 30 min.

Preparation of ^{99m}TcN–DBODC–DMSEt

A total of 0.5 mL of saline containing $^{99m}TcO_4^-$ (activity ranging from 1.85 to 185 MBq) was added to a vial containing 0.05 mg of $SnCl_2 \cdot 2H_2O$, 0.5 mg of PDTA and 5.0 mg of SDH in a freeze-dried form.[9] The resulting solution was kept at room temperature for 10–15 min. A total of 5 mg of DMSEt dissolved in 0.1 mL ethanol and 5 mg of sodium bis(2-ethoxyethyl)carbamodithioate dissolved in 0.1 mL water were added to the solution and then the pH was maintained at 8.0 by adding sodium hydroxide solution (0.1 M). The reaction vial was maintained at room temperature for 30 min.

Separation and radiochemical analysis

^{99m}TcN–DBODC–DMSEt was separated and evaluated by HPLC. HPLC experiments were performed by using a SHIMADZU System with an SCL-10Avp HPLC pump system and a Park radioflow detector. The column (Kromasil, 250 × 4.6 mm, 5 μm) was eluted at a flow of 1.0 mL/min using a linear gradient system (time/%B) (0/0), (30/100), (40/100), (45/0) of acetonitrile with 0.1% trifluoroacetic acid (B) and water with 0.1% trifluoroacetic acid (A).

Determination of partition coefficient for the complex

The lipophilicity of ^{99m}TcN–DBODC–DMSEt with a radiochemical purity of at least 95% was determined by partitioning the complex

Table 1. The stability of ^{99m}TcN–DBODC–DMSEt at different times (mean value ± SD, $n = 3$)

Time (h)	0	0.5	1	2	3	4	6
RCP (%)	96 ± 2.5	97 ± 1.9	95 ± 2.2	95 ± 1.8	94 ± 2.1	94 ± 2.7	93 ± 3.5

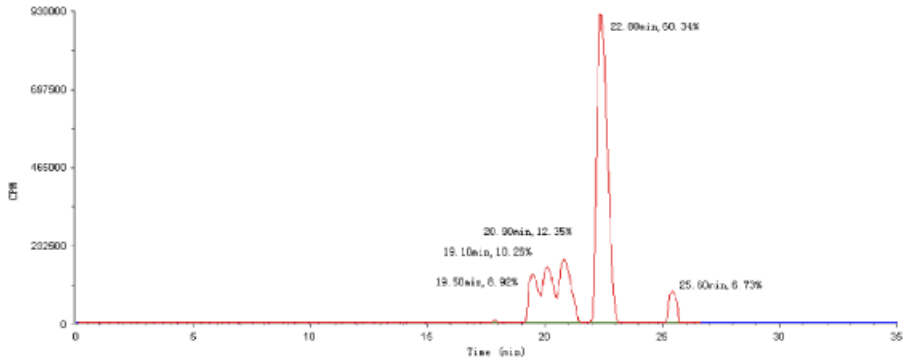


Figure 8. The stability of ^{99m}TcN–DBODC–DMSEt in the solution of plasma (120 min). This figure is available in color online at www.interscience.wiley.com/journal/jlcr.

Table 2. The results of biodistribution in mice for the ^{99m}TcN–$(DBODC)_2$, ^{99m}TcN–$(DMSEt)_2$ and ^{99m}TcN–DBODC–DMSEt (ID%/g ± SD, $n = 3$)

Tissue	Groups[a]	Post-injection time (min)			
		5	15	30	60
Heart	I	2.30 ± 0.13	1.36 ± 0.17	0.62 ± 0.04	0.34 ± 0.06
	II	3.49 ± 0.52	1.84 ± 0.27	0.81 ± 0.10	0.34 ± 0.10
	III	4.22 ± 1.15	3.67 ± 0.84	1.29 ± 0.42	0.89 ± 0.22
Liver	I	3.98 ± 0.33	3.72 ± 0.41	3.54 ± 0.98	3.28 ± 0.59
	II	7.08 ± 1.49	4.89 ± 1.26	4.68 ± 1.24	3.46 ± 0.77
	III	17.25 ± 3.44	17.43 ± 4.32	12.52 ± 3.14	10.29 ± 3.02
Lungs	I	1.93 ± 0.42	1.48 ± 0.05	1.09 ± 0.04	0.72 ± 0.17
	II	4.42 ± 0.76	2.61 ± 0.41	2.27 ± 0.52	0.95 ± 0.24
	III	4.92 ± 1.24	4.75 ± 1.36	3.04 ± 1.17	2.15 ± 0.46
Blood	I	0.87 ± 0.04	0.80 ± 0.19	0.60 ± 0.05	0.32 ± 0.12
	II	5.48 ± 1.16	3.24 ± 0.50	2.35 ± 0.40	0.92 ± 0.22
	III	13.57 ± 3.45	3.54 ± 1.06	3.17 ± 0.95	2.24 ± 0.72
Kidneys	I	4.46 ± 0.25	2.75 ± 0.62	1.55 ± 0.02	1.52 ± 0.08
	II	14.49 ± 3.25	9.44 ± 2.26	6.08 ± 1.04	5.14 ± 0.79
	III	4.97 ± 1.34	3.26 ± 1.05	2.41 ± 0.72	2.25 ± 0.67
Brain	I	0.29 ± 0.01	0.21 ± 0.02	0.08 ± 0.01	0.06 ± 0.01
	II	0.24 ± 0.06	0.11 ± 0.03	0.08 ± 0.03	0.03 ± 0.01
	III	0.24 ± 0.05	0.14 ± 0.07	0.06 ± 0.03	0.05 ± 0.02
Muscle	I	1.47 ± 0.20	0.74 ± 0.21	0.38 ± 0.16	0.33 ± 0.04
	II	1.78 ± 0.35	0.84 ± 0.15	1.02 ± 0.21	0.27 ± 0.08
	III	2.12 ± 0.64	1.59 ± 0.43	1.28 ± 0.44	0.77 ± 0.29
Bone	I	1.09 ± 0.13	0.63 ± 0.26	0.43 ± 0.09	0.34 ± 0.11
	II	3.18 ± 0.67	2.60 ± 0.61	1.21 ± 0.42	1.46 ± 0.25
	III	1.68 ± 0.41	3.06 ± 1.14	1.25 ± 0.37	0.83 ± 0.24
Spleen	I	1.96 ± 0.46	0.80 ± 0.14	0.73 ± 0.26	0.51 ± 0.10
	II	2.79 ± 0.86	1.63 ± 0.25	0.69 ± 0.27	0.58 ± 0.21
	III	3.24 ± 1.21	3.37 ± 1.05	2.21 ± 0.35	1.12 ± 0.39

[a]I, ^{99m}TcN–$(DBODC)_2$; II, ^{99m}TcN–$(DMSEt)_2$; III, ^{99m}TcN–DBODC–DMSEt.

between 1-octanol and saline (pH = 7). The test tube containing 1-octanol, saline and the complex is vortexed at room temperature for 5 min and then centrifuged at a high speed for 10 min. A 0.2 mL aliquot of both phases is pipetted into another test tube and counted in a well counter. The partition coefficient P was calculated using the following equation: P = (cpm in octanol − cpm background)/(cpm in water − cpm background). The partition coefficient was measured several times and the mean value was taken as the final partition coefficient of the complexes to be determined. The final partition coefficient was expressed as log P.

Electrophoresis

Electrophoresis was performed on chromatography paper strips (10×1 cm^2) impregnated with the electrolyte solution and at a potential difference of 150 V. The analyses were run for different times (90–180 min), the developed electrophoresis strips were left to dry and each of them was divided into three parts (cathode, origin and anode). The radioactivity of each part was determined by a well-type gamma counter. The relative percentage of radioactivity distribution of each part was calculated.

Stability study

The stability of the ^{99m}TcN–DBODC–DMSEt was assayed by measuring the RCP by HPLC at different times (0.5, 1, 2, 3, 4,6 h. The RCP was measured three times at each time interval and the mean value of the measurements was used as the final RCP) after preparation in a phosphate buffered solution (pH = 7.4) and the solution of plasma at room temperature.

Biodistribution studies

^{99m}TcN–DBODC–DMSEt was separated by HPLC and the acetonitrile and water were removed under vacuum. After adding saline to the sample, the complex (about 740 kBq in 0.1 mL solution) was injected through the tail vein into Kunming mice (18–22 g, female, obtained from the Animal Center of Beijing Medical University). The mice were sacrificed at 5, 15, 30 and 60 min post-injection. Selected organs were weighed and counted. The accumulated radioactivity in the tissue of organs was calculated in terms of the percentage of the injected dose per gram organ (%ID/g).

Conclusion

A simple method for the preparation of ^{99m}TcN–DBODC–DMSEt is described. *In vivo* studies in mice showed that there was no specific uptake in the organs except for a higher uptake in liver, lungs and kidneys.

Acknowledgement

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