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HPLC ANALYSIS OF TECHNETIUM(I)-99m LABELLED $C_{60}(OH)_{22}$

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Abstract

Fullerenols, water-soluble polihydroxylated fullerenes, are very important kinds of fullerene derivatives because it is suitable for biological study. In order to get convenient substance for studies (*in vivo* and *in vitro*) we investigate possibilities of labeling fullerenol. The HPLC results performed by isocratic HPLC, confirmed that hydrophilic organometallic $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor allows forming of ^{99m}Tc(I) complexes with fullerenol.

Introduction

Fullerenes have some potential effects, e.g. specific cleavage of DNA, antiviral activity, inhibition of HIV protease and photodynamic therapy [1], so it has become a challenging research field at present. Fullerenols, water-soluble polihydroxylated fullerenes, are very important kinds of fullerene derivatives because they are suitable for biological study. The biological behaviour of fullerenol derivatives shows their considerable potential for medical application.

Radiolabelled fullerenol is suitable for many different investigations (radiochemical purity, pharmacokinetics and biodistribution). Technetium-99m is still the radionuclide of choice because of his ideal physical properties ($T_{1/2}$=6.02h, Eγ=141keV) for many applications in nuclear medicine. For radiopharmaceuticals preparation it was often used like technetium pertechnetate (TcO_4^-), which have to be reduced in lower oxidation state. Besides this labelling approach, it is possible to use hydrophilic organometallic $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor to form Tc(I) radiopharmaceuticals based on the tricarbonyltechnetium (I) core [2].

Experimental

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ ion was prepared by addition of 1 ml of ^{99m}Tc-pertechnetate (20 – 100 mCi $^{99m}TcO_4^-$ eluted in saline from ^{99m}Tc-generator, Vinča Institute) to a penicillin vial with lyophilized form of 7.15 mg sodium carbonate, 4.5 mg sodium boranocarbonate, 2.85 mg sodium tetraborate. The samples of fullerenol, produced at the University of Novi Sad [3] were prepared by dissolving in water appropriate amount of substances for obtaining solution with 1.5 mg fullerenol/ml. The pH of solutions was adjusted at about 9.0 and 5.5. ^{99m}Tc-carbonyl fullerenol complexes were prepared by addition of 0.5ml of fullerenol solutions to a proper volume of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor with appropriate pH values to get fullerenol: carbonil ratio 1:1; 1:3 and 1:9. The vials were heated for 30 min in boiling water bath.

The quality control of the obtained $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor (pH = 10 - 11) was performed by the gradient HPLC (Liquid Chromatograph, Hewlett Packard 1050, S/N with UV and Ray test gamma flow detector) on RP C18 column (250 x 4.6 x 5 mm). The solutions of 0.05 M triethylammonium phosphate (TEAP) of pH = 2.25 and methanol were used as mobile phases. The labelling efficiency for ^{99m}Tc-carbonyl tagged fullerenol was determined in isocratic HPLC with 90 % TEAP : 9 % H_2O : 1 % CH_3OH, pH = 2.25 as mobile phase (flow rate 0.7 ml / min) at room temperature.

Results and Discussion

In this paper the results of the conditions and possibilities investigation of water soluble fullerenol $C_{60}(OH)_{22}$ labelling with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ as precursor for ^{99m}Tc(I) were presented.

For determination of radiochemical purity of all ^{99m}Tc-labelled compounds the standard paper (Whatman No1) and instant thin layer chromatography (ITLC-SG) with two solvents (acetone and saline) were used.

The same chromatographic methods for investigation of $^{99m}Tc(CO)_3$-$[C_{60}(OH)_{22}]$ labelling yields were used. The obtained results have shown that applied chromatographic methods could not separate labelled fullerenol from radiochemical impurities like $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor or free $^{99m}TcO_4^-$.

The quality control performed by HPLC method show good separation impurities from radiolabeld fullerenol. The results of ^{99m}Tc-carbonyl tagged of fullerenol, as well as $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor, obtained with heating of the samples in boiling water for 30 min, were presented as HPLC chromatograms, in Fig. 1 and Fig. 2 respectively.

The retention time values (R_t) for $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and $^{99m}TcO_4^-$, obtained by gradient HPLC with 0.05 M triethylammonium phosphate (TEAP) of pH = 2.25 and methanol as mobile phases (flow rate 0.7 ml/min, room temperature) were 4.736 min and 12.624 min respectively. HPLC quality control results of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor have shown that the radiochemical purity of precursor was higher than 95 %. The HPLC results performed by isocratic HPLC with 90% TEAP, 9 % H_2O and 1% CH_3OH as eluense, with flow 0.7 ml/min, confirmed that hydrophilic organometallic $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor allows forming of Tc(I) complexes with fullerenol. The retention time value (R_t) for $^{99m}Tc(CO)_3$-fullerenol (pH around 9.0, carbonyl : fullerenol = 1 : 1 v/v), was 15.923. The labelling yield was 95.35%, with 4.65% of free, not bound $[^{99m}Tc(CO)_3]^+$ as radiochemical impurities. If the labelling was performed at pH around 5.5, better results were obtained for samples with carbonyl : fullerenol = 3 : 1v/v then 9 : 1v/v. If ration was 9 : 1v/v, the labelling yield was about 20%, but if ration was 3 : 1v/v the labelling yield was more then 65%.

The lipophilicity measurements for ^{99m}Tc(I)-labelled fullerenol were done by solvent extraction method with n-octane equilibrated with 0.15 mol dm^{-3} phosphate buffers (pH=3.5-7.5) at room temperature. The fullerenol labelled by $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor gave compound with hydrophilic character.

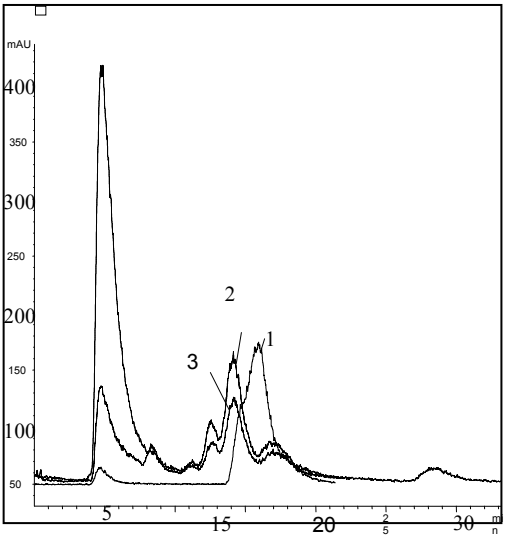

Figure 1. HPLC chromatogram : fullerenol : Tc(I) = 1:1v/v (1); 1:3 (2) and 1:9 (3);

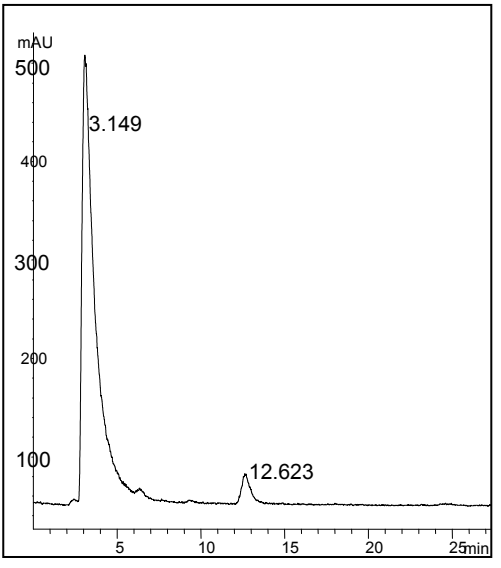

Figure 2. HPLC chromatogram of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor

Conclusion

The obtained results have shown that applied standard paper (Whatman No1) and instant thin layer chromatography (ITLC-SG) methods with two solvents (acetone and saline) could not separate labelled fullerenol from radiochemical impurities. The quality control performed by HPLC method shows good separation impurities from radiolabeld fullerenol. The labelling yield was more than 95%.

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