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Synthesis of Platinum-195m Radiolabelled *cis*-Diammine(1,1-cyclobutanedicarboxylato) platinum(II) of High Radionuclidic Purity

Kenichi KAWAI[1], Yoshiko TANAKA[1], Yukihiro NAKANO[1],
Wilhelm EHRLICH[2] and Mitsuhiko AKABOSHI[1]

Research Reactor Institute, Kyoto University[1], Kumatori-cho, Osaka-590, Japan
Robert Koch Institute[2], D-1000 Berlin 65, Germany

SUMMARY

An experimental method is described for the synthesis of ^{195m}Pt-radiolabeled *cis*-diammine(1,1-cyclobutanedicarboxylatio) platinum(II) (carboplatin). 10 mg of 95 % enriched ^{194}PT was irradiated for 75 h in the hydraulic conveyer of KUR at a thermal neutron flux of approx.8.15×10^{13} $n \cdot cm^{-2} \cdot sec^{-1}$, and the ^{195m}Pt-radiolabeled carboplatin was synthesized and purified using HPLC (column; C_{18}-ODS). The chemical yield was higher than 59.1 %, its chemical purity was greater than 99.3 %, the radionuclidic purity was nearly 100 % and the specific activity was 6.0 MBq$\cdot$ mg^{-1} carboplatin.

Key words: *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin), ^{195m}Pt, HPLC

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INTRODUCTION

Since Rosenberg and his colleages(1) demonstrated strong antitumor activity of *cis*-diamminedichloroplatinum(II), (CDDP), CDDP has found widespread application in cancer therapy(2,3,4). However, its toxic side effects such as vomiting, neurotoxicity, ototoxicity, and mainly its nephrotoxicity as well as its low solubility in water limit the dose that can be given to patients(5,6,7). Therefore, in recent years, many analogs have been synthesized with improved properties, especially with regard to toxicity and solubility(8,9). One of these is the newly developed antitumor agent carboplatin, a coordination complex of platinum with cyclobutanedicarboxylato and ammonia(10). Before the introduction of the new drug into therapy, risk assessments of its biological and toxicological effect have to be made. Pharmacokinetic studies concerning the distribution and metabolism of, for example, carboplatin in cells and animals are regularly requested. For these studies the synthesis and utilization of radiolabeled carboplatin is essential.

Since 1988, we have been engaged in improving the methods for the synthesis of radiolabeled CDDP(11,12). Recently, we described a method for the synthesis of ^{195m}Pt-radiolabeled CDDP(13), and more recently, -DWA2114R(14) using HPLC. The HPLC technique enabled us to produce these ^{195m}Pt-compounds from only small amounts of starting materials (less than 10 mg-Pt). In the present paper, we report on the synthesis of ^{195m}Pt-radiolabeled carboplatin and its purification using HPLC. To increase the chemical purity of the product, we synthesized ^{195m}Pt-carboplatin indirectly, namely from $^{195m}PtCl_4$ *via* ^{195m}Pt-CDDP.

MATERIALS AND METHODS

All the synthetic procedures are shown schematically in Fig. 1.

Synthesis of ^{195m}Pt-radiolabeled CDDP. The method for CDDP synthesis is described elsewhere(13). In brief, 10 mg of 95 % enriched ^{194}Pt (purchased from Oak Ridge National Laboratory, USA) was irradiated in the hydraulic conveyer of the KUR at a

^{194}Pt metal (10 mg)

↓ Reactor irradiation (8.15×10^{13} n·cm^{-2}·sec^{-1}, 75 hr)

^{195m}Pt metal

↓ Dissolved in 1 ml aqua regia, Evaporated and finally dissolved in H_2O (1 ml)

^{195m}Pt(IV)

↓ Reduction using hydrazine

^{195m}Pt(II)

↓ Reaction with 3 N ammonium hydroxide (slow heating)

^{195m}Pt(II)-CDDP (crude)

↓ Separation and purification HPLC (OHpak B-2004)

^{195m}Pt-CDDP (Final volume: 8 ml)

↓ Reaction with cyclobutanedicarboxylate at 90 ℃, for 30 min

^{195m}Pt-carboplatin

Separation and purification HPLC (C_{18}-ODS)

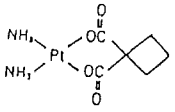

Fig. 1. Scheme for the synthesis of ^{195m}Pt-carboplatin

thermal neutron flux of approx. 8.15×10^{13} n·cm^{-2}·sec^{-1} for 75 h. After 3 d cooling for eliminating undesired radioactivities due mainly to ^{191}Pt and ^{197}Pt, synthesis of ^{195m}Pt-radiolabeled CDDP was carried out in the following manner. The sample (10 mg ^{195m}Pt-metal) was dissolved in 1 ml aqua regia, the solvent removed by repeated evaporation, and then the sample dissolved in 1 ml H_2O. Thereafter the sample was stored for 30 min at 80 ℃ with a molar equivalent of hydrazine to reduce Pt(IV) to Pt(II). The remaining HCl was removed by evaporation. ^{195m}Pt-radiolabeled CDDP was synthesized from the $H_2{}^{195m}PtCl_4$ by slow heating with 3 M NH_4OH at 80 ℃ for 1 h. At the final step of the synthesis, the solution (final volume: 20 ml) was applied to a

HPLC-column (Shodex, OHpak B-2004, 50 cm length x 2 cm diameter) attached to a Shimadzu HPLC-apparatus especially designed for preparative use. Elution was carried out using millipore-filtered H_2O. The injection volume was 2 ml for one injection and the elution rate was 5 $ml \cdot min^{-1}$.

Synthesis of ^{195m}Pt radiolabeled carboplatin. To the aqueous solution of ^{195m}Pt-CDDP (about 8 mg-CDDP in 8 ml-H_2O), two molar equivalents of silvercyclobutanedicarboxylate were added, and the mixture heated at 90 ℃ for 30 min. ^{195m}Pt-radiolabeled carboplatin, thus synthesized, was separated and purified using HPLC. The preparative column C_{18}-ODS (5 cm x 20 cm, Gasukuro Kogyo Inc.) was used. Elution was carried out using H_2O with a flow rate of 5 $ml \cdot min^{-1}$. The detection of ^{195m}Pt-carboplatin was accomplished by a radioanalyzer (Aloka TCR-5011) and UV-detector (Jasco-875 UV) both connected to the HPLC apparatus.

RESULTS AND DISCUSSION

Figure 2 shows the HPLC analysis of the reaction mixture. There appear to be two peaks, one small and the other more prominent, in the radiochromatogram. The first one (retention time; 2.45 min) represents CDDP and the second (8.90 min) carboplatin. From the figure, the yield for carboplatin obtained based on the Pt-atom is higher than 96.8 %. However, as the yield for CDDP synthesis was typically 61 %, the total yield for carboplatin synthesis is estimated to be more than 59.1 %.

The 8.9-min fraction was collected, concentrated and rechromatographed using the same HPLC-condition (Fig. 3). From this figure, the chemical purity was determined to be greater than 99.3 %. Though thin-layer chromatography was applied separately to the inspection of the chemical purity (silica gel 60 F254 plate and isopropanol:water = 7:3), the observed purity was always higher than 99.3 % (data not shown).

Figure 4 demonstrates the γ-ray spectrum of ^{195m}Pt-radiolabeled carboplatin measured with a Ge(Li)-detector attached to a multichannel analyzer. It can be seen that

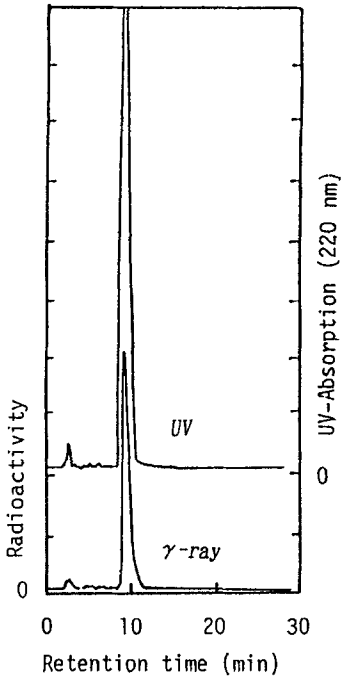

Fig. 2. HPLC-elution pattern of several Pt-compounds Sample: 10 μl specimen taken out at the final step of the synthesis

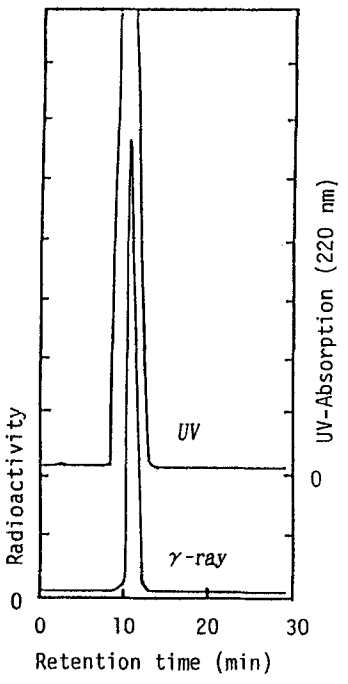

Fig. 3. HPLC-analysis of synthesized ^{195m}Pt-carboplatin Sample: 100 μl ^{195m}Pt-carboplatin (total volume was 20 ml in H_2O)

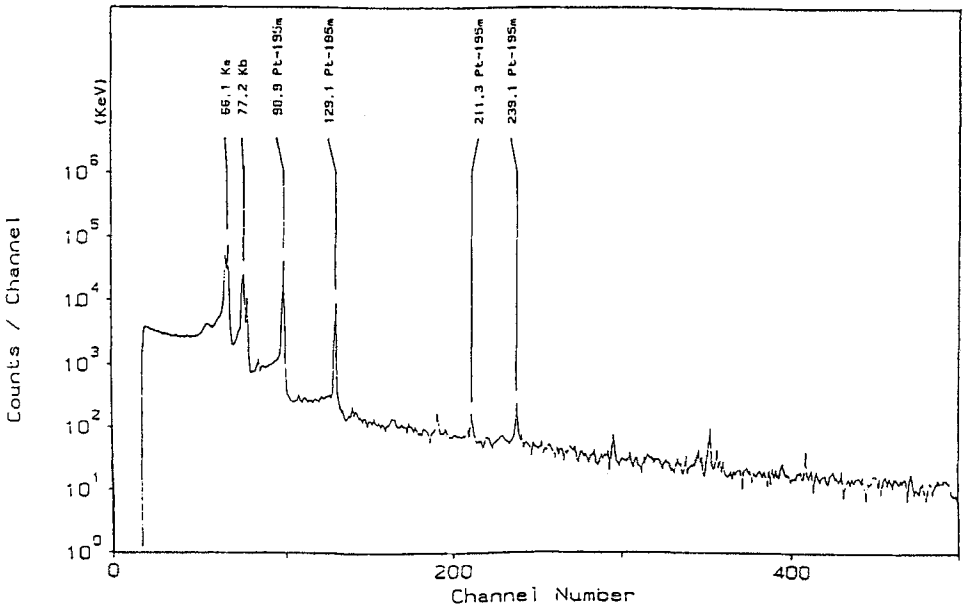

Fig. 4. Gamma-ray spectrum of ^{195m}Pt-radiolabeled carboplatin

the spectrum consists of almost pure ^{195m}Pt (kα, kβ, 98.9, 129, 211 and 239 keV γ -rays). ^{198}Au derived from the β-decay of ^{198}Pt which is expected to coexist in the original material, may be eliminated by the synthetic procedure. From the figure the radionuclidic purity of ^{195m}Pt-carboplatin was estimated to be nearly 100 %.

Few papers have been published on the synthesis and utilization of ^{195m}Pt-radiolabeled carboplatin (15,16). However, as no detailed information about the properties of synthesized ^{195m}Pt-carboplatin, except for overall yield which was estimated to be in the range of 48 %, is given in those reports, we can not compare the chemical and radionuclidic purity between the two results. Thus the advantage of the present method is the higher yield (59.1 %) and the lower amount of the starting material (only 10 mg compared with 50 mg(15)) required. The present results are summarized in Table 1. This is the first report on the synthesis of ^{195m}Pt-radiolabeled carboplatin using HPLC. Because of its high chemical and radionuclidic purity, and specific activity, it will be a very useful tool for examining the biological effects of carboplatin, and hence, the mechanism(s) of the biological effects of CDDP and other Pt-compounds.

Table 1. Summary of ^{195m}Pt-Carboplatin Synthesis

Chemical yield (%)	59.1
Chemical purity (%)	99.3
Radionuclidic purity (%) ca.	100
Specific activity (MBq.mg^{-1})	6.0

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