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Synthesis of Endohedral ^{133}Xe-Fullerenol by Using Higher Fullerene

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Hydrophilic endohedral ^{133}Xe-fullerenols [^{133}Xe@$C_{76}(OH)_x$ and ^{133}Xe@$C_{84}(OH)_x$] were synthesized from hydrophobic endohedral ^{133}Xe-fullerenes. The yields were found to depend on the solubility of endohedral ^{133}Xe-fullerenols in *o*-dichlorobenzene and water phases, reflecting the number of OH groups of the product. The endohedral ^{133}Xe-fullerenols stored in 0.9% NaCl solution at 20 °C were stable enough for the use in nuclear medicine.

1. Introduction

Endohedral fullerenes encapsulating a radionuclide within the fullerene cage are of current interest as radiopharmaceuticals for therapy and diagnosis.[1–6] To realize these medical purposes, hydrophilic fullerene derivatives, which allow *in vivo* investigations, have to be synthesized. We synthesized hydrophilic endohedral ^{133}Xe-fullerenols [^{133}Xe@$C_{60}(OH)_x$ and ^{133}Xe@$C_{70}(OH)_x$][7] which would be applied to the therapy of bone cancer, because ^{133}Xe emits β rays with the maximum energy of 0.346 MeV and fullerenol is concentrated in bone tissues.

For therapeutic purposes, the separation of endohedral ^{133}Xe-fullerenes from empty fullerenes is necessary, because the endohedral ^{133}Xe-fullerene derivatives not containing empty fullerenes could effectively concentrate in target tissues. In the previous work, however, we did not succeed in separating ^{133}Xe@C_{60} from C_{60} and ^{133}Xe@C_{70} from C_{70}.[8,9] Recently, we produced endohedral higher ^{133}Xe-fullerenes such as ^{133}Xe@C_{76} and ^{133}Xe@C_{84} by implantation of ^{133}Xe ions into C_{76} and C_{84} fullerene targets. It was easy to separate endohedral higher ^{133}Xe-fullerenes from empty fullerenes by the high performance liquid chromatography (HPLC).[10] The present work aims at the synthesis of hydrophilic endohedral ^{133}Xe-fullerenols [^{133}Xe@$C_{76}(OH)_x$ and ^{133}Xe@$C_{84}(OH)_x$] from the endohedral higher ^{133}Xe-fullerenes (^{133}Xe@C_{76} and ^{133}Xe@C_{84}). To obtain optimum conditions of the synthesis, yields of the endohedral ^{133}Xe-fullerenols are examined as a function of reaction time of hydroxylation of carbon atoms in the fullerene cage. In addition, the extraction behavior of the endohedral ^{133}Xe-fullerenols from *o*-dichlorobenzene to water phases are investigated.

2. Experimental

2.1. Production of endohedral higher ^{133}Xe-fullerenes. The endohedral higher ^{133}Xe-fullerenes (^{133}Xe@C_{76} and ^{133}Xe@C_{84}) were synthesized in a manner as described in detail in our previous paper.[8] Fullerene targets for ion implantation were prepared by vacuum evaporation of fullerenes (C_{76} or C_{84}) on Ni foil. Xenon-133 ions were implanted into the targets with an isotope separator (Takasaki Institute, JAEA) at an acceleration energy of 30 keV. After the ion implantation, the fullerenes on the target foil were dissolved in *o*-dichlorobenzene. The solution was filtered through a membrane filter of 0.2 μm in pore size (Millipore, JGWP) to remove insoluble materials. The filtrate was purified by HPLC with a Cosmosil Buckyprep column of 4.6 mm i.d. × 250 mm long. The purified solution was used to synthesize endohedral ^{133}Xe-fullerenols.

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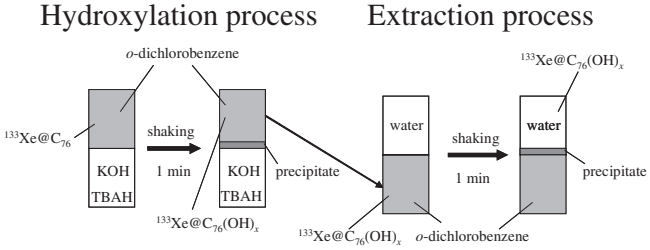

Figure 1. A Schematic diagram of a synthetic procedure for ^{133}Xe@$C_{76}(OH)_x$. The same procedure was also used for ^{133}Xe@$C_{60}(OH)_x$, ^{133}Xe@$C_{70}(OH)_x$, and ^{133}Xe@$C_{84}(OH)_x$.

2.2. Synthesis of endohedral higher ^{133}Xe-fullerenols. Hydroxylation of endohedral higher ^{133}Xe-fullerenes to ^{133}Xe@$C_{76}(OH)_x$ and ^{133}Xe@$C_{84}(OH)_x$ was carried out in a manner as described in our previous paper.[7] The method comprises two processes: hydroxylation and extraction (Figure 1).

In the hydroxylation process, *o*-dichlorobenzene solution containing ^{133}Xe@C_{76} or ^{133}Xe@C_{84} was shaken for 1 min with 14 M KOH solution containing tetrabutylammonium hydroxide (TBAH, 40% in water) in a polypropylene centrifuge tube. After shaking, the mixture was centrifuged (9000 rpm, 5 min) to form three fractions of *o*-dichlorobenzene phase, KOH phase, and precipitate in between the *o*-dichlorobenzene and KOH phases. The *o*-dichlorobenzene and KOH phases were separately transferred into another polypropylene centrifuge tube, and evacuated in a vacuum desiccator to release gaseous ^{133}Xe from the solution. The ^{133}Xe radioactivity in each fraction of the *o*-dichlorobenzene phase, the KOH phase, and the precipitate remaining in the centrifuge tube was measured by γ-ray spectrometry with a germanium detector.

In the extraction process, water was added to the *o*-dichlorobenzene containing hydroxylated products, and then the mixture was shaken for 1 min to extract ^{133}Xe@$C_{76}(OH)_x$ and ^{133}Xe@$C_{84}(OH)_x$ into the water phase. After centrifugation, each fraction was evacuated in a vacuum desiccator to release gaseous ^{133}Xe. The radioactivity of ^{133}Xe in each fraction was measured by γ-ray spectrometry.

3. Results and Discussion

Yields of endohedral ^{133}Xe-fullerenols calculated as a percentage of the ^{133}Xe radioactivity extracted into the water phase against the initial ^{133}Xe radioactivity of endohedral higher ^{133}Xe-fullerenes are summarized in Table 1, together with those of ^{133}Xe@$C_{60}(OH)_x$ and ^{133}Xe@$C_{70}(OH)_x$.[7] Here listed are the

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TABLE 1: Yields of endohedral ^{133}Xe-fullerenols through hydroxylation for 1 min and extraction for 1 min

Fullerene species	Yield /%
C_{60}	43
C_{70}	18
C_{76}	21
C_{84}	11

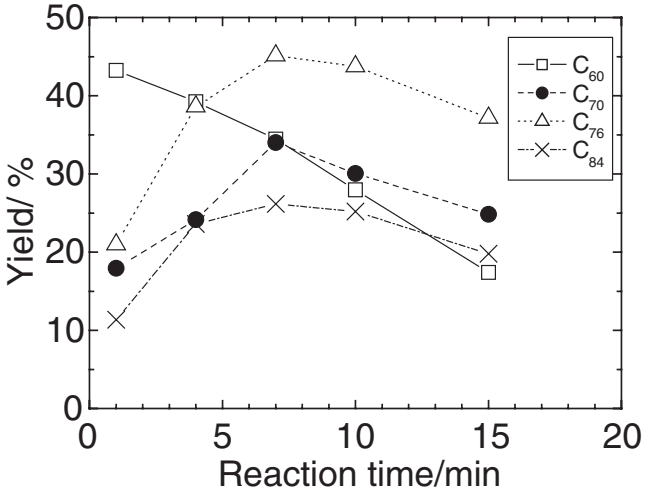

Figure 2. Yields of endohedral ^{133}Xe-fullerenols as a function of reaction time of hydroxylation. The extraction time is fixed to 1 min.

yields obtained at 1 min extraction after hydroxylation for 1 min. The yield decreases with increasing number of carbon atoms in a fullerene, although slight disorder is seen between $^{133}Xe@C_{70}(OH)_x$ and $^{133}Xe@C_{76}(OH)_x$. The yields should depend on the number of hydroxyl groups of endohedral ^{133}Xe-fullerenols synthesized here.

To obtain optimum conditions, the yields of the endohedral ^{133}Xe-fullerenols were examined as a function of reaction time of hydroxylation from 1 to 15 min, the extraction time being fixed to 1 min. As shown in Figure 2, the yield of $^{133}Xe@C_{60}(OH)_x$ decreases monotonically with an increase of the reaction time, whereas the yields of $^{133}Xe@C_{70}(OH)_x$, $^{133}Xe@C_{76}(OH)_x$, and $^{133}Xe@C_{84}(OH)_x$ increase up to 7 min and then decrease. Here, it is to be noted that the sum of distribution of ^{133}Xe in the organic (*o*-dichlorobenzene) phase and precipitate in the hydroxylation process were almost constant, and higher than 90% irrespective of the reaction time of hydroxylation. The distribution of ^{133}Xe in the aqueous (KOH) phase was about 5%. The endohedral ^{133}Xe-fullerenols once produced in the organic phase did not migrate to the aqueous phase, suggesting that the fullerenols synthesized here should be insoluble in 14 M KOH solution. The gaseous ^{133}Xe released from endohedral ^{133}Xe-fullerenes was about 5% or lower in the present work.

Changes in the distribution of ^{133}Xe in the organic phase and precipitate in the hydroxylation process are shown in Figures 3(a) and 3(b) as a function of the reaction time of hydroxylation. As shown in Figure 3(a), the distributions of ^{133}Xe as $^{133}Xe@C_{70}(OH)_x$, $^{133}Xe@C_{76}(OH)_x$, and $^{133}Xe@C_{84}(OH)_x$ in the organic phase are as high as 90% at short reaction time but decrease after 7 min. On the contrary, the distributions of ^{133}Xe in the precipitate increase after 7 min as shown in Figure 3(b). The fact suggests that endohedral higher ^{133}Xe-fullerenols once produced should change to the precipitating species at longer time of hydroxylation. The precipitating species might be a hydrophilic endohedral fullerenols insoluble in *o*-dichlorobenzene with a large number of hydroxyl groups.

To get an insight into the extraction process, we observed

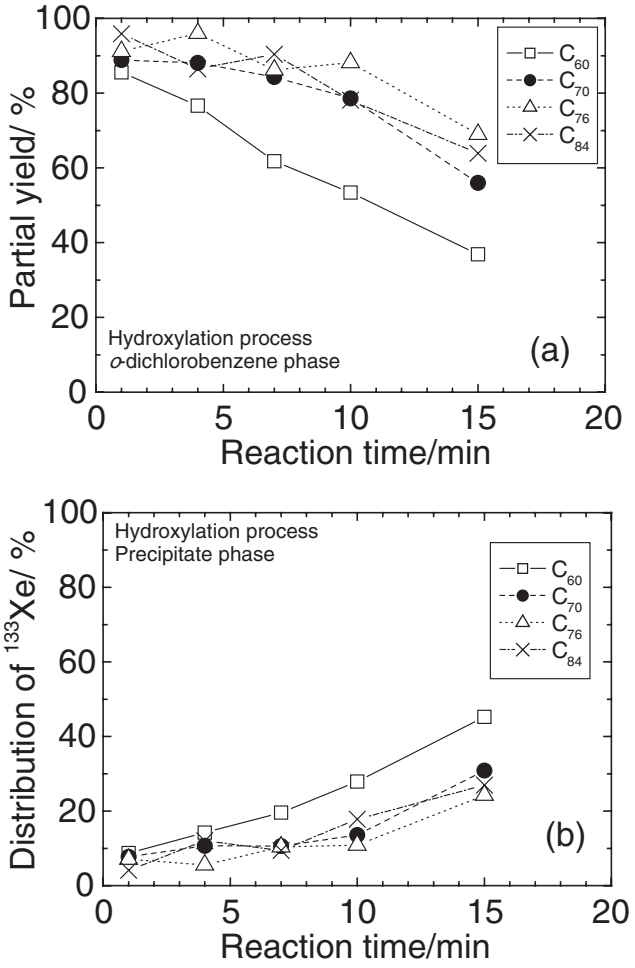

Figure 3. (a) Partial yields of endohedral ^{133}Xe-fullerenols obtained in the *o*-dichlorobenzene phase in the hydroxylation process as a function of reaction time of hydroxylation. (b) Distribution of ^{133}Xe in precipitate in the hydroxylation process as a function of reaction time of hydroxylation.

changes in the partial yield that was defined as a percentage of the endohedral ^{133}Xe-fullerenols extracted into the water phase relative to the initial amount of endohedral ^{133}Xe-fullerenols produced in the *o*-dichlorobenzene phase through hydroxylation. The partial yields of the endohedral ^{133}Xe-fullerenols extracted into the water phase are shown in Figure 4(a) as a function of reaction time of hydroxylation, the extraction time being fixed to 1 min. As shown in Figure 4(a), the partial yields of $^{133}Xe@C_{70}(OH)_x$, $^{133}Xe@C_{76}(OH)_x$, and $^{133}Xe@C_{84}(OH)_x$ increase from 20% or less to 40% or more in 7 min and then become almost constant thereafter. In contrast, the distributions of ^{133}Xe in the water-insoluble precipitate (Figure 4(b)) are as high as 60–75% for the endohedral higher ^{133}Xe-fullerenols synthesized in short-time hydroxylation, and decrease gradually to smaller values in 7 min. They become almost constant after 7 min. In this extraction process, the distributions of ^{133}Xe in gaseous phase were about 15% or lower, and in the organic phase were about 5% or lower.

The increase of the yields of $^{133}Xe@C_{70}(OH)_x$, $^{133}Xe@C_{76}(OH)_x$, and $^{133}Xe@C_{84}(OH)_x$ up to 7 min (Figure 2) is explained as follows. Endohedral higher ^{133}Xe-fullerenols that are highly soluble in *o*-dichlorobenzene are synthesized through hydroxylation up to 7 min (Figure 3(a)). These fullerenols with rather small number of hydroxyl groups are still hydrophobic and insoluble in the water phase at the reaction time of 1 min (Figure 4(a)). According to Li et al.,[11] fullerenols with less hydroxyl groups than 10 is insoluble in water. As the reaction time increases, the hydroxylation proceeds to afford hydrophilic species soluble in water. After 7 min, endohedral ^{133}Xe-fullerenols with a large number of hydroxyl groups are

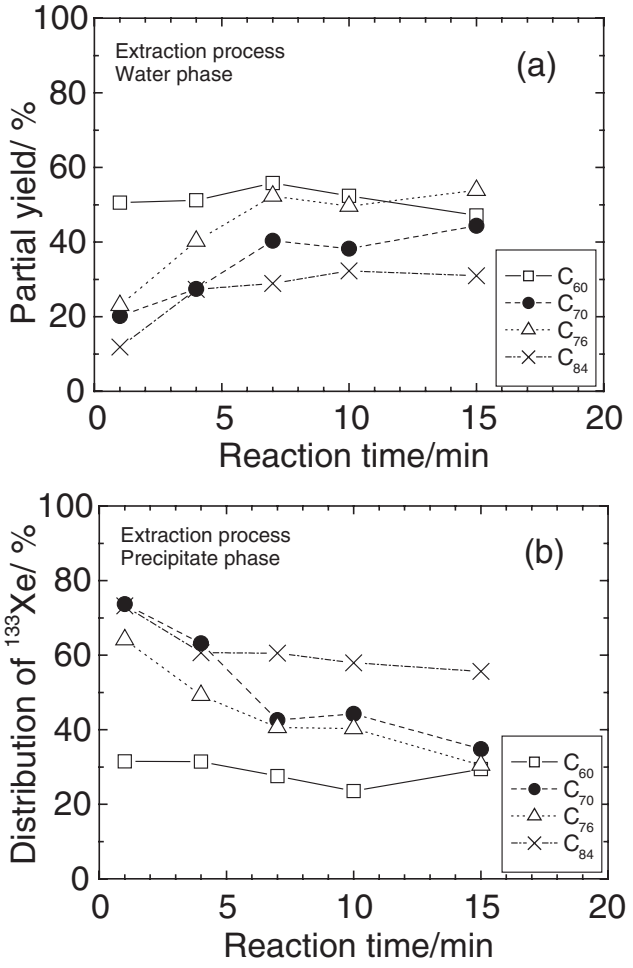

Figure 4. (a) Partial yields of endohedral ^{133}Xe-fullerenols extracted into water phase as a function of reaction time of hydroxylation. (b) Distribution of ^{133}Xe in precipitate in the extraction process. The partial yield (a) and the distribution of ^{133}Xe (b) were defined as a percentage of the endohedral ^{133}Xe-fullerenols extracted into the water phase or in the precipitate relative to the initial amount of endohedral ^{133}Xe-fullerenols produced in the *o*-dichlorobenzene phase through hydroxylation.

produced. These fullerenols are hydrophilic and less soluble in *o*-dichlorobenzene, but extracted well in the water phase.

The monotonic decrease seen in the yield of ^{133}Xe@$C_{60}(OH)_x$ (Figure 2) is explained by considering that the rate of hydroxylation to afford hydrophilic ^{133}Xe@$C_{60}(OH)_x$ with a large number of hydroxyl groups would be higher than that of endohedral higher ^{133}Xe-fullerenols. In fact, the partial yield of ^{133}Xe@$C_{60}(OH)_x$ soluble in *o*-dichlorobenzene decreased monotonically as the hydroxylation reaction proceeded (Figure 3(a)). Consequently, the optimum reaction time of hydroxylation is to be 7 min for ^{133}Xe@$C_{70}(OH)_x$, ^{133}Xe@$C_{76}(OH)_x$, and ^{133}Xe@$C_{84}(OH)_x$, and that for ^{133}Xe@$C_{60}(OH)_x$ is 1 min.

Under the optimum condition, the yields of ^{133}Xe@$C_{60}(OH)_x$, ^{133}Xe@$C_{70}(OH)_x$, ^{133}Xe@$C_{76}(OH)_x$, and ^{133}Xe@$C_{84}(OH)_x$ were 43%, 34%, 45%, and 26%, respectively. When we synthesized these endohedral ^{133}Xe-fullerenols by using the method of Cagle et al.,[5] the corresponding yields were as low as 1.2%, 2.1%, 12%, and 17%. In addition, most of the encapsulated ^{133}Xe atoms were found to be released from the fullerene cage in the latter case where it took 12 h for hydroxylation.

For medical applications, the stability of ^{133}Xe@$C_{60}(OH)_x$, ^{133}Xe@$C_{70}(OH)_x$, ^{133}Xe@$C_{76}(OH)_x$, and ^{133}Xe@$C_{84}(OH)_x$ synthesized here was examined in saline solution. When the endohedral ^{133}Xe-fullerenols were stored in 0.9% NaCl at 20 °C, the decomposition or release of ^{133}Xe was less than 10% even after 5 days for every endohedral ^{133}Xe-fullerenol. The fact suggests that the endohedral ^{133}Xe-fullerenol products obtained here would be favorable for nuclear medicine. In particular, the medical use of ^{133}Xe@$C_{76}(OH)_x$ and ^{133}Xe@$C_{84}(OH)_x$ would be highly promising because ^{133}Xe@C_{76} and ^{133}Xe@C_{84} are easily separated from empty fullerenes (C_{76} and C_{84}) by HPLC before the hydroxylation.[10]

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