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Separation of Carrier-Free Holmium-166 from Neutron-Irradiated Dysprosium Targets

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Holmium-166 (^{166}Ho, $t_{1/2}$ = 26.4 h) is utilized in radiotherapeutic applications such as radioimmunospecific pharmaceuticals, bone marrow ablation, and radiation synovectomy. High specific activity ^{166}Ho can be obtained from the decay of dysprosium-166 (^{166}Dy, $t_{1/2}$ = 81.5 h). Dysprosium-166 is produced by the ^{164}Dy[n,γ]^{165}Dy-[n,γ]^{166}Dy reaction in a nuclear reactor. The applicability of reversed phase ion-exchange chromatographic methods was demonstrated for the separation of carrier-free ^{166}Ho from milligram quantities of $^{164}Dy_2O_3$ irradiated targets. An efficient and quantitative separation was achieved utilizing a metal-free HPLC system with Dowex AG 50WX12 or Aminex-A5 cation exchangers and α-hydroxyisobutyric acid (α-HIBA) as the eluent (0.085 M, pH = 4.3 adjusted with NH_4OH). The Aminex-A5 column gave a separation factor of ~10^3 between Ho and Dy. Subsequent to the acidic destruction of the Ho–HIBA complex, Ho^{3+} was further purified on a small cation-exchange column from acidic chloride solutions. The separation was achieved within 2 h, with a 95% overall radiochemical yield for carrier-free ^{166}Ho with a Dy breakthrough of <0.1%.

Holmium-166 (^{166}Ho) is utilized in medical radiotherapeutic applications[1–7] because of its physical properties, which include high-energy β radiation [$E_{\beta 1}$ = 1855 keV (51%), $E_{\beta 2}$ = 1776 keV (48%), and $E_{\beta av}$ = 666 keV], a 26.4-h half-life, and decay to a stable daughter. In addition, ^{166}Ho has chemical characteristics suitable for protein labeling with bifunctional chelates. Holmium-166 also emits low-intensity and low-energy γ-rays (80.5 keV, 6%) which are suitable for imaging. Due to the absence of high-energy γ-rays in its decay, ^{166}Ho may be used for outpatient therapy without significant external radiation to other individuals.

Although ^{166}Ho with moderate specific activity can be produced by the ^{165}Ho[n,γ]^{166}Ho reaction, its radionuclidic parent, ^{166}Dy ($t_{1/2}$ = 81.5 h), can serve as a source of high specific activity ^{166}Ho. Dysprosium-166 is produced by double neutron capture reaction on ^{164}Dy. In certain applications, such as protein labeling, the use of a high specific activity radioisotope is essential. In addition, generator-produced ^{166}Ho is free from 1200-y ^{166m}Ho, which is unavoidably coproduced with ^{166}Ho by the ^{165}Ho[n,γ] reaction.

Successful separation of rare earths has been achieved by reversed phase ion-exchange chromatography with cation-exchange resins such as Dowex AG 50W and Aminex-A5 and sodium or ammonium salts of α-hydroxyisobutyric acid (α-HIBA) as complexing agents.[8–10] Yoshida and Haraguchi[11] used a strong cation-exchange resin IEX-210SC for the separation of several rare earths with ammonium lactate as the mobile phase. Elbanovski et al.[12] purified yttrium from heavy lanthanides (mainly Dy and Ho) by use of Wofatit KPS cation exchanger and Tiron (disodium salt of pyrocatechol-3,5-disulfonic acid) as eluent. Dynamic ion-exchange chromatography was recently used for rapid separations of rare earths.[13–15] Separation of nanogram amounts of the rare earths Y, Th, and U was performed on the HPLC 5-mC_{18} reversed phase column with mobile phase consisting of ammonium *n*-octylsulfonate (as dynamic exchanger), α-HIBA, and methanol.[13] On-column formation of nitrilotriacetato complexes of rare earths in a reversed phase ODS column in the presence of 1-octanesulfonate provided a high-resolution chromatographic system for the separation of individual rare earth elements at room temperature.[16] Purification of Y concentrate from heavy lanthanides on various types of anion exchangers in iminodiacetate form has been studied.[17] Separations without complexing agents (e.g., mixtures of methanol and nitric acid) were also reported.[18,19]

Partition chromatography offers a possibility for separating small amounts of both light or heavy rare earths using tributyl phosphate (TBP) as the stationary phase and 11–15 M HNO_3 or

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HCl as the mobile phase[20,21] or bis(2-ethylhexyl) hydrogen phosphate (HDEHP) as an extracting agent from 0.2 to 5 M HNO_3.[22,23] Partial separation of mixtures of rare earths with electrophoresis on paper and acetylcellulose films in a medium of α-HIBA,[24,25] and with high-voltage capillary electrophoresis,[26,27] has also been reported. Solvent extraction techniques have been used extensively for separation and preconcentration of rare earths.[28,29]

In spite of the rather large number of publications on rare earth separations, only a few address the separation in a micro–macrocomponent system, where microscopic amounts (≤1 μg) of one member of the lanthanide series are separated from macroscopic amounts (≥1 mg) of another member. Because of the uniform chemistry exhibited throughout the lanthanide series, the separation factors between the adjacent members are very small. For example, separation of 1 μg of Tb from 110 mg of Er was studied by partition chromatography using a HDEHP/Kieselguhr column.[23] In this case, the separation factor between the two rare earths was ~17. Under similar conditions, the separation factor for the Dy–Ho pair was only 2.2.[23] Yasumi et al.[30] reported separation of carrier-free ^{163}Ho produced by the ^{164}Dy[p,2n] reaction from milligram amounts of a Dy–metal target. An initial separation of a ^{163}Ho fraction from the dissolved target material was carried out on the AG 50Wx8 cation exchanger using 0.48 M α-HIBA as eluent. Further purification of the ^{163}Ho fraction from various radioactive impurities required ~20 h. Separation of carrier-free ^{166}Ho from Dy_2O_3 targets with partition chromatography using HDEHP or TBP as stationary phase and 3–12 M HNO_3 as mobile phase was unsatisfactory for biomedical applications of ^{166}Ho.[31]

This paper demonstrates the applicability of reversed phase LC and HPLC ion-exchange chromatography for separation of carrier-free ^{166}Ho from neutron-irradiated $^{164}Dy_2O_3$ targets. In addition, the method described herein defines a selective and convenient means to prepare ^{166}Ho in high radionuclidic purity and in a solvent suitable for radiolabeling of pharmaceuticals.

EXPERIMENTAL SECTION

Reagents and Materials. Isotopically enriched ^{164}Dy (97%) as Dy_2O_3 was obtained from ORNL and Medgenix Diagnostics GmbH. The cation-exchange resins Dowex AG 50Wx4 (H^+ form, 100–200 mesh, 106–250 μm, 1.1 mequiv/mL), AG 50Wx8 (H^+ form, 100–200 mesh, 106–250 μm, 1.7 mequiv/mL), and AG 50WX12 (H^+ form, 200–400 mesh, 53–106 μm, 2.1 mequiv/mL) and Aminex-A5 (Na^+ form, cross-linkage 8%, 132 μm) were purchased from Bio-Rad Labs. The resins were stored in H_2O, and AG 50W resins were converted to the Na^+ form before use. The α-HIBA (98%), Arsenazo III [2,7-bis(o-arsenophenolazo)-1,6-dihydroxynaphthalene-3,6-disulfonic acid, 99.99%], and Dy_2O_3 (99.9%) were supplied from Aldrich. All other inorganic chemicals were of reagent grade.

Radiochemical Reagents and Radioactivity Measurements. Targets consisting of 5–10 mg of $^{164}Dy_2O_3$, encapsulated either in a quartz ampule or in a titanium can, were irradiated in the hydraulic tube of the ORNL high-flux isotope reactor (HFIR) at a thermal neutron flux of 2.0×10^{15} n·s^{-1}·cm^{-2} for 1 or 8 days or in the pneumatic tube of the ANSTO high-flux Australian reactor (HIFAR) at 5×10^{13} n·s^{-1}·cm^{-2} for 12 h. Following irradiation, the target was allowed to cool for 2 days, then was dissolved in 5 mL of 9 M HCl, and evaporated to dryness and the residue was dissolved in 1 mL of 0.01 M HNO_3. Dy–carrier solution of 58 mg/mL was prepared using the same procedure. Mixtures of active and inactive solutions were prepared as needed for the separations.

γ-Spectrometry was performed using a Ge(Li) detector coupled to a PC-based MCA. The 80.5-keV (6%) γ-rays of ^{166}Ho and 82.4-keV (13%) γ-rays of ^{166}Dy were used for detection. Our detection limit for ^{166}Dy was estimated to be 70 pCi. All radioactive samples and standards were counted in 10-mL vials in a constant liquid geometry. Percent activities were calculated relative to the external standards.

Apparatus. The ^{166}Ho/^{166}Dy separation was performed using a metal-free Isco Model 2350 HPLC pump, Rheodyne Model 9125 syringe injector, and Isco Retriever-500 fraction collector. The determination of Dy was performed on a DMS 100 UV/visible spectrophotometer.

Column Preparation. The following columns were prepared for separation of ^{166}Ho from Dy mixtures:

Column A. A 0.8 × 20 cm glass column was packed with 100–200 mesh AG 50W resin of varying cross-linkage (Na^+ form) and operated at 87 °C (melting point of α-HIBA).

Column B. A 1.8 × 50 cm glass column was packed with 200–400 mesh of AG 50WX12 resin and was operated at room temperature except as noted.

Column C. A 0.4 × 15 cm HPLC metal-free column was packed with the same type of resin as column B.

Column D. A 0.4 × 25 cm HPLC metal-free column was packed with Aminex-A5 cation-exchange resin. Columns C and D were always operated at room temperature.

Column E. A 1.3 × 2.1 cm glass column was packed with the same type of resin as column B. Column E was used for purification of ^{166}Ho from the complexing agent after separation from Dy.

Separation of ^{166}Ho from Dy. All columns were preequilibrated with the appropriate eluent before injection of the Ho/Dy samples. Column A was eluted at a flow rate of 0.6–1.0 mL/min with 0.2–0.3 M aqueous solution of α-HIBA at pH = 4.28–4.63 adjusted with NH_4OH. The column loads were typically 1 mL in volume in which the contents of ^{164}Dy varied between 0.2 and 3 mg in 0.01 M HNO_3. Column B was eluted at a rate 0.8 mL/min (once at 1.6 mL/min) with 0.132–0.2 M α-HIBA at pH = 4.2–4.3 adjusted with NaOH, except for 0.132 M samples, where the pH was adjusted with NH_4OH. In this case, the sample volume was 150 μL with Dy contents of 200–400 μg in 0.01 M HNO_3. Columns C and D were eluted with 0.066–0.132 M α-HIBA at pH

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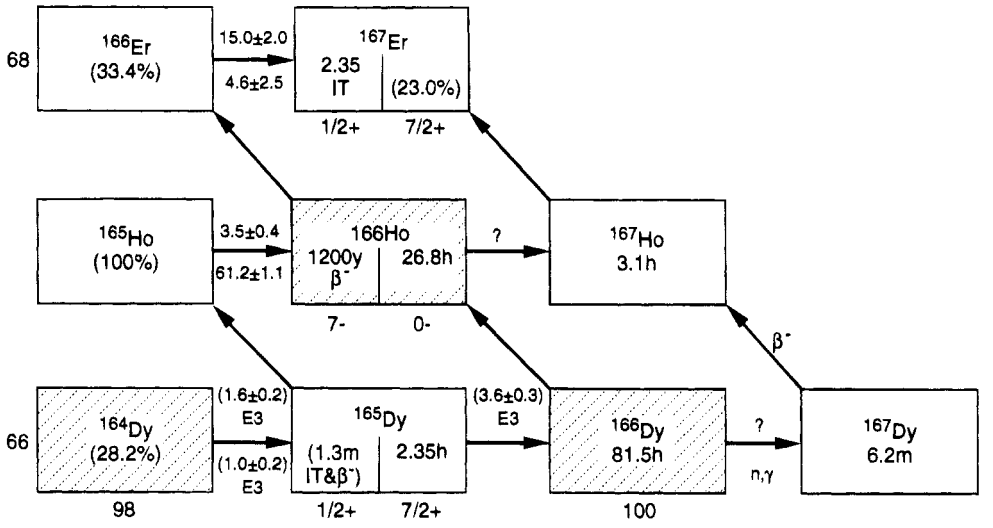

Figure 1. Scheme for production of ^{166}Ho and ^{166}Dy in a nuclear reactor.

Table 1. Reactor Productions of ^{166}Dy[a]

reactor/irradiation position	neutron flux ($n \cdot s^{-1} \cdot cm^{-2}$)	T_{irr} (days)	^{166}Dy Yield (mCi/mg of ^{164}Dy)
HFIR-HT[b]	2.0×10^{15}	8	3.5×10^3
		1	2.2×10^3
HIFAR-PT[c]	5×10^{13}	0.5	~2

[a] Targets were 97% isotopically enriched $^{164}Dy_2O_3$. [b] HFIR-HT, ORNL high-flux isotope reactor, hydraulic tube. [c] HIFAR-PT, ANSTO high-flux australian reactor, pneumatic tube.

= 4.2–4.3 adjusted with NH_4OH. In certain experiments, ^{166}Ho was eluted with 0.085 M α-HIBA and Dy with 0.132 M. Volume of samples were 150 μL in which the contents of ^{164}Dy varied between 200 μg and 2.5 mg in 0.01 M HNO_3.

Purification of ^{166}Ho from α-HIBA after Separation from Dy. The combined fraction containing ^{166}Ho (usually 25–30 mL) was acidified with 1 M HCl to pH = 2 and loaded on column E preequilibrated with 1 M HCl. The column was washed with 6 × 10 mL portions of 1 M HCl to remove α-HIBA. These fractions were evaporated to dryness for further analysis. Purified ^{166}Ho was eluted from the column at a rate 2 mL/min with 12 × 2 mL portions of 6 M HCl.

Spectrophotometric Determination of Stable Dy. For calibrations, solutions of $DyCl_3$ and Arsenazo III were buffered at pH = 4.0 with 0.01 M acetate buffer at an ionic strength of 0.1 (NaCl) and mixed in the Dy:Arsenazo III ratio of 1:1. The absorbance was monitored at 660 nm. Free Arsenazo III absorbs only slightly at this wavelength and pH ($\epsilon = 650\ cm^{-1} \cdot M^{-1}$) while the 1:1 and 1:2 lanthanide complexes of Arsenazo III have extinction coefficients of 3.5×10^4 and $5.0 \times 10^4\ cm^{-1} \cdot M^{-1}$, respectively.[32] The detection limit of Dy was estimated to be ~2 μg/mL. For determination of Dy contents in carrier-free ^{166}Ho fraction, solutions containing ^{166}Ho (6 M HCl) were evaporated to near dryness. The residue was dissolved in 1 mL of 0.01 M acetate buffer at ionic strength of 0.1, and Dy concentration was determined.

RESULTS

Production of ^{166}Dy. Dysprosium-166 is produced by double neutron-capture on ^{164}Dy in a nuclear reactor (see Figure 1). Table 1 summarizes the experimental yields of ^{166}Dy. The yields of ^{166}Dy, produced in the hydraulic tube of the ORNL HFIR was 2.2 and 3.5 Ci/mg of ^{164}Dy (the initial mass of ^{164}Dy) for 1 and 8 days of irradiation, respectively. The relative reduction of the yield for the 8-day irradiation is due to the very large effective decay constant ($\lambda + \phi_n\sigma$) of the short-lived intermediate nuclei, ^{165}Dy, which results in substantial target depletion. The low yield from

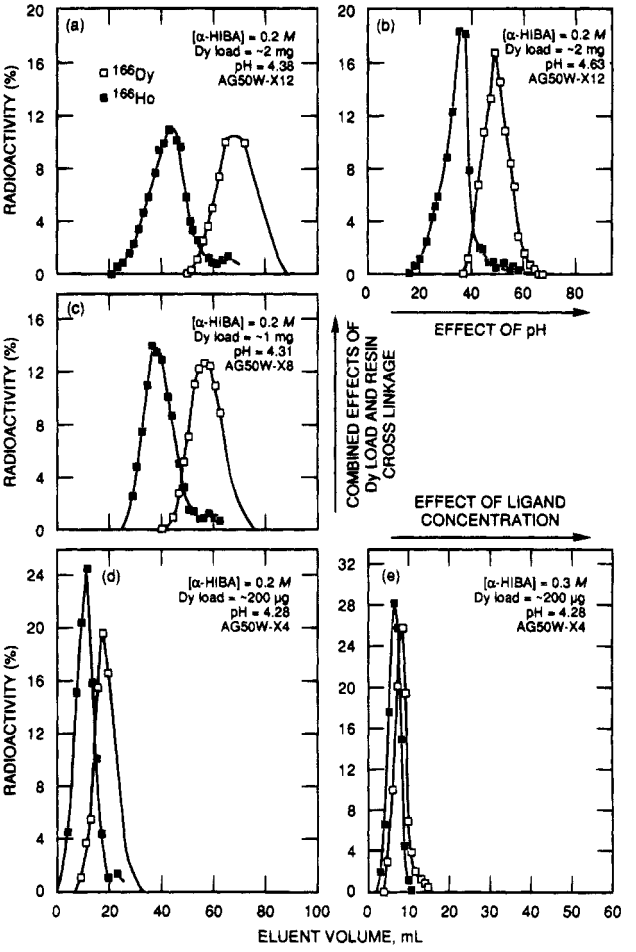

Figure 2. Separation of ^{166}Ho/Dy mixtures using column A (AG50W, 100–200 mesh, 0.8 × 20 cm at 87 °C): influence of pH (a, b), combined effects of Dy load and degree of resin cross-linkage (a, c, d), and effect of ligand concentration (d, e).

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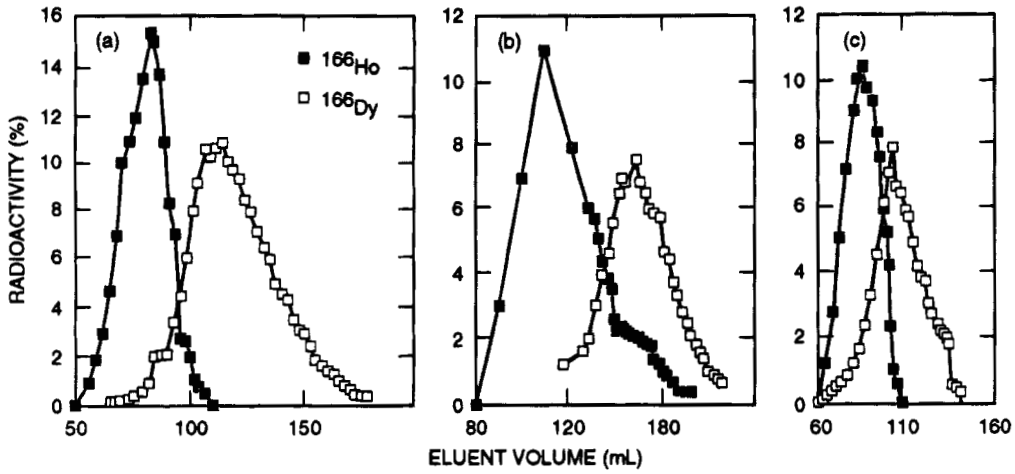

Figure 3. Effect of temperature on separation of ^{166}Ho/Dy mixtures using column B (AG 50WX12, 200–400 mesh, 1.8 × 50 cm): [α-HIBA] = 0.2 M; Dy load, 400 μg); T (°C) = (a) 25, (b) 37, and (c) 57.

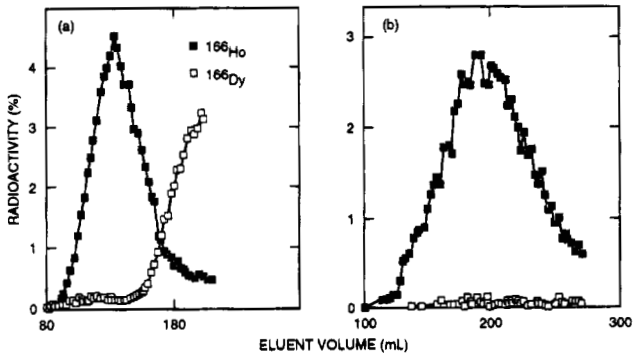

Figure 4. Combined effects of [α-HIBA] and Dy load on separation of ^{166}Ho/Dy mixtures (column B): Dy load, (a) 200 (b) 400 μg; [α-HIBA] = (a) 0.145 and (b) 0.132 M.

the ANSTO HIFAR simply reflects the 40-fold reduction in the neutron flux (in the double-neutron-capture process, yield is proportional to the square of the neutron flux).

^{166}Ho/Dy Separation. The results from the first series of experiments employing column A, depicted in Figure 2, define the following general conditions for the separation of ^{166}Ho from Dy on AG 50W resins: [α-HIBA] < 0.2 M; pH in the range from 4.3 to 4.6; resin cross-linkage 8 and 12. Separations were performed at 87 °C and a flow rate of 0.6–1.0 mL/min.

Variables studied with the larger column B include flow rate, temperature, and concentration of α-HIBA (see Figures 3 and 4). The optimal flow rate for column B was found to be 0.8 mL/min. The separation was not quantitative at higher flow rates. The retention time and full width at half-maximum (fwhm) of peaks decreased at higher temperature (Figure 3), but it appears that under similar conditions, better separation was achieved at lower temperature. Figure 4 illustrates the combined effects of α-HIBA concentration and Dy load. At pH = 4.3 and T = 25 °C, reducing the concentration of α-HIBA from 0.145 to 0.132 M improved the separation, even though the Dy load increased from 200 to 400 μg. The substitution of NH_4OH for NaOH, which was used for pH adjustment, may have also contributed to improvement of separation. Figure 4b shows that a quantitative separation between carrier-free ^{166}Ho and 400 μg of Dy was achieved when the concentration of α-HIBA was decreased to 0.132 M. The fraction of Dy in ^{166}Ho, in this case, was estimated to be <4%, or 16 μg by UV measurement. Due to the large size of column B, however, complete separation of ^{166}Ho fraction took ~6 h.

The results obtained from column C are shown in Figure 5. In this case, the column was eluted at p_{sys} = 200 psi at a flow rate of 0.8 mL/min. Optimization of the elution parameters was carried out in the range of α-HIBA concentrations from 0.066 to 0.132 M at pH = 4.27. As shown in Figure 5, 0.085 M α-HIBA provides almost complete separation of ^{166}Ho from 1.5, 2.0, and 2.5 mg of Dy (panels a–c of Figure 5, respectively). The fractions of Dy in ^{166}Ho fractions were 1.7% when Dy load on the column was 1.5 mg and 2.7% for Dy loads of 2.0 and 2.5 mg. In all three cases, complete elution of ^{166}Ho required ~1 h. In the cases of 2.0- and 2.5-mg Dy loads, after elution of ~96% of ^{166}Ho activity, Dy was stripped off the column with 0.132 M α-HIBA (Figure 5b,c).

The results obtained from column D (Aminex-A5) are shown in Figure 6. Separations of ^{166}Ho from 1.5–2.5 mg of Dy were performed via elution with 0.085 M α-HIBA. As shown, an increase in system pressure from 700 to 1400 psi resulted in significant improvement of the separation efficiency of the column. The back pressure in column D increased slightly in each pass as a result of improving the packing conditions. Similar to column C, complete elution of ^{166}Ho fraction required ~1 h followed by Dy elution with 0.132 M α-HIBA.

Preparation of ^{166}Ho in Ionic Form after Separation from Dy. The results of separation of ^{166}Ho in ionic form from the α-HIBA complexing agent on column E are shown in Figure 7. As seen, ^{166}Ho was strongly retained by the resin when the column was washed with 6 × 10 mL of 1 M HCl and then was stripped off the column with 24 mL of 6 M HCl. Upon evaporation, the column washes 1–4 showed decreasing amounts of α-HIBA, while washes 5 and 6 were visibly clear. Spectrophotometric analysis of $^{166}Ho^{3+}$ in 1 mL of 0.01 M acetate buffer at 0.1 ionic strength detected ~35 μg of Dy after separation from 2.5 mg of Dy on an AG 50W column (column C) and ≤2 μg after separation on Aminex-A5 (column D).

DISCUSSION

The separation scheme described here is based on reversed phase ion-exchange chromatography, where Ho and Dy are partitioned between the cation-exchange resin (AG 50W and Aminex-A5) and the mobile phase containing the weakly complexing ligand α-HIBA at pH = 4.3–4.6. As a consequence of "lanthanide contraction" and smaller ionic radii, the complex of α-HIBA with Ho has slightly higher thermodynamic stability than

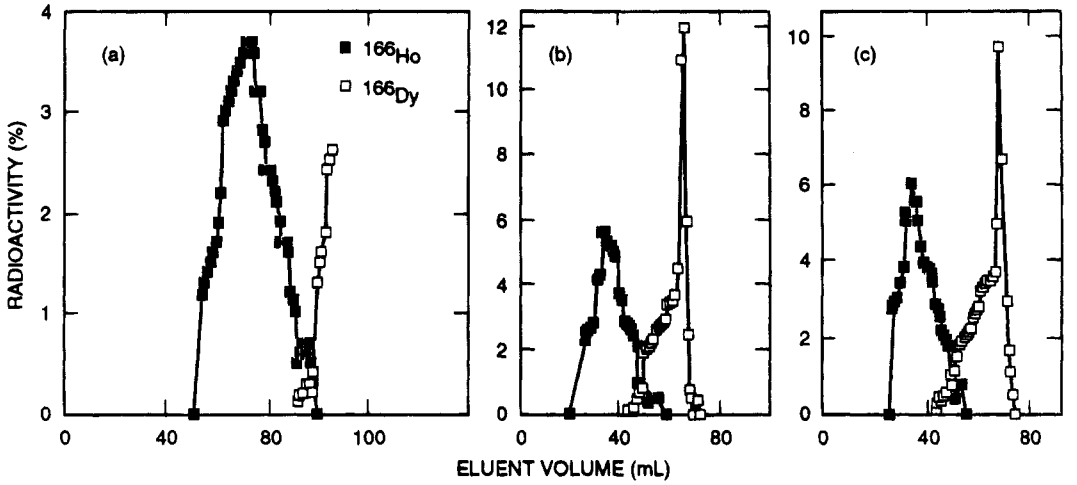

Figure 5. Separation of ^{166}Ho from 1.5–2.5 mg of Dy on a metal-free HPLC using column C (AG 50WX12, 200–400 mesh, 0.4 × 15 cm) via elution with 0.085 M α-HIBA at pH = 4.27 and p = 200 psi: Dy load (mg), (a) 1.5, (b) 2.0, and (c) 2.5. Dy was stripped off the column with 0.132 M α-HIBA from (a) 0, (b) 59, and (c) 63 mL.

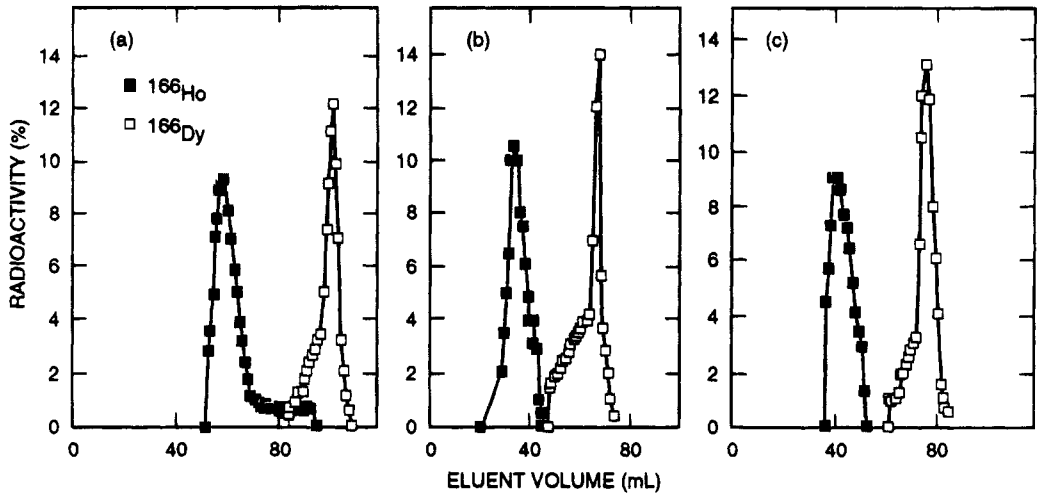

Figure 6. Separation of ^{166}Ho from 1.5–2.5 mg of Dy on a metal-free HPLC using column D (Aminex-A5, 0.4 × 25 cm) via elution with 0.085 M α-HIBA at pH = 4.27: Dy load, (a) 1.5, (b) 2.0, and (c) 2.5 mg; system pressure, (a) 750, (b) 1000, and (c) 1400 psi. Dy was stripped off the column with 0.132 M α-HIBA from (a) 92, (b) 62, and (c) 70 mL.

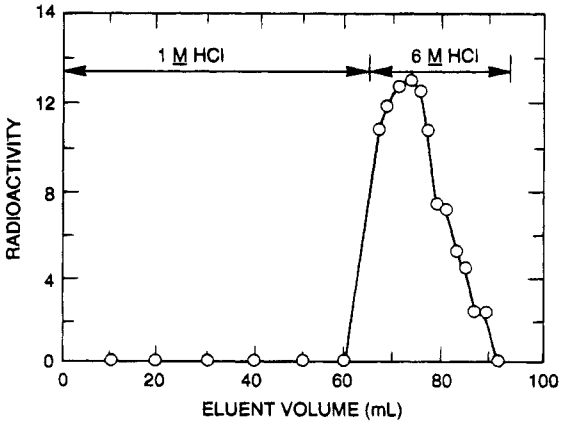

Figure 7. Elution profile of carrier-free ^{166}Ho from column E (AG 50WX12, 200–400 mesh, 1.3 × 2.1 cm) with HCl solutions.

that with Dy, and the elution pattern is reversed with Ho being eluted first. The log β (overall stability constant) of Ho and Dy complexes with α-HIBA are 7.67 and 7.24 at 0.1 ionic strength, respectively.[9] The technique has been used extensively for separation of various members of lanthanides, but it is believed that the present study is the first demonstration of the applicability of this technique for the separation of carrier-free ^{166}Ho from milligram quantities of Dy, where carrier-free ^{166}Ho is produced from β^- decay of ^{166}Dy. Under optimum conditions of [α-HIBA] = 0.085 M, pH = 4.27 (adjusted with NH_4OH), T = 25 °C, and flow rate of 0.8 mL/min, quantitative separation between Ho and Dy was achieved in a metal-free HPLC column containing AG 50WX12 resin (0.4 × 15 cm, 200–400 mesh) operated at 200 psi. In this case, the separation factor between Ho and Dy, calculated as the ratio of (% recovery of Ho)/(% recovery of Dy), was ~60. At higher column pressure, better separation was obtained at lower α-HIBA concentration. A much larger column (1.8 × 50 cm), containing the same resin and operating just above the atmospheric pressure, was not as effective as the above noted smaller HPLC column. Under similar experimental conditions as above, Aminex-A5 owing to its smaller particle size (~13 μm) provided the best resolution. A separation factor of ~950 between Ho and Dy was obtained with an Aminex-A5 column operated at 1400 psi. Further separation of the purified ^{166}Ho from α-HIBA was achieved with a small column of AG 50WX12 (200–400 mesh) from 1 M HCl solution followed by elution of the ionic Ho^{3+} from the column with 6 M HCl.

In conclusion, the possibility of obtaining carrier-free ^{166}Ho from decay of ^{166}Dy was demonstrated. It was shown that, within a narrow pH range and ligand concentration, it is possible to separate carrier-free ^{166}Ho with high yield (95%) from milligram quantities of neutron-irradiated Dy target by use of a HPLC system. The entire separation process was achieved within 2 h and the separation factor between Ho and Dy was ~950 (corresponding to a Dy breakthrough of ~0.1%) in a single pass. These results provided a basis for development of a ^{166}Dy → ^{166}Ho biomedical generator system, which is currently under evaluation.

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