Radiochim. Acta **90**, 411–415 (2002)

Radiochemical separation of no-carrier-added radioniobium from zirconium targets for application of ^{90}Nb-labelled compounds

By S. Busse, J. Brockmann and F. Rösch*

Institut für Kernchemie, Johannes Gutenberg-Universität, D-55128 Mainz, Germany

(Received October 24, 2001; accepted in revised form February 19, 2002)

Positron emitter ^{90}Nb */ Radiochemical separation / Extraction / Ion exchange*

Summary. The radioisotope ^{90}Nb is a positron emitter with a relatively high positron branching of 51% and a rather low β^+-energy of $E_{\text{mean}} = 662$ keV ($E_{\text{max}} = 1.5$ MeV) with potential for application in positron emission tomography (PET). Its half-life of 14.6 h makes it especially promising for the quantitative investigation of biological processes with slow distribution kinetics. An advantageous production route of ^{90}Nb is the $^{\text{nat}}$Zr$(p,n)^{90}$Nb process. To separate nca (no-carrier-added) radioniobium from macroscopic amounts of irradiated zirconium targets, an effective two step separation procedure was developed. A liquid-liquid extraction using N-benzoyl-N-phenylhydroxylamine (BPHA) in $CHCl_3$ was applied first to separate the bulk of the zirconium matrix with a Zr separation coefficient of about 10^4, followed by anion-exchange chromatography for high grade purification of nca radioniobium. A sufficiently high decontamination factor of $> 2 \times 10^7$ and an overall radiochemical yield of nca ^{90}Nb of about 70% was achieved within about 3 hours.

Introduction

The radioisotope ^{90}Nb is a new positron emitter with potential for application in the synthesis of new radiopharmaceuticals for positron emission tomography (PET). It has a relatively high positron branching of 51% with a rather low β^+-energy of $E_{\text{mean}} = 662$ keV and $E_{\text{max}} = 1.5$ MeV. Its half-life of 14.6 h makes it promising in particular for the investigation of biological processes with slow distribution kinetics, *e.g.* for the non-invasive and quantitative detection of tumours and metastases using ^{90}Nb-labelled peptides or antibodies.

An advantageous production route of ^{90}Nb at cyclotrons providing proton energies of at least 11 MeV is the $^{\text{nat}(90)}$Zr$(p,n)^{90}$Nb-process. Investigations of this nuclear reaction over the most relevant proton energy region between 7.5 and 19 MeV indicate, that ^{90}Nb can be produced with batch activities of the order of 10 GBq and in high isotopic purity [1], which would be adequate for potential application of this new positron emitter in nuclear medicine.

However, for the synthesis of potential ^{90}Nb-radiopharmaceuticals an effective chemical separation of nca (no-carrier-added) radioniobium from macroscopic amounts of zirconium targets is necessary. Although methods for the separation of nca ^{95}Nb from nca ^{95}Zr have already been developed in the context of the recovery of fission products, the separation of nca radioniobium from macro amounts of Zr was hardly investigated before [2]. The aim of this study was therefore to develop a separation method, providing nca radioniobium in high radiochemical yields and in high chemical purity, necessary for subsequent labelling syntheses.

Materials and methods

Chemicals

Zirconium granules of 1–3 mm diameter (ChemPur, 99.8%, Karlsruhe, Germany) were used as target material for the production of ^{95}Nb. The upper limits of contamination (in ppm) were Nb (50), Hf (250), Fe (200), Cr (200), Al (40), Ti (20), Ni (20), Cu (20), V (20), W (200), rare earth elements (50). N-benzoyl-N-phenylhydroxylamine (BPHA) was used in extraction experiments (purchased from Merck, Darmstadt, Germany). Anion-exchange experiments were performed using Aminex A27 resin (Biorad, Ca, USA). Other reagents were standard chemical compounds of analytical grade purchased from Merck, Darmstadt, Germany.

Production of ^{95}Nb

In the separation experiments ^{95}Nb ($T_{1/2} = 35$ d) was employed as a model nuclide. It was produced *via* the ^{94}Zr$(n,\gamma)^{95}$Zr $\xrightarrow{\beta^-}$ ^{95}Nb reaction. In analogy to the production of the positron emitter ^{90}Nb *via* the ^{90}Zr(p,n)-process using a cyclotron, the irradiation of $^{\text{nat}}$Zr in a nuclear reactor provides nca radioniobium in a bulk sample of a zirconium target. Thus, the reactor nuclides ^{95}Zr and ^{95}Nb represent an adequate model system to study their chemical separation.

To produce ^{95}Nb ($T_{1/2} = 64$ d) *via* the ^{94}Zr$(n,\gamma)^{95}$Zr $\xrightarrow{\beta^-}$ ^{95}Nb process typically 1 g of $^{\text{nat}}$Zr was irradiated first at the TRIGA research reactor, Mainz, Germany, with a thermal neutron flux density of $\Phi = 4 \times 10^{12}$ s^{-1} cm^{-2}. However, higher activities were produced at the BER-2 reactor, Berlin, Germany, with a thermal neutron flux density of $\Phi = 4 \times 10^{14}$ s^{-1} cm^{-2}. In the latter case, an irradiation lasting 5.2 days resulted in about 1.4 GBq of ^{95}Zr. Approaching

---

*Author for correspondence (E-mail: frank.roesch@uni-mainz.de).

the transient equilibrium between ^{95}Zr and ^{95}Nb, the maximum daughter activity of 0.68 GBq ^{95}Nb was generated after 67 d.

Measurement of radioactivity

For the determination of radiochemical separation efficiencies relative activity measurements were performed. Ge(Li) or HPGe detectors were used additionally for γ-ray spectrometry of ^{95}Nb and ^{95}Zr and the other radionuclides involved in the experiments, cf. Table 1.

Spectroscopy

The contamination of radioniobium with zirconium after the separation process was determined independently by UV-VIS spectroscopy and ICP-MS.

Arsenazo III is well known in inorganic chemistry as a complexing agent for ionic zirconium. The resulting complex has an absorption band in the visible region with a maximum at 675 nm, which is suitable for the quantification of Zr(IV), since uncomplexed arsenazo III hardly absorbs at this wavelength. A calibration curve for the quantitative determination of Zr was achieved as follows: various aliquots ($x = 100$, 60, 50, 40 and 20 µl) of a solution of 10 µg/ml Zr in hydrochloric acid (5% by weight) were added to 2 ml of an arsenazo III stock solution (0.543 mg/ml 5% HCl) followed by an addition of $(1000 - x)$ µl of 5% HCl. After 10 minutes these solutions were transferred to glass cuvettes of 1 cm diameter and the extinction at 675 nm was determined using a Varian UV-VIS spectrometer Cary 50 Bio. The detection limit of the described method was approximately 0.1 µg Zr.

Smaller traces of Zr in radioniobium were quantified by ICP-MS at the Institut für Radiochemie, Garching, Germany, with a Perkin Elmer & SCIEX spectrometer Quadrupol ICP-MS ELAN 5000. The samples of the separated radioniobium were prepared to contain 1 M HF. The detection limit for Zr in these experiments was about 0.5 ng.

Distribution coefficients (K_d)

Distribution coefficients K_d for ^{95}Nb were determined for Dowex 1×8 (200–400 mesh) anion exchange resin in batch experiments. A stock of the resin was thoroughly rinsed with desalted water and then dried at 40 °C to constant weight. For the distribution experiments, 1 g amounts of the dried resin were suspended in 10 ml of the desired solution in polyethylene vessels. After a swelling time of 10 min under continuous shaking the suspensions were spiked with nca ^{95}Nb and kept in motion for another 24 hours. After this period of time the solution was separated from the resin by filtration through polyethylene filters. The activity in aliquots of 5 ml of the solution was determined by γ-ray spectrometry leading to the K_d values through $K_d = (A - A_l)/A_l \cdot V_l/m_{resin}$; ($A$: spike activity, A_l: activity in solution, V_l: volume of solution, m_{resin}: mass of resin).

Dissolution of Zr

The irradiated ^{nat}Zr-granules (200 mg, 2.19 mmol) were transferred in a 100 ml polyethylene beaker containing 2 ml water. Under vigorous stirring and cooling on a water bath, 0.63 ml of 48% hydrofluoric acid (17.5 mmol) were added in small portions.

Liquid–liquid extraction

After dissolution of the irradiated Zr, 5 ml of 37% HCl and 3.4 ml saturated boric acid (2.7 mmol) were added. The ^{95}Nb fraction was extracted with 5 ml of 10^{-2} M of N-benzoyl-N-phenylhydroxylamine (BPHA) in $CHCl_3$ by extensively stirring the two phases in a tetrafluoroethylene funnel for three minutes. The two phases were separated and the aqueous phase was washed with 3 ml of $CHCl_3$. The unified $CHCl_3$ phases were transferred to another extraction funnel and washed consecutively with 2 ml of 9 M HCl/0.001 M HF and 2 ml of 9 M HCl. The re-extraction into an aqueous phase was carried out according to [3] with 5 ml aqua regia for 20 minutes. This aqueous phase was washed three times with 2 ml of $CHCl_3$.

Ion exchange

The aqueous phase derived from the extraction process was evaporated to dryness. The residue was dissolved in 0.5 ml of 0.25 M HCl/0.1 M oxalic acid and adsorbed on a small anion exchange column (Aminex A27, 20 × 1.7 mm). After washing with 0.1 ml of 37% HCl, the adsorbed Zr was eluted with 0.2 ml of 9 M HCl/0.001 M HF with a flow of

Table 1. Relevant data for the production of ^{95}Nb in a nuclear reactor.

Nuclide	Abundance [%]	Decay mode	$T_{1/2}$	γ-energy [keV]	Intensity [%]	$\sigma_{n,th}$ [10] [b]
^{90}Zr	51.45	stable	–	–	–	≈ 0.014
^{91}Zr	11.22	stable	–	–	–	1.2
^{92}Zr	17.15	stable	–	–	–	0.2
^{93}Zr		β^-	1.5×10^6 a	–	–	≈ 2
^{94}Zr	17.28	stable	–	–	–	0.049
^{95}Zr	–	β^-	64.02 d	724.2 756.7	43.7 55.4	–
^{95}Nb	–	β^-	34.98 d	765.8	99.9	–
^{96}Zr	2.76	pseudo stable	3.9×10^{19} a	–	–	0.020
^{97}Zr	–	β^-	16.9 h	507.6 1148	5.1 2.6	–
^{97}Nb	–	β^-	1.2 h	657.9	98.5	–

10 μl/min. Subsequently the ^{95}Nb fraction was eluted with 0.1 ml 6 M HCl/0.01 M oxalic acid.

Results and discussion

To make ^{90}Nb an appropriate radiolabel for applications in nuclear medicine, the specific activity of a potential radiopharmaceutical has to be of the order of 50 GBq ^{90}Nb/μmol. The requirement of an adequate separation process was to reduce the macroscopic amounts of ^{nat}Zr to the level of nca radioniobium. Since the ratios of the masses corresponding to 10 MBq ^{95}Nb (reactor nuclide) or 1 GBq ^{90}Nb (cyclotron nuclide) and an assumed target mass of 200 mg ^{nat}Zr are $\mathrm{Nb/Zr} \approx 3 \times 10^{-8}$ and 6×10^{-8} respectively, it was necessary to reach separation factors of the order of $> 10^7$. The concept of an effective separation was to eliminate first the bulk of the zirconium matrix, followed by a high grade purification.

Dissolution of irradiated zirconium targets and liquid–liquid extraction

Liquid–liquid extraction is often an effective method to separate two species, especially when the separation of large amounts of material is considered. To dissolve the inert metal zirconium, hydrofluoric acid is most appropriate. The fluoride anions in the resulting solution, however, exhibit a high affinity for the highly charged cations Zr(IV) and Nb(V). Thus, high HF concentrations in the solution reduce the extraction yield of nca $^{95(90)}$Nb. It is known, that this undesired effect can be partly suppressed by increasing the concentration of HCl in the solution [4]. To reduce the content of free fluoride in the aqueous phase, the effect of adding boric acid was investigated.

Lyle *et al.* [3] showed that ^{95}Nb is readily extracted with a solution of N-benzoyl-N-phenylhydroxylamine (BPHA) in $CHCl_3$ from a hydrofluoric and hydrochloric acid solution containing ^{95}Nb and ^{95}Zr in no-carrier-added amounts. Because in this system no-carrier-added Nb is extracted into an organic phase, it was assumed that it is applicable to extract ^{95}Nb from macroscopic amounts of Zr, too.

Irradiated samples of Zr were dissolved in a minimum of HF. With an eightfold excess of HF over Zr a solution was obtained after one day. Having dissolved macroscopic amounts of Zr with HF, it is not possible to exactly determine the concentration of free fluoride in solution, because Zr-species like $[\mathrm{Zr(OH)}_x\mathrm{F}_y]^{n-}$ of different composition are present. In Fig. 1 the extraction yield of ^{95}Nb from 0.02 M $[\mathrm{Zr(OH)}_x\mathrm{F}_y]^{n-}$-solutions in the absence of boric acid and with a $B(OH)_3$ concentration of 0.08 M as a function of the HCl concentration is shown.

The extractability of ^{95}Nb decreases continuously for $[\mathrm{HCl}] < 8$ M, cf. Fig. 1. In the presence of 0.08 M $B(OH)_3$ this undesired reduction of the extraction yield of ^{95}Nb begins to occur only for $[\mathrm{HCl}] < 3$ M. Thus, the employment of boric acid reduces the amount of hydrochloric acid, which has to be added to the extraction system. The resulting smaller volume of the aqueous phase makes an effective stirring with a likewise small volume of the organic BPHA-phase easier. The achieved extraction yield under optimum conditions is $> 99\%$.

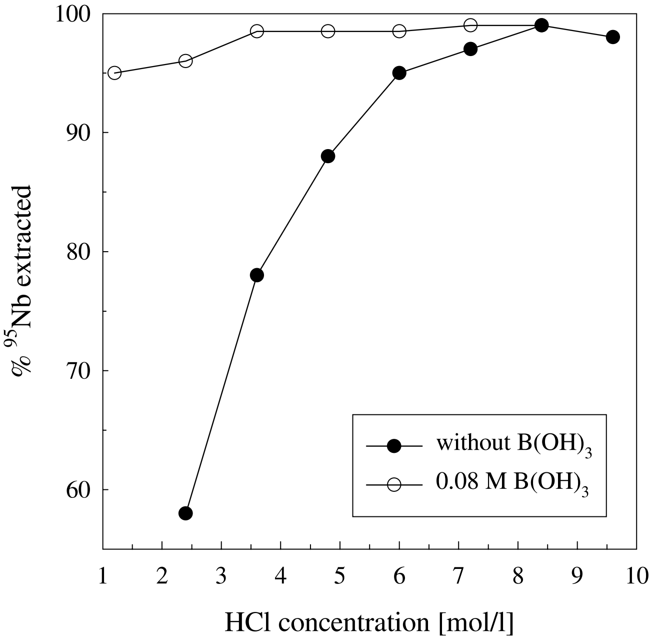

Fig. 1. Influence of boric acid on the extraction yield of ^{95}Nb with a solution of 0.02 M BPHA in $CHCl_3$ from a solution of 0.02 M $[\mathrm{Zr(OH)}_x\mathrm{F}_y]^{n-}$ in dependence of the concentration of HCl ($t_{\mathrm{extraction}} = 3$ min).

From the experimental results a routine extraction protocol was derived as follows: To 2 ml water in a 100 ml polyethylene beaker 200 mg ^{nat}Zr-granules (2.19 mmol) are given. Under stirring and cooling on a water bath 0.63 ml of 48% hydrofluoric acid (17.5 mmol) are added in small portions. Having dissolved the zirconium (approximately 15 min) 5 ml of 37% HCl and 3.4 ml of a saturated boric acid solution (2.7 mmol) are given to the solution. This aqueous phase is extracted with 5 ml of 10^{-2} M BPHA in $CHCl_3$ by extensively stirring the two phases in a tetrafluoroethylene funnel for three minutes. The two phases are separated and the aqueous phase is washed with 3 ml of $CHCl_3$. The unified $CHCl_3$ phases are transferred to another extraction funnel and washed consecutively with 2 ml of 9 M HCl/0.001 M HF and 2 ml of 9 M HCl. The re-extraction into an aqueous phase is carried out according to [3] with 5 ml aqua regia for 20 minutes. This aqueous phase is washed three times with 2 ml of $CHCl_3$ (approximately 15 min).

The described process takes about two hours and the yield of ^{95}Nb varies between 70% and 80%. The average overall content of Zr in the ^{95}Nb-fraction, determined independently by UV-VIS spectroscopy and ICP-MS, is 21–26 μg with respect to the initial 200 mg ^{nat}Zr granules. The separation coefficient thus achieved is $9 \pm 2 \times 10^3$ (Table 2) and is comparable to that found for the separation of ^{95}Nb from carrier-free Zr of 10^4 [3].

Anion exchange

To reduce the remaining content of Zr further, an anion-exchange step was introduced. The conditions for the adsorption of nca ^{95}Nb and nca ^{95}Zr on strongly basic polymeric resins from a 0.25 M HCl/0.1 M oxalic acid solution [5] and the elution of nca ^{95}Zr with a 0.001 M HF/9 M HCl [6] from such an anion-exchange material were adapted

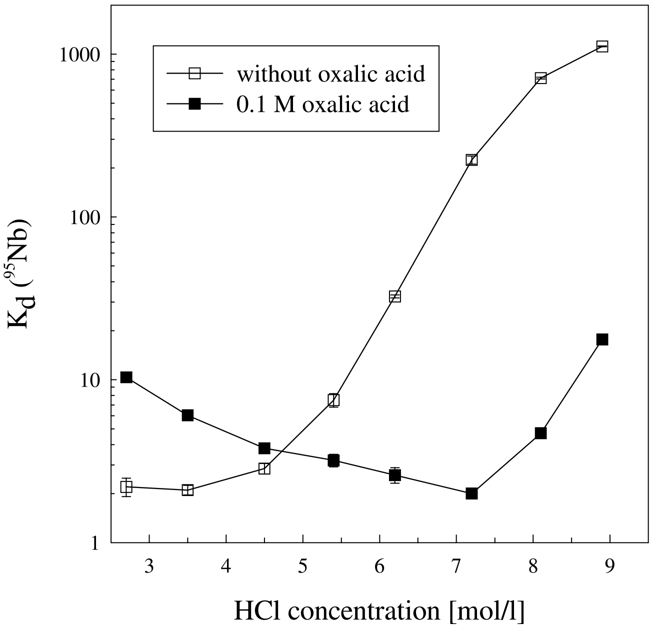

Fig. 2. Distribution coefficients (K_d) of ^{95}Nb for Dowex 1×8 (200–400 mesh) in dependence of the concentration of HCl and the presence of oxalic acid.

from the literature and evaluated for the separation of nca ^{95}Nb from macroscopic amounts of ^{95}Zr on Aminex A27 or Dowex 1×8.

To optimise the conditions for the final elution of nca ^{95}Nb the influence of oxalic acid was studied, due to the known high affinity of Nb for oxalic acid [7]. For this purpose the distribution coefficients K_d for ^{95}Nb on Dowex 1×8 (200–400 mesh) were determined in batch experiments. The results are summarised in Fig. 2.

The increase of the K_d values for higher concentrations of pure HCl can be explained by the formation of anionic species of Nb and is in good agreement with the results from Bunney *et al.* [8]. In the presence of 0.1 M oxalic acid, however, the K_d is different. As can be also seen in Fig. 2, for [HCl] < 4.5 M the respective K_d values are larger and for [HCl] > 4.5 M smaller than in pure HCl. Following the results from Fig. 2, comparable conditions for the elution of ^{95}Nb are either 3 M HCl in the absence of oxalic acid or 6 to 7 M HCl in the presence of 0.1 M oxalic acid. For the preparation of nca Nb stock solutions the latter conditions are recommended, because oxalic acid prevents the uncontrolled adsorption of nca Nb on surfaces, which is a typical phenomenon of nca niobium in the absence of a complexing species [9].

In practice, the aqueous phase derived from the extraction process is evaporated to dryness, the sample is taken up in 0.5 ml of 0.25 M HCl/0.1 M oxalic acid and adsorbed on a minimised Aminex A27 column (20 × 1.7 mm). After washing with 0.1 ml of conc. HCl, the adsorbed Zr is eluted with 0.2 ml of 9 M HCl/0.001 M HF and ^{95}Nb is subsequently eluted with 0.1 ml of 6 M HCl/0.01 M oxalic acid.

The anion exchange procedure takes about one hour and the nca radioniobium yield varies between 90 and 95%. The amount of zirconium still present in the nca Nb fraction was

Table 2. Yields and attained decontamination factors of the subsequent separation steps.

Separation step	Yield of ^{95}Nb [%]	Zr-content [μg] relative to 0.2 g Zr	Decontamination factor
extraction	> 99	21–26^{b,c}	$9 \pm 2 \times 10^3$
re-extraction	70–80		
anion-exchange	90–95	0.005–0.01^{c}	$3 \pm 1 \times 10^3$
total^{a}	62–75	0.005–0.01^{c}	$1 \pm 3 \times 10^7$

a: including dissolution of the target and evaporation to dryness of the re-extracted solution;
b: UV-VIS-spectroscopy;
c: ICP-MS

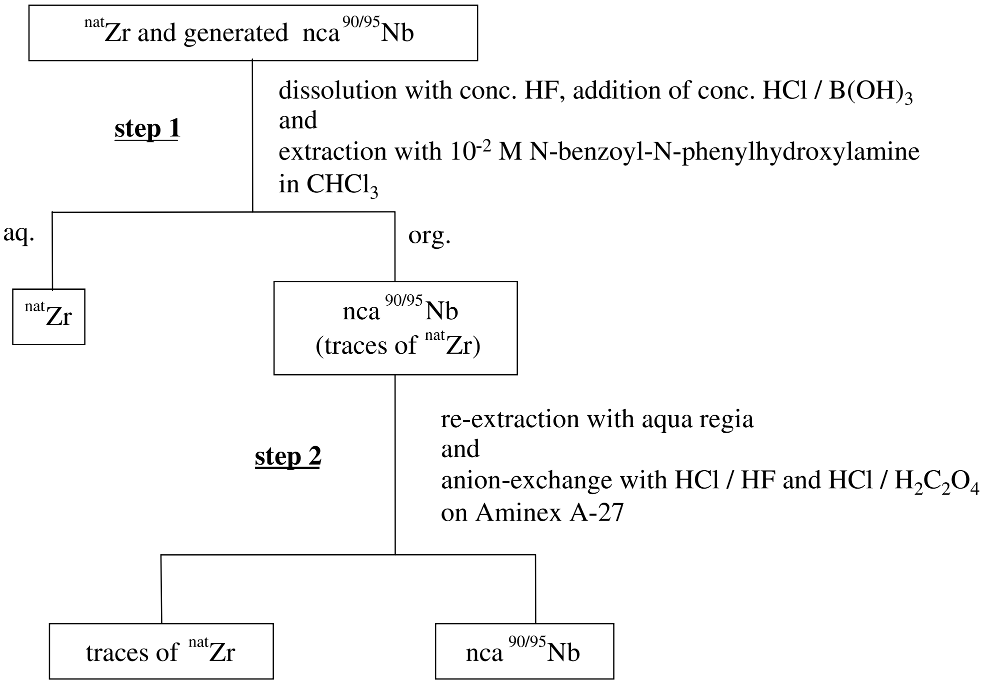

Fig. 3. Concept for the separation of nca $^{90/95}$Nb from macroscopic amounts of ^{nat}Zr.

determined by means of ICP-MS as 5–10 ng. With respect to the mean value of 23 μg of Zr adopted from the extraction step the decontamination factor of this anion exchange purification step is 2.3×10^3 to 4.6×10^3. Compared to the 200 mg of Zr used in the beginning, the overall separation factor is $3 \pm 1 \times 10^7$ cf. Table 2.

A flow sheet of separation process developed, is given in Fig. 3. The whole process takes about three hours.

Conclusion

To separate nca radioniobium as produced from the irradiation of Zr targets, a two step procedure was developed. It is capable of separating Nb at nca level from macroscopic amounts of Zr within about 3 hours with a sufficiently high decontamination factor of about 10^7 and an overall radiochemical yield of about 70%. Regarding the highest specific activity of nca radioniobium required, the amount of stable niobium present in the zirconium target remains an essential parameter.

Following the presented data it should be possible to separate ^{90}Nb produced *via* the $^{90}\mathrm{Zr}(p, n)$ process in a satisfactory yield and sufficient chemical purity to label biomolecules for medical application. However, adequate bifunctional chelators are required for its labelling to peptides and proteins.

Acknowledgment. Acknowledgement is made to the crew of the research reactor BER-2 at the Berlin Hahn Meitner-Institut in Berlin for the irradiation. The authors wish to thank PD Dr. Th. Probst of the Institut für Radiochemie, Garching, for the ICP-MS-measurements.

References

1. Busse, S., Rösch, F., Qaim, S. M.: Cross section data for the production of the positron emitting niobium isotope ^{90}Nb *via* the $^{90}\mathrm{Zr}(p, n)$-reaction. Radiochim. Acta **90**, 1 (2002).
2. Lahiri, S., Mukhopadhyay, B., Das, N. R.: Studies on liquid–liquid extraction of no carrier-added ^{91,92,96}Nb and ^{93m}Mo isotopes produced in α-particle activated zirconium target with HDEHP. Radiochim. Acta **83**, 93 (1998).
3. Lyle, S., Shendrikar, A. D.: A convenient carrier-free separation of niobium from zirconium. Radiochim. Acta **3**, 90 (1964).
4. Cristallini, O., Dupetit, G. A.: On the carrier-free extraction of niobium with N-benzoyl-phenyl-hydroxylamine. Radiochim. Acta **4**, 172 (1965).
5. Speecke, A., Hoste, J.: La separation niobium-tantale en melieu chloro-qxalique sur echangeur d‘anion. Talanta **2**, 332 (1959).
6. Kraus, K. A., Moore, G. E.: Anion exchange studies. 1. Separation of zirconium and niobium in HCl-HF mixtures. J. Am. Chem. Soc. **73**, 9 (1951).
7. Ahrens, H., Kaffrell, N., Trautmann, N., Herrmann, G.: Decay properties of neutron-rich niobium isotopes. Phys. Rev. C **14**, 211 (1976).
8. Bunney, L. R., Ballou, N. E., Pascual, J., Foti, S. C.: Quantitative radiochemical analysis by ion exchange. Anion exchange behaviour of several metal ions in hydrochloric, nitric and sulphuric acid solutions. Anal. Chem. **31**, 324 (1959).
9. Schubert, J., Richter, J. W.: The use of ion exchange for the determination of physicochemical properties of substances, paticularly radiotracers, in solution. III. The radiocolloids of zirconium and niobium. Colloid Sci. **5**, 376 (1950).
10. Pfennig, G., Klewe-Neenius, H., Seelmann-Eggebert, W.: *Chart of Nuclides*. 6th ed., Karlsruhe (1998).