Alternative rapid separation strategy for isolation of no-carrier added $^{90}\text{Nb}$ from Zr target, for application in \textit{immuno-PET}.

Valery Radchenko$^1$, Dmitry Filosofov$^2$, Nikolai Lebedev$^2$, Olga Bochk$^2$, Frank Roesch$^1$

$^1$ Institute of Nuclear Chemistry, Johannes Gutenberg-University Mainz, Fritz-Strassmann-Weg 2, D-55128 Mainz, Germany;

$^2$ Laboratory of Nuclear Problems, Joint Institute of Nuclear Research, Joliot-Curie 6, 141980, Dubna, Russian Federation.

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Immuno-PET

- Labeling of monoclonal antibody (mAb) with positron emitting radionuclides for tracking, visualization, and measurement of tumor gene expression
Requirements for *immuno*-PET nuclides

- a physical half-life paralleling the biological half-life of the antibody or antibody fragment

- a preferably low $\beta^+$ energy to allow high-resolution PET imaging

- a high positron branching with no or weak accompanying irradiation ($\beta^-$, $\gamma$) to offer high-sensitive PET imaging while reducing the radiation burden of the patient

- the availability of the radionuclide, *i.e.* an efficient production route
Motivation

- Relative high positron branching (53%)
- Rather low $\beta^+$ energy ($\beta^+_{\text{mean}} = 0.35 \text{ MeV}$)
- Intermediate half-life 14.6 hours

make $^{90}\text{Nb}$ a promising radionuclide for application in *immuno-PET.*
• successful labeling of monoclonal antibody (Rituximab) with $^{90}$Nb (90% labeling after 1 hour incubation at RT)

• high *in vitro* stability (less than 7% degradation after 9 days incubation in FCS at 37°C)

• proved suitability of $^{90}$Nb for *immuno*-PET

• **however**: previous separation strategy was too complicated and time consuming (more than 4 hours) for routine *in vivo* application

• **consequently**: fast and more efficient alternative separation strategy was developed
Production of $^{90}\text{Nb}$

Target holder

Target station

Zirconium discs $\varnothing$ 10 mm
Irradiation parameters

- \( E_p : 17.5 \text{ MeV} \) (at first foil)
- Current: 5 \( \mu \text{A} \)
- Irradiation: 60 min
- Batch: 724.6 MBq
- Production yield 144.9 MBq/\( \mu \text{A} \cdot \text{h} \)

Radionuclide purity EOB

<table>
<thead>
<tr>
<th>Isotopes</th>
<th>(^{90}\text{Nb}) 14.6 h</th>
<th>(^{89}\text{Zr}) 78.4 h</th>
<th>(^{92m}\text{Nb}) 10.2 d</th>
<th>(^{95}\text{Nb}) 35 d</th>
<th>(^{96}\text{Nb}) 23.4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>96.76</td>
<td>0.29</td>
<td>1.79</td>
<td>0.42</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Previous separation strategy

Dissolution of Zr target

Extraction

Aqueous phase containing bulk amount of Zr

Organic phase containing n.c.a. Nb with rest of Zr

Back-extraction

Organic phase containing trace of Zr

Aqueous phase containing n.c.a. Nb with trace of Zr

Anion-exchange

F1 Loading
F2 Washing
F3 Elution of Zr
F4 Elution of Nb

• Stepwise addition of 0.63 ml conc. HF to 2 ml of water under ice-cooling
• Addition of conc. HCl (5 ml) and B(OH)_3 (3.4 ml)
• Extraction with 2·10^-2 M N-benzoyl-N-phenylhydroxylamin (BPHA) (5 ml)
• Back-extraction with 5 ml aqua-regia (5 ml)
• Anion-exchange:
  • resin Aminex-27 15 ± 2 μm
  • column 20X1.5 mm
  • F1 loading
  • F2 100 μl 10 M HCl
  • F3 200 μl 9 M HCl/0.001 M HF
  • F4 200 μl contain Nb in 6 M HCl/0.01 M oxalic acid
Alternative separation strategy

- irradiated zirconium metal dissolved in 2 mL conc. HF

- cation exchange (Dowex® 50X8) column to filtrate unsolved particles and absorb possible contamination of 2+ and 3+ cations

- transfer to anion exchange (AG® 1X8) column
- absorb $^{90}$Nb from hydrofluoric solution
- zirconium passed trough
- 5 mL of 28 M HF to reduce Zr contamination
- 1 mL 1 M HCl to remove HF traces
- elution of $^{90}$Nb with 700 $\mu$L 6 M HCl / 1% H$_2$O$_2$
• 700 μL of mixture 6 M HCl / 1% H₂O₂ heated 5 min. at 120 °C

• 700 μL of 12 M HCl added to increase HCl concentration

• mixture loaded on UTEVA® column

• 5 mL 5 M HCl to remove Zr traces

• 200 μL 1 M oxalic acid passed through

• final elution of ⁹⁰Nb with 200 μL 1 M oxalic acid
Results

Irradiated Zr target

28 M HF

K⁺

A⁻

UTEVA®

<table>
<thead>
<tr>
<th>Separation step</th>
<th>Separation yield %</th>
<th>Decontamination factor (ICP-MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolution</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Cation+anion</td>
<td>99</td>
<td>0.97 ·10⁵</td>
</tr>
<tr>
<td>UTEVA</td>
<td>95</td>
<td>3.36 ·10⁸</td>
</tr>
</tbody>
</table>

- total separation 1.5 hours
- separation yield 95%
- decontamination factor > 10⁸
- < 0.77 ng of Zr
- ⁹⁰Nb in final fraction appropriate for labeling conditions (200 μL 1 M oxalic acid)
Semi-automated separation module

- decreasing dose burden
- separation yield 90%
- faster separation (1 hour)
- similar decontamination factor $10^8$
Comparison of both separation strategy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>New separation strategy</th>
<th>Old separation strategy</th>
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</thead>
<tbody>
<tr>
<td>Separation time (h)</td>
<td>1-1.5</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>Separation yield %</td>
<td>90-95</td>
<td>79-81</td>
</tr>
<tr>
<td>Decontamination factor Zr/Nb</td>
<td>$10^8$</td>
<td>$10^7$</td>
</tr>
<tr>
<td>Automation</td>
<td>available</td>
<td>difficult</td>
</tr>
<tr>
<td>Final fraction</td>
<td>200 µL 1 M ox. acid (easy labeling protocol)</td>
<td>200 µL 6M HCl/ 0.01 M ox. acid (complicated neutralization procedure)</td>
</tr>
</tbody>
</table>
Deferoxamine (Df): chelator of choice

- best complexation with Nb compared to other chelators (DOTA, TETA, DTPA and EDTA)
- successful complexation of Nb at RT
- high stability of *Nb-Df complexes and *Nb-Df-Octreotide
- clinically approved chelator
- well established conjugation chemistry for $^{89}$Zr
Conjugation of Df to mAb

Product identification:
- Macrocyclics Product ID: B-705
- Molecular weight: 752.9 g/mol
- Purity: ≥ 94%
- Desferroxamine-p-SCN
- Molecular Formula: $C_{33}H_{52}N_{8}O_{8}S_{2}$
- Appearance: white solid
Labeling of Monoclonal Antibody

Monoclonal antibody (IMAB362) as proof-of-principle.

100 µg of mAb (modified with desferrioxamine) labeled with 10 MBq of $^{90}$Nb 1 hour at room temperature and pH 6.8

Results:

- Labeling yield more than 85% (HPLC, ITLC)
- Specific activity $> 85$ MBq/mg (comparable with $^{89}$Zr 180 MBq/mg)
- After purification (PD-10) more than 97% of product
- Product stable (90%) at room temperature and at incubation in FCS at 37°C for 5 days
Summary

- **aim:**
  New separation strategy of $^{90}$Nb appropriate for *in vivo* evaluation of biomolecules (*immuno*-PET)

- **efficient:**
  90 - 95\% of $^{90}$Nb with a decontamination factor of Zr / Nb of $> 10^8$

- **fast:**
  1 - 1.5 hours (almost four times faster than with previous separation strategy)

- **semi-automated module**

- **labeling $^{90}$Nb-mAb:**
  > 85\% after 1 h incubation at RT.
  specific activity > 85 MBq/mg

- **stability *in vitro*:**
  High, <10\% of degradation after 5 days of FCS incubation at 37 °C
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Thank you for your attention!