Separation and purification of no carrier added arsenic from bulk amounts of germanium being adequate to radiopharmaceutical labeling chemistry.

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Introduction: The element arsenic is well known as the favorite poison of the Savellis, the Borgias and Agatha Christie [1]. Another application of arsenic radioisotopes might be its use as radioactive probe in sub-toxic trace amounts for biological or medical purposes. The tracer concept of radiopharmaceutical chemistry allows the application of no carrier added (nca) amounts of radioactive isotopes (e.g. of arsenic) that are used for labeling of interesting biological carriers like monoclonal antibodies (mab) and the imaging of their biological behavior *in vivo*.

Experimental: *Dissolving the target:* The germanium target (100-200 mg) was placed in a quartz distillation apparatus (Fig. 1) and 4 ml *aqua regia* was added. The apparatus was heated to 120°C and during this time the irradiated germanium metal dissolved.

Distillation of $GeCl_4$: After complete dissolution of the target the temperature was maintained at 120°C for the distillation (Fig. 1) of $GeCl_4$. For acceleration of this process a stream of Argon was bubbled through the solution. Over a period of 1.5 h additional 6 ml of HCl (10 M) were added. After completed clearance the solution was condensed to less than 500 µl.

Anion exchange: The distillation solution was filled to 500 µl with 10 M HCl. The solution was transferred onto an anion exchange column (3*100 mm, AG1X8) in the chloride form and eluted with 500 µl fractions of 10 M HCl. Arsenic *As(V) was eluted in the fractions 2 and 3 (Fig. 2). After 10 fractions the eluent was switched to 0.1 M HCl for removal of gallium, germanium and zinc isotopes.

Reduction of As(V) to As(III), extraction into CCl_4 and back extraction into PBS-buffer: Fractions 2 and 3 were combined (1 ml solution) and mixed with 50 mg CuCl. The mixture was heated at 60 °C for different periods ranging from 5 to 120 minutes, with 60 minutes finally applied for the batch experiments. The As(III) was extracted twice with 500 µl CCl_4. Combined CCl_4 fractions were extracted with 500 µl PBS-buffer containing 25 mM EDTA and 0.5 M hydroxylamine.

Speciation of As(III) and As(V)

One of the key steps for labeling of mab with arsenic isotopes is the availability of *As(III) in the labeling solution. Care should be taken that the separated samples are used for labeling immediately or stored frozen in an atmosphere of argon to prevent the *As(III) from re-oxidation. The final fraction in 500 µl PBS contained > 95 % *As(III).

Labeling of Bevacizumab: The 500 µl of the purified radioarsenic solution in the PBS fraction was combined with 500 µl of Bevacizumab solution (1.25 mg, 8 nmol). 10 µl of TCEP (10 mg/ml, 420 nmol) were added and the mixture was allowed to stand at room temperature for 1 h.

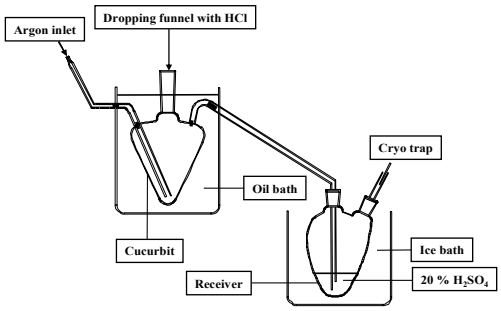

Figure 1. Schematic drawing of distillation apparatus

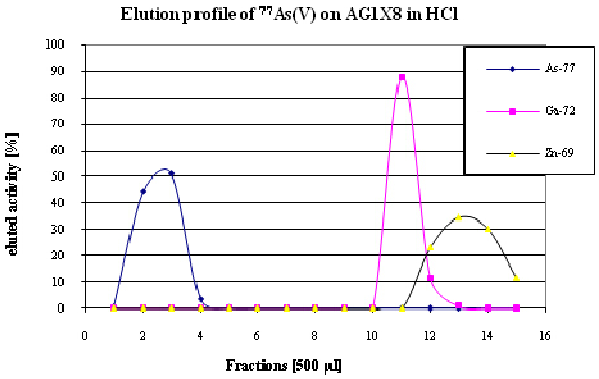

Figure 2. Elution profile of ^{77}As(V) distillate for reactor irradiated Ge target.

Results: Metallic germanium and ^{77}As(V) were first separated by distillation with an average separation factor of $2*10^4$. This was followed by purification with anion exchange chromatography for separation of the remaining germanium and radioactive trace amounts of ^{69m}Zn and ^{72}Ga formed during irradiation. The overall separation for germanium/arsenic was > $1•10^6$. The overall yield of *As(III) from the target to the final 500 µl PBS fraction was > 40%. Labeling of antibodies was successfully exemplified with the monoclonal antibody Bevacizumab providing labeling yields of > 99 % after 1 h incubation at room temperature.

References

[1] Zhu, J., et al., *How acute promyelocytic leukaemia revived arsenic.* Nat Rev Cancer, 2002. **2**(9): p. 705-13.

Acknowledgement

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