Speciation of nca As(III)/(V) and determination of antibody labeling yield by radio-TLC

M. Jahn[1], F. Rösch[1]

[1] *Institute of Nuclear Chemistry, University of Mainz, Fritz-Strassmann-Weg 2, D-55128 Mainz, Germany*

Introduction: The use of no-carrier-added (nca) radioarsenic isotopes for labeling of interesting biomolecules like monoclonal antibodies (mab) needs a fast and easy to handle method for the determination of its oxidation state and labeling yield. The labeling strategy of mabs with radioactive arsenic isotopes like reactor produced nca ^{77}As (β^--emitter) or cyclotron produced nca ^{74}As (β^+-emitter) consists of its reaction with free sulfhydryl groups that are present inside the mab. Therefore the mab needs to be modified with additional SH-groups by the SATA pathway [1] or by reduction of endogenous cystein-disulfide bonds with TCEP (tris(2-carboxyethyl)phosphine) [2].
The determination of the As oxidation state is very important, because As(III) is the reactive form, whereas As(V) is not able to react with free sulfhydryl groups. The determination of labeling yields of antibodies can be performed with HPLC methods by using size exclusion columns (Fig. 1). This method is very effective but also time consuming because one single run needs 10-20 min. With TLC methods it is possible to follow the reaction progress at shorter time points by spotting 0.5 µl of the reaction mixture on a TLC plate. Therefore a method that was developed for macroscopic amounts of carrier added ^{76}As was modified and applied to the nca $^{74/77}$As system [3].

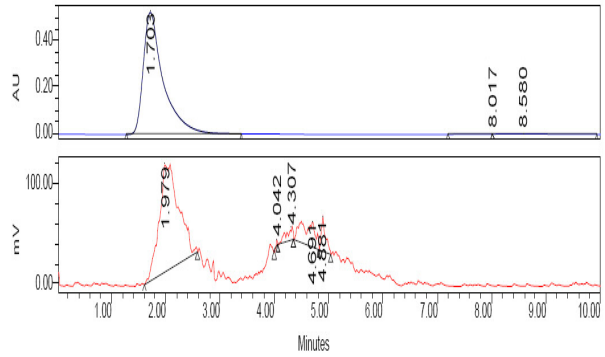

Fig. 1: SEC-HPLC-chromatogram of mab in UV (1.7 min) and radioactivity (2 min: labeled mab; 4 min: unreacted nca ^{77}As)

Experimental: a) 0.5 µl of nca ^{74}As(III) in PBS buffer are speckled on a Si-60 TLC plate. 50 µl of this solution are treated with 0.5 µl of a H_2O_2 solution (35%) to oxidize ^{74}As(III) to ^{74}As(V). 0.5 µl of this solution are speckled on the same TLC plate. TLC is developed with 0.01 M sodium tartrate / methanol (3:1) as mobile phase.
b) 0.5 µl of a reaction solution of ^{77}As and the mab Bevacizumab (1.25 mg / 500 µl PBS) are speckled on a Si-60 TLC plate. TLC is developed with 0.01 M sodium tartrate / methanol (3:1) as mobile phase.

In both cases a) (Fig. 2) and b) (Fig. 3) the TLC plates are analyzed with an Instant Imager of Packard Canberra for the determination of R_f-values.

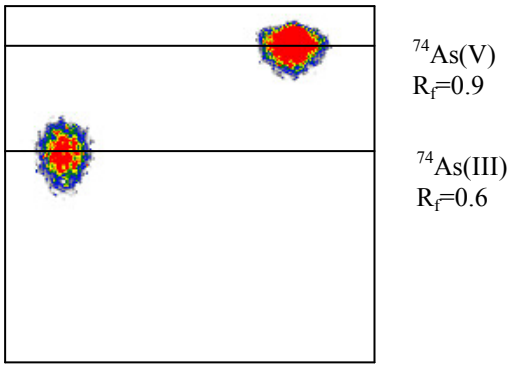

Fig.2: Radio-TLC of nca ^{74}As(III) and nca ^{74}As(V)

Results: The R_f-values of nca *As(III) and *As(V) have been determined to 0.6 and 0.9 (Fig. 2). Under these TLC conditions (0.01 M sodium tartrate / methanol in value (3:1) as mobile phase) a sample of labeled Bevacizumab has the R_f-value of 0.

Discussion: The TLC method for speciation of nca *As (III)/(V) is useful for the development of new separation methods for arsenic isotopes from a germanium matrix. A lot of publications deal with the assumption of reducing As(V) without the experimental proof of concept. It is essential for labeling chemistry to be sure to have the reactive form *As(III) in solution. This method will easily help to test reducing agents for their ability on reducing *As(V) and to optimize the amounts that needs to be applied. In addition it was shown that a labeled fraction of *As-labeled antibody Bevacitumab shows completely different R_f-values than both, *As(III) and *As(V). This makes the TLC method useful for the determination of labeling yields of mab's. The results of the TLC method are in good correlation with yields determined by SEC-HPLC (Fig. 1). But the method allows shorter intervals for investigation if labeling kinetics as far as one HPLC run needs 10-20 minutes.

References:

1. Jennewein, M., et al., *In vitro Stability and Immunoreactivity of ^{74}As[SATA]Vatuximab®.* Annual Report, Institute of Nuclear Chemistry, University of Mainz, 2004.
2. Jahn, M., et al., *Labeling of an anti-VEGF monoclonal antibody with radioactive arsenic isotopes.* Journal of Labelled Compounds and Radiopharmaceuticals, 2007. **50**(Supplement 1): p. S48.
3. Schmidt, A., J. Brockmann, and F. Rösch, *Chromatographische Trennung von AsIII, AsV und 2-Nitrophenylarsonsäure.* Annual Report, Institute of Nuclear Chemistry, University of Mainz, 2000.