Budapest University of Technology and Economics
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Production of carrier-free arsenic isotopes for medical, biochemical and environmental purposes

Summary of PhD thesis

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1. Introduction and objective

Almost everyone thinks of poison when it comes to arsenic, it is less known that it has long been used as a medicine as well.

Labeled arsenic-containing radiopharmaceutical candidates have already been prepared [1], although these are still in experimental phase. Radiopharmaceuticals apply an isotope which is attached to a biomolecule that reaches an abnormal organ or tissue through a targeted biological process or interaction in the body [2].

These special drugs are used for diagnostic and/or therapeutic purposes in cancer. In the case of a diagnostic test, information is obtained about the organ (its location, size, density etc.) where the radiolabelled biomolecule was enriched. In such cases, beta-positive emitting and "pure gamma" mesomer-state isotopes are often used, e.g. ^{18}F, ^{99m}Tc and ^{11}C [3].

In therapeutic applications radiation emitted by the isotope performs targeted cell destruction [4], which means "surgical intervention in the dimension of elementary particles" [5]. In this case, radiopharmaceuticals contain the beta-negative emitters, such as ^{131}I, ^{90}Y and [6].

A new area of research and application is theranostic use of radiopharmaceuticals. This means diagnostic and therapeutic procedures are performed with a single radiopharmaceutical. To enable this the radiopharmaceutical have to be labeled with a nuclide showing both decay properties simultaneously [7,8]. It allows personalized treatments to be developed, which creates a new opportunity in nuclear medicine. The number of isotopes which can be used in theranostics are limited, that is why the production of arsenic-labeled radiopharmaceuticals is an innovative research, because ^{72}As and ^{74}As are suitable candidates as a "theranostic pair".

In my research, I aimed to produce an arsenic-labeled biomolecule which is a suitable radiopharmaceutical candidate for theranostic purposes.

The test irradiations and the experimental irradiations were performed in the Training Reactor at the Budapest University of Technology and Economics. One of my tasks was to optimize the irradiation conditions in the reactor. During my PhD work in Aachen, Rhine-Westphalia, I was also able to work with the ^{74}As isotope produced by cyclotron in the Department of Nuclear Medicine at the University Hospital of Rhine-Westphalia in Aachen. The isotope was produced at the Jülich Research Center and transferred from there to the University of Aachen.

Another goal was to develop a new separation technique for the ^{77}As / ^{77}Ge system produced in the Training Reactor, which will also be applicable in the case of cyclotron produced isotopes.

A complete study of the quantitative reduction reaction of the separated arsenic fraction, where both arsenite and arsenate may occur, is lacking in the literature. Therefore, the systematic investigation and optimization of the reduction conditions of irradiated arsenic was also an important part of my research work.

Another one of my goals was to optimize the labeling reaction with the arsenic isotope, because the systematic study of the labeling reactions with the isotope is not complete in the literature.

2. References

2.1 Characterization of arsenic isotopes

Arsenic has a stable isotope with mass number is 75. It belongs to the group of so-called pure elements. Its isotopes are playing an increasingly important role among radioisotopes, including nuclides with a half-life less than a second (^{87}As, $T_{1/2}$=0.48 s), some seconds (^{84}As, $T_{1/2}$=4.5 s) and some minutes (^{79}As, $T_{1/2}$=9.01 min) half-life. These can be used primarily for experimental purposes or nuclear physics research. The half-lives of isotopes ^{77}As ($T_{1/2}$=38.83 h) and ^{74}As ($T_{1/2}$=17.77 d) can be measured in days. These radionuclides can be used for medical applications. I have worked with these isotopes in my research.

^{74}As can be generated by the following nuclear reactions: ^{74}Ge(p,n)^{74}As, ^{73}Ge(d, n)^{74}As and ^{nat}Ge(p,x)^{74}As in a small cyclotron. Details of irradiation with naturally occurring germanium target have been reported by Basile [9,10].

Another major advantage of this isotope is that it has a low positron energy ($E_{\beta+}$ =128 keV), which allows for high local resolution in imaging processes.

^{77}As is 100% beta emitter, it can be used as a therapeutic radiopharmaceutical e.g. synovectomy, (which means the surgical removal of the synovial membrane by chemical means or radiation), radioimmunotherapy, or intravascular radiation therapy (intravascular irradiation). A further advantage of arsenic-77 is that it can be produced carrier-free from natural germanium in reactors by a nuclear reaction of ^{76}Ge(n,γ)^{77}Ge with adequate efficiency without enrichment of germanium dioxide. The resulting germanium-77 decays to the isotope arsenic-77 with a half-life of 11.3 hours. This isotope can also be prepared in cyclotron from an -enriched germanium target by the ^{76}Ge(d, n)^{77}As nuclear reaction [1].^{77}As/^{77}Ge system for optimizing appropriate radiochemical separation techniques, thanks in part to the gamma line of ^{77}Ge (E_{max} = 264.44 keV) and the gamma line of ^{77}As (E_{max} = 239.01 keV), allows each separation step to be monitored gamma spectroscopically; on the other hand, the developed method can be applied to arsenic isotopes produced by cyclotron.

2.2 Separation of arsenic from irradiated germanium target

Several methods for the separation of arsenic from germanium dioxide target are already known, one of them is flotation [11]. The oldest separation procedure was based on distillation, this was successfully used as early as 1942 to separate arsenic [12]. Anion exchange chromatographic separation has been used since the last century [13], and several new methods have been developed since then. Liquid-liquid extraction separation can also be found in the early literature on arsenic/germanium separation [14, 15, 16, 17]. Thin layer chromatography has been used mainly to determine arsenic components in different oxidation states [18]. There has even been a review of separation techniques for arsenic [19] and for germanium [20].

Distillation methods

The distillation method is one of the oldest radiochemical separation techniques used. Most methods are based on the distillation of $AsCl_3$. Most of the methods are based on the distillation of $AsCl_3$. In the case of the As / Ge system, the separation is performed in several steps, where the arsenic is first oxidized to the less volatile As(V) state, the germanium is separated by distillation, and then the arsenic is reduced back to the As(III) state, it is separated from the remaining germanium by fractional distillation.

Smales and Pate [21] first used a distillation method to separate radioactive arsenic from a germanium target using a ^{76}As tracer. The method is automated and suitable for the detection of 10^{-9} g As, but the system is not closed, so volatile components may can be removed during separation. Jahn et al. [22] used distillation as part of an off-line coupled technique to separate most of the Ge from the arsenic in the form of $GeCl_4$ then to purify the arsenic by ion exchange column chromatography. The method works with good efficiency, but needs several steps and it is time consuming The Fassbender group used distillation to produce a radioarsenic target [23], and a later publication [24] reported that $AsCl_3$, together with the 6 M HCl-water azeotrope, distilled at a lower temperature than the boiling point of arsenic trichloride (BP=130.2 °C, Lide 2009), the distillation takes place in several steps with adequate efficiency (90%).

Another method of dry distillation [25] used by Tolmachev and Lundqvist to separate positron decaying arsenic from germanium dioxide is essentially a thermochromatographic process. The method was promising, but the chemical purity was inadequate.

Jennewein used a distillation method for the ^{72}As /^{72}Se generator, which was also based on the distillation of $AsCl_3$ [38].

Based on the literature, the most commonly used separation method is the distillation of volatile $GeCl_4$ from HCl solution [26, 27]. As a further step, anion exchange chromatography can be used to reduce germanium in the arsenic fraction.

Ion exchange separation methods

Ion exchange chromatography is one of the earliest methods used to separate an arsenic isotope from a germanium target [13].

Most ion exchange separation is performed by column chromatography with commercially available anion exchange resins. High acid concentrations are generally used in separation (>8 M HNO_3, HF, and HCl), but these cannot be used for isotopic labeling for human use [13].

In addition to conventional ion exchange resins, separations with new adsorbents, such as zirconium dioxide [29] or nanocrystal-based ion exchangers embedded in a polymer can also be found in the literature [30].

However, no HPLC separation method for radioactive arsenic/germanium separation is found in the literature. Nevertheless, HPLC has been used in the past to separate arsenic, and several comprehensive articles can be found on the subject, however it is about the separation of organic arsenic components [31, 32, 33].

Slejkovec published an HPLC-based method for the separation of ^{76}As-labeled arsenic molecules [34].

The focus is on the organic components because the two different oxidation states of arsenics elute in one peak. However, no HPLC separation method for radioactive arsenic / germanium separation is found in the literature.

The method developed by Schindewolf and Irvine uses a highly reproducible, strong anion exchange resin (AG-1X8) applying hydrogen fluoride eluent. In this case, arsenite is not bound to the column, germanium and arsenate are bound [13].

Separation of arsenite/arsenate was performed by Basile using this anion exchange resin [10]. The description of the method for non-radioactive components is attributed to Faris, who determined the adsorption of 50 elements in hydrogen fluoride medium [35]. Faris data can be used well to design a separation. In Jahn's doctoral dissertation, the literature on early ion exchange (1950–1960) is detailed with references [22]. He developed a mixed acid medium (HF/HCl) method on an AG-1X8 column, where at high HCl concentrations $GeCl_6^{2-}$ and $AsCl_4^-$ are bound until $AsCl_5$ elutes [22]. The method is suitable for determining the oxidation ratio, but the arsenic must be oxidized to pure fraction.

The behavior of As and Ge on the AG-1X8 column is also known in hydrochloric acid medium, at high HCl concentrations germanium and arsenite are bound until arsenate elutes [22]. He used a separation based on the Bartyzel ^{73}As /^{69}Ge /^{67}Ga system [36]. Korkish and Feik also used a strong anion exchange column, but they used a mixture of hydrochloric acid and acetic acid as eluent which are volatile solvents, so separation requires a closed system [37].

Jennewein used a strong cation exchange column to separate ^{72}Se / ^{72}As [38], Sterlow studied the sorption of arsenite and arsenate on an AG®MP-50 cation exchange column, but these show little sorption on this column [38].

There is no cation exchange-based separation for the radioactive As/Ge system in the literature, as both elements occur in anionic form [23,39,40].

Reduction of arsenic

Most of the arsenic fractions obtained in the separation contain the arsenate form. It can be used directly for various applications, such as environmental measurements [40]. Producing arsenic-based radiopharmaceuticals, arsenic must be in the As (III) oxidation state to enable the labeling reactions [1].

There is an extensive literature on the reduction of arsenate, but most of these relate to inactive conditions [41], such as the reduction of arsenic in a drinking water sample [42]. Reductions have even been performed with Escherichia coli [43] and cerium [44], also under inactive conditions [45].

Limited literature is available for the reduction of radioactive arsenic. Jahn summarized the literature on the reduction of arsenate to arsenite. In his doctoral dissertation, he studied several reducing agents (Fe(II)sulfate, ferrocene, sodium hypophosphite, cysteamine, mercaptoethanol, hydroxylamine, hydrazine, oxalic acid, and sodium iodide) [22].

According to Jennewein, NaI proved to be the best reducing agent, KI was used and it was given different results [38]. In addition, sulfur dioxide [10,46] and copper(I)chloride have also been used in the reduction of the As/Ge system [47]. Elteren et al. used $Na_2S_2O_3$ and KI for arsenic reduction [48] and Billinghurst's group also used KI [49].

The corresponding reduction reaction is important to carry out the labeling step. There is literature on the reduction of arsenic, but these are either non-quantitative reactions or have other disadvantages. A comprehensive publication on radioactive arsenic reduction is absent in the literature.

Arsenic-labeled biomolecules

Several radioisotopes of arsenic (^{72}As, ^{74}As, and ^{77}As) are also suitable for use in nuclear medicine. ^{72}As and ^{74}As are excellent for diagnostic use, and ^{77}As is a promising candidate in radioimmunotherapy [50, 51].

A possible labeling strategy is to attach arsenite to a biomolecule containing a thiol group by a strong covalent bond [52, 53]. It is well-known in the literature. For the first arsenic-labeled compound, ^{74}As isotope was prepared in carrier-free form by cyclotron irradiation using germanium target. ^{74}As-labeled monomethyl-arsonic acid and dimethyl-arsinic acid [54,55] have been used in various biodistribution studies, as well as inorganic arsenite and arsenate [56,57] also 76-arsenic-labeled dimethylarsino-penicillamine and dimethylarsino-mercaptoethanol [58].

Monoclonal antibodies have also been labeled with arsenic [47, 38], Jennewein labeled two functional globulins, ch3G4 and Rituxan (Rituximab) with arsenic, which have been successfully used as experimental radiopharmaceuticals in animal experiments. Jahn labeled a special protein called bavituximab, an antibody that binds to fats on the surface of tumors, with arsenic [59].

A compound containing trithiol (4-ethyl-2,6,7-tritia-1-arsabicyclo [2.2.2] octane) [60] has also been successfully labeled with arsenic as a nanobio material in medical imaging [61].

A polymer based on N-(2-hydroxypropyl) methacrylamide was prepared and used for a labeling reaction [44]. It is an interesting fact and coincidence that this molecule is already used as a polymeric therapeutic [62].

Monoclonal antibody (TRC105) and thiol-modified mesoporous silica nanoparticles (MSN-SH) were labeled with arsenic by Ellison et al[63].

In addition, an arsenic labeling reaction with lipoic acid has been published [63], the emphasis was not on optimizing labeling conditions but on potential applications.

3. Experimental methods

Reactor and cyclotron irradiation

The ^{77}As isotope can be produced easily and carrier-free in nuclear reactors by (n,γ) nuclear reaction from a $^{nat}GeO_2$ target. In my experiments, I used 95-105 mg GeO_2 per irradiation, (at 100 kW, with a neutron flux of 2.7×10^{12} $cm^{-2}s^{-1}$, for a period of 2-6 hours).

The ^{74}As isotope was prepared from 300 mg of GeO_2 powder (Puratronic®, 99.999%) by proton irradiation with the ^{nat}Ge (p, x) ^{74}As nuclear reaction in BC1710 type baby cyclotron (9 hours, ~ 3 μA current and 16.5 MeV proton energy).

Dissolution experiments

The main goal of my inactive dissolution experiments was to investigate germanium dioxide dissolution. I performed the dissolution studies with an inactive sample, using

literature data [64]. Dissolution experiments of the irradiated sample were designed on the basis of the inactive test experience.

Azeotropic distillation

I distilled large amounts of germanium in the form of $GeCl_4$ with an azeotropic mixture of hydrochloric acid and water at low pressure and room temperature, well below the boiling point corresponding to atmospheric pressure [65]. The redistilled germanium chloride was immediately condensed with liquid nitrogen [66].

Anion exchange column chromatogrpahy, distribution coefficient, ratio measurement

I investigated the distribution coefficient of germanium on various resins frequently used in radiochemistry (DOWEX 1 * 8, UTEVA and TRU, and Sr resin) in order to develop a new separation method using the results [67]. I developed a new column chromatography anion exchange separation method for the separation of As produced by reactor irradiation from germanium. Using this, I also measured the arsenite/arsenate ratio as a function of cooling times.

Anion Exchange Liquid Chromatography

I developed a liquid chromatographic method using a column containing an anion exchange resin coupled to an analytical HPLC system [68].

Reduction

In my work, I performed a comprehensive study to systematically optimize the reduction reaction conditions of the selected reducing agent (investigated areas: selection of appropriate reducing agent, optimal reducing agent concentration, optimal reaction time, appropriate reaction temperature, and solution pH) [69].

Labeling

The conditions of the selected model molecule (lipoic acid) labeling reaction were systematically investigated in my experiments. I developed a new HPLC method using a monolithic column to separate the labeled lipoic acid from the matrix components [69].

4. Results

Irradiation experiments

Data from experiments performed to optimize irradiation time are summarized in Table 1.

Duration of irradiation (h)	**Activity at the end of irradiation /100 mg GeO_2 (kBq)**
0.5	$4.63 \cdot 10^5$
1	$9.13 \cdot 10^5$
1.5	$1.35 \cdot 10^6$
2	$\mathbf{1.78 \cdot 10^6}$

6	$4.72 \cdot 10^6$

1. Table: Optimization of irradiation

It can be seen from Table 1. that the two-hour irradiation is optimal. The two-hour irradiation was also determined by the technical conditions (specific reactor time, 8-hour working day). Therefore, for subsequent experiments, irradiations were performed for 2 h at 100 kW with a neutron flux of 2.7 10^{12} cm^{-2} s^{-1} using the tube mail start-receive station, producing $1.8 \cdot 10^6$ Bq (1.8×10^4 Bq/mg) ^{77}Ge by irradiating 100 mg of $^{nat}GeO_2$. I optimized the production of ^{74}As based on Basile's work, our results were consistent with his. As it can be seen from the figure in the article [10], it is advisable to let it cool the irradiated sample for 13 days, at which time the ratio of ^{74}As activity to contaminants is optimal. We cooled the sample for 10 days before processing. Table 2. summarizes the activities measured at the end of irradiation and the activities measured after a cooling period of 10 days (arsenic-74 and major contaminants).

Isotope	$\mathbf{A_{EOB}}$	$\mathbf{A_{th=(10\ day)}}$ **[Bq]**
^{67}Ga	1.9 MBq	0.2 kBq
^{72}As	11.3 MBq	1.0 kBq
^{74}As	14.8 MBq	9.9 MBq
^{71}As	1.5 MBq	1.1 MBq

2. Table: Activity of As isotopes measured at the end of cyclotron irradiation and after 10 days of cooling time

Dissolution experiments

Table 3 summarizes my measurement results. Best dissolution material is concentrated hydrogen-fluoride, but it is not always usable, e.g. for HPLC separation or in case of subsequent human use.

Materials	$\mathbf{L_{GeO2}}$
cc. HF	400 mg/ml
0.1M NaOH	33.3 mg/ml
H_2O	4.2 mg/ml

3. Table: GeO_2 dissolution data based on my own measurements

The dissolution experiments of the already irradiated GeO_2 sample were performed according to Table 3. The acidic and alkaline solutions were used several times in my later experiments. When dissolved in water, the concentration of irradiated germanium dioxide can not get high enough to enable detection in all relevant fractions of the studied separation methods.

Azeotropic distillation

I developed a simple separation method to separate ^{77}As from ^{77}Ge, the principle of the method being low temperature separation based on the partial pressure difference between $GeCl_4$ and $AsCl_3$.

For the separation, I used a special two-fingered glass jar, the graphic outline of which is shown in Figure 1. Irradiated GeO_2 dissolved in 2 ml of 2 M KOH was placed in one finger

section. This was frozen using liquid nitrogen and HCl was added to the sample, (final c_{HCL}=6 mol/dm^3). The acid layer also was frozen and then the whole system was placed under vacuum. After that the system was allowed to warm up to room temperature to combine the acidic and alkaline fractions. Meanwhile, the other part of the glass jar, was cooled with liquid nitrogen. The system was under vacuum for 1.5 hours until the separation was complete. Separation can be accelerated by placing the finger part 1 in a 30° C water bath. The $GeCl_4$ co-distilled with the azeotropic mixture of hydrochloric acid and immediately condensed into the other part of the glass vessel, which was cooled with liquid nitrogen. The entire system was evacuated to 10^{-4} bar pressure.

The condensed fraction was warmed to room temperature, the system was brought to atmospheric pressure, and 200-200 µl of both fractions/finger sections were pipetted, which were measured on the gamma detector as point sources.

I removed the arsenic fraction from one finger of the glass jar, leaving the germanium in the other finger, a new saturation cycle was created due to the decay of the ^{77}Ge isotope. By swapping the room temperature and frozen finger sections, the Ge/As separation can be repeated after waiting three ^{77}Ge half-lives in the same glass device, as shown in Figure 1. The advantage of the new method is that the separation takes place in a closed system and no heating is required during the process. $GeCl_4$, which is volatile at room temperature, is separated into the second finger portion of the glassware, which is under continuous cooling, so that the arsenic fraction can be safely removed from the glassware.

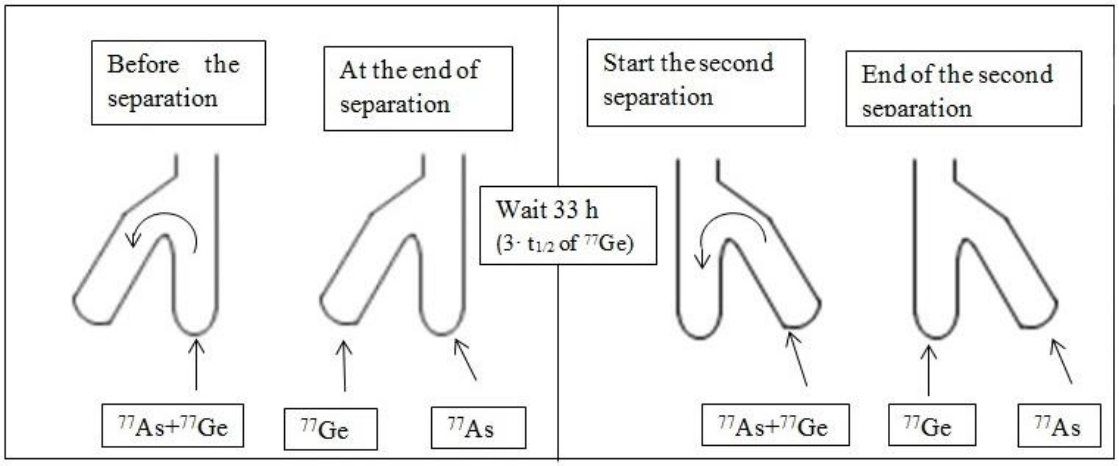


Figure 1: Azeotropic distillation

Anion exchange column chromatography

I developed a simple anion exchange chromatographic method to separate ^{77}As from ^{77}Ge, the principle of the method is based on the different distribution coefficients of GeF_6^{2-} and AsF_4^-.

Sample preparation is relatively simple and the separation method is fast, column conditioning can be performed in parallel with sample preparation and optional reduction steps, so the entire process takes up to two hours from sample dissolution to collection of the separated arsenic fraction.

100 mg of irradiated $^{nat}GeO_2$ target was dissolved in 0.25 ml cc. HF, this takes place with stirring for five minutes at room temperature. The clear solution was diluted with distilled

water to give a concentration of 1 M for HF. The solution was separated in two and a solution containing 50 mg of GeO_2 was applied to each column.

A column containing Dowex® 1X8 anion exchange packing (chloride form, 100-200 mesh) was used for the separation. I filled the column (l = 4 cm d = 0.8 cm) myself, I closed the filled column with quartz wool.

Then I conditioned the column with 2·6 ml of distilled water and then with 6·6 ml of 1 M HF solution. The capacity of the column is 1.2 meq/ml, so I poured half of the sulphite solution in HF onto the column and the other half onto a column prepared and conditioned in the same way.

While stirring, Na_2SO_3 was added to the solution so that the sulphite concentration was also 1 M for the solution. Na_2SO_3 reduced the total amount of arsenate in the solution to arsenite. The sample was stirred for an additional 45 minutes at room temperature for the reduction to occur, with the last five minutes at 60 °C. After heating, I checked the pH of the solution with pH paper (pH = 6). After adding 6 drops of concentrated HF solution, I measured the pH of the solution again (pH = 1).

The load was poured onto the column, the dripping solution was collected in a plastic well test tube, and the column was washed with 3×2 ml of 1 M HF solution and then with 4×2 ml of cc HF solution. Each fraction was collected in additional well tubes, from which I later pipetted 200-200 µl and measured on the gamma detector as a point source. The method has the advantage of automation and standardization, as well as the possibility of using different eluents or eluent mixtures to optimize the separation.

Anion Exchange Liquid Chromatography

I developed a new liquid chromatographic method to separate the ^{77}As isotope from ^{77}Ge. The irradiated sample was dissolved in KOH to give a final concentration of 50 mM KOH for alkali. The injected sample was separated by the following buffer gradient method: HPLC column was conditioned with 50 mM NaOH for 50 minutes at a flow rate of 1 ml/min after a 10-minute aqueous wash at a flow rate of 1 ml/min. 500 µl of sample was injected, which was separated by the following control procedure: 50 mM NaOH was applied for 8 min; then a linear gradient was chenged from 8-16 minutes from NaOH to 50 mM HCOOH. The flow rate was 1 ml/min throughout. After detection, the peaks of the chromatogram were collected in fractions and detected with a gamma spectrometer. I did not use the UV detector because neither arsenic nor germanium has UV absorption [37]. The developed liquid chromatographic method was successfully applied to the $^{nat}GeO_2/^{74}As$ system as well.

The separation method is fast, and due to the automation of the liquid chromatography system, the column can be conditioned in parallel with the sample preparation steps, so the whole process takes 1.5 hours from sample dissolution to collection of the separated arsenic fraction.

Reduction

I systematically examined the conditions of the arsenic reduction reaction (selection of the appropriate reducing agent, optimal reducing agent concentration, optimal reaction time, appropriate reaction temperature and pH of the solution) and examined the volume dependence and compared the two best reducing agents. Reduction with 1 M Na_2SO_3 at 95 °C

for 20 minutes at pH=1.1 quantitatively reduces arsenate with an efficiency above 99%. Quantitative reduction of arsenic opens the door using arsenic isotopes in nuclear medicine, but may also be the key to the successful implementation of each separation method.

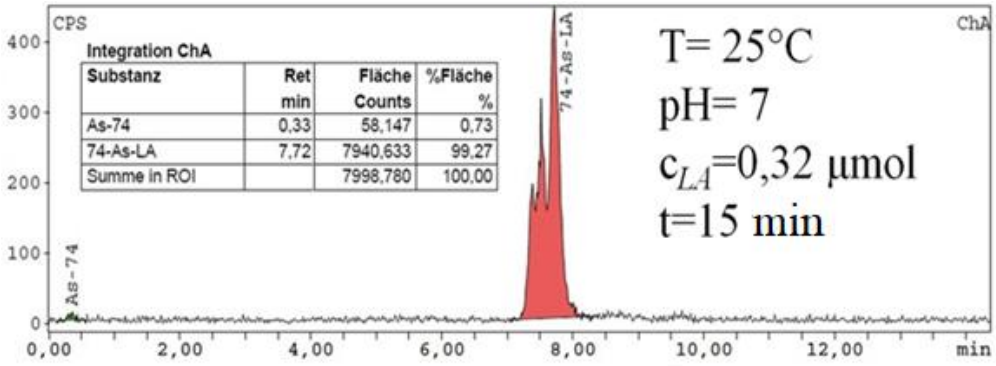
Labeling

I systematically examined the conditions of the labeling reaction of lipoic acid with arsenic (solution pH, concentration of the applied lipoic acid, reaction temperature, and reaction time) and determined the optimal parameters.

Reduced lipoic acid is a good model molecule due to its free thiol groups. The labeled lipoic acid is suitable for biomedical use because it is found in the mitochondria of cells and is a cofactor for several enzymes. The labeling reaction proceeds rapidly at neutral pH and room temperature with a labeling efficiency over 99%.

Description of HPLC method

Similary to the control of the reduction efficiency, the labeling efficiency was determined by analytical HPLC method. For the ^{74}As-lipoic acid analysis, I used a Chromolith (50×3 mm) type reverse phase monolithic chromatography column. In this case, I used a reversed-phase monolithic column, which allows rapid separation due to the high flow rate used (3 ml / min). Thus, arsenic-labeled lipoic acid can be simultaneously selectively and efficiently separated from the contaminant components.



1. Figure 2: ^{74}As-labeled lipoic acid chromatogram containing optimal lbeling reaction parameters

5. Theses points

1. I developed a separation method to separate ^{77}As from an irradiated GeO_2 target, first applying the low-temperature separation method based on the partial pressure difference of $GeCl_4$ and $AsCl_3$. The method can separate large amounts of arsenic (MBq) from the irradiated target and works like a generator because radioactive arsenic is repeatedly generated in the pure germanium fraction so that the separation can be repeated. [2]

2. I was the first to develop a liquid chromatographic method for the separation of radioactive ^{77}As from germanium dioxide using a column containing an anion exchange resin coupled to an analytical HPLC system [68]. Most ion exchange methods use high concentrations of toxic acids in the separation, which is not permissible for materials designed for later human use.

With the sodium hydroxide and acetic acid system, I was able to develop a separation method where arsenic fraction can be used directly for a labeling reaction. A further advantage of the method is that it also separates the arsenite-arsenate forms, so it can be used to monitor the efficiency of the reduction reaction described previously. [3]

3. I was the first to systematically investigate the parameters of the reduction reaction of ^{77}As-arsenate irradiated with the reactor. In my experiments, I selected the optimal reducing agent, examined the optimal concentration, the optimal reaction time, the ideal reaction temperature, and the optimal pH of the solution. The reproducible reduction reaction allows the irradiated arsenite/arsenate system to be reduced to a single oxidation state component so that the arsenic fraction obtained during the separation can be used 100% to label a biomolecule. [4]

4. I was the first to systematically investigate the conditions of the labeling reaction of lipoic acid with radioactive arsenic. During the experiments, the pH of the solution, the applied lipoic acid concentration, the reaction temperature and the reaction time were optimized. Determining the labeling efficacy of labeled lipoic acid I developed a new liquid chromatography method using a reversed phase monolithic column that separates labeled lipoic acid from inorganic components in one step and detects the pure fraction. Using a reproducible labeling reaction, I prepared an experimental radiopharmaceutical candidate that can be used in pre-clinical studies. [4]

6. Application or possibility of application

Biomolecules labeled with arsenic isotopes are promising radiopharmaceutical candidates. First step in this process is the production of the required isotope by cyclotron or reactor. Prior to the experiments, it is necessary to plan the irradiation conditions, calculate the irradiation time and the amount of target material required. Separation of the pure arsenic fraction from the irradiated germanium target is the next step, requiring an optimized radiochemical separation technique. After adjusting the appropriate oxidation state of arsenic, the labeling reaction may follow. The model molecule must be carefully chosen, because not only the labeling must be effective, but also the subsequent possible use must be considered at this stage.
The long-term goal of my work is to be able to use arsenic-labeled lipoic acid as a radiopharmaceutical after performing the appropriate tests.

7. Publications

7.1 Publications related to the PhD thesis

1. Szűcs Zoltán, Kovács Béla, **Oláh Zita**, Dóczi Rita, Moumita Maiti, Susanta Lahiri, Chapter 64. Automated unit for separation of arsenic with iron doped calcium alginate, One Century of the Discovery of Arsenicosis in Latin America (1914-2014) As 2014 Proceedings of the 5th International Congress on Arsenic in the Environment, May 11-16, 2014, Buenos Aires, Argentina

2. **Oláh Zita**, Szűcs Zoltán, Varga Zoltán, Dóczi Rita: Development of ^{77}Ge/^{77}As parent-daughter system for periodic removal of 77As for environmental sanitation and biochemical purposes, Radiochim. Acta. 103, 871-877. https://doi.org/10.1515/ract-2015-2387 **IF=1,414, FI=2**

3. **Oláh Zita**, Kremmer Tibor, Andreas Vogg, Varga Zoltán, Szűcs Zoltán, Bernd Neumaier, Dóczi Rita, 2017. Novel ion exchange chromatography method for nca arsenic separation. Appl. Radiat. Isotopes 122, 111 -115. https://doi.org/10.1016/j.apradiso.2017.01.008 **IF=1,270, FI=3**

4. **Oláh Zita**, Andreas Vogg, Tibor Kremmer, Szűcs Zoltán, Varga Zoltán, Dóczi Rita, 2019. Optimization of the reduction of 74As(V) to 74As(III) and of the labelling of dithiol dihydrolipoic acid. Appl. Rad. Isotopes 149, 75-82. https://doi.org/10.1016/j.apradiso.2019.04.004 **IF=1,270**

5. Balatoni-**Oláh Zita**: Izotópok a gyógyítás szolgálatában: Arzén reneszánsza. Élet és tudomány 71:(29) pp. 905-907. (2016)

7.2 Other publications related to the thesis

1. **Oláh Zita**, Szűcs Zoltán, Dóczi Rita, Arzén izotópok elválasztása új, folyadékkromatográfiás módszerrel és új jelölési stratégia kidolgozása. In: Szentmiklósi László (szerk.) Őszi Radiokémiai Napok 2016. Balatonszárszó, Magyarország, 2016.10.10-2016.10.12. Budapest: Magyar Kémikusok Egyesülete (MKE), 2016. pp. 47-51. (ISBN:978-963-9970-69-4)
2. **Oláh Zita**, Szűcs Zoltán, Dóczi Rita, ^{77}As izotóp elválasztása $^{77}GeO_2$ targetből környezetvédelmi és orvos biológiai alkalmazásokhoz. In: Szentmiklósi László (szerk.) Őszi Radiokémiai Napok 2015. Balatonszárszó, Magyarország, 2015.10.19-2015.10.21. Budapest: Magyar Kémikusok Egyesülete (MKE), 2015. pp. 64-68.(ISBN:978-963-9970-59-5)

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1. **Oláh Zita**, Szűcs Zoltán, Varga Zoltán, Dóczi Rita, A new method for separation of radioarsenic from germanium oxidetarget for medical applications In: 6th Alpe-Adria Medical Physics Meeting. Budapest, Magyarország, 2014.05.29 2014.05.31.pp. 67-68.
2. **Oláh Zita**, Szűcs Zoltán, Andreas Vogg, Z. Varga Zoltán, Dóczi Rita, Developments of nca. ^{77}As radioisotope production In: Jahrestagung der AG Radiochemie / Radiopharmazie. Bécs, Ausztria, 2013.09.12-2013.09.14.pp. 21-56. (Jahrestagung der AG Radiochemie / Radiopharmazie)
3. **Oláh Zita**, Andreas Vogg, Kremmer Tibor, Dóczi Rita:Arzén-74 izotóp: új potenciál a teranosztikában. In: Bujtás Tibor, C Szabó István Csige István, Déri Zsolt, Elek Richárd,

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4. **Oláh Zita**, Szűcs Zoltán, Dóczi Rita, Az arzén-77 izotóp germánium-dioxid targettől való elválasztásának vizsgálata: Magyar Orvosfizikai Társaság Szimpóziuma Budapest, Magyarország, 2013.11.29. 2013. 6 p.
5. **Oláh Zita**, Andreas T. Vogg, Dóczi Rita Arzénizotóppal jelölt vegyületek a diagnosztikában és az endoterápiában. Magyar Orvosfizikai Társaság XVII. konferenciája. Pécs, Magyarország, 2012.09.28-2012.09.29. Paper B11. (Magyar Orvosfizikai Társaság XVII. konferenciája)
6. Balatoni-**Oláh Zita**: Arzén izotópok a nukleáris medicinában/Arsenic isotopes in nuclear medicine In: Kozák, Dóra; Tóth, Gábor; Molnár, Dániel (szerk.) XI. Szent-Györgyi Albert Konferencia: Célkeresztben a rák Budapest, Magyarország : Budapesti Műszaki és Gazdaságtudományi Egyetem, (2017) pp. 73-74.

7.4 Education materials

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7.5 Other publications

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2. **Oláh Zita,** Integrált irányítási rendszer bevezetésének tapasztalatai a BME Nukleáris Technikai Intézetben, Minőség és megbízhatóság, 67. évfolyam 2013/6 304-307.

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