

**Short thesis for the degree of doctor of
philosophy (PhD)**

**Cyclotron produced ^{52}Mn and $^{155,156}\text{Tb}$
radioisotopes production and
purification as well as ^{52}Mn application
for phenotyping corn with miniPET
camera and gamma spectrometer**

by Károly Brezovcsik

Supervisor: Dr. Zoltán Szűcs



UNIVERSITY OF DEBRECEN
Doctoral School of Chemistry

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Introduction and objectives

Radioisotopes are widely used because they can be easily detected even in extremely small amounts, and radioactive decay is not affected by any external circumstances (e.g. temperature, pressure, pH, etc.). This enables their use in various biological systems because they do not affect the observed processes. In addition to radioisotopes found in nature, they can also be produced artificially, thus increasing the possible number of elements that can be used.

They are used in many areas, such as radiation sterilization, imaging, soil moisture measurement, etc., but perhaps the most commonly well-known is their medical use. In medical practice, they are used for non-invasive diagnostics and therapy that do not require a surgical process. Gamma and positron-emitting radioisotopes are used for diagnostics, while negative beta, alpha, and Auger electron-emitting radioisotopes are used for therapy. With their help, tumors and pathological changes can be detected or cured that cannot be done by other procedures. Diagnostic-therapeutic radioisotope pairs are used and, from this point of view, terbium is a special element, since its 4 different radioisotopes can be used for both diagnostics ($^{152,155}\text{Tb}$) and therapy ($^{149,161}\text{Tb}$).

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Since radioisotopes can be easily detected even in extremely small amounts, they are ideal for nutrient uptake and circulation studies in plants. Macro-elements (carbon, nitrogen, oxygen, phosphorus) and micro-elements (boron, zinc, iron, copper, nickel, manganese, molybdenum, chlorine) essential for plants can be traced with radioisotopes and can be tested even with non-invasive methods (e.g.: positron emission tomograph, PET camera). Based on the changes in the speed and quantity of the uptake of the elements required as nutrients, we can distinguish and categorize (phenotype) the different types of a given plant. In general, the necessary radioisotopes are produced artificially, for example with particle accelerators. The first step in radioisotope production using particle accelerators is the production of the target object for the irradiation, which can be done using several methods: tablet pressing, electrolysis, sedimentation, and evaporation. When choosing the method, the thickness of the desired target object and the properties of the elements used are important considerations. The target must have sufficient mechanical stability to withstand the conditions (high temperature) occurring during irradiation. The second step is chemical processing, during which the produced radioisotope is chemically separated from the target material. After purification, the produced radioisotope can be used alone or linked to some molecule.

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During my doctoral work, I dealt with the production of various radioisotopes, the preparations necessary for their production, and then the purification and use of the produced radioisotopes. I focused on two elements, manganese, and terbium. Both elements have radioisotopes that are suitable for medical use (^{52}Mn , $^{149,152,155,161}\text{Tb}$), so working with them is important for the development of radiopharmaceuticals. In the case of terbium radioisotopes, my goal is to produce suitable targets for cross-section measurements and to separate terbium(III) from high-mass (150-300 mg) gadolinium(III) while minimizing the size of the column.

Manganese(II) appears as an essential micronutrient in plants and using this, I developed a new method for categorizing corn hybrids and testing their stress-resistant properties, with the help of which the more resistant type can be selected more quickly.

Experimental methods

In the case of the $\text{Gd}^{3+}/\text{Tb}^{3+}$ and $\text{Tb}^{3+}/\text{Dy}^{3+}$ separations, fractions collected into 10 ml vials. While during the plant experiments, the chopped plants were wrapped, and their weight was measured. In both cases, the quality and quantity measurements were carried out using a Canberra-type HPGe gamma spectrometer. In the spectra, I used the γ -line of ^{156}Tb at 534.32

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keV, ^{159}Gd at 363.55 keV, ^{159}Dy at 58.00 keV, and ^{52}Mn at 744 keV for the evaluations. I used the Nucleus-II software to evaluate the spectra.

I followed the distribution and movement of radiomanganese in the plant samples with the MiniPET-3 positron emission tomography (PET) made at the Nuclear Research Institute, whose software was also controlled by me. I evaluated the images with the BrainMOD program.

Abbreviations

PET – positron emission tomography

SPECT – single photon emission computer tomography

PEG - Polyethylene glycol

α -HIBA - 2-Hydroxy-2-methylpropionic acid

New scientific results

1 Radioterbium was produced, which may be suitable for later medical research.

In medical practice, radioisotope diagnostic-therapeutic pairs are used in radiopharmaceuticals. It is a big advantage if the same element can be used for both. Terbium is a special element whose radioisotopes include types that can be used both for

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diagnostics, ^{155}Tb (for SPECT examinations), ^{152}Tb (for PET examinations), and for therapy, $^{149,161}\text{Tb}$. Terbium radioisotopes can be produced artificially with a particle accelerator in the case of $^{149,152,155}\text{Tb}$, and with neutron irradiation in the case of ^{161}Tb . Terbium radioisotopes are most often produced from gadolinium targets by these nuclear reactions: $^{152}\text{Gd}(p,n)^{152}\text{Tb}$, $^{155}\text{Gd}(p,n)^{155}\text{Tb}$, $^{152}\text{Gd}(p,4n)^{149}\text{Tb}$, $^{160}\text{Gd}(n, \gamma)^{161}\text{Gd} \rightarrow ^{161}\text{Tb}$. During my work, I mainly focused on radioterbium cleaning after irradiation.

1.1 Thin target from gadolinium was made by electrodeposition for cross-section measurements.

The first step in terbium isotope research is the optimization of cyclotron production. Thin targets, a few μm thick, are required for the cross-section measurements. In order to have as few interfering isotopes as possible in the irradiated sample during the cross-section measurements, the gadolinium was electrolyzed on an Al backing. 2.5 μm thick targets were made by my method, which resulted in mechanically stable gadolinium targets. The targets survived test irradiations with a 15 MeV energy proton beam. Cross-section measurements were also made with the targets produced by me.

1.2 A separation method was developed which could purify the generated radioterbium(III) from a large mass (303 mg) of gadolinium(III) target while minimizing the size of the column used.

During the chemical experiments, pressed tablets were made of 175-350 mg of Gd_2O_3 used for irradiation. I developed a new method for the separation of radioactive terbium(III) produced during irradiation from 151.5-303 mg of gadolinium(III) on 250x4.6 mm (analytical) and 115x15 mm size columns, with AG50W-X8 cation exchange resin. As an eluent, I used α -HIBA, well known from the literature, in different concentrations, 0.2 mol/dm³ for Tb^{3+} and 0.5 mol/dm³ for Gd^{3+} . The separation efficiency was 85% for the smaller column and 93% for the larger column, measured by radioanalytical methods. At the end of the process, both the terbium- and gadolinium-containing fractions can be purified from α -HIBA using the same cation exchange resin. The final product will be in the form of terbium(III) chloride.

The generated radioterbium can be quickly purified with the method, and the starting material, gadolinium, can also be recovered in the form of gadolinium(III) chloride. When using enriched starting material, recovery of the used material is also important.

1.3 A separation method was developed which could purify the generated radioterbium(III) from radiodysprosium(III) and radiogadolinium(III) at the same time.

Dysprosium is also produced from gadolinium during the production of ^{161}Tb with neutrons. The generated radioterbium must be cleaned of both pollutants. Using my method, I separated radioterbium(III) from 151.5 mg of gadolinium(III) and a trace amount of radiodysprosium(III) simultaneously on a 115x15 mm cation exchange column with AG50W-X8 resin filling. I managed to get 98% of the total amount of radioterbium without contamination.

Another possible use of the method is the production of ^{155}Tb via the radioisotope ^{155}Dy from ^{159}Tb . During the separation, it was possible to obtain 34% of the dysprosium produced without contamination, the amount of which can be increased with a second purification step.

2 A method was developed for phenotyping corn seedlings using ^{52}Mn , a positron-emitting radioisotope.

Manganese is one of the essential micronutrients for corn. During the experiments, I used the positron-emitting radioisotope manganese-52, which gave me the opportunity to

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use a miniPET camera and a gamma spectrometer. With the help of a miniPET camera, I was able to non-invasively observe the uptake of manganese(II) in corn. I supplemented these measurements with gamma spectrometer measurements, which is already an invasive procedure, but it can detect the radioisotope in much smaller quantities than the miniPET camera. After developing the experimental parameters necessary for the process, I tested two different corn hybrids, the 'P9903' (type 1) and 'Armagnac' (type 2) varieties.

2.1 Cr³⁺/Mn²⁺ separation was optimized for my own system to purify ⁵²Mn.

The manganese-52 radioisotope was produced from chromium metal by nuclear reaction $^{52}\text{Cr}(p,n)^{52}\text{Mn}$. After dissolving of the irradiated target with hydrochloric acid, the Cr³⁺/Mn²⁺ system was separated on AG 1-X8 anion exchange resin with a concentrated hydrochloric acid eluent. I optimized the purification, during which the manganese radioisotope was obtained pure after one separation on a 115x15 mm column. The method is suitable for processing 400 mg of irradiated chromium target.

2.2 Manganese(II) circulation in corn seedlings was investigated using a miniPET camera and a gamma spectrometer

My goal was to map and display the route of manganese(II) in corn seedlings. For this, plants compatible with the MiniPET camera were needed, so I examined them at the young age of 3 leaves. The method was checked with gamma spectrometric measurements. It was possible to observe the dynamics of manganese(II) uptake of different species. During the experiments, the stems of the two different hybrids appeared on the PET images. Based on these, type 1 delivered the manganese(II) radioisotope to the stem faster. After 3.5 hours, the entire stem is visible, while in the case of type 2 this only happens after 6 hours. At the 12-hour endpoint, the images taken of type 1 show that it has accumulated a greater amount of activity than type 2.

The accumulated manganese(II) activity in the leaves can be measured with gamma spectrometer. In this case, there is a clearly visible difference in the distribution of manganese(II) in the leaves of the two hybrids. In the case of type 1, manganese(II) appears in every leaf and reaches the leaves faster than in the case of type 2, which shows a delay of 4 hours compared to the type 1.

My method is able to differentiate between corn seedlings with both a miniPET camera and a gamma spectrometer, and based on the results, phenotyping can be performed on the corn seedlings.

2.3 The change in manganese(II) uptake was observed through different corn hybrids using a miniPET camera and a gamma spectrometer under drought stressed conditions.

A difference between the uptake of manganese(II) of the different maize hybrids even under ideal conditions was already demonstrated with the help of PET images and gamma spectrometer measurements. In order to select the one that is more resistant to drought among the two plants, new experiments were conducted with corn seedlings, where drought was simulated by applying PEG. Different concentrations of PEG in the nutrient solution were used, from mild to strong dryness: 5-10-20 % (m/m). At all PEG concentrations, hybrid type 1 performs better than type 2. In the case of a high PEG content of 20%, a small amount of manganese(II) uptake can be observed even in the leaves of type 1, while in the case of type 2, with a PEG content of 10%, no accumulation of manganese(II) can be detected in the leaves at 6 hours and no well measurable radiomanganese(II) can be

found in the leaves at 10 hours. These differences were also reflected in the PET images and gamma spectrometry results. The procedure is suitable for quickly and cost-effectively selecting among possible varieties based on their drought tolerance.

2.4 The effect of changes in environmental conditions (temperature, light intensity) on the uptake of manganese(II) were observed by corn seedlings with gamma spectrometer.

Changes in environmental conditions (such as temperature and light) also have an effect on manganese(II) uptake by maize seedlings. Lower manganese(II) content can be detected in individuals tested at 17°C, lower than the 25°C used previously and during rearing, while in those tested at a higher temperature, 35°C, a small excess of radiomanganese(II) is shown after 6 hours. At 10 hours, a similar amount of radiomanganese(II) can be detected in the leaves of the seedlings tested at 17 °C and 35 °C. Based on these, long-time elevated temperatures are not ideal either.

In the night phase, manganese(II) accumulation is slower than during the day phase and based on my measurements, most of the manganese(II) reached the oldest leaf. Approaching the daytime phase, the accumulated manganese(II) begins to

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distribute similarly to the individuals examined during the day phase. Under the influence of 2 hours of light, the distribution and amount of manganese(II) are similar to that of the specimens examined with full light exposure at the 6-hour point. The effect of reduced light is not as great as that measured during the temperature change or during the night phase, however, here too, a difference in the distribution of manganese(II) in the leaves can be seen compared to those examined in full light.

Based on these, my method is suitable for monitoring manganese(II) uptake as a result of environmental changes and, based on these, for categorizing corn seedlings according to new parameters.

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Publications

List of publications related to the dissertation

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1. Z. Kovács, F. Szelecsényi, and K. Brezovcsik, “Preparation of thin gadolinium samples via electrodeposition for excitation function studies,” J. Radioanal. Nucl. Chem., vol. 307, no. 3, pp. 1861–1864, Mar. **2016**, doi: 10.1007/s10967-015-4399-4.
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List of other publications

1. E. Sarkadi-Priboczki, M. Varga, I. Valastyán, K. Brezovcsik, A. Fenyvesi, and J. Molnár, “Compound-Specific Imaging of Methanol Fuel Cell for Performance and Degradation Studies Using a Mini Positron Emission Tomograph,” Chemistry–Methods, vol. 1, no. 7, pp. 315–322, **2021**, doi: 10.1002/cmtd.202000054.
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3. F. Tárkányi, S. Takács, F. Ditrói, Z. Szűcs, K. Brezovcsik, A. Hermanne, A. V. Ignatyuk, “Activation cross sections of gamma-emitters produced in deuteron induced reactions on 209 Bi up to 50 MeV,” Eur. Phys. J. A, vol. 57, no. 7, **2021**, doi: 10.1140/epja/s10050-021-00548-y.
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Conference participation

List of presentations related to the dissertation

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1. Brezovcsik Károly, Pribóczki Éva, Fenyvesi András, Varga Máté, Molnár József, Veres Szilvia, Szűcs Zoltán: Kukoricapalánták mangánfelvételének nyomon követése ^{52}Mn radioizotóppal, Őszi Radiokémia Napok, Balatonszárszó, 2018
2. Brezovcsik Károly, Szűcs Zoltán, Pribóczki Éva, Fenyvesi András, Varga Máté, Német Brigitta: PET felvételek készítése növényekről mini pozitron emissziós tomográfiával, Őszi Radiokémia Napok, Balatonszárszó, 2017
3. Károly Brezovcsik, Zoltán Kovács, Ferenc Szelecsényi: Production of radioactive terbium isotopes for medical use, 5th International Nuclear Chemistry Congress, Sweden, 2017
4. Brezovcsik Károly, Szelecsényi Ferenc, Kovács Zoltán: Radioaktív terbium izotópok előállítása orvosi felhasználásra, Őszi Radiokémia Napok, Balatonszárszó, 2016

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List of publications related to the dissertation

Foreign language scientific articles in international journals (3)

1. **Brezovcsik, K.**, Veres, S., Molnár, J., Fenyvesi, A., Szűcs, Z.: Comparison of manganese uptake and transport of maize seedlings by mini-PET camera.
Appl. Radiat. Isot. 160, 1-6, 2020. ISSN: 0969-8043.
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List of other publications

Foreign language scientific articles in international journals (9)

4. Tárkányi, F., Takács, S., Ditrói, F., Szűcs, Z., **Brezovcsik, K.**, Hermanne, A., Ignatyuk, A. V.: Activation cross sections of gamma-emitters produced in deuteron induced reactions on ^{209}Bi up to 50 MeV.
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