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Czech Technical University in Prague
Faculty of Nuclear Sciences and Physical Engineering

RNDr. Ján Kozempel, Ph.D.

Výzkum a vývoj cílených radiofarmak na bázi alfa zářičů

Research and development of radiopharmaceuticals for targeted alpha particle therapy

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Summary

The therapy of oncological diseases with alpha particle emitting nuclides belongs to the fastest developing fields of nuclear medicine. The basic aspects and specific issues of labelling with alpha particle emitters in general and the possibilities of ^{223}Ra use in targeted alpha particle therapy are discussed in this work, together with the possible use of inorganic nanoparticles as prospective nuclear recoil resistant carriers of nuclides decaying by several alpha particle emissions.

Souhrn

Terapie onkologických onemocnění radionuklidy emitujícími alfa částice patří k nejrychleji se rozvíjejícím oblastem v oboru nukleární medicíny. V této práci jsou diskutovány základní aspekty a specifické otázky značení s alfa zářiči a možnosti použití ^{223}Ra pro cílenou alfa částicovou terapii, včetně možnosti využití anorganických nanočástic jako potenciálních nosičů alfa zářičů, které alespoň částečně eliminují efekt atomové odrazu, zejména u nuklidů dezintegrujících kaskádou alfa rozpadů.

Keywords

Radium; nanoparticles; nuclear recoil; nuclear medicine; targeted alpha particle therapy; radiopharmacy

Klíčová slova

Radium; nanočástice; atomový odraz; nukleární medicína; cílená alfa částicová terapie; radiofarmacie

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1. Introduction

Application of radiation and radionuclides in the therapy of oncologic diseases started soon after their discoveries at the end of 19th and the beginning of 20th century. The use of alpha particle emitting nuclides (e.g. ^{226}Ra) was however limited to sealed radioactive sources for a long time. These sources were used in brachytherapy since their decay characteristics and the lack of proper radionuclide targeting did not allowed their use as open sources. *Thorotrast* – stabilised colloidal suspension of $^{232}ThO_2$ was used in radiology as an excellent contrast agent though with late (20-30 years) adverse effects causing severe oncological diseases due to long-term radiation burden (**Kadrnka et Rossier** 1931). Thanks to the fast development of nuclear sciences novel nuclides and their preparation routes were discovered and the portfolio of nuclides suitable for medical use widened. Eventhough the research was mainly focused on the preparation of β^- emitters, e.g. ^{131}I for the thyroid tumour therapy (**Miller** et al. 1948), also the research of alpha particle emitters was ongoing (e.g. the discovery of ^{211}At (**Corson** et al. 1940). Initial optimism in the use of alpha emitters however faded, because of difficulties in the proper dose targeting, difficult nuclide chemistry, *in vivo* labelled compounds stability and unwanted late adverse effects, like in ^{224}Ra therapy of ankylosing spondylitis and other diseases, reported in long-term studies following ^{224}Ra therapy (e.g. **Wick** 1993).

Rediscovery and potential use of antibody conjugate labelled with ^{211}At and ^{212}Bi in the treatment of cancer was proposed in the ´90s (**Wilbur** 1991).

Recently, the first modern approved radiopharmaceutical preparation based on ^{223}Ra was introduced by Bayer AG. The $^{223}RaCl_2$ in physiological saline solution started to be used for metastatic castration-resistant prostate cancer, symptomatic bone metastases and unknown visceral metastatic disease therapy, targeting to bone and bone metastases. Eventhough such simple radionuclide targeting system did not brought significant innovation, it may be considered as very important breakthrough in the field of *TAT*.

Further, several $^{225}Ac/^{213}Bi$ labelled compounds were developed for the therapy of various diseases, e.g. the prostate cancer based on prostate specific membrane antigen targeting moiety (**Kratochwil** 2016). Eventhough the nuclear recoil and release of daughter ^{213}Bi was not eliminated and may cause serious late adverse effects, the complete response of two patients was observed in this preliminary report bringing immediate benefits for the patients. Further, ^{227}Th (**Ryan** 2017) or ^{212}Pb (**Kasten** 2017) were applied in preclinical studies.

This lecture focuses on the research and development of nanocarriers based on hydroxyapatite, TiO_2 and Fe_3O_4 materials that are at least partially recoil-resistant and allow advanced targeting and multimodal imaging in *TAT* follow-up. Present status in the field as well as an outlook to the future developments is given.

2. Nuclides

2.1. General overview

Alpha emitters have advantage of commonly used β emitters in the high linear energy transfer, massive ionization and quite short range of the alpha particles in tissues, which provide high biological effectiveness. Schematic comparison of ranges of various particles with the same energy is depicted in **Figure 1**.

Figure 1. Schematic comparison of ranges of various particles with the same kinetic energy of 1 MeV in a muscle tissue.

Wide range of nuclides that undergo alpha decay is available. Characteristics, production routes and status of their use in nuclear medicine of selected alpha emitters are summarized in **Table 1**. Among naturally-occurring nuclides offering the possibility of construction of a radionuclide generator, nuclides prepared using accelerators or nuclear reactors production routes were also included.

Table 1. Summary of properties of selected alpha particle emitters.

Radionuclide system	Half-life	E_{amax}/ E_{chain} [MeV]	Preparation	Status	Reference
^{149}Tb	4,12 h	3,97	$^{152}Gd(p,4n)^{149}Tb$	Research	(**Beyer** 2002) (**Steinber** 1968)
^{211}At	7,2 h	5,87	$^{209}Bi(a,3n)^{211}At$	Clinical trials	(**Lebeda** 2005) (**Back** 2017)
^{229}Th / **^{225}Ac** // ^{213}Bi	7340 r 10 d 46 m	5,83 / 27,62	^{229}Th decay $^{226}Ra(p,2n)^{225}Ac$	Clinical trials	(**Morgenstern** 2005) (**Apostolidis** 2005)
^{227}Ac / ^{227}Th / **^{223}Ra**	27 r 18 d 11 d	5,87 / 26,70	$^{227}Ac/^{227}Th/^{223}Ra$ generator	Clinical praxis	(**Larsen** 2000) (**Henriksen** 2001) (**Guseva** 2004) (**Shiskin** 2011)
^{228}Th / **^{224}Ra** // ^{212}Bi	1,9 r 3,7d 61 m	5,69 / 27,54	$^{228}Th/^{224}Ra$ generator	Research	(**Schwarz** 2002) (**Šebesta** 1974)

2.2. Single alpha emitters

Nuclides decaying by a single alpha particle emission (not creating decay chains) simply decay to stable nuclei decay products and thus the recoiling atoms do not generate radioactive deposits and unwanted radiation burden. On the other hand, the applied activity necessary to achieve the same dose as in case of chain decaying nuclides is much higher (approx. 5 vs. 27 MeV /decay).

Astatine-211 could be prepared on a cyclotron via the $^{209}Bi(a,3n)^{211}At$ reaction. Short half-life of 7,2 h slightly limits the use of ^{211}At for labelling of biomolecules with slower kinetics *in vivo*. On the other hand, its decay is favourable for the fast dose delivery. Similar chemical properties to iodine allow to use the same labelling methods (e.g. electrophilic substitution, oxidative destannylation, etc.).

Novel prospective alpha emitting nuclide of ^{149}Tb was recently proposed for *TAT* (**Beyer** 2002). Even though its availability is limited, advantageous decay properties favour this promising radio-metal for further studies. Standard *Ln* chelators may be easily used to label various biomolecules.

Bottom members of decay chains belong to the last group of single alpha particle emitters. Some α and β^- emitters decaying into radioactive nuclei may be also considered. They typically form short-lived decay chains, like ^{213}Bi, ^{212}Pb and others. In such way, the risk of radioactive burden spread may be at least partially reduced (e.g. **Kasten** 2017).

2.3. Chain alpha emitters

As mentioned in previous chapters, due to the short range and high *LET* of alpha particles, the overall energy deposited by decaying mother nuclide in a short decay chain ranges up to some units of MeV. Thus the total dose and the therapeutical efficiency is much higher compared to a single alpha particle decay. Such so-called *in vivo* generators however suffer from the recoils release from the targeting molecules (e.g. **Jaggi** 2005). Contrary to this, advantageous reasons for employing such nuclides are their good availability, suitability for long-term therapeutical applications and irradiation of larger tissue volumes employing diffusing alpha emitters radiation therapy (*DART)* that may induce antitumor immunity against experimental metastatic tumors (**Confino** 2015).

First *Food and drug administration (FDA)* and *European medicines agency (EMA)* approved alpha emitter was ^{223}Ra. It could be obtained from natural decay series of ^{235}U or by neutron irradiation of ^{226}Ra and subsequent decay of ^{227}Ra to ^{227}Ac, which is a mother nuclide of ^{227}Th and ^{223}Ra respectively. Other radionuclide systems include the $^{225}Ra/\ ^{225}Ac/^{213}Bi$ and the $^{228}Th/\ ^{224}Ra///\ ^{212}Pb$. These systems allow construction of radionuclide generators that is favourable for nuclear medicine applications.

2.4. Radium-223

Radium-223 (T = 11,4 d) is a member of natural uranium-actinium decay-chain, preceded by soft β^- emitter, the ^{227}Ac (T = 21,7 y) and ^{227}Th (T = 18,7 d). The ^{227}Ac decay scheme is shown in **Figure 2**. As it can be clearly seen ^{223}Ra could be very useful in *TAT* thanks to its decay properties. Total released energy reaches approx. 27 MeV in one complete decay, distributed over four α and two β^- emissions. The only longer-lived daughter nucleus is ^{211}Pb (T = 36,1 min). Due to coordination chemistry of radium the labelling of biomolecules is still undeveloped and the search for proper radium chelator is ongoing.

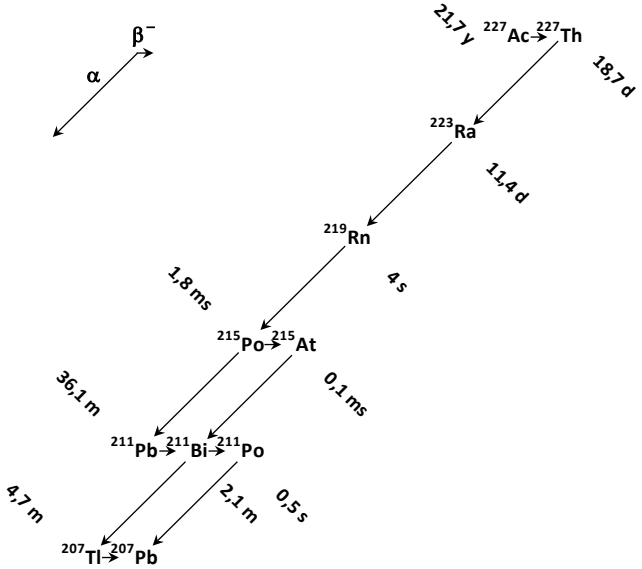

Figure 2. Decay scheme of ^{227}Ac.

While the separation of ^{227}Ac, the mother nuclide of ^{227}Th and ^{223}Ra, from natural sources or uranium mine tailings is problematic, it could be easily prepared in a reaction sequence:

$$^{226}Ra(n,\gamma)^{227}Ra \xrightarrow{\beta^-} {}^{227}Ac \xrightarrow{\beta^-} {}^{227}Th \xrightarrow{\alpha} {}^{223}Ra$$

Pure ^{223}Ra could be isolated from $^{227}Ac/^{227}Th$ system employing extraction (e.g. tetraalkyldiglycolamide resins) or ion-exchange chromatographic methods. The construction of a radionuclide generator is possible, however, the generator sorbents (mainly organic) suffer from intense radiations and degrade over time due to radiolysis. The shelf-life of such generators is then limited. Other possibility is the stripping of ^{227}Ac and its storage in a separate stock vial.

3. Carriers for targeted alpha therapy

3.1. Nuclear recoil effect

As a consequence of the momentum conservation in the alpha decays, the major part of the decay energy is distributed as a kinetic energy among daughter nucleus and the emitted alpha particle. This could be expressed by a simplified relation:

$$E_{recoil} = E_{\alpha} \frac{m_{\alpha}}{M_{recoil}}$$

In case of ^{223}Ra decay (see **Figure 3**) the recoil energy of daughter ^{219}Rn reaches the energy of some 107 keV and the α-particle bears some 5,871 MeV. Assuming that typical chemical bond energy lies in the interval of 1-10 eV, it is clear that no chemical bond would remain preserved and daughter atom would immediately escape from its carrier. Further, highly ionized nascent daughter atoms ionize its surroundings causing significant damage. E.g. the charge of nascent ^{219}Rn ranges up to 25+ (**Wieclavik** & **Perrin** 1968).

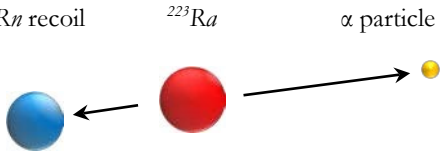

Figure 3. Schematical drawing of ^{223}Ra decay.

3.2. Small molecules

Small molecules typically have fast *in vivo* kinetics that is essential for *TAT* applications. However, as mentioned in previous chapter, the nuclear recoil effect limits their use to single alpha particle emitters or emitters with very short-lived decay chain to avoid spread of daughter radioactive nuclei and irradiation of healthy tissues. Surprisingly this fact is very often neglected and the effects of radioactive recoils are not subject of investigation, eventhough the late adverse effects were demonstrated (e.g. **Wick** 1993).

Various compounds and chelators (e.g. *DOTA*, *DTPA*, *CHXA-DTPA*, macrocyclic ligands, phophonates) to target on a molecular level were developed for the therapy of tumor diseases, e.g. ^{225}*Ac-PSMA-617* (**Kratochwil** 2016), ^{227}*Th-3-hydroxy-N-methyl-2-pyridinone* labelled *mAb* (**Ryan** 2017) or ^{212}*Pb* labelled *mAb* (**Kasten** 2017) and many others were applied in preclinical studies. Proper chelator for radium is still under development. Eventhough the nuclear recoil and the release of daughter radioactive nuclei was not eliminated, even not quantified, immediate benefits for the patients were observed in some cases. However, it was too soon to observe any late adverse effects and careful and comprehensive studies have to be undertaken in the future.

Astatine as the only halogen used in *TAT* was used in many studies to label proteins, *mAbs*, small molecules, etc., e.g.: *^{211}At-methylene blue* (**Brown** 1986), [*^{211}At*]-*MABG* (**Batra** 2014) or *^{211}At-MX35-F(ab`)(2)* (**Back** 2017). Unfortunately the carbon-astatine bond is the weakest of the halogen-carbon bonds and organic compounds of astatine are quite unstable *in vivo* and undergo enzymatic dehalogenation and astatine accumulation in thyroid. This disadvantage lead to the development of advanced carriers based on astatine-metalloid/metal bonds (e.g. **Wilbur** 2001, **Kučka** 2005, **Ostrowski** 2017) pointing towards nanocariers (**Hartman** 2007).

3.3. Nanocarriers

The two main reasons for applying nanosized particulate carriers in *TAT* are given by their physicochemical properties. Firstly, the size of the *NPs* mediates the passive tumor targeting by the enhanced permeation and retention effect (*EPR*) (**Maeda 2012**) and secondly, the stopping power of the *NPs* material should be sufficient to retain at least partially daughter radioactive recoils. Thus both single alpha emitters and nuclides decaying in a chain could be applied. Simulation of *^{219}Rn* ion recoils using SRIM 2013 code (**Ziegler** 2013) in **Figure 4** shows clearly the need of proper nuclide – material – purpose selection.

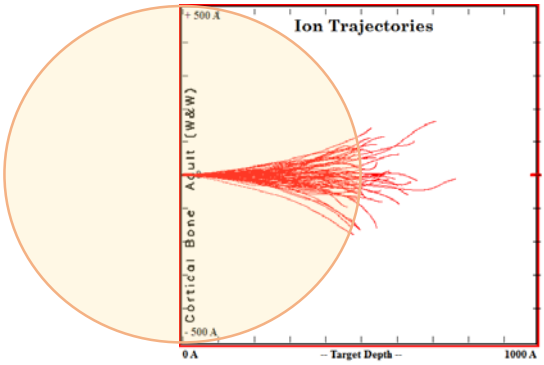

Figure 4. Track simulations of 100 keV *^{219}Rn* recoils from a point source (500 repetitions, unidirectional zero-degree angle) in an *ICRU* adult cortical bone (ϱ = 1.92 g/cm^3). It could be clearly seen that *^{219}Rn* recoils partially escape from a nanoparticle of 100 nm diameter, thus denser material or a larger particle have to be used.

Various nanocarriers were developed for use in *TAT*, like nanozeolites for *^{224}Ra* and *^{225}Ra* (**Piotrowska** 2013), *$LaPO_4$ NPs* for *^{225}Ac* (**Woodward** 2011) and others.

4. Nanocarriers preparation and labelling

4.1. Hydroxyapatite

Hydroxyapatite (*HAp*) was selected as a first choice material because of its biocompatibility, clinical use and natural occurrence in bone tissue, together with chemical similarity of calcium to radium (**Salberg** 2005). The *HAp NPs* were prepared by the aqueous precipitation of calcium salts solutions by phosphate at pH ≈ 9-11. Two strategies of *HAp NPs* labelling were chosen. Firstly, the sorption of ^{223}Ra on the ready-made *HAp NPs*, secondly the intrinsic incorporation of ^{223}Ra into *HAp NPs* structure at precipitation. Such approach was used in all further studied *NPs*. Labelling yields of *HAp NPs* with various diagnostic nuclides and radium are summarized in **Table 2**. The motivation for selection of other nuclides was given by the possible theranostical use. The shape of the prepared *NPs* was tuned by the addition of polymeric surfactants resulting in needle-like and spherical nanocrystals (see **Figure 5**).

Table 2. Labelling yields of *[^{223}Ra]HAp NPs*; *N* - number of trials.

Nuclide	Labelling strategy	Yield	*N*
^{68}Ga	Sorption	94.5 ± 0.4	21
^{18}F	Sorption	84.2 ± 1.3	5
^{99m}Tc	Sorption	94.7 ± 1.3	8
^{99m}Tc	Intrinsic	94.8 ± 0.7	16
^{223}Ra	Sorption	94.7 ± 1.6	16
^{223}Ra	Intrinsic	98.8 ± 1.2	10

However, the true *in vivo* stabilities were not sufficient enough with several tents of percent of activity release. On the other hand, the hydroxyapatite allows its further modification by interstitial ions doping (Ba^{2+}, Sr^{2+}, etc.) or surface coating and may be useful for some particular nuclear medicine applications.

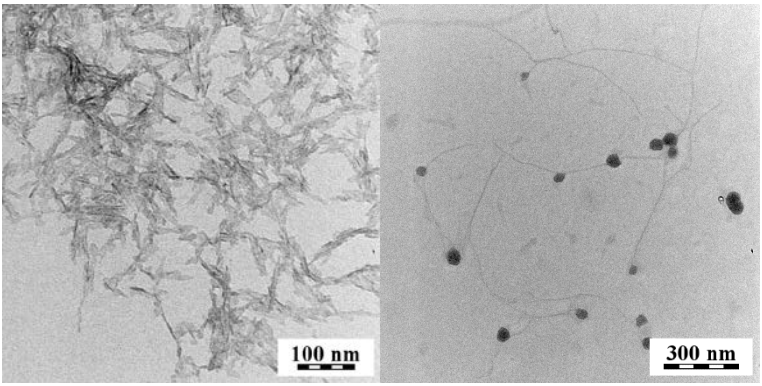

Figure 5. Two electron micrographs of needle-like (left) and spherical (right) *HAp NPs* with polymeric fibres.

4.2. Titanium dioxide

Titanium dioxide is well-known material with favourable sorption properties for many elements (e.g. **Lieser** 1979). Thus in its nanoparticulate form it was a material of our main interest for ^{223}Ra labelling. The *TiO_2 NPs* were prepared by the hydrolysis of *titanium t-butoxide*. Electron micrograph of prepared *TiO_2-NPs* is shown in **Figure 6**.

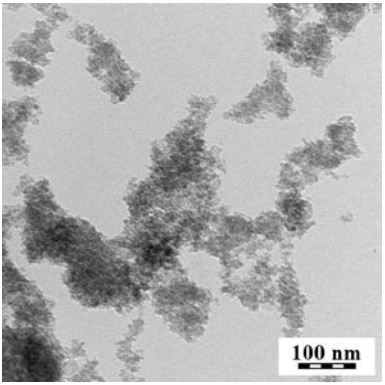

Figure 6. Electron micrograph of *TiO_2-NPs*.

Practically quantitative yields of *TiO_2 NPs* labelling were obtained with ^{99m}Tc and ^{223}Ra. The labelling results for both sorption and intrinsic strategies were comparable and were summarized in **Table 3**.

Table 3. Labelling yields of *[^{223}Ra]TiO_2 NPs*, Number of trials = 6 in all cases.

Nuclide	Labelling strategy	Yield [%]
^{99m}*Tc*	Sorption	98.4 ± 0.5
^{99m}*Tc*	Intrinsic	97.6 ± 0.7
^{223}*Ra*	Sorption	98.6 ± 0.5
^{223}*Ra*	Intrinsic	99.1 ± 0.3

The *in vivo* stability study was performed in *CD1-Foxn^{1nu}* ♀ mice model bearing *HT-29* colorectal adenocarcinoma tumor xenograft. The labelled *NPs* were applied by intratumoral injection and the activity washout was measured over 14 days. The *[^{223}Ra]TiO_2 NPs* showed good *in vivo* stability with very low activity washout (see **Figure 7**).

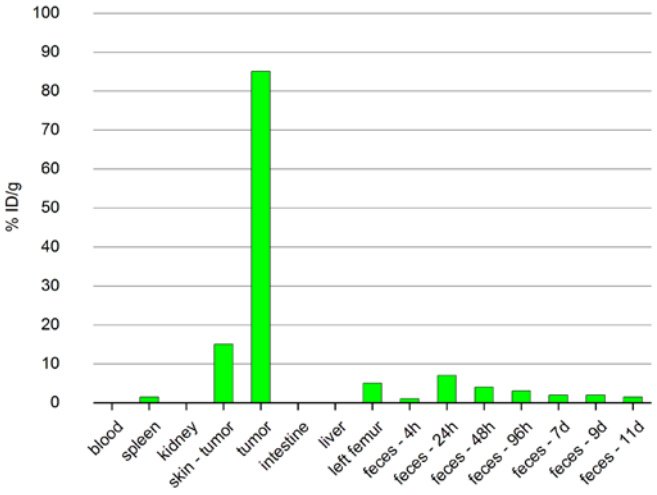

Figure 7. Results of *in vivo* stability study of *[^{223}Ra]TiO_2-NPs* in a mice model, the sample was applied *via* intratumoral injection and the activity release was measured over 14 days.

4.3. Iron(II, III) oxide

Iron(II, III) oxide *NPs* (also called as superparamagnetic iron oxide nanoparticles – *SPIONs*) were prepared in order to explore the possibility of magnetic force targeting employing external magnetic field (**Li** 2014). The Fe_3O_4 *NPs* were synthesised using co-precipitation method. Only the sorption strategy was used for ^{223}Ra labelling. Well-defined and almost spherical shape *NPs* were obtained (see **Figure 8** and **9**).

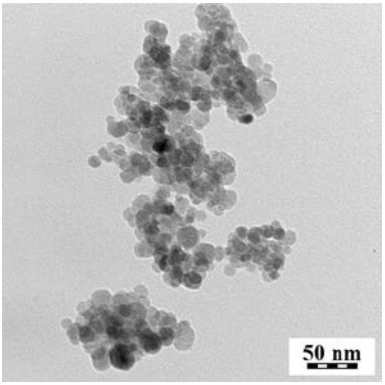

Figure 8. Electron micrograph of Fe_3O_4 *NPs*.

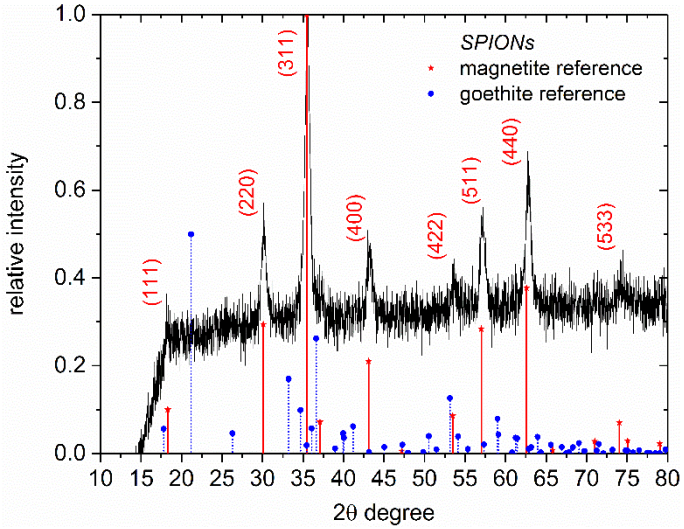

Figure 9. *XRD* pattern of Fe_3O_4 *NPs*.

Besides the preparation of *Fe_3O_4 NPs*, also the mechanism of radium uptake was studied and analysed. The *Fe_3O_4 NPs* were selected as a model system. In this model we have found that the radium uptake by the *Fe_3O_4 NPs* corresponds well with the sorption and surface complexation, rather than the precipitation mechanism. As it could be seen in **Figure 10** the initial *^{223}Ra* uptake on the surface (edge-sites) is subsequently followed by a slower process of *^{223}Ra* incorporation into interstitial positions of *Fe_3O_4 NPs* (layer-sites).

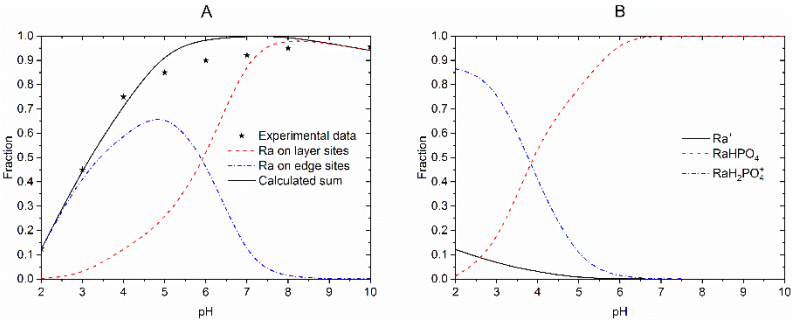

Figure 10. Relative abundances of given species versus pH. A - Radium experimental and calculated data; B - Radium speciation on edge-sites.

5. Conclusions

The progress in the field of *TAT* is obvious. The recent results of several studies indicate immediate patient benefits when applying radiopharmaceuticals labelled with alpha emitters. This would certainly speed-up further development of novel radiopharmaceuticals for *TAT*. Main efforts in further research would focus on the preparation of biocompatible and recoil resistant carriers of alpha particle emitters, eliminating the risk of late adverse effects.

In our studies we have shown that successful labelling of simple inorganic *NPs* (hydroxyapatite, *TiO_2* and *Fe_3O_4*) with *^{223}Ra* opens novel way of chain alpha particle emitters stabilization. Some of these nanocarriers were stable *in vivo* and this approach seems to be very promising for the use in *TAT*. Controlled release of radium *in vivo* may be useful for application in diffusing alpha emitters radiation therapy (*DART*).

Such labelled nanoconstructs seem to be the only system that withholds the daughter nuclides recoil and radiation damage and ensure their retention in nanoparticles. In this way the proper dose delivery may be achieved. Also we have shown that novel targeting modalities (e.g. external magnetic field) may bring opening of novel class of radiopharmaceuticals.

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RNDr. Ján Kozempel, Ph.D.

Curriculum vitae

Born on March 29, 1980

2003 Mgr. degree in Nuclear chemistry, Charles University in Prague

2004 RNDr. degree in Nuclear chemistry, Charles University in Prague

2005 Marie-Curie fellow at Joint Research Centre, European Commission, Ispra, Italy (6 months)

2006 junior researcher at Nuclear Physics Institute, Academy of Sciences of the Czech Republic

2009 scientific and technical support officer at the Joint Research Centre, EC, Ispra, Italy (3 years)

2010 Ph.D. degree in Organic chemistry, Charles University in Prague

2013 assistant professor Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University in Prague

Ján Kozempel specializes in radiopharmaceutical chemistry, nuclear chemistry, nanotechnology, and applied nuclear physics.

He supervises and co-supervises 3 Ph.D. and more than 10 master and bachelor students. The works of his students received various national and international awards - Anna Bajzíková (2014), Ekaterina Kukleva (2015), Petra Mičolová (2015), Lucie Kománková (2016), Eva Málková (2017), Kateřina Fialová (2017).

He teaches "Organic chemistry I. & II.", "Radiopharmaceuticals", "Toxicology", "Syntheses with Isotopes", "Structural Analysis", "Practice in Radiation Technology in Biology and Medicine" at the Faculty of Nuclear Sciences and Physical Engineering of Czech Technical University in Prague and "Nuclear Chemistry" at the Faculty of Science of Charles University in Prague. He is also a member of Master`s and Bachelor`s degree committees in Nuclear chemistry.

He authored or co-authored more than 25 publications in international peer-reviewed and impacted journals and three book chapters. He has H-index 8, sum of times cited without self-citations of 190 by Clarivative analytics WoS. He also authored two Czech patents and two utility models.

He is a member of Czech Chemical Society (1999), International Society of Radiopharmaceutical Sciences (2012), Czech Society of Nuclear Medicine (2014) and The Union of Czech Mathematicians and Physicists (2015).

Since 2013 he acts as a reviewer for the *Journal of Radioanalytical and Nuclear Chemistry*, the *Applied Radiation and Isotopes* and other journals. He also acts as a project reviewer of the Technology Agency of the Czech Republic (2012).
Ján Kozempel was a principal investigator of two projects from the Ministry of Education Youth and Sports of the Czech Republic, grants No.:

- LK21310 Methods of ^{226}Ra transmutation and separation of irradiation products (2013-2015)

- C120d Laboratories for radiopharmaceuticals and labelled compounds teaching (2014).

He was author and co-investigator of one project from the Technology Agency of the Czech Republic, grant No.:

- TA03010027 RadiAcT (2013-2016) Recyclation of Ra-226 and novel technologies exploiting Ac-227.

Since 2016 he is co-investigator of the Health Research Agency of the Czech Republic and the Ministry of Education youth and sports of the Czech republic and the European Union project, grants No.:

- 16-30544A „New multistage nanodiagnostics for imaging of tumor diseases and prediction of antiangiogenic therapy effectiveness“

- CZ.02.1.01/0.0/0.0/15_003/0000464 Centre of advanced photovoltaics

He was also principal investigator of two grants of CTU in Prague, grants No.:

- SGS14/084/OHK4/1T/14 Use of radiometals in nuclear medicine

- SGS15/094/OHK4/1T/14 Radionuclide carriers for targeted therapy and diagnostics

He co-investigated also 3 grants from the Joint Institute of Nuclear Research in Dubna, Russian Federation, grants No.: 04-5-1076/2013, 04-05/2016, 03-02-1100/2017.

He was a member of research teams of 5 other grants and he also performed contract research R&D projects for Triskem International SAS, Bruz, France and NPI AS CR v.v.i. Řež, Czech Republic.

Selected publications

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