Study of $^{223}$Ra uptake mechanism on hydroxyapatite and titanium dioxide nanoparticles as a function of pH

Petra Suchánková, Ekaterina Kukleva, Karel Štamberg, Pavel Nykl, Martin Vlk and Ján Kozempel

The mechanism of $^{223}$Ra uptake on hydroxyapatite and titanium dioxide nanoparticles was studied as a function of pH. Both materials are widely used in food industry and medicine. They offer properties suitable for labelling with medicinal radionuclides, particularly for targeted radionuclide therapy. The selected isotope, $^{223}$Ra, is an alpha emitter widely used in targeted alpha particle therapy due to high-dose delivery in very small tissue volume, nevertheless the results are applicable for any radium isotope including $^{226}$Ra. The study was performed in the pH range 4.5 to 12 for hydroxyapatite nanoparticles and 2 to 12 for titanium dioxide nanoparticles in Britton–Robinson buffer solution. Both nanomaterials at pH 6 and higher showed that over 95% of the radium has been sorbed. According to the applied chemical equilibrium model, the most important species playing a role in sorption on the edge-sites were $\text{RaCO}_3$, $\text{RaPO}_4^{-}$, $\text{RaHPO}_4$ and $\text{Ra}^{2+}$ and $\text{RaH}_2\text{PO}_4^{+}$ on layer-sites. All experiments were conducted under free air conditions and no negative impact of CO$_2$ was found. The surface complexation model was found suitable for describing radium uptake by the studied hydroxyapatite and titanium dioxide nanomaterials.

Conveniently, the novel blood vessels are abnormal and leaky, so the nanocarriers can get stuck in the tumor tissue. The second one is active targeting by functionalization of the drug carrier surface. The carriers can be modified by specific ligands or antigens and the drugs are delivered to the target tissue due to their bond to the required receptor. The basic principles of nanocarriers’ preparation for the targeted alpha particle therapy (TAT), particularly bearing the alpha-emitting nuclides that decay in series, were described previously. Accordingly, hydroxyapatite ($n$HAp) and titanium dioxide nanoparticles ($n$TiO$_2$) could be applied as useful vehicles for radionuclide delivery in case of radiolabelling with an appropriate radionuclide, such as $^{18}$F, $^{68}$Ga, $^{99m}$Tc etc. for diagnostic purposes or $^{223}$Ac, $^{211}$Bi, $^{177}$Lu, $^{223}$Ra, $^{186}$Re, $^{90}$Y and others for therapeutic purposes. Nowadays, there are several publications dealing with radiolabelling of $n$HAp and $n$TiO$_2$. For example, there are published studies, where the $n$HAp is labelled by alpha therapeutic radionuclide $^{222}$Ra. Other radionuclides used for labelling were $^{134}$Cs or $^{90}$Sr, where the $n$HAp was applied for remediation of contaminated waters by anthropogenic radionuclides. Available published data for radiolabelling of $n$TiO$_2$ are limited. There are some studies dedicated to labelling of TiO$_2$ with $^{48}$V or $^{125}$I for in vivo toxicological studies and with $^{225}$Ac for targeted alpha radionuclide therapy. This article is focused on the study of $^{223}$Ra sorption on $n$HAp and $n$TiO$_2$. The published data on radion sorption using

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Introduction

Hydroxyapatite (HAp) and titanium dioxide are materials intensely studied and widely used for their favorable properties in many fields. Due to this, the range of their applications is extended from foodstuffs, cosmetics and sunscreen creams through environmental decontamination to medicine, which is our area of interest. Both materials are biocompatible and have relatively low clinical toxicity (LD$_{50}$HAp (oral, rat) over 23 g kg$^{-1}$; LD$_{50}$TiO$_2$ (oral, rat) over 10 g kg$^{-1}$), which is the underlying assumption for using them for patient applications. Some advantages of radionuclide sorption are the following properties: high specific surface area, radiation stability and size. Apart from the previously mentioned reasons, ease of synthesis on a nanoscale size is another argument for selection of these two materials.

Our main area of interest is specifically the possibility of the application of the chosen nanomaterials as a drug carrier system for diagnostic, therapeutic or theragnostic radionuclides. The targeting of the nanoparticles to the required tissue can be enabled by two mechanisms. The first one is passive targeting due to the Enhanced Permeability and Retention effect (EPR effect). This effect exploits the size of the drug carriers. Tumor tissue grows fast and stimulates angiogenesis. The mechanism of $^{223}$Ra uptake on hydroxyapatite and titanium dioxide nanoparticles was studied as a function of pH. Both materials are widely used in food industry and medicine. They offer properties suitable for labelling with medicinal radionuclides, particularly for targeted radionuclide therapy. The selected isotope, $^{223}$Ra, is an alpha emitter widely used in targeted alpha particle therapy due to high-dose delivery in very small tissue volume, nevertheless the results are applicable for any radium isotope including $^{226}$Ra. The study was performed in the pH range 4.5 to 12 for hydroxyapatite nanoparticles and 2 to 12 for titanium dioxide nanoparticles in Britton–Robinson buffer solution. Both nanomaterials at pH 6 and higher showed that over 95% of the radium has been sorbed. According to the applied chemical equilibrium model, the most important species playing a role in sorption on the edge-sites were $\text{RaCO}_3$, $\text{RaPO}_4^{-}$, $\text{RaHPO}_4$ and $\text{Ra}^{2+}$ and $\text{RaH}_2\text{PO}_4^{+}$ on layer-sites. All experiments were conducted under free air conditions and no negative impact of CO$_2$ was found. The surface complexation model was found suitable for describing radium uptake by the studied hydroxyapatite and titanium dioxide nanomaterials.

Conveniently, the novel blood vessels are abnormal and leaky, so the nanocarriers can get stuck in the tumor tissue. The second one is active targeting by functionalization of the drug carrier surface. The carriers can be modified by specific ligands or antigens and the drugs are delivered to the target tissue due to their bond to the required receptor. The basic principles of nanocarriers’ preparation for the targeted alpha particle therapy (TAT), particularly bearing the alpha-emitting nuclides that decay in series, were described previously. Accordingly, hydroxyapatite ($n$HAp) and titanium dioxide nanoparticles ($n$TiO$_2$) could be applied as useful vehicles for radionuclide delivery in case of radiolabelling with an appropriate radionuclide, such as $^{18}$F, $^{68}$Ga, $^{99m}$Tc etc. for diagnostic purposes or $^{223}$Ac, $^{211}$Bi, $^{177}$Lu, $^{223}$Ra, $^{186}$Re, $^{90}$Y and others for therapeutic purposes. Nowadays, there are several publications dealing with radiolabelling of $n$HAp and $n$TiO$_2$. For example, there are published studies, where the $n$HAp is labelled by alpha therapeutic radionuclide $^{222}$Ra. Other radionuclides used for labelling were $^{134}$Cs or $^{90}$Sr, where the $n$HAp was applied for remediation of contaminated waters by anthropogenic radionuclides. Available published data for radiolabelling of $n$TiO$_2$ are limited. There are some studies dedicated to labelling of TiO$_2$ with $^{48}$V or $^{125}$I for in vivo toxicological studies and with $^{225}$Ac for targeted alpha radionuclide therapy. This article is focused on the study of $^{223}$Ra sorption on $n$HAp and $n$TiO$_2$. The published data on radion sorption using
chosen nanoparticles are quite limited and available papers are focused mainly on $^{226}$Ra behavior in uranium mill tailings and the articles studied only iron compounds as a goethite or ferrihydrite, or environmental minerals.\textsuperscript{25-27}

The $^{223}$Ra is a radionuclide used for TAT as radium chloride \cite{Xofigo}\textsuperscript{18,28-30}. Due to the cascade of emitted alpha particles, it could provide accurate high-dose irradiation of the target tissue (range of the alpha particles approx. 10 cells) without damage to the organism, if appropriate targeting strategy is applied. Nowadays, Xofigo\textsuperscript{8} is used for the therapy of metastatic prostate cancer, but number of its applications decreases. Possible fields and ways of its applications could be significantly wider due to the capture of recoil nuclei by the nanoparticles and advantages of $^{223}$Ra. Furthermore, $^{223}$Ra can be obtained from a radionuclide generator $^{227}$Ac/$^{227}$Th/$^{223}$Ra,\textsuperscript{28-31} which can probably guarantee availability of the radionuclide in the hospitals. Main introduced disadvantage of $^{223}$Ra are recoil nuclei appearing during the radioactive decay. They have considerable energy, which means, that daughter nuclei are escaping from chemical bonds and are distributed into the organism due to their chemical and biological properties. In the case of $^{223}$Ra ($\beta$-emitter, $T_{1/2} = 11.4$ days), its daughter decay products with half-lives longer than one minute are: $^{211}$Pb ($\beta$-emitter, $T_{1/2} = 36.1$ min), $^{211}$Bi ($\beta$-emitter, $T_{1/2} = 2.1$ min) and $^{207}$Tl ($\beta$-emitter, $T_{1/2} = 4.8$ min).\textsuperscript{34}

The aim of this work is focused on the study of $^{223}$Ra sorption as a function of $pH$. The mechanism of radium uptake by $n$HAp and $n$TiO$_2$ in Britton–Robinson buffer solution (BRB) was modelled and absorbed species were studied. For describing the surface complexation systems, chemical equilibrium model (CEM) was used. Modelling programs and codes for study of sorption mechanism requires protonation constants, ion-exchange constants and total concentration of edge-sites and layer-sites, which were determined experimentally via titration method and corresponding models.\textsuperscript{25-37}

**Experimental**

**Materials**

All chemicals were of analytical grade purchased from Merck Millipore (Germany) and were used without further purification: sodium hydroxide, tetraethyl ortho-titanate (TBOT), 2-propanol (IPA), ammonium hydroxide, phosphoric acid, nitric acid, methanol, boric acid, acetic acid, calcium nitrate tetrahydrate and diammonium hydrogen phosphate. Demineralized water of 18 M$\Omega$ cm$^{-1}$ was obtained from water purification system (Millipore, USA). The activities of the samples were measured with a well-type NaI(Tl) crystal detector (Capintec, USA). For mixing of samples, Stuart SSM3 rocker (Cole-Parmer Ltd, United Kingdom) was used and the separation was made on VWR Micro Star 12 centrifuge (VWR International, LLC, USA). Gamma spectra were recorded on Canberra Packard HPGe detector (USA) under GammaVision software.

**Britton–Robinson buffer solution**

For Britton–Robinson buffer solution preparation in the pH ranging from 2 to 12, two stock solutions were mixed in appropriate ratio. The first one was 0.2 M sodium hydroxide and the second one was the mixture of 0.04 M phosphoric acid, 0.04 M boric acid and 0.04 M acetic acid.

**Preparation of $^{223}$Ra stock solution**

The $^{223}$Ra was eluted from $^{227}$Ac/$^{227}$Th/$^{223}$Ra generator, which was prepared at our laboratory based on the study published by Guseva et al.\textsuperscript{33} The column of the generator was filled with 0.5 g of Dowex-1 $\times$ 8 and $^{227}$Ac in equilibrium with its decay products was loaded on it. The elution was provided by 0.7 M nitric acid in 80% methanol solution for the separation of $^{223}$Ra from $^{227}$Ac and $^{227}$Th. The eluted $^{223}$Ra(NO$_3$)$_2$ solutions were dried and reconstituted with deionized water. Possible breakthrough of parent radionuclides was checked by gamma spectrometry and was not observed in the eluate.

**Sorbent materials preparation**

The sorbent preparation was described by Kukleva et al. in detail.\textsuperscript{35} In this article only brief procedure is mentioned.

For hydroxyapatite nanoparticles preparation, 1.2 M Ca(NO$_3$)$_2$, (24 mL) was added into 0.5 L of demineralized water. It was necessary to set and maintain the pH of the mixture to 11 with ammonium hydroxide. Afterwards, the solution of calcium nitrate was stirred and 0.7 M (NH$_4$)$_2$HPO$_4$ (24 mL) was added dropwise. The mixture was left overnight under stirring, washed three times with demineralized water (20 mL) and then dried under vacuum.

The titanium dioxide nanoparticles were prepared by the dropwise addition of the mixture of TBOT (1 mL) in IPA (4 mL) into demineralized water in ultrasonic generator and was stirred for 30 minutes. Prepared $n$TiO$_2$ were washed three times with demineralized water (20 mL), once with IPA (10 mL) and then dried under vacuum.

**Sorption experiments**

Experiments were performed in pH range from 4.5 to 12 for $n$HAp and from 2 to 12 for $n$TiO$_2$. All samples were in triplets and were prepared in a following way: 2 mg of $n$HAp or 5 mg of $n$TiO$_2$ were dispersed in 1 mL of BRB. Then 50 $\mu$L of $^{223}$Ra(NO$_3$)$_2$ was dosed into each sample vial (pH of Ra solution was adjusted before adding to the sample by diluted ammonium hydroxide to the approx. sample pH in order not to exceed BRB’s buffering capacity). Added radioactivity was ranged between 1.4 and 2.4 kBq. All samples were shaken for 24 hours, centrifuged and the supernatant was quantitatively removed. Then samples were dispersed in 1 mL of demineralized water in order to ensure same geometry of measurement. All samples and all supernatants were measured. All experiments were accomplished under free air conditions and at the laboratory temperature.

In the same manner, sorption of pure $^{223}$Ra (without addition of nanomaterials) on the vial walls was studied in pH range from 2 to 12.

**Modelling of the $^{223}$Ra uptake**

First of all, calculations of $^{223}$Ra speciation diagrams were performed based on stability constants of Ra$^{2+}$ mainly. In the
case of unavailable Ra constants, Ba$^{2+}$, Sr$^{2+}$ and Ca$^{2+}$ were used and were chosen due to their similar chemical properties. The stability constant values were taken from the Hatches database used in the geochemical code PhreeqC.\textsuperscript{38}

Then the model based on the surface complexation theory was constructed and incorporated into the simulation code. According to the surface complexation theory, it is supposed that there are two types of surface functional groups: edge-sites and layer-sites. Their concentrations were already calculated based on experimental data.\textsuperscript{35-37} For further modelling protonation constants of edge-sites, ion-exchange constant of layer-sites and the total concentration of both sites were used as input data.

The software product FAMULUS\textsuperscript{39} and our code Praspec6.fm (code package STAMB 2017) were used for the speciation calculations. The corresponding code, Pramg6ZM.fm (code package STAMB 2017), is based on the Newton–Raphson multidimensional nonlinear regression used for experimental data fitting. It has to be added, that the number of complexes, which can be used in calculation, is selectable. The appropriateness of the fit is evaluated by the χ²-test, and the values of χ² are used to the calculation of criterion WSOS/DF (weighted sum of squares divided by degrees of freedom).\textsuperscript{40} It holds, if $0.1 < WSOS/DF < 20$, then there is a good agreement between the experimental and the calculated data.

### Results and discussion

As mentioned above, published studies\textsuperscript{25-27} were focused only on $^{226}$Ra and the sorption was studied only on a few materials.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ra$^{2+}$</th>
<th>Ca$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_3^{2-}$</td>
<td>2.50/–8.30</td>
<td>3.15/–8.14</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>–0.10</td>
<td>–0.29</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>2.75</td>
<td>2.31</td>
</tr>
<tr>
<td>OH$^-$</td>
<td>–13.49</td>
<td>–12.78</td>
</tr>
</tbody>
</table>

For the medicinal purposes, the attention was paid to the sorption of $^{223}$Ra in BRB and it was experimentally studied on nHAp in the pH range of 4.5–12 and of 2–12 for nTiO$_2$. Studied pH ranges were different due to dissolution of the HAp under pH 4.5 while TiO$_2$ is stable in a wide pH range. The BRB consists of sodium compounds derived from phosphoric, boric and acetic acids – the speciation of which strongly depends on the pH value. Not only the corresponding $^{223}$Ra complexes have to be taken into account, but also the hydroxo- and carbonate-complexes especially if the pH is higher than 7 due to dissolution of atmospheric CO$_2$.

Sorption of $^{223}$Ra on superparamagnetic iron oxide nanoparticles (nFe$_3$O$_4$, SPIONs) was studied earlier in our laboratory,\textsuperscript{42} therefore, in the current work the results of $^{223}$Ra sorption on nHAp and nTiO$_2$ were compared with SPIONs.

### Calculation of speciation diagram for $^{223}$Ra in Britton–Robinson buffer solution

At first, it was necessary to draw attention to the fact, that the present state of stability constants knowledge for radium complexes was limited. The reliable values of studied complexes were available only for RaOH$^-$ and RaCO$_3$. Therefore, stability constants were taken into consideration for elements with similar chemical properties, namely for Ba, Sr and Ca. Unfortunately, data availability for Ba was also not sufficient, for Sr it was slightly better, but constants for acetic and boric acids complexes were also not available. Therefore, stability constants for Ca with above mentioned ligands were taken in addition to Ra hydroxo- and carbonate-complexes constants. The similarity of Ra and Ca complexation is demonstrated in Table 1 on the values of stability constants ($\beta$) and solubility product (SP)\textsuperscript{38,41} (the values hold for $I = 0$).

The calculation of speciation diagrams were performed for the following composition of aqueous phase: $5.00 \times 10^{-3}$ mol L$^{-1}$ Ra(NO$_3$)$_2$; $\sum_{[H_2PO_4]} = 4.00 \times 10^{-2}$ mol L$^{-1}$; $\sum_{[H_2BO_3]} = 4.00 \times 10^{-2}$ mol L$^{-1}$; $\sum_{[CH_3COOH]} = 4.00 \times 10^{-2}$ mol L$^{-1}$; $pCO_2 = 3.16 \times 10^{-3}$ at, ionic strength $I = 0.15$. It deals with sodium salts of above-mentioned acids in a case of higher pH values.

The values of stability and dissociation constants ($I = 0$) are summarized in Table 2 and the results of calculation are shown.

### Table 2  The values of stability and dissociation constants ($I = 0$)\textsuperscript{38,41}

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Stability constant</th>
<th>log $K$</th>
<th>Dissociation constant</th>
<th>log $K$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_3PO_4^-$</td>
<td>$K = [CaH_2PO_4][\text{Ca}\cdot H_2PO_4]$</td>
<td>1.28</td>
<td>$K = [HPO_4][\text{H}\cdot PO_4]$</td>
<td>12.36</td>
</tr>
<tr>
<td>$HPO_4^{2-}$</td>
<td>$K = [CaHPO_4][\text{Ca}\cdot HPO_4]$</td>
<td>2.68</td>
<td>$K = [H_2PO_4][\text{H}_2\cdot PO_4]$</td>
<td>19.70</td>
</tr>
<tr>
<td>$PO_4^{3-}$</td>
<td>$K = [CaPO_4][\text{Ca}\cdot PO_4]$</td>
<td>6.46</td>
<td>$K = [HPO_4][\text{H}_2\cdot PO_4]$</td>
<td>21.93</td>
</tr>
<tr>
<td>$H_2BO_3^-$</td>
<td>$K = [CaH_2BO_3][\text{Ca}\cdot H_2BO_3]$</td>
<td>1.80</td>
<td>$K = [HCO_3][\text{H}\cdot CO_3]$</td>
<td>10.33</td>
</tr>
<tr>
<td>OH$^-$</td>
<td>$K = [RaOH][\text{Ra}\cdot OH]$</td>
<td>–0.5</td>
<td>$K = [HCO_3][\text{H}_2\cdot CO_3]$</td>
<td>16.68</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>$K = [RaCO_2][\text{Ra}\cdot CO_2]$</td>
<td>2.50</td>
<td>$K_p = [PCO_2][\text{H}_2\cdot CO_3]$</td>
<td>18.60$^*$</td>
</tr>
<tr>
<td>Ac$^-$</td>
<td>$K = [CaAc][\text{Ca}\cdot Ac]$</td>
<td>1.18</td>
<td>$K = [HAc][\text{H}\cdot Ac]$</td>
<td>4.76</td>
</tr>
<tr>
<td>(Ac$^-$)$_2$</td>
<td>$K = [Ca(2Ac)][\text{Ca}\cdot (2Ac)]$</td>
<td>4.00</td>
<td>$K = [H_2BO_3][\text{H}\cdot H_2BO_3]$</td>
<td>–9.24</td>
</tr>
<tr>
<td>(Ac$^-$)$_3$</td>
<td>$K = [Ca(Ac_3)][\text{Ca}\cdot (3Ac)]$</td>
<td>4.45</td>
<td>$K = [HBO_3][\text{H}\cdot HBO_3]$</td>
<td>–12.74</td>
</tr>
<tr>
<td>(Ac$^-$)$_4$</td>
<td>$K = [Ca(Ac_4)][\text{Ca}\cdot (4Ac)]$</td>
<td>3.60</td>
<td>$K = [BO_3][\text{H}\cdot HBO_3]$</td>
<td>–13.79</td>
</tr>
<tr>
<td>SP</td>
<td>$K = [Ra][\text{CO}_3]$</td>
<td>–8.30</td>
<td>$K = [H][\text{OH}]$</td>
<td>–14</td>
</tr>
</tbody>
</table>

$^*$ $pCO_2$ [at] – partial pressure of CO$_2$, atmospheric $pCO_2 = 3.16 \times 10^{-3}$ [at], Ac = CH$_3$COO$^-$. 

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in Fig. 1. Complexing reactions and corresponding complex compounds were incorporated in the code Praspec6.fm (Table 2). Under the given conditions, only five of them can be regarded as more important, namely, the complexing reactions with \( \text{PO}_4^{3-} , \text{HPO}_4^{2-}, \text{H}_2\text{PO}_4^- \), \( \text{CO}_2^{2-} \) and \( (\text{CH}_3\text{COO})_2 \). The calculation of possible precipitation of \( \text{RaCO}_3 \) was also considered in code Praspec6.fm, however, due to the very low Ra concentration, precipitation could not occur.

### Modelling of experimental data as a function of pH

Chemical equilibrium model was used to describe the surface complexation systems. It consisted of two groups of equations. The first one characterized the protonation and ion-exchange behavior of sites (eqn (1)–(3)) and the second one the sorption of individual species including the balance equations (eqn (4)–(12)). The values of sorption constants, \( K \) (eqn (4)–(10)) were obtained during iterations.

The equations (eqn (1)–(12)) listed below were incorporated in the regression function of the code Pramg6zp.fm. The protonation reactions (eqn (1) and (2)) on edge-sites were:

\[
\text{SO}^- + \text{H}^+ \leftrightarrow \text{SOH}, \quad K_{S1} = [\text{SOH}]/([\text{SO}^-] \cdot [\text{H}^+]) \tag{1}
\]

\[
\text{SOH} + \text{H}^+ \leftrightarrow \text{SOH}_2^+ , \quad K_{S2} = [\text{SOH}_2^+]/([\text{SOH}] \cdot [\text{H}^+]) \tag{2}
\]

The Na\(^+\)/H\(^+\) ion-exchange reaction (eqn (3)) on layer-sites was:

\[
\text{XNa} + \text{H}^+ \leftrightarrow \text{XH} + \text{Na}^+ , K_{\text{ex}} = ([\text{XH}] \cdot [\text{Na}^+] )/([\text{XNa}] \cdot [\text{H}^+]) \tag{3}
\]

The sorption reactions (eqn (4)–(10)) were:

\[
\text{SO}^- + \text{Ra}^{2+} \leftrightarrow \text{SORa}^+ , K_1 = [\text{SORa}^+]/([\text{SO}^-] \cdot [\text{Ra}^{2+}]) \tag{4}
\]

\[
\text{SO}^- + \text{RaCO}_3 \leftrightarrow \text{SORaCO}_3^- , K_2 = [\text{SORaCO}_3^-]/([\text{SO}^-] \cdot [\text{RaCO}_3]) \tag{5}
\]

\[
\text{SO}^- + \text{RaPO}_4^- \leftrightarrow \text{SORaPO}_4^-^2 , K_3 = [\text{SORaPO}_4^-^2]/([\text{SO}^-] \cdot [\text{RaPO}_4^-]) \tag{6}
\]

\[
\text{SO}^- + \text{RaHPO}_4^- \leftrightarrow \text{SORaHPO}_4^- , K_4 = [\text{SORaHPO}_4^-]/([\text{SO}^-] \cdot [\text{RaHPO}_4]) \tag{7}
\]

\[
2\text{XH} + \text{Ra}^{2+} \leftrightarrow \text{X}_2\text{Ra} + 2\text{H}^+ , K_5 = ([\text{X}_2\text{Ra}] \cdot [\text{H}^+])^2/([\text{XH}]^2 \cdot [\text{Ra}^{2+}]) \tag{8}
\]

\[
\text{XH} + \text{RaH}_2\text{PO}_4^+ \leftrightarrow \text{XRaH}_2\text{PO}_4^- + \text{H}^+ , K_6 = ([\text{XRaH}_2\text{PO}_4^-] \cdot [\text{H}^+])/([\text{XH}] \cdot [\text{RaH}_2\text{PO}_4^+]) \tag{9}
\]

\[
\text{SO}^- + \text{Ra}(\text{Ac})^- \leftrightarrow \text{SORa}(\text{Ac})^-^2 , K_7 = [\text{SORa}(\text{Ac})^-^2]/([\text{SO}^-] \cdot [\text{Ra}(\text{Ac})^-]) \tag{10}
\]

The balance equations (eqn (11) and (12)) were:

\[
\sum \text{SOH} = [\text{SO}^-] + [\text{SOH}_2^+] + [\text{SOH}] + [\text{SORa}^+] + [\text{SORaCO}_3^-] + [\text{SORaPO}_4^-^2] + [\text{SORaHPO}_4^-] + [\text{SORa}(\text{Ac})^-^2] \tag{11}
\]

\[
\sum \text{X} = [\text{XNa}] + [\text{XH}] + 2[\text{XRa}] + [\text{XRaH}_2\text{PO}_4^-] \tag{12}
\]

Following input data were also needed: the composition of liquid phase, the phase ratio \( \text{V/m (L kg}^{-1}) \), the values of stability and dissociation constants of Ra complexes and \( \text{CO}_2 \) atmospheric pressure (Table 2), protonation constants, ion-exchange constant and total concentration of edge-sites \( \sum \text{SOH} \) and layer-sites \( \sum \text{X} \) (Table 3). All data in the Tab. 3 were described in detail in publication Kukleva et al.\textsuperscript{23}

### Table 3 Constants describing the surface protolytic properties of nHAp, nTiO\(_2\) and SPIONs respectively\textsuperscript{23,42}

<table>
<thead>
<tr>
<th>Constant</th>
<th>Units</th>
<th>nHAp</th>
<th>nTiO(_2)</th>
<th>nFe(_3)O(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protonation constant - ( K_{S1} )</td>
<td>[L mol(^{-1})]</td>
<td>5.12 \times 10(^{14})</td>
<td>2.31 \times 10(^6)</td>
<td>9.65 \times 10(^6)</td>
</tr>
<tr>
<td>Protonation constant - ( K_{S2} )</td>
<td>[L mol(^{-1})]</td>
<td>1.19 \times 10(^8)</td>
<td>1.84 \times 10(^4)</td>
<td>9.13 \times 10(^5)</td>
</tr>
<tr>
<td>Ion-exchange constant - ( K_{\text{ex}} )</td>
<td>[\text{--}]</td>
<td>3.01 \times 10(^6)</td>
<td>5.67 \times 10(^7)</td>
<td>3.60 \times 10(^7)</td>
</tr>
<tr>
<td>Concentration of edge-sites - ( \sum \text{SOH} )</td>
<td>[mol kg(^{-1})]</td>
<td>5.10</td>
<td>0.20</td>
<td>0.05</td>
</tr>
<tr>
<td>Concentration of layer-sites - ( \sum \text{X} )</td>
<td>[mol kg(^{-1})]</td>
<td>0.15</td>
<td>0.67</td>
<td>0.09</td>
</tr>
<tr>
<td>Specific surface area</td>
<td>[m(^2) kg(^{-1})]</td>
<td>1.17 \times 10(^8)</td>
<td>3.30 \times 10(^5)</td>
<td>1.09 \times 10(^5)</td>
</tr>
</tbody>
</table>
The results are summarized in Table 4 and Fig. 2 and 3. Experimental data were in a good agreement with calculated values of radium uptake as a function of pH on both edge- and layer-sites (Fig. 2A and 3A). The results of the modelling of the nHAp and nTiO$_2$ labelling with $^{223}$Ra support the concept that the radium uptake can be described with the surface complexation model type of CEM and both edge- and layer-sites were involved in sorption mechanism (Fig. 2 and 3). It means, in our opinion, that labelling corresponds better with sorption than with co-precipitation mechanism.

The sorption efficiency ($Y\%$) was calculated based on the equation

$$ Y\% = \frac{A_{NP}}{A_{NP} + A_{aq}} \times 100\% $$

where the $A_{NP}$ is the activity of the centrifuged and secondary dispersed nanoparticles [cps], the $A_{aq}$ is the activity of the supernatant [cps]. The $A_{NP}$ and the $A_{aq}$ were measured under the same conditions and no time correction was applied.

The sorption efficiency on nHAp was 95 ± 5% in the pH range from 5 to 12 (Fig. 2 and 5). It could be caused by relatively high sorption capacity or high specific surface area of nHAp (117 m$^2$ g$^{-1}$) and by the composition of used aqueous solution (BRB). Fig. 2 shows that the most important sorption reactions going on edge sites were RaCO$_3$ (eqn (4), minor), RaPO$_4$ (eqn (6)) and RaHPO$_4$ (eqn (7)), and on layer-sites were Ra$^2+$ (eqn (8)) and RaH$_2$PO$_4$ (eqn (9)). On the base of speciation diagrams (Fig. 1), the greater role of PO$_4^{3-}$, CO$_3^{2-}$ and CH$_3$-COO$^-$ ligands were expected, but this supposition especially in a case of acetic anion was not confirmed. However, the role of HPO$_4^{2-}$ seems to be underestimated.

In the case of nTiO$_2$ sorption efficiency was about 100% in pH range from 3 to 12 and decreased to 75% at lower pH values (Fig. 3 and 5), which could be also caused by high sorption capacity.

### Table 4 Resulting values of constants $K$[1–7] of sorption reactions (eqn (4)–(10)) for nHAp and nTiO$_2$

<table>
<thead>
<tr>
<th>Eqn</th>
<th>nHAp</th>
<th>nTiO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4)</td>
<td>$K[1] = 2.44 \times 10^8$</td>
<td>$K[1] = 4.13 \times 10^{11}$</td>
</tr>
<tr>
<td>(9)</td>
<td>$K[6] = 6.60 \times 10^6$</td>
<td>$K[6] = 2.34 \times 10^4$</td>
</tr>
<tr>
<td>(10)</td>
<td>$K[7] = 2.81 \times 10^8$</td>
<td>$K[7] = 4.88 \times 10^{11}$</td>
</tr>
</tbody>
</table>

WSOS/DF 0.21 0.18

![Fig. 2](image) Sorption on nHAp: (A) $^{223}$Ra uptake vs. pH; (B) uptake of given species vs. pH.

![Fig. 3](image) Sorption on nTiO$_2$: (A) $^{223}$Ra uptake vs. pH; (B) uptake of given species vs. pH.
capacity or high specific surface area (330 ± 10 m² g⁻¹) of nTiO₂, and also due to the composition of aqueous phase. The main role in sorption reactions on layer-sites played Ra²⁺ itself (eqn (8) and Fig. 3). On edge-sites the most important species seems to be Ra²⁺ (eqn (4)), RaCO₃ (eqn (5)), RaHPO₄ (eqn (7)) and Ra(Ac⁻)₂ (eqn (10)) (Fig. 4). On the base of speciation diagrams, the greater role of PO₄³⁻ (Fig. 1A) and CH₃COO⁻ (Fig. 1B) ligands were expected, however this expectation were not verified. One of the most interesting result is that surface complexation model type of CEM is suitable as a describing model of studied nTiO₂ radium uptake, as well as of nHAp.

Certainly, the question considering presence of atmospheric CO₂ also needs to be taken into account. Relatively high concentrations of carbonates in samples with pH greater than 8–9 could affect the results, therefore, it might be better to use inert atmosphere for clearer experiments. In spite of this, the CO₂ and HCO₃⁻ were included in calculations.

Radium sorption properties of nHAp and nTiO₂ were compared also with SPIONs (Fig. 4 and 5). From comparison, it is evident that all three materials have relatively high sorption affinity to Ra¹⁰⁶ under the studied conditions. It is necessary to keep in mind that low initial radium concentrations were used (approx. 2 kBq, 5 × 10⁻¹² M). Although there were some differences in the values of parameters of edge-sites (K₁, K₂, ∑SOH), of layer-sites (Kₓ, ∑X) and of the specific surface area, sorption yields for all three materials were high. Additional
distinction of nHAp towards nTiO₂ and SPIONs was its lower chemical stability in acidic aqueous solutions. Regarding its possible application in vivo, it should not play any significant role.

The values of nHAp’s and nTiO₂’s K₅ (equilibrium constant of reaction eqn (1)) indicated the relatively greater shift of nHAp’s protonation reaction (eqn (1)) to the right in comparison to the nTiO₂. In relation to K₅ values, the deprotonated species SO₄⁻ did not exist in pH lower than 6 in the case of nHAp and lower than 5 for nTiO₂. This fact played a certain role in ion-exchange reactions (eqn (3)) and surface complexation reactions (eqn (4)–(7) and (10)).

Comparing all three materials, it could be said that all of them have comparable properties in the context of Ra sorption (Fig. 5 and 6). Hydroxyapatite nanoparticles as well as SPIONs showed high yields of Ra on nanoparticles of nHAp, nTiO₂ and SPIONs by means of the surface complexation model type of CEM, in spite of the fact, that studied materials were not similar.

Conclusions

Studied materials nHAp and nTiO₂ had shown relatively high sorption affinity to Ra(III) under the studied conditions and the radiolabelling yields were over 95% in a wide pH range. Based on the calculations and modelling it was found, that the main role in sorption in the case of nHAp played RaCO₃, RaPO₄⁻, RaHPO₄, Ra²⁺ and RaH₂PO₄⁻. In the case of nTiO₂ the main role in sorption reactions played Ra²⁺ itself, RaCO₃, RaHPO₄ and Ra(AC)₂. Furthermore, it was found, that presence of CO₂ did not interfere high sorption yields, what could be important to take into account for further experiments.

This paper shows possibility to use nHAp and nTiO₂ nanoparticles as a useful vehicle for ²²²Ra delivery for targeted alpha therapy. So, it could be concluded, that nHAp and nTiO₂ are suitable nanomaterials for medicinal usage due to high sorption properties in a pH range required for medicine, radiation stability and biocompatibility. Another important benefit is labelling procedure simplicity, where radium chloride in a liquid form is mixed with ready-made particles under laboratory temperature without any inert atmosphere. Obviously, this study is preliminary and further investigations of labelling kinetics and in vitro stability in biologically relevant media are necessary.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the grants of NV16-30544A (Czech Health Research Council), C.Z.02.1.01/0.0/0.0/15_003/000046 (EU and the Ministry of Education, Youth and Sports of the Czech Republic), and SGS19/194/OHK4/3T/14 (Czech Technical University in Prague).

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