

## Brief Communications

### Modified hydroxyapatite as a carrier for $^{69m}\text{Zn}$

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Sorption of zinc ions and  $^{69m}\text{Zn}$  radionuclide proceeds much more rapidly on hydroxyapatite modified with zinc ions (HAP-Zn) than on unmodified HAP. The sorption can be described by a pseudo-second order model and probably follows an exchange mechanism, as evidenced by the transfer of calcium ions into the mother liquor during the process. Sorbed  $^{69m}\text{Zn}$  participates in a slow reverse isotope exchange with the solution containing non-radioactive zinc ions.

**Key words:** hydroxyapatite, modification with zinc ions, reverse isotope exchange,  $^{69m}\text{Zn}$  radionuclide.

The delivery of radionuclides into the body is key for the medical use of radiopharmaceuticals. Hydroxyapatite (HAP), which is widely used as an implant and is a biocompatible and biodegradable component, has attracted the attention of researchers.<sup>1</sup> The parameters of sorption of zinc ions on HAP under different experimental conditions are known.<sup>2–6</sup> However, since radionuclides are used, as a rule, in a carrier free mode, process of sorption of the potential diagnostic radionuclide  $^{69m}\text{Zn}$  on HAP, previously modified with zinc ions (HAP-Zn), is of interest. It can be assumed that this material would sorb the

target radionuclide more rapidly, this being an important advantage when working with short-lived isotopes. Moreover, if the rate of additional ion exchange turns out to be comparable or even higher than the rate of sorption, the degree of labeling will also increase. Desorption processes, as well as reverse isotope exchange, can be of considerable importance during sorption on HAP- $^{69m}\text{Zn}$ .

The purpose of this work is to reveal the parameters characterizing the processes of sorption—desorption of the  $^{69m}\text{Zn}$  radionuclide and to explain the role of direct and reverse isotope exchange in its binding and retention.

## Experimental

**Radionuclide  $^{69m}\text{Zn}$**  was obtained on a microtron using the reaction of  $^{71}\text{Ga}(\gamma, np)^{69m}\text{Zn}$  with an electron energy of 55 MeV. The dissolution of the target and the separation of the radionuclide was carried out according to the procedure described earlier<sup>7</sup> using double liquid extraction with methyl isobutyl ketone. The separation was monitored by gamma spectrometry (Canberra GC 3020, USA) using the 438 keV line. The obtained specific activity of the final aqueous solution (pH 6.8) was  $34.2 \pm 1.7$  kBq mL<sup>-1</sup>, the radionuclide purity was 96.9%, the activity of  $^{65}\text{Zn}$  was negligible.

**Sorption of  $^{69m}\text{Zn}$  on HAP-Zn.** A solution of  $^{69m}\text{ZnCl}_2$  (carrier free, 0.3 mL) in distilled water (5 mL) was added to a zinc nitrate solution ( $C = 0.83$  g Zn L<sup>-1</sup>, 10.7 mL). The specific activity of newly prepared solution was  $933 \pm 9$  Bq mL<sup>-1</sup>. Then the obtained solution (0.9 mL) was added to a suspension of HAP-Zn (0.3 mL, the sorbent was synthesized according to the procedure described earlier<sup>4</sup>), stirred on a shaker (Sky Line, Shaker S-3L, 170 rpm) and, after specific periods of time, the phases were separated using a centrifuge (Eppendorf, Centrifuge 5418, 4 min, 10000 g). Portions (1.1 mL) of the residual solution were selected, and the activity of this solution and sorbent was measured on a  $\gamma$ -spectrometer. In all the experiments, the radionuclide activity was recalculated taking into account the half-life.

**Desorption of Zn<sup>69m</sup> from HAP-Zn into a physiological solution.** A 0.83 g Zn L<sup>-1</sup> zinc nitrate solution (2.4 mL) and distilled water (5 mL) were added to an aqueous solution of carrier free  $^{69m}\text{Zn}$  (0.1 mL). The concentration of the obtained  $^{69m}\text{Zn}(\text{NO}_3)_2$  solution was 0.277 g Zn L<sup>-1</sup>, the specific activity when newly prepared was  $455 \pm 25$  Bq mL<sup>-1</sup>. The sorption of  $^{69m}\text{Zn}$  on HAP-Zn was carried out first. For this, the previously obtained  $^{69m}\text{Zn}(\text{NO}_3)_2$  solution (0.45 mL) and water (0.45 mL) were added to a HAP-Zn suspension (0.3 mL) and placed on a shaker. After 25 min, the phases were separated by centrifugation (4 min, 10000 g), an aliquot (1.1 mL) of the residual solution was sampled, and physiological solution (1.1 mL) was added to the sorbent. The system was resuspended, placed on a shaker, and the phases were separated as described above after specific intervals. Portions (1.1 mL) of the residual solution were selected, and the activity of the sorbent and the residual solution was measured on a  $\gamma$ -spectrometer.

**Reverse isotope exchange of  $^{69m}\text{Zn}$  from HAP-Zn to a zinc nitrate solution.** Sorption was carried out first, following the procedure described earlier. After a complete phase separation (see the previous section), an aliquot (1.1 mL) of the residual solution was sampled, and the previously obtained zinc nitrate solution (1.1 mL) was added to the sorbent. The system was resuspended, mixed on a shaker, and the phases were separated after specific intervals. Portions (1.1 mL) of the residual solution were sampled, and the activity of the sorbent and the residual solution was measured on a  $\gamma$ -spectrometer, taking into account the half-life.

**Sorption of inactive zinc ions on HAP-Zn.** To compare the characteristics of the processes of sorption of active and inactive zinc on HAP-Zn and to reveal the role of isotope exchange, we carried out an experimental investigation of the sorption of inactive zinc in the form of a zinc nitrate solution on HAP-Zn.

**Kinetics of sorption of zinc ions on HAP-Zn.** A HAP-Zn suspension (0.5 mL) and distilled water (2.5 mL) were added to an aqueous solution (1 mL) of zinc nitrate with a concentration

$C = 0.83$  g Zn L<sup>-1</sup> in test tubes. The tubes were placed on a shaker, and the phases were separated by centrifugation after the completion of mixing. The residual solution was separated from the precipitate, and the final concentration of zinc ions was determined spectrophotometrically using a calibration plot.

**Kinetics of calcium ions release during sorption of zinc ions on HAP-Zn.** To study the mechanism of binding of zinc with HAP-Zn, the amount of calcium entering into the residual solution during the sorption of zinc ions on HAP-Zn was determined. The content of calcium in aqueous solutions was determined using an analytical kit Calcium-KFK (Agat, Russian Federation) according to the instructions recommended by the manufacturer. Photometry of the solutions was carried out on a Shimadzu UV-1280 spectrophotometer (Japan) at a wavelength of 575 nm.

## Results and Discussion

The kinetic curves of zinc sorption on HAP-Zn in the presence and in the absence of a radioactive label are shown in Fig. 1. With allowance for measurement error, it can be assumed that all the experimental results fall on a single curve. It can be seen that the sorption of  $^{69m}\text{Zn}$  from a zinc nitrate solution on HAP-Zn proceeds very rapidly and reaches a plateau in less than 20 min. For convenience of the subsequent comparisons, both in the case of sorption of a radionuclide with a carrier and of non-radioactive zinc, the degree of sorption ( $\alpha$  (%)) was used, which was calculated using the following equations:

— for non-radioactive zinc:

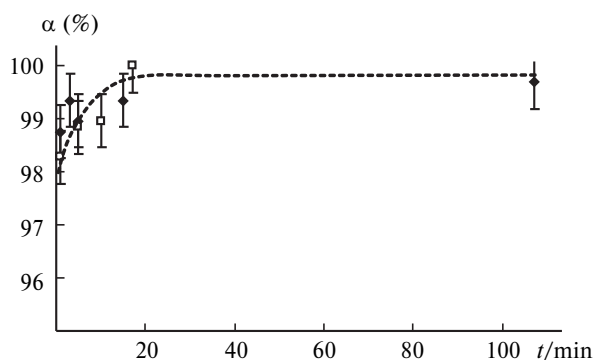
$$\alpha = (C_{\text{Zn}}^{\text{in}} - C_{\text{Zn}}^{\text{r}}) / C_{\text{Zn}}^{\text{in}} \cdot 100\%;$$

— for a radionuclide with a carrier:

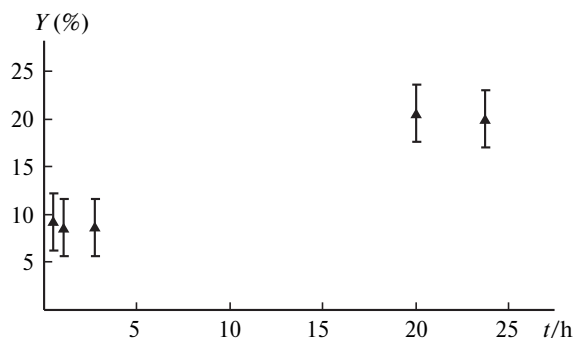
$$\alpha = (A^{\text{in}} - A^{\text{r}}) / A^{\text{in}} \cdot 100\%,$$

where the indices "in" and "r" signify "introduced" and "residual", respectively.

Experiments investigating the desorption of  $^{69m}\text{Zn}$  from HAP-Zn into a physiological solution showed that less than 5–6% is desorbed in 1 day. With a  $^{69m}\text{Zn}$  half-life



**Fig. 1.** Kinetics of zinc sorption on HAP-Zn in the presence (white squares) and in the absence (black diamonds) of a radioactive label ( $^{69m}\text{Zn}$ );  $\alpha$  is the degree of sorption.



**Fig. 2.** Reverse isotope exchange of  $^{69m}\text{Zn}$  with HAP-Zn into a zinc nitrate solution with  $C = 0.83 \text{ g Zn L}^{-1}$ ;  $Y$  is the isotope yield (% of introduced).

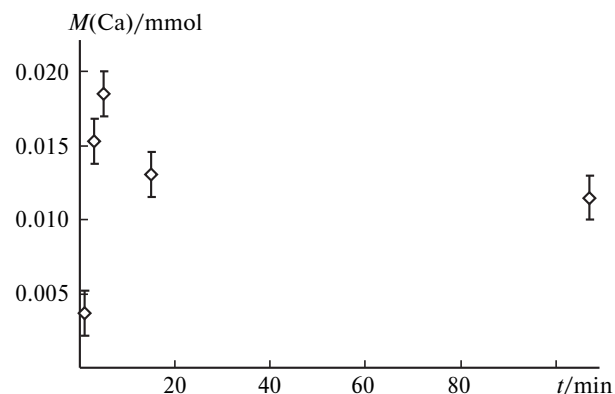
equal to 13.8 h, this value of desorption will not lead to an additional dose load on the organism.

On studying the  $^{69m}\text{Zn}$  releasing from the sorbent HAP-Zn into the zinc nitrate solution, it was found that reverse isotope exchange is possible (Fig. 2). Within 1 day, 18–20% of  $^{69m}\text{Zn}$  is transferred from the sorbent into the mother liquor. The degree of reverse isotope exchange ( $F$ ) was estimated and found to reach 20–25% in 1 day.

For comparison, we characterized the sorption of inactive zinc on HAP-Zn (see Fig. 1), which also proceeded rapidly and reached a plateau ( $32.2 \text{ mg Zn (g HAP-Zn)}^{-1}$ ) in less than 20 min.

The parameters obtained by processing this kinetic curve using pseudo-first and pseudo-second order models are given in Table 1. In this case, the pseudo-first order equation is applicable for the description of adsorption only in those cases, where the rate of the entire process is limited by the rate of diffusion of the adsorbate from the solution to the interface. Obviously, the pseudo-second order model suits better the kinetics of sorption of zinc ions on HAP-Zn. This model assumes the presence of a "second component", *i.e.*, it includes interaction with the active site of the sorbent, for example, ion exchange. The correspondence of the obtained results to the pseudo-second order model is confirmed by the exchange mechanism of zinc sorption on HAP-Zn, which includes the substitution of calcium with zinc in the structure or on the surface of the sorbent.

The kinetics describing transfer of calcium from the sorbent into mother liquors during zinc sorption on HAP-Zn is shown in Fig. 3. There is a very rapid increase



**Fig. 3.** Kinetics of calcium release into mother liquors during zinc sorption on HAP-Zn;  $M(\text{Ca})$  is the amount of calcium liberated into the solution.

in calcium concentration in the mother liquor at the beginning of sorption, followed by a distinct fall, which can be explained by reverse sorption of calcium ions on HAP-Zn by a different mechanism.

A comparison of the kinetic patterns of zinc ion sorption on HAP-Zn in the presence of  $^{69m}\text{Zn}$  and without it showed that they are practically the same (see Fig. 1), and no additional contribution to drug labeling due to isotope exchange is observed (or it is insignificant). The reason may be that sorption on HAP-Zn proceeds more rapid ( $\sim 20 \text{ min}$ ) than on HAP ( $\sim 90 \text{ min}$ )<sup>2,5</sup> and the rate of isotope exchange low.

In conclusion, it was found that modified HAP-Zn, similar to a conventional HAP, almost irreversibly sorbs zinc ions from solutions. However, the rate of this process is much higher in our case, which is very important for binding the short-lived  $^{69m}\text{Zn}$ . The rate of isotope exchange is much lower than the rate of sorption, and, as a result, additional labeling does not take place. The sorption mechanism is mainly ion-exchange, specifically exchange with calcium, and the kinetics can be described by a pseudo-second order model. When using a sorbent such as this carrier of radionuclide  $^{69m}\text{Zn}$ , there are practically no losses due to the desorption of the radionuclide. However, the release of the radionuclide into the medium containing non-radioactive zinc ions due to reverse isotope exchange is possible, although its rate is low. It can be inferred that HAP-Zn is a promising carrier for the short-lived radionuclide  $^{69m}\text{Zn}$ , since it can bind the radionuclide

**Table 1.** Kinetic characteristics of zinc (in the form of a zinc nitrate solution) sorption on HAP-Zn, obtained on describing sorption using pseudo-first and pseudo-second order models

Order	$k$	$\Gamma_e/\text{mg Zn (g HAP-Zn)}^{-1}$	$R^2$
1	$1.17 \cdot 10^{-2} \text{ min}^{-1}$	32.2	0.8023
2	$0.42 \text{ g HAP-Zn (mg Zn)}^{-1} \text{ min}^{-1}$	32.3	1.0000

to the sorbent more rapidly than a conventional HAP, and losses of isotopes during desorption or isotope exchange are minimal, which is important for any radiopharmaceutical agent.

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This paper does not contain descriptions of studies on animals or humans.

The authors declare no competing interests.

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