

## SPECIAL ISSUE ARTICLE

# Direct monitoring of immediate release of Zn from zinc-doped bioactive glass

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## Funding information

European Union's Horizon 2020, Grant/Award Number: N°739566

## Abstract

Zinc-doped bioactive glass prepared by conventional melting was tested in simulated body fluid (SBF) to quantitatively determine zinc ion release at the early stage of dissolution of bioactive glass. The analysis of the leaching solution was performed in a flow-through arrangement, with the reaction cell connected directly to the nebulizer of the ICP OES. Possible formation of zinc silicate and zinc phosphate as alteration products precipitating from SBF solution along with hydroxyapatite was evaluated using the PHREEQC modeling code. Direct monitoring in the first 10-min interval of contact of the glass with SBF contributed to determination of the immediate quantity of zinc leached from the glass and confirmed burst release of zinc from material, with a maximum after ~4.5 min of contact with the SBF.

## KEYWORDS

dissolution, SBF solution, zinc-based glass, zinc stability

## 1 | INTRODUCTION

Zinc is a vital element that our body uses in countless ways. It is necessary for the activity of enzymes,<sup>1,2</sup> critical for the development and function of immune cells, and its supplementation decreases oxidative stress markers and generation of inflammatory cytokines.<sup>3</sup> Besides other positive aspects, zinc as an essential micronutrient distributed throughout the body, participates in cell division and growth.<sup>4</sup> When designing amorphous biomaterials for bone regeneration, it is therefore frequently selected as the ion with potential therapeutic effect, and incorporated into the structure of bioactive materials.<sup>5–7</sup> Release of zinc from a material ensures its delivery to proteins/enzymes to fulfill its desired therapeutic role. Expected higher

durability of zinc-containing bioactive glasses in aqueous solutions is usually insufficient to achieve stable release of Zn<sup>2+</sup> or to prevent complete dissolution of glass. The amount of Zn<sup>2+</sup> released from glass can thus achieve levels cytotoxic to human osteoblasts.<sup>8</sup> Therefore, determination of immediate concentrations of zinc ions is crucial, and selection of adequate test conditions needs to be carefully considered.

Generally, Zn<sup>2+</sup> cations in aqueous solution tend to be coordinated by six water molecules to form the hexaquo [Zn(H<sub>2</sub>O)<sub>6</sub>]<sup>2+</sup> and tetraquo [Zn(H<sub>2</sub>O)<sub>4</sub>]<sup>2+</sup> complexes. In natural aqueous systems, some anions can potentially act as ligands and bind to zinc to form other complexes.<sup>9</sup> Water-based solutions used in laboratory-designed experiments and simulating human plasma contain besides

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usual cations, such as  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , also chlorides, hydrogen carbonate ( $\text{HCO}_3^-$ ), hydrogen phosphate ( $\text{HPO}_4^{2-}$ ), and sulfate ( $\text{SO}_4^{2-}$ ) anions. Proper planning and execution of experiments is crucial for detection of the immediate or cumulative true amount of zinc ion released from material by quantitative analysis, especially when toxicity is considered. Static tests are most frequently used to determine the ion release during bioactivity studies of different bioactive materials.<sup>10,11</sup> However, testing under dynamic conditions provides more accurate picture about dissolution behavior of such material by minimizing the extent of precipitation reactions. Direct monitoring of concentrations of dissolved ions released from a glass in line with an analytical instrument was documented either in an acidic solution or a neutral solution only by a few authors.<sup>12–14</sup> Blochberger et al.<sup>14</sup> directly measured ion release profiles with the optical emission spectrometer with inductively coupled plasma (ICP OES) for Bioglass 45S5 model glass system, at low (pH 4) and in the TRIS solution. High Zn concentrations released from bioactive glasses have been proved to be cytotoxic in vitro: it is therefore important that the amount of Zn released from a biomaterial under physiological conditions simulating human plasma is sufficiently low.<sup>8</sup> To the best of our knowledge, there exist no papers dealing with direct measurement of zinc ion released from a bioactive glass to the solutions of simulated body fluid (SBF).

In this work, zinc-doped bioactive glass was tested under standard static conditions and dynamic conditions in the SBF. Release of calcium and phosphorus from such glasses with reported bioactivity and therapeutic effect were expected to trigger precipitation of hydroxyapatite from SBF. In this paper, neither bioactivity nor the mechanism of dissolution of zinc-containing glass will be discussed. Our primary interest was the identification of the test conditions under which both the immediate and cumulative zinc amount possibly released into the SBF solution can be reliably and quantitatively determined by elemental analysis. This work also draws attention to the fact that the incorporation of therapeutic ions into HAp or formation of other zinc-containing phases is generally not considered in discussing dissolution of zinc-based bioactive glasses.

## 2 | MATERIALS AND METHODS

### 2.1 | Glass doped with Zn

The core glass composition is well known under abbreviation 45S5. The glass contains (in mol%):  $\text{SiO}_2$  46.1,  $\text{P}_2\text{O}_5$  2.6,  $\text{CaO}$  25.6,  $\text{Na}_2\text{O}$  24.3, and it is considered less durable

in aqueous solutions. It was doped with 3 mol% of ZnO and prepared by conventional melt-quenching method. The following reagents of 99.0%–99.8% purity were used:  $\text{SiO}_2$ ,  $\text{CaCO}_3$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{Na}_5\text{P}_3\text{O}_{10}$ , and ZnO (Centralchem, Prague, Czech Republic). The reagents were mixed and homogenized for 1 h. The glass batch was gradually heated at the rate of  $10^\circ\text{C}/\text{min}$  to the temperature of  $1350^\circ\text{C}$  and melted for 2 h. The melt was periodically stirred to assure homogeneous mixing of all species. The glass melt was then poured onto a stainless steel plate, annealed at  $541^\circ\text{C}/1$  h, and then gradually cooled down to room temperature. The glass was then ground and sieved to the fraction  $\leq 25 \mu\text{m}$ . The glass was labeled as Zn-45S5 for presenting and discussing the results of ion release.

Durability of the Zn-containing glass powder (Zn-45S5) was tested under dynamic conditions and evaluated through release of ions from the glass structure and compared to ion release at static conditions.

### 2.2 | Characterization and test methods

#### 2.2.1 | Surface area

Nitrogen adsorption/desorption isotherms were measured (ASAP2020, Micromeritics ASAP 2020 Plus Physisorption, Norcross, GA, USA) at a temperature of  $-196^\circ\text{C}$ . Before the nitrogen adsorption/desorption measurements, each sample was degassed at  $100^\circ\text{C}$  for 3 h. The Brunauer–Emmett–Teller (BET) model was applied to determine the specific surface areas of the samples.

#### 2.2.2 | Static test conditions

Powder sample with particle size  $\leq 25 \mu\text{m}$  weighted 30 mg was tested in SBF freshly prepared according to the protocol of Kokubo and Takadama<sup>15</sup> under static and dynamic conditions at  $37^\circ\text{C} \pm 0.5^\circ\text{C}$ . Surface-to-volume ratio  $\sim 0.7 \text{ m}^{-1}$  was used for the static test. During the test, the sample was incubated with an agitation speed of 120 rpm, with sampling times of 0.5, 2, 4, and 24 h until 3 and 7 days. For each time point, the sample was in contact with SBF in closed vessel without removal or addition of fresh solution. After sampling, concentrated  $\text{HNO}_3$  was added to adjust pH to  $\leq 2$  to stabilize ions in dissolved form. For each sampling time, three parallels were measured. The same procedure was applied to *blank* sampling where no glass powder was in contact with SBF. Afterwards, the concentration of zinc and other released ions was measured by ICP OES spectrometry.

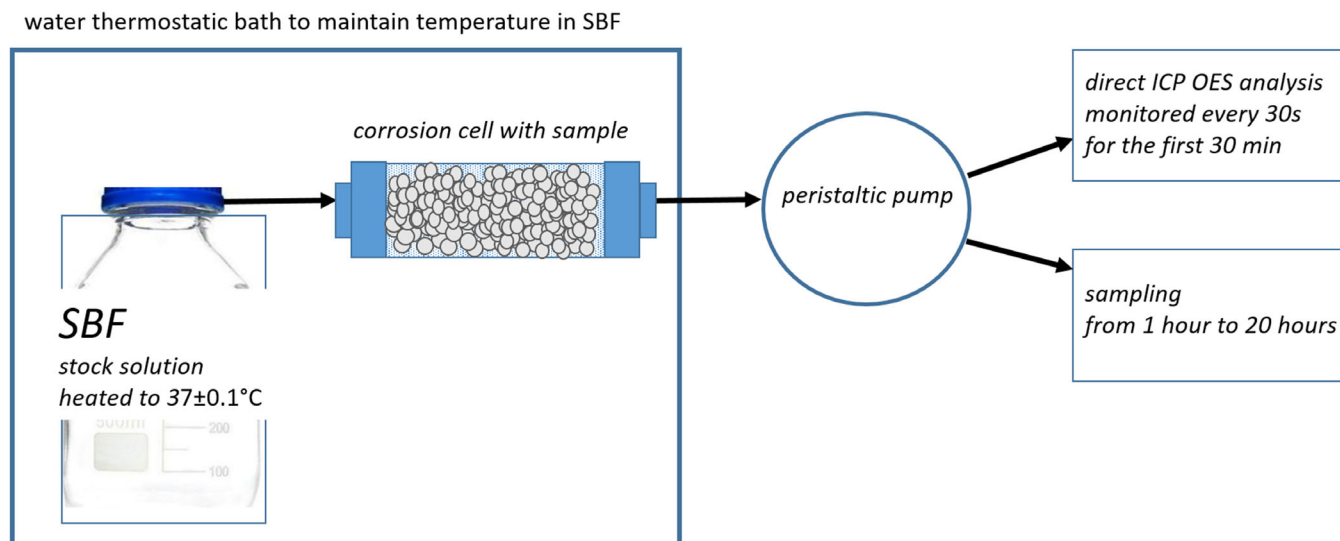


FIGURE 1 Scheme of dynamic test

### 2.2.3 | Dynamic test conditions

Under dynamic testing conditions (Figure 1), the aqueous solution continuously flowed through the sample placed in a corrosion cell with a volume of ~1.2 ml. The constant temperature  $37^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$  in the corrosion cell was maintained by tempering the stock SBF solution using thermostatic bath (Argolab WB12, Kushan, China). To detect the concentration of zinc ions reliably, the limit of quantification (LOQ) of the element had to be achieved or exceeded. This was achieved by adjusting the SBF flow rate to 0.3 ml/min to ensure laminar flow and sufficient retention time for interaction of glass with the solution and detectability of dissolved zinc ion on quantitative level. Considering that zinc-containing glasses are more durable than the undoped 45S5 glass, ~500 mg of the tested Zn-45S5 powder was used to measure the amount of dissolved zinc reliably at/or above the LOQ. The solution was sampled periodically every 30 s in the first 30 min, followed by sampling in 10-min intervals for the next 5 h, with final sampling after 20 h.

The reference 45S5-based bioactive glass is expected to be less durable when in contact with aqueous solutions. To obtain a complex picture and not to underestimate the maximum zinc quantity released from glass at the very beginning of the contact of glass with SBF (starting from the first minutes), an additional short-term dynamic test was also carried out.<sup>16</sup> During this test, the sampling tube was connected directly to nebulizer of the ICP OES to quantitatively monitor (referring to the calibration solutions of known concentration of zinc) the amount of dissolved zinc directly, starting from the first 3 min. The optimization and synchronization of the ICP OES instrument to dynamic test setup is in detail described earlier.<sup>16</sup>

### 2.2.4 | The conditions of the ICP OES measurement

The elemental analysis was carried out by ICP OES (Agilent 5100 SVDV, Agilent Technologies, Inc., USA) after stabilization of the solution with concentrated nitric acid to ~pH 2 prior the analysis. The results are expressed as the mean  $\pm$  standard deviation (SD) of zinc concentration in moles per liter. A series of four calibration solutions was prepared to obtain a linear correlation between the intensity of spectral line of a particular element and its concentration. The reference standards (Analytika, Prague, Czech Republic) certified for ICP techniques were diluted to prepare the stock calibration solutions. To deal with nonspectral interferences, the internal standardization technique with scandium was used. The precision of the analysis for all required ions expressed as RSD% was below 5%. Axial viewing of plasma was used to measure Zn, P, and Si concentrations in the SBF solution. Calcium concentration was determined using a radial view (8 mm). The ICP expert software (Agilent Technologies, Inc., USA) conditions were set to read the analytes' concentration every 30 s.

### 2.3 | PHREEQC modeling

The PHREEQC<sup>17</sup> software package was used to simulate processes possibly occurring in the studied system under static conditions, involving equilibrium and irreversible reactions. Equilibrium reactions for the calculations are defined by specifying a solution and a reactant (glass) put in the beaker. After complete dissolution of reactant in solution, solution composition changes and is expressed as the ion product (IAP). For each zinc-based phase in

the database, a solubility product constant ( $K$ -equilibrium constant) is defined, which is equal to the product of concentrations of the ions involved in the equilibrium, each exponentiated to the power of its stoichiometric coefficient in the equilibrium equation.<sup>17,18</sup> Possible relationship between the solution changes represented by IAP and  $K$  is specified by saturation indices ( $SI = IAP/K$ ). If  $SI$  is higher than zero, the solution is supersaturated with respect to a respective mineral. In case of  $SI < 0$ , the solution is undersaturated with respect to the mineral phase, and it is in equilibrium with the phase if the  $SI = 0$ . LLNL database was used for calculation of saturation indices and selection of mineral phases. The database uses thermodynamic data assembled by the Lawrence Livermore National Laboratory, which is the only available database containing the data for amorphous  $\text{SiO}_2$ . Jiříčka<sup>19</sup> used this geochemical code modeling to simulate the dissolution of silicate-based glasses and to predict the thermodynamically most stable phases precipitated from solution.

In this work, the preparation of SBF solution was simulated first. Then the amount of Zn-45S5 (Ca0.083-Na0.164-P0.034-Si0.149-Zn0.011-O0.560) as the input reactant was calculated, considering the maximum normalized released quantity of ion ( $nl_i$ ), using Equation (1), to simulate the dissolution of glass in modeled SBF:

$$nl_i = c_i V / x_i \quad (1)$$

where  $c_i$  is the concentration of a leached element  $i$  in  $\text{mg}/\text{dm}^3$ ,  $V$  is volume of SBF in  $\text{dm}^3$ , and  $x_i$  is the weight fraction of the released element  $i$  in the glass. The primary aim of using PHREEQC was to predict the formation of zinc-containing alteration products possibly precipitating from the SBF solution, which, to the best of our knowledge, cannot be determined by any single analytical method. All possible reactions of zinc with the anions from the SBF (chloride, phosphate, silicate, etc.) were allowed to possibly precipitate from the solution during the virtual experiment modeling static dissolution conditions.

### 3 | RESULTS

#### 3.1 | Identification of time interval of maximum zinc release based on static test results

The specific surface of powdered Zn-45S5 sample obtained from BET analysis was  $0.496 \text{ m}^2/\text{g}$ . The amount of powder corresponding to the total surface area of  $0.25 \text{ m}^2$  was

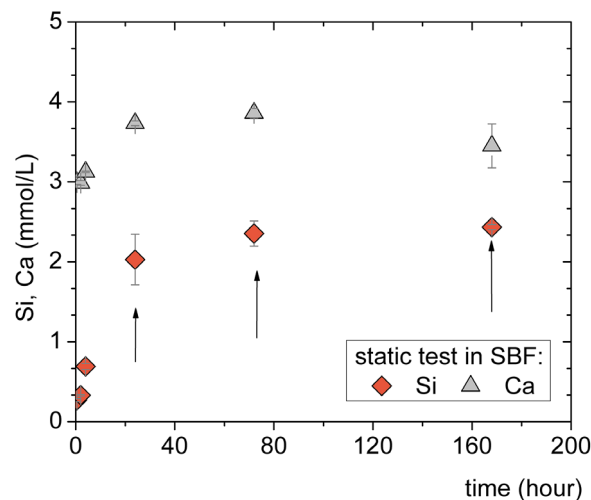
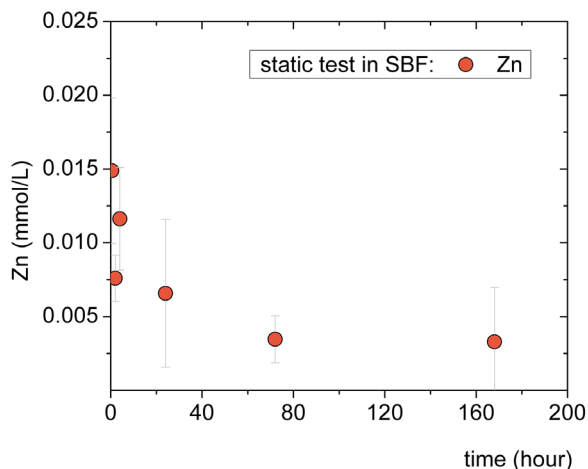


FIGURE 2 Silicon and calcium release from Zn-45S5 glass in simulated body fluid (SBF). The arrows indicate the steady-state ion release

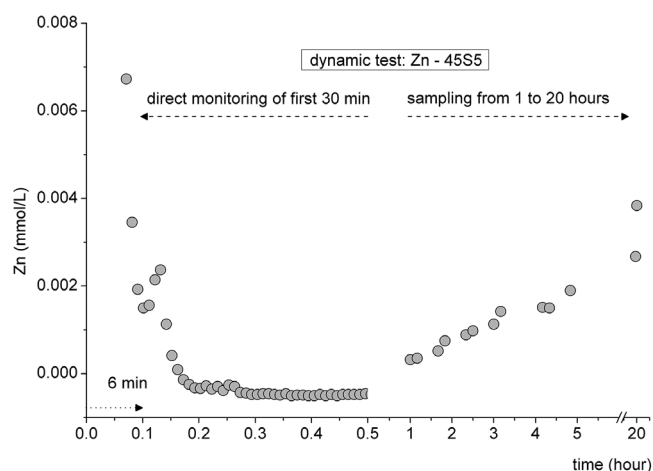
placed to the corrosion cell with the volume of 1.2 ml. Figure 2 shows the time dependences of concentrations of silicon and calcium leached from the Zn-45S5 glass into SBF and documenting the attack of glass structure by SBF and resulting in calcium and silicon release. The concentration of calcium at the end of the test time interval (~170 h) is higher than the concentration of calcium (2.34 mmol/L) determined in SBF at the beginning of the test. Phosphorus concentration measured in the first 4 h and ranging from 1.15 to 1.2 mmol/L was slightly (5%–9%) higher comparing to P quantified in the SBF before the test ( $1.10 \pm 0.02 \text{ mmol/L}$ ). After 24 h of immersion, P was depleted from the solution with the lowest concentration (0.19 mmol/L) achieved after 7 days. In the static test, a plateau for silicon and calcium release from the first day of the test is observed, which is a typical behavior for very reactive glasses.<sup>20</sup> In other words, steady-state release of Ca and Si is observed. The results of the static test also show a burst release of zinc after 30 min, which is then also followed by its steady-state release after 24 h (Figure 3). This observation motivated us to a more detailed investigation of the first 30-min time interval to determine and validate the true maximum amount of zinc, which can be possibly released from the tested glass. The SBF after partial dissolution of glass is assumed to be undersaturated with respect to all Zn-based phases. The probability of precipitation of various mineral phases using PHREEQC modeling code was done by allowing precipitation of all possible zinc-containing alteration products from SBF solution, including chlorides, phosphates, silicates, ZnO, and hydroxyapatite.



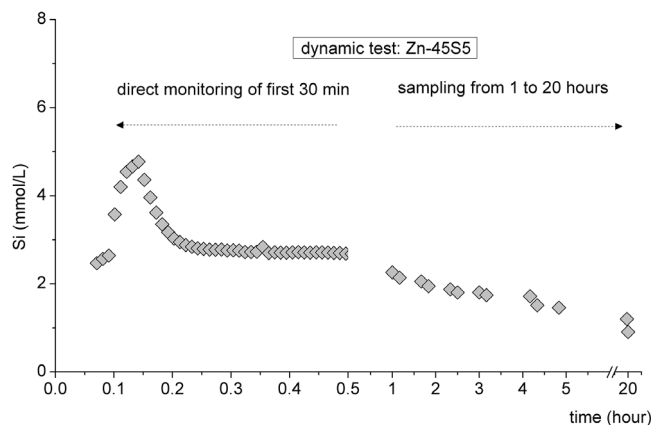
**FIGURE 3** Burst release of Zn at the beginning of the static test, followed by gradual decrease of Zn concentration at the latter stages of the test

### 3.2 | Determination of the immediate zinc amount released from Zn-doped glasses

Dynamic test conditions are advantageous for obtaining the information about actual/true detectable concentration ( $\geq$ LOQ) of dissolved ions in any particular time interval through eliminating or minimizing the oversaturation of solution, resulting in precipitation of new phases. It is expected that under dynamic conditions, the complexation of zinc ions, as well as the reactions with other ions will be partially eliminated. The initial period of 30 min was monitored by direct measuring of Si and Zn ions leached from the glass by ICP OES (Figures 4 and 5) together with calcium and phosphorus (Table 1). A burst release



**FIGURE 4** Direct monitoring of zinc release during 30 min in simulated body fluid (SBF) continued by determination of concentration profile of zinc up to 20 h



**FIGURE 5** Direct monitoring of silicon release during 30 min in simulated body fluid (SBF) continued by determination of concentration profile of silicon up to 20 h

of zinc was observed already from the first monitored minute, then suddenly decreased below the LOQ of ICP OES (0.06 mg/L). A sudden decrease from 0.007 mmol of Zn/L to the concentration  $<$ LOQ was observed within  $\sim$ 10 min of the test. Detectable quantity of zinc was determined again after 1 h of leaching with gradual increase until 20 h of the test (Figure 4). There are two possible explanations of the observation: either a layer with low permeability is formed on the surface, slowing down dissolution of the glass, or local oversaturation with respect to the new phase (zinc silicate, Figures 4 and 5) is achieved in the reaction cell. The respective ions are depleted from the solution and bound in a new phase, which might again trigger the dissolution of the glass.

The total quantity of released zinc 0.039 mmol/L was calculated as the cumulative value of zinc concentrations measured for the whole period of 20 h. Partial precipitation of silicate phases cannot be excluded, as partial depletion of Si from the solution was also observed, starting after 10 min of the test (Figure 5). In contrast to phosphorus, which was gradually depleted from the solution from the beginning of the test with the values below its initial concentration in SBF ( $1.10 \pm 0.02$  mmol/L), the experimentally determined concentration of calcium increased simultaneously with Si in the initial 8-min period (Table 1, measured values). The fact that Zn-45S5 glass is bio-active lead to the conclusion that Ca and P leached into the SBF in the first minutes, might trigger partial precipitation of the desired apatite phase.

### 3.3 | PHREEQC modeling

The experimental results indicate that zinc and silicon are depleted from the solution both under static and dynamic

**TABLE 1** Comparison of experimentally determined concentrations of dissolved ions with output results from PHREEQC modeling code together with SI of the mineral phases measured and calculated for Zn-45S5 glass dissolution

Time (min)	Reactant amount <sup>a</sup> (mol) Zn-45S5	Measured (mmol/L)				PHREEQC output results							
		Ca	Zn	P	Si	pH	mmol/L				SI		
							Ca	Zn	P	Si	HAp	Zn <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> ·2H <sub>2</sub> O	Zn <sub>2</sub> SiO <sub>4</sub>
4.5	1.9E-8	2.9	0.0067	0.48	2.5	7.5	2.6	0.00	1.0	0.1	8.0	0.2	0.9
5	3.5E-7	2.7	0.0035	0.62	2.6	9.0	3.2	0.08	1.2	1.1	13.8	4.5	8.7
7.5	5.3E-7	3.2	0.0021	0.19	4.5	9.2	3.5	0.12	1.4	1.6	14.6	4.7	9.6
8	1.3E-6	3.9	0.0023	0.18	4.7	9.7	4.9	0.31	2.0	4.1	16.5	4.5	11.1
8.5	2.0E-6	4.7	0.0011	0.16	4.8	10.0	6.0	0.47	2.4	6.2	17.5	3.8	11.5
9	2.5E-6	5.2	0.0004	0.18	4.4	10.3	7.0	0.59	2.8	7.9	18.1	2.9	11.9
9.5	2.9E-6	5.7	<LOQ	0.20	4.0	10.4	7.7	0.69	3.1	9.1	18.5	2.2	11.7
10	3.3E-6	6.1	<LOQ	0.20	3.6	10.6	8.3	0.78	3.4	10.3	18.9	1.5	11.7

Abbreviations: HAp, hydroxyapatite; SI, saturation indices.

<sup>a</sup>Dissolved glass.

conditions. Moreover, direct monitoring of concentration profiles of Zn, P, and Si revealed that the first 10 min of interaction of glass with SBF is crucial for clarification of possible precipitation of Zn- and Si-containing solid phases or any other Ca- and P-containing phases. Especially in the beginning of the test, the amounts of any precipitated phases are so low, that their experimental detection and identification is virtually impossible. For this reason, possible reaction products were identified with the use of the PHREEQC modeling. The amounts of Zn-45S5 glass reactant shown in Table 1 were input to PHREEQC simulated SBF and let to virtually react first to determine the concentration of Zn, P, Ca, and Si, at which the solution will be supersaturated with respect to zinc silicate and zinc phosphate (chlorides, sulfates, and carbonates were also included) formation. Apart from the hydroxyapatite phase, further discussion covers zinc silicates and zinc phosphates only, because they predominantly appeared in the output results for the zinc-containing phases. The experimental and calculated results are summarized in Table 1.

The saturation indices shown in Table 1 are in all cases higher than 0, indicating that after complete dissolution of the quantity of glass determined for the particular time interval,  $n_l = f(t)$ , SBF solution becomes supersaturated both toward hydroxyapatite (HAp), zinc phosphate ( $Zn_3(PO_4)_2 \cdot 2H_2O$ ), and zinc silicate ( $Zn_2SiO_4$ ). Further dissolution of glass can easily trigger their precipitation, resulting in significant depletion of Zn, Si, Ca, and P from the solution. Gradual decrease in measured concentration of ions (Zn and P) was observed already after 5 min of dynamic leaching, indicating that even under the conditions of dynamic test, zinc silicate and/or zinc phosphate may partially precipitate from the very beginning of the test. The concentrations of Zn determined experimentally

in the solution under dynamic conditions are lower than the equilibrium concentrations estimated by the PHREEQC modeling code, and in the latter stages of the experiment fall below the LOQ of the used experimental method. This indicates that the actual amount of Zn released from glass can be significantly underestimated. Although in static regime, the simulations indicate the probabilities of formation of various phases (based on their SI values), which under favorable conditions (oversaturation), can occur as secondary precipitation products. Lower SI values were calculated for zinc phosphate than for zinc silicate, indicating the reaction of phosphorus with calcium to form HAp is more favored.

## 4 | DISCUSSION

Stability of zinc ions dissolved in the simulated body solution buffered with Tris was evaluated. A weak Zn–ligand complex<sup>8</sup> can be formed in the solution containing 0.05 M of Tris, which can significantly influence the amounts of measured Zn in samples not stabilized by the addition of an acid to ~pH 2 and cause a deviation from the actual value of dissolved zinc species.

A static test was performed with Zn-45S5 glass immersed in SBF to determine the concentration–time profiles (dissolution curves) of respective ions. The results in Figures 3 and 4 indicate that Zn is leached preferentially, with a strong release at the very beginning of the test, followed by immediate decrease of its concentration. In the contrary, the leaching of Si is gradual, followed by a plateau in the latter stages of the static test. Based on these results, we can assume that silicon might trigger precipitation of zinc from SBF solution. Depletion of phosphorus from the solution after 24 h and reaction with calcium and  $OH^-$  ions might

trigger HAp formation, as documented previously.<sup>14,20,21</sup> An additional test was therefore performed under dynamic conditions in the initial 30-min interval for Zn-45S5 glass, with direct monitoring of the amounts of dissolved Si and Zn. An increase of Si concentration during the initial 10 min of the test (Figure 5) and a maximum of Zn concentration (0.0067 mmol/L) after 4.5 min (Figure 4) was observed. After that, the content of Zn decreased under the LOQ of the used analytical method, indicating possible chemical reaction with silicon and precipitation of Zn-containing phases from the solution. Chemical modeling of glass dissolution using PHREEQC code, dissolving the quantity of Zn-45S5 glass assessed from the experimental data, supports the hypothesis that zinc silicate phase might precipitate from SBF solution along with hydroxyapatite predominantly before formation of zinc phosphate. Based on the calculated SI values, these phases are favored over the formation of zinc phosphate. The data in Table 1 indicate that the actual amount of zinc released from glass is higher than the concentration measured by ICP OES, and the stage when solution is oversaturated with respect to  $Zn_2SiO_4$  and  $Zn_3(PO_4)_2 \cdot H_2O$  is readily achieved. In other words, precipitation of zinc-containing phases is very likely to occur. The hypothesis of preferential formation of zinc silicate is supported by the higher saturation indices of silicate compared to phosphate and can also be supported by the fact that the determined zinc concentrations in the acidified medium tests were higher than at the physiological pH.<sup>22</sup> Because in an acidic environment, Si is bound in the form of amorphous  $SiO_2$ , it is not available for the formation of zinc silicate.

These findings put in jeopardy the hitherto routinely used static tests used for evaluation of the amounts of therapeutic ions from bioactive glasses. Second, most authors report the amounts of the elements leached from the solution under static conditions after relatively long times, often days.<sup>22,21</sup> Our results document that under such conditions, the amount of released Zn can be severely underestimated. Zinc tends to be burst released in the first few minutes of exposure of glass to the SBF, quickly reaching concentration levels, which in combination with Si and P also release from the glass and/or available from SBF, can cause supersaturation with respect to formation of zinc silicate and zinc phosphate. Zinc silicate and zinc phosphate may thus precipitate from the solution, along with hydroxyapatite, although direct experimental evidence for its formation is missing. The glass powder sample partially/or fully dissolves under the applied test conditions and the amount required for X-ray diffraction powder analysis is usually not available after the test. The contents of Zn reported by other authors released from glass under static conditions, and measured after hours, even days, are then much lower than the actual contents of Zn released from

the glass matrix. In extreme cases, the amounts of therapeutic ions released from glass at the initial stage of the test may exceed toxicity level but stay undetected for the reasons outlined above. The extension of the conclusions obtained for Zn in this work to other ions with potential therapeutic effect will be the subject of further study.

## 5 | CONCLUSION

Zinc-based glass prepared by conventional melting was tested in SBF to evaluate the stability of zinc ions leached from bioactive glass. Direct monitoring of Zn content under dynamic test conditions confirmed burst release of zinc from material with a maximum after ~4.5 min. PHREEQC modeling code enabled a prediction of Zn-based mineral phase/s, which at certain concentration of released ions could precipitate from the solution. The modeling confirmed that the solution is supersaturated with respect to zinc silicate and zinc phosphate, and thus the release of silicon from the glass can trigger preferential formation of zinc silicate, along with hydroxyapatite. The in vitro testing of the release of bioactive glasses doped with ions with possible therapeutic ions can thus lead to significant underestimation of the actual amount of the therapeutic ions leached from the glass, even if the test is carried out under dynamic conditions.

## ACKNOWLEDGMENTS

This paper is a part of the dissemination activities of the project FunGlass. This project has received funding from the European Union's Horizon 2020 research and innovation program under Grant Agreement No. 739566 and is also created in the frame of the project Centre for Functional and Surface Functionalized Glass (CEGLASS), ITMS code is 313011R453, operational program research and innovation, co-funded from European Regional Development Fund. Financial support of this work by the project VEGA 1-0191/20 is gratefully acknowledged. The authors would like to thank Lukáš Münster and Jakub Ševčík from the Centre of Polymer Systems, Tomas Bata University, Zlín, Czech Republic for performing the BET analysis of the tested glass system and Martin Michálek from FunGlass centre, Alexander Dubček University of Trenčín, Slovakia for melting glass sample.

## CONFLICT OF INTEREST


The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

Conceptualization: Dagmar Galusková. Methodology: Dagmar Galusková and Hana Kaňková. Validation of data:

Dagmar Galusková and Hana Kaňková. Formal analysis: Hana Kaňková, Anna Švančárková, and Lenka Buňová. Investigation: Dagmar Galusková and Hana Kaňková. Data curation: Hana Kaňková, Anna Švančárková, and Lenka Buňová. PHREEQC calculations: Hana Kaňková. Writing the original draft: Dagmar Galusková. Reviewing and editing: Dagmar Galusková, Dušan Galusek, and Hana Kaňková. Visualization: Dagmar Galusková. Project administration: Dagmar Galusková and Dušan Galusek. Funding acquisition: Dagmar Galusková and Dušan Galusek. All authors have read and agreed to the published version of the manuscript.

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**How to cite this article:** Galusková D, Kaňková H, Švančárková A, Buňová L, Galusek D. Direct monitoring of immediate release of Zn from zinc-doped bioactive glass. *Int J Appl Glass Sci.* 2022;1–8. <https://doi.org/10.1111/ijag.16568>