Inline measurement of leachates from bioglass immersion tests

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The use of simulated body fluid (SBF) for bioactivity testing has exploded in bio community. The SB fluid was developed initially to evaluate surface structural changes of glass-ceramics used to manufacture artificial vertebrae, ileum, tooth roots, and bioactive material used to repair hard tissues such as artificial middle-ear bone and maxillofacial implants [1]. Variety of tests are performed with SBF solution including apatite-forming ability test, leaching or immersion tests. What do these tests have in common is evaluation of dissolved species, particularly ions transferred from immersed biomaterial into SBF solution [2]. The formation of apatite on a material dipped in SBF is proved on the basis of decrease of concentration of calcium and phosphate ions in the SBF. Except from Ca and P other elements can be present in solution depending on the chemical composition of biomaterial and kinetics of its dissolution. The bio-glass structure is composed from network formers: silicon, boron, phosphorus, as well as modifiers: alkali or alkaline earth elements. A variety of therapeutic ions e.g. Co, Cu, Sr, Zn or ions (Y, Ce) can be also incorporated into bioglass structure. Immersion tests reported in scientific literature usually rely on evaluation of the dissolution in only few time intervals, e.g 1, 3, 7 and 14 days, not taking into account the fact that the first hours of leaching often provide valuable information on leaching kinetics and represent the most important part of the dissolution curve. In this short communication, the experimental procedure for inline measurement of leachates from bioglass immersion tests is discussed and the limits of quantification for therapeutic ions in SBF solution are presented.

EXPERIMENTAL PART

For the *flow-through* test the thermostatic bath, corrosion cell, peristaltic pump and tubing were assembled and connected directly to the introduction system of the optical emission spectrometer (ICP OES, VARIAN MPX). Internal standard scandium (10 mg/L) was included in inline sampling in order to deal with non-spectral interferences. SBF buffer solution was prepared according to the protocol described by Kokubo and colleagues [2] at 37 °C and pH 7.4. Limit of detection for therapeutic ions together with silicon and boron, the elements not dissolved in SBF solution, was determined. Limit of quantification with precision calculated as RSD% was considered to be < 5%. Dissolution of the borosilicate glass prepared in FunGlass centre by Dr. Si Chen labelled as BG10B4Co was used for optimization of the experimental parameters of inline measurement of leachates. The pump rate and replicate time was considered and the following parameters were selected respectively: 10rpm (~0.7 mL/min) and 60 s. Inline tests were conducted with 500 mg of bioglass sample soaked in 10 mL volume cell, through which continuously flew fresh SBF solution at the temperature held constant at 37±1 °C. Concentration of Ca, P, B, Si and Co as function of time was directly monitored using ICPExpert software. The total amount of leached elements (*Q)* is determined by the following equation [3]:

$$Q_i^t = c_i \frac{F}{m} \Delta t + Q_i^{t-\Delta t} \qquad (1),$$

where c_i is concentration of particular leached element in time *t*, *F* is the flow rate. Values of the initial leaching rate may be evaluated from experimental time dependencies of Q_i.

RESULTS AND DISCUSSION

The reference SBF solution was scanned for 45 min period of time. Limits of quantification were calculated as 10x of the value of standard deviation from all recorded data (Table 1).

Table 1: *Limits of detection (LOD) and limits of quantification (LOQ) for selected elements in the simulated body fluid.*

element [nm]	B *249.772*	Co *228.615*	Cu *324.754*	Si *288.158*	Sr *216.596*	Sr *421.552*	Zn *202.548*
LOD	0.007	0.02	0.02	0.04	0.008	0.001	0.02
LOQ	**0.02**	**0.07**	**0.06**	**0.1**	**0.03**	**0.004**	**0.05**

The data from the flow through test of the Co-containing borosilicate bioglass 10B4Co were collected in the time interval of ~ 70 min. Chemical durability of selected bioglass was tested against SBF solution. Deionized water was used as the reference medium. Time dependence of released ions calculated according to equation (1) for deionized water and SBF solution is shown respectively, in the Fig. 1 and 2.

Fig. 1: *The total amount of leached elements from bioglass directly measured in deionized water.*

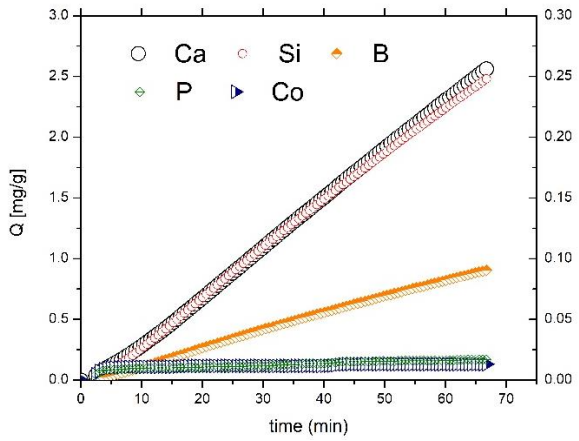
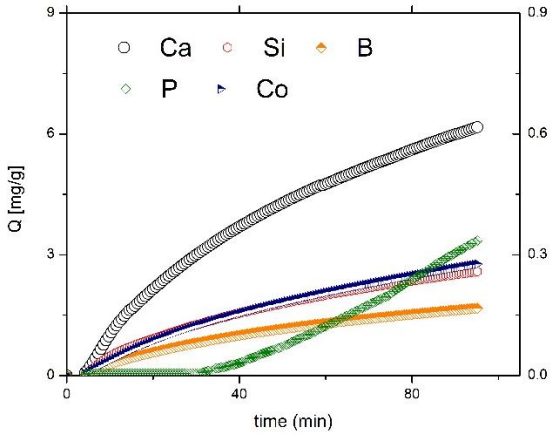


Fig. 2: *The total amount of leached elements from bioglass directly measured in SBF solution.*



The lowest detectable leached amount 10 and 5 mg/L for Ca and P, respectively, which differentiate with 5% confidence from the content of calcium and phosphorus in SBF was included in the calculations. For deionized water the kinetic performance is linear (Fig. 1), while for SBF solution nonlinear time dependence of total leached amount for Ca, B and Si are plotted in Fig. 2. Discussion related to the rate of dissolution and characterization of kinetic curves is beyond the scope of this article.

CONCLUSIONS

Inline measurement of the concentration profile for the *flow-through tests* was optimized for studying dissolution curves of borosilicate bioglass. Time dependent dissolution curves were plotted in order to study chemical durability of bioglass in deionized water as well in simulated body fluid solution. Limits of quantification with precision of less than 5% (RSD) were determined for analysis of therapeutic ions in SBF.

Keywords: bioglass, kinetics, SBF

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