

THE EFFECT OF TRIS-BUFFER ON THE LEACHING BEHAVIOUR OF BIOACTIVE GLASS-CERAMICS

JAN HLAVÁČ, DANA ROHANOVÁ, ALEŠ HELEBRANT

Department of Glass and Ceramics, University of Chemical Technology 166 28 Praha 6, Czech Republic

Received 13. 4. 1994

The leaching of bioactive glass-ceramics of the apatite-wollastonite type in water and in a simulated body fluid was studied using standard tests with granular specimens and exposures at 37 °C. The solutions were analyzed for Ca and SiO₂ and the evolution of both concentrations with time was examined in comparison with the CaO/SiO₂ ratio. A considerable increase in the rate of leaching was found in buffered solutions due to the effect of TRIS on the conditions for the deposition of a hydroxyapatite protective layer on the leached surface.

INTRODUCTION

Bioactive glasses and glass-ceramics can become attached to the bone tissue due to the formation of a joining layer of hydroxyapatite which results from a chemical reaction at the interface between the bone and the biomaterial. The blood plasma participates on the reaction mechanism as a transport medium and as a source of hydroxyapatite (HA) components – mainly calcium and phosphorus. The release of calcium ions into the body liquid by extraction from the biomaterial increases their concentration beyond the solubility limit and enables the precipitation and deposition of HA. For this reason the leaching behaviour of bioactive materials in a suitable simulated liquid has been studied in vitro by many authors (1–7).

During the leach tests, the changing pH values of the solution can affect the rate of the solid-liquid interaction. There are several possibilities how to eliminate that effect: 1) by periodical or continuous exchange of the leachant, 2) using pH stat titration technique (8, 9) or 3) using suitable buffers. The last method is the most simple and most common but involves the possibility of affecting the leaching rate by the chemical reactivity of the buffer (10, 11).

TRIS, i.e. tris(hydroxymethyl)aminomethane, has been frequently used as a buffer in studies of the leaching of bioactive glasses and glass-ceramics in solutions simulating the composition of blood plasma (simulated body fluid – SBF). The effect of TRIS on Si and Ca release from a glass-ceramic material was studied in this work.

EXPERIMENTAL

Leaching tests were performed with specimens of bioactive glass-ceramics supplied by Lasak Co*. The material was composed of apatite, wollastonite, whitlockite and the residual glass phase. The standard

*Lasak Co., producer of biomaterials, Prague 6, Papírenská 25

granular specimens were prepared of 0.315–0.50 mm grain size, equivalent to a surface area of approx. 110 cm²/g and exposed to the leaching attack of the simulated body fluid (SBF) having the following composition: 142,0 Na⁺, 5,0 K⁺, 1,5 Mg²⁺, 2,5 Ca²⁺, 148,0 Cl⁻, 4,2 HCO₃⁻ and 1,0 HPO₄²⁻ (mmol/l). TRIS was added to the solution in the amount of 7,47 g/l and pH of the solution was adjusted by addition of HCl to 7,45 (room temperature) or 7,25 (37 °C). All of the leaching exposures were carried out at 37 °C. The so-

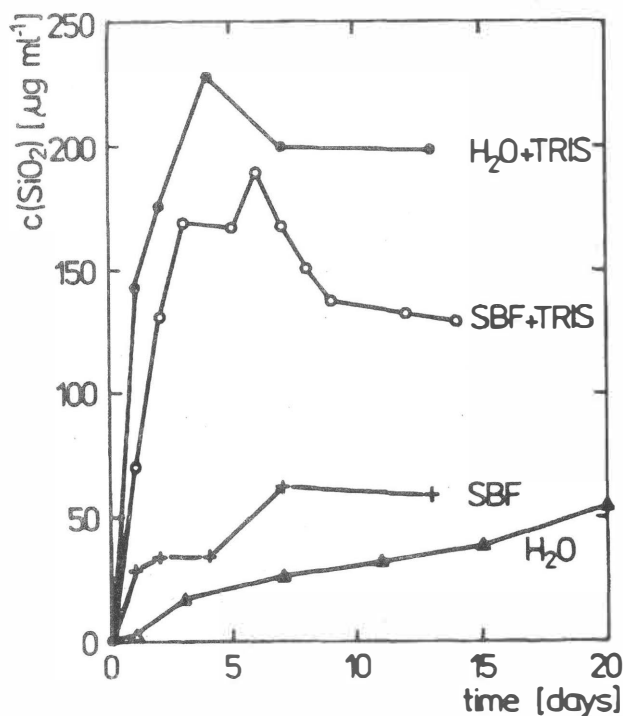


Fig. 1. SiO₂ concentration in the solution after leaching of the glass-ceramics in a buffered and a non-buffered SBF, and in distilled water.

lutions were then analyzed using AAS for Ca and the silicon-molybdate photometric method for SiO_2 .

Similar leaching experiments were performed in SBF without any buffer addition and in distilled water, again with or without the TRIS buffer. During all leaching exposures, the interaction of the specimens with the liquid occurred, under static conditions, with a surface to volume ratio of $1.1\text{--}1.5\text{ cm}^{-1}$. Besides the concentration changes, pH was measured after leaching in the resulting solutions.

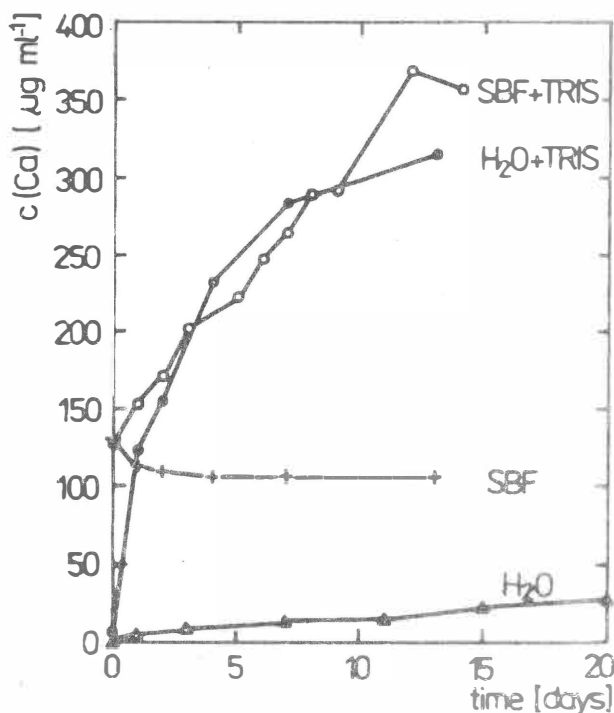


Fig. 2. Calcium concentration in the solution after leaching of the glass-ceramics in a buffered and a non-buffered SBF, and in distilled water.

RESULTS AND DISCUSSION

The silica concentrations in solution in terms of time are shown in Fig. 1. The results for distilled water depend very considerably on the presence of TRIS, the dissolution being more extensive in the buffered solution, although the pH was changing only within the range 7.45–8.1.

In the case of SBF, there was also a higher value of dissolved SiO_2 in the presence of TRIS, with an apparent trend to supersaturation. Both curves approached to a constant value which was higher about two times in the buffered solution.

The concentration change of Ca is shown in Fig. 2. The release of Ca into distilled water was higher by an order of magnitude in the presence of TRIS. Similarly, the release of Ca into buffered SBF was more

extensive and high values of Ca concentration in the solution were attained continuously, whereas in non-buffered SBF a lower constant concentration level was established. The original value of $\text{pH} = 7.45$ was increased to 8.0 and 8.5 in buffered and non-buffered SBF solutions, respectively.

According to common experience, the changes in pH between 7.5 and 8.5 cannot be considered to be a direct cause of the differences shown above; moreover, they should act in the opposite direction. The following hypothetical explanation can be offered, based on a widely accepted mechanism of leaching which was confirmed to be valid also for the glass-ceramics studied in the present paper (13):

During interaction of silicate materials with aqueous solutions a leached layer is formed on the surface by selective extraction of cations which are transferred from the material into the solution by diffusion through this layer of gradually increasing thickness. This process can be accompanied by deposition of a secondary film on the reaction surface from the supersaturated solution [12, 14]. The film then retards the diffusion as well as the surface dissolution of silica. The two compounds followed in this work (i.e. Ca and SiO_2) represent diffusing and dissolving species, respectively.

The following mechanism of TRIS involvement comes into consideration: TRIS is known as a compound forming complexes with various cations including Ca^{2+} . In the case of interaction between the bioactive material and SBF, calcium ions released from the material are strongly bound into a soluble complex so that they are not available for deposition of a surface layer exhibiting a protective effect.

Several aspects should be considered in this connection: the ratio of CaO to SiO_2 in the solution after leaching, the solubility product for hydroxyapatite, and finally, the amount of CaO which can be bound into the complex with TRIS.

The CaO/ SiO_2 weight ratio in the solution is shown in Fig. 3 where CaO is expressed as the change of its concentration in comparison with the actual one in the case of SBF. In non-buffered solutions, the CaO/ SiO_2 value is low and remains at an approximately constant level. A considerable increase of the ratio occurs in buffered solutions. The values of CaO/ SiO_2 indicate that congruent dissolution does not occur in the systems followed and that, consequently, depleted layers of different compositions are formed in different solutions. The permeability of these layers for diffusing species can also vary.

The following conclusions can be made from a comparison of the CaO/ SiO_2 ratio with the concentration changes of Ca and SiO_2 :

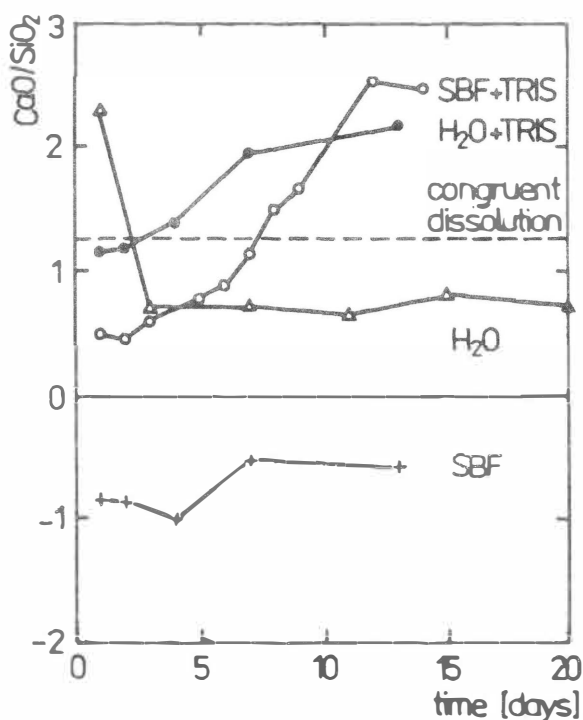


Fig. 3. The weight ratio of CaO to SiO₂ in the solutions after leaching (Δ CaO related to the original concentration in the solution).

- In distilled water (without TRIS), CaO remains chemically bound in the surface as calcium phosphate or silicate (the silica concentration in the solution increases whereas CaO/SiO₂ remains constant and the Ca concentration does not show any great change – see Figs. 1–3).
- In SBF without TRIS, the CaO/SiO₂ ratio has a negative, nearly constant value, probably as a result of the CaO consumed in HA formation. In agreement with this conclusion, the concentration of Ca decreases with time (see Fig. 2).
- The increase in the CaO/SiO₂ ratio in terms of time in TRIS-buffered solutions (in both distilled water and SBF) corresponds with the continuous increase of Ca (Fig. 2) and with the increase in silica content to a supersaturated value followed by a decrease to the final saturated limit (Fig. 1). A steep increase in both concentrations at the beginning of the interaction indicates that calcium ions preferably enter the complex and are not available for HA layer formation which would otherwise suppress the leaching.

The deposition of hydroxyapatite layer on the reaction surface represents an important process influencing the leaching rate in a way associated with pH of the solution: The rate of growth of HA layer is a function of relative supersaturation $S = (I/K)^{1/18}$

[15] where I is the ion activity product and K the solubility product in respect to hydroxyapatite. Assuming that calcium in SBF is in the form of Ca²⁺ only and $pK = 117.2$ [16], then at 37 °C ($pH = 7.3$), $pI = 94.38$ and $S = 18.5$. At $pH = 7.9$ the supersaturation is twice as high ($pI = 89.1$ and $S = 36.4$). Unfortunately there is a lack of equilibrium data on the TRIS-calcium complex but assuming that 50% of calcium is bound in the complex, supersaturation decreases to approx. 66% ($pI = 97.4$ and $S = 12.6$). This means that relatively small changes of the solution pH result in a considerable change of supersaturation and consequently, the HA layer growth is slower in the buffered solutions.

The results indicate that leaching of biomaterials in simulated liquids brings about some doubt concerning the reliability of leaching tests in vitro for conclusions to be made on the bioactivity of various materials in vivo. Moreover, the SBF used represents only the inorganic part of the blood plasma. In order to find out if albuminous compounds of the plasma can affect the leaching behaviour, experiments were carried out using the SBF enriched with albumin. The results shown in Fig. 4 indicate that the leaching behaviour of the material studied is very similar in both the SBF+TRIS and SBF+albumin solutions. Therefore a good prediction capability can be attributed to the

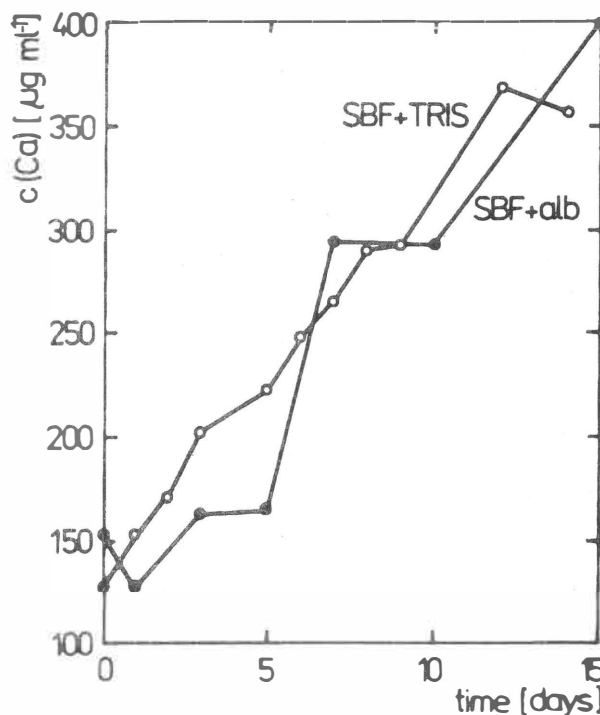


Fig. 4. Calcium ion concentration in the solution after leaching of the glass-ceramics in buffered SBF and in SBF + albumin.

tests described above, at least for the type of material examined in the present study.

CONCLUSIONS

The presence of TRIS-buffer in the solution can considerably increase the rate of leaching of bioactive glass-ceramics of the apatite-wollastonite type by aqueous solutions. The effect of TRIS is based on binding calcium ions into a soluble complex by a reaction competitive with HA formation. In this way, the buffer suppresses the deposition of a secondary film on the leached surface exhibiting a protective effect. At the same time, the buffer keeps the pH of the solution at a lower level which, owing to a strong dependence of supersaturation on pH, results in a reduced tendency of the solution to HA precipitation. Both effects act in the same direction, i.e. increase the intensity of leaching.

References

- [1] Weinstein A. M., Klawitter J. J., Cook S. D.: *J. Biomed. Mat. Res.* 14, 23 (1980).
- [2] Klein C. P. A. T., Driessen A. A., deGroot K.: *Biomaterials* 5, 157 (1984).
- [3] Hench L. L. et al.: *J. Biomed. Mat. Res.* 23, 685, (1989).
- [4] Kokubo T.: *J. Noncryst. Sol.* 120, 138, (1990); *Biomaterials* 12, 155 (1991).
- [5] Kokubo T., Ito S., Huang Z. T.: *Biomed. Mat. Res.* 24, 331 (1990).
- [6] Anderson O. H.: *The Bioactivity of Silicate Glass*. PhD Thesis, Dep. Chem. Eng., Abo Akad. Univ., Finland 1990.
- [7] Ducheyne P. et al.: *Bioceramics*, Vol. 4 p. 135. (Proceed. 4th Int. Symp. on Cer. in Med.), London 1991.
- [8] Mc Grail B. P., Pederson L. R., Peterson D. A.: *Ph. Chem. Gl.* 27, 59 (1986).
- [9] Pederson L. R. et al.: *Ph. Chem. Gl.* 34, 140 (1993).
- [10] Klein C. P. A. T. et al.: *Biomaterials* 11, 509 (1990).
- [11] Anderson O. H., Yrjas K. P., Karlsson K. H.: *Bioceramics*, Vol. 4, p. 127 (Proceed. 4th Int. Symp. on Cer in Med.), London 1991.
- [12] Ohura K. et al.: *J. Biomed. Mat. Res.* 25, 357 (1991).
- [13] Rohanová D.: PhD Thesis, Inst. Chem. Technol., Prague 1993.
- [14] Helebrant A., Tošnerová B.: *Glass Technol.* 30, 220 (1989).
- [15] Mann S., Webb J., Williams R. J. P.: *Biomaterialisation*. VCH Verlagsgesellschaft, Weinheim 1989.
- [16] Driessens F. C. M.: *Mineral Aspects of Dentistry*. S. Karger, Basel 1982.

Submitted in English by the authors

VLIV PUFRU TRIS NA LOUŽENÍ BIOAKTIVNÍ SKLOKERAMIKY

JAN HLAVÁČ, DANA ROHANOVÁ, ALEŠ HELEBRANT

Ústav skla a keramiky, Vysoká škola
chemicko-technologická Technická 5, 166 28 Praha 6

Bylo studováno loužení bioaktivní sklokeramiky apatit-wollastonitového typu ve vodě a v simulované tělní kapalině (SBF) za použití statických testů se standardní drtí, s expozicí na 37 °C. Ve výlužích byl pak stanoven obsah Ca^{2+} a SiO_2 a výpočtem poměr CaO/SiO_2 .

Byl nalezen podstatný rozdíl mezi výsledky získanými v nepufrovaných roztocích a v roztocích obsahujících pufr TRIS (tris-hydroxymethyl-aminomethan). Z výsledků plyne, že přítomnost TRISu může podstatně zvýšit rychlost loužení sledované sklokeramiky ve vodných roztocích (viz obr. 1–4). Vliv TRISu je přičítán tomu, že váže vyloužený vápník do rozpustného komplexu a tím omezuje tvorbu hydroxyapatitového povlaku. Současně se udržuje pH pufrovaného roztoku na nižší hodnotě, což vzhledem k silné závislosti součinu rozpustnosti pro hydroxyapatit rovněž redukuje tendenci roztoku k precipitaci HA. Oba tyto efekty zvyšují rychlost loužení tím, že omezují tvorbu vrstvy, jež má z hlediska vyluhování a rozpouštění ochranný účinek.

Obr. 1. Koncentrace SiO_2 ve výluhu ze sklokeramiky v pufrovaném a nepufrovaném SBF, a v destilované vodě.

Obr. 2. Koncentrace vápníku ve výluhu v pufrovaném a nepufrovaném SBF, a v destil. vodě.

Obr. 3. Hmotnostní poměr CaO:SiO_2 ve výluhu (obsah CaO uveden jako rozdíl od původní koncentrace v roztoku).

Obr. 4. Koncentrace vápenatých iontů ve výluhu v pufrovaném SBF a v SBF s přísadkou albuminu.