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Study of yttrium containing bioactive glasses behaviour in simulated body fluid

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Abstract The influence of yttrium oxide on the bioactivity of glasses in the system SiO_2-Na_2O-P_2O_5-CaO-B_2O_3-K_2O-MgO was studied in a simulated body fluid (SBF). Two series of glasses with different bioactivity were investigated. The reaction layers formed on the surface of the exposed glasses were evaluated by means of back scattered electron imaging of scanning electron microscopy equipped with energy dispersive X-ray analysis (BEI-SEM/EDXA).

The concentration of Y, Ca and P released from the glasses into SBF, during 21 days was determined using inductively coupled plasma-emission spectroscopy ICP-AES and inductively coupled plasma-mass spectroscopy ICP-MS.

Introducing yttrium in the selected bioactive glass tended to diminish the bioactivity of the glasses. The thickness of the calcium phosphate layer decreased with increasing yttrium oxide content. The same effect was also observed when yttrium oxide partially replaced only calcium, magnesium and phosphorous oxide in the precursor glass.

The data show that we can produce bioactive glasses with yttrium oxide as a component. By suitable tailoring of the rest of the glasses the yttrium effect on the glass behavior in SBF should be possible to control and thus produce yttrium containing glasses with desired bioactivity.

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Introduction

Since discovery by Hench in 1969 [1], bioactive glasses have been intensively investigated due to their successful use in various clinical applications. The unique feature of bioactive glasses is their chemical and biological *in vivo* activity [2]. *In vitro*, the bioactivity of a glass can be shown by the formation of a calcium phosphate rich layer on the glass surface when the glass is exposed to physiological fluids [3, 4, 5].

Biodegradable glasses containing neutron activable cations have also been investigated for applications in the *in situ* radiotherapy of cancer [6, 7]. Internal radionuclide therapy using ^{90}Y labelled microspheres represents an alternative for cancer treatment especially for cancers where the response to chemotherapy and external radiotherapy is poor like liver cancer [8, 9].

The aim of internal radionuclide therapy is to achieve the delivery of maximal radiation to the tumour, while minimising irradiation of surrounding tissues. This can be obtained by direct injection of a radionuclide into a tumour cavity or into the artery supplying a tumour. Glass microspheres are attractive candidates for carrying the radionuclide in the tumour for delivery of high doses of radiation [10]. Since the tumours are irradiated *in situ* high localized beta radiation replaces the more penetrating gamma radiation of external beam treatment systems. Currently, both glass and resin based microspheres with radioactive yttrium are applied for treatment of liver metastases and beneficial effects are reported [11–16].

The non-biodegradable radioactive glass microspheres remain as impurities in the target area after the radioactivity decays [16]. However, dissolution and elimination of the glasses from the body after their deactivation would be desirable.

The present study proposes the manufacture of biodegradable glass microspheres incorporated with yttrium potentially

useful for radionuclide therapy of cancer. For the study, five experimental bioactive glasses and two bioactive glasses with documented *in vitro* and *in vivo* activity [2, 3] were used. The bioactivity of the glasses and the effects of the yttrium oxide on their bioactivity were studied *in vitro* by immersing them in a simulated body fluid (SBF) [2]. The chemical durability of the glasses was investigated under *in vitro* conditions and the concentration of yttrium released from the glass was determined.

Experimental

For the study, seven different bioactive glasses (Ylänen et al.) were manufactured, two of them HB1 and LB1 showing documented *in vitro* and *in vivo* bioactivity. Yttrium containing glasses were manufactured by adding different amount of yttrium oxide to the oxide mixture and by partially substitution of only calcium, magnesium and phosphorus oxide by yttrium oxide (LYS sample). The compositions of the yttrium containing glasses and the host bioactive glasses are given in Table 1 and Table 2 (H series—glasses with high bioactivity, and L series—glasses with low bioactivity). The two bioactive glass series, with high and low bioactivity, were chosen in order to obtain glass samples with different bioactivity and biodegradation rate.

The glasses were prepared by mixing analytical grades of Na_2CO_3, K_2CO_3, MgO, $CaCO_3$, $CaHPO_4(2\,H_2O)$, Y_2O_3 and commercial Belgian quartz sand and melting the batches in a Pt-crucible at 1360°C for 3 hours. For the glasses with high yttrium oxide content the melting temperature was 1450°C.

The glasses were cast, annealed, crushed and remelted to improve homogeneity.

The bioactivity tests were carried out by immersing glass plates in a simulated body fluid (SBF) with a pH close to the body fluid value [2]. For each sample two rectangular pieces ($3.18 \times 0.9 \times 0.15$) cm were immersed in SBF at 37°C for 72 hours in order to evaluate the thickness of the reaction layers formed on their surface. The plates were placed in a polystyrene container with 17 ml SBF that led to a ratio between glass surface area (SA) and SBF volume (V), SA/V= 0.4 cm^{-1} [17]. After 72 hours the samples were taken out from the fluid, rinsed with millipore water and ethanol, dried and embedded in acrylic resin. After hardening the samples were sectioned and polished. The polished surface was coated with a thin layer of carbon for scanning electron microscopy BEI-SEM/EDXA.

The thickness of the reaction layers formed on the host bioactive glasses and on the glasses containing yttrium was measured by means of BEI-SEM. All measurements were performed on six randomly selected locations of each sample. EDX analysis was used for the elemental identification of the reaction layers formed on the glass surface.

In vitro chemical durability tests were carried out by immersing glass powder with powder particle diameters less than 45 μm in SBF for up to 21 days at 37°C. The solutions

Table 1 Composition of the yttrium containing bioactive glasses (% wt).

Glass	SiO_2	Na_2O	P_2O_5	CaO	B_2O_3	K_2O	MgO	Y_2O_3
HY1	48.15	5.45	3.64	18.18	0	10.91	4.55	9.09
HY1.1	46.09	5.22	3.48	17.39	0	10.43	4.35	13.04
HY2	43.91	13.04	0.87	15.22	0.87	13.04	0	13.04
HY3	43.91	17.39	0	15.22	0	6.52	3.91	13.04
HY4	44.35	21.74	0	13.04	2.61	0	5.22	13.04
LY1	46.09	5.22	1.74	19.13	0.87	9.57	4.35	13.04
LY2	51.96	4.35	0	13.04	2.61	9.78	5.22	13.04
LY3	51.74	4.35	0	21.74	0	6.52	2.61	13.04
LY2.1	47.8	4	0	12	2.40	9	4.8	20
LYS	53	6	2	10	0	12	2	15

Table 2 Composition of the host bioactive glasses (% wt).

Glass	SiO_2	Na_2O	P_2O_5	CaO	B_2O_3	K_2O	MgO
HB1	53	6	4	20	0	12	5
HB2	50.5	12	1	17.5	1	15	0
HB3	50.5	20	0	17.5	0	7.5	4.5
HB4	51	25	0	15	3	0	6
LB1	53	6	2	22	1	11	5
LB2	59.75	5	0	15	3	11.25	6
LB3	59.5	5	0	25	0	7.5	3

were stirred four times in a day without exchange of the solutions. Taking into account that the ratio of exposed surface to the volume of the solution influences the reactions, both for glass pieces and glass powder the ratio SA/V was kept close to 0.4 cm^{-1}.

After separating the powder from the solution, the powder was rinsed, dried and embedded in acrylic resin for analysing by BEI-SEM/EDXA.

After each immersion stage the concentration of Y, Ca and P ions in the immersion solutions was measured using an inductively coupled plasma emission spectrometer (ICP-AES). The yttrium released into the immersion solutions was also analysed by ICP-MS. The variation of the Ca and P concentration was calculated as the difference (Δc) between the concentration of the element as found soluble in the immersion solution after the exposure and the concentration of the element in the initial SBF.

The pH of the solutions was measured with an electronic pH-meter.

The *in vitro* dissolution stability was investigated on glass grains with 315-500 μm diameter [18]. 200 mg of glass grains were immersed in 25 ml SBF for up to 14 days at 37°C. The procedure was similar to that used for powder samples. The relative weight loss of the samples in SBF was determined as a ratio between the measured mass change of the glass after the time t and the initial mass of the glass grains.

Results

The BEI-SEM analyses revealed two reaction layers formed on the surface of the host bioactive glasses after 3 days immersion in SBF at 37 °C. Figure 1 shows the BEI-SEM results for the bioactive glass HB1. According to EDXA spectra the grey thick layer on the glass surface (spot 2) consisted of a silica rich layer and the uppermost white layer is an image of calcium phosphate (spot 1).

While on the bioactive glasses two reaction layers of different composition were identified, for the glasses containing yttrium only one reaction layer was formed on the glass surface after 3 days of immersion in SBF. This reaction layer is a calcium phosphate layer rich in yttrium, confirmed by EDX analysis (Fig. 2). Figure 2 shows the BEI-SEM micrograph and EDX analysis for the HY1.1 sample obtained by adding yttrium oxide to the HB1 bioactive glass composition.

Si rich layer was not detected on yttrium containing glasses. However, a layer consisting of a mixture containing both silica and calcium phosphate was identified on one

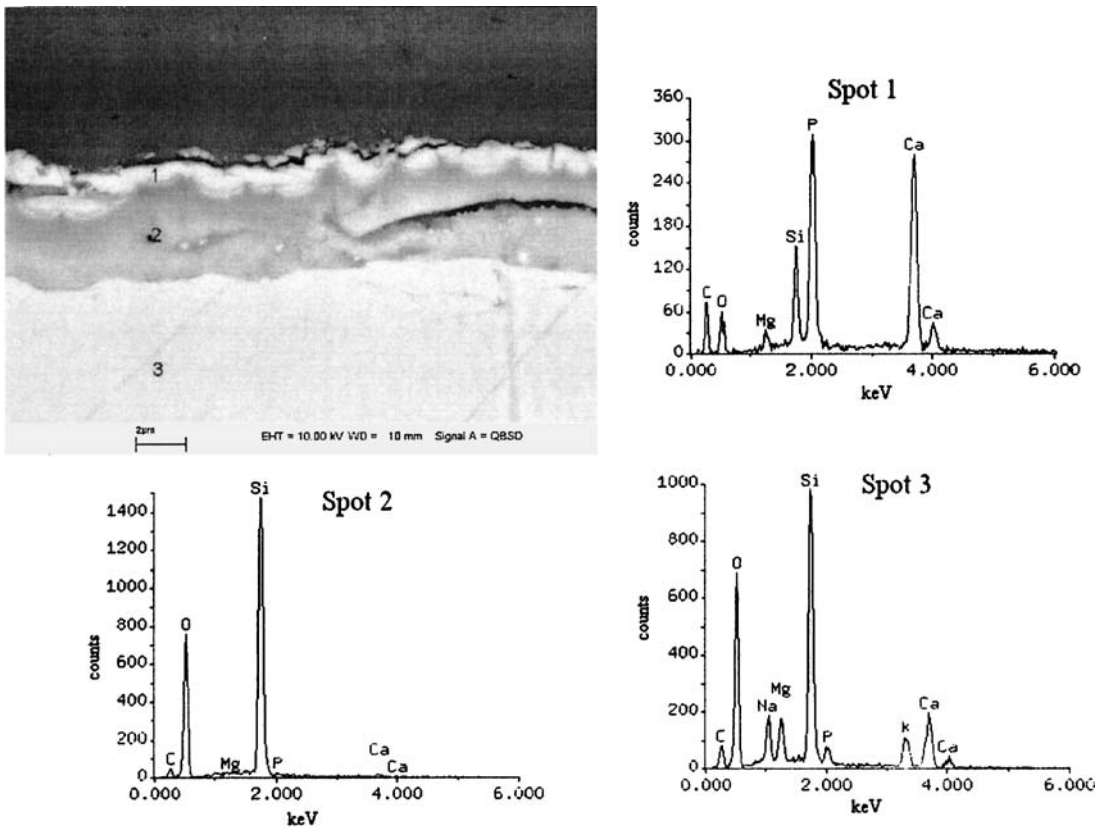


Fig. 1 BEI-SEM micrograph and EDXA spectra for HB1 glass after 3 days immersion in SBF. According to EDXA: spot 1—CaP layer, spot 2—Si-rich layer, spot 3—glass.

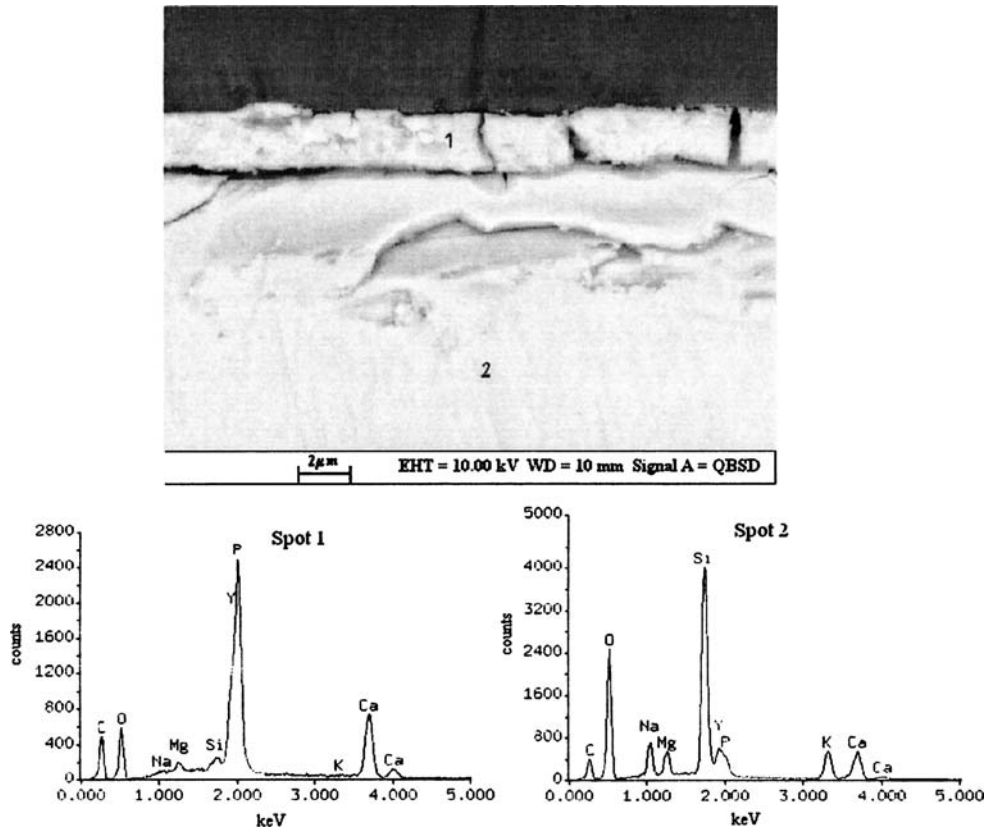


Fig. 2 BEI-SEM micrograph and EDXA spectra for HY1.1 glass after 3 days immersion in SBF. According to EDXA: spot 1—CaP layer, spot 2—glass.

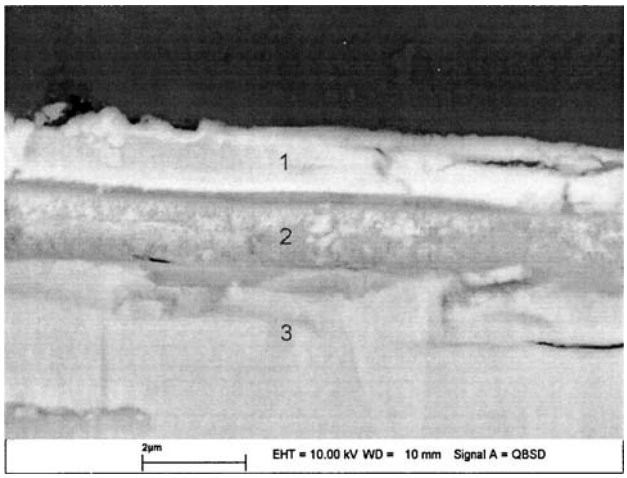


Fig. 3 BEI-SEM micrograph for HY2 glass after 3 days immersion in SBF. According to EDXA: spot 1—CaP layer, spot 2—mixture of Si and CaP layer, spot 3—glass.

of the yttrium containing glasses HY2 (Fig. 3). This layer was more clearly observed on the glass powder immersed in SBF for up to 21 days (Fig. 4).

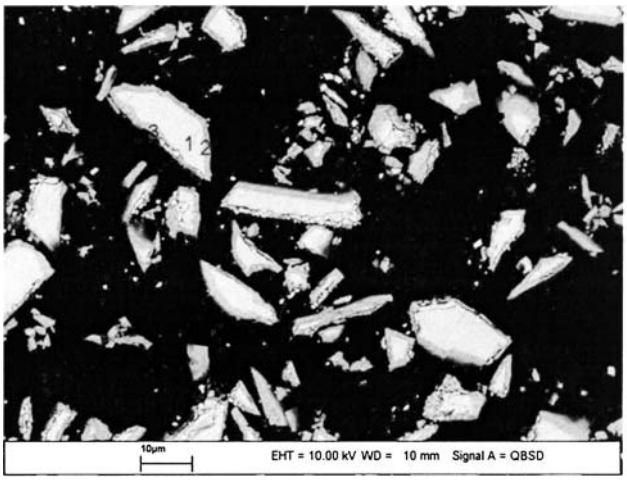
Calcium phosphate layer formed after 3 days on the surface of all samples (Fig. 5) except the glass with the highest yttrium content. After 7 days, calcium phosphate layer was developed also on the glass with the highest yttrium content. The tendency to form a calcium phosphate layer is retarded by a progressive addition of Y_2O_3 to the reference composition. The substitution of calcium, magnesium and phosphorous oxide by Y_2O_3, in LYS glass also influences the bioactivity of the glass but it doesn't suppress the calcium phosphate layer formation.

On the glasses of H series (Table 1) a thicker calcium phosphate layer can be observed as compared with the glasses of L series (Fig. 5). The thickness of the calcium phosphate layer formed on the yttrium containing glasses is thicker than on the host bioactive glasses.

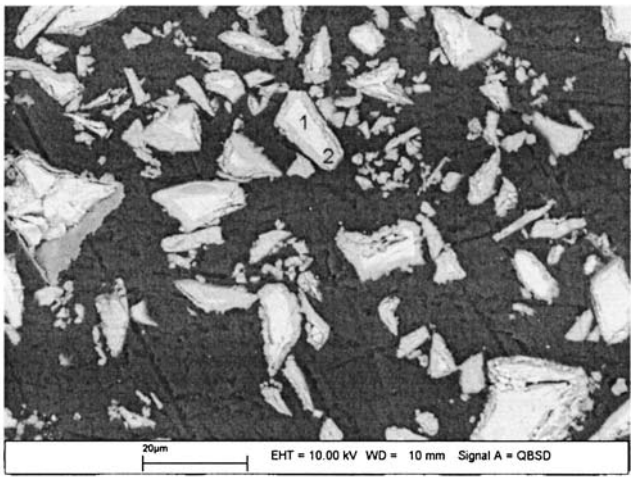
The time dependence of the relative weight loss for yttrium containing glasses is illustrated in Figure 6. Not surprisingly, the glasses with high bioactivity show faster solubility when compared to the glasses with low bioactivity. The lowest weight loss was found for the glass with the highest yttrium content. The weight loss occurs fast during the first stage of the immersion but after 7 days the rate of the weight loss is decreased slightly.

The pH measurements (Fig. 7) show an increase in the first 7 days. After 7 days no significant changes in the pH value was recorded.

The weight loss measurements do not reveal the material or ions species leached from glass into the solution or whether the glasses dissolve uniformly [10]. For *in vivo* use of radiotherapeutic glasses it is very important that there



(a)



(b)

Fig. 4 BEI-SEM micrograph for the HY1.1 glass powder after: (a) 3 days immersion in SBF. According to EDXA: spot 1—glass, spot 2—mixture of Si and CaP layer, spot 3—CaP layer. (b) 21 days immersion in SBF. According to EDXA: spot 1—glass, spot 2—CaP layer.

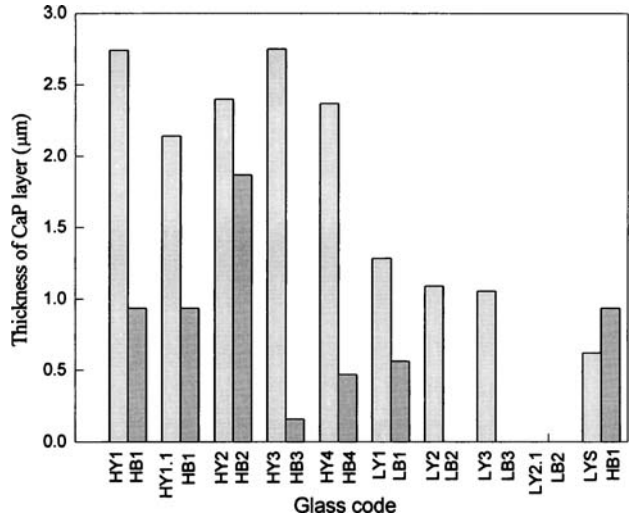
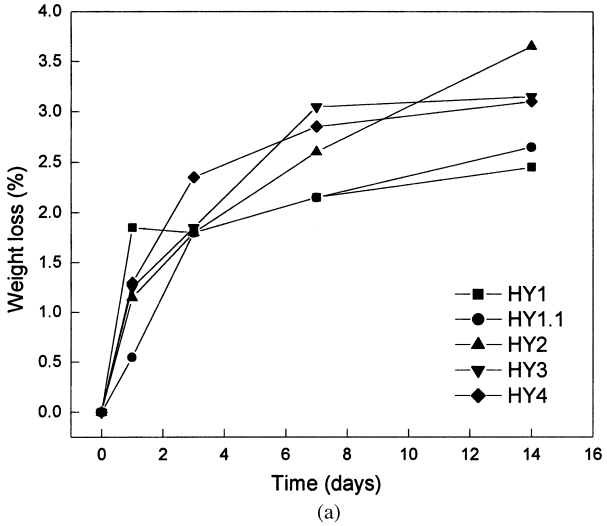
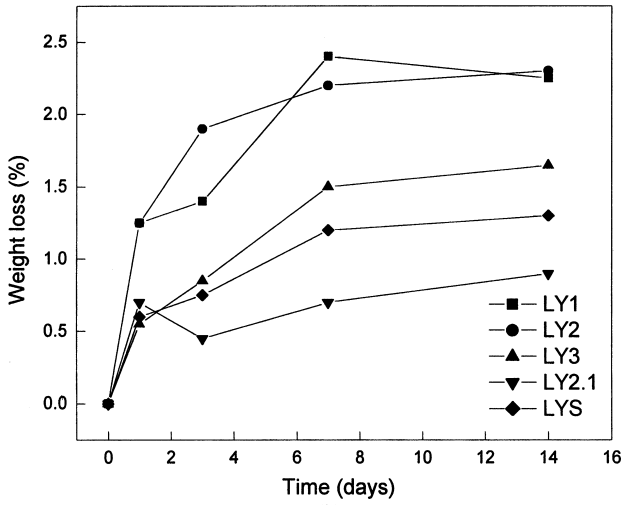


Fig. 5 The thickness of CaP layer for host bioactive (black) and Y containing glasses (grey), after 3 days immersion in SBF.



(a)



(b)

Fig. 6 Time dependence of weight loss: (a) H series and (b) L series. (The solid line is only a guide for the eye.)

is no mobilization of the radionuclides from the glass matrix. For the investigated samples the interest was focused on the neutron activable yttrium. Elemental analysis of the solutions collected from the glass powder was made by ICP-AES.

Figure 8 illustrates the variation of the Ca and P concentration versus the immersion time for two glass samples belonging to H series and two glass samples belonging to the L series. The concentration of P in SBF significantly decreases with the growth of calcium phosphate layer, while the concentration of Ca increases during the first stage of dissolution. The amount of Ca released from the glasses with high bioactivity is greater than that from the glasses with low bioactivity.

The Ca concentration in the solution collected from the glass with 15 and 20% Y_2O_3 was lower than the initial concentration of Ca in SBF. This could be assigned to the

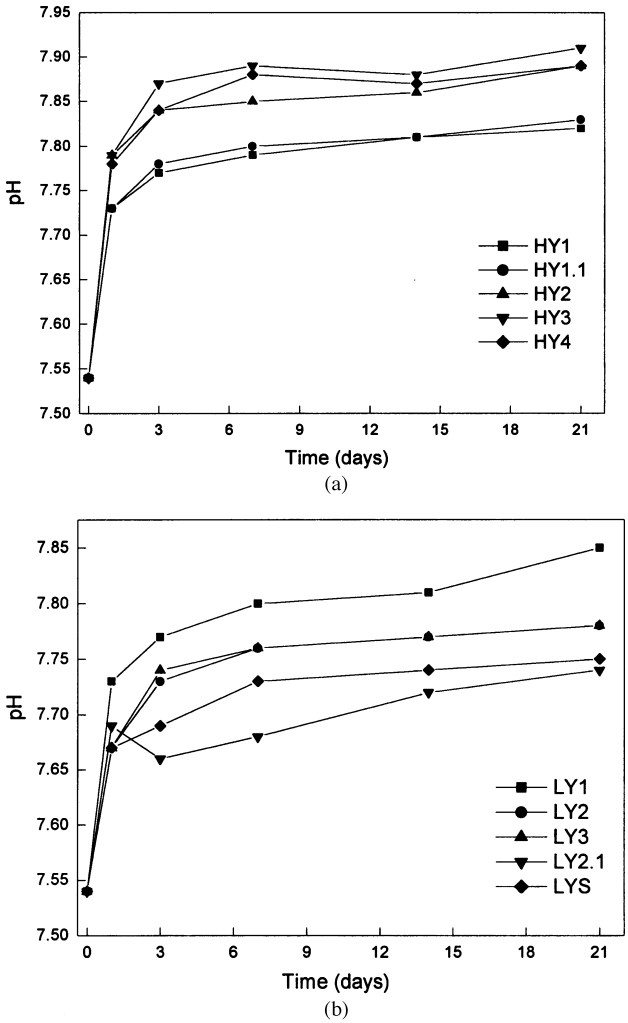


Fig. 7 pH values of the corrosion solution (glass powder) versus the immersion time: (a) H series and (b) L series. (The solid line is only a guide for the eye.)

precipitation of calcium and phosphorous from SBF on the glass surface. The immersion solutions do not contain detectable amounts of yttrium. The ICP-AES method indicates that these are less than 0.03 mg/1 after up to 21 days immersion time. The levels of the yttrium in the SBF are summarised in Table 3.

Discussion

The bioactivity of bioactive glass is characterised by the rate of calcium phosphate formation on their surface. It can be shown *in vitro* by exposing the glass in a simulated body fluid. The calcium phosphate rich layer is formed on the glasses surface through precipitation of Ca^{2+} and $PO_4{}^{3-}$ ions leached from the glass and those originating in the SBF solution. A detailed description of the reactions occurring on the bioactive glass under *in vitro* and *in vivo* conditions was given by Hench and Wilson [4]. According to their results, the initial stage is a dealkalisation of the glass surface, which results in the formation of a silica gel layer. Due to the diffusion of calcium and phosphorus from the glass mass onto its surface a calcium phosphate layer starts to build up.

Both thickness, growth rate and dissolution rate are depended on the glass composition, from of the glass body and, if crushed glass, the particle size. The glass degradation process is influenced by pH value, stirring rate, and S_A/V ratio [3, 19, 20].

With respect to the composition and thickness of the reaction layers there is a significant difference in the reaction layers on the host bioactive glasses and on the yttrium containing glasses. The components leached from glasses may react with the components in the SBF. One observes that the addition of yttrium to the bioactive glasses blocks the development of a Si rich layer. By increasing the Y_2O_3 content in the glass, the thickness of the calcium phosphate layer is reduced. The greater the Y_2O_3 content the thinner the calcium phosphate layer suggesting lower bioactivity of the glass. On the other hand the thickness of the calcium phosphate layer is not an absolute indicator for the bioactivity of a glass, as can be observed in the case of host bioactive glasses. The change in the chemical composition of the SBF was used as a bioactivity indicator of yttrium containing glasses.

The data obtained from the solution analysis point out a very small release of yttrium in SBF, less than 0.03 mg/l after 21 days (the half life time of radioactive yttrium is around 2.6 days). The low concentration of yttrium in SBF indicates a very low mobility of neutron activable yttrium from the glass and reaction layers during the immersion time that supports the idea of using bioactive glasses for radiotherapeutic glasses.

The concentration of P diminished due to the migration of P onto the glass surface to form a calcium phosphate layer. The evolution of the cations concentration in SBF obtained from ICP-AES measurements is in good agreement with the development of calcium phosphate layer and with the pH value of SBF.

For immersion times up to 14 days the yttrium containing glasses from the high bioactivity series exhibit a higher weight loss than those from the low bioactivity series. An increase in the amount of Y_2O_3 resulted in lower weight loss of all the glasses. This behaviour suggests an increase in the structural stability of the yttrium containing glasses. This may be explained by the field strength of the Y^{3+} ion, which is higher than the field strength of the other glass network modifier cations entering the glass [21–23]. In Table 4 the radius [22] and the ionic field strength of the network modifier cations are reported.

The increase of the pH value of the SBF during the dissolution tests can be assigned to the cations release from glasses

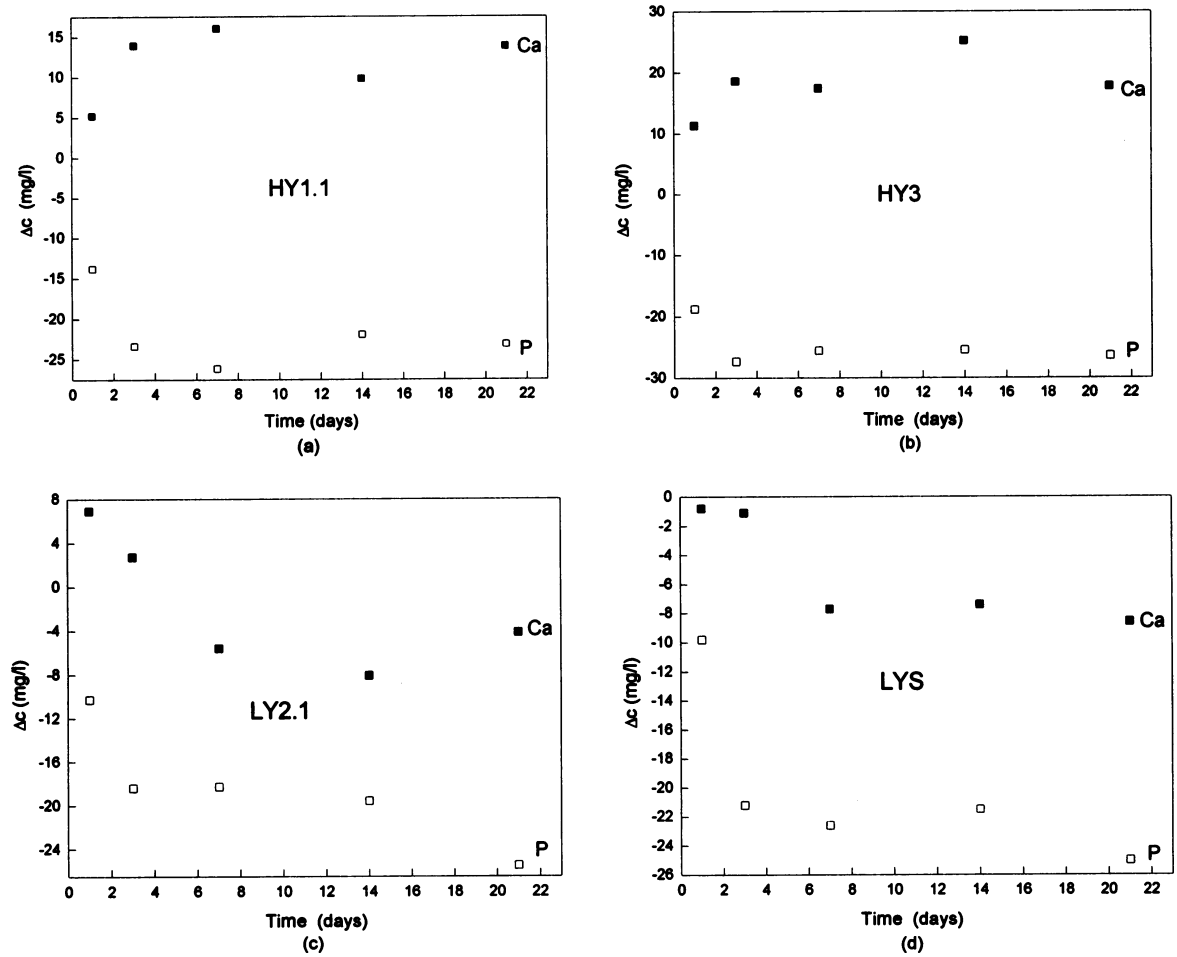


Fig. 8 P and Ca concentrations variation in the corrosion solution (glass powder) versus immersion time: (a, b) H series and (c, d) L series.

Table 3 Yttrium concentration (mg/l) in SBF after different immersion times.

Glass/Time	1d	3d	7d	14d	21 d
HY1	<0.01	<0.01	<0.01	<0.02	1.10^{-5}*
HY1.1	<0.03	<0.01	18.10^{-5}*	<0.01	4.10^{-6}*
HY2	<0.03	<0.01	<0.01	1.10^{-6}*	<0.01
HY3	<0.03	<0.01	<0.01	<0.02	<0.01
HY4	<0.02	<0.01	<0.01	<0.01	<0.01
LY1	<0.02	<0.01	<0.01	<0.01	<0.01
LY2	<0.03	<0.01	<0.01	<0.01	<0.01
LY3	<0.03	<0.01	<0.0	6.10^{-6}*	29.10^{-5}*
LY2.1	<0.02	<0.01	<0.01	<0.01	<0.01
LYS	<0.02	<0.01	<0.01	<0.01	3.10^{-6}*
Detection limit ICP-AES	0.01				
Detection limit ICP-MS*	9.10^{-6}				

*Measured by ICP-MS

by an ions exchange mechanism with H^+ or H_3O^+ ions from solution. The pH changes are due to the dissolution of the glass surface during the immersion in SBF. The biodegradation of the glass in SBF may accomplish due to the reactions occurring on the glass surface.

Further research work is needed in development of controlled biodegradation behaviour of the glasses for longer immersion time and in the dosimetric analysis of other neutron activable cations in the glass after neutron activation in a nuclear reactor.

Table 4 Radius and ionic field strength of the network modifiers cations, Z/r^2.

Cation	Shannon ionic radius [22] (Å)	Ionic field strength Z/r^2 ($Å^{-2}$)
Ca (2+)	1.12	1.59
Mg (2+)	0.89	2.52
Na (1+)	1.18	0.72
K (1+)	1.51	0.44
Y (3+)	1.02	2.88

Conclusions

The yttrium release from both yttrium glass series is less than 0.5% from the initial amount of yttrium in the investigated conditions, that denotes a low solubility of the neutron activable yttrium cations. The addition of yttrium oxide to the bioactive glass samples decreases but does not suppress the bioactivity of the glasses. A layer rich in calcium, phosphorus and yttrium is formed on the samples surface. The data obtained from elemental analysis of the immersion solutions suggest that a calcium phosphate layer is formed due to the reaction of the glass with the SBF implying not only the release of cations from glass but also the deposition of Ca^{2+} and PO_4^{3-} from the SBF on the glass surface. The results show that the reactions occurring on the glass surface in a SBF are depended on Y_2O_3 content of the bioactive glass.

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