EVALUATING THE PHASE COMPOSITION OF THE SURFACE OF BIOACTIVE GLASSES FOLLOWING THEIR INTERACTION WITH SIMULATED BODY FLUID, WITH THE USE OF X-RAY DIFFRACTION ANALYSIS

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Following interaction with simulated body fluid, apatite and calcite were identified on the surface of bioactive glasses. The phase composition of the glass surface layer after elution is affected by the experimental conditions. At a high ratio of sample surface area (S) to the volume of the leaching solution (V), calcite was identified in the surface layer, whereas a low ratio (a large volume of the solution) had led to the formation of apatite. From the point of view of crystallography, the apatite was not well developed, and its detection limit by the X-ray diffraction method was less than 5%. The calcite was well developed. Phosphorus-free glasses exhibited a higher tendency to calcite formation than the phosphorus containing ones. Suitable experimental conditions with respect to the S/V ratio have to be chosen in the "in vitro" testing to ensure availability of phosphorus in the solution throughout the exposure, as otherwise calcite would eventually precipitate on a thin layer of apatite.

INTRODUCTION

X-ray diffraction analysis is used as one of the methods of assessing the changes in the surface of bioactive materials resulting from their implanting in live organism ("in vivo" testing), or following their interaction with model solutions ("in vitro" testing). The method was used in studying the surface of bioactive glasses after "in vitro" testing, e.g. by Kokubo at al. [1] who were able to identify apatite in the surface layer of the so-called Hench's glass 45S5 already after one day's exposure to a solution simulating the body fluid. In the diffraction pattern, apatite was characterized by broad diffraction peaks indicating that it was present in the form of small crystallites, or had a defective structure resembling that of apatite in natural bones. In study [2], the layer formed on the surface of a bioactive glass after reacting with a model solution was likewise shown, by X-ray diffraction analysis, to contain apatite, and possibly also calcite or hydroxyapatite with a defective structure.

The X-ray diffraction method was also employed in studying the surface of bioactive glass-ceramic materials [3, 4]. In the surface of apatite-wollastonitic glass ceramics, which had been implanted in rat bone, Kitsugi et al. [3] identified hydroxyapatite. Apatite, structurally different from the apatite phase constituting a component of the glass-ceramic material, was also determined in the surface of this type of glass-ceramics in "in vitro" tests (7 days leaching in simulated body fluid). Formation of apatite was also observed on another type of glass-ceramics (Ceravital) after an "in vivo" test (implanting in rabbit's bone) as well as after "in vitro" tests [4]. In both cases the hydroxyapatite was structurally defective and contained CO_3^{2-} ions. The X-ray diffraction analysis was likewise used in other studies, e.g. [5, 6].

The present contribution had the aim to investigate the crystalline products forming on the surface of bioactive glasses by reactions with model solutions under various experimental conditions.

EXPERIMENTAL

Samples

Four types of bioactive glasses prepared in the laboratory were used (Table I), namely glasses containing phosphorus (Hench's glass 45S5 and P50 glass) and phosphorus-free glasses (S45, S50). The measurements were carried out on both ground and compact samples. Ground glass of the 0.315 - 0.5 mm grain size fraction was prepared by the standard method according [7], while the compact samples were cut into the desired dimensions and polished to optical-grade gloss.

Table I. Composition of the glasses

Glass designation	Composition (wt. %)			
	SiO_2	Na_2O	CaO	P_2O_5
45S5*)	45.0	24.5	24.5	6.0
P50	50.0	22.0	22.0	6.0
S45	45.0	27.5	27.5	0
S50	50.0	22.5	22.5	0

*) "Hench's glass"

Leaching of the glasses in model solutions

The aqueous solution designated SBF (Simulated Body Fluid) in the literature, whose composition (Table II) corresponds to the inorganic component of the human plasma, was used as the base model solution. Another solutions were SBF free of HPO_4^{2-} ions and SBF free of HCO_3^- ions. All of the model solutions were buffered with tris(hydroxymethyl)aminomethane and HCl addition to pH 7.25 at 37 °C.

Table II. Ionic composition of SBF (simulated body fluid) Content (mmol l^{-1})

Na^+	K^+	Mg^{2+}	Ca^{2+}	HCO_3^-	Cl^-	HPO_4^{2-}
142.0	5.0	1.5	2.5	4.2	148.8	1.0

The samples were leached under static conditions at 37 °C for 6, 13 and 35 days. With the compact samples, the ratio of sample surface area to leaching solution volume (S/V) was chosen at 0.01 cm^{-1} and 0.1 cm^{-1} respectively. With the ground samples, 1 g of the sample was leached in 70 ml or 1000 ml of the solution. This, on the assumption that the grain size fraction employed (0.315 - 0.5 mm) had a surface area of approx. 110 $cm^2\,g^{-1}$ as given in the literature, corresponded to an S/V ratio of 1.57 cm^{-1} and 0.1 cm^{-1} respectively.

Following exposure to the model fluid and drying, the samples were treated to allow for X-ray diffraction analysis.

Analyses of surfaces by the X-ray diffraction method

The surface layer of reaction products was separated from the ground samples by brushing through a sieve, and then ground to a grain size of less than 0.2 mm. The ground sample was applied onto a microscope slide coated in advance with a thin layer of clear white vaseline. The compact samples were measured without any pretreatment.

The measurements were carried out on the Seifert XRD 3000 P diffractometer, using CoKα radiation and graphite monochromator. Background analysis and peak analysis (using the SPEKTRA 1 program by the Seifert Company) were performed on all samples. The diffraction patterns were evaluated according to the PDF 2 database [8].

RESULTS AND DISCUSSION

Phase composition of the products of reaction of bioactive glasses with model solutions

As indicated by data from the literature, the surface of bioactive glasses reacts with body fluids or their model solutions yielding apatite, probably one with a defective structure.

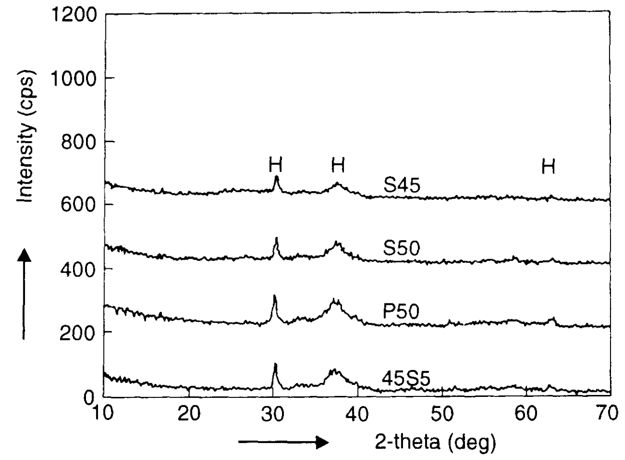

Figure 1. Diffraction patterns of compact glass samples after leaching in the base SBF solution (6 days, S/V = 0.01 cm^{-1})

With all of the studied samples where apatite was determined, the most intensive lines in the diffraction patterns corresponded to the most intensive lines of tabellated hydroxyapatite $Ca_5(PO_4)_3OH$ with interplanar spacings d: 2.814, 2.778, 2.720, 3.44 [Å]. Figure 1 shows the diffraction patterns of compact glass samples leached for 6 days in the SBF base solution (S/V = 0.01 cm^{-1}). An apatite phase was identified in the surface layer of all these samples. On account of the diffuse expansion and overlapping of the diffraction lines it was difficult to determine exactly what type of apatite was involved. The phases of apatite type considered have identical or very similar interplanar distances and do not differ perceptibly in their intensities. Fluoroapatite can be ruled out in view of the chemical composition of the glasses and that of SBF. The phases designated hereafter as hydroxyapatite or only apatite may be an apatite or chloroapatite phase or a phase of the $Ca_5(PO_4)_3(OH,Cl)$ type, but most probably it is a phase of the carbonate-hydroxyapatite type [3, 4, 10]. (The hydroxyapatite lines in the Figures are marked with the letter H.)

In order to determine the detection limits, hydroxyapatite prepared in the laboratory (of 95 % purity) was measured as a standard sample. Its diffraction pattern is shown in Figure 2 jointly with a diffraction pattern of a ground sample of the original 45S5 glass, and a model mixture of this glass with 5 % of the synthetic hydroxyapatite. The diffraction pattern of the model mixture shows faintly only peaks of the most intensive hydroxyapatite lines. As the synthetic hydroxyapatite was well crystallized, the low intensities of the peaks in the mixture diffraction pattern indicate that the content of 5 % is close to the detection limit. When taking into account that with samples of glasses leached in the model solutions (in particular for short exposure periods) the hydroxyapatite is not likely to be well developed, in the case of the actual exposed samples it may be assumed that the detection limit of apatite will be shifted towards concentrations higher than 5 %.

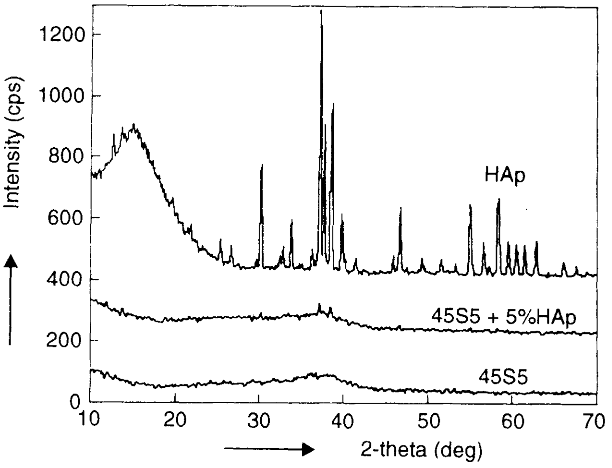

Figure 2. Diffraction patterns of hydroxyapatite (HAp) and its mixture (5%) with glass 45S5

Apart from apatite, also calcite was identified in the surface layer of the glasses under certain conditions of leaching (cf. below). In the evaluation of the diffraction patterns, its main lines with the following interplanar spacings d were considered: 3.035, 2.285, 2.095, 1.913 [Å].

As an example, Figure 3 shows diffraction patterns of ground samples of glass S45, which was free of phosphorus, after leaching (S/V = 1.57 cm^{-1}) in a solution which was likewise phosphorus-free but contained HCO_3^- ions. As expected, calcite was identified in the surface layer of this glass after all the times of exposure, and the intensity of its lines increased in terms of the time of leaching. (Calcite lines in the Figures are marked with letter C.)

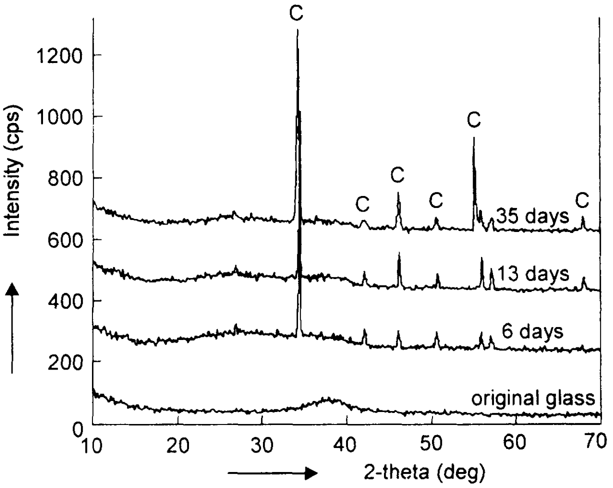

Figure 3. Ground samples of glass S45 after leaching in a solution free of HPO_4^{2-} ions (S/V = 1.57 cm^{-1})

Calcite was also often identified in the surface of the glasses following their reaction with solutions containing phosphorus. It appeared in particular whenever a high S/V ratio had been employed (a small volume of the solution) and after longer times of exposure. The subject matter of the effect of the conditions of exposure is dealt with below in more detail.

Apart from apatite and calcite, no other crystalline phases were found in the surface layers of the leached glasses in question.

The effect of leaching conditions on the phase composition of the surface layer

As already mentioned above, either apatite alone or apatite jointly with calcite, or calcite alone were identified in the surface layer of the leached glasses. The occurrence of these phases depended on the conditions of the leaching process as well as on the composition of the glass in question.

In the case of phosphorus containing glasses, apatite was identified even after leaching in a phosphorus-free model solution. In this instance, only phosphorus leached out of the glass during its reaction with the model solution was obviously available for the formation of apatite in the surface layer.

Formation of apatite was found to take place in particular whenever a high S/V ratio (small volume of the leaching solution) was employed, and for the most part with the ground glass samples. It may be assumed that under these conditions the reaction producing apatite in the surface layer will quickly exhaust the phosphorus from the solution, and calcite will then precipitate on the thin layer of apatite already formed on the surface. The amount of apatite involved is obviously below the detection limit, so that it cannot be distinguished in the diffraction patterns. The results obtained by leaching ground glass samples in the base SBF solution for six days are shown as illustration in Figure 4. Apatite was not identified in any of these samples. The main calcite lines were identified in the surface layer of phosphorus-free glasses, and no crystalline products were found in the surface layers of glasses containing phosphorus.

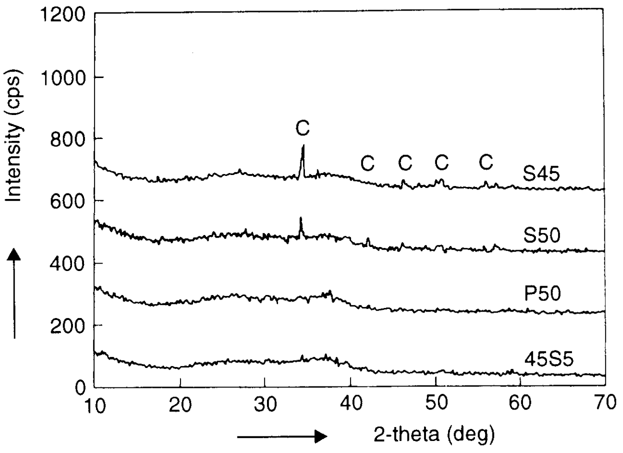

Figure 4. Ground samples of glasses after leaching (6 days) in base SBF solution at a high S/V ratio (1.57 cm^{-1})

The tendency towards calcite formation was exhibited more by phosphorus-free glasses that by those containing phosphorus. Figure 5 shows diffraction patterns of ground sample of glass P50 leached in the base solution at the highest S/V ratio employed (1.57cm^{-1}) for periods of 6 to 35 days. Figure 6 shows the corresponding diffraction diagrams for glass S50. At this high S/V ratio, apatite was not reliably identified in any of the samples. However, calcite was found in the phosphorus-containing glass (P50) after the longest time of exposure (35 days, Figure 5). In contrast to this, it was identified in the surface of phosphorus-free glass (S50) after all of the times of exposure followed (even the shortest ones)(Figure 6).

With the use of the lower S/V ratio (leaching into a larger volume of the solution), apatite or a mixture of apatite with only indications of calcite was identified in the surface layers of all the glasses.

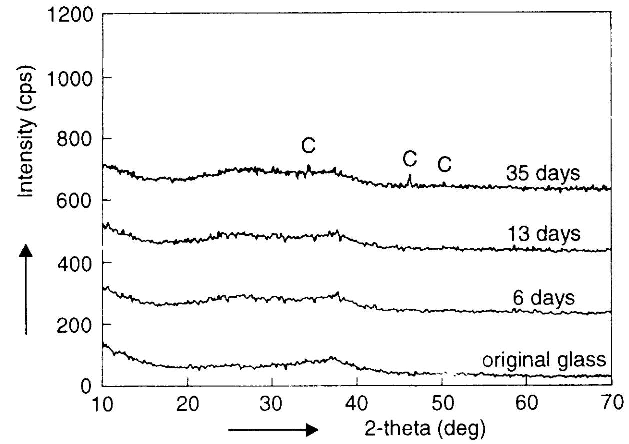

Figure 5. Ground samples of glass P50 after various times of leaching in base SBF solution (S/V = 1.57 cm^{-1})

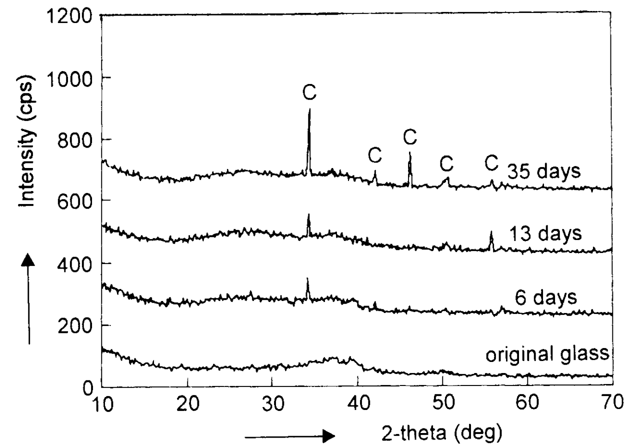

Figure 6. Ground samples of glass S50 after various times of leaching in base SBF solution (S/V = 1.57 cm^{-1})

Apatite was also identified in the product of spontaneous precipitation of the SBF base solution, found in the form of sediment at the vessel bottom after a long standing (weeks or months). Figure 7 shows a diffraction pattern of this precipitate, jointly with diffraction patterns of ground glasses P50 and S50 leached for 13 days in the model solutions (S/V = 0.1cm^{-1}). A comparison of these diffractograms shows that the diffraction pattern of apatite precipitated spontaneously from solution is similar to that of the leached samples. It is also obvious that with phosphorus-containing glasses the main lines of apatite obtained by exposure to the base solution are more distinct than in the case of phosphorus-free glasses. There is also a certain difference between the diffraction pattern of P50 glass leached in the base solution containing phosphorus and that of the same glass exposed to the phosphorus-free solution.

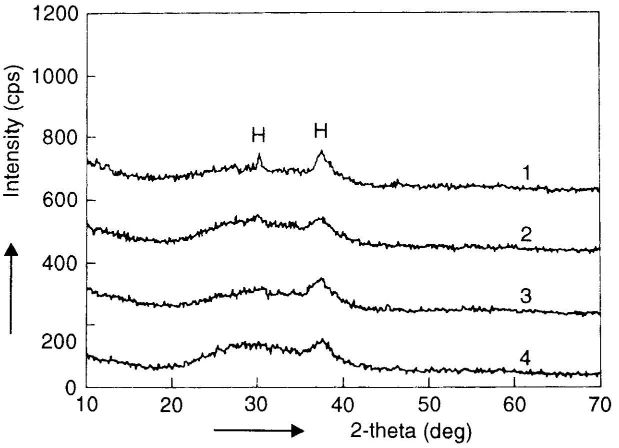

Figure 7. A comparison of diffraction patterns of ground glass samples leached for 13 days at $S/V = 0.1\ \mathrm{cm}^{-1}$ with a diffraction pattern of the products of spontaneous precipitation of SBF
1 - product of spontaneous precipitation of SBF
2 - glass P50, solution free of HPO_4^{2-}
3 - glass P50, base SBF solution
4 - glass S50, base SBF solution

CONCLUSION

The X-ray diffraction analysis showed that apatite, and under certain conditions also calcite, are formed on the surface of bioactive glasses by reactions with solutions simulating the composition of the inorganic component of human plasma.

The apatite thus formed is not crystallographically well developed and X-ray diffraction analysis does not allow the exact type of apatite involved to be identified. Its detection limit by X-ray diffraction analysis is likely to be higher than 5 %.

Apart from apatite, also calcite, which is crystallographically well developed, will form on the surface of bioactive glasses under certain conditions. The formation of calcite and thus also the phase composition of layers on the surface of leached glasses are influenced by experimental conditions. Leaching at a high ratio of sample surface area to the volume of the leaching solution leads to early exhausting of phosphorus from the solution as a result of the formation of apatite on the glass surface. Only a thin apatite layer is formed on the surface of glass, onto which calcite is then precipitated. The amount of apatite formed may thus be below the detection limit of the X-ray diffraction method, so that it will not appear in the diffraction pattern which would only show the calcite lines. This situation may occur in particular with ground samples which have a large specific surface area.

On the other hand, when the leaching takes place in a large volume of the solution, the phosphorus is not likely to become exhausted from the solution and apatite alone, or apatite with just traces of calcite main lines was identified in the surface layer of the leached glasses. The tendency towards calcite formation was exhibited more by glasses that were free of phosphorus than by those containing phosphorus.

The results of the present study indicate that in "in vitro" tests it is necessary to select suitable experimental conditions with respect to an adequate amount of the leaching solution ensuring a continuous supply of phosphorus in solution throughout the duration of the experiment. Failure to comply with this requirement leads to precipitation of calcite on the surface of glass as soon as the supply of phosphorus in the solution has been exhausted. In any case, exposure to the effects of a solution which no longer contains any phosphorus would be a poor simulation of the actual "in vivo" conditions.

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HODNOCENÍ FÁZOVÉHO SLOŽENÍ POVRCHU BIOAKTIVNÍCH SKEL PO INTERAKCI SE SIMULOVANOU TĚLNÍ TEKUTINOU RTG DIFRAKČNÍ ANALÝZOU

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Na povrchu bioaktivních materiálů se po reakci s tělními tekutinami nebo jejich modelovými roztoky vytváří vrstva bohatá na vápník a fosfor, podle údajů v literatuře vzniká apatit s defektní strukturou.

RTG difrakční metodou byly analyzovány povrchy bioaktivních skel soustavy Na_2O-CaO-SiO_2 (obsahujících fosfor i bez fosforu) po loužení v simulované tělní tekutině (SBF) při teplotě 37 °C po dobu 6 až 35 dnů. Vzorky skel (drťové i celistvé) byly louženy za statických podmínek při různém poměru velikosti povrchu vzorku k objemu loužícího roztoku. RTG analýza byla provedena na difraktometru Seifert XRD 3000 P, difraktogramy byly vyhodnoceny podle databáze PDF 2.

Na povrchu loužených skel byl identifikován apatit, v řadě případů také kalcit. Apatit není krystalograficky dobře vyvinut a je obtížné přesně určit, o jaký druh se jedná. Pravděpodobně jde o fázi karabonát-hydroxyapatitového typu. Mez jeho detekce RTG analýzou bude zřejmě vyšší než 5%. Vedle apatitu se za určitých podmínek tvoří na povrchu také kalcit, krystalograficky dobře vyvinutý. Tvorba kalcitu a tím fázové složení povrchové vrstvy loužených skel je ovlivněno podmínkami experimentu. Při loužení za vysokého poměru velikosti povrchu vzorku k objemu loužícího roztoku (*S/V*) může dojít brzy k vyčerpání fosforu v roztoku a na povrchu tenké vrstvy apatitu se pak dále sráží kalcit. Při loužení v dostatečně velkém objemu roztoku byl v povrchu skel identifikován apatit. Sklon k tvorbě kalcitu vykázala více skla bez obsahu fosforu, než skla obsahující fosfor.

Při testech "in vitro" je třeba volit vhodné podmínky experimentu z hlediska objemu loužícího roztoku, aby byla zaručena přítomnost fosforu po celou dobu experimentu. Jinak se na povrchu skel sráží po vyčerpání fosforu kalcit.