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In vitro Studies of Bioglass Material by X-Ray Diffraction and Solid-State MAS-NMR[1]

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Abstract—Bioactive glass 46S6 has been elaborated by melting method at high temperature. "In vitro" experiments of 46S6 glass were carried out by soaking in a simulated body fluid for different times. The kinetics of chemical reactivity and the bioactivity of this biomaterial was investigated by X-ray diffraction and elucidated by using an original structural analysis method based on solid-state MAS-NMR. After in vitro assays, X-ray diffraction confirmed the high bioactivity of bioglass 46S6. The ^{29}Si MAS-NMR spectra showed the emergence of two new species: $Q^3_{Si}(OH)$ and Q^4_{Si} which are characteristic of the dissolution of vitreous network of 46S6 glass while the ^{31}P MAS-NMR spectra highlighted the formation of new component attributed to hydroxycarbonate apatite. The bioglass 46S6 have presented the rapid formation of a biological active hydroxycarbonate apatite layer after soaking in simulated body fluid fluid.

Keywords: bioactive glass, bioactivity, hydroxyapatite, ^{31}P MAS-NMR, ^{29}Si MAS-NMR

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INTRODUCTION

Bioactive glasses are a group of reactive surface glass-ceramic biomaterials used as implant materials in the human body to repair and replace diseased or damaged bone. Their bioactivity is based on the ability to form a biological hydroxycarbonate apatite layer when they are immersed in a physiological solution or implanted in human body. The formation of apatite layer promotes the adhesion of bone tissues and permits an intimate bone-bonding with the implants. Consequently, the bone architecture is repaired and reconstructed [1–2].

The aim of this work is to elaborate the 46S6 bioglass by melting method. The in vitro kinetics of chemical reactivity and bioactivity of this biomaterial were investigated by X-ray diffraction and by the main focus on using the nuclear magnetic resonance spectroscopy (NMR).

MATERIALS AND METHODS

Elaboration of 46S6 Glass

The bioactive glass with chemical composition 46 wt % SiO_2, 24 wt % CaO, 24 wt % Na_2O and 6 wt % P_2O_5 (46S6) was prepared by melting method according to E. Dietrich's method [3]. The starting materials: calcium silicate ($CaSiO_3$), sodium metasilicate (Na_2SiO_3) and sodium phosphate ($NaPO_3$) were weighted and mixed homogeneously. Then, they were heated in a platinum crucible according to the following steps. First, the temperature was ramped to 900°C. This temperature was kept at 900°C for 1 h to decompose the chemical constituents. Furthermore, the temperature was risen and kept at 1300°C during 3 h to melt the reactive mixture. Afterward, the melted bioactive glass was poured into preheated brass moulds and annealed for 4 h at the glass transition temperature (about 536°C) to remove the residual mechanical stress. After cooling to room temperature, the bulk glasses were ground into powder and sieved to achieve the bioactive glass particles with size less than 40 μm.

In vitro Assays in SBF Solution

The "in vitro" study of 46S6 glass was carried out by soaking 100 mg of material samples into 200 mL of simulated body fluid (SBF) which has acidity and mineral composition similar to that of human blood plasma. The SBF solution was prepared by dissolving the chemical reagents of NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$ and $CaCl_2$ into deionised water and buffering with $(CH_2OH)_3CNH_2$ and 6 M HCl to obtain pH value of 7.4 according to

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Table 1. Concentrations of the SBF solution, (10^{-3} mol L^{-1})

	Ions						
	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}
SBF	142.0	5.0	2.5	1.5	148.8	4.2	1.0
Plasma	142.0	5.0	2.5	1.5	103.0	27.0	1.0

Kokubo's technique [4]. The ionic concentrations of this solution are shown in Table 1. The powder samples were immersed in SBF solution and maintained at body temperature (37°C) under controlled agitation 50 rpm during 1, 3, 7, 15 days.

Physico-Chemical Characterizations

X-ray diffraction measurements were realized on Bruker D8 Advance diffractometer. Powder samples were mixed homogeneously with cyclohexane and dropped on the surfaces of plastic tablets. Then, these tablets were dried to remove the solvent and introduced into diffractometer. The XRD data were acquired in the range of $2\theta = 10°–70°$ with a scanning speed of 1°/min. The aim of this work consists particularly on the use of solid-state MAS-NMR method to investigate the kinetic of chemical reactivity and the bioactivity of 46S6 glass after the "in vitro" assays. The ^{29}Si and ^{31}P MAS-NMR spectra were measured on a Bruker Avance 300 spectrometer (7T). Material samples were packed in zirconium rotors with a diameter of 2.5 mm, and spun at the magic angle of 54.7° with a spinning frequency of 15 MHz. The deconvolution of the MAS-NMR spectra was performed on the dmfit2010 software [5].

RESULTS AND DISCUSSION

X-Ray Diffraction Characterization

Figure 1 shows the XRD diagrams of 46S6 glass before and after soaking in SBF. The XRD diagram of hydroxyapatite is presented as reference to evaluate the bioactivity of material as a function of immersion time [JCPDF 09-432 card]. The XRD diagram of the initial glass did not show any evidence of crystalline phase and confirm the amorphous character of glass. It presents a diffraction halo with centre at $2\theta = 32.5°$. After 3 days of soaking in SBF, the immersed 46S6 glass is still amorphous material and the characteristic signs of a mineral phase of apatite did not appear yet. After 7 days, two characteristic peaks of hydroxyapatite at $2\theta = 26°$ and 32° are shown. They correspond respectively to the (002) and (211) reflection planes in the structure of hydroxyapatite crystals. This result confirms the bioactivity of 46S6 glass. These two apatite characteristic peaks became more intensive after

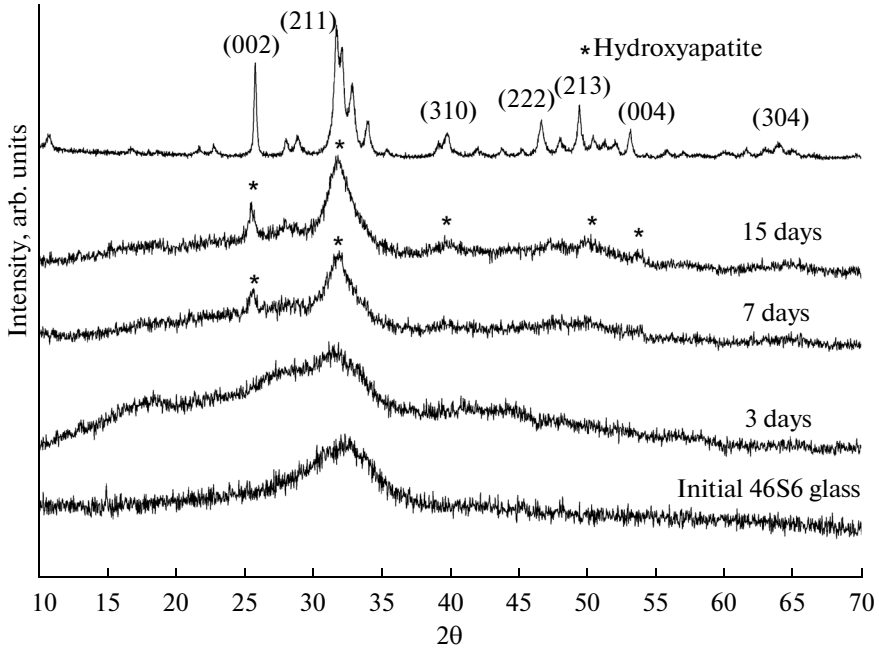

Fig. 1. XRD diagrams of 46S6 glass before and after soaking in SBF.

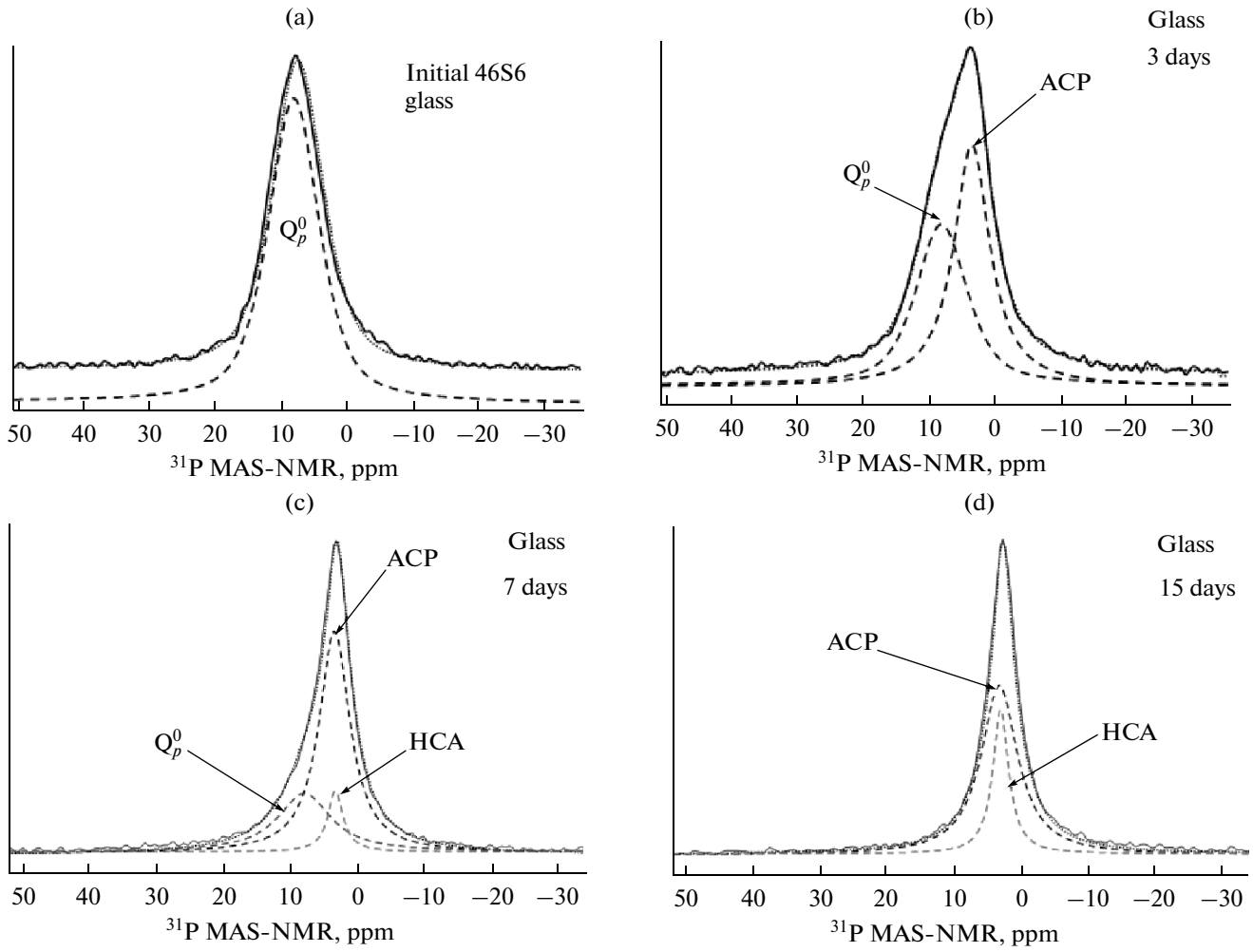

Fig. 2. ^{31}P MAS-NMR spectra of 46S6 glass: (a) initial 46S6 glass, (b) after 3 days, (c) after 7 days and (d) after 15 days of soaking in SBF solution.

15 days of immersion. On the other hand, four other small peaks at $2\theta = 40°$, 46.7°, 50° and 53° are also exhibited. They are respectively attributed to the (310), (222), (213) and (004) reflection planes in the crystallographic system of the hydroxyapatite. This result indicates that the amount and the crystalline quality of apatite layer formed on the surface of 46S6 glass compound increases with time of soaking in SBF solution.

^{31}P MAS-NMR Characterization

Figure 2 presents the ^{31}P MAS-NMR spectra of 46S6 glass before and after soaking in SBF solution. Table 2 lists the data of the different components after the spectral deconvolutions. The ^{31}P MAS-NMR spectrum of the initial 46S6 glass shows only one resonance (Fig. 2a). Its chemical shift (δ) and full width at half-maximum (FWHM) are characteristic of the phosphate tetrahedra PO_4^{3-} without bridging oxygen, called orthophosphate [6–8]. We use Q_P^0 code for the tetrahedrons PO_4^{3-} in the initial 46S6 glass to differentiate between it and another phosphorus species in the same orthophosphate environment. After 3 days of immersion in SBF solution, the quantity of Q_P^0 decreases strongly due to the release of phosphorus from the glassy network under the active effect of the SBF solution. On the other hand, a new resonance is observed as shown in Fig. 2b. This resonance is assigned to an amorphous calcium phosphate (ACP) in according to the previous researches [9–12], in which the synthetic ACP revealed a chemical shift (δ) around 3 ppm and a FWHM in the range 4.4–6.9 ppm. This result is in agreement with the previous analyses by X-ray diffraction (Fig. 1), where the 46S6 glass was still amorphous material after 3 days of immersion. It is also consistent with the bioactivity mechanism of bioactive glass, described by [1–2], where there is the deposit of the Ca^{2+} and PO_4^{3-} ions to

Table 2. ^{31}P MAS-NMR data of 46S6 glass before and after soaking in SBF solution

Soaking time	^{31}P MAS-NMR data of 46S6 glass					
0 day	$Q_P^0 = 100\%$					
	δ, ppm 7.57	FWHM, ppm 9.19	–		–	
3 days	$Q_P^0 = 45.17\%$		ACP = 54.83%			
	δ, ppm 7.54	FWHM, ppm 9.52	δ, ppm 3.01	FWHM, ppm 6.65	–	
7 days	$Q_P^0 = 28.43\%$		ACP = 62.92%		HCA = 8.65%	
	δ, ppm 7.61	FWHM, ppm 9.56	δ, ppm 3.10	FWHM, ppm 5.60	δ, ppm 2.96	FWHM ppm 2.80
15 days	–		ACP = 72.20%		HCA = 27.80%	
			δ, ppm 3.13	FWHM, ppm 6.61	δ, ppm 2.94	FWHM, ppm 2.81

form an ACP on the surface of glass after soaking in SBF solution. After 7 days of immersion the amount of Q_P^0 continuously decreases strongly while an increase of ACP and an appearance of new resonance are observed (Fig. 2c). The new resonance has the δ and FWHM characterized to hydroxycarbonate apatite (HCA) in agreement with the previous studies [12, 13], in which the HCA obtained after "in vitro" assays exhibited a chemical shift about 3 ppm and a full width at half-maximum from 2 to 2.8 ppm. This result is in accordance with the XRD analyses, where the 46S6 glass revealed two characteristic peaks of hydroxyapatite after 7 days of soaking in SBF solution. It also agrees with the bioactivity mechanism of bioactive glass [1, 2], in which the ACP evolves into crystalline HCA with the increase of immersion time. After 15 days of immersion in SBF solution, the Q_P^0 species are disappeared while the HCA component increases quickly (Fig. 2d). The disappearance of Q_P^0 units confirms their complete migration out of the glassy network into the SBF solution. The increase of HCA quantity corresponds to the progressive crystallization of the ACP to form the crystals HCA.

^{29}Si MAS-NMR Characterization

Figure 3 shows the ^{29}Si MAS-NMR spectra of 46S6 glass before and after soaking in SBF solution. Table 3 shows the data of the different species after the spectral deconvolutions. The spectrum of glass after 1 day did not show because it is similar to the one after 3 days. The deconvoluting spectrum of the initial 46S6 glass shows two resonances. The first chemical shift is assigned to Q_{Si}^2 species (SiO_4 tetrahedron is linked into the vitreous network *via* two bridging oxygens). The second chemical shift is characteristic of Q_{Si}^3 species (SiO_4 tetrahedron is linked into the vitreous network *via* three bridging oxygens) [5–7]. The chemical neutrality around the non-bridging oxygens of Q_{Si}^3 tetrahedra is respected by the preferential presence of Na^+ cations, is shown as $Si(OSi)_3(O\cdots Na)$. The non-bridging oxygens of Q_{Si}^2 species are rather combined with Ca^{2+} cations and Na^+ remaining cations. These two

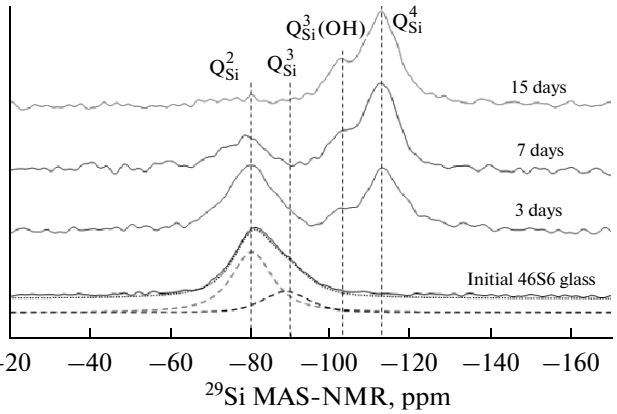

Fig. 3. ^{29}Si MAS NMR spectra of 46S6 glass before and after soaking in SBF solution.

combinations could be expressed as $Si(OSi)_2(O_2\cdots Ca)$ and $Si(OSi)_2(O\cdots Na)_2$ [5–7]. The ^{29}Si MAS-NMR spectrum of 46S6 glass shows some changes as a function of soaking time in SBF solution. This characterizes the structural modification of the vitreous matrix due to the chemical reaction between glass and SBF solution. In fact, the quantities of Q^2_{Si} and Q^3_{Si} species decrease gradually while two new components $Q^3_{Si}(OH)$ and Q^4_{Si} are emerged on the spectrum of 46S6 glass after in vitro assays. The $Q^3_{Si}(OH)$ characterizes the tetrahedral environment of Si with three bridging oxygens and one hydroxyl group [14, 15]. The Q^4_{Si} is characteristic of the silicon in tetrahedral environment with four bridging oxygens. This silicon environment corresponds to pure silica (SiO_2) [16–18]. The emergence of two new components $Q^3_{Si}(OH)$ and Q^4_{Si} is the witness of the dissolution of glassy network. These obtained results are attached to the progressive chemical reactions between bioactive glass and the physiological fluid SBF, discovered by Larry Hench et al. [1, 2]. The first is the dealkalization process through the interdiffusion/ion-exchange of Na^+, Ca^{2+} out of the glass and H_3O^+ into the glass to form the Si–OH groups. Then, the reactions of the breaking of Si–O–Si bridging links occur at the interface glass-liquid SBF which lead the loss of soluble silica $Si(OH)_4$. The pursuit of two above reactions is the condensation and repolymerization of surface silanols to form SiO_2-rich layer, expressed by the increase of species $Q^3_{Si}(OH)$ and Q^4_{Si}. These key reactions are illustrated folowing steps 1–4 below. After 15 days of immersion in SBF solution, all species of Q^2_{Si} and Q^3_{Si} are disappeared. This confirms the end of the chemical reactions 1, 2 and 3. The ^{29}Si MAS-NMR spectrum of 46S6 glass shows only two species $Q^3_{Si}(OH)$ and Q^4_{Si}. Their percentages as shown in Table 3 confirm the formation of a SiO_2-rich gel layer on the surface of glass after 15 days of immersion:

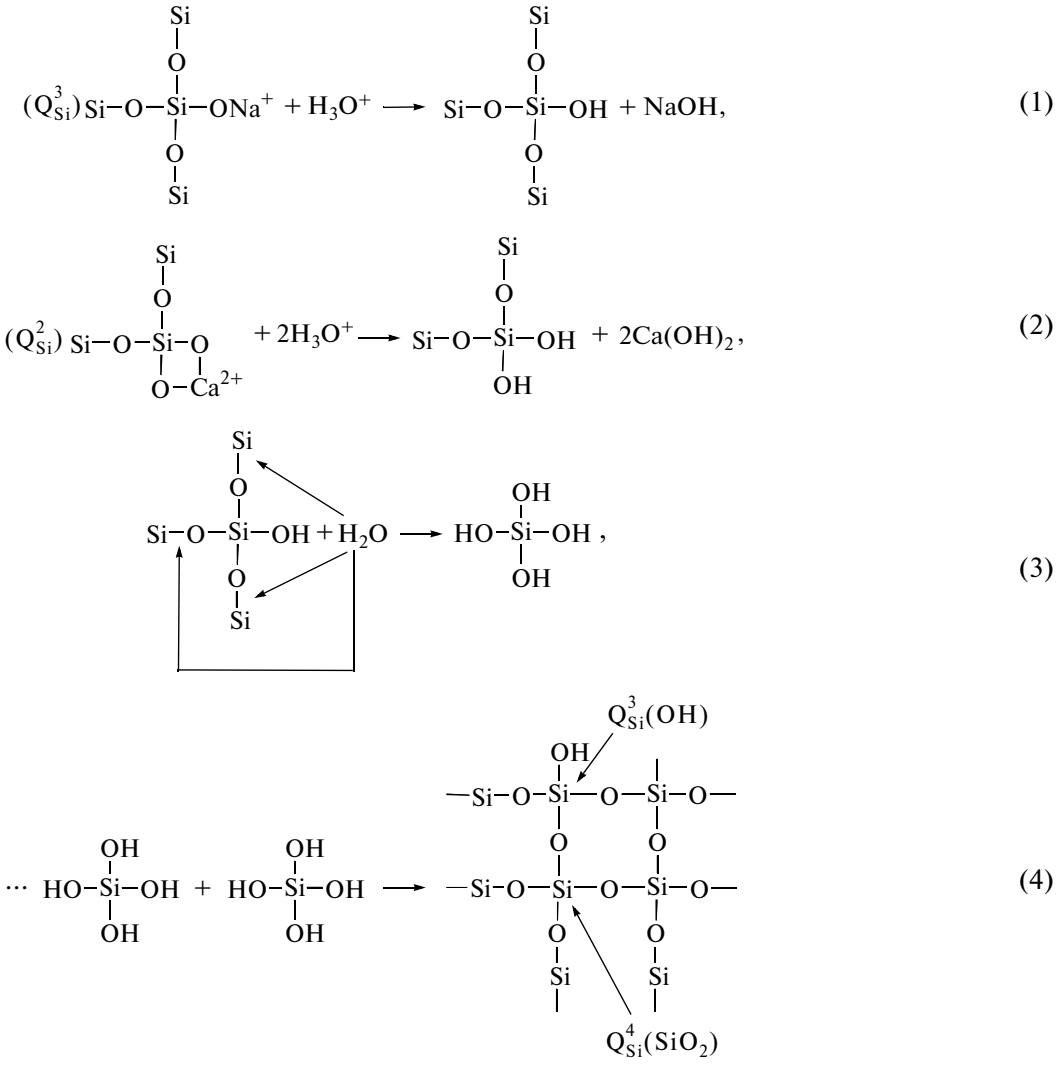

Table 3. ^{29}Si MAS-NMR data of 46S6 glass before and after soaking in SBF solution

Soaking time	^{29}Si MAS-NMR data of 46S6 glass									
	Q^2_{Si}		Q^3_{Si}		$Q^2_{Si} + Q^3_{Si}$	$Q^3_{Si}(OH)$		Q^4_{Si}		$Q^3_{Si}(OH) + Q^4_{Si}$, %
	δ, ppm	%	δ, ppm	%		δ, ppm	%	δ, ppm	%	
0 day	−80.3	79.5	−89.2	20.5	100		0		0	0
3 days	−80.0	56.4		0	56.4	−102.5	6.1	−113.2	37.5	43.6
7 days	−79.1	28.0		0	28.0	−102.3	17.0	−112.5	55.0	72.0
15 days		0		0	0	−102.0	24.7	−112.5	75.3	100

CONCLUSION

Solid-state ^{29}Si and ^{31}P MAS-NMR proved to be very sensitive technique to elucidate the in vitro chemical reactivity and bioactivity of 46S6 glass. ^{29}Si MAS-NMR showed the emergence of two new components $Q^3_{Si}(OH)$ and Q^4_{Si} which characterizes the dissolution of the vitreous network of 46S6 glass after in vitro assays. ^{31}P MAS-NMR highlighted the formation of new component attributed to hydroxycarbonate apatite. This technique combined with X-ray diffraction confirmed the high bioactivity of synthetic 46S6 bioglass.

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