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Doping influence on the interaction between a bioactive glass and a simulated physiological solution: chemical and EPR tests

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The adhesion between bioactive glass and metal is improved by the addition of small amounts of transition ion oxides. Because it is difficult to carry out *in vivo* tests to check with continuity, chemical information about the interactions of the different doped glasses with living tissues, the behaviour of the different doped bioactive glasses in a liquid simulating physiological conditions was studied. Chemical and EPR analyses show that the mineralization phenomena around the bioglasses in the tissues also take place at an inorganic level. This simulation shows that the ions released from the different vitreous systems are correlated with the adopted doping. In particular an increase in doping with iron involves a decrease in the release rate of the other ions. Furthermore the doping agents allow a control of the release rate and consequently the obtainment of the best biological adaptability.

1. Introduction

Bioactive glasses are a class of biomaterials suitable for coating metallic prostheses. Previously we ascertained a need to dope the base glass composition [1–3] to obtain and improve the adhesion between bioactive glass and metal. Now it has become necessary to study the role of an addition of small amounts of different oxides (among them transition ones) within the bioactive glass composition when these are implanted in the bone tissues. It is, however, very difficult to carry out *in vivo* tests to check detailed information about these interactions: consequently we decided to study the behaviour of different doped bioactive glasses in a liquid simulating physiological conditions. Yet this kind of test differs from the *in vivo* one where the possibly involved components of the liquid solution (enzymes, proteins, amino acids and so on) are more numerous, and also because living tissue acts in an "open" system, characterized by the possibility to remove and eject all foreign substances that in *in vitro* tests (with a floating liquid) remain or accumulate. However, a test on the chemical inorganic interactions could give an idea of the mechanism of transformation undergone by bioactive glass when really implanted in a living tissue, even though vital interactions are absent.

2. Materials and methods

The bioactive glass samples were prepared by mixing the base composition oxides as reported in Table I with doping agents in the amounts reported in Table II and then melting the mixture in platinum crucibles at 1350° C. When this glass-making temperature was reached, it was held steady for 2 h. The melt was put in a graphite crucible, preheated at 600° C. Small blocks of the obtained glasses, when cooled, were finely ground in a mill with Al_2O_3 balls to obtain a granulometry around 3600 mesh cm^{-2}. Samples of 500 mg of the obtained powder were tested and dipped in 200 ml of Ringer solution in a Moplen (isotactic polypropilene; Montecatini–Edison S.p.A, Milan, Italy) beaker covered by Parafilm, and maintained at constant temperature (36 ± 1° C) in a thermostatic device. No variation in the quantity of H_2O was permitted during the entire experiment. Ringer solution (simulating a physiological environment) produced by the Merck Co. had the following composition ($g l^{-1}$): NaCl = 2.25, KCl = 0.105, $CaCl_2$ = 0.045, $NaHCO_3$ = 0.05. For each kind of bioactive glass six samples were prepared, five of them for interaction with Ringer solution according to prefixed times (7, 14, 30, 60 and 90 days) and one for electron paramagnetic resonance (EPR) analyses at zero time. At the expiration date, the liquid of each sample was filtered, obtaining a solution diluted 1 : 1 with twice-distilled water. All the solutions were analyzed by an atomic absorption spectrometer to test for silicon, calcium, sodium, iron, aluminium, chromium, nickel, copper and cobalt, making reference to titrated solutions with all Ringer constituents as well.

EPR analyses were carried out both on the unattacked vitreous samples and on the vitreous samples drawn from each solution after filtering. These samples were then washed by decantation with a little twice-

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TABLE I Starting base compositions

Base composition	Starting components (wt %)			
	SiO_2	$CaCO_3$	Na_2CO_3	P_2O_5
System A (used for all the samples except the $KRVI_{17}$	32.94	32.01	30.66	4.39
System B (used for the preparation of $KRVI_{17}$ alone)	28.45	29.19	38.09	4.27

distilled water then dried naturally. The measurements were taken in the X band at ~9.1 GHz. The spectra of un-attacked samples were interpreted as a simple overlapping of signals with a relative weight, related to the concentrations of the paramagnetic ions, as reported in Table II. This is reliable in that the dopant cations were very diluted, having a very large average distance between them.

The following attributions were adopted to solve by computational procedures the spectra:

(i) to Fe^{3+} ions a symmetric signal ($g \simeq 2.00$, $\Delta H \simeq 600$ G) and an asymmetric one ($g_{eff} \simeq 4.27$) as reported in the literature [4];

(ii) to Cu^{2+} ions a symmetric signal ($g \simeq 2.10$, $\Delta H \simeq 120$ G) and two asymmetric ones ($g_{\parallel} \simeq 2.20$, $\Delta H_{\parallel} \simeq 300$ G, $g_{\perp} = 2.05$, $\Delta H_{\perp} = 50$ G the first; $g_{\parallel} \simeq 2.30$, $\Delta H_{\parallel} \simeq 120$ G, $g_{\perp} = 2.06$, $\Delta H_{\perp} = 70$ G the second) as reported in the literature [5];

(iii) to Cr^{3+} ions a signal with $g \simeq 1.98$ and to Ni^{2+} a signal with $g \simeq 2.20$.

3. Results

The results of the chemical analyses are reported in Table III. The kinetic trends for silicon, calcium and sodium are displayed in Fig. 1. The plots of Fig. 2 show the correlations Si–Na and Cr–Na against time. Iron is surprisingly stable in the glass, present only in negligible amounts in the liquid (up to 0.1 p.p.m. for sample AFE 8, corresponding to 8 wt % Fe_2O_3 doping). Nickel, like aluminium, is practically constant in the solution, showing a small leaching from the glass at the beginning of the experiment, and a slow decrease of this concentration in the liquid against time, down to negligible levels. The concentration variations for Al^{3+} and Ni^{2+} seem to follow those of Ca^{2+}.

The EPR measurements show that the chemical attack of the Ringer solution causes the rapid growth of a Gaussian-shaped signal with a more pronounced asymmetric trend towards the lowest magnetic fields, having $g_{eff.} = 2.00 \pm 0.6$, $\Delta H_{eff.} = 900 \pm 200$ G and whose amplitude and width show small fluctuations from one sample to another. Since the shape of the signals coming from paramagnetic cations is not modified, it can be excluded that the chemical attack causes modifications in the interactions among dopant cations or in the symmetry of the molecular sites filled by them. Well detailed trends of the EPR signal against attack time were not possible, because only the signals of the samples attacked more than 30 days were significantly different from the signals of the un-attacked samples.

4. Discussion

The ionic release trends from these glassy systems show some common similarities and some differences. The similarities make reference principally to the trends of silicon and sodium leaching. A possible reason for the different leaching rate of the ions at the interface as a function of different doping could be attributed to slightly diverse ratios between surface development and volume of the grains due to different glass flaking. The differences among the computed ratios cannot justify the big release variations seen in time on purely geometric arguments. Looking at the fitting trends of Cr^{3+} and Ca^{2+} releases, a hypothesis linked to a kind of catalytic interference in the inner glass bulk may be proposed, that would act in comparing the doping ions with themselves and with the others constituting the glass system. To date the reason for such a catalytic action is not clear. It is possible to suggest some hypotheses as, for example, the constitution of more stable molecules within the vitreous system detaining certain ionic species, a close approach of the glass molecular network, or the occlusion of eventual small channels. All those hypotheses could make ionic diffusion towards the external liquid difficult. On the basis of Figs 1 and 2 it can be deduced that, as a function of the quantity of iron present in the glassy system, the release rates of Na^+ and Si^{4+} decrease. This behaviour is contrary to that observed for a pure ternary Na_2O–Fe_2O_3–SiO_2 system [6]. The presence and amount of P_2O_5 probably modifies these properties of the system; Fe^{3+} is evidently stabilized, as can be deduced by the release values of Table III. This stabilization seems to be reflected in all the elements constituting the system.

TABLE II Additions of doping oxides to the base compositions reported in Table I

Sample code	Base composition	Doping oxides added (wt %)									
		B_2O_3	Al_2O_3	NiO	Fe_2O_3	Cr_2O_3	CuO	Co_2O_3*	Ta_2O_5	Sb_2O_3	La_2O_3
A	A										
AFE 2	A				2.0						
AFE 8	A				8.0						
AKRA 15	A		2.0	1.0	6.0	0.6			1.0		0.5
AKRA 17	A	0.6	2.0		2.4	0.2	0.1		0.6	0.1	0.1
AKRA 18	A				8.0	0.2		0.1			0.1
$KRVI_{17}$	B	0.6	2.0		2.4	0.2	0.1		0.6	0.1	0.1

*During firing this decomposes to CoO at about 900° C.

TABLE III Concentration of some elements into Ringer solution

Sample	Days	Concentration (p.p.m. ±2%)								
		Si	Ca	Na	Fe	Al	Cr	Ni	Cu	Co
Ringer solution		0	16.3	899	0.00	0.00	0.00	0.00	0.00	0.00
A	7	49	26.5	939						
	14	55	22.4	947						
	30	116	7.0	1012	< 0.05					
	60	146	5.3	1129						
	90	146	7.8	1065						
AFE 2	7	35	21.9	923	< 0.05					
	14	47	20.9	944	< 0.05					
	30	63	10.9	970	< 0.05					
	60	96	11.4	1024	0.06					
	90	129	6.8	1055	< 0.05					
AFE 8	7	22	18.0	924	0.05					
	14	28	16.1	929	< 0.05					
	30	36	18.9	949	< 0.05					
	60	62	7.4	993	< 0.05					
	90	84	2.6	1044	0.06					
AKRA 15	7	17	18.3	910	< 0.05	0.10	0.36	0.06		
	14	25	17.6	928	< 0.05	0.20	0.52	0.15		
	30	27	18.6	952	< 0.05	0.09	0.67	0.14		
	60	40	14.1	1005	< 0.05	0.10	1.32	0.17		
	90	64	5.7	990	0.05	< 0.05	1.42	0.06		
AKRA 17	7	24	21.6	917		< 0.05	0.14	0.06		
	14	30	19.5	932		0.26	0.15	0.20		
	30	55	6.7	958	< 0.05	0.07	0.30	0.20	< 0.05	
	60	58	22.4	985		0.40	0.31	0.10		
	90	92	5.4	1015		< 0.05	0.59	0.06		
AKRA 18	7	21	17.8	919			< 0.05	0.08		0.10
	14	28	16.3	939			< 0.05	0.17		< 0.05
	30	43	14.9	1000	< 0.05		0.06	0.13		< 0.05
	60	52	11.9	1013			0.07	0.11		< 0.05
	90	68	3.5	1056			0.09	0.05		< 0.05
$KRV1_{17}$	7	27	28.0	933		0.24	0.29	0.07		
	14	31	24.0	942		0.30	0.29	0.20		
	30	46	11.0	963	< 0.05	0.12	0.42	0.10	0.05	
	60	49	20.0	996		0.47	0.48	0.10		
	90	94	3.6	1062		0.05	1.05	0.05		

Generally Ca^{2+} is shown to decrease in the solution with Na^+ emission from the glass. This can be linked to a Ca^{2+} absorption within the glass to substitute the released Na^+; it is also possible that Ca^{2+} precipitates as carbonate on to the surface of the same vitreous grains. This last hypothesis of the precipitation of $CaCO_3$ comes from the fact that the adopted Ringer solution does not contain phosphates but only bicarbonate. Consequently it is difficult to hypothesize a phosphate precipitation on the basis of the small possible extent of PO_4^{2-} coming from the glassy system.

Particularly for the AKRA 17 and $KRV1_{17}$ systems, there is, probably, a first step in which Ca^{2+} begins to emerge from the glass together with Na^+; however the phenomenon goes on only a few days, after which Ca^{2+} returns to the glass through some kind of precipitation. Such Ca^{2+} behaviour is in agreement with that verified by other authors [7] on other vitreous systems and using other spectroscopic tests. Their hypothesis concerns the formation of a calcium silicate of *para*-wollastonite type ($CaSiO_3$), fixed on the attacked glass surface.

The chromium release, which is decisive for biological acceptability [8], increases significantly with time, with the exception of AKRA 18 and AKRA 15.

Concerning the EPR analyses, the signal developing after chemical attack was attributed by us to the formation of paramagnetic sites, whose shape and width were caused by dipolar interactions with a very low exchange. This kind of interaction is typical of centres which occur at the surface due to local distorsions of the molecular network, caused by atomic substitutions such as those between Na^+ and H^+ ions. The greater intensity of the corresponding signal, observed in the samples having Fe_2O_3 amounts greater than 4 wt %, occurs because of the overlapping of the Fe^{3+} coordination signal. In fact, further additions of Fe^{3+} arrange themselves progressively in new octahedral sites [4] whose EPR signal (with $g \sim 2$) overlaps that of the said local distortions. So it may be wrong to think of observing an apparent contribution due only to the intensity of these sites. On the plots in Fig. 2 a certain correlation between the increase in Na^+ and Si^{4+} may be seen for a wide range.

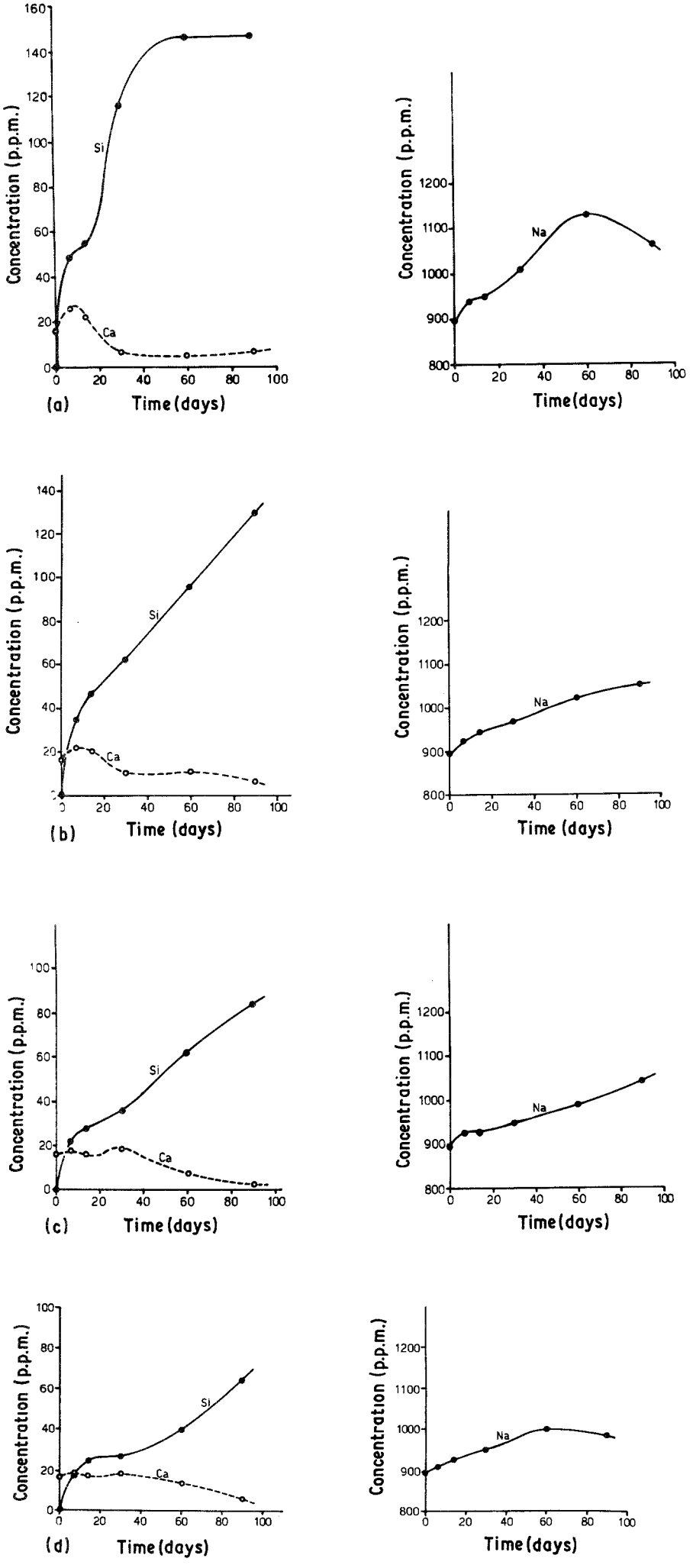


Figure 1 Silicon, calcium and sodium concentrations in the Ringer solution against time: (a) Glass system A, (b) AFE 2, (c) AFE 8, (d) AKRA 15, (e) AKRA 17, (f) AKRA 18, (g) $KRV1_{17}$.

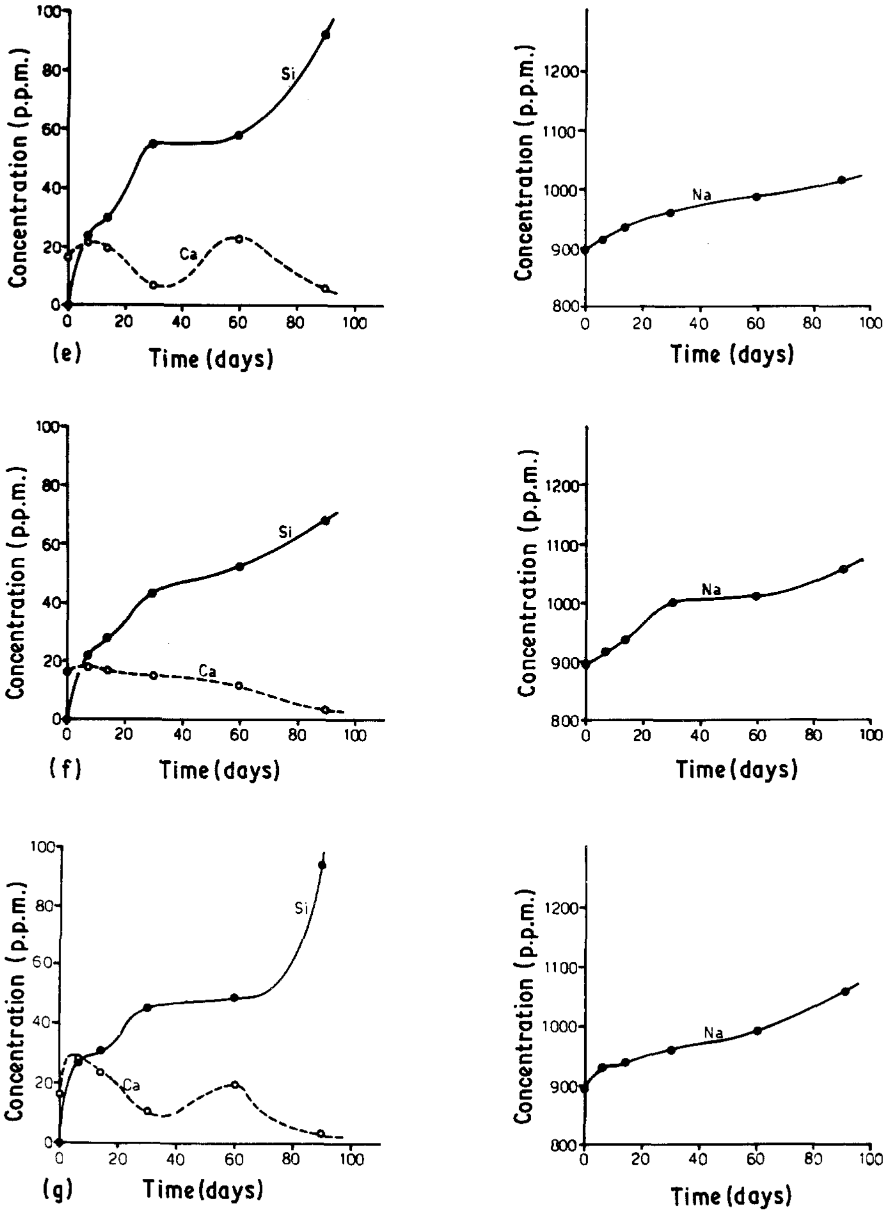
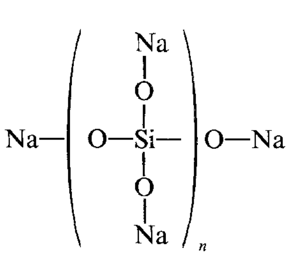


Figure 1 Continued.

In the case of doping with only Fe_2O_3 the diagrams show a straight line in the range from about 7 to 90 days. Since the slopes of this line are practically equal, it is possible to hypothesize a partial emission of sodium silicate following the exchange between Na^+ and H^+ and/or the emission of other types of sodium salts constituted by the vitreous system components, always preserving, obviously, the electroneutrality in the two systems. From slopes of the straight lines a molar ratio $Na^+/Si^{4+} = 2.4$ was deduced, that allows us to imagine the production of polymeric silicate molecules (like Na_2SiO_3). By writing the generic silicate molecule as



$= Na_2O \cdot (Na_2SiO_3)_n$

a molar ratio of 2.4 may be computed when $n = 5$.

Some correlations between Na^+ and Cr^{3+}, similar to those verified for Na^+ and Si^{4+}, are deducible for AKRA 17 and $KRV1_{17}$ samples. The existence of all these correlations seems to suggest the presence of selective leaching. Therefore it is useful to calculate the Schmidt parameter $\alpha = (Y_s/X_s)/(Y_g/X_g)$, where Y_s, Y_g are the SiO_2 moles and X_s, X_g the Na_2O ones in the solution (subscript s) and in the glass (subscript g). We arrive at values ranging from 0.005 to 0.055 (also depending upon the attack time); for such values selective dissolution is effectively forecasted [9].

From the α values it is also possible to compute the amount of silica $\varepsilon = Y_s(1 - \alpha)/\alpha$ at disposal for the formation of silica gel on the the surface of the glass grains, hypothesizing that the ratio between Na_2O and SiO_2 remains steady within the glass. The trends of ε against time are reported in Fig. 3, computed as mean values for all the glassy systems having the same system A base composition (Table I). Apart from the obvious difference between the $KRV1_{17}$ system and the AKRA ones, it is also important to look at the quite constant thickness of silica gel film formed in only about ten days.

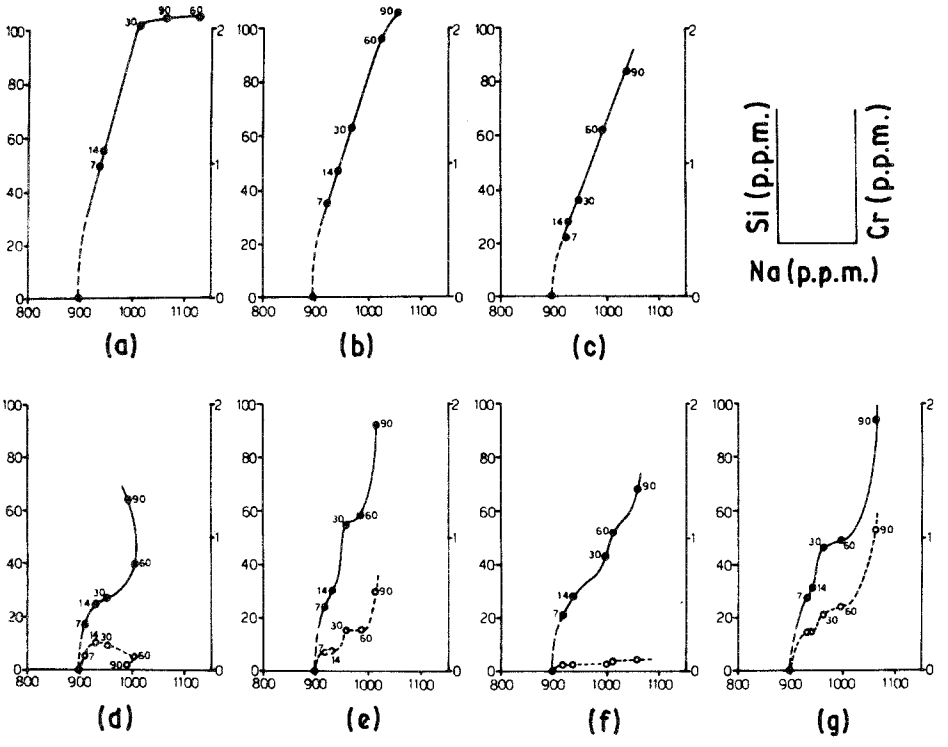


Figure 2 Correlations between (●) silicon and (○) chromium with the release of sodium. The numbers of the days of contact with the Ringer solution are indicated near the experimental points. (a) Glass system A, (b) AFE 2, (c) AFE 8, (d) AKRA 15, (e) AKRA 17, (f) AKRA 18, (g) $KRV1_{17}$.

5. Conclusion

The simulation of behaviour with time in these kinds of vitreous doped systems shows that the mineralization phenomena around bioglasses in tissues take place at an inorganic level too. The kind of simulation adopted, even though operated on a static solution, shows interesting correlations in the release behaviour of the different vitreous systems. Release rates linked to the adopted doping combination are noted. Particularly, the increase in the quantity of iron within the vitreous system involves a decrease in the release rate of the analysed ions. Furthermore, the doping agents show that it is possible to control the release rate and consequently obtain the best biological adaptability.

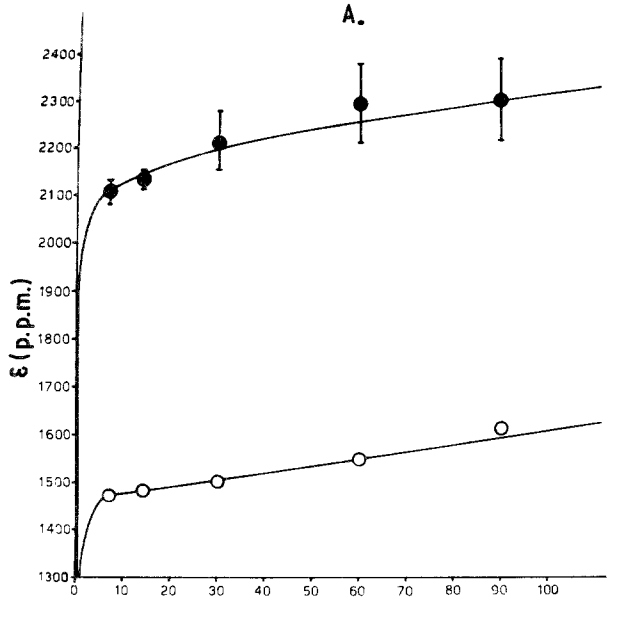


Figure 3 Computed values of ε (see text) at the prefixed expiration times. (●) Values averaged for all the samples with the same base glass A (Table II); the ε value of sample A at 60 days of treatment is indicated by the labelled point above the curve. (○) $KRV1_{17}$ samples.

Further information comes from the observed decrease in the Ca^{2+} content in the original solution. Such a decrease can be correlated with the presence of bioactive glass. The Ca^{2+} decrease was attributed to the precipitation of calcium carbonate on the surface of the vitreous grains, but the formation of calcium silicate was not excluded. It is difficult in this kind of experiment to imagine a formation of calcium phosphates, although more insoluble, due to very low concentration of PO_4^{3-} that can be acquired. In other cases, when phosphates are present in a greater amount, a precipitation of phosphates may take place, localized on the glass surface, instead of or co-precipitating with carbonates.

This simulation seems to lead to the conclusion that the hydroxylapatite layer, formed on the surface of biological glasses implanted in living bone tissue, is probably due to the precipitation of insoluble ionic salts whose components are coming from outside and not inside the glass. This precipitation is triggered by the emission of Na^+ ions from the glass.

Some information on molecular structure modifications of the glass due to this release and the ionic exchange, was verified through EPR analyses. In particular, in the attacked samples a signal around $g \sim 2$ appears, substantially attributable to unstable paramagnetic sites coming from a molecular network contraction related to the Na^+–H^+ substitution.

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