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Phase separated borosilicate glasses for dental applications : mixture design in relation with the ion release in acid condition and microstructure

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Verres borosilicatés à séparation de phase pour applications dentaires. Formulation de la composition en relation avec la dissolution des ions en milieu acide et la microstructure.

Phase separated borosilicate glasses for dental applications. Mixture design in relation with the ion release in acid condition and microstructure.

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Résumé

La recherche de matériaux dentaires efficaces est une préoccupation constante de toute l'histoire de la dentisterie. Avec l'émergence de meilleurs produits, le développement de matériaux toujours plus innovants s'est imposé. De plus, la combinaison de différentes sciences comme la chimie, la biologie, la physique et l'ingénierie a permis de mieux connaître les exigences liées à la restauration dentaire. Le projet BIODENSOL est un projet de recherche et de mobilité doctorale entre l'Université de Lyon et LUCIDEON Ltd (Stoke-on-Trent, Royaume-Uni), financé par la Commission européenne. Le projet a été conçu pour favoriser les relations entre la recherche académique et les applications commerciales, pour améliorer les innovations médicales et associer des chimistes de l'état solide avec des praticiens hospitaliers en contact direct avec les patients ayant des problèmes dentaires. Ce projet proposait trois thèses de doctorat permettant de répondre aux conséquences des caries et d'érosion de l'émail par les aliments acides, l'idée de base étant de favoriser la reminéralisation pour éviter ces problèmes. Les recherches ont évolué de manière indépendante selon trois voies distinctes examinant trois matériaux différents qui pourraient amener des solutions potentielles. Le sujet principal de cette thèse concerne l'étude de poudres de verre borosilicaté présentant une séparation de phases et pouvant être incorporées dans des ciments verre ionomères. Les verres borosilicatés sont des matériaux prometteurs qui ont été largement étudiés pour des applications biomédicales, comme par exemple les échafaudages dans les tissus mous où la réparation osseuse. Par analogie avec les verres silicatés développés par Hench en 1969, qui sont reconnus pour leur bioactivité et leurs propriétés antimicrobiennes, les verres borosilicatés pourraient intéresser la dentisterie. Le système de verre étudié ici est un verre basé sur l'association de 5 constituants, $\text{SiO}_2\text{-K}_2\text{O-B}_2\text{O}_3\text{-CaO-Al}_2\text{O}_3$, qui a une forte tendance à la séparation des phases.

L'objectif de ce travail est d'élaborer par fusion/trempe puis caractériser une série de nouvelles formulations de verres borosilicatés, puis de comprendre le mécanisme et la cinétique de dissolution en relation avec leur microstructure et leur composition. La variation des proportions de chaque constituant est déterminée par l'approche des plans d'expérience. L'utilisation d'un traitement thermique pour favoriser la séparation des phases en vue d'influencer le taux de libération cationique a été spécialement étudiée. Le processus de dissolution des borosilicates dans une solution aqueuse neutre (fluide corporel simulé) ou dans une solution acide (simulant des scénarios où des bactéries ou des aliments acides sont présents) fournit des informations sur le type et la concentration des espèces libérées par le verre. Différentes compositions ont été étudiées dans lesquelles SiO_2 et K_2O sont fixés tandis que les autres éléments varient afin d'évaluer l'effet sur la séparation de phases. Les cinétiques de dissolution des ions B-, Si-, Ca-, K- et Al- peuvent alors être reliées à la chimie du verre et à la microstructure.

La séparation de phases amorphes (APS) provoque la séparation d'une phase unique initialement homogène en deux phases ou plus de compositions différentes. Le degré d'interconnectivité des deux phases vitreuses dépend de la nature du mécanisme de séparation de phases. Les verres élaborés sont optiquement transparents, puis deviennent plus ou moins opalescents suite à un traitement thermique. Le degré d'opacité est clairement dépendant de la proportion des éléments entrant dans la composition du verre. Le schéma de la libération d'ions implique que l'une des phases est plus réactive et sensible à l'attaque ; elle sera dissoute plus rapidement du verre. L'autre phase restera dans le ciment, améliorant les propriétés mécaniques du matériau de restauration. A cet effet le mélange de la poudre de verre avec un acide poly acrylique commercial a effectivement montré des propriétés mécaniques et bioactives intéressantes. Ces résultats ouvrent une perspective de recherche prometteuse pour les applications de restauration dentaire.

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Mots clés : Borosilicates, Ciment verre ionomère, Libération d'ions, Séparation de phase, Propriétés mécaniques, Propriétés biologiques.

Abstract

The research for efficient dental materials has been a constant throughout the history of dentistry. As better materials emerged, the development of ever more innovative materials has been pushed forward. Moreover the combination of different sciences such as chemistry, biology, physics and engineering has provided better knowledge to the demanding requirements of the dental restorations. The BIODENSOL project is a European commission funded mobility research project managed by the University of Lyon and LUCIDEON Ltd, Stoke-on-Trent, UK. The project is designed to help building relations between academic and commercial research to enhance medical innovations and associates solid state chemists and hospital practitioners in direct contact with the patients with dental problems. This project proposes three PhD studies to address the problems of caries and enamel erosion by acidic foods (leading to sensitive teeth) encouraging re-mineralization to help avoid these problems. The researches independently evolved in three different routes investigating three different materials that could provide potential solutions. The main objective of this thesis concerns the study of Borosilicate phase separated glass powders for glass ionomer cements. As silicate bioglasses developed by Hench in 1969, regarding their bioactivity and antimicrobial properties, borosilicates glasses are promising materials and have been widely studied for biomedical applications for scaffolds in soft tissues and for bone repair and could be of interest in dentistry. The glass system studied here is a borosilicate glass ($\text{SiO}_2\text{-K}_2\text{O-B}_2\text{O}_3\text{-CaO-Al}_2\text{O}_3$) with a strong tendency to phase separate.

The objective of this work is to characterize a series of the novel borosilicate formulations and to understand the mechanism and kinetic of dissolution related to their microstructure and composition. The use of thermal treatment to promote phase separation as a means of influencing the rate of ion leaching was especially studied. The dissolution process of borosilicates in neutral body solution (simulating body fluid) or in acid solution (simulating scenarios where bacteria or acid foods are present) provides information regarding the type and concentration of species released by the glass. Different compositions have been investigated in which SiO_2 and K_2O are fixed while the other elements are varied in order to assess the effect on the phase separation. As the novel borosilicates produced with the melt-quenching technique is immersed in an aqueous environment, B-, Si-, Ca-, K- and Al- species are released to different degrees as a function of the time. Ion leaching trends can be related to the glass chemistry and microstructure.

It has been shown that the amorphous phase separation (APS) causes an initially homogeneous single phase to separate into two or more phases of different compositions. The degree of interconnectivity of the two glass phases depend on the nature of the phase separation mechanism. This process can occur by a nucleation and growth process which gives isolated spherical particles or by spinodal decomposition where an interconnected structure is obtained. It is significant that before a heat-treatment the glasses are optically

clear, but turn opalescent to different degrees following a heat treatment depending on the wt% of the elements in the composition. The pattern of the ion release implies that one of the phases is more reactive and susceptible to acid attack and will be leached out from the glass earlier. The other phase will remain in the cement improving the mechanical properties of the dental restorative material. Moreover, the mixing of the glass powder with a commercial poly(acrylic acid) has shown interesting mechanical and bioactive properties.

This work showed how the ions leaching are influenced by the glass composition and the heat-treatment. The study of the behaviour of the borosilicate in different acid solutions and the elements leached out could be a promising investigation for the analysis of the properties of the final dental restoration.

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Key words: Borosilicates, Glass ionomer cements, Ions release, Phase separation, Mechanical properties, Biological properties.

List of works of Lizzi Federico

Publications:

- *Mechanical characteristic and biological behaviour of implanted and restorative bioglasses used in medicine and dentistry: A systematic review* F. Lizzi, C. Villat, N. Attik, P. Jackson, B. Grosgeat, C. Goutaudier. Dental Materials 33 (2017) pp. 702-712.
- *Investigation of phase separation and ion release of borosilicate glass powders in hard acidic conditions.* F. Lizzi, C. Villat, N. Attik, P. Jackson, I. Campbell, I. Mokbel, B. Grosgeat, C. Goutaudier. Submitted.

Presentations:

- Poster presentation: *Borosilicate phase separated glasses: incidence of the addition of K, Al and Ca*
JEEP 2016 - Journées d'Étude des Équilibres entre Phases, Paris (France), 23-25th March 2016
- Oral presentation: *Borosilicate phase separated glass for GICs: incidence of the addition of K, Al and Ca.*
UKSB 2016 - UK Society for Biomaterials, London (United Kingdom), 30th June – 1st July 2016.
- Oral presentation: *Solubility of borosilicate phase separated glasses under acid conditions.*
JEEP 2017 – 43th Conference on Phase Equilibria, Barcelona (Spain), 22-24th March 2017.
- Poster presentation: *Borosilicate Phase-Separated Glasses for GICs: Ion-Release under Acid Condition.*
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- One week training at James Kent Group, Fenton (United Kingdom), 25-29th July 2016.
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- Industrial work: Setting up a method for the preparation of a novel toothpaste containing bioactive glass and production of 2 Kg of that material for an external company, 1st July - 7th August 2017.

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- Non-scientific course attended: “Histoire des sciences et épistémologie”. Course held by Philippe JAUSSAUD. 24 hours, January-April 2016.
- Scientific course attended : “Méthodologie des plan d’expérience”. Course held by Prof. Pierre LANTERI. 20 hours, January-March 2016.
- Poster presentation at Annual meeting of “Graduate School of Chemistry” at University of Lyon, 12th April 2016.
- Participation at “Fête de la Science” at the University of Lyon, 12-14th October 2016.
- Participation at “matinée LMI-MATEIS” for networking and collaboration between different laboratories. 13th April 2017.
- Followed the work of an I.U.T. student, introducing him to specialized instruments and to the researches activities. April-June 2017.
- Set up the initial organization for the Biodensol final symposium expected for the 7th of November.

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1. INTRODUCTION

1.1 Origins: from dental cements to glass ionomer cements

Dental diseases are as old as human beings. Dental cares became a need as soon as the first civilizations developed. Surely, it was very different from the dentistry that we know now, but teeth extractions and prosthesis applications have been proved to be common practices for the Greeks and the Etruscan populations. In ancient Greece, Hippocrates and Aristotle wrote about dentistry (500-300 BC) especially about treating carious teeth, while the Etruscans practiced dental prosthetics using gold crowns and fixed bridgework. During the Tang Dynasty in ancient China (618 AD) experimentations with melted silver and tin, gave birth to the first known dental amalgams. In 1728, Pierre Fauchard, often called the 'Father of Modern Dentistry' wrote 'The Surgeon Dentist' in which he explained how to fill a tooth cavity with lead, tin and gold.

The research for efficient dental materials has been a constant throughout the history of dentistry. As better materials emerged, the development of ever more innovative materials has been pushed forward. Moreover, the combination of different sciences such as chemistry, biology physics and engineering has provided better knowledge to the demanding requirements of the dental restorations.

Back in the present, dental diseases are still a major problem that affects nearly 100% of the population in the world. To preserve teeth for longer amongst an ageing population, there is a strong need to focus attention on filling carious teeth with sustainable and bioactive materials, prevention of decay, remineralisation of early lesions and minimal invasive dentistry.

Different kinds of materials can be used; among them dental cements are used for many purposes for example to attach crowns and inlays to teeth but also to directly fill cavities. The clinical application depends on the composition of the cements. They are all formed with two principal components: an inorganic powder and an acid. The reaction between these two elements is the setting reaction that brings about the hardening of the cements. They can be classified depending on the type of powder/liquid present in its composition.

Historically, the first formulation was discovered in 1855 by Sorel (Sorel 1855) and was composed of zinc oxide and phosphoric acid (Nicholson J. 1998). Actually, the zinc powder was first combined with different amounts of inorganic and organic liquids, until the use of an aqueous solution of phosphoric acid was noted as giving the best results. Sorel's cement is known as zinc phosphate cement and was fully developed at the end of the 19th century. Their properties make them suitable for luting crowns and inlays. Another material that can be classified as phosphate-bonded cement is the dental silicate cement. In this case, the powder is an ion leachable calcium aluminosilicate glass that is mixed together with a liquid very similar to the one used for the zinc oxide cements. The glass powder is composed principally of SiO_2 and Al_2O_3 that provides metal ions for the setting reaction, while other elements are used in varying amounts (some examples are shown in the tables 1, 2 and 3). The setting mechanism has been fully studied by Wilson (Wilson 1991) and demonstrates

the importance of the aluminium ions due to their role in making the glass more suitable for the acid attack. The SiO₂ network alone is very resistant to acid but, if Al₂O₃ is added, Si-O-Al bridges are formed and the different Si⁴⁺ and Al³⁺ charges provides an increasing global negative charge in the glass network. This will be balanced by other cations present in the composition (Ca²⁺, Na⁺) and the overall effect will destabilize the glass structure. H₃O⁺ ions from the acid penetrate and disrupt the glass network with the subsequent liberation of cations for the formation of the cement. The studies of the reaction mechanism and the powder composition improve the dental silicate cements as a restoration but still new formulations brings to more advanced cements. These are the ionic polymer cements that are introducing the basics principles for the most recent dental restorations. The reaction mechanism involves acid decomposable metal-oxides or aluminosilicate glasses (as reported in the tables 1, 2 and 3) and an aqueous solution of poly (alkenoic acid). The formation of cross-linked ionic bonds derived from the ions leached from the glasses lead to the use of the name glass ionomer cements (GIC). These dental cements differs from the zinc oxide known as the zinc polycarboxylate cements, and they are usually composed of calcium aluminosilicate glasses. For this reason they are also called ASPA (Alumino-Silicate PolyAcrylate). The latter are of major importance for dentistry as they provide a stronger adhesive effect towards enamel and dentin. The glass formulation is quite complex and employs ion-leaching elements as for the silicate dental cements. The first generation of this material was developed by Wilson and Kent in 1969 and it was prepared mixing the aluminosilicate glass powder with an aqueous solution of poly (acrylic acid).

Table 1. Compositions of Zinc phosphate cements (Nicholson J. 1993).

Zinc phosphate cements	Composition (%w/w)		
ZnO	89.4	90.3	88.9
MgO	3.2	9.6	8.0
Al ₂ O ₃	6.8	-	1.5
SiO ₂	0.6	-	1.6

Table 2. Compositions of the glass component in dental silicate cements (Dickson J. 1972).

Dental silicate cements	Composition (%w/w)		
SiO ₂	41.6	38.8	31.5
Al ₂ O ₃	28.2	29.1	27.2
CaO	8.8	7.7	9.0
Na ₂ O	7.7	8.2	11.2
F	13.3	13.8	22.0
P ₂ O ₅	3.3	3.0	5.3
ZnO	0.3	2.9	-

Table 3. Compositions of the glass component in modern glass ionomer cements (Nicholson J. 2009).

Glass ionomer cements	Composition (%w/w)			
SiO ₂	35.0	32.2	29.9	24.9
Al ₂ O ₃	29.7	27.3	16.6	14.2
CaO	26.2	3.0	-	-
CaF ₂	9.1	37.5	34.3	12.8
Na ₃ AlF ₆	-	-	5.0	-
AlF ₃	-	-	5.3	11.0
AlPO ₄	-	-	9.9	24.2
NaF	-	-	-	12.8

1.2 Hench's glass

At the same of time of Wilson, in 1969, Hench et al. (Hench 2006) developed a new material for medical applications; creating a solid base for the following 40 years of research in the bone/tissue regeneration field. 45S5 was the first bioactive glass generated, with a composition showing an excellent biocompatibility (fig.1). Its composition by weight is: 45% SiO₂, 24.5% Na₂O, 24.5% CaO and 6% P₂O₅. This material is able to bond with bone and stimulates bone growth due to hydroxy-carbonate apatite (HCA) formation. This type of apatite is chemically and structurally very similar to the mineral phase of hard tissues.

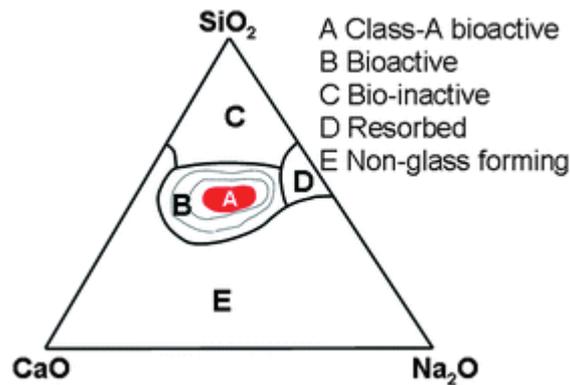


Figure 1 Triangular diagram representing the bioglass composition (Hench 2006).

The original 45S5 bioglass (BG) has been already used in different materials for repairing bone defects in the jaw and orthopaedics. Bioactive glass grafts were originally developed for replacing ear bones and alveolar bone defects around teeth; the products used were based on particles rather than a monolithic shape, i.e. PerioGlas® and NovaBone®. Micro and nano-particles have superior bioactive behaviours due to a larger specific surface area that allows a faster ion release. Bioactive coatings are likewise very important for metallic implants because they have the potential to improve their performance by providing strong bonding to the host bone and to the resin cement (Moezzizadeh M. 2017). Nevertheless,

45S5 bioglasses are applied also to non-permanent materials especially used in dentistry. BG-pastes are favourable for the treatment of dentin hypersensitivity (Bakry A.S. 2011), enamel demineralization (Bakry A.S. 2014) and for tooth bleaching (Deng M. 2013). Finally, bioactive glasses have some satisfying characteristics as a scaffold material for bone tissue engineering. However despite excellent biological properties, mainly osteoblast proliferation and differentiation induced by the released ions by the material, bioglasses are brittle materials that are easily cracked. This low strength and fracture toughness prevents their use for load-bearing implants (Thompson I.D. 1998). The development of new glass compositions with improved mechanical properties is a challenging objective and the trend is to incorporate different elements to obtain better biological and physical characteristics. Crystallinity significantly changes the fracture characteristics of glasses. This opens the way for glass-ceramics as offerings with improved mechanical properties. On the other hand, the introduction of crystalline phases could decrease the bioactivity. Several attempts have been made to preserve the amorphous structure of the glass with the addition of silver, magnesium, strontium, boron, zinc, aluminium, fluoride, potassium, gallium, barium and zirconia. Addition of silver (Balamurugan A. 2008) and boron (Liu X. 2009) have been investigated in order to improve the strength and develop antibacterial and antimicrobial materials; magnesium has stimulatory effects on the growth of new bony tissues (Dietrich E. 2008); calcium is shown to be responsible for osteoblast proliferation (Maeno S. 2005), while elements like zirconia improve the mechanical properties but decrease the bioactivity behaviour (Kasuga T. 1992).

Several families of bioactive glasses have been more precisely investigated:

- Silicate-based glasses, like 45S5, are glasses where silica (SiO_2) is the classic network-former. The basic unit is the SiO_4 tetrahedron capable of sharing up to 4 oxygen atoms with other such tetrahedral units or other elements.
- Phosphate-based glasses in the system $\text{CaO-Na}_2\text{O-P}_2\text{O}_5$ have the tetrahedral structure formed by PO_4 units and the phosphate group has a charge of 5^+ (Abou Neel E. A. 2009). Therefore, they contain at least one terminal oxygen that limits connectivity to x_3 P-O-M linkages and so the reactivity of the structure that gives unique dissolution properties in aqueous-based fluids for these types of glasses.
- Borate-based glasses (Kaur G. 2014) are based on a B_2O_3 network that can occur both in triangular and tetrahedral coordination but mostly in the triangular form for vitreous compounds. Borate glasses show potential in bone regeneration owing to a potential for complete conversion to apatite through a series of dissolution-precipitation reaction similar to those of 45S5 bioglass.

This thesis work is focused on the research of improved dental restorative materials. Since their requirements are day by day more demanding, the need for restorations with good manipulative properties and adequate working time together with the ability to set and harden rapidly once placed in position, are extremely important. Moreover, dental

restorative materials should adhere to tooth material and are required to function in the sensitive but hostile environment of the mouth. A wide range of dental materials have been formulated. One of the most important classes of materials used in dentistry is that of the dental cements. Currently, they are used in a variety of clinical dental situations such as restorative, lining, luting and sealing materials. Dental cements are based on the hardening reaction between a powdered solid and a viscous hydrogen-bonded liquid, more details of this reaction will be provided in chapter "State of the Art" of this thesis.

The 45S5 bioglass has been studied extensively by many researchers and yet still some of its properties are not fully satisfying for the final purpose, both in medicine as in dental applications. This investigation relates to formulation of a new borosilicate glass composition for enhancing the mechanical properties of a dental restorative biomaterial. The properties of this class of glasses falls in between those for silicate and borate glasses and they could be an ideal candidate to avoid problems encountered with the materials studied so far. Moreover, the tendency of the borosilicate glasses to phase separate is hypothesized as extremely important in regulating the ions dissolution. This phenomenon (that will be explained in details in the chapters of the thesis) causes a homogeneous single amorphous phase to separate into two or more amorphous phases of different compositions. The two phases will act differently in distinct chemical environments. One of the phases will be more reactive and susceptible to certain conditions such that it will be dissolved from the glass earlier; potentially delivering ions useful for the cells stimulation (Deng M. 2013) or remineralisation over time. The other phase will largely remain in the cement improving the mechanical properties of the dental restorative material. However, this phase may release re-mineralising ions over time, especially if exposed to acidic conditions provided by food / bacteria.

The study will also help the understanding of the reaction mechanism of the dental cements. The dissolution of the borosilicate glass is a fundamental factor that is strongly related to the ion release and the biomaterial behaviour. Finally, new ideas and new developments of the bio-glasses can be assumed by this work that has as a final objective the innovation of the dental cements in the frame of the BIODENSOL project.

1.3 BIODENSOL project

BIODENSOL is a European commission funded mobility research project (FP7-PEOPLE-2013-ITN) managed by the University of Lyon and LUCIDEON Ltd, Stoke-on-Trent, UK. BIODENSOL focuses on developing novel bioactive materials for dental restoration and regeneration. This project proposes three PhD studies to address the problems of caries and enamel erosion by acidic foods (leading to sensitive teeth) encouraging re-mineralization to help avoid these problems. The researches independently evolved in three different routes investigating three different materials that could provide potential solutions. These materials are: Phosphate glasses as fillers in dental scaffolds, Mesoporous bioactive glasses

(MBGs) as fillers in dental adhesives and finally the objective of this thesis that is Borosilicate phase separated glass powders for GICs.

The project is designed to help building relations between academic and industrial research to enhance medical innovations. For this reasons the studies were divided between the two locations: University of Lyon and Lucideon Ltd in Stoke-On-Trent, with a displacement every 6 months for a total of 18 months in each site. The advantage was to work on the PhD project in two different environments the University and the industrial one. It was also relevant for the experience to be involved in external activities related to the industry (working on side projects with ceramics materials in this case) and be familiar with commercial jobs.

1.4 Targeting an ideal composition

In the scientific literature, it is very hard to find an article that deals with the issue of how the properties of the cement correlate with the glass composition. Establishing composition-structure-property relationship of the cements could improve knowledge of the material and help in the design of new and improved glass ionomer cements. Understanding and being able to control the degree of crosslinking of the glass particles within the polysalt matrix will enable control over cement properties and so the development of glass polyalkenoate cements with improved properties. The concentration of ions released into solution will determine the ions available for the cross-linking and so the set cement properties. A literature review gives some important information to guide the choice of the elements in the novel glass composition arises.

As previously mentioned, the setting reaction is known to involve the acid hydrolysis of the glass network, leading to the release of aluminium and calcium cations (Nicholson J. 1998) which react with the carboxylic groups of the polyacid resulting in the hardening process of the cement. This reaction can be influenced by the other elements present that can cause, for example, impediments for the cross-linking with the polysalt matrix.

In fact, in commercial glasses, such as G338 (tab.4), there are significant quantities of phosphorus, fluorine and other alkali metals that are interfering the hardening process.

Table 4 Composition of the commercial glass G338 (Nicholson J. 2009)

Component	%w/w
SiO ₂	24.9
Al ₂ O ₃	14.2
AlF ₃	11.0
CaF ₂	12.8
NaF	12.8
AlPO ₄	24.2

The fact that AlPO_4^- species are present in the cement matrix, as demonstrated by Kent and Wilson (Kent B. 1969), provides evidence that phosphate competes with the carboxylate group for the cross-linking cations. The introduction of phosphate can also enhance the glass degradation (Ray 1978) as already well-known for the easily degraded phosphate glasses. In the case that the PO_4 tetrahedra are not charge-balanced by an AlO_4 tetrahedra, one of the oxygens attached to the phosphorus will form a double bond which cannot be resonance stabilised leading to hydrolytically unstable phosphorus oxygen bonds.

Aluminium contained in glass filler particles was deemed to be an important factor for cement setting ability (R. Pires 2009, Munhoz T. 2010) but it was found that the role of that component and its ratio with silicon it is not so significant (Griffin S. 1999). Actually the presence of both aluminium and phosphorous inhibits the number of cations available for the cross-linking. Aluminium release has been cited as causing a mineralisation defect thereby inhibiting the action of osteoid cells in glass polyalkenoate cements intended for orthopaedic applications (Carter D. 1997). Aluminium is also recognized as a neurotoxin and the cause of some neurological diseases, such as Alzheimer's and Parkinson's diseases (Zatta P. 1999). The results of this study are therefore important in relation to the design and development of low aluminium or aluminium-free polyalkenoate cements. It is a challenging goal because of the positive role aluminium plays in the hardening process but several attempts has already been made substituting the aluminium with other ions (Zn^{2+} , Mg^{2+} , Sr^{2+} , Ce^{3+}) as explained in the “background and information” chapter.

Sodium will compete with the other cations to bind with the carboxylic groups. Fluorine, despite its positive role in inhibiting caries, can influence the crystallization of the glass system during the melting process if present in sufficient quantities (Griffin S.G. 2000).

The melt-quenched borosilicate glass presented in this study is a novel composition. It is composed of: $\text{SiO}_2\text{-B}_2\text{O}_3\text{-K}_2\text{O-CaO-Al}_2\text{O}_3$. Each element introduced has a specific individual role, but the overall compositions have been varied in a way that enables the effect of two or more element combinations to be established. Therefore, multiple analyses were carried out to investigate the effect of the Al:B ratio for the degree of phase separation and of the B:Ca ratio for the influence on ions leaching.

In the borosilicate system, silicon is known to be essential for metabolic processes associated with the formation and calcification of bone tissue (Carlisle 1981). In the early stages of bone matrix calcification, high silica contents have been reported and this will cause a precipitation of hydroxyapatite (Damen J.M. 1992) resulting in the enhancement of bone formation. It is not a very soluble ion and it can remain longer in the dental restoration probably affecting the mechanical properties of the material in which it is applied. The borosilicate comprises boron too, that helps induce the phase separation and the creation of chemically durable and non-durable phases. Furthermore boron possesses very interesting properties. It is thought to play a regulatory role on other elements such as calcium (also present in the borosilicate glass) and hence a role in bone metabolism (Naghii M.R. 1993).

Boron has in fact been linked with preventing calcium loss and bone demineralization in post-menopausal women and boron supplementation has been shown to reduce the effects of vitamin D deficiency in chicks (King N. 1991). Boron also affects bone regulating hormones involved in bone growth and bone turnover (Palacios 2006). In a recent publication, it was reported that the addition of B_2O_3 (5–10 mol%) to calcium phosphate glass systems increased the tensile strength from ~450 MPa to ~1200 MPa (Sharmin N. 2014). It has also been reported that the incorporation of B_2O_3 (up to 5mol%) to the same glass system showed favourable cell metabolic activity, proliferation and morphology (Sharmin N. 2013). The antimicrobial effect of this element is gaining much more attention in the research articles as proved by the studies of Prasad et al. (M.P. Prasad 2014) and of Gorriti et al. (Gorriti M. 2009). Finally due to the use of boric acid in poly(vinyl) adhesive for improving the thickening action it is hypothesized that boron could have an essential role in the cross-linking of the cement. For this reason attempts at substituting boron with aluminium have been investigated in this work. The concentration of aluminium was generally maintained at a low level in glasses compositions with some even designed without aluminium.

Potassium is considered a useful desensitizing agent (Matis B. 2007). It is also useful to reduce the tendency of a glass to crystallize (note that crystallisation can bring about a lower biocompatibility). A new glass composition, inspired by the 45S5 Bioglass[®], was formulated by substituting the sodium oxide with potassium oxide and the results proved that the thermo-mechanical properties, as well as the in vitro response of the two glasses were comparable (Cannillo V. 2009). Due to the difference in ionic radius the ion release increased with increasing potassium for sodium substitution causing changes in the packing of the glass network, described by glass molar volume and oxygen density (Brukner R. 2016). The choice of an alkali ion of larger ionic radius (K for Na in this case) and also the lower electronegativity was employed to expand the borosilicate network, allowing a faster ion release.

Calcium, as one of the main components in the biological mineral phase apatite ($Ca_{10}(PO_4)_6(OH)_2$), obviously plays an essential role in bone formation and resorption. Low calcium concentrations are suitable for osteoblastic proliferation, differentiation and extra-cellular matrix (ECM) mineralization (Maeno S. 2005). Moreover, calcium plays an important role in bone remodelling by directly activating an intracellular mechanism in osteoblastic cells (Marie 2010). As a network modifier, it will influence the glass structure; nevertheless it is important to know the specific concentration of this ion when released into aqueous media as it is implicated in the mechanism of interaction with the polyacid for the cement formation.

This manuscript will explore the subject just introduced and will analyse every single aspect in detail following this structure:

- The first part called State of the Art will refer to the most recent development in this specific scientific field. There will be a complete description of theoretic concepts related to the glass structure and how they are related to the dental application.
- The second part called Materials and methods will describe the techniques and methodologies employed to explore the mixture design, the elaboration and characterization of the glasses in the system $\text{SiO}_2\text{-B}_2\text{O}_3\text{-K}_2\text{O-CaO-Al}_2\text{O}_3$
- The third part is related to the analysis and discussion of the results obtained. Three main aspects are here identified. The elaboration of the borosilicate glasses and their characterization, the experiments on the ions leaching and surface microstructure and finally the mechanical and biological properties of the potential restorative material.
- The conclusion will finally resume the main founding and limitation of the research proposing solution for future studies.

2. STATE OF THE ART

2.1 Mineral glasses

One definition of glass is as follows:

“A glass is an inorganic product of fusion which has cooled to the rigid condition without crystallizing” (1945, American society for testing materials)

This definition can be regarded as incomplete, because glasses can be obtained using other techniques like sol-gel processing. For this reason a glass can also be defined as “a non-crystalline solid that exhibits the phenomenon of glass-transition”, to include a wider range of amorphous materials.

In this thesis, glasses were obtained with the melt-quenching technique, so the material can be defined with the first definition. The technique is the most ancient method for fabricating glasses and will be discussed in details in the next chapters.

2.1.1 Glass structure: The random network theory

This theory so called Zachariasen model, considers glass networks to be composed of polyhedra of the same type found in crystals but arranged randomly between each other's, basically giving a structure with no long-range order. X-ray diffraction on glasses confirms the fact that no crystal peaks are detectable and a characteristic halo is only observable. Zachariasen developed his theory by observing that the mechanical properties of an inorganic glass are quite similar to its corresponding crystal form. This led him to hypothesize that the internal forces generated by the structure should be approximately similar.

The oxides forming the glass can be of two different types: network former or network modifiers. To be part of the first group they have to fulfil some criteria:

1. An oxide tends to form a glass if it easily forms polyhedral groups as the smallest building units.
2. Oxygens and others anions (such as S^{2-} , F^-) should form linkages with no more than two Metal atoms.
3. Polyhedra must share only corner with each other, not edges, not faces.
4. For a three-dimensional network at least three corners of each polyhedral must be connected with neighbour polyhedral.

The network formers are providing the strong part of the glass, it is possible to see them as the skeleton of the glass network. The glass formers are elements of high ionic potential forming very strong bonds with the oxygen. They are usually Si, B, P, Ge, As, Be oxides with a coordination number of generally 3 or 4. Oxides with the formula MO and M_2O are not satisfying these criteria and they are actually not able to form a glass alone. These kinds of oxides are called network modifiers and they are alkaline earth oxide and alkali metal such as: Na, K, Ca and Ba with coordination number generally bigger than 6. These elements are

generally of relative low ionic potential. The addition of the modifiers oxides result in breaking the Si-O-Si bond (in silicate glasses) with the formation of two Si-O⁻ species that are counterbalanced by the presence of the cations from the modifiers oxide that preserve the neutrality of the overall structure (figure 2). In the figure it is possible to observe the effect of Na⁺ in altering the glass structure. The degree of polymerisation (proportional to the number of Si-O-Si bonds) is reduced causing a lower melting point, higher thermal expansion and a lower viscosity (the addition of the modifiers make the glass manufacturing easier). The glass is also more susceptible to acid attack resulting in the exchange of the modifying cations with the protons of the acid solution.

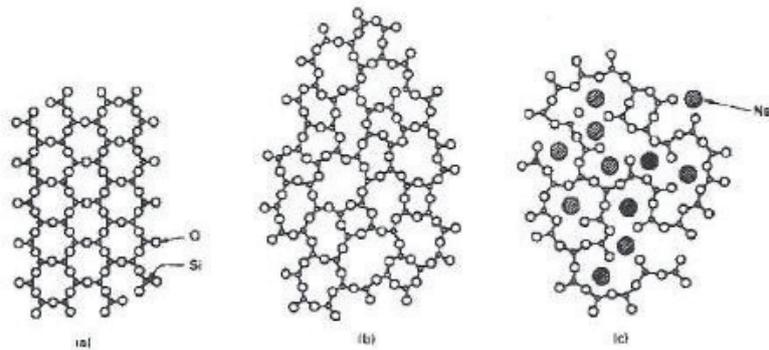


Figure 2. a) Crystal structure of Silica, b) Amorphous structure, c) Amorphous structure with the addition of modifiers oxides.

2.1.2 Amorphous phase separation

Some glasses are not homogeneous (at least less homogeneous than others if it is considered that all glasses present a little inhomogeneity in their structure). The glass system could be composed of two or more non-crystalline phases derived after the phase-separation. This phenomenon brings to the formation of a glass with different physical and chemical properties and also with a different glass appearance that will change depending on the process and type of phase separation. In 1979, Barry et al. (Barry T. 1979) noted that the ionomer glasses are separated in two phases, one of which have more affinity for the acid and will be attacked earlier than the other.

In the liquid phase, phase separation can occur above or below the liquidus temperature. In the first case it is called stable immiscibility (like for example between oil and water) while in the second case the separation is known as metastable immiscibility (fig.3).

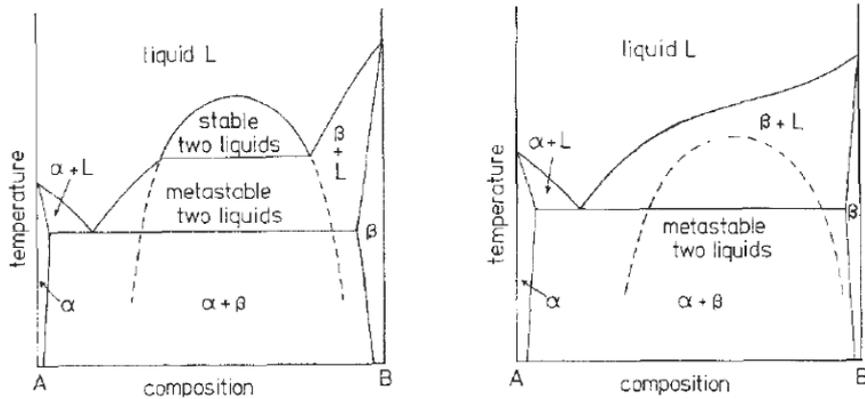


Figure 3. Stable and metastable two-liquid immiscibility and subliquidus metastable immiscibility (James 1975)

When the glass enters in the immiscibility region on cooling or by heating at appropriate temperatures, the rates of diffusion are so low that there is insufficient time for a visually detectable separation to be formed. For this reason the phenomena can be seen thanks to the electron microscope in the majority of the cases. Sometimes the separation can be made coarse enough for the glass to become opalescent. Two or more phases can have a different refractive index inducing the light scattering that cause the colour changing. The appearance of the glass can be also due for two types of separation that can occurs. The study of the microstructure of the phase separated glasses make it possible to determine two different mechanisms that explain the phenomena: one corresponds to the formation of a continuous and interpenetrating phase into the other and the second occurs as a result of the second phase comprising isolated droplets. The first is called spinodal decomposition while the latter nucleation and growth (fig. 4).

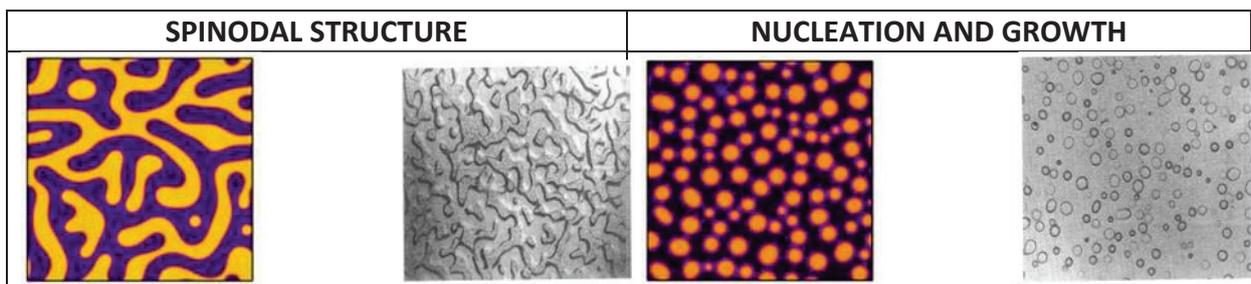


Figure 4 The two different mechanism of phase separation: spinodal structure and nucleation and growth (Vogel 1985).

The phase separation is a thermodynamic phenomenon that is strongly related to the free energy of the system (fig. 5). When different compounds are mixed the total free energy (ΔG_{mix}) is related to both enthalpy and entropy by the equation: $\Delta G_{mix} = \Delta H_m - T\Delta S_m$. Entropy is related to the system disorder; after mixing the system becomes more disordered and ΔS_m always increases, causing ΔG_{mix} to become more negative. If the enthalpy is also negative, as shown in the figure, the ΔG_{mix} is much more negative and the mixing is the most favoured process as the free energy is reduced. If the enthalpy is positive, the mixing is unfavourable and the system will tend to separate into the different compositions. ΔH_m is related to the

bond energy of the mixture, it will be positive if the bonds formed are less stable than the bond of the singular composition before the mixing. Phase separation will occur because the free energy is reduced by the demixing process.

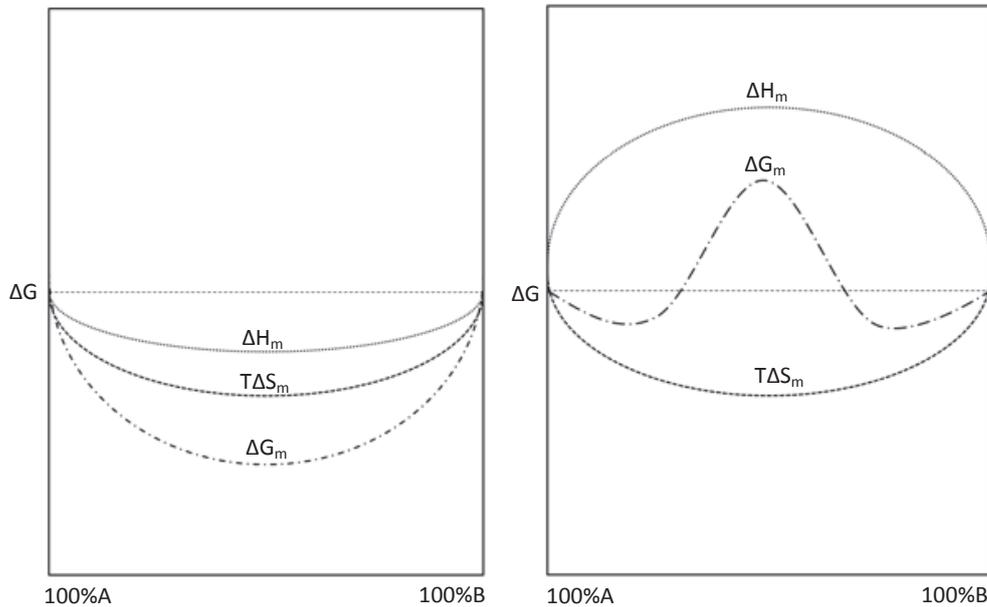


Figure 5. Relationship between the variables that define the free energy in a system of two components.

The two different mechanisms of phase separation are explained from a thermodynamic point of view in figure 6. In the graph is represented the immiscibility dome related to the changes in free energy. It is possible to distinguish the area between the point X_3 and X_4 in which the system will spontaneously separate into a spinodal structure. This because any fluctuation of the binary components would result in a reduction of ΔG and the separation can occur without an activation energy. In outer regions, between X_1 , X_3 and X_4 , X_2 , any small fluctuations in the components would cause the free energy to increase and thus result in a disadvantageous situation. The phases will tend to be separate and in that case the nucleation and growth process will bring to the formation of a phase separated droplet structure in which one phase will totally surround the other composed of small nuclei. The temperature is an important factor that strongly influences the phase separation. At low temperature the limits of the immiscibility dome are wide apart from each other, but when the temperature increases they become increasingly close together eventually reaching T_c which is the consolute temperature at which the components of the binary mixture are miscible in all proportions.

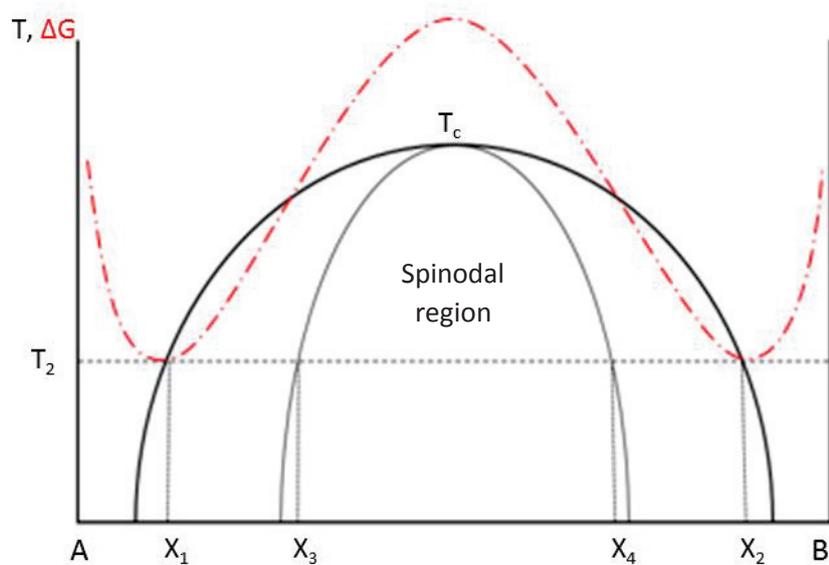


Figure 6. The study of the free energy (red line) trace out an immiscibility dome (black line) related to the temperature that evidence the spinodal region.

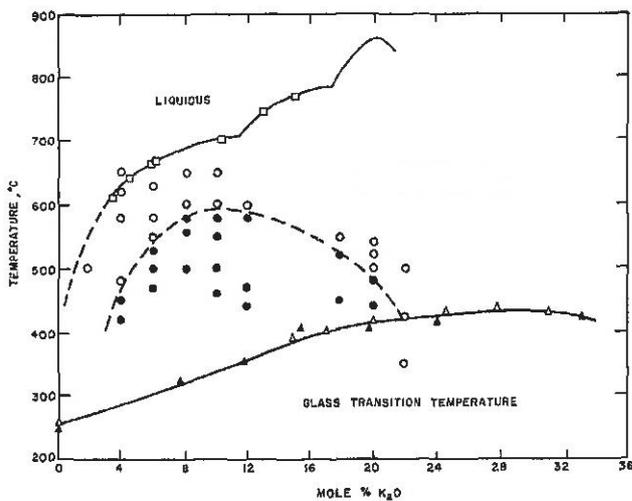
It has been known that borosilicates can be phase separated below the liquidus temperature. Thanks to this property this glass system is widely used in industry and the most well-known product that makes use of the phenomenon is Vycor glass. It is probably also one of the first applications to exploit phase separation and is used for creating silica ware by a porous glass route. Hood and Nordberg (H. Hood 1938) developed a process to phase separate a glass composed of $\text{Na}_2\text{O}-\text{B}_2\text{O}_3-\text{SiO}_2$ choosing a composition near the centre of the immiscibility dome to create the spinodal microstructure after a thermal treatment at 600°C . The resulting glass is composed of a silica-rich phase and a sodium-borate-rich phase that are interconnected. The chemically weaker second phase is easily dissolved under acidic conditions. For that reason, after the glass has been heat treated it is immersed in an acid bath to cause the complete dissolution of the most soluble phase. The Vycor glass at the end of the process is a 96% pure SiO_2 porous glass that can be used as said before for silica ware but also for the preparation of specialised diagnostic products, for the immobilisation of enzymes in fixed-bed reactors and for filters employed in protein separation. Basically they can be used whenever porous glasses for filtration include rigidity, chemical inertness, high temperature capability, superior thermal shock resistance and controlled micro-porosity are needed.

A range of different compositions in the $\text{R}_2\text{O}-\text{B}_2\text{O}_3-\text{SiO}_2$ system, where $\text{R} = \text{Na}, \text{K}$ or Li , exhibit phase separation when heat-treated with an appropriate thermal treatment, each phase being continuous and presenting the spinodal structure.

If two phases are thermodynamically stable above the melting temperature we have stable phase separation known as liquation. If the two phases exist only below the liquidus then

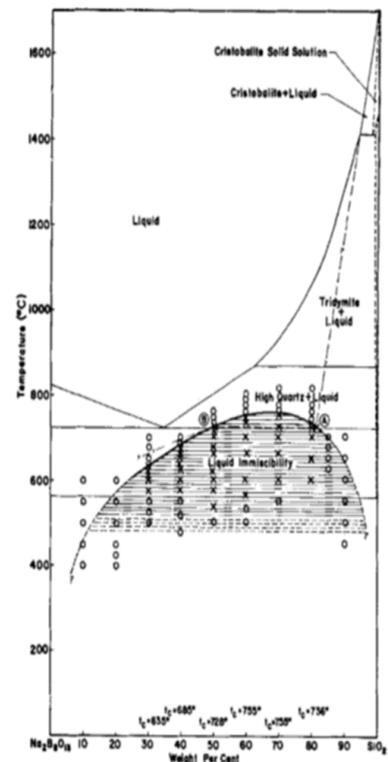
we have metastable phase separation (fig. 7). In the last case a single phase glass can be obtained by rapid cooling. Subsequent heat treatment of this glass at a lower temperature than the one used for melting can then bring about phase separation. In the borosilicate system typically metastable phase separation takes place without exhibiting liquation. Progressive separation upon heating between the T_g and liquidus temperatures results in one of two mechanism of separation: 1) Isolated droplets of one phase in the prevailing matrix by nucleation or 2) spinodal interconnected structure (Hlavac 1983). The temperature of the heat treatment was selected after consulting the literature for different glass systems exhibiting phase-separation and the focussing on sodium-borosilicate behaviour.

a)



- Above phase boundary
- Below phase boundary

b)



c)

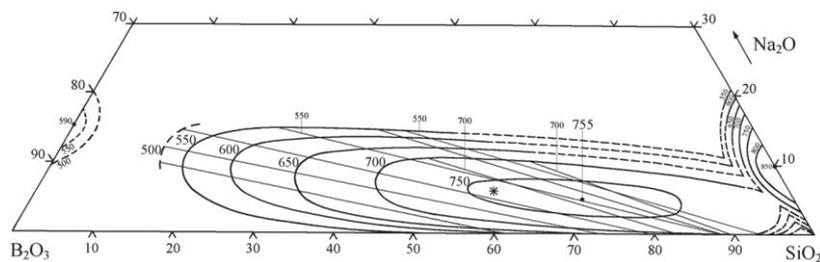
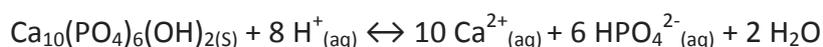


Figure 7 Phase diagram showing subliquidus miscibility gap for different systems: a) B_2O_3 - K_2O (Shaw R. 1968) b) $Na_2B_4O_{13}$ - SiO_2 (Rockett T. 1965) c) Na_2O - B_2O_3 - SiO_2 . (Rockett T.J: 1965)

Atsuo et al. (Yasumori 2013) reported spinodal phase separation in the CaO-Al₂O₃-SiO₂ ternary system but did not describe compositions or heat treatment regimes. The CaO rich phase was easily leached. Moawad (Moawad M. 2007) investigated phase separation in 45S5 glass, producing spinodal separation by heat treatment at 670°C (a temperature very close to that chosen in this study). Samples were then leached in 1M HCl at 85°C with 50ml of acid employed per gram of sample. As previously stated, Rafferty (Rafferty A. 2000) studied phase separation in ionomer cements of the generic composition SiO₂-Al₂O₃-P₂O₅-CaO-CaF₂. Glasses were shown to separate into two phases with an optimum nucleation temperature around 700°C.

2.2 Bioactive glasses

Usually, when we consider the word “glass” we rapidly connect it with common objects like windows, drinking vessels, optic instruments or at least artistic artefacts. In reality, this material known since thousands of years, ancient as much as the Egyptian civilization has evolved and used in always more advanced applications. Especially in the second half of our century, the study of glasses brought to apply this material in an environment that not so easily can cross our mind: the human body and especially in the mouth. The oral environment is very aggressive and poses many challenges to restorative materials due to a large variety of factors. Acids are generated by bacteria in plaque following the metabolism of fermentable carbohydrates derived from foods and drinks, causing erosion processes. An acid commonly found under these conditions is lactic acid, a breakdown product from bacterial attack on sugars. These lactic acid solutions may have a pH of 4.0 or less, whereas human saliva typically has a pH in the range 5.5-7.5. Whilst saliva can be useful as a buffer solution, sometimes it is over-powered. Moreover the human saliva creates a wet environment that adds a difficult circumstance to the restorations. The mineral component of the tooth enamel is calcium hydroxyapatite with the formula Ca₁₀(PO₄)₆(OH)₂. Defects and caries are caused by the dissolution of this material at low pH. A natural re-mineralization caused by the ions in the saliva make the following reaction exists as an equilibrium:



Finally abrasion and attrition are generated by physical effects. Restorations are expected to last many years while being subjected to many different mechanical forces and chemical attack.

As explained in the introduction, was Professor Larry Hench from University of Florida that introduce the concept of bioactive glass after his discovery of the 45S5 glass in 1969. The use of glass for biomedical applications is due to the active role in the body, stimulating the natural healing of hard and soft tissues. The glass network, with its degradation in the body environment, will provide useful ions for tissue regrowth and the active stimulation of cells

for the production of new tissue. This will also help in forming a strong bond between the biomaterial and the living bone tissue providing long lasting implants.

Bonding to bone and tissues is well documented and demonstrated by a large series of bioactive glass compositions (Gerhardt L. 2010, A. Macon 2015). The mechanism of bone bonding enables the bioglass to develop an adherent interface with tissues that resists substantial to mechanical forces. In many cases, the interfacial strength of adhesion is equivalent to or greater than the cohesive strength of the implant material or the tissue bonded to the bioactive implant. Five steps have been described for bone-bonding mechanism(Jones 2013):

- Step 1: fast release of Na^+ and Ca^{2+} exchanged with the H_3O^+ ions present in the solution, causing the hydrolysis of the silica groups and a rapid increase of the pH.
- Step 2: network silica is attacked by hydroxyl group causing the formation of $\text{Si}(\text{OH})_4$ in the solution.
- Step 3: Silanols ($-\text{Si}-\text{OH}$ groups) form a silica rich layer on the surface thanks to re-polymerization reactions.
- Step 4: Ca^{2+} and PO_4^{3-} migrate to the just formed silica surface forming a $\text{CaO}-\text{P}_2\text{O}_5$ film on top.
- Step 5: $\text{CaO}-\text{P}_2\text{O}_5$ film crystallize and incorporate other ions from the solution (such as OH^- and CO_3^{2-}) will form a HCA layer.

The hydroxyapatite (HA) layer can be formed on the surfaces of biomaterials in an acellular and protein-free solution called simulated body fluid (SBF). The HA formed is very similar to the bone mineral in its composition and structure. The evaluation of HA-forming ability on implant materials in SBF is useful for evaluating its in vivo bone-bonding ability. Applied in glass ionomer cement could improve their bonding within the tooth. When a bioactive material is implanted in a living body, a thin layer rich in Ca and P forms on its surface. The material then connects to the living tissue through this apatite layer without a distinct boundary and providing a strong bond. With this bioactivity test this hydroxyapatite layer can be reproduced on the surfaces of materials in SBF and the formation of HA can be evaluated with scanning electron microscopy.

The five stages previously described are illustrated in figure 8. A rapid release of soluble ionic species from the glass into the interfacial solution induces surface reactions. A high surface area-hydrated silica and polycrystalline hydroxy carbonate apatite (HCA) bilayer is formed on the glass surface within hours in a 45S5 glass. The bioactivity is obtained because the reaction layers enhance the adsorption and desorption of growth factors. The macrophages are attached to the surface and their role is important because they are required to prepare the implant site for tissue repair. Finally stem cells and synchronized proliferation and

differentiation of the cells act on the surface of bioactive materials to create new bone (Boyd D. 2005).

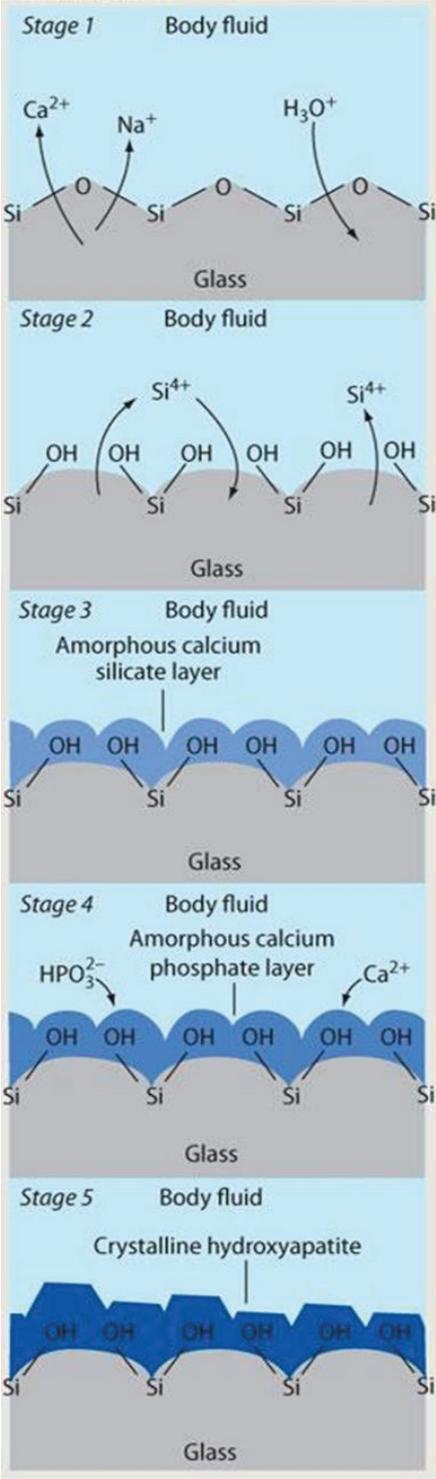


Figure 8 Five step mechanism of HA formation on the surface of a melt-quenched bioactive glass.

2.3 Glass Ionomer Cements (GICs)

Since their development during the late 1960s, glass ionomer cements (GICs) have been of great interest as dental restorative material. Several features such as good adherence to dentin, good biocompatibility and proper working and setting time make GICs the ideal material for dental restorations.

GICs are set through an acid-base reaction between a mineral phase and a weaker soluble polymer. Typically, an aqueous poly(acid), such as poly(acrylic acid), reacts with an ionomer glass. The glass composition is designed to be degraded by the relatively weak acid causing the formation of ionically cross-linked acidic polymer chains between the multivalent counter ions leached from the glass and the carboxylic group of the poly(acid). The result is a self-hardening process that ends up with a hard dental cement.

2.3.1 Composition and chemistry

GICs were invented by Wilson and Kent in 1969 at the Laboratory of the Government Chemist in London, United Kingdom. These first materials had very poor properties compared with the ones available today.

Setting occurs by an acid base reaction that involves the carboxylic group of the polymer crosslinked by the multivalent counterions leached from the glass. The hydrogens on the carboxyl groups of the poly(acid) are progressively replaced thanks to an exchange mechanism, which regulates the hardening process. The glass leaching is also an important factor to consider because it determines the ions present into the solution. The ionomer glasses are complex, and often contain two phases, either completely or partially separated (R.G. Hill 1988). The setting reaction is also very complicated including different factors, and involves not only the formation of ionically cross-linked acidic polymer chains, but also hydration processes and long-term effects caused by slower reactions occurring also after the initial setting process.

The setting mechanism has been intensively studied but there are still different interpretations of the overall process. M. Khoroushi in her review (Khoroushi M. 2013) divides the reaction in 4 stages:

1. Decomposition of the glass: where the glass particles are attacked by the acid provoking the release of the metallic cations and the formation of a silica gel layer at the surface of the particles.
2. Gelation: The ion exchange mechanism decreases the proton concentration in the solution resulting in a pH increase. This condition promotes the ionization of the carboxylate groups that induce the uncoil of the poly(acid) chains. The linear configuration brings them to react quickly with the metallic cations. This causes the increase in viscosity that is the main effect of this stage.

3. Hardening: the cross-linking of the polymer chains by the cations leads to the hardening of the cement. At the end of this stage the material will be formed by leached glass particles surrounded by the polysalt matrix containing cross-links.
4. Maturation: the reaction continues after the setting. Ions are still released from the glass but also directly from the cement to the tooth and the buccal environment. In this stage the mechanical properties increase (due to the stabilization of the inter-molecular forces) although much of the strength is achieved at 24 hours of reaction.

According to Sidhu et al. (Sidhu S.K. 2016) the reaction mechanism take place in two main steps.

1. Formation of ionic crosslinks, resulting from the glass releasing ions such as Ca^{2+} , Na^+ , and this is responsible for the immediate hardening process
2. A second step that is still a crosslinking process, this time involving Al^{3+} ions. It is very slow and takes approximately one day to finish.

These two steps are followed by further reactions that collectively account for the process "Maturation". They are associated with the increase of strength as also reported in the previous interpretation.

It is possible to notice that the main aspects of the reaction mechanism are pretty well described, but the details about the ions involved in the process are still unclear. There is much research going on in this field and lots of data can be consulted to better clarify.

The setting reaction involves, in the first common stage, the acid hydrolysis of the Si-O-Al bonds of the glass network that leads to the release of aluminium and calcium cations as described in fig. 9.

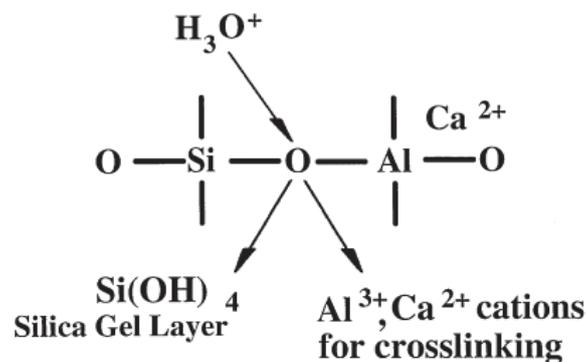


Figure 9. Hydrolysis of silicon-oxygen-aluminium bond.

Early studies demonstrate that aluminium and calcium (as major constituents) were the first ions to be leached out from the glasses (Crisp S. 1974). Although spectroscopy measurements revealed that the initial setting resulted in the formation of only calcium poly(acrylate). This compares with the formation of aluminium poly(acrylate), which is delayed by one hour. This was postulated to be due to differences in morphologies between the Ca^{2+} and the Al^{3+} ions. The aluminium ion, which is fully hydrated, was seen as less

mobile than the calcium ion. In more recent studies ^{27}Al MAS-NMR spectroscopy has been employed to better investigate the transformation of the aluminium cations occurring during setting. Muñoz et al (Munhoz T. 2010) show that in the initial glass, aluminium is mostly present in a four coordination or tetrahedral state, Al(IV), and it switches to a six coordination or octahedral state, Al(VI), when crosslinking the polymeric chains. It is interesting to observe that the conversion of aluminium is largely complete between 1 and 6 hours of ageing. For that reasons calcium ions are the first leached ions to be part of the setting mechanism.

Aluminium has also been shown to be released from conventional glass ionomer cements after the setting reaction. This is a cause for concern as there are several well-known toxic effects in humans, including adverse effects on the central nervous system, skeleton and hematopoietic system.

Usually three principal components form the base composition for the ionomer glass in GICs: SiO_2 , Al_2O_3 and CaO . In addition the glasses can contain P_2O_5 and Na_2O , resulting in a composition very similar to the one of Hench's bioglasses. Moreover CaF_2 can be added as a source of fluoride - very important for teeth remineralisation. Phosphorous, fluorine and alkali metals tend to inhibit the crosslinking process. Phosphate groups and fluoride ions will both compete with the carboxylate groups for the crosslinking cations. Sodium ions will compete with aluminium and calcium for the crosslinking with the carboxylate groups. The sodium is poor at cross-linking compare to ions with higher valence number, this avoid the multiple ionic bound with different carboxyl group. In figure 10 is show how Ca^{2+} ions ionically cross-links two carboxyl groups.

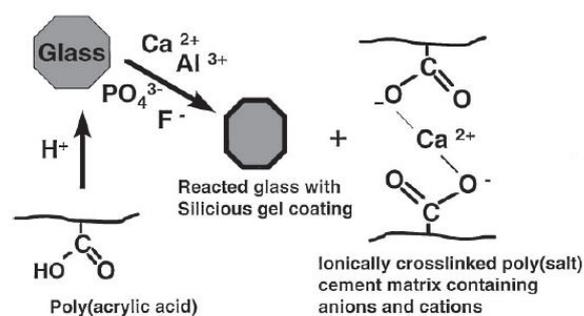


Figure 10. Setting reaction diagram with the principal ions involved in the reaction.

2.3.2 GICs properties

The characteristics of clinical relevance in a GIC include adhesive properties to tooth structure, biocompatibility, fluoride release and a low thermal expansion coefficient that is similar to that of the tooth. The chemical adhesion of GIC to enamel and dentin is achieved

by the displacement of phosphate ions in the enamel surface, by carboxylate groups from the polymer chain (fig. 11). Electrical neutrality is maintained by the displacement of calcium ions with the phosphate ions (Wilson A.D. 1983). During the maturation process its ionic exchange continues and enhances the bond strength with dentin and enamel.

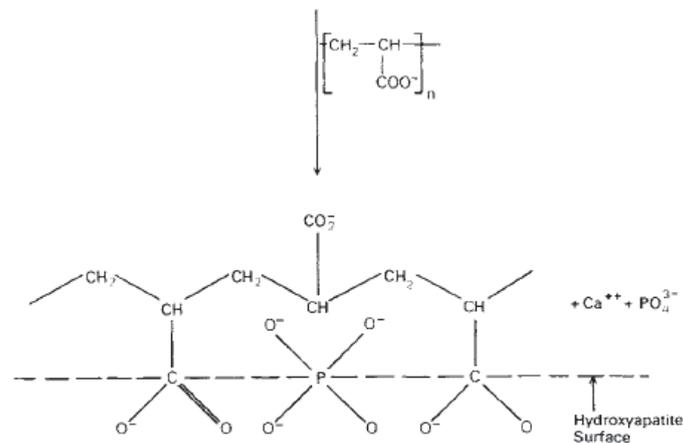


Figure 11. Schematic representation of the adhesion mechanism of ionomer cement to the hydroxyapatite surface.

One of the most important features of these cements is their biocompatibility with both enamel and dentine. GICs seems to be less biocompatible with the tooth pulp to which it can cause pulpal irritation (Hume W.R. 1988). However, since Glass-Ionomers cements are generally used either with a lining material or on intact dentin layer this finding is not thought to be significant. There was also evidence that dentin acted as a barrier for the diffusion of toxic components from the cement, thereby protecting the pulp under normal clinical conditions. There are three main aspects that enhance their biocompatibility:

- 1) GIC setting reactions have one of the smallest exotherms compared to other dental cements (Crisp S. 1978). This is very important from a clinical point of view because excessive heat developed during the reaction may damage the tooth structure and pulp.
- 2) The neutralization is rapid enough to avoid any irritation that can be caused by the polymeric acids. The poly (acrylic) acid is a weak acid with a pKa of 4.5-5 and the reaction with the glass particles generate a quick neutralization (few minutes).
- 3) The species leached from the glass are generally beneficial for the tooth tissue and can stimulate odontoblast cells growth and proliferation. An important role for this aspect of the biocompatibility is associated with the design of glass compositions that leach the correct ratio of ionic species for assisting the remineralisation. For example the calcium release favours osteoblast proliferation, differentiation and extracellular matrix (ECM) mineralization (Maeno S. 2005).

The fluoride release contributes to bacterial inhibition. The anticariogenic effect of glass ionomer cements is mostly due to this property (Forsten 1998). As the metabolism by bacteria in the mouth environment causes the release of acidic species and caries, fluoride acts to enhance the remineralisation of enamel and dentin assisting in the development of a structure that is highly resistant to caries. Dimensional changes during the GIC setting and lack of adaptation of the restoration to the cavity can create marginal leakage. This phenomenon is strictly influenced by the moisture sensitivity. It is noticed that the need to maintain a water balance in GICs is important, especially in the early stage of the setting-reaction. Waterproof coatings have been used, but the risk of limiting the fluoride release is present (Hattab F.N. 2001).

Despite these excellent properties, limitations in their applications may result from the low mechanical strength and toughness. The principal mechanical properties that characterize a restorative material are: elastic modulus, compressive strength, flexural strength and surface hardness (Vickers hardness). The Vickers hardness value of tooth enamel (274.8 ± 18.1 HV) is approximately 4.2 times greater than that of dentin (65.6 ± 3.9 HV) while no significant differences are observed among the elastic modulus (E) values (1338.2 ± 307.9 MPa for enamel and 1653.7 ± 277.9 MPa for dentin) (Zhang Y.R. 2014). Amalgams have an elastic modulus of 1437.5 ± 507.2 MPa and a Vickers hardness value of 90 HV. The E values for dental resins are lower (833.1 ± 92.4 MPa) while Vickers hardness values (86.3–124.2) are marginally higher (Keyoung J.C. 2014). In the study of Shintome et al. (Shintome L. 2009) the highest micro-hardness values were obtain for Fuji IX with 97.79 ± 3.71 VH (table 5). A comparison of flexural and compressive strength for different commercial GICs is shown in the table 6.

Table 5. Glass ionomer formulations and their Vickers hardness measured after 7 days.

Product name	Powder	HV (24hours)	HV (7 days)
Fuji IX (GC)	Alumino fluoro silicate glass	89.85 ± 3.1	97.79 ± 3.71
ChemFlex (Dentsply)	Strontium, aluminum, fluoride, silicate	52.52 ± 3.57	64.68 ± 1.70
Magic Glass ART (Vigodent)	Radiopaque fluoraluminum silicate crystals	49.18 ± 2.88	50.90 ± 1.96
Vidrion R (SS White)	Sodium fluorosilicate calcium and aluminum, barium sulfate	35.08 ± 1.65	42.55 ± 1.76

Table 6. Comparison of the mechanical properties between different commercial GICs. (FS= flexural strength. CS= compressive strength).

Product name	FS (Mpa)	CS(Mpa)	Author
Fuji II	26,1	202	(D. Xie 2000)
	14,8	161,0	(Moshaverinia A. 2008)
	2,0/15,3	/	(Irie M. 2008)
	30,8/23,0	/	(Bapna M.S. 2002)
Fuji IX	22,6/15,4	/	(Iazzetti G. 2001)
	29,2	211	(Lucksanasombool P. 2002)
	42	236	(Peez R. 2006)
Ketac Molar	1,9/19,3	/	(Irie M. 2008)
	19,7/33.0/35	/	(Lohbauer U. 2003)
	51	244	(Peez R. 2006)
	21,2	301	(D. Xie 2000)

2.3.3 GICs: Strategies for the development and innovations

Glass-ionomer cements possess certain exceptional properties that make them useful as restorative and adhesive materials. Some limitations (including brittleness, poor fracture toughness and sensitivity to moisture in the early stages of the placement) reduce the applications of these materials.

Several attempts have been made in the last decade to improve the properties of the glass ionomer cements. Investigations into this restorative material are essential to succeed in the delivery of better products. Different directions have been taken in order to study separately the different aspects.

As already highlighted, aluminium has a double effect in the material: it appears to be fundamental for the setting mechanism but at the same time it is recognized as a neurotoxin (JG 1990) and cause of some neurological diseases, such as Alzheimer's and Parkinson's disease. Therefore one of the aspects investigated most extensively has been the development of aluminium-free ionomer glasses. Replacing it with iron showed good biocompatibility (Nourmohammadi J. 2010) but the glasses lose their amorphous nature. Hurrell-Gillingham et al. (Hurrell-Gillingham K. 2006) substituted aluminium with iron - one trivalent metal cation for another. They developed a Fe₂O₃ based glass-ionomer cement with the purpose of avoiding Al³⁺ release. They concluded that it was possible to develop the cements from all the Fe₂O₃-based ionomer glasses, but that aluminium contamination was always present. Zinc oxide is considered a good candidate but only for orthopaedic

applications. The zinc-silicate ionomer glasses studied by Boyd et al. (Boyd D. 2005) with a composition in w% of: 5 CaO, 53 ZnO, 42 SiO₂ exhibited handling properties and flexural strengths comparable to conventional GICs. However, it has been proved that the higher release of zinc ions creates adverse effects to the metabolic activity of murine osteoblastic cells (Brauer D.S. 2011). In a more recent study, Kim et al. (Kim D. 2014) developed an aluminium-free glass ionomer cement doped with magnesium and strontium. Both ions are acting as network modifiers and enhanced the bioactivity. Strontium in particular increased the mechanical properties and bone cell proliferation rate whilst maintaining a proper setting behaviour.

Shape and size of the glass particles have been also studied in order to improve the mechanical properties. Kobayashi et al. (Kobayashi M. 2000) proposed that short glass fibres composed of CaO-P₂O₅-SiO₂-Al₂O₃ can function as a reinforcing agent of glass-ionomer cement. Larger diametral tensile strength and flexural strength compared with set cements devoid of glass short fibres has been detected. Nano-granular glass particles with a median size of 0.73 µm and 6.02 µm were made in the Caluwé et al. study (De Caluwé T. 2014). Smaller particles have higher surface area. As such, during the reaction with the poly(acid), the contact area between the powder/liquid components is increased to enhance the setting and mechanical properties of GICs. More precisely in the study cited it is proved that the setting time shortens and compressive strength and elastic modulus increase in formulations containing nano-granular glass particles.

Tartaric acid is nowadays incorporated in the majority of the glass ionomer cements. Since initial studies in 1972 it has been seen that the introduction of this additive modifies the cement forming reaction, thus improving manipulation, extending working time, and sharpening the setting rate. Therefore it is worth exploring ways to optimize the liquid part of the cement: changing the microstructure of the acrylic acid copolymers could be a possible route to improving GICs. Different research areas can be summarized as follows:

- Study amino acids for functionalization of the acrylic acid copolymers in order to increase the level of crosslinking with the ions leached from the glass.
- Develop new polymers for the visible light cured versions of GIC in order to avoid the use of toxic components in example the Poly(methyl methacrylate).
- Looking to change directly the copolymer microstructure via a new synthetic route to obtain improved GIC.

In-depth investigations of these routes can be useful in formulating GIC with improved properties.

Another aspect that is gaining more and more interest for making new glass ionomer cement, is the addition of additional fillers to the glass powder in ionomer glasses. Actually the first attempt was made by Simmons in 1983, trying to increase the strength of conventional GICs with the addition of amalgam alloy powder (Simmons 1983). The result was a commercially available product called Miracle Mix (MM, GC Corporation, Japan).

However the simple addition of amalgam powder did not exhibit promising results in term of mechanical properties.

It is around 2004, that the first articles have been published on the addition of bioactive glass to the GIC. In particular, Yli-Urpo and her group investigated this area and the different characteristics generated like compressive strength (Yli-Urpo H. 2005), ions release (Yli-Urpo H. 2004) and tooth remineralisation (Yli-Urpo H. 2005). Experimentally the materials were made by mixing the commercial GICs powders with the 45S5 bioactive glass powders. The final study gave good results only from the bioactivity point of view. The mechanical properties seem to be compromised by the addition of bioactive glasses. Several studies have used various chemical composition of bioglasses for example Xie et al. (Xie D. 2008) used the S53P4 formula (in w%: 53SiO₂, 23Na₂O, 20CaO, 4P₂O₅) while Wollenweider et al (Wollenweider M. 2007) used NBG with 45S4 formulation (in w%: 44.7SiO₂, 27.6CaO, 22.8Na₂O, 4.9P₂O₅) and Perioglass (Novabone) with 45S5. The same conclusions were drawn for all the studies.

Hydroxyapatite (HA) has been used as filler for enhancing the biological properties. It has excellent biological behaviour, its composition and crystal structure are similar to those of the apatite found in human dental structures and the skeletal system. Gu et al. (Gu Y. 2005) found that the combination of crystalline HA with GIC glass did not affect compressive strength significantly but they found that amounts > 12% (w/w) had an adverse effect on the mechanical properties. Increased values were found for HA/ZrO₂ additions in the same study. ZrO₂ has been widely used for the toughening and strengthening of brittle HA and bioglass in biomedical applications (Biological reactivity of zirconia-hydroxyapatite composites). In an article from Moshaverinia et al. (Moshaverinia A. 2007) nano-HA and nano-FA (fluoroapatite) were incorporated into commercial glass-ionomer powder (Fuji II GC). It was concluded that the experimental dental cements improved both mechanical properties and bond strength to the dentin. In this case the influence of two factor is considered important: the fillers particles size and their chemistry. Proving that working on different aspect at the same time could be a promising area of investigation.

Metallic additives such as silver, gold, titanium and palladium have been investigated as fillers in GICs. Silver and gold presented higher strength compared to the conventional glass-ionomer cements, however, these modified GICs are difficult to polish and their aesthetic is poor.

The current literature lack of studies that demonstrates how the mechanical properties of the glass-ionomer cements are unlikely to be enhanced alone by the addition of reinforcing particles and fillers such as bioactive glass fillers, glass fibers or metals fillers. Just few attempts has been made with glass composition that differs from the conventional GICs. More interest should be concerned in this direction too. It has been proved by recent articles that dental cements set also with non-classical glass compositions. The study of the properties of these materials can bring new information to the subject in order to obtain a

new innovative material with better properties. More details will be added in the next chapter.

2.4 Borosilicate glasses

Boric oxide is the second most important glass-forming oxide. It can be used without silica, forming borate glasses employed for very technical purposes, but in general it is used in conjunction with silica to give the borosilicate glasses.

Silica and boric oxide glass formers have very different physical and chemical properties as described in table 7.

Table 7 Comparison between silica and boric oxide properties.

SILICA (SiO₂)	BORIC OXIDE (B₂O₃)
High melting point ($\approx 1700^{\circ}\text{C}$)	Easy melting ($\approx 450^{\circ}\text{C}$)
High viscosity	Low viscosity
High chemical resistance	Soluble in water

Borosilicates glasses are of technological interest because they have many applications. This is due to their lower thermal expansion, good chemical resistance and high dielectric strength.

Borosilicate glasses and more precisely alkali borosilicates compositions are the basis of many practically important glasses used in the chemical industry, optics, optoelectronics, the atomic industry, etc. Pyrex glasses are one of the most known applications of borosilicate glasses. It was developed in the early years of the 20th century to satisfy the demand for a material with high thermal resistance. This property, coupled with excellent chemical durability, makes Pyrex the ideal material for laboratory products. Because of its low expansion characteristics, Pyrex borosilicate glass is often the material of choice for reflective optics in astronomy applications.

Borosilicates glasses are a promising material that have been widely studied for biomedical applications such as scaffolds in soft tissue repair and various bone repair scenarios. Although silicate glasses have received major attention for dental restorative materials, borosilicate glasses have recently been developed for similar applications (Neve A. D. 1992). A few studies already demonstrate some attempts in using borosilicates for dental cements with enhanced bioactivity and antimicrobial properties realized through the species released from those glasses. The objective of this work is to characterize a series of novel borosilicate glasses and to understand the mechanism and kinetics of dissolution related to their microstructure and composition. The use of thermal treatment to promote phase separation as a means of influencing the rate of ion leaching will be especially studied. The dissolution

process for borosilicates in neutral body solution (simulating saliva) or in acidic environments (simulating scenarios where bacteria or acid foods are present) provides information regarding the type and concentration of ionic species released by the glass. As the novel borosilicates generated in this work (see next section for compositional information) react in an aqueous environment, B-, Si-, Ca-, K- and Al- species are liberated to different degrees as a function of the time. Ion leaching trends can be related to the glass chemistry and microstructure. The dissolution rate of the different elements leached out will stimulate theories for which of these species are more or less involved in the mechanism of cement consolidation. For example boron, leached out as boric acid, could have a fundamental role in the crosslinking with the poly(acid) chains in addition to its excellent biological properties. Moreover it can potentially substitute for aluminium that has so far been considered as essential for the hardening of the cement due to its high (+3) oxidation state. Silica that remains in the less soluble phase could enhance the mechanical properties, the concept being that the chemically durable phase remains as a continuous layer that maintains mechanical properties over time. Studies on the mechanical properties discussed later on in the thesis, will provide evidence for the relationship between elements leached and the microhardness / flexural strength delivered in the cured composite.

2.5 Ion release and glass dissolution

The dissolution rate of the bioactive glass into specific solutions is one key characteristic for selecting glasses for different applications. The concentration of each ion in solution varies with the composition, surface area of the glass particles, temperature and pH of the solution.

The elements that dissolve from the glass will control the hardening process of the glass ionomer cement formation, due to the reaction with the poly(acrylic acid). During and after the maturation of the cement some of the ions will then activate process related to the tooth remineralization and influence antimicrobial behaviour. HA precipitation is pH dependent so some of the ions from the glass might cause a pH change that helps the HA deposition process. For understanding completely the glass ionomer cement forming ability and its subsequent performance, it is essential to study the degradability of the glass powder in acid solution. The action of the different ions released from bioactive glasses is very well documented in a review from Hoppe et al. (Hoppe A. 2011).

Ion release kinetics from bioactive glasses has been extensively studied in vitro. Usually the studies are carried out in static conditions using approximately neutral solutions like simulated body fluid (SBF), phosphate buffered solution (PBS) and tris(hydroxymethyl)amino methane solution (Tris). Alternatively acidic solutions like nitric acid (HNO_3), lactic acid ($\text{C}_3\text{H}_6\text{O}_3$) and acetic acid (CH_3COOH) can be used. The choice of the solution used is strictly dependent on the type of study that the authors decides to perform and the oral conditions the experimenter wishes to replicate. After the immersion (the temperature is always

constant at 37°C) the ions are collected at different time-points and analysed using ionized coupled plasma (ICP) spectroscopy.

The study of glass dissolution in solutions similar to those present in the human body have, as the main objective, the creation of preliminary test data to evaluate performance in potential new implantation materials. For example Cluppera et al. (Clupper D. 2002) observed the formation of crystalline hydroxyapatite (HA) on the surface of 45S5 bioglass immersed in SBF. The formation of HA is reported in the majority of the studies and it is an indication of the biocompatibility of the glass.

Nourmohammadi et al. (Nourmohammadi J. 2007) investigated the dissolution of an aluminosilicate powder (destined for use in a glass ionomer cement) into a dilute acetic acid solution. This work was undertaken as the acid degradation and consequent ion leaching from the glass was deemed crucial for the cement forming ability.

Most of the studies are focused on gaining an appreciation of the material behaviour in vitro. It is desirable to develop materials with sufficient bioactivity and controllable degradation behaviour to meet the requirements for a biomaterial. The absorption should be gradual and concurrent to the replacement with the host bone or the induced remineralisation.

In silicate glasses the degradation in biological environments brings about the release of ions such as Na^+ and Ca^{2+} that are responsible for initiation of HA layer formation that is able to bound tightly to the bone. The resulting amorphous calcium silica layer formed at the surface of the glasses is one of the steps of the mechanism leading to the formation of crystalline hydroxyapatite. It is also confirming the high biocompatibility of silicate glasses of which 45S5 is considered the baseline composition as documented in numerous studies (Wilson J. 1981). Although a network former, silicon can be leached out as silicic acid $\text{Si}(\text{OH})_4$. It is partially involved into the HA layer formation and it is also harmlessly excreted in soluble form through the urine (W. Lai 2002). The biological environment is significantly influenced by ion dissolution and pH changes occurs in loco as a resulting of this phenomenon. The addition of different glass modifiers results in the production of other types of silicates such as 13-93 (6wt% Na_2O , 12wt% K_2O , 5wt% MgO , 20wt% CaO , 53wt% SiO_2 , 4wt% P_2O_5) that has already been approved for in vivo uses in Europe because of its similar biological properties to 45S5; this is despite presenting a slower degradation rate.

Phosphate glasses have unique dissolution properties due to their different structure. When they form 3D binary oxides, phosphorous can only share three out of its four oxygens. This fact limits their connectivity because phosphate anions should contain at least one terminal oxygen. Therefore the rigidity, related with the interatomic forces, is less in phosphate glasses in respect to silicate glasses. When mixed with alkali metals oxides, phosphate glasses contain fewer cross-links but a higher number of terminal oxygens resulting in more flexibility of the orientation of the structural units. Their dissolution is considered to be dependent on three factors that are similar to some of the factors important for the silicate

glasses (Bunker B.C. 1984). These are the structure of phosphate network, the ions exchanged between the glass and the solution and the kinetics of phosphate hydrolysis. The first factor is related to the number of cross-links and is defined as the ratio that exists between the numbers of PO_4 groups attached to other P atoms via one, two or three bridging oxygens (BO). The addition of modifier oxides results in the creation of Non-Bridging Oxygens (NBO) and a consequent faster dissolution of the phosphate network. The ions exchanged are regulated by acid/base reactions. Ions such as Na^+ and Ca^{2+} lie along the phosphate network chains in different positions that determine their capability to be exchanged with the solution. The acid/base reactions aid glass dissolution by disrupting the ionic interaction between the chains. Finally the hydrolysis reaction will break the phosphate chains into orthophosphate group. The rate of hydrolysis are slow if compared to glass dissolution rates and the pH has a fundamental role in accelerating this type of reaction.

In borosilicate glasses chemical durability generally decreases with an increase in boron concentration relative to the silicon concentration. High alkali concentrations in borosilicate as well as in silicates, promotes the formation of NBOs in the network structure that induce a decrease in durability. When compared to silicate glass, borate and borosilicate bioactive glasses were found to degrade faster and completely convert to HA because of their low chemical durability. When a proton coming from the solution, substitutes for an alkali ion in a borosilicate it causes an important change to the network structure: 4-coordinated borate units become a trigonal borate unit increasing the corrosion rate of the glass, as confirmed by the faster leaching rate of B and K compared to Si. This is documented in the literature (B.C. Bunker 1986, A. Ledieu 2004) but also confirmed in this work.

When a borosilicate is immersed in a solution two predominant borate species are detected, boric acid $\text{B}(\text{OH})_3$ and tetrahydroxyl borate anions $\text{B}(\text{OH})_4^-$. At biological pH, the $\text{B}(\text{OH})_3$ is the stable species in solution. Apparently, with different acidic solutions the pH in the presence of borosilicate ions increases. This was confirmed by the studies with the nitric acid in this work. In fact, Fu et al. (Fu Q. 2010) reported that if the solution is SBF, the pH will increase too and lead to $\text{B}(\text{OH})_4^-$ species formation. Boric acid is a weaker acid compared with phosphoric acid associated with the SBF solution (or directly from the body saliva), and the consumption of PO_4^{3-} ions associated with the release of BO_3^- ions from the glass results in the increase of the solution pH.

The strong affinity for a pair of electrons in boric acid and other borate compounds makes them valuable for adhesive applications like many other fine chemicals. Boric acid's acidity is due to the acceptance of an electron pair rather than by proton dissociation. This is why the weakly acidic and electron deficient boric acid accepts an OH^- ion from the solution and forms borate anions $\text{B}(\text{OH})_4^-$. This anion in its tetrahedral form undergoes bridging and creates cross-links with any polyol, cellulose, polysaccharide, glycoprotein, etc. The interaction of simple carbohydrates with borates is well known. Therefore, boron in boric acid and borates forms a hydrogen bond network with a compound containing poly-hydroxy groups (fig. 12). This is the basis for its importance in adhesives. Boron's use in adhesives

shows that it plays primarily a structural crosslinking role. In crosslinked adhesive products, borate ions are all bound within the adhesive matrix. The presence of this compound released from the glass could also be relevant for enhancing the hardening process in glass ionomer cements.

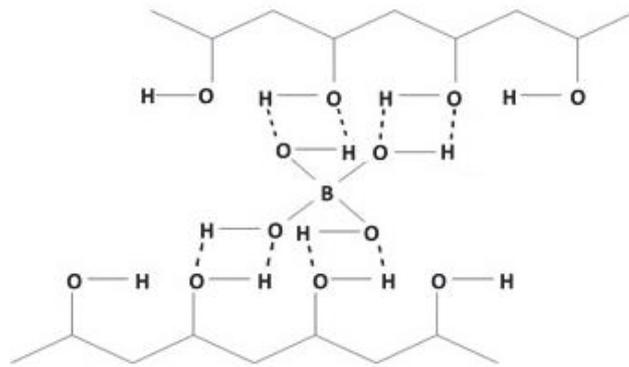


Figure 12. Borate ion crosslinking with the hydroxyl group of a polymer chains. The hydrogen bond formation is highlighted.

2.6 From silicates bioactive glasses to borosilicates

The bioactivity of biomaterials has been defined as “the ability to bond with host bone tissue” and includes the ability to encourage apatite formation. To be classified for biomedical applications, a bioglass has to meet three requirements. The first requirement is that the material must be biocompatible; it means that the organism should not treat it as a foreign object. Secondly, the material should be resorbable; it should be degraded or dissolved by the organism to allow it to resume natural functioning. Thirdly, the material should have appropriate mechanical properties; for the replacement of load bearing structures, the material should possess equivalent or greater mechanical stability than the natural tissue to ensure high reliability of the graft. The Hench 45S5 glass still shows the best bioactivity, but more recent work has shown that certain compositions in other glass-forming systems, such as borate glass and borosilicates are also bioactive. The conversion to hydroxyapatite (HA) has been observed also for borate and borosilicate glasses with a similar mechanism postulated to that proposed for the 45S5 (fig. 13).

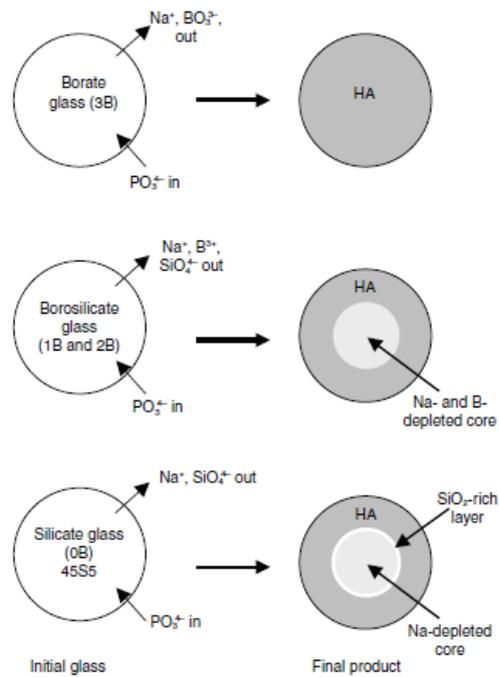


Figure 13. HA conversion mechanism of borate, borosilicate and silicate glasses.

Huang et al. (Huang W. 2006) investigated the dissolution of a 45S5 glass in which the silicon is partially and totally replaced by boron. The experiments were performed in a phosphate solution and it was observed that some borate glasses converted completely to HA and at a faster rate than 45S5 glass. Boron has a three coordination number in glasses and it cannot form easily a 3-dimensional network as the one formed by silica. For this reason borate glasses have a lower chemical durability and a faster dissolution rate. Borosilicate dissolution rates fall, predictably, between the two glass types, as they are composed of both trigonal borate and tetrahedral silica units. Whereas the low chemical durability of some borate glasses has been known for decades, it is only recently that the potential of borate glasses in biomedical applications has been explored. The rate of conversion to HA can be tailored in relationship to the regeneration of new bone tissues in order to improve the resorbability of the biomaterial. In fact, for final applications a gradual resorption of the implanted biomaterials and the simultaneous replacement of the biomaterials by host bone is required.

The biocompatibility of borate and borosilicates is has also been exploited in different studies that proved their ability in enhancing the cell proliferation and differentiation. Marion et al. (Marion N. W. 2005) showed how porous borate glass can present excellent cytocompatibility and the capacity of osteogenic promotion of human mesenchymal stem cells. Cell lineage have survived up to two weeks of seeding on porous borate glass disks, suggesting that porous borate glass provides a suitable environment for cell attachment and proliferation. Bioactive glass scaffolds with the composition (mol%): 22 CaO, 6 Na₂O, 8 MgO, 8 K₂O, 18 SiO₂, 36 B₂O₃, and 2 P₂O₅, were prepared by Fu et al. (Fu H. 2009). Biocompatibility

tests showed the enhanced proliferation of bone marrow stromal cells, as well as the proliferation and function of murine MLO-A5 cells, an osteogenic cell line, proving the potential of the borosilicates as biomaterials applications.

Few researches demonstrate that acceptable working and setting times may be achieved with systems other than silicate ones. Neve et al. (Neve A. D. 1992) have manufactured a novel aluminoborate glass system, for use in GICs. The formulation was composed of: B_2O_3 , $SrCO_3$, Al_2O_3 and ZnF. The powders were mixed with freeze dried poly(acrylic acid) in varying proportions, and the cement forming ability of these mixes was assessed by the measurement of working and setting times. It was found that the alumina content has a major effect on the reactivity of the aluminoborate cements; zinc and strontium in the glasses exhibit more complex reactivity. Probably this was due to the differences in the rate of extraction of the cations or their mobility into the network structure during the hardening process. In the end these glasses are capable of forming cements with acceptable characteristic from a clinical point of view. In the work of Shen et al. (Shen L. 2014) borate-based glasses were evaluated as a primary component of GICs. The borate glass compositions developed in this study possessed several important features: they are entirely free of aluminium, they contain silver in the ionic state (which is desirable for antibacterial applications) and they contain zinc, strontium and/or titanium. Some of the compositions were able to produced cements with good working and setting times. However the mechanical properties of the borate-based GICs considered were not sufficient for structural applications. They may still be of use in applications where minimal load bearing and/or rapid resorption is required. It was found that TiO_2 helps in controlling the rheological properties, giving the longest working times and the shortest setting times for high wt% loadings into the composition.

One way to improve the biological and mechanical properties of the GICs may be by controlling the composition of the glass component. To do this knowledge of (i) the processes that lead the glass formation and (ii) how changes versus time to the structure of the glass affect its properties is required.

2.7 Review article: Mechanical characteristic and biological behaviour of implanted and restorative bioglasses used in medicine and dentistry.

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Mechanical characteristic and biological behaviour of implanted and restorative bioglasses used in medicine and dentistry: A systematic review

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ABSTRACT

Objective. Nowadays bioactive glasses are finding increasing applications in medical practice due to their ability to stimulate re-mineralisation. However, they are intrinsically brittle materials and the study of new compositions will open up new scenarios enhancing their mechanical properties and maintaining the high bioactivity for a broader range of applications. This systematic review aims to identify the relationship between the composition of bioactive glasses used in medical applications and their influence on the mechanical and biological properties.

Methods. Various electronic databases (PubMed, Science Direct) were used for collecting articles on this subject. This research includes papers from January 2011 to March 2016. PRISMA guidelines for systematic review and meta-analysis have been used. 109 abstracts were collected and screened, 68 articles were read as relevant articles and a total of 22 papers were finally selected for this study.

Results. Most of the studies obtained enhanced mechanical properties and the conservation of bioactivity behaviours; although a lack of homogeneity in the characterization methods makes it difficult to compare data.

Significance. New compositions of bioactive glasses incorporating specific ions and the addition in polymers will be the most important direction for future researches in developing new materials for medical applications and especially for dentistry.

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1. Introduction

In 1969, Hench et al. [1] developed a new material for medical applications; creating a solid base for the following 40 years of research in the bone/tissue regeneration field. 45S5 was the first bioactive glass generated, with a composition showing an excellent biocompatibility. Its composition by weight is: 45% SiO₂, 24.5% Na₂O, 24.5% CaO and 6% P₂O₅. This material is able to bond with bone and stimulates bone growth due to hydroxy-carbonate apatite (HCA) formation. This type of apatite is chemically and structurally very similar to the mineral phase of hard tissues.

Bonding to bone and tissues has been well documented and investigated by a large series of bioactive glass compositions [2,3]. The mechanism of bone bonding enables the bioglass (BG) to develop an adherent interface with tissues that resists mechanical forces. In many cases, the interfacial strength of adhesion is equivalent to or greater than the cohesive strength of the implanted material or the tissue bonded to the bioactive implant. Five steps have been described for bone-bonding mechanism in bioactive glass [4]:

- Step 1: fast release of Na⁺ and Ca²⁺ ions which are exchanged with the H₃O⁺ ions present in the solution. A rapid increase of solution pH develops.
- Step 2: network silica is attacked by hydroxyl groups causing the formation of Si(OH)₄ in the solution.
- Step 3: Silanols (–Si–OH groups) form a silica rich layer on the surface thanks to re-polymerization reactions.
- Step 4: Ca²⁺ and PO₄³⁻ migrate to the newly formed silica surface forming a CaO–P₂O₅ film on top.
- Step 5: CaO–P₂O₅ film crystallize and incorporate other ions from the solution (such as OH⁻ and CO₃⁻) will form a HCA layer.

For the treatment of bone defects or dental trauma as well as diseases such as osteoporosis, cancer and infectious diseases, it is essential to develop new active materials that are able to interact with host surroundings, enhancing and directing complete tissue healing, repair and regeneration. Synthetic biocompatible materials are used to replace damaged tissues but the weakness of some chemical, biological and/or physical properties results in implant failure that requires retreatment.

The original 45S5 bioglass has been already used in different materials for repairing bone defects in the jaw and orthopaedics. Bioactive glass grafts were originally developed for replacing ear bones and alveolar bone defects around teeth; the products used were based on particles rather than monolithic shape, i.e. PerioGlas[®] and NovaBone[®]. Micro and nano-particles have superior bioactive behaviours due to a larger specific surface area that allows a faster ion release. Bioactive coatings are likewise very important for metallic implants because they have the potential to improve their performance by providing strong bonding to the host bone and to the resin cement [5]. Nevertheless, 45S5 bioglasses are applied also to non-permanent materials especially used in dentistry. BG-pastes are favourable for the treatment of dentin hypersensitivity [6], enamel demineralization [7] and for tooth bleaching [8]. Finally, bioactive glasses have satisfying characteristics as a scaffold material for bone tissue engineering, but the application of glass scaffolds for the load-bearing bone defects repair is often limited by their low mechanical strength and fracture toughness.

Despite the excellent biological properties, mainly osteoblast proliferation and differentiation induced by the released ions by the material, bioglasses are brittle materials that are easily cracked. This low strength and fracture toughness prevents their use for load-bearing implants [9]. The development of new glass compositions with improved mechanical properties is a challenging objective and the trend is to incorporate different elements to obtain better biological and physical characteristics. Crystallinity significantly changes the fracture characteristics of glasses. This opens the way for glass-ceramics as offerings with improved mechanical properties. On the other hand, the introduction of crystalline phases could decrease the bioactivity. Several attempts have been made to preserve the amorphous structure of the glass with the addition of silver, magnesium, strontium, boron, zinc, aluminium, fluoride, potassium, gallium, barium and zirconia. Addition of silver [10] and boron [11] have been investigated in order to improve the strength and develop antibacterial and antimicrobial materials; magnesium has stimulatory effects on the growth of new bony tissues [12]; calcium is shown to be responsible for osteoblast proliferation [13], while elements like zirconia improve the mechanical properties but decrease the bioactivity behaviour [14].

In recent years, bioactive glass particles have been introduced as fillers in conventional composites for tissue

engineering, mixing them with polymers such as polylactic acid (PLA), polyglycolic acid (PGA) and their copolymer poly L (lactic-co-glycolic) acid (PLGA). Composite scaffolds for bone tissue engineering have been studied to obtain materials that impart better mechanical and physiological properties to the host tissue. Polymer/bioactive glass composite scaffolds show especially an increase in bioactivity which is achieved by the bioglass inclusion. The degree of bioactivity is adjustable by the volume of fraction, size, shape and arrangement of this inclusion. It has been shown that increased volume fraction and higher surface areas to volume ratio favour higher bioactivity; incorporation of fibres as fillers (instead of particles) enhances the mechanical properties. The dissolution of the bioactive glass should result in nucleation and growth of a crystalline HCA layer on the surface of the polymer scaffold, which should further affect the polymer degradation behaviour in addition to provide the required osteoconductivity. Thus, a combination of polymers and bioactive glass phases result in promising composite scaffolds for (bone) tissue engineering. Finally, it is clear that the chemistry and physical properties of the added polymer and bioactive glass have a significant incidence on mechanical properties and consequently in material degradation [15].

Several families of bioactive glasses have been more precisely investigated:

- Silicate-based glasses, like 45S5, are glasses where silica (SiO_2) is the classic network-former. The basic unit is the SiO_4 tetrahedron capable of sharing up to 4 oxygen atoms with other such tetrahedral units or other elements. Silicon has a charge of 4+.
- Phosphate-based glasses in the system $\text{CaO-Na}_2\text{O-P}_2\text{O}_5$ have the tetrahedral structure formed by PO_4 units and the phosphate group has a charge of 5+ [16]. Therefore, they contain at least one terminal oxygen that limits connectivity and so the reactivity of the structure that gives unique dissolution properties in aqueous-based fluids for these types of glasses.
- Borate-based glasses [17] are based on a B_2O_3 network that can occur both in triangular and tetrahedral coordination but mostly in the triangular form especially vitreous compounds. Borate glasses show potential in bone regeneration owing to its complete conversion to apatite through a series of dissolution-precipitation reaction similar to those of 45S5 bioglass.

The purpose of this literature review is to assess the mechanical and biological properties of bioactive glasses used for dental and medical applications regarding their compositions.

2. Materials and methods

PRISMA guidelines for systematic review and meta-analysis have been used for this review manuscript [18]. A literature search was performed using several electronic databases (PubMed, ScienceDirect) on articles published in the last 5 years, to identify the most recent developments on the subject. The research took place on 1st March 2016 in the Faculté d'Odontologie, Université Claude Bernard Lyon 1, Lyon, France.

To be included, papers had to consist of both physical properties and biological properties or at least physical properties as these studies had to provide valid information in order to compare the various results obtained in the different studies. Language restrictions were not applied. Furthermore, these studies had to meet the following criteria:

1. Bioactive glasses used in medicine.
2. Evaluation of mechanical and biological properties.

The terms used in the search (in Title/Abstract) were as follows:

("Bioglass" AND "Biological properties")

OR

("Bioglass" AND "Physical properties").

Review articles and short communications were excluded. Two reviewers (FL and CV) screened accurately and independently all the titles and abstracts. The full texts of all the articles in accordance with the inclusion criteria (by consensus) were collected and reviewed. Also all the reference citations were screened for any relevant publications that might have been missed by the electronic search and that could be relevant for the current report. Finally, a consensus between the two readers was reached to determine which studies met the selection criteria.

3. Results

3.1. Study selection

A total of one hundred and nine abstracts were collected after an electronic database search with the selected terms (Fig. 1). After the first screening, sixty-eight publications were excluded based on the title and abstract because they did not meet the inclusion criteria. The full texts of the forty-one remaining studies were read. Nineteen of them did not meet the inclusion criteria:

- Ag and Sr substituted bioactive glass for the investigation of antibacterial effects [19,20];
- Focused on cells growth, proliferation and protein interactions [21–27];
- Studies evaluating only the bioactivity and hydroxyl apatite (HA) formation [28–34];
- General characterization and synthesis of bioglass powders [35,36];
- Mechanical properties of a bioactive ceramic β -TCP [37].

The references of the twenty-two remaining publications were screened carefully for additional studies that might have been missed by the electronic search (no paper found). Finally, no other articles have been included in the present review.

3.2. Studies aim

All the twenty-two remaining articles were classified based on different scientific themes present in each of them and related to the influences on the mechanical/biological properties. These properties describe the reactions to physical forces applied directly to their surfaces. Mechanical properties occur

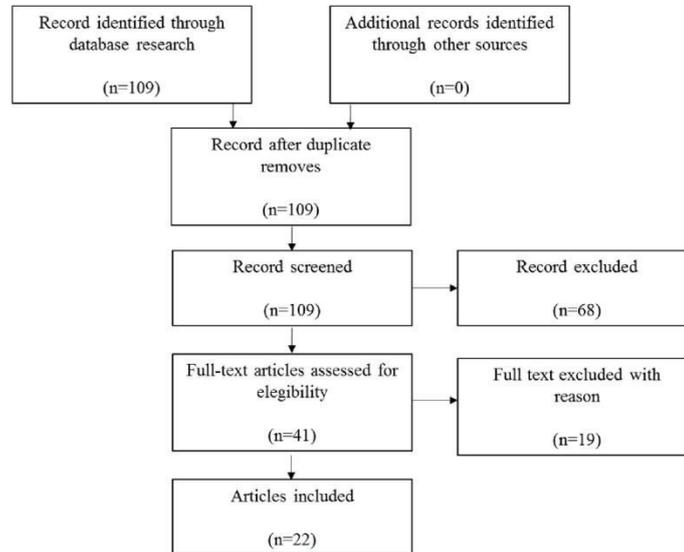


Fig. 1 – Flow diagram.

Table 1 – Physical properties reported in the articles.

Number of tests	Physical properties	Related articles
1	Compressive strength Tensile strength Elastic modulus Flexural strength Knoop microhardness Vicker's hardness	[47–49] [50] [51–54] [55,56] [57] [58,59]
2	Vicker's hardness + elastic modulus Compressive strength + elastic modulus Tensile strength + elastic modulus	[38,39] [40–44] [45]
3	Elastic modulus + tensile strength + compressive strength	[46]

as a result of the physical properties inherent to each material, and are determined through a series of standardized mechanical tests. For this reason, the methods used for determining the mechanical properties have also been listed. Moreover, the impact of polymers addition to the glass filler and the bioactivity identified with the different materials has been evaluated.

3.3. Testing objectives

Most of the articles selected describe the effects of one specific property. However, some authors investigated more than one test to provide a more complete characterization (Table 1). In fact, two properties are studied in 8 articles [38–45] and in one case three properties have been reported [46].

3.4. Testing methodology

The universal testing machine, able to measure the tensile strength and the compressive strength of the materials is used

Table 2 – Tests methodologies of the studies.

Type of test	Related articles
Universal testing machine	[40–52,56]
Indentation hardness test	[38,39,57–59]
Ultrasonic method	[39]
Archimedean method	[53,55]
Electro force biodynamic test	[54]

in most of the articles (n = 14). In five studies, indentation hardness tests were used to calculate Vicker's hardness and Elastic modulus. The Archimedean method was used in two studies to obtain flexural strength and elastic modulus. Just one paper used an Electro Force Biodynamic Test Instrument for the mechanical characterization of biomaterial specimens in contact with simulated body fluids (SBF) solution. The testing methods are detailed in Table 2.

Table 4 – Bioactivity tests performed.

Bioactivity tests	Related articles
Immersion in simulated body fluid (SBF)	[38,39,41–46,49,52,55,59]
Immersion in Dulbecco's Modified Eagle Medium (DMEM)	[48]
Immersion in tris buffer	[58]
None	[40,47,50,51,53,54,56,57]

3.5. Compositional features, mechanical properties and applications

Considering all the twenty-two studies, ten of them are investigating the direct influence of different compositions of glasses on mechanical properties [38,39,47,48,51,53,55,56,58,59]. Most of them are investigating the silicates system, only one [47] is studying a porous silicate BG mixed with hydroxyapatite (HA). Regarding the final applications, they are divided into: bone tissue regeneration [40,43,46,48,53–54], tooth abrasion [51] and both bone and dental application [47,50]. The remaining twelve articles point out the effect given by polymers addition to different glass systems. The majority of them is still centred on Silicate systems except for two studies [46,57] that investigate respectively HA/CaCO₃ and grounded mineral trioxide aggregates (MTA). These twelve articles are mostly focused on applying the material in scaffolds and composites for bone tissue engineering while, only two articles are dealing with dental field [52,57]. The articles are listed in Table 3.

3.6. Testing the bioactivity & the biocompatibility

The bioactivity of biomaterials has been defined as “the bond ability with host bone tissue” that includes the ability of enhancing the apatite formation. Developing new bioactive glasses for load bearing bone repair applications, requires an understanding of the bioglasses bonding process to living bone and recognizing the best methods to test bonding abilities (Table 4). One method is to evaluate the apatite-formation ability of bioglasses in different solutions; in the articles screened, the most used solution is the Simulated Body Fluid (SBF), while only two articles use Dulbecco's Modified Eagle Medium (DMEM) and Tris buffer solution [48,58]. The majority of the bioactivity tests are performed on composites and SBF is still the preferred solution used. Another method is to investigate *in vitro* bone cell response to bioglasses (Table 5).

The evaluation for the efficacy of a method for *in vitro* biocompatibility of bioglasses depends on the cell proliferation, metabolic activity and cell attachment that are strictly related to the glass composition. The apatite formation methods after dissolution are useful approaches to evaluate the *in vitro* bioactivity of silicate-based bioglasses. *In vitro* cell based assays are also valuable indicators for bioglasses bioactivity, but relevant standard materials should be considered as reference controls.

4. Discussion

Glasses used for biomedical or clinical applications are known as bioactive. The articles used for this purpose, consider bone, dental tissue regeneration or replacement. To be classified for biomedical applications, a bioglass has to meet three requirements. The first requirement is that the material must be biocompatible; it means that the organism should not treat it as a foreign object. Secondly, the material should be resorbable; it should be degraded or dissolved by the organism to allow it to resume natural functioning. Thirdly, the material should have appropriate mechanical properties; for the replacement of load bearing structures, the material should possess equivalent or greater mechanical stability than the natural tissue to ensure high reliability of the graft. As the Hensch 45S5 glass still shows the best bioactivity, now the research is mainly focused on enhancing the mechanical properties of the bioactive glasses keeping the 45S5 important characteristics.

4.1. Testing objectives and methodology

In the majority of the articles (65%) the Universal testing machine is used for measuring the mechanical properties. Commonly, it is used to test the tensile strength, compressive strength and elastic modulus. Most of these studies performed at least two of the aforementioned tests, giving broader information about the properties; compressive strength and elastic modulus are the main properties examined [40–44]. Only in the study presented by Simpson et al. [46], were all three mechanical properties measured. The aim of this study was to compare four different fillers in a PLGA matrix. In order to compare different bioglasses or composites accurately, it is also necessary to use the same production method and testing regime because differences in these will result in variations in material and mechanical properties.

Regarding the elastic modulus, that describes the relative stiffness or rigidity of a material, it can be calculated using the Universal testing machine. However, as alluded to earlier, it can be obtained also with other methods. Bachar et al. [39] and Bellucci et al. [38] used the indentation hardness testing machine and measured the elastic modulus respectively by the Knoop indentation method given by Marshall et al. [60] and by the depth-sensing indentation technique proposed by Oliver et al. [61]. With the first method, the elastic modulus is obtained by a mathematical conversion of the hardness; the decrease in length of the indent minor diagonal is caused by elastic recovery of the material and so the conversion is related to an hardness:elastic modulus ratio. The second method used is based on the unloading process deformation, assumed to be both elastic and plastic in nature as the permanent hardness impression forms; it is a technique that is very sensitive to local defects, such as pores. The elastic modulus is also calculated by the Archimedean method by Lin et al. [53], values being obtained from the density of the bioactive glasses. It can also be calculated using the Electro force biodynamic Test (see the work of Marelli et al. [54]) where the aim is to simulate *in vivo* conditions (immersion in SBF solution) whilst evaluating the mechanical properties at different

Table 3 – Mechanical characteristics related to the bioactive glass compositions of the 23 selected articles.

BG system	Composition features	Effect on mechanical properties	Application	Articles
Si–Ca–Na–P–K–Mg	Infiltrated with PCL Infiltrated with PLA	Improved elastic modulus and compressive strength	Hard tissue regeneration and bone tissue engineering	[40] ^a
Si–Ca–Na–P	Addition of Ba	Improved flexural strength	Bone substitution	[55]
Si–Ca–Na–P–K	BG + HA (80 wt%: 20 wt% ratio)	Improved Vicker's hardness & elastic	Composites for bone tissue repair and regeneration	[38]
Si–Ca	Mixed with PCL	Improved elastic modulus	Composite for tissue engineering	[45] ^a
Si–Ca–Na–Al–P–Mg–Fe	Coated with P3HB	Improved compressive strength and compressive modulus increasing immersion time of the polymer,	Scaffolds for bone tissues engineering	[41] ^a
Si–Ca–P–Sr	Addition of PCL	Improved compressive strength and elastic modulus increasing Sr concentration and PCL addition	Composite for tissue engineering	[50] ^a
Si–Ca–Na–P Grounded MTA	Incorporated into dental adhesives	Reduction of Knoop microhardness values after prolonged DPBS storage	Dental restorative and bonding adhesive	[57] ^a
CaCO ₃ HA 4S5 ICIE4	Addition of PLGA	Lower compressive and tensile Young's modulus Improved Compressive strength, Compressive and Tensile Young's modulus	Bone substitute material for orthopaedics	[46] ^a
Si–Ca–P	Increase Si content	Improved Young's modulus with higher Si content	Bone regeneration	[51]
Si–Ca–P	Addition of PLGA	Improved mechanical properties	Bone tissue engineering	[43] ^a
Si–Ca–P	Mixed with PVA, chitosan and collagen	Improved compressive strength and compressive modulus for a 1:1 PVABG/ChiCol ratio	Composited scaffolds for bone replacement application	[42] ^a
Si–Ca–Na	Addition of nitrogen	Improved microhardness & elastic modulus	Orthopaedic and dental	[39]
Si–Ca–Na–P–F	Na ₂ O content variation	Reduction of Vicker's hardness linearly with increasing Na ₂ O content	Air abrasion, tooth remineralization	[58]
Si–Ca–Na–Sr–Zn	Mixed with PEGDMA	Improved in compressive strength and Young's modulus values	BG composite hydrogels as bone graft substitutes in cancellous bone defects	[44] ^a
Si–Ca–Na–P	Mixed with PDLLA	The mechanical properties were not significantly influenced	PDLLA/BG membranes for the treatment of periodontal defects	[52] ^a
BG + HA	Addition of Al ₂ O ₃	Improved compressive strength with increasing Al ₂ O ₃	Orthopedic and dental	[47]
Si–Ca–Na–P	Foaming sol–gel synthesis	Reduced compressive strength with increasing porosity	Scaffolds for bone tissues engineering	[48]
Si–Ca–Na–P	Thermal treated	Improved bone load-bearing capacity	Bone graft substitutes	[56]
Si–Ca–Na–P	Increasing CaO/P ₂ O ₅ ratio	Improved elastic modulus	Prosthesis or bone implantation	[53]
Nano Si–Ca–Na–P	Incorporated in dense collagen gels	Improved compressive modulus	Hydrogel scaffolds for bone tissue engineering	[54] ^a
Si–Ca–Na–P–Ag	Scaffolds produced using the foam replication technique	Improved compressive strength of scaffolds	Scaffolds with therapeutic and antibacterial potential for bone tissue engineering	[49] ^a
Si–Ca–Na–P	Addition of Na ₂ O	Reduction of Vicker's hardness with increasing Na ₂ O content	Bone prostheses	[59]

PCL, polycaprolactone; PLA, polylactic acid; P3HB, poly-3-hydroxybutyrate; PLGA, poly(lactic-co-glycolic acid); PVA, polyvinyl alcohol; PEGDMA, poly(ethylene glycol) dimethacrylate; PDLLA, poly(D,L-lactic acid).

^a Studies using polymers.

Table 5 – Biocompatibility tests.

Biocompatibility tests	CELL type	Results	Articles
Hemolysis assay (ISO 10993-4)	None	Improved biocompatibility	[55]
NR test, XTT test, BrdU test, ISO 10993-5, ISO 10993-12	BALB/3T3, MLO-Y4	No negative effects in cell viability and growth	[38]
ALP assay, Western blotting, quantitative RT-PCR, <i>In Vivo</i> test	MC3T3-E1	Excellent osteogenic features	[51]
CellTiter96Aqueous one solution cell proliferation assay, ALP assay, Western blot, quantitative RT-PCR	hBMSCs	Promotes cell attachment	[43]
MTT assay, ALP assay	UMR-106	Improved biocompatibility	[42]
AlamarBlue [®] assay, ALP assay	hPDL, hBMSC	Positive impact in the adhesion, proliferation, differentiation and mineralization	[52]
AlamarBlue [®] assay (ISO 10993)	MG63	No significant cytotoxicity	[48]
Live/dead assay, AlamarBlue [®] assay, ALP assay	MC3T3-E1	Maintained cell viability, high ALP production	[54]
Live/dead assay	HPDLCs	No dead cells, low silver conc. maintain cell viability and attachment	[49]
ALP assay, AlamarBlue [®] assay, (ISO 10993-5)	MG63	Improved biocompatibility for bioglass containing Ca	[59]
None	None	None	[39–41,44–47,50,53,56–58]

time-points. Overall, the Elastic modulus has been studied in thirteen articles and can be obtained using all the methods described above; it is a key property for every kind of material. However, comparisons between workers should be considered with caution because the obtained values for the mechanical properties can be affected by the specific measurement technique applied. Test standardization is needed to avoid these variations.

Four articles in this review perform the Vicker's hardness test [38,39,58,59]. In this test the aim is to generate cracks at the extremities of the indent, calculating the fracture resistance from the dimensions of the indent diagonals and of the crack lengths. The Knoop hardness test [57] has also been applied. Here, small indentations are made to evaluate specific regional differences in micro-hardness; in the aforementioned study [57] samples are associated with mineral deposition over time.

4.2. Compositional features, mechanical properties and applications

Although most of the studies describe methods used to achieve particular mechanical properties, there is a lack of consistency in the range of information obtained from each paper. In contrast, the glass composition used is always well detailed even though the researches are still focused especially on silicate systems—just one article studied Calcium Phosphates [46] and no Borate glasses are investigated. Between the ten articles focusing only on bioactive glass composition; eight analysed the effect of different elements added to a silicate system [38,39,47,51,53,55,58,59] and two articles investigated the effect of synthesis methods [48,56].

The addition of Barium, Nitrogen, Calcium, Silicon and Aluminium into the glass composition has a direct positive

influence on the mechanical properties. The flexural strength increases by adding Barium, because it acts as a sintering additive accelerating the melting, giving more homogeneity in the glass and more rigidity. Ba²⁺ ions possess a larger ionic radius than Silicon ions, and this substitution allows the formation of a denser network in the glass structure [55]. Nitrogen increases microhardness and elastic modulus similarly influencing the network structure; substituting the trivalent N³⁻ ions for the divalent O²⁻ ions affects the contraction of the network and therefore reduces the molar volume and increases the density. This all improves the mechanical properties [39]. Lin et al. [53] found that increasing the CaO/P₂O₅ ratio correlates to a direct increase of the elastic modulus caused by the cohesion (effect of Ca²⁺ ions). The addition of Silicon and Aluminium, that are both network former elements, respectively improves the Young's modulus and the compressive strength [47,51].

Sodium content was shown to decrease the Vicker's hardness as confirmed by two articles in this systematic review. This is due to the Na⁺ cations forming weaker ionic linkages between two non-bridging oxygens in the glass structure consequently resulting in a less rigid glass network [58]. When HA is added to the bioglass powder, Vicker's hardness and elastic modulus increase [38].

The synthesis method also influences the properties of the final bioactive glass; foaming sol-gel synthesis delivers an enhanced porosity and consequently a weaker compressive strength for a scaffold for bone replacement [48]. On the other hand, a thermal treatment just after the synthesis can improve the load-bearing capacity of a biosilicate but also promote the nucleation of crystals producing a glass-ceramic like product [56].

In the other twelve articles among the twenty-two analysed, the influence of the addition of polymers has been

investigated, forming a relatively new class of bioactive materials called bioglass-based composites. These composites combine the mechanical and bioactivity properties of glass with the polymer's flexibility and elasticity resulting in enhancing characteristics for the final application. 83% of the studies ($n = 10$), for hard tissue engineering and bone substitution in orthopaedic claimed superior performance. The inclusion of bioglass particles in a polymer matrix is a recent and very advantageous direction for improving the mechanical, biological and physiological properties; the number of the publication in this field increased in recent years-ten articles [40–46,50,52,57] were released after the 2013, while only two [54,59] were reported between 2011 and 2012.

Polycaprolactone (PCL) is the most used polymer for bioactive glass composites and is studied in three papers [40,45,50] in this review. The incorporation of a bioglass system in PCL polymer induced elastic modulus increase.

Poly(lactic acid (PLA) and its copolymer poly-L-lactic-co-glycolic acid (PLGA) have also been mixed with bioactive glass powders to deliver improved mechanical properties [40,43,46]. The poly(D,L-lactic acid) (PDLLA) added to bioglass powders has been used to develop scaffold membranes for the treatment of periodontal defects but does not influence the mechanical properties; on the contrary, it has a positive impact on the biocompatibility of the material by enhancing cell proliferation and adhesion [52].

Nanocomposite films and coatings have also been developed to improve the mechanical properties of biocompatible scaffolds. Polymers can be used as a reinforcing agents, as in the study of Montazeri et al. [41] which covers a nBG (nano-bioglass) scaffold with poly-3-hydroxybutyrate (P3HB), increasing the compressive strength and compressive modulus which is directly proportional to the immersion time in the polymer. Recently, Moezzizadeh et al. [5] found that using bioglass as coating on zirconia implants enhance the bond strength to resin cement. This increase could be attributed to the higher surface roughness in the etched bioglass-coated surface.

Using a natural biopolymer in addition to bioglass powders is also a positive strategy for enhancing both mechanical properties and biocompatibility of the biomaterial. In this systematic review, two authors investigated the effects of collagen along with a mixture of poly(vinyl alcohol) (PVA) [42] and collagen in a compressed and dense form [54]. In the first study the mixture of a well-known biocompatible and apatite-forming biopolymer (chitosan/collagen) and PVA, with good elastic properties is added to a bioactive glass in order to obtain a scaffold with increased compressive modulus and compressive strength. In the study of Marelli et al. [54], the combination of dense collagen and bioglass powder was useful for producing scaffolds with improved compressive strength and good apatite formation ability.

The incorporation of bioglass powders into a polymer matrix enhances the mechanical properties, most of all the compressive strength and elastic modulus; this is evidenced in the majority of the studies considered ($n = 10$, 83%).

Knowledge of the role played by bioglass composition is fundamental for understanding trends in the mechanical properties and biological behaviour of the final biomaterials. The strong relationship between the variations of mechan-

ical properties associated with bioactive glass composition is essential to solve problems related to the development of improved materials.

4.3. Bioactivity & biocompatibility assessment

In 65% of the papers studied, the bioactivity of the glasses was investigated *in vitro* by examining apatite formation on the surface of glasses treated in different solutions. Twelve authors opted for SBF, an aqueous solution which mimics the (acellular and protein-free) human blood plasma. Chen and Thouas [48] used Dulbecco's Modified Eagle's medium (DMEM), a modification of Basal Medium Eagle (BME) that contains a four-fold higher concentration of amino acids and vitamins plus additional supplementary components. Tris buffer is used by Farooq et al. [58] to investigate the apatite forming ability of the glasses in a solution containing no Ca^{2+} and PO_4^{3-} ions, designating conditions similar to that presents in human saliva. Whatever the type of solution, that is chosen to be related to the clinic application, the bioactivity is always confirmed. It is common in all the articles that the hydroxycarbonate apatite layer formed (HCA) is confirmed using FTIR, XRD and SEM techniques. Bellucci et al. used micro-Raman spectroscopy for this purpose in his experimental protocol [38].

40% of the articles perform both a bioactivity test and a biocompatibility test. It can be noticed that once the apatite-forming ability (the essential condition for a biomaterial to bond with living bone) is confirmed, the biocompatibility is also established.

There is a huge variety of biocompatibility tests carried out by the authors considered in this systematic review. The most used tests are the Alkaline Phosphatase Assay (ALP) assay and the Alamar Blue[®] assay for cell metabolic activity and proliferation. The two tests are almost always coupled and this combination leads to a complete evaluation for *in vitro* cells testing. Direct references to ISO procedures are adopted by four articles [38,48,55,59] and specifically deal with the biological evaluation of medical devices referring to ISO 10993-4, ISO 10993-5 and ISO 10993-12. Proper methodologies for blood testing, cytotoxicity testing and samples preparation are detailed.

The cells types and the culture systems are very important for comparing results and it is possible to observe a large variety of choices between the authors of the studies considered, especially for the cellular model. The majority of the studies use Minimum Essential Medium (α -MEM) or Dulbecco's Modified Eagle Medium (DMEM) as culture medium. The most used cells are primary human cells, preferred in five articles [43,48,49,52,59]: adult human bone marrow stromal cells, human periodontal ligament cells, human osteoblast-like MG63 cells and human periodontal ligament stromal cells. Mouse cells are used for testing the biocompatibility in three articles [38,51,54]. Specifically, mouse embryonic fibroblast cell lines, murine long bone osteocyte-like immortalized cell line and murine calvarial osteoblast cells were used. Finally, rat osteoblast-like UMR-106 cells are used by Pon-On et al. [42]. Regarding work with primary human cells, the authors make equal use of osteoblast and fibroblast-like types; considering

all the articles studied, the fibroblast-like cells are the most used.

Generally the bioactivity tests are simple and cheap tools to preliminary investigate the bioactivity of biomaterials and it is interesting to see that the totality of the studies using both the tests demonstrate both bioactivity and biocompatibility for their biomaterials. Cytotoxicity tests based on *in vitro* cell culture are fundamental to ascertain the biocompatibility of new materials intended for biomedical applications. Although complex and time consuming analyses can be avoided, it is important to perform at least one test to check this feature. Several articles do not provide any information about the biological behaviour of their biomaterials.

5. Conclusion

Bioactive glasses have been proposed for a wide range of medical applications. Most of the studies so far have evidenced the enhancement of mechanical and biological properties; though there is still a lack of a definitive and satisfactory solution for highly biocompatible load-bearing materials in medicine and dentistry. The current aims in bioglass research must be framed in this area. The addition of HA to a Silicate bioglass powder achieves a higher Vicker's hardness, elastic modulus and introduces no negative effect regarding the biocompatibility. Barium and silicon enhance both mechanical properties and biological behavior. These elements/compound provide higher flexural strength, Young's modulus, compressive strength and improved biocompatibility via osteogenic effects and early osteo-conduction stage. Mixing bioglass with polymers (PLGA, PVA-chitosan/collagen, and PDLA) mainly enhances the elastic modulus and compressive strength as mechanical properties and improves the biocompatibility by enhancing cell attachment and proliferation. Finally, scaffolds prepared with the foam replication technique produced an increase in the compressive strength and maintained cell attachment and cell growth. The investigation of new bioglass compositions and the bioglass-based composites offer a very promising area for future research, which should be conducted with more comparable methods and significant results.

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3. MATERIALS & METHODS

The glasses studied in this thesis are based on the quinary system $\text{SiO}_2\text{-B}_2\text{O}_3\text{-K}_2\text{O-Al}_2\text{O}_3\text{-CaO}$ and were obtained by melt-quenching technique. In this chapter, a factorial experimental design will introduce how the set of samples was built. Then a first part is dedicated to the description of the methods used for developing the compositions of the borosilicate glass powders and how they were produced with the melt-quenching technique and the heat-treatment, with insight related to the raw materials and their behaviours.

A second part will explain the concepts behind the techniques used for the preliminary characterization of the glass powders obtained and how they are applied to the study of the borosilicates. Refractive index calculation, X-ray powders diffraction and differential thermal analysis were the techniques used for this purpose.

A third part is referred to the surface studies for the investigation of different microstructures found in the glasses induced by the heat-treatment. Optical microscopy and scanning electron microscopy are the most important techniques used and in this section will be describe how the samples were prepared for these analysis.

The last two parts are addressed to tests developed for the analysis of the ions release of the glasses in acid conditions and to the mechanical and bioactive properties connected with the final material mixed with a commercial poly(acrylic acid).

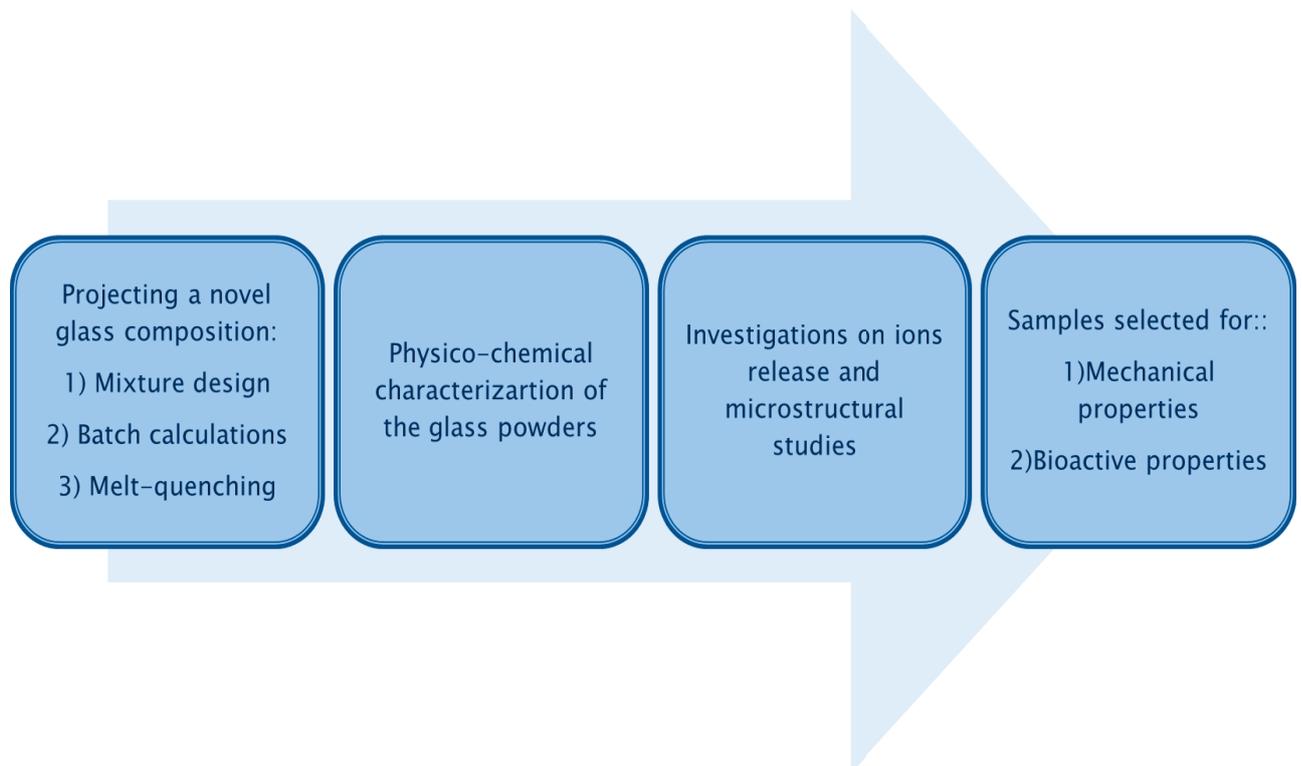


Figure 14 Overall scheme of the operations

3.1 Projecting and manufacturing of the borosilicate glass composition

3.1.1 Mixture design using an experimental design

Designs of experiments are useful tools that allows efficient organization of the experiments required in scientific research or industrial process optimisation (J. Goupy 2001). The complete understanding of this method should start by learning the essential notions that describe the area of the study and should follow with the choice of the most appropriate strategy for the purpose of the research.

The “response” is the property measured in final components delivered in every experiment. It is dependent from different variables that are called “factors” (i.e. Time, Temperature & Concentration). If the influence of just one factor is studied, its variations are limited between two (upper and lower) limit values (see figure 15).

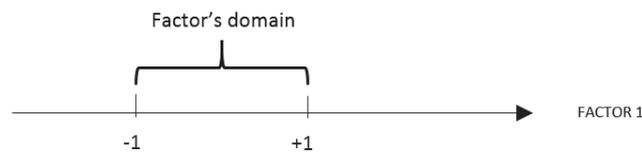


Figure 15 The factor domain includes all the values between the low (-1) and the high (+1) levels which are coded values without unit, i.e. (-1) corresponds to the minimum and (+1) to the maximum of the natural variable.

The domain of the factor includes all the values that a factor can assume. If there is a second factor it is also defined by a domain of values between an upper and a lower limit. The experimental area is in such a way defined by the space created by two axis. An experimental point is defined by the coordinates of the two axis in this manner (see figure 16)

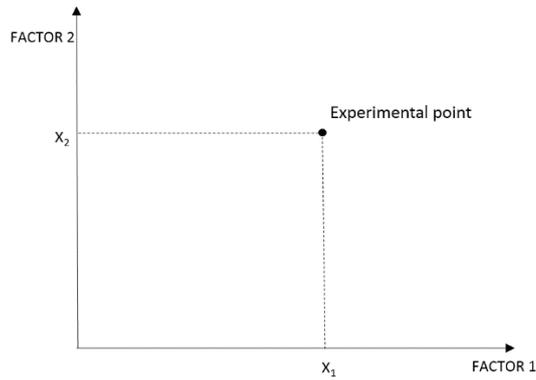


Figure 16 Representation of the experimental point into the experimental space.

The study domain is the zone of the experimental space chosen at the beginning of the research. The study domain groups the factor's domain:

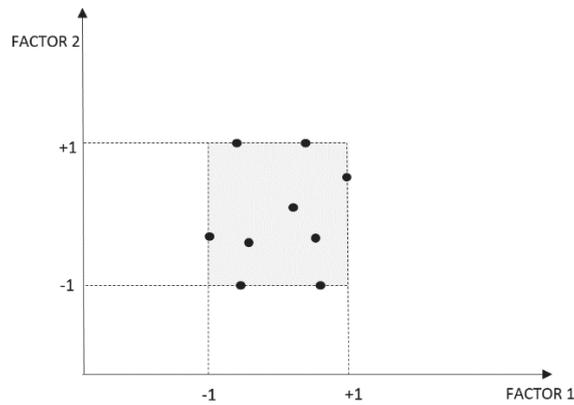


Figure 17 Experimental points defined into the experimental space determined by the researcher.

The response surface is graphically defined by an axis that is orthogonal to the experimental area.

The principal objective of the design of experiment is to obtain the most optimal response possible from the lowest number of experiments undertaken.

From a mathematical point of view the response can be represented as a polynomial model:

A first degree model is used for a screening design $y = a_0 + \sum a_i x_i$

A synergic model is used for factorial design $y = a_0 + \sum a_i x_i + \sum a_{ij} x_i x_j$

A quadratic model for surface response designs

$$y = a_0 + \sum a_i x_i + \sum a_{ij} x_i x_j + \sum a_{ii} x_i^2$$

where

y = response as measured during the experience

x_i = represents the coded value of the factor i (given by the experimenter)

a_0, a_i, a_{ij}, a_{ii} = coefficients of the mathematical model calculated in relation with the response of the experiment.

From a statistical point of view the random nature of the response involves an experimental error for each measurement that creates dispersion inside the values of the results.

An experimental design is associated with a model to get the matrix of the model, called X ; e is the vector of errors and y the vector of responses.

$$y = Xa + e$$

The resolution of this system of equations is obtained by linear regression using the least squares method:

$$\hat{a} = (X^T X)^{-1} X^T y$$

where

\hat{a} = vector coefficient

X^T = transposed matrix of X

Case of experiments with mixtures: mixture design

In this work a mixture design of experiments was selected.

In the general mixture problem, the measured response is assumed to depend only on the proportions of the ingredients present in the mixture.

The studied factors in a mixture design are the proportions of the components of the mix. If x_i represents the relative molar proportion or w_i the relative weight amount of the component i , the sum of the proportions of all the components satisfies this relationship:

$$\sum_{i=1}^{i=n} x_i = 100\% \text{ or } \sum_{i=1}^{i=n} w_i = 100\%$$

This relationship is called fundamental limit of the mix.

With three components the simplex factor space is an equilateral triangle. The pure products are found at the vertices of the triangle. The binary mixtures are found along the sides. Points inside the triangular area represent a ternary mixture.

For a mixture with more than three components a regular polyhedron is used. The borosilicate glass studied in this work is a quinary system in which two of the components are displayed together as they are fixed at a certain value ($\text{SiO}_2 = 45\text{wt}\%$; $\text{K}_2\text{O} = 15\text{wt}\%$). For this reason a regular tetrahedron will be used to represent the overall system studied (fig. 18 (a)), and 3 factors are sufficient to describe the study domain.

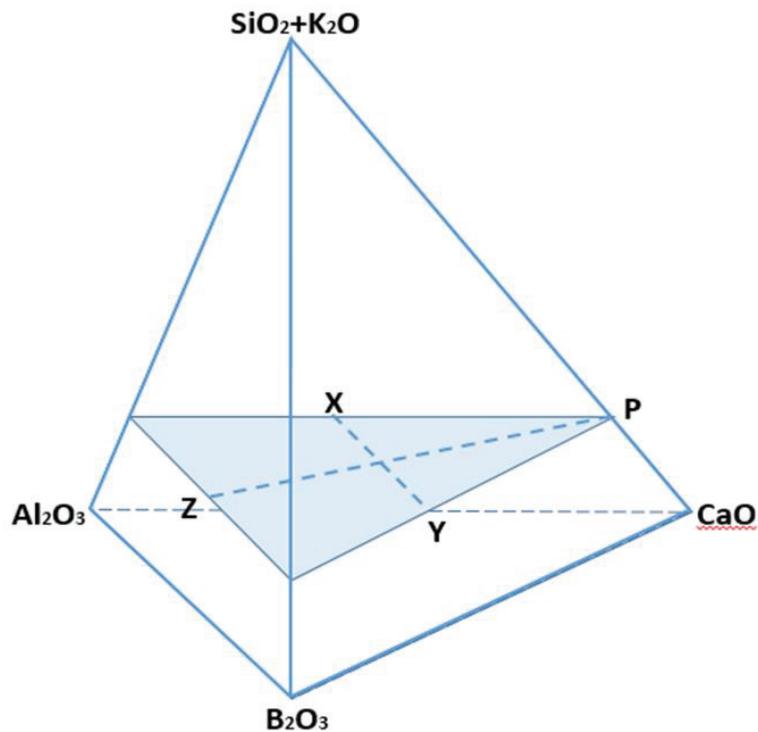


Figure (a) Quinary system represented with a tetragonal polyhedron. Several isoplethic sections were studied: 4 sections with a constant Al/B ratio (along the line ZP) and 3 sections with a fixed Ca content (along the line XY).

Different mathematical models are used for the response with 3 factors:

A first degree model $Y = b_1X_1 + b_2X_2 + b_3X_3$

A second degree model $Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$

A special cubic model $Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$

The higher the degree, the higher is the number of the points that should be used in the experimental area to determine the coefficients. The resolution of this system of equations is always obtained by linear regression using the least squares method:

$$\hat{b} = (X'X)^{-1}X'y$$

\hat{b} = coefficient

X' = transposed matrix of X

Taking into account all the previous information, 14 glass compositions were formulated to study a specific area on the triangle defined by the three factors (Fig 18 (b) and Table 8). The glasses have to satisfy the fundamental limit relationship of the mixture design. Keeping in mind that $\text{SiO}_2 + \text{K}_2\text{O}$ is a constant equal to 60w%, the remaining 40w% will be the fixed value that have to be reached by the sum of the other elements.

The constraints have been fixed in order to describe the experimental domain:

- B_2O_3 : 20-40w%
- CaO : 6-15w%
- Al_2O_3 : 0-11,5w%
- $\text{SiO}_2 = 45\text{w}\%$; $\text{K}_2\text{O} = 15\text{w}\%$

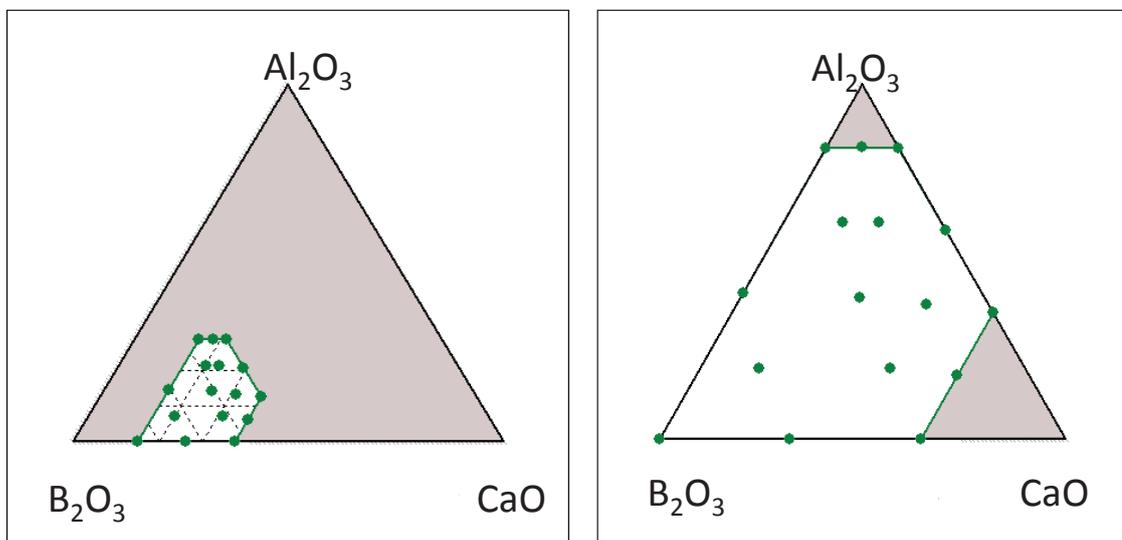


Figure 18 (b) Representation of theoretical experiments (DOE) from (NEMROD) Software-L.P.R.A.I, Marseille

Some compositions run were replicates that give a more robust response by providing information on standard deviations for repeat runs. Some compositions were

central points between the factor's domains while others are deliberately chosen on the isopleth lines to maintain a ratio of two elements constant and study how one single element affects the properties of the glass. Moreover, three final compositions were added as experimental points external to the study domain in order to collect information on glasses belonging to the isopleth lines but without at least one of the three variable elements. The response studied was defined by the kinetics of ions leaching of every glasses.

Table 8 Composition of the experimental borosilicate glasses (w%)

Glass	SiO ₂	K ₂ O	B ₂ O ₃	Al ₂ O ₃	CaO
PSBS 1	45,0	15,0	22,5	7,1	10,4
PSBS 2	45,0	15,0	26,2	3,4	10,4
PSBS 3	45,0	15,0	25,0	0,0	15,0
PSBS 4	45,0	15,0	20,0	11,5	8,5
PSBS 5	45,0	15,0	20,0	5,0	15,0
PSBS 6	45,0	15,0	26,2	3,4	10,4
PSBS 7	45,0	15,0	25,9	8,1	6,0
PSBS 8	45,0	15,0	34,0	0,0	6,0
PSBS 9	45,0	15,0	30,3	0,0	9,7
PSBS 10	45,0	15,0	25,9	8,1	6,0
PSBS 11	45,0	15,0	34,4	5,6	0,0
PSBS 12	45,0	15,0	30,0	10,0	0,0
PSBS 13	45,0	15,0	22,5	11,5	6,0
PSBS 14	45,0	15,0	22,5	2,5	15,0
PSBS 15	45,0	15,0	29,1	4,8	6,1
PSBS 16	45,0	15,0	21,4	7,1	11,5
PSBS SBK	45,0	15,0	40,0	0,0	0,0

Taking into account that SiO₂ and K₂O are constant the data were transformed by calculating the Janecke's coordinates that allow glass compositions to be represented on a triaxial diagram regardless of constant amount of SiO₂ and K₂O (Table 9 and Fig 19).

$$B_2O_3 w_J = m(B_2O_3) / (m(B_2O_3) + m(Al_2O_3) + m(CaO))$$

$$Al_2O_3 w_J = m(Al_2O_3) / (m(B_2O_3) + m(Al_2O_3) + m(CaO))$$

$$CaO w = m(CaO) / (m(B_2O_3) + m(Al_2O_3) + m(CaO))$$

Where:

w^J= coordinate on the Janecke triangle for each components

m= weight percentage of the glass components.

Table 9 Janecke coordinates calculate in percentage.

Samples	Janecke's coordinates w ^j		
	B ₂ O ₃ %	Al ₂ O ₃ %	CaO %
PSBS 1	56,14	17,96	25,90
PSBS 2	65,55	8,48	25,98
PSBS 3	62,50	0,00	37,50
PSBS 4	50,00	28,75	21,25
PSBS 5	50,00	12,50	37,50
PSBS 6	65,55	8,48	25,98
PSBS 7	64,70	20,30	15,00
PSBS 8	85,00	0,00	15,00
PSBS 9	75,68	0,00	24,32
PSBS 10	64,70	20,30	15,00
PSBS 11	86,00	14,00	0,00
PSBS 12	75,00	25,00	0,00
PSBS13	56,25	28,75	15,00
PSBS14	56,25	6,25	37,50
PSBS 15	72,85	11,89	15,25
PSBS 16	53,57	17,68	28,75

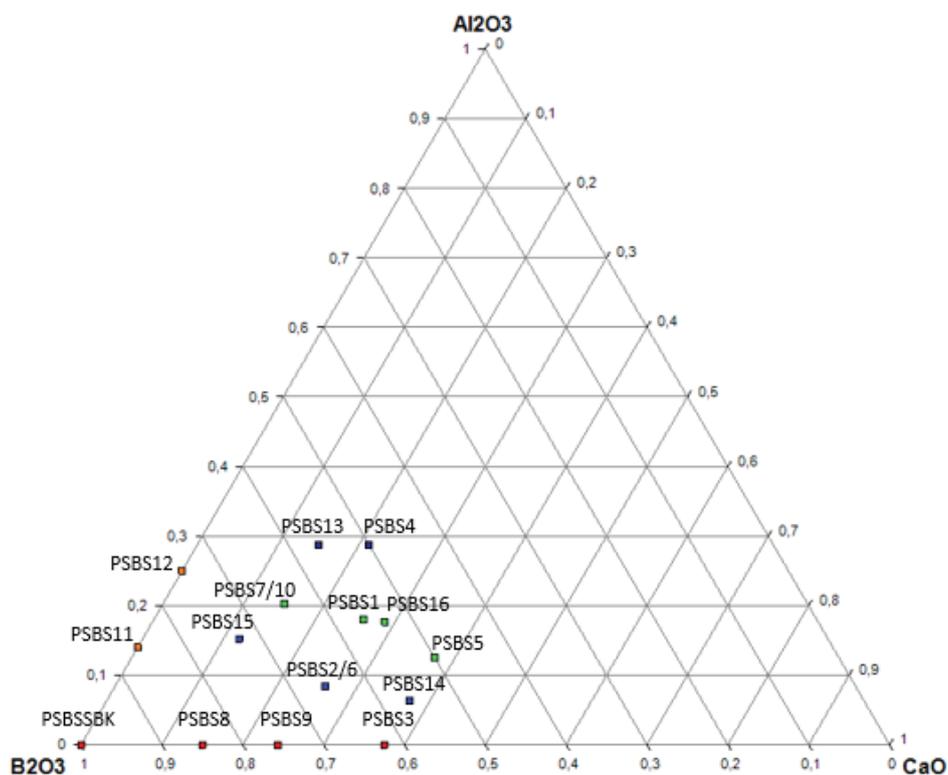


Figure 18 Ternary diagram with the representation of PSBS samples (Janecke's coordinates)

3.1.2 Batch calculations

All the series of glass compositions based on $\text{SiO}_2\text{-B}_2\text{O}_3\text{-K}_2\text{O-Al}_2\text{O}_3\text{-CaO}$ were prepared from raw materials purchased by different manufacturers: MICROSIL M10.2 (SIBELCO Benelux 99% pure SiO_2), H_3BO_3 , K_2CO_3 (ALTAIR Chimica s.p.a.), $\text{Al}_2(\text{OH})_6$ (Industrial mineral service) and CaCO_3 (Ben Bennett Jr Ltd).

A complete list of the raw materials used can be found in table 10.

Table 10 Raw materials used for the novel borosilicate system

RAW MATERIALS	DESCRIPTION	CHEMICAL/PHYSICAL PROPERTIES	SUPPLIER (EXAMPLE)
<i>Sand, silica, SiO_2</i>	It is the major constituent of most glasses. French, English and Holland sands are famous for their purity. This will permit the manufacture of good and colourless glasses. MICROSIL®M4.1T is produced by iron-free grinding and accurate sieving. Silica of >99% purity was used in these studies	Silica confers high viscosity, good chemical resistance and a low coefficient of thermal expansion.	Sibelco Benelux
<i>Potassium carbonate, K_2CO_3</i>	It is also known as potash or pearl ash, appears as a white powder or as colourless solid crystal.	It is similar to Na_2O , in action, acting to depolymerise silica glass networks. It lowers glass melting points, raises thermal expansion and improves the appearance or brilliance of the glass. It also helps maintain high electrical resistance.	Altair chimica s.p.a.
<i>Boric acid, H_3BO_3</i>	Boron containing minerals such as borax and boric acid, most commonly originate in dried salt lakebeds of deserts or arid areas (such as Death Valley, CA, Turkey, and China) or other geographic regions that expose similar deposits (such as the Andes Mountains in South America). Boric acid crystals are white and odourless.	Facilitates the melting operation, providing a lower viscosity glass during melting. Mechanical strength and scratch hardness are also increased in the final glass.	
<i>Lime, Calcium carbonate, CaO</i>	The Derbyshire deposits in Great Britain are very famous for their good quality. The raw	Gives great fluidity to the molten glass. High concentrations may	Ben Bennett Jr Ltd

	material used in these studies comes from there, dried and pulverised for the best purity. Other sources are calcite and dolomite, the latter contains also magnesium.	cause devitrification.	
<i>Hydrated alumina</i> , $Al_2(OH)_6$	Alumina can be introduced as one of the oxide components present in (e.g.) feldspar or as calcinated alumina. It has a marked effect on durability and provoking high viscosity. It appears as a white crystalline powder.	Produces glasses with high viscosity and high durability.	Industrial mineral services

The choice of a particular batch material depends primarily on the oxides required in the glass. It is very important to calculate the correct amount of raw materials, taking into account the appropriate conversion factors associated with chemical changes during heating. Any organic components can burn off completely or they can leave unwanted residual carbon in the glass if the combustion is incomplete. This causes reduction reactions that generate unwanted colorations in the melted glass; carbonates, sulfates, sulphites, nitrates, nitrites change to oxides during heating and no C, S, N should remain in the final glass structure. Fluorides, chlorides, bromides and iodides convert to an equivalent amount of oxide; there is usually little trace of F, Cl, Br, and I left in the glass due to their volatility; gases such as CO_2 are produced by decomposition (e.g. of K_2CO_3) and result in a relevant loss of weight in the final glass. There are four ways of expressing a glass composition each of which is valid and useful for different purpose:

1. Weight % oxide: results from XRF analysis are in this form and can be useful for comparing batches and samples.
2. Mole % oxide: sometimes useful when investigating different behaviour and development of new formulae.
3. Sager formula: useful shorthand for comparing compositions, sometimes referred to as "unity" formula. It shows the mathematical relationship between oxides in the glass that are "formers", "modifiers" and "intermediates"
4. Batch recipe: useful in preparation and specification for production. It is not ideal for describing the exact final compositions due to variations between raw material sources and minor compositional changes (e.g. due to volatilisation; or dissolution of elements from the crucible) during melting.

When the chemical composition of the glass to be melted is known, the batch is calculated from the amount of each oxide and carbonates required. The conversion of a glass composition into a batch composition is a simple mathematical calculation that is simplified by the use of conversion factors (G_f). These are basically depending on the type of raw

materials and oxides used for the studied composition. Table 11 demonstrates how to calculate the amount of each raw material in grams for a total 100 grams batch ready to be melted.

To calculate the conversion factor the following equation has to be use for each component:

$$G_f = \frac{\text{molar weight (oxide)}}{\text{molar weight (raw material)}}$$

Table 11 Batch recipe calculations for PSBS1.

Oxides	w% oxide	Raw Material	Conversion Factor	Part by weight	100g Batch recipe
SiO ₂	45	SiO ₂	1	45	33
K ₂ O	15	K ₂ CO ₃	0,68	22,06	16,17
B ₂ O ₃	22,5	H ₃ BO ₃	0,563	39,89	29,24
Al ₂ O ₃	7,1	Al ₂ (OH) ₆	0,654	10,98	8,05
CaO	10,4	CaCO ₃	0,561	18,47	13,54

Table 12 Conversion from weight% to mole% for PSBS1 sample.

Oxides	w% oxide	Molecular weight (oxide)	Parts by mol	mol% oxide
SiO ₂	45	60,1	0,749	50,4
K ₂ O	15	94,2	0,159	10,7
B ₂ O ₃	22,5	69,62	0,323	21,7
Al ₂ O ₃	7,1	101,96	0,07	4,7
CaO	10,4	56,1	0,185	12,5

The Seger formula separates the mole parts of the oxide groups into three sets as follow:

RO, R ₂ O	R ₂ O ₃	RO ₂
Alkali, Alkaline earths	Amphoteris	Acidic
Glass modifiers	Intermediates	Glass formers

In some cases B₂O₃ is included as amphoteric but it will be consider as glass former.

The Sager formula represents the molar ratio of its constituents. In order to compare glasses on an equal basis the Seger formula is converted to the “unity” formula. This is achieved by

ratio of the alkali oxides to 1 or unity, dividing the oxides by the total amount coming from the glass modifiers group (table 13).

Table 13 Calculation for the Sager formula

RO, R ₂ O	R ₂ O ₃	RO ₂
K ₂ O (0,159)	Al ₂ O ₃ (0,07)	SiO ₂ (0,749)
CaO (0,185)		B ₂ O ₃ (0,323)
Sum (0,344)		

Table 14 Sager formula example for PSBS1

RO, R ₂ O	R ₂ O ₃	RO ₂
K ₂ O (0,46)	Al ₂ O ₃ (0,20)	SiO ₂ (2,18)
CaO (0,54)		B ₂ O ₃ (1,07)
Sum (1)		

The “unity” formula can be used to compare formulae, oxide ratios and so on. It is sometimes used to describe compositions where other information such as the raw materials selected may be considered confidential.

3.1.3 Melt quenching and Heat treatment

All the weighed raw materials have to be placed into a suitable crucible. In this work, a quartz crucible was selected for the purpose. Usually silica crucibles are the most recommended because of their thermal shock resistance, low reactivity and low cost. Since glasses often contain silica as a major component, contamination from a pure silica crucible will be minimal.

The melting is thus carried out by placing the batch inside the crucible and heating the crucible in an electric furnace. The melting temperature to be used is determined by consulting the phase diagram for related glasses. Theoretically the principles for the glass melting are:

- Reaction between the components of the raw materials mix
- Dissolution of solids in the primary melt
- Degassing the melt
- Chemical homogenisation of the melt.

Glass batches were prepared by mixing dry raw materials from the recipes described and sieving to ensure thorough blending. Glass batch (100 grams) melting took place in a quartz

crucible at 1375°C, in an electric furnace. After melting for approximately 2 hours at around 1350°C to reduce bubble in the glass generated by gas evolution, the melts were poured into a metal mould to produce glass bulk. The pouring of the melted glass is performed on to a clean steel plate to avoid any contact with water and thus the initiation of unwanted premature glass leaching reactions that are required when activating the GIC product (fig.20).



Figure 19 Glass melting procedure: quartz crucible placed in the kiln, checking the glass melt and pouring it to obtain the frit.

The time held at temperature chosen to induce phase separation could also be an important factor. For this reason, different trials at 5, 10, 20 and 40 hours have been made to establish the best conditions for phase separation. The results section provides details of the different experiments conducted. Once the phase separation heat treatment methodology was fully optimized, all experimental glasses were heat treated in this way using an electric intermittent laboratory kiln. Samples were heated (175°C/hour) to a single selected

temperature (700°C) and held for 20 hours. The kiln was allowed to cool naturally from the dwell temperature (see figure 21).

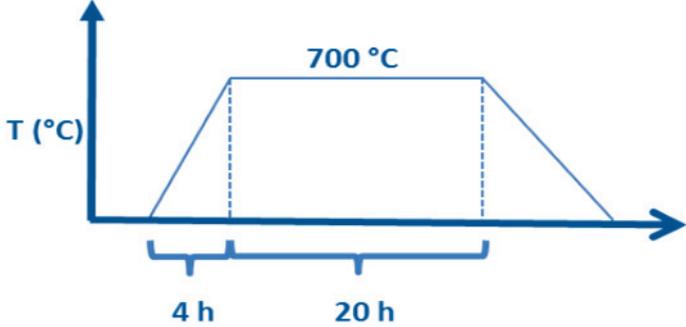


Figure 20 Procedure used for the heat treatment.

3.1.4 Particle size distribution analysis

Particle size influences many properties of particulate materials and is a valuable indicator of quality and performance. Smaller particles dissolve more quickly, influencing the biocompatibility of bioglasses and lead to higher suspension viscosities than larger ones. The technique of laser diffraction is based on the principle that particles passing through a laser beam will scatter light at an angle that is directly related to their size: large particles scatter at low angles, whereas small particles scatter at high angles. The measurable size ranges from 50 [nm] to 1000 [μm]. Suspensions are mostly prepared with water as in this case, but other solvents can also be used (ethanol, isopropanol, or octane). The particle size distribution was measured by the Malvern Mastersizer 3000 laser diffraction particle size analyser. The glass powders were evaluated with a Dv99 of less than 50 μm and a Dv50 of less than 6,9 μm . D50 means that the diameter where 50% of the particles have a smaller size that on a volume basis is the value reported. The particle dimension was compared to that one of a commercial product called GC Fuji IX GP[®] (GC corporation, Tokyo, Japan).

3.2 Characterizations of the borosilicate glasses

3.2.1 Refractive index:

- calculated with the Appen method

The refractive index can be calculated if the nature and the concentration of the different glass components are known. There are two possible ways but the most efficient and used one is the so called “Appen method” and calculated with the following equation (Appen 1956):

$$n_d = \sum_{i=1}^n \frac{n_{d,i} c_i}{100}$$

$n_{d,i}$ = factor referred to the glass oxide

c_i = oxide concentration in mol%

Table 15 Factors necessary to calculate the refractive index of glasses by the composition.

Oxides	$n_{d,i}$
SiO ₂	1.475
K ₂ O	1.575
B ₂ O ₃	1.71
CaO	1.73
Al ₂ O ₃	1.52

- measured with the Becke line test

It is an immersion technique in which the refractive index in solid samples is compared with the index of a known liquid (Allen 1985). Particles of the sample are mounted in the reference liquid and the index of the PSBS8 and 16 glasses are compared with that of the liquids. If the solid index is higher, a liquid of higher index have to be used, if the solid is lower vice versa. The method continues with these repetitions until a match is obtained. To compare the index the Becke line test is used. The Becke line test uses the contrast of the particle in the liquid and the presence of a halo around the particle for the index comparison. When particle and liquid are exactly matched, it is not possible to detect the particle (although there may be inclusions and cleavage marks that are visible). This impossibility is due to the fact that there is no refraction of light at the interface between the liquid and solid, and hence, no contrast. When the liquid and solid refractive indices are close but not exactly the same, then you will see some particle contrast as well as a bright or dark halo around the particle. This bright halo is called the Becke line and the direction it moves when raising the focus of the microscope (generally by lowering the stage) is the mechanism for determining refractive index. The Becke line travels toward the material with the higher index.

The precise method followed during the measurement was:

1. Placing a small amount of the glass sample (fine particulate) on a glass slide and covering it with a small coverglass.
2. Placing a small amount of the reference liquid next to the coverglass and allowing the liquid to flow into the sample using capillary action.
3. Inserting the sample onto the microscope stage
4. Closing down the substage iris.
5. Note the contrast of the particles. With very high contrast the Becke line test may be ambiguous and it may be better to mount in two liquids much higher and much lower than the current one.
6. Start raising the focus of the microscope (lower stage generally) while observing a particle of interest.
7. If the bright halo (Becke line) is moving toward the particle, its index is greater than that of the liquid and the next liquid used should have a higher index.
8. If the bright halo (Becke line) is moving toward the liquid, the particle index is lower than that of the liquid and the next liquid used should have a lower index.
9. Placing next liquid based on the degree of contrast. If the contrast is low, is better chosing a liquid close in value with the current one and vice versa.
10. Continue changing liquids until a match is achieved. Verify by testing the next highest and next lowest index values.

These are caused by refraction effects, and concentrate light near the edges of the glasses. The Becke line method is a good methodology because comparing the refractive index of the oil with that of the glass it is possible to determine clearly the RI of the PSBS samples. When the stage is moved up or down, the Becke line(s) also move in and out of the glass, towards or opposite to the oil. The direction of movement of the Becke Line is determined by lowering the stage with the Becke Line always moving into the material with the higher refractive index. In the figure 30 is shown where the Becke line is moving with the help of a black arrow that indicate the direction of the shift. The Becke Line can be considered to form from a cone of light that extends upwards from the edge of the mineral grain (fig. 22). Increasing the distance between the stage and the objective lenses the Becke lines will move to the material with the highest refractive index. Decreasing the distance between the stage and the objective lenses the Becke line will move to the material with smaller refractive index.

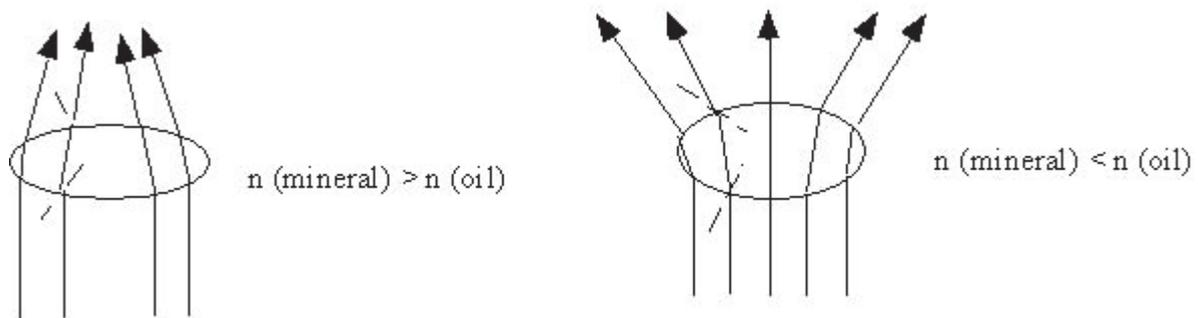


Figure 21 Becke line formation.

3.2.2 X-rays powder diffraction

Powder X-ray diffraction (XRD) data were collected with a Bruker diffractometer with graphite monochromator using $\text{CuK}\alpha$ radiation (fig. 23). Data were collected in the range of $2^\circ < 2\theta < 60^\circ$ in 0.05° steps. This technique is used to determine if the glass powder is fully amorphous before and after the heat treatment. The amorphous glass does not have long-range atomic order and therefore produces only broad scattering peaks. A crystal is composed of periodically arranged atoms in a 3D space. On the other hand amorphous materials do not possess that periodicity and atoms are distributed randomly in the 3Dspace. The scattering of the X-rays by atoms is the point to be considered in this case. When there is a periodic arrangement of atoms the X-rays will be scattered only in certain directions when they hit the formed lattice planes (formed by atoms). This will cause the high intensity of the peaks (the width of the peaks depends on other variables). With an amorphous phase the X-rays will be scattered in many direction leading to a large bump distributed in a wide range instead of high intensity peaks.

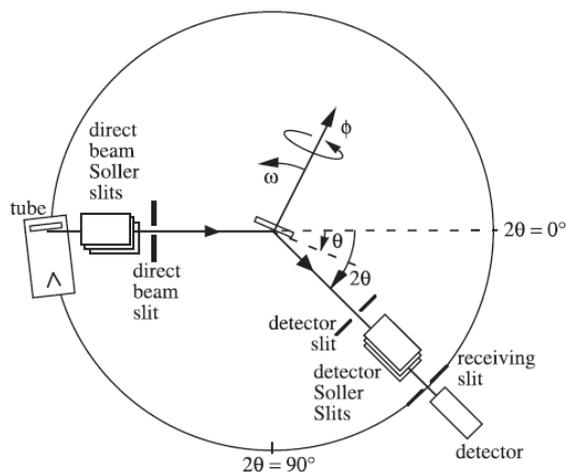


Figure 22 The detector moves in a circle around the sample and its position is recorded as the angle 2θ . The detector records the number of X-rays observed at each angle 2θ . The X-ray intensity is usually recorded as “counts” or as “counts per second”.

3.2.3 Differential thermal analysis

Thermal behaviour was analysed using differential thermal analysis (DTA). The instrument used is a Mettler Toledo TGA/DSC 2 in the platforme d’analyse thermique directed by Doctor Chiriac Rodica. The measurements were conducted with a heating rate of $20^{\circ}\text{C}/\text{min}$ and using nitrogen as both protecting and purge gas. Approximately 50 mg of glass powder was put into an alumina crucible and gently pressed to ensure good heat transfer. The powders were scanned over the 30 to 1200°C range in order to obtain data on the principal transformations associated with each glass samples, such as glass transition, crystallization and melting. The heat capacity (C_p) is measured by the instrument and allows the calculation of different phenomena.

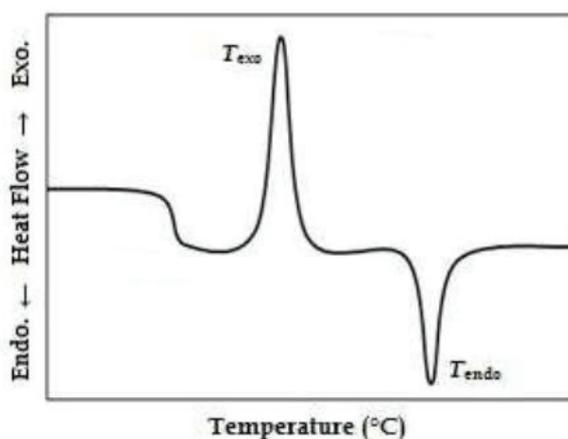


Figure 23 A representative thermal analysis thermogram.

When a reaction occurs during thermal analysis, the change in heat content and in the thermal properties of the sample is indicated by a deflection in the thermogram that can be positive or negative as indicated in the figure 24.

At the T_g the plot shifts suddenly. This means that the system is getting more heat flow. A change in the heat capacity of the glass is detected as the glass transitions. This results in an endothermic peak indicative of a phase change.

Crystallization happens when the sample reaches a given temperature where viscosity allows for an ordered re-arrangement of atoms into crystalline structures. The temperature is called T_c and at this moment the glass will give off heat resulting in a decrease in the heat flow. This is represented by an exothermic peak (Kissinger 1957).

Once the sample has passed the T_c during heating, there is eventually a further thermal transition associated with melting. At T_m the glass begins to melt and flow. In this case the thermocouple under the crucible containing the sample is producing a lot of heat in order to both melt the glass and keep the temperature rising at the same rate as the reference pan.

3.3 Ions leaching, dissolution experiments & surface analysis

3.3.1 Static dissolution experiments

Static dissolution experiments were performed for all the PSBS samples. The glass powders (0,4 g each) were mixed with a 0,003M HNO₃ solution (40 mL) in a falcon tube and placed in a reactor vessel at 37°C (thermostatically controlled) as shown in figure 25. At different time points the solutions were centrifuged and collected for the ion coupled plasma (ICP) measurements to determine the ions released for each samples.

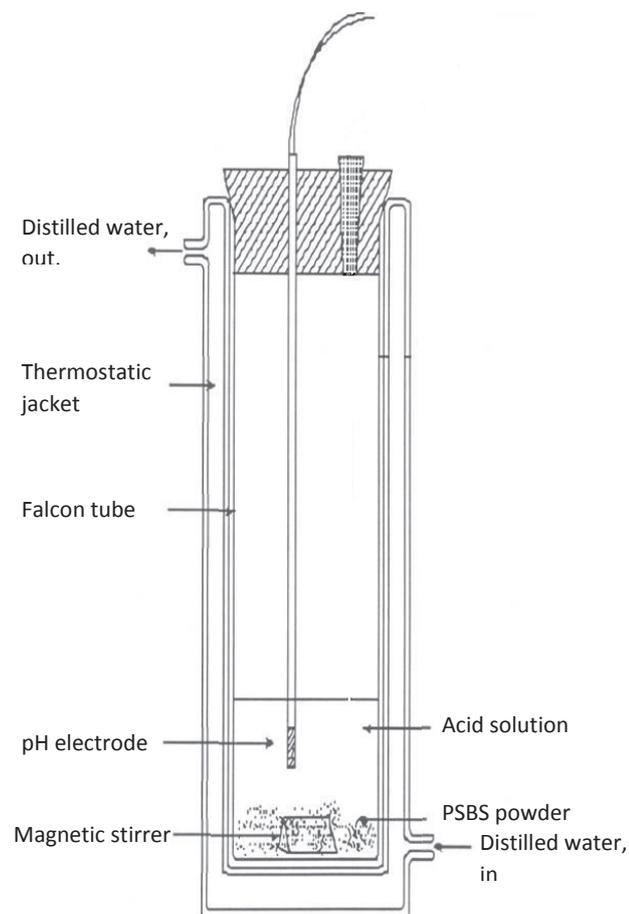


Figure 24 A falcon tube is inserted into a reaction vessel that is controlled by a thermostat regulating the temperature at 37°C.

3.3.2 Inductively coupled plasma atomic emission spectrometry

The elements detected and the corresponding absorption wavelengths are reported in table 17. Calibration curves were obtained by preparing multi-elements standard solutions containing B, Ca, K and Si in the concentrations reported in the table 16 (dilutions were made in HNO₃ solutions). Three replicates were collected for each measurement. The result obtained for the same element from different emission lines were averaged and presented as % dissolution, calculated as the ratio between the concentration measured in solution and the maximum concentration that could be obtained if total dissolution of the glass occurred.

Table 16 Standard concentration for ICP measurements.

Element	St 1	St 2	St 3	St 4	St 5
Si	10.4107	21.2556	34.3286	60.1571	81.1483
B	10.1793	19.8089	29.7970	60.3272	80.2119
K	9.8642	19.9033	30.1274	50.3756	70.3344
Ca	10.2147	19.7464	29.1073	50.6244	70.1392

Table 17 Elements detected with ICP measurements and respective emission lines.

Element	Emission lines
B	208.956, 249.678, 249.772 nm
Ca	317.993, 373.690 nm
K	766.49, 769.897 nm
Si	250.690, 251.611, 288.158 nm

3.3.3 Optical microscopy

With reflected or transmitted light modes and different magnifications available the optical microscope is a very useful technique for a preliminary screening of the surface of samples. For the best results, the sample should be polished properly following an adapted procedure dependent on the type of material studied.

Sample preparation: Mounting

Mounting of glass bulk specimens is usually necessary to allow them to be handled easily. It also minimises the amount of damage likely to be caused to the specimen itself. The mounting material used should not influence the specimen as a result of chemical reaction or mechanical stresses. A cold-setting resin of methyl methacrylate (Resine mecaprex KM-U) was used.

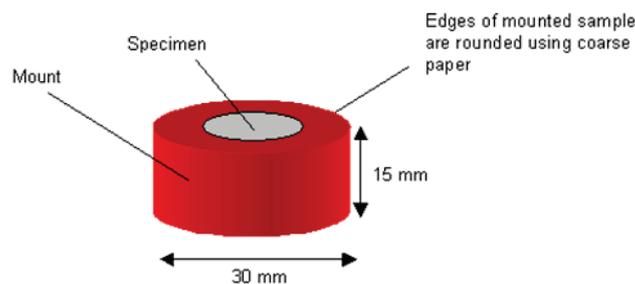


Figure 25 Specimen on resin for optic microscopy

Grinding

Surface layers damaged by cutting must be removed by grinding. Mounted specimens are ground with rotating discs of abrasive paper.

Polishing

Polishing discs feature soft cloth impregnated with abrasive diamond particles and an oily lubricant. A TISSEDIAM 40 μm was used with a 3 daN pressure. The plate speed was 300 rpm and the sample holder speed was 135 rpm. The polishing was performed over 180/240 seconds.

The next step was performed in a NWF+ 9 μ disc and then on a NWF+ 3 μ disc. For both the polishing steps the speed of the plate was 150 rpm, the speed of the sample holder was 135 rpm and the time was 180/240 seconds.

The last disc used was NV pre-wet with deionized water and Al_2O_3 suspension in order to obtain a flat, level surface to be examined optically. This last step should be avoided in case of elemental analysis with EDX because it can contaminate the sample surface.

3.3.4 Scanning electron microscopy & Energy dispersive x-ray spectrometry analysis

The morphology of the samples was examined using an FEI Quanta FEG 250 Scanning Electron Microscope before and after an acid treatment. SEM imaging of the samples was conducted using Backscattered Electron Imaging; using this imaging mode the brightness of a given feature is proportional to its mean atomic number. Imaging and analysis conditions were 15 kV accelerating voltage and 10mm working distance. Microanalysis results are tabulated on a semi-quantitative, normalised, elemental weight basis. Light elements such as lithium and boron are not quantified by this analytical technique. Carbon is also omitted.

Two different conditions were chosen to compare the dissolution at body temperature and at higher temperature to speed up the leaching process. The normal condition was

replicated with a 1 M HNO₃ etching solution at 37°C for 48 hours. The harsher conditions were simulated with the same 1 M HNO₃ solution for 48 hours but at 90°C. Then the PSBS glass samples were rinsed with deionized water and carbon coated before the analysis. The 10nm thick carbon coating is a conducting surface applied to the samples which has a minimal influence on x-ray intensities due to its low atomic number but is useful to provide a path for the incident electrons to flow to the ground.

3.3.5 BET analysis

The Brunauer-Emmett-Teller (BET) method is the most widely used procedure for the determination of the surface area of solid materials and involves the use of the BET equation. The glass powder porosity and surface area was checked for the heat treated samples before and after one week of dissolution into an HNO₃ solution 0,003M at 37°C. The samples were weighted (~23 grams) and placed in a reaction probe. Two probes at the time can be connected to the instrument while a third probe is placed empty and used as a control. The heating program consists of a ramp that takes the samples from ambient temperature to 150°C for eliminate any water present inside the sample. Drying and degassing follows. A Dewar with liquid nitrogen is then placed on the platform below the three test probes. The BET method is based on the adsorption of the N₂ on the surface. The amount of gas adsorbed as a function of pressure allows the determination of the surface area as well as information on pore shapes. Superficial area, pore size and pore volume were measured for the samples before and after the heat treatment.

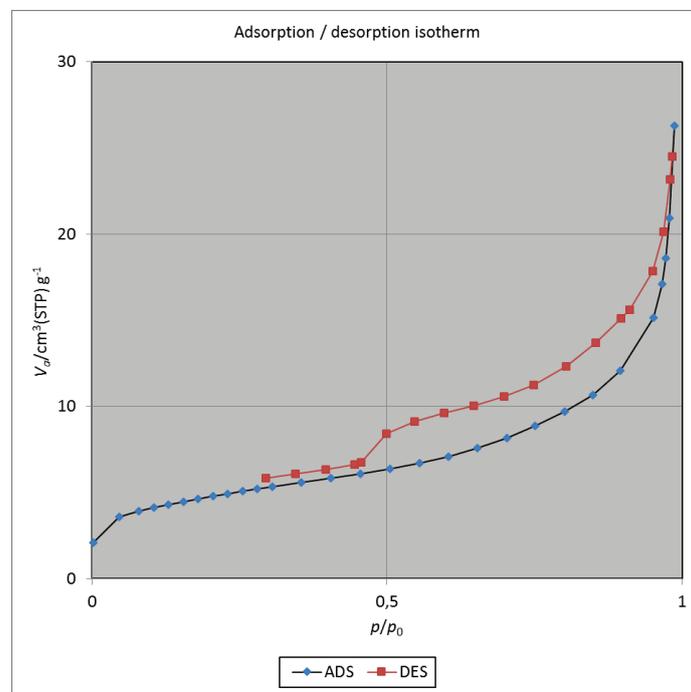


Figure 26 Typical adsorption/desorption isotherm obtain with BET analysis (PSBS1).

3.4 Mechanical properties analysis

3.4.1 Sample preparation

The mechanical properties were applied on two PSBS glass powder samples mixed with the poly(acid) liquid of a commercial glass ionomer cement (FUJI IX[®]GP, GC Europe N.V, Leuven, Belgium). The same instruction given by the producer of FUJI IX[®] were used for the mixing and the powder:liquid ratio was 1spoon:2drops. Figure 28 indicates the test pieve dimensions.

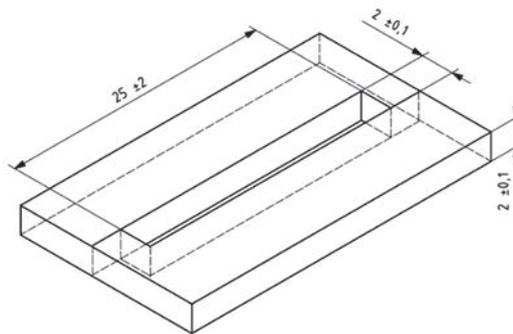


Figure 27 Mould shape and dimension used for mechanical tests.

3.4.2 Microhardness test

The indentation technique consists of the placement of an indenter (usually diamond) to the material surface, with a known force applied (see figure 29). The contact area between the material and the indenter is then measured directly or indirectly. From the classical point of view, the interpretation of the contact area is evaluated with the help of a microscope optic. The area is calculated by measuring the diametre of the fingerprint indent left on the surface of the tested material. In the case of a Vickers tests as used in the present study, the shape of the indenter is pyramidal. Currently, the indentation method is the only technique used to measuring the mechanical properties at high definition. The Vickers microhardness (measured in Kg/mm²) is obtained with the relation:

$$HV = 1854,4 \times P \times d^{-2}$$

P: charge applied in grams.

d: average of the length of the two diagonals ($d_1 + d_2 / 2$)

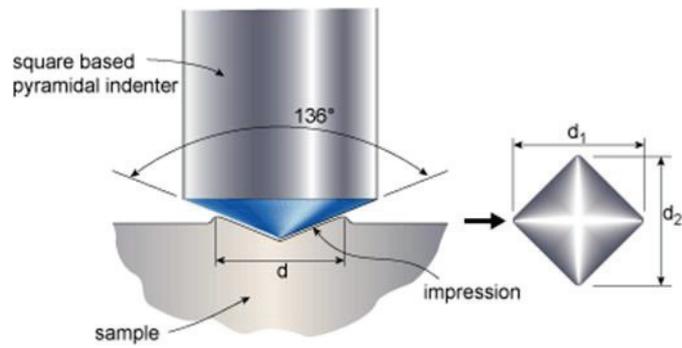


Figure 28 Vickers hardness indenter.

3.4.3 Flexural strength

A three point bend test consists of placing the sample bar horizontally upon two points prior to applying a force to the top of the sample through a single point so that the sample flexes only in the middle. The flexural strength is defined as the maximum stress at the outermost fiber on either the compression or tension side of the specimen. It is calculated with the following equation:

$$\text{Flexural strength} = \frac{3FmI}{2bh^2}$$

where

Fm: Force (Newton)

I: length (20 mm)

b: width

h: thickness

3.5 Bioactivity characterization

3.5.1 Simulated body fluid (SBF) preparation

Kokubo and his colleagues (A. Macon 2015) developed an acellular simulated body fluid that has inorganic ion concentrations similar to those of human extracellular fluid, in order to reproduce formation of apatite on bioactive materials *in vitro*. This fluid can be used not only for evaluation of bioactivity of artificial materials *in vitro*, but also coating of apatite on various materials under biomimetic conditions. The simulated body fluid is often abbreviated as SBF or Kokubo solution. The ion concentrations of SBF are given in Table 18.

Table 18 Comparison between SBF solution and human blood plasma ions concentration.

Ion	Concentration (mmol/dm ³)	
	Simulated body fluid (SBF)	Human blood plasma
Na ⁺	142.0	142.0
K ⁺	5.0	5.0
Mg ²⁺	1.5	1.5
Ca ²⁺	2.5	2.5
Cl ⁻	147.8	103.0
HCO ₃ ⁻	4.2	27.0
HPO ₄ ²⁻	1.0	1.0
SO ₄ ²⁻	0.5	0.5

The pH of SBF is adjusted to pH 7.25 at 36.5°C, by using 50 mM (=mmol/dm³) of tris(hydroxymethyl)aminomethane and approximately 45 mM of HCl.

For the preparation of SBF, 750 mL of ultra-pure water were placed in a 1000 mL beaker (polyethylene beaker is preferred). The water should be stirred continuously with the temperature at 36.5°C. The beaker is preferably placed on a clean bench and covered with cling film, to avoid dust pick-up

The chemicals given in the table 19 have to be individually dissolved into the water in turn until reagent number 8. Each reagent has to be completely dissolved before adding the next one. The Addition of reagent number 9 should be done incrementally (< 1g at a time), in order to avoid local increases in pH of the solution.

The stability of the solution obtained has to be examined. 50 mL of the solution was stored in a polystyrene bottle and placed in an incubator at 36.5°C. After 2-3 days, it is suggested to visually assess whether the solution has any precipitation or not. If there are not precipitations the solution can be used for experiments.

Table 19 Order of reagents for the SBF solution preparation.

Order	Reagent		Amount
#1	NaCl	Assay min. 99.5%, Nacalai tesque, Kyoto, Japan	7.996 g
#2	NaHCO ₃	Assay (after drying) min. 99.5-100.3%, Nacalai tesque, Kyoto, Japan	0.350 g
#3	KCl	Assay min. 99.5%, Nacalai tesque, Kyoto, Japan	0.224 g
#4	K ₂ HPO ₄ · 3H ₂ O	Assay min. 99.0%, Nacalai tesque, Kyoto, Japan	0.228 g
#5	MgCl ₂ · 6H ₂ O	Assay min. 98.0%, Nacalai tesque, Kyoto, Japan	0.305 g
#6	1 kmol/m ³ HCl	87.28 mL of 35,4% HCl is diluted to 1000 mL with volumetric flask	40 cm ³
#7	CaCl ₂	Assay min. 95.0%, Nacalai tesque, Kyoto, Japan Use after drying at 120 °C for more than 12 hours	0.278 g
#8	Na ₂ SO ₄	Assay min. 99.0%, Nacalai tesque, Kyoto, Japan	0.071 g
#9	(CH ₂ OH) ₃ CNH ₂	Assay (after drying) min. 99.9%, Nacalai tesque, Kyoto, Japan	6.057 g
#10	1 kmol/m ³ HCl	See above	Appropriate amount for adjusting pH

3.5.2 Bioactivity test ISO 23317:2014

The hydroxyapatite (HA) layer can be formed on the surfaces of biomaterials in an acellular and protein-free solution called simulated body fluid (SBF). The HA formed is very similar to the bone mineral in its composition and structure. The evaluation of HA-forming ability on implant materials in SBF is useful for evaluating its in vivo bone-bonding ability.

Glass disks were prepared by melting prepared PSBS glass samples and pouring the melt into graphite moulds (pre-heated at 300°C). The conditions used in this process are useful to avoid any cracks during the disk formations that can be due to the high stress for pouring the disk in a very compressed disk mould. The disks obtained were then heat treated to induce the phase separation. One glass disk was made with a 45S5K glass (representing the Hench composition but with sodium replaced by potassium) to have, as a control, a well known bioactive glass. The surface area was calculated for every sample by measuring the diameter with a micrometer while the thickness was fixed at 0.2 cm. This area was used to calculate the volume of SBF necessary for each specimen with the following formula:

$$V_S = 100\text{mm} \times S_A$$

Where

V_S : volume of SBF in mm^3

S_A : surface area of the specimen.

The disks were then fixed with a blue tac support into reaction tubes (see figure 30 below) and placed inside an incubator at 37°C . After different time periods, the specimens were taken out from the SBF and gently rinsed with distilled water. The disks were then dried in a desiccator without heating as preparation for the SEM-EDX analysis.

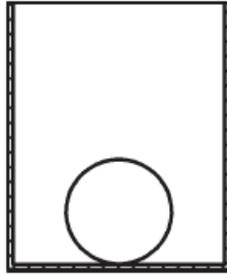


Figure 29 Disk fixed perpendicularly to the bottom of the reaction tube.

4. GLASS ELABORATION AND CHARACTERIZATION

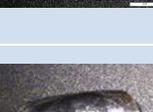
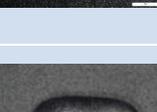
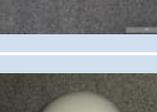
4.1 Projecting and manufacturing of the borosilicate glass composition

4.1.1 Melt quenching and heat-treatment

All the glasses were melted and heat treated in accordance with the procedure described in the previous chapter of the thesis (3. Materials and Methods). The results are shown in the following table (table 20).

Table 20 Pictorial representation of Borosilicate glasses before and after the heat treatment plus associated melting temperature and viscosity observations

SAMPLE NAME	BEFORE Heat Treatment	AFTER Heat Treatment	Melting Temperature (°C)	Observations of the melt
PSBS1			1300	Viscous
PSBS2			1250	Fluid
PSBS3			1250	Fluid (smoky boron)
PSBS4			1350	Medium Viscosity
PSBS5			1300	Viscous
PSBS6			1250	Fluid

PSBS7			1250	Viscous
PSBS8			1200	Fluid (smoky boron)
PSBS9			1250	Fluid (smoky boron)
PSBS10			1280	Viscous
PSBS11			1250	Fluid
PSBS12			1300	Highly viscous
PSBS13			1325	Medium viscous
PSBS14			1300	Viscous
PSBS15			1200	Viscous
PSBS16			1250	Viscous

PSBSSBK			1200	Fluid (smoky boron)
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Viscosity is one of the key properties of a glass melt that determines its behaviour in the initial stage of the manufacturing process. Viscosity is dependent on the chemical composition. The effect of the individual components also depends on the overall composition of the glasses. Generally, SiO_2 and Al_2O_3 as network formers increase viscosity (due to enhanced M-O connectivity) whereas alkalis reduce it. Glasses with high CaO content usually exhibit a drop in the viscosity curve (viscosity Vs. temperature). Mixing alkalis generally lowers the viscosity more readily and the phenomenon is called mixed-alkali-effect. B_2O_3 decreases viscosity at temperature approaching glass melting, increasing it at lower temperatures. The difference between the viscosity of high boron-containing glasses and silicon-containing glasses is explained because silica is a fully connected tetrahedral network whilst a boric oxide network is only triangularly connected. The variation of the viscosity with temperature is important as it is necessary to attain a certain viscosity to allow molten flow from the crucible to the steel plate. This ensures fast quenching and so avoidance of any crystal formation.

It is important to note that during the melting process, samples without alumina (PSBS SBK ; 8 ; 9 and 3) suffered a probable strong loss of boron.

It is significant that after initial melting / quenching the glasses are often optically clear, but turn opalescent following subsequent heat treatment at 700°C . This trend is dependent on the weight % of the elements in the composition. Opalescent colouration originates from the scattering of light caused by the coexistence of glass phases with dissimilar refractive indexes while the transparent-like colour could be derived from the presence of just a low percentage of the secondary phase dispersed in the main one or the co-existence of two glass phases with the same refractive index.

If the glasses obtained after heat treatment are compared within the triangular diagram given by the Janecke's coordinates, it becomes apparent that different regions are detected (figure 31). The samples highlighted with red points belonging to the isopleth without aluminium appear to be transparent. The samples emphasized with green points belonging to the isopleth $w^I = 25\% \text{ Al}$ become opalescent after the heat treatment except for the sample without calcium in the composition (orange spots). The samples found in the area between and above the two isopleths and highlighted with blue spots ($w^I = 15\% \text{ Al}$ and $35\% \text{ Al}$) assume a partial opalescent colour.

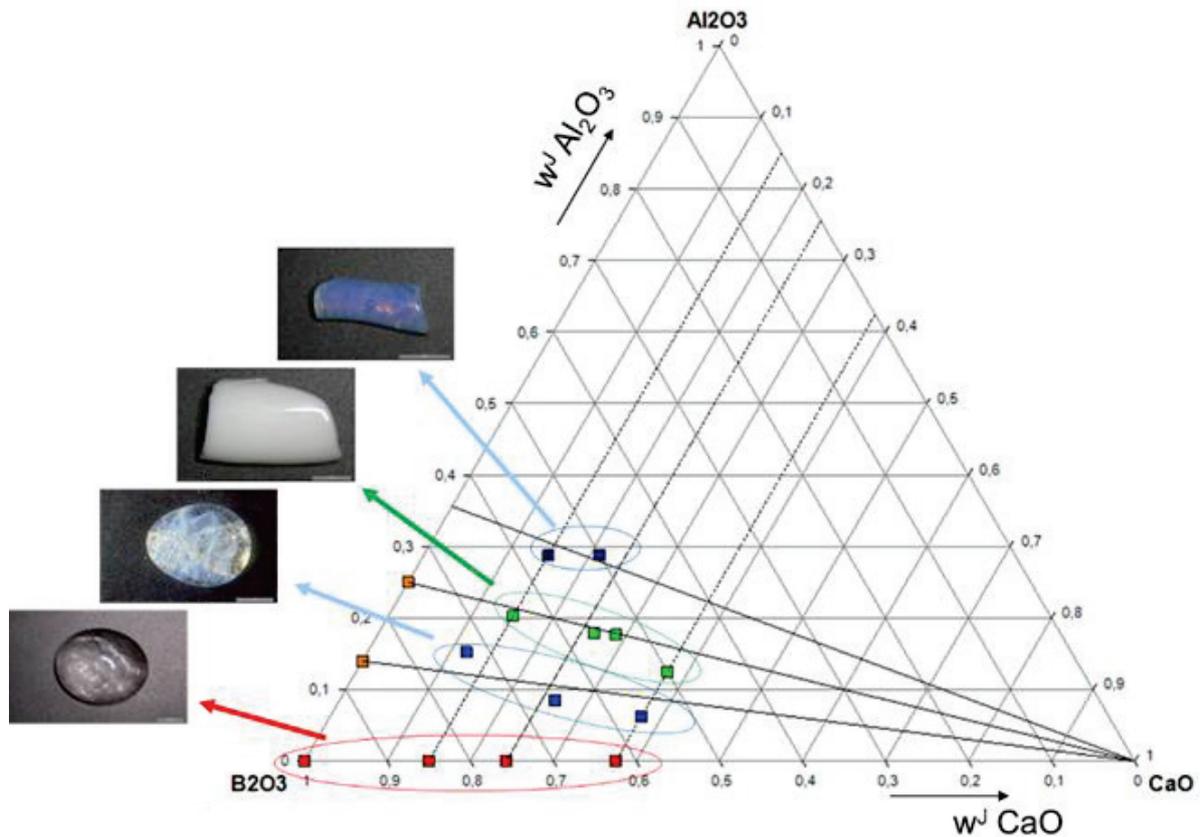


Figure 30 Ternary diagram showing the samples composition in the quinary system (SiO_2 and K_2O are fixed). The composition is influencing the opacity of the samples. Several isopleth sections were studied: 4 sections with a constant Al/B ratio and 3 sections with a fixed Ca content.

4.1.2 Refractive index

The refractive index was calculated for all the glasses assuming them to be one phase before the heat treatment because at that moment the samples are considered composed of one single transparent phase and the Appen method can be applied. As comparison, the SiO_2 rich Pyrex glass has an index equal to 1.470.

Table 21 PSBS samples and their refractive index calculated for the melt quenched samples

Samples	n_d
PSBS1	1.571
PSBS2-6	1.576
PSBS3	1.583
PSBS4	1.562
PSBS5	1.577
PSBS7-10	1.566
PSBS8	1.581
PSBS9	1.582
PSBS11	1.567
PSBS12	1.560
PSBS13	1.561
PSBS14	1.581
PSBS15	1.572
PSBS16	1.571
PSBSSBK	1.577

4.1.3 Becke line test

Many trials have been done but here some of the most interesting figures were collected. The idea was to show a comparison between the calculated refractive index before the heat treatment and the RI measured after the heat treatment. The result clearly indicates that the RI decrease in both cases. PSBS8 change from 1,58 to 1,51 after the heat treatment while PSBS16 change from 1,57 to 1,52. While it was clear that for PSBS16 there would have been a change in the refractive index because of the colour change (transparent to opalescent) after the treatment, also the PSBS8 seems to present changes that were not detected just by observing the appearance of the glass that remains transparent also after the heat treatment. No information about the phase separation (two different refractive index in both glasses) was detected because the phenomenon has to be studied at a microstructural level.

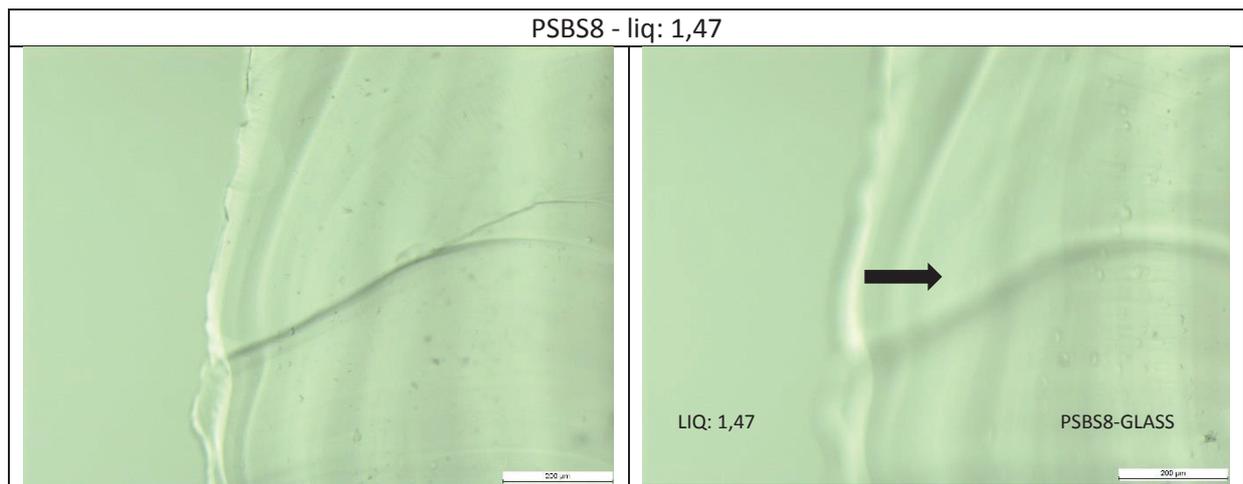


Figure 31 Becke line observed on the left figure for PSBS8. Becke line movement through the glass as indicated by the black arrow.

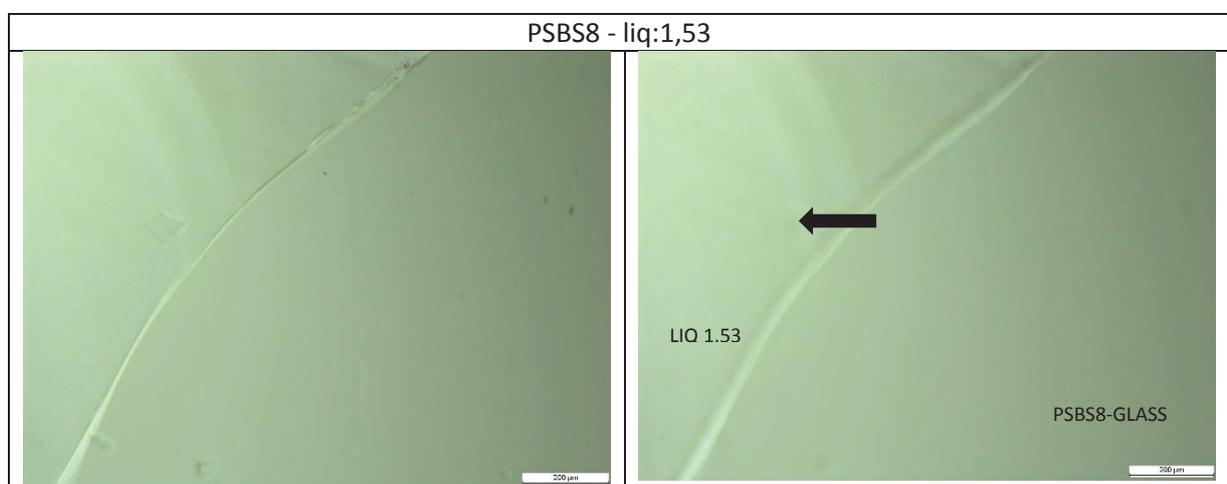


Figure 32 Becke line observed on the left figure for PSBS8. Becke line movement through the liquid as indicated by the black arrow.

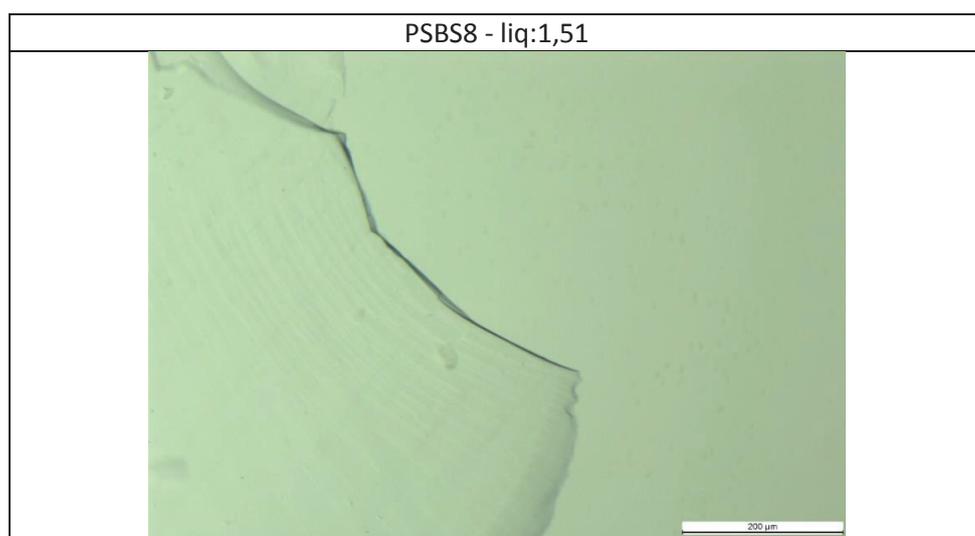


Figure 33 Becke line is not detected. The refractive index of the liquid correspond to the one of the PSBS8.

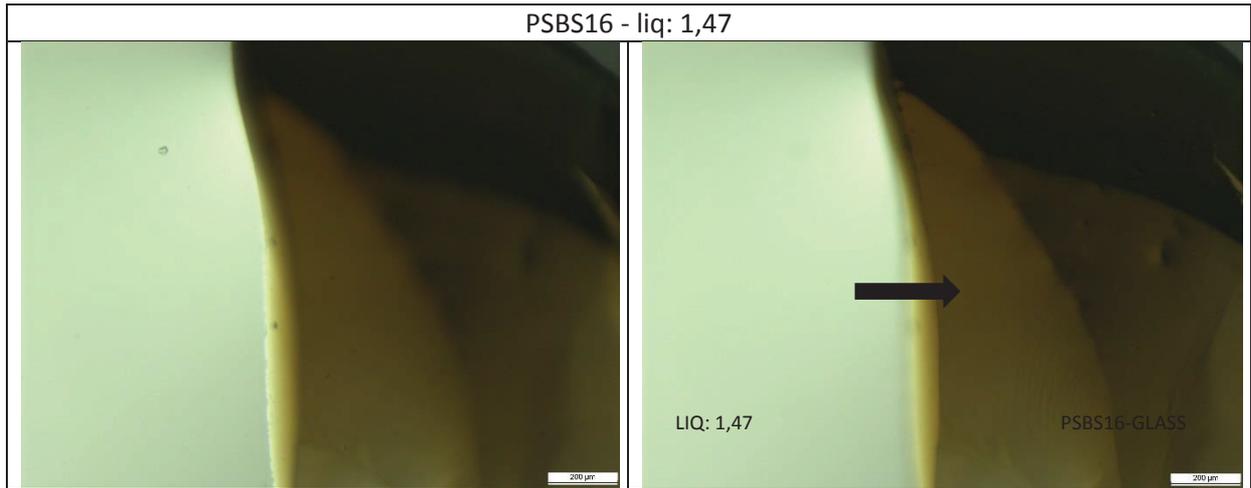


Figure 34 Becke line observed for PSBS16 in the left figure. Becke line shifting through the glass in the right figure

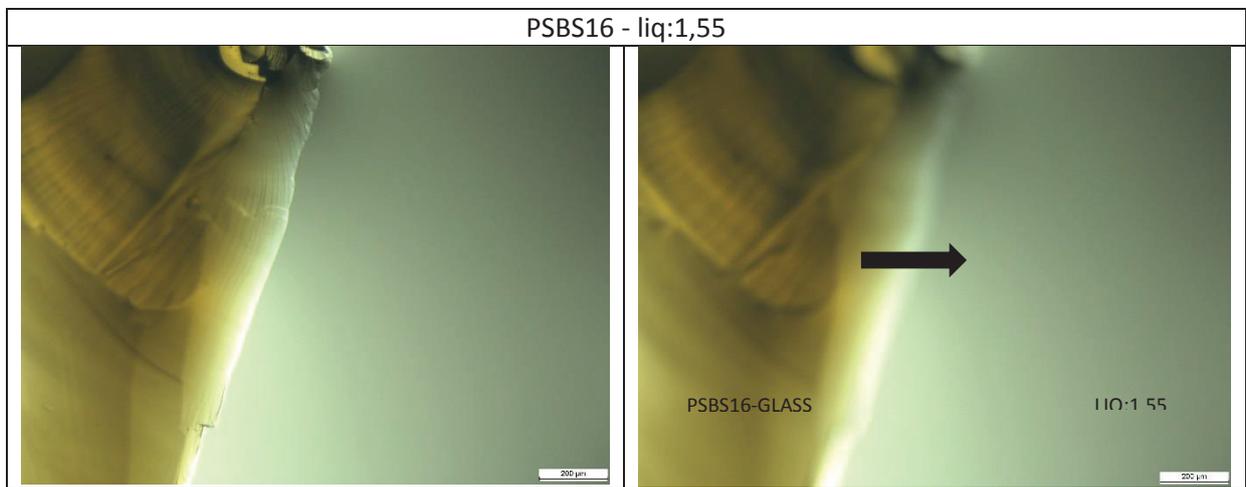


Figure 35 Becke line observed for PSBS16 in the left figure. Becke line shifting through the liquid in the right figure.

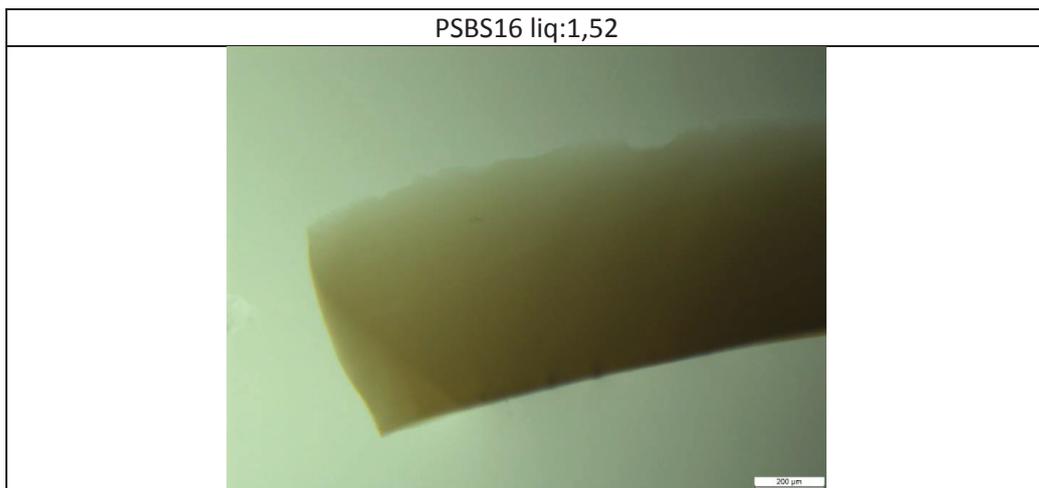


Figure 36 Becke line not detected. The refractive index of the liquid correspond to the one of the PSBS16.

4.1.4 Particle size distribution

From the analysis of each glass powder sample after the Tema[®] milling, the following figures were obtained showing the distribution of the particle size. The first result is related to the commercial GIC powder called GC FUJI IX[®] that is used as a reference to compare with the other measurements.

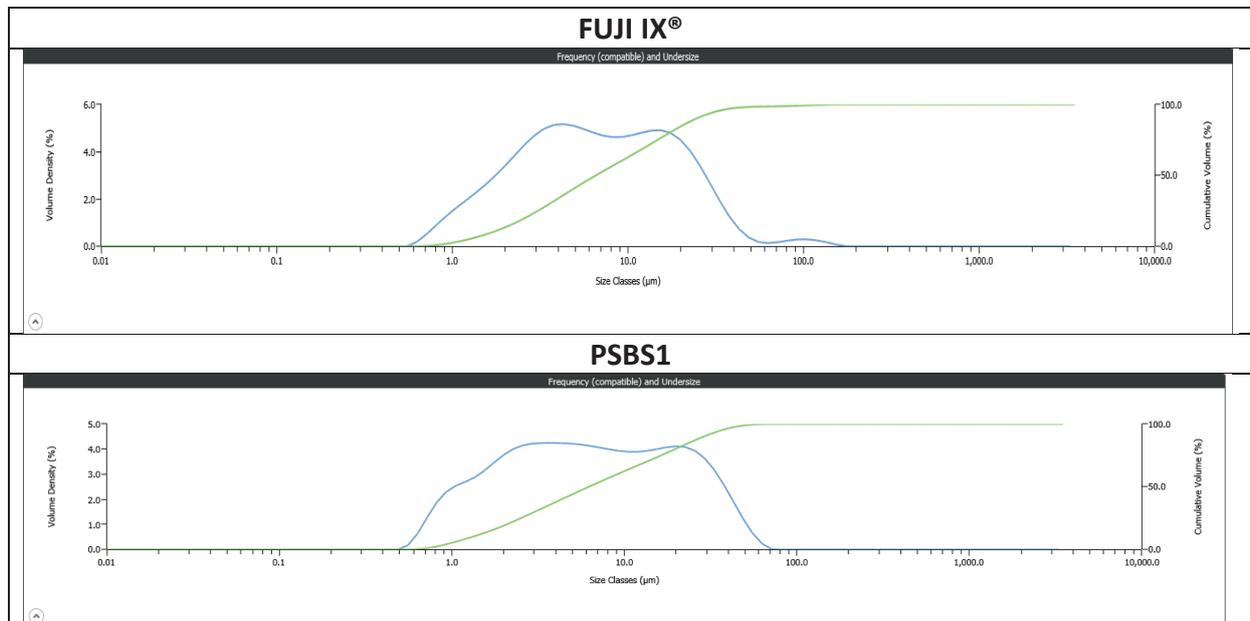


Figure 37 Particles size distribution of the commercial FUJI IX and the PSBS1 sample. The other graphs are reported in the annex 1.

The distribution graphics are useful for extrapolating the values of the particles size at key percentages values (tab. 22). Dv10 represents the dimension of the population at 10 percent, while Dv50 and Dv90 respectively at 50 and 90 percent. The majority of the glasses present a D90 of less than 50µm while all the samples exhibit a particle size below 100 µm for the 90 percent of the population. The samples that present Dv90 higher than 50 µm were sieved to give more homogeneity to the results of the characterization. The differences in particle size in this case is due to the milling time. As it was not homogeneous and constant for all the samples, at least the same range of particle size was collected.

Table 22 Values representing the particle size of the 10, 50 and 90 percent of the distribution of the PSBS glass powders.

	D10(μm)	D50(μm)	D90(μm)
FUJI IX®	1.69	6.54	24.4
PSBS1	1.31	6.22	28.9
PSBS2	1.64	9.67	38.9
PSBS3	1.5	7.28	31.8
PSBS4	1.21	5.45	26
PSBS5	1.31	6.21	30.8
PSBS6	1.53	9.54	39.9
PSBS7	1.29	6.09	30.1
PSBS8	1.51	8.8	38.3
PSBS9	1.35	7.98	33.5
PSBS10	1.42	7.5	33.8
PSBS11	2.65	19.9	87.6
PSBS12	2.16	18.8	86.7
PSBS13	2.88	24.2	100.7
PSBS14	2.08	21.1	99.1
PSBS15	2.48	21.8	99.1
PSBS16	1.75	8	36.4
PSBSSBK	1.6	9.05	40.9

4.2 Structural characterizatoin

4.2.1 X-ray powder diffraction

Powder X-ray diffraction (XRD) analysis was performed for every sample after the heat treatment and for PSBS1 only before and after the thermal process in order to observe possible differences in the structure.

All the XRD patterns show a characteristic amorphous shape, since they exhibit a broad formless “bump”, in lieu of distinctive Bragg peaks when the structure is well ordered (tab. 23). More detailed analysis allows evaluation of the percentage amorphous material in every sample; this revealed a consistent 100.0 wt% amorphous except for the PSBS SBK that presents three unknown peaks corresponding to a 1.2 wt% of crystalline phase. It is likely the crystalline part is composed of Cristobalite (high temperature SiO₂ crystalline form) but the small peaks doesn't permit an accurate identification. The absence of Al and Ca in the composition may cause a premature crystallization due to a lower viscosity and a more opportunity for molecular arrangement as regular crystalline phases; indeed this is supported by observing the DSC curves of the PSBS3 in the next section (table 24 where the samples without Al are more prone to crystallization).

Table 23 Percentage of amorphous phase in the PSBS heat treated samples.

SAMPLE	AMORPHOUS PHASE (w%)
PSBS1 (melt quenched)	100.0
PSBS1	100.0
PSBS2-6	100.0
PSBS3	100.0
PSBS4	100.0
PSBS5	100.0
PSBS7-10	100.0
PSBS8	100.0
PSBS9	100.0
PSBS11	100.0
PSBS12	100.0
PSBS13	100.0
PSBS14	100.0
PSBS15	100.0
PSBS16	100.0
PSBS SBK	98.8

The extent to which Calcium content influences the structure dimension is interesting. The XRD results of the glasses without Al₂O₃ are compared in figure 39. The superposition of the resulting spectra shows a 2θ angle shift of the typical amorphous halo. It has been found to be related to the CaO addition in the sample compositions (Table 24). Calcium cations are not forming the main part of the glass structure, and the introduction of those oxides called “modifiers” result in an alteration of the glass structure (Aboutaleb D. 2012). The higher the addition of CaO in the composition, the greater is the formation of non-bridging oxygens (NBO) that are causing a reduction in the borosilicate network connectivity. By augmenting the Calcium into the borosilicate network the Si-O bonds tend to break (de-polymerization) and the oxygen atoms negatively charged are attracted from Si atoms (δ+) causing smaller range distances between the atoms (d-spacing). Figure 39 and table 24 illustrate the effect of network modifiers into the glass structure.

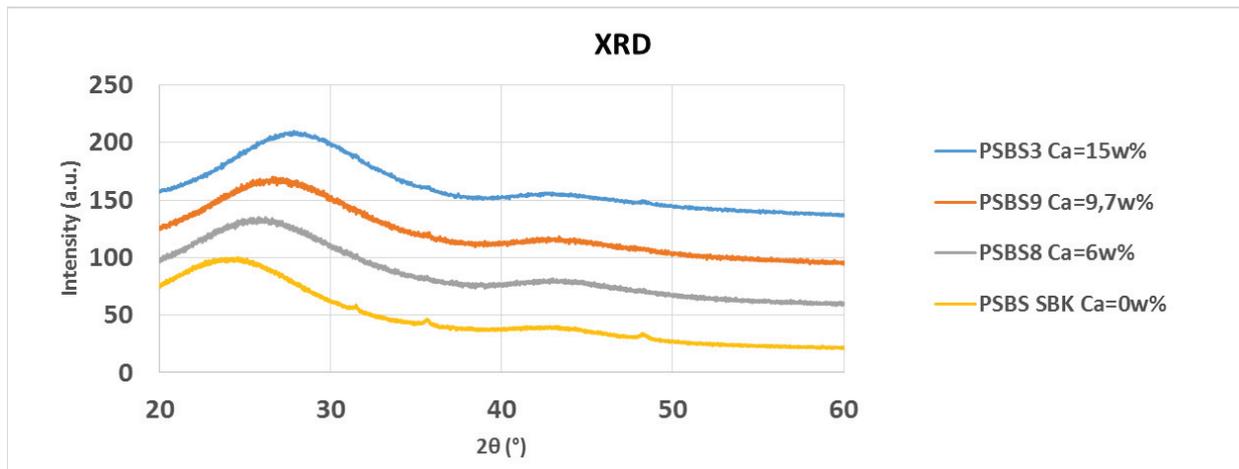


Figure 38 XRD pattern of the samples without Al₂O₃.

Table 24 The diffraction parameters in relation with the calcium w% in different PSBS glass samples without alumina.

	PSBS SBK	PSBS 8	PSBS 9	PSBS 3
2θ	24,55°	25,80°	26,64°	27,83°
d-spacing	3,69	3,41	3,29	3,19
Ca (w%)	0	6	9,728	15
B (w%)	40	34	30,272	25

Aluminium has not the same effect of calcium in modifying the glass structure. Comparing the XRD patterns of the glasses without calcium in the composition PSBSSBK, PSBS11 and PSBS12 the right shift of the glass halo is not evidenced (fig. 40). Thus it is probably taking part of the structure as a network former instead of being a network modifier. In fact, alumina is an intermediate oxide close enough in size so that Al³⁺ can replaces Si⁴⁺ in network sites:

Al³⁺ (CN=4) ~0.39Å

Si⁴⁺ (CN=4) ~0.26Å

Al₂O₃ additions increase the connectivity of an alkali modified glass by replacing NBO's with cross-linking Al-O-Si bonds (fig. 41).

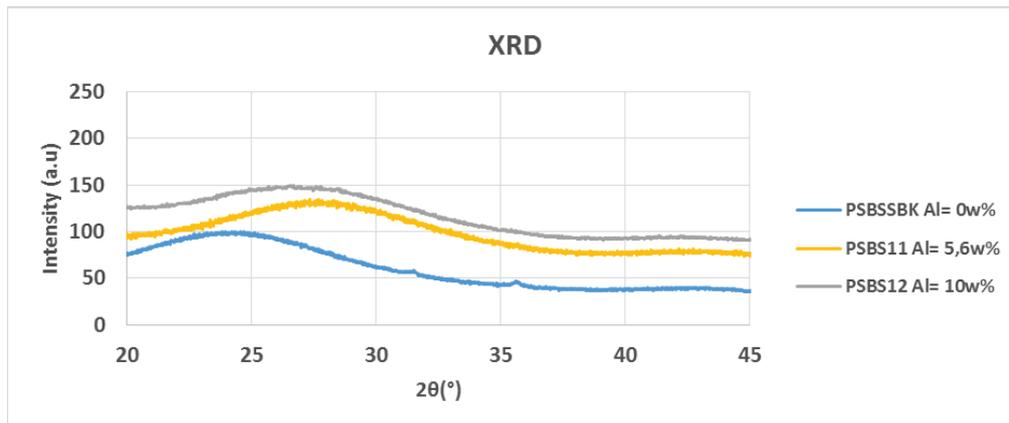


Figure 39 XRD pattern of the samples without CaO

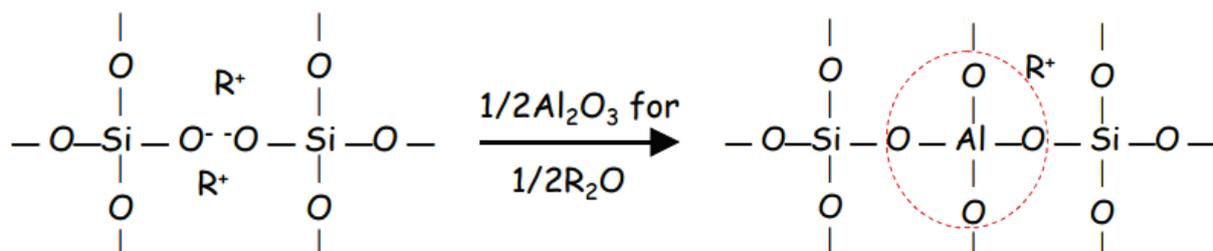


Figure 40 Effect of Al₂O₃ addition into a silicate network.

Table 25 The diffraction parameters in relation with the aluminium w% in different PSBS glass samples.

	PSBS SBK	PSBS 11	PSBS 12
2θ	24,55°	26,80°	26,06°
d-spacing	3,69	3,53	3,45
Al (w%)	0	5,6	10

4.2.2 DSC analysis

Studying the phase separation within the ionomer glass is crucial since it will help in understanding the structure-cement properties relationship.

One manner of studying it is demonstrate that the glass has more than one glass transition temperature (T_g). Differential scanning calorimetry (DSC) provides a reliable method for detecting existing T_gs in a glass as reported by Pedersen (Pedersen M. 2015). During the quenching, if the melt is cooled quickly enough to avoid crystallization, the process will result is an amorphous solid. Below the T_g the viscosity is so high that is avoided the rearrangement of the glass network into a crystal structure. Only after this point the initial

formation of nuclei bring to a final crystallization. There are also other method to calculate the T_g with the help of theoretical calculation as the ones propose by Andersson and O'Donnell (Andersson 1992, O'Donnell 2011) but compositional limitations doesn't allow to apply this formulation to the borosilicate glass system studied in this work.

The samples analyzed were: PSBS1 melt-quenched, PSBS1 heat-treated, PSBS3 melt-quenched, PSBS3 heat-treated, PSBS4 heat-treated and PSBS SBK. These samples were selected because representative of glasses that become opalescent, transparent, semi-opaque and contain maximum, medium and minimum amount of aluminium in the composition.

The DSC curves (fig. 42-45) revealed the various glass transition temperatures corresponding to an endothermic event, where a change in heat capacity is depicted by a shift in the baseline. It is considered the softening point of the material or the melting of the amorphous regions in the glass. The principal glass transition is detected for all the samples around 600°C, the other glass transition have to be further investigated because are smaller phenomena probably attribute to smaller phases. In any case should be considered as multiple phase system with at least two principal phases detected experimentally. The amorphous phase separation could have form a system consisting of a silica glass matrix in which a borate rich phase is dispersed but still other analysis have to be done to evaluate this hypotesis (SEM analysis in the chapter "surface analysis"). Only for PSBS3 heat-treated was observed an exothermic peak around 800°C. The exothermic peak arises when a glass arranges into a more ordered structure characteristic of crystals. This process give off heat as proven by a rapid change in the heat flow.

The last phenomena present in each curve represents the melting temperature T_f.

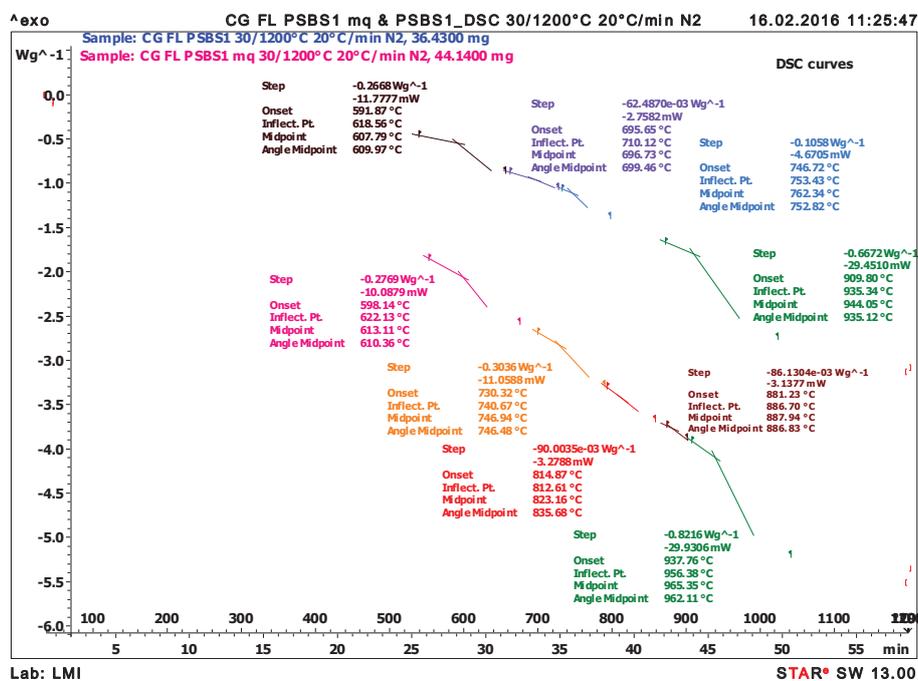


Figure 41 DTA traces for PSBS1 melt quenched (pink line, above) and PSBS1 heat treated (blue line, below).

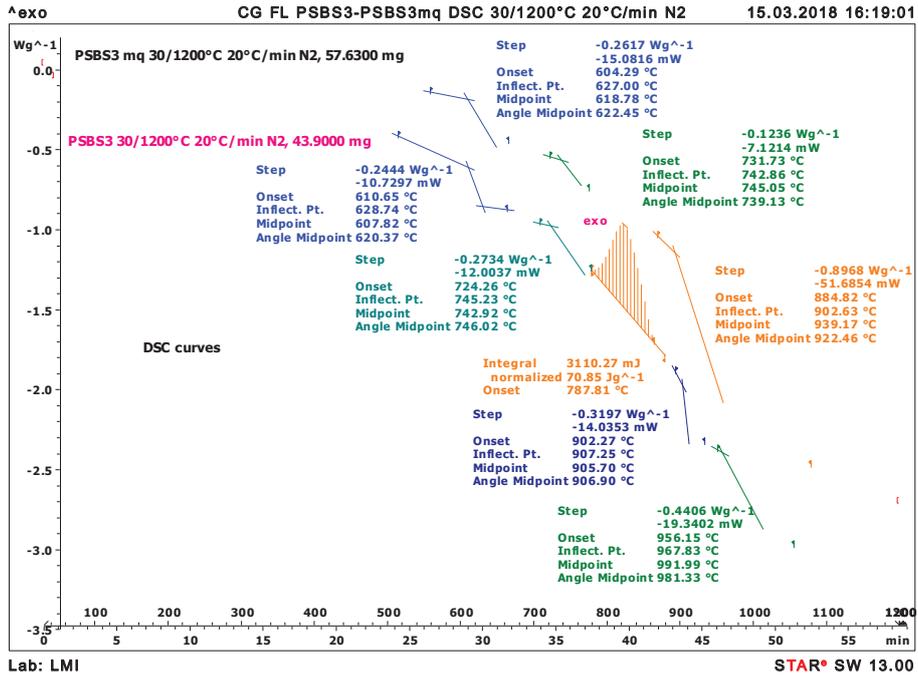


Figure 42 DTA traces for PSBS3 melt quenched (pink line, above) and PSBS3 heat treated (blue line, below).

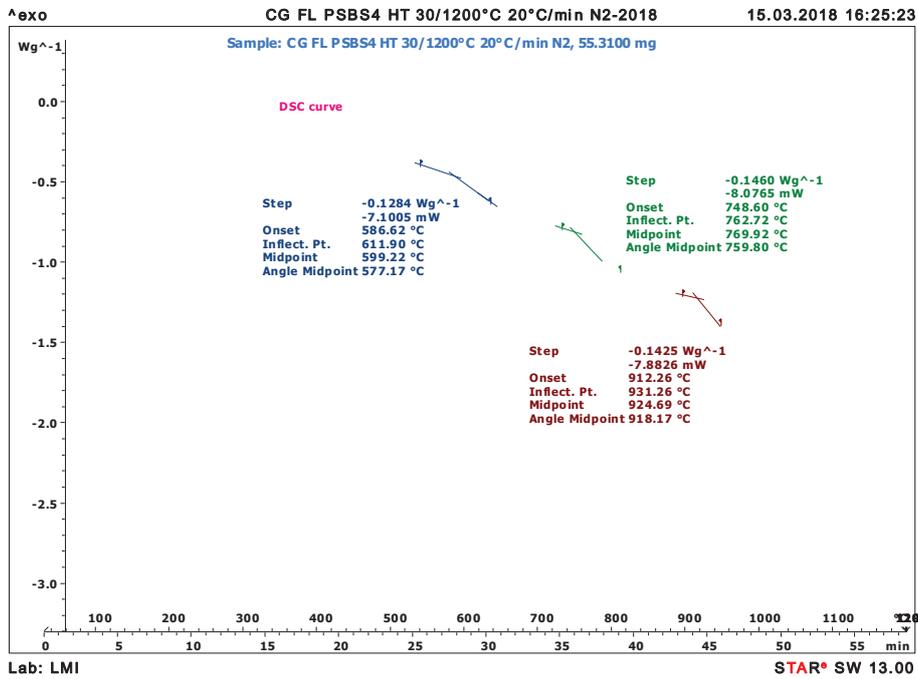


Figure 43 DSC trace for PSBS4 heat treated.

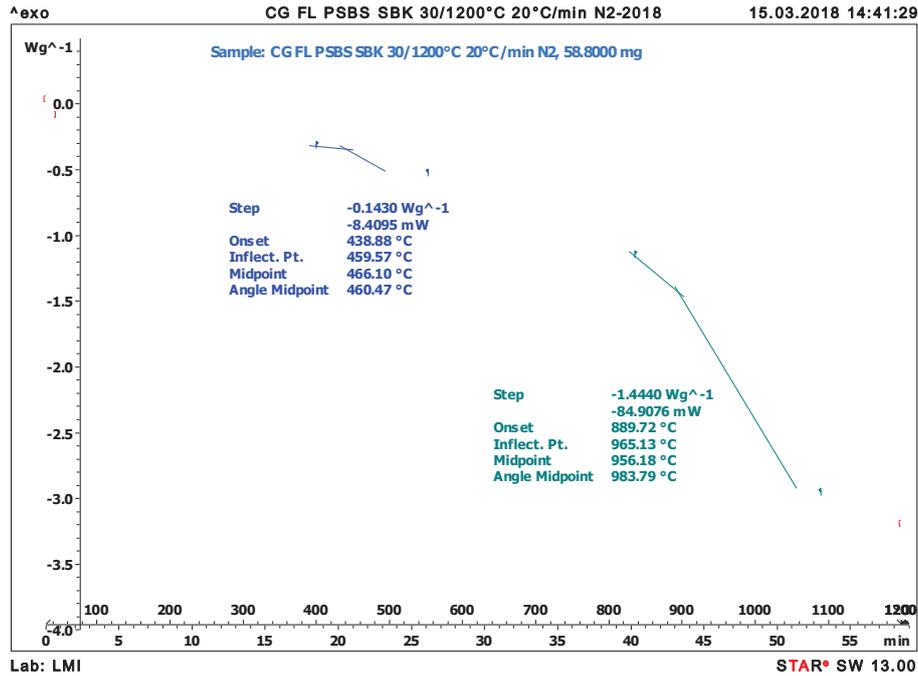


Figure 44 DSC trace for PSBSBK heat treated.

A clear first T_g was observed for all the glasses at temperatures between 586-610°C for glasses with at least four components. While the second glass transition it is observed at higher temperatures for the PSBS samples in a range between 695-748°C. The glass formation is possible whenever there is sufficient level of glass “network formers”. In these glasses SiO₂ level is constant whilst the B₂O₃ content varies between the glasses. The “network modifiers” can also participate in glass formation acting to modify the glass properties. For this reason the data presented in the following table (table 26) are useful to compare the results obtained in term of thermal history and the original composition of the different glass samples. From the scientific literature one T_g value for the 45S5 have been reported to as: 532°C (Bretcanu O. and R. Conradt 2009). The bioglass 45S5 contains higher amount of network modifiers (24,5 wt% for both CaO and Na₂O) in comparison with borosilicate glasses and the network can be considered less connected. This can be determined with the comparison of the different T_g. Lower T_g values are assumed to be related to less compact glass network in which the mobility of the atoms tend to re-arrange into crystal structure due the the lower viscosity.

Table 26 Thermal analysis results obtained with DTA technique of T_g and T_c compared with the glass compositions.

SAMPLE	T_{g1} (°C)	T_{g2} (°C)	T_c (°C)	Al_2O_3 (w%)	CaO (w%)	B_2O_3 (w%)
PSBS1 Mq	591.8	746.7	n.o.	7.2	10.4	22.4
PSBS1 HT	598.1	730.3	n.o.			
PSBS3 Mq	604.3	731.7	n.o.	0	15	25
PSBS3 HT	610.7	n.o.	787.8			
PSBS4	586.6	748.6	n.o.	11.5	8.5	20
PSBSSBK	438.8	n.o.	n.o.	0	0	40

The interesting observation is that only “PSBS3 heat treated” presents a T_c in its thermogram at 787.8 °C. In the next section the crystallization phenomenon of the “PSBS3 glass heat-treated” sample at 800°C will be investigated in detail.

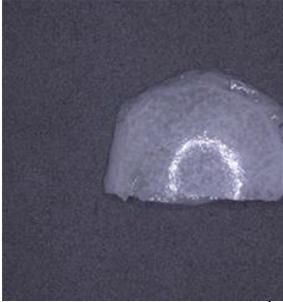
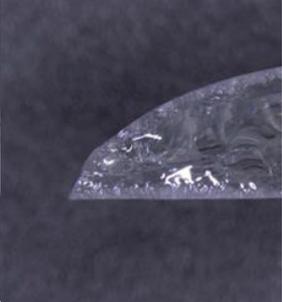
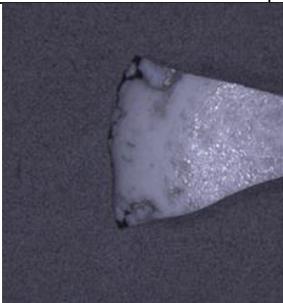
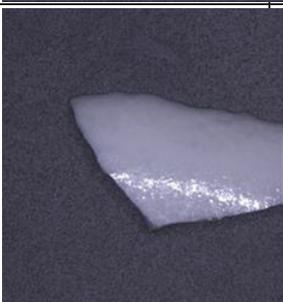
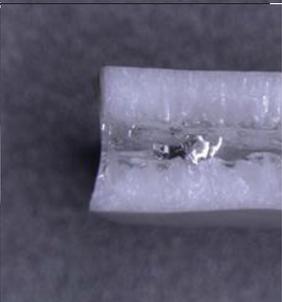
Glass transition studies are important from the point of view of understanding the mechanism of glass transformations. The T_{g1} values of PSBS1 and PSBS3 appear to be increased by the thermal treatment indicating the kinetic nature of the glass transition. If the comparison is made for all the samples after the heat-treatment it is possible to also note that T_{g1} shifts to higher temperatures with increasing CaO amount in the composition. The T_{g2} values after the heat-treatment is detected only for samples PSBS1 and PSBS4. Probably a T_{g2} peak is also present in PSBS3 but the crystallization peak doesn't allow the detection and it is probably the indication of a second phase formed that is more prone to crystallize.

4.2.3 Crystallization studies on PSBS3

After DSC analysis revealed an exothermic peak at 800°C, a heat treatment at different time-points was performed to investigate the phase crystallized at a temperature higher than the original heat treatment. Only the phase-separated PSBS3 sample shows this phenomena, probably indicating that one of the phases present after the first heat treatment at 700°C is more prone to crystallization. An XRD study can then bring an understanding of the composition of this phase.

The experiment started with the PSBS3 phase-separated samples heated at 800°C for 5h, 10h and 20h. Pictures of the resulting sectioned samples were taken with the optical microscope and are showed in table 27 below.

Table 27 PSBS3 heat-treated at 800°C showing the development of the crystalline phase.

Time (hours)	Temperature (°C)	Sample surface	Sample section
5	800		
10	800		
20	800		

The crystallization of an amorphous solid is a complex process involving simultaneous nucleation and growth of crystallites (Kalb 2009). Crystallization is initiated by crystal nucleation that can occur spontaneously or may be induced artificially. In this case it is induced by the heat provided by the thermal treatment and it is dependent on the heating time. The nucleation process is followed by growth of the crystal nuclei to macroscopic dimensions. . In PSBS3 glass the crystallization begins from the external surface and develops through to the deeper areas of the glass bulk. The crystalline phases were revealed by XRD analysis (fig. 46) and are shown in table 28.

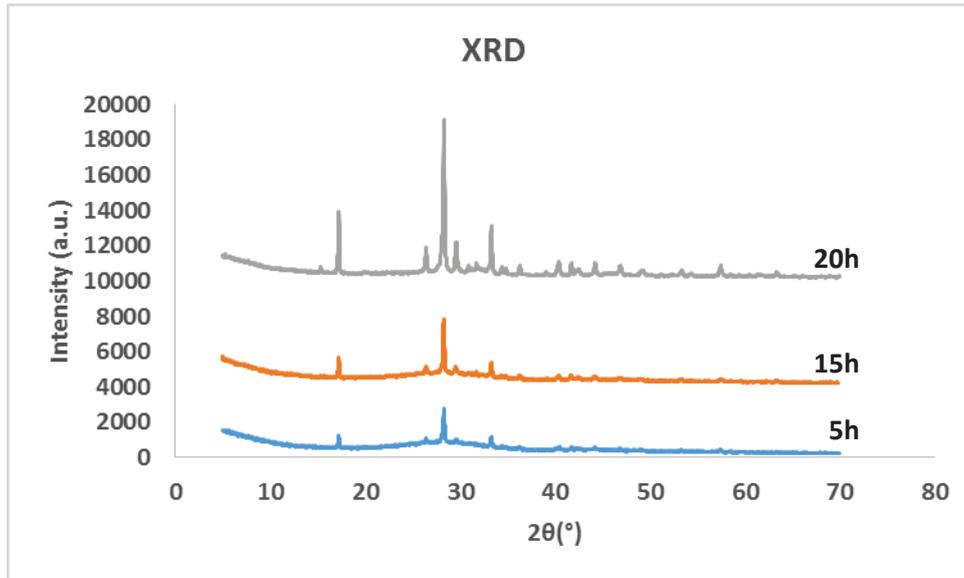


Figure 45 XRD patter of PSBS3 heat-treated at 800°C at different time-points: 5h, 15h and 20h.

Table 28 Weight% of the crystalline phases formed with the heat treatment of PSBS3 at 800°C.

Phases:	Content of phases (w%)		
	5 hours	10 hours	20 hours
Potassium Boro-Silicate (KBSi_2O_6)	16.0	23.6	57.0
Calcium Borate (CaB_2O_4)	3.9	6.9	22.1
Tri-Calcium Silicate (Ca_3SiO_5)	N.d.	N.d.	2.2
Amorphous	80.1	69.5	18.8

Different types of alkali borate crystal structures are found at lower heating times. The first one KBSi_2O_6 has a molar ratio $\text{SiO}_2/\text{K}_2\text{O}$ equal to 4, i.e similar to that the glass composition (4.6 for all PSBS). This phase may be due to the boron losses during the melting process and start to crystallize during the thermal treatment.

The second one CaB_2O_4 is one of the four solid phases of the binary system $\text{CaO}-\text{B}_2\text{O}_3$. In the Janecke triangular representation (fig. 31) CaB_2O_4 is located at 44.6% Ca just on the right side of PSBS 3 composition.

After 20 hours of thermal treatment a silicate phase start to crystallize, it is rich of calcium and can be identified as the mineral phase called Alite Ca_3SiO_5 . The majority of the glass volume at 20 hours (see picture in table 28) has experienced crystallization.

The phenomenon of crystallization in PSBS 3 could be interpreted as the combination of

- the boron losses during the fusing process ;
- the higher amount of CaO in its composition, as also demonstrated in a study conducted by Zhou et al (X. Zhou 2009) where it was proven that the increase in the crystallization of a $\text{CaO}-\text{SiO}_2-\text{B}_2\text{O}_3$ glass ceramic is related to the increased CaO amount in the composition ;
- the absence of Al_2O_3 in the composition of the glass: alumina doping in borosilicate glasses is considered to enhance glass formation ability (Araujo R.J. 1982), the glasses without aluminium in this study are more prone to crystallization.

4.3 Ions leaching, dissolution experiments & surface analysis

Due to their amorphous structure, glasses are less dependent on a specific stoichiometry compared to crystals. For that reason, it is possible to incorporate various amounts of network modifiers into their composition with each element being useful for a specific physiological activity. The borosilicate system studied so far, in addition, can be induced to structural modification with a thermal process.

When glasses are immersed in a solution, three main processes occur: ion exchange, dissolution and precipitation. The ion exchange occurs especially in the glass surface where cations from the glass are exchanged with H_3O^+ from the surrounding solution:



The dissolution results in an increase in the pH that cause the disruption of the glass network with the breaking of the Si-O-Si bonds:



Finally, the precipitation of some of the ions can form salts in solution or form a kind of gel layers on the surface of the glass particles. Hench proposed the formation of a calcium phosphate-rich layer in his explication of the hydroxyapatite formation (Boyd D. 2005). Essentially the glasses start a degradation process in which ions will be continuously released from their structures. This property makes them very interesting as agents for controlled release of actives.

Altering the glass composition and controlling its reactivity once immersed in solution is challenging, but studies can provide valuable information on what species are leached and when (Elgayar I. 2005). In this chapter dissolution studies following by ICP analysis are employed to elucidate the mechanism of ions release and how this correlates with glass composition and the microstructure formed after the heat treatment and leaching.

4.3.1 Dissolution in acid medium

4.3.1.1 pH measurement associated with ion dissolution and heat-treatment

The PSBS samples have been compared by measuring the pH change in an acid solution. This allows initial theories to be developed concerning the exchange reaction between the glass ions and the ions present in the solution. As borosilicate glass particles or scaffolds dissolve, the pH increases and the concentrations of the predominant species in solution will vary according to the pH.

pH evolution as a function of time has been analysed for the samples before and after heat-treatment Fig. 47 shows a typical example of curve.

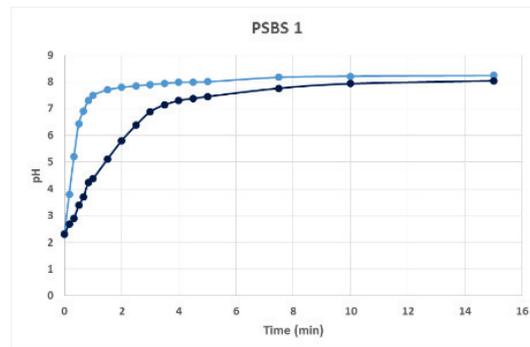


Figure 46 pH variation in the HNO_3 acid 1M monitored in the first 15 minutes of reaction for the PSBS samples before (dark blue line) and after (light blue line) the heat treatment.

All glasses show the characteristic pH increase typical of the bioactive glasses when dispersed in aqueous media. The glass particles started to leach ions as soon as they get in contact with the acid solution as observed also by Zhang et al. (D. Zhang 2008). A rapid increase in pH that takes place during the first minutes, after which the pH increases only slightly (Fig. 50). This phenomenon is due to the ionic exchange between the network modifiers and the HNO_3 solution. At 5 minutes, the pH had increased from 2 to 8 resulting in a fast dissolution process. After the fast pH rise it stabilizes to values between 8 and 9. Hydrolysis of the silica network is known to occur at higher pH values (usually above pH 10), resulting in congruent dissolution of silicate glasses (Hench L.L. 1978). The greatest pH increase is observed for the samples with higher calcium concentration (PSBS3 and PSBS5) confirming that the exchange of the alkaline earth ions with the protons in the solution is responsible for the pH increase (given that potassium ion concentration is constant). Comparing the pH variations of the PSBS samples this result is confirmed especially observing the isophlets with constant amount of aluminium. In the three isophlets in figure 48 with $W_j = 0\%$, 15% and 25% Al, the samples with the lower increase in pH are the ones without calcium in the composition. Observing this specific isopleth: $W_j = 0\%$ Ca (fig. 49) the increasing in aluminium concentration result in a decrease in the pH evolution rate confirming that the durability is also increasing with higher aluminum concentration in the glass composition.

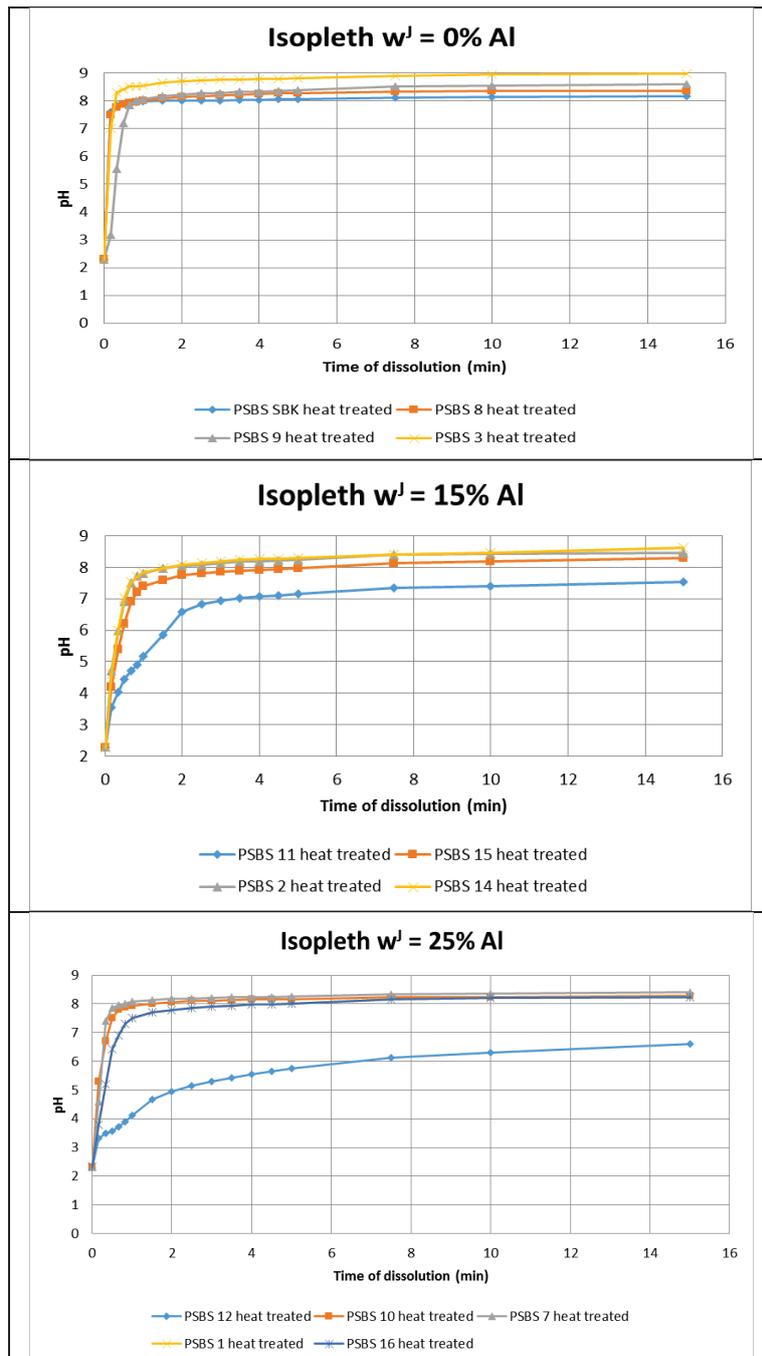


Figure 47 Comparison of the pH evolution in the isopleths with constant amount of calcium.

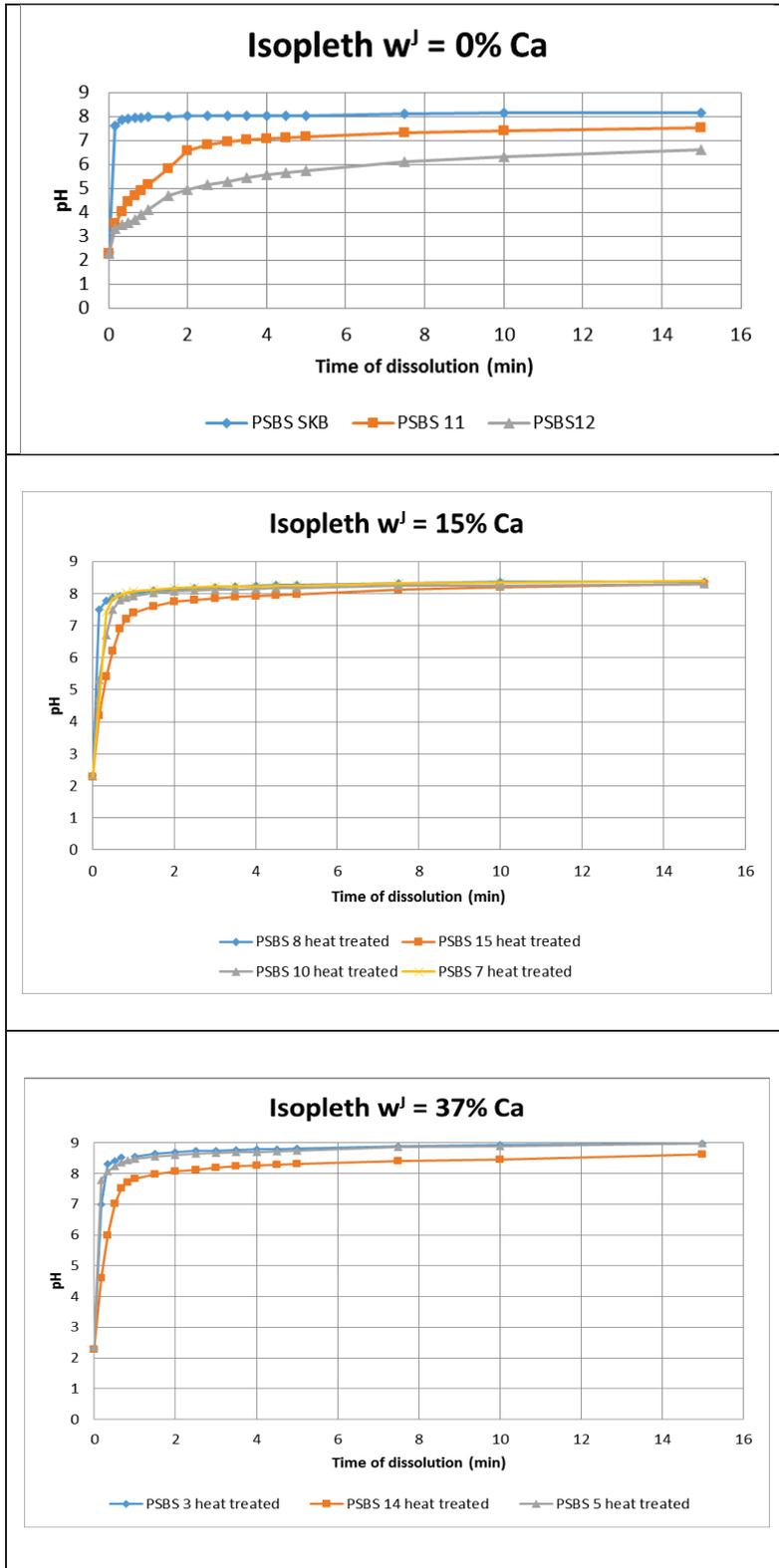


Figure 48 Comparison of the pH evolution along the isopleths with constant aluminium concentration.

4.3.1.2 pH measurement associated with weak and strong acid media

For PSBS1, PSBS8, PSBS16 and PSBSSBK the pH was also measured with a weak acid, the poly(acrylic acid) (from Sigma Aldrich, 35 wt. % in H₂O, mol wt: average Mw ~250000). The comparison of the evolution of the pH between the HNO₃ and the poly(acrylic acid) shows the same trend for all the samples. In fact the acid involved in the dissolution influence the pH increasing, especially if the acid is weak the rise in the pH will be less pronounced because of the low exchange between the glass ions and the protons in solution. This experiment want to confirm that the dissolution is affect by the type of solution and consequently the pH variation is different (fig. 50).

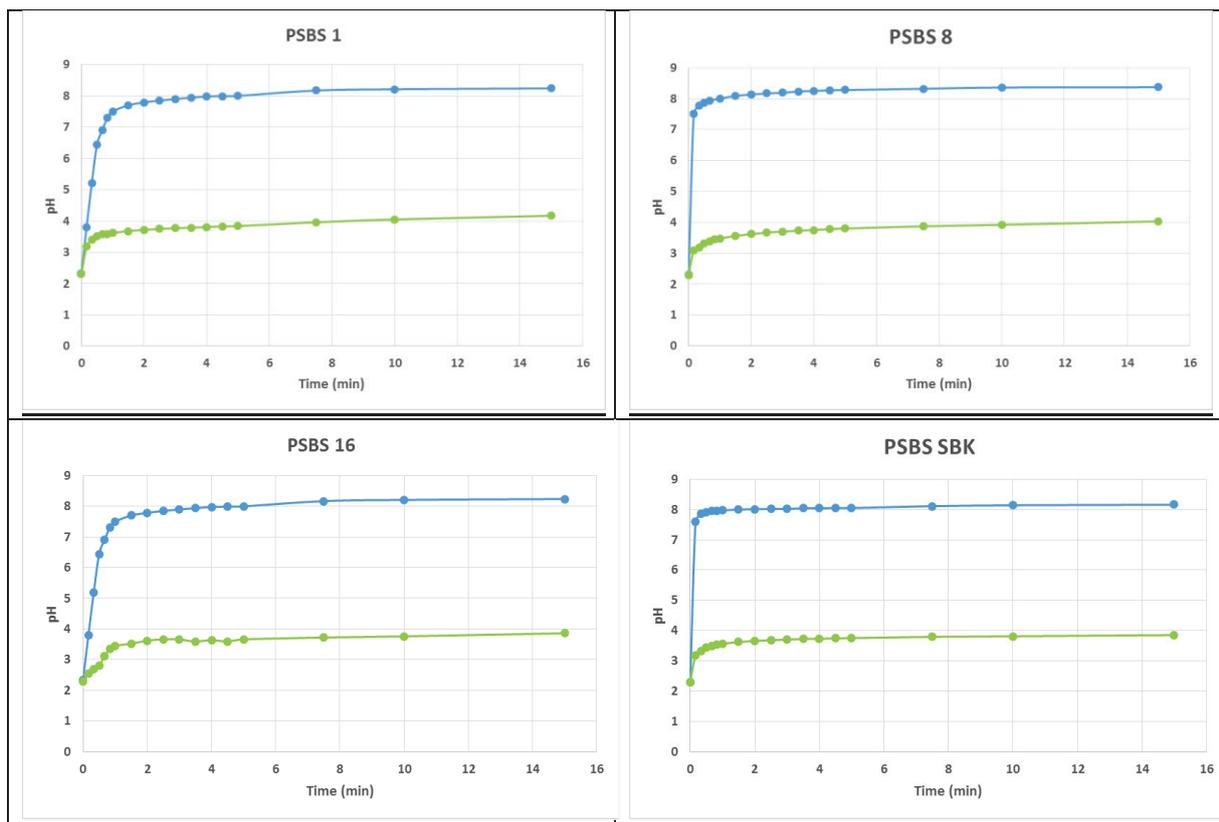


Figure 49 pH measurement of PSBS 1, PSBS 8, PSBS 16 and PSBS SBK in two different acids: HNO₃ 1M (blue line) and poly(acrylic acid) (green line).

pH neutralization is of recent interest in the characterization of self-adhesive resin luting materials because it has been related to mechanical properties (Zorzin J. 2012). Also Ferracane et al. (Ferracane J.L. 2011) reported that a hydrophilic behaviour due to the low pH value in the cured material could affect the mechanical properties. However the studies cited monitor the pH change in the final product, directly putting the pH probe in contact with a cement disk suspended in a solution providing a similar condition to that found in the mouth (37°C and acid environment). The studies reveal that the pH neutralization is not consistent when comparing different commercial materials. There is potentially much work to be performed to investigate different conditions and considering also the fact that contact between the tooth substrate can play an additional role to the acid-base interaction of the materials. The results obtained so far show values close to some of the materials presented

in literature but the most useful data should come from the final cement mixed with the poly(acid). Finally also, effects in term of antimicrobial activity could well be related to the pH neutralization ability of the restoration

4.3.2 Analysis of the ion release

Identification of the ion released has been done by ICP analysis as function of time during the first hours of de dissolution process. The glass powders were immersed in the acid solution, that was than collected at different time-points. Fig. 51 shows a typical example of Si, K, B and Ca evolution. The results were converted from ppm to the molar percentage of the element dissolved in solution (Ex. [Elem]%). This was done to better compare the results amongst glass compositions that contain different amount of oxides in their compositions.

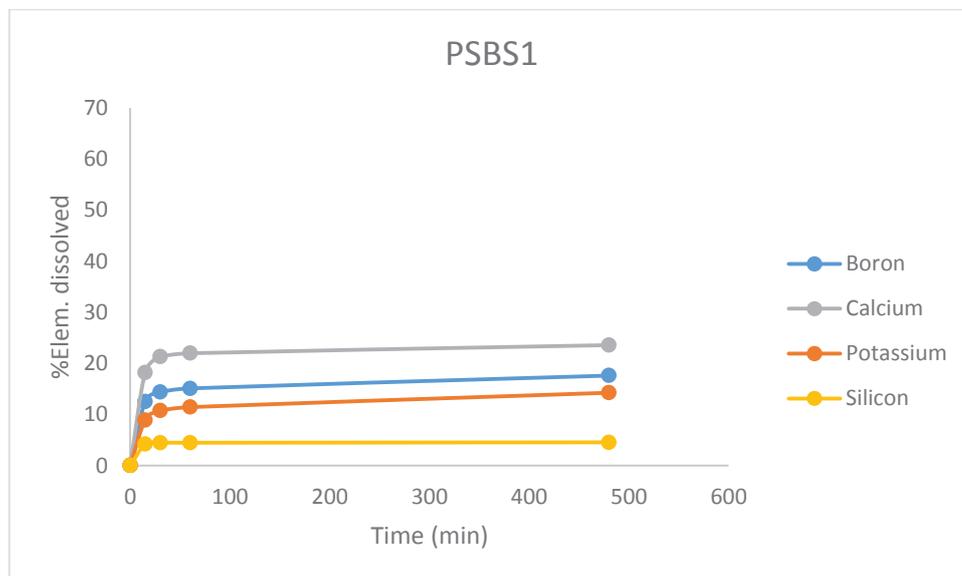
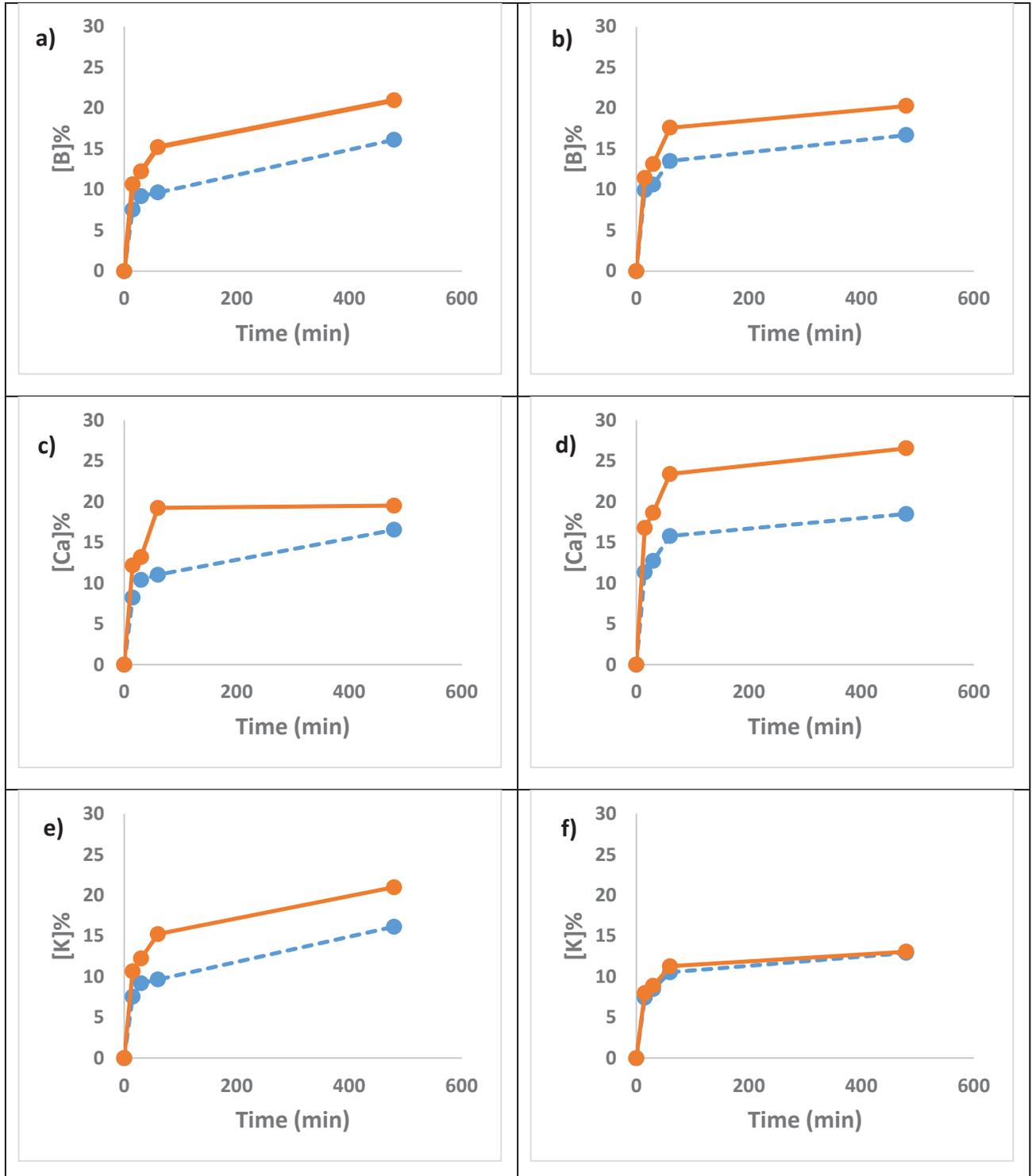
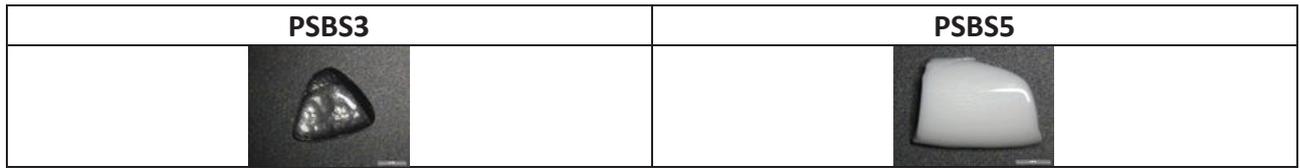


Figure 50 Example of PSBS1 ions release in the first 8 hours of dissolution in HNO₃.

4.3.2.1 Effect of the heat-treatment

The first assay was made for two different glasses both before and after the heat treatment to observe the effect on ion leaching. PSBS3 and PSBS5 were selected for this test because they present distinct appearance (the first is transparent while the second is opalescent) and because they have the same amount of calcium in their composition.



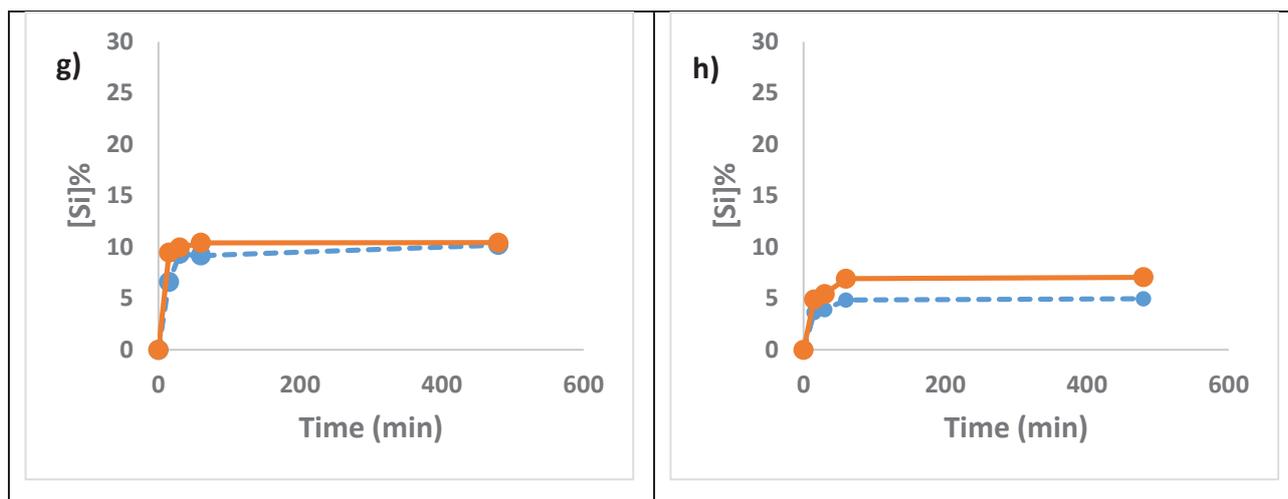


Figure 51 Comparison of a transparent PSBS3 and an opalescent PSBS5 glass sample (graphs on the left and right respectively). The comparison has been made within a heat-treated sample (solid line) and a melt-quenched sample (dotted line) between the leaching of different ions: boron (a,b), calcium (c,d), potassium (e,f) and silicon (g,h). The ordinates axis presents the percentage of the available element in the glasses dissolved into the acid solution.

The ions concentration profiles suggest that the dissolution rate of B, Ca, K and Si is enhanced by the heat treatment. Moreover, it is evidenced that different leaching rates exist between the opal and clear samples. In PSBS3 potassium ions are released with higher kinetic rate than PSBS5 ($\Delta K/\Delta t = 0.71$ Vs. 0.53 K%/min), while for calcium ions the opposite trend is observed ($\Delta Ca/\Delta t = 0.8$ Vs. 1.12 Ca%/min).

4.3.2.2 Comparison of the dissolution kinetics: PSBS8 and PSBS16

The comparison of samples PSBS16 and PSBS8 respectively with and without Al_2O_3 reveals a different ion leaching behaviour during time (Fig. 53). PSBS 16 (opalescent glass, 15 wt% of Al_2O_3) releases calcium at a higher kinetic rate ($\Delta Ca/\Delta t = 2.982$ Ca%/min). For PSBS8 (transparent glass, without Al_2O_3) the release of potassium and boron is congruent and happens at a faster kinetic rate than calcium ($\Delta K/\Delta t = 2.744$ K%/min; $\Delta B/\Delta t = 2.66$ B%/min). Silicon release is very low for both the glass samples. This is to be expected as it is a network former and not prone to leaching. Boron and potassium are dissolved much more quickly than silicon. Fast potassium release is to be expected but boron, like silicon can play a network former role and shouldn't be release as a weaker phase. The formation of NBO weakens the structure of glass and makes the species soluble and more accessible to water. The depolymerisation of the silica network by the creation of NBO generates larger cavities, this favours the invasion of nitric acid solution and the exit of the soluble elements. Two mechanisms of dissolution are hypothesized for PSBS8; after 125 minutes in HNO_3 , a rapid increase in the ions release is detected. It has been proved that in a bulk sample of the same glass, the structure is starting to degrade in the same condition. The phenomenon is not

detected in PSBS 16 where after 125 minutes seems that the ions releasing reaches a plateau.

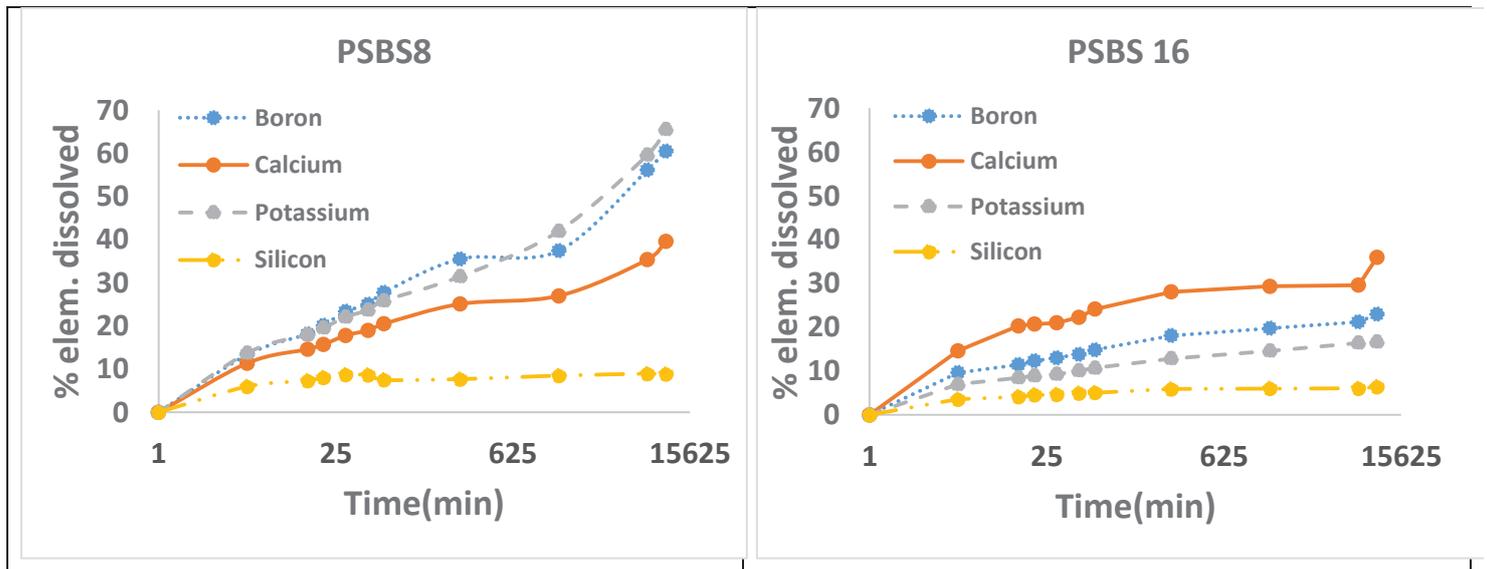


Figure 52 Kinetic study of the ion release: comparison between transparent (PSBS8) and opalescent (PSBS16) glasses. Data are presented in a logarithmic time scale. The release of boron, calcium, potassium and silicon ions are detected at different time-points.

The PSBS16 leaching mechanism starts with a relatively high release of Ca^{2+} in the first 25 minutes of dissolution. As time passes, the amount of Ca^{2+} released reaches a plateau, the diffusion of the ions into the solution only slightly increase. This is probably due to a higher glass durability that maintains intact the calcium channel/rich calcium phase and permits a constant release of this element due to the effect of the nitric acid on the glass surface. In PSBS8 the ion leaching behaviour is different. In the first minutes a fast release of K^+ and B^{3+} (release as borate ions) is observed, probably belonging to a separate phase more susceptible to acid attack. After 125 minutes a rapid increase of the glass dissolution is seen due to a faster degradability of the structure. The sample without Al_2O_3 had the highest solubility and this plays an important role when considering the calcium release after the first 125 minutes. The structure is dissolved and the calcium and the other ions are released faster. The two different tendencies explained above are similar for the PSBS samples belonging to the two main type of glasses: opalescent and transparent.

4.3.2.3 Effect of the composition

The entire set of glasses was then analysed under the same conditions and with the same time-points. The heat treated samples were used to study the compositional effect on the ions leaching. In this case the results are expressed as kinetic rate, and so as the percentage

of element dissolved in solution every minute. To make the calculations the value obtained at 15 minutes was considered because this time-scale is felt to be the most pertinent in terms of initial setting in the cement where cross-links between poly(acrylic acid) and released ions start to form.

The effect of the composition will be described across different aspects in the following section and a discussion at the end of the chapter will give an overall description of the data obtained.

Glasses without CaO ($W^I = 0\% \text{ Ca}$)

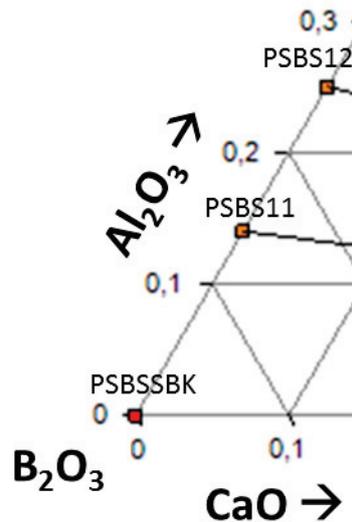


Figure 53 Part of the triaxial diagram indicating the position of the studied samples: PSBSBK, PSBS11 and PSBS12.

The three glasses without calcium PSBSBK, PSBS11 and PSBS12 (fig. 54) were compared in terms of ions release. With silicon and potassium fixed in the composition, aluminum is the only variable factor influencing the experiment in these samples.

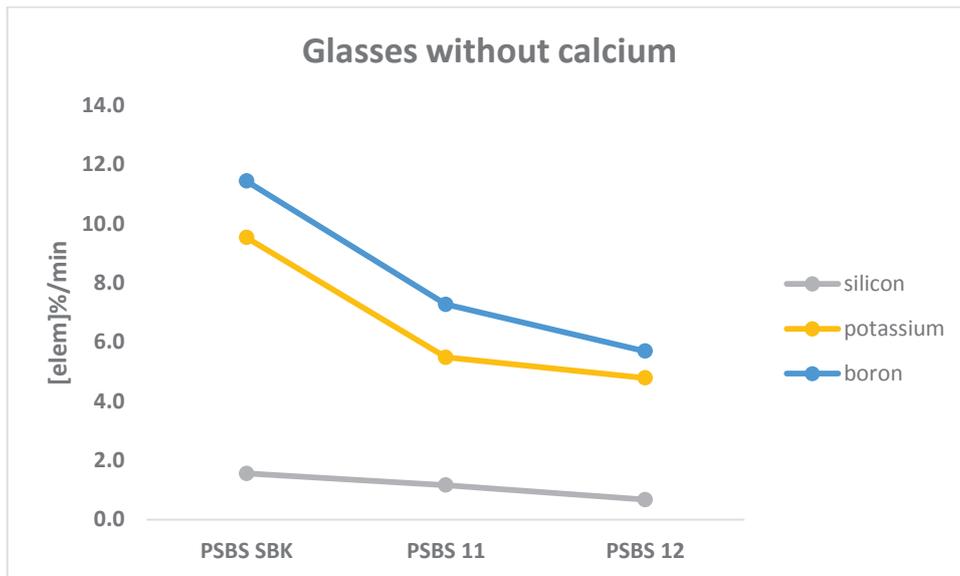


Figure 54 Release of boron, potassium and silicon from samples without calcium.

Calcium ions are not present in these glasses and the only network modifier is thus K_2O . It has been previously observed, thanks to XRD analysis, that calcium ions have a role in modifying the base structure built by the network formers (fig. 39). The mobility of network modifiers ions is affected by the openness of the glass network. Calcium ions tend to depolymerise the glass structure by the formation of NBO. The absence of CaO in the composition focusses the attention on the effect of aluminium. Increasing the w% of Al_2O_3 in the composition is associated with a decrease in the level of ions in solution after immersion. Former ions like boron and silicon, but also modifying ions like potassium are leached out at a slow kinetic rate as aluminium wt% levels increase. Consequently the glass becomes more durable with aluminium in the network. The explanation is related to its network forming nature (in the presence of Si, B oxides) that reduces the number of NBO, increasing the network connectivity and increasing durability. Overall this results in a decrease in the hydrolysis rate for the glass (Bunker 1994). Moreover alumina was proved to enhance glassification, with XRD studies demonstrating that glasses without aluminium tend to crystallize more ready with a specific thermal treatment.

Glasses without Al_2O_3 ($W^I = 0\%$ Al)

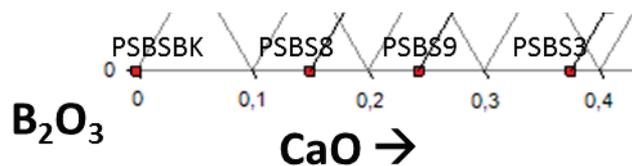


Figure 55 Part of the triaxial diagram indicating the position of the studied samples: PSBSSBK, PSBS11 and PSBS12.

Four glasses, PSBSSBK, PSBS8, PSBS9 and PSBS3 (fig. 56) - all of them without aluminium - are compared in this section. With the silicon and potassium concentrations are fixed, ion leaching trends will be interpreted in terms of the calcium concentration in their composition (fig. 57).

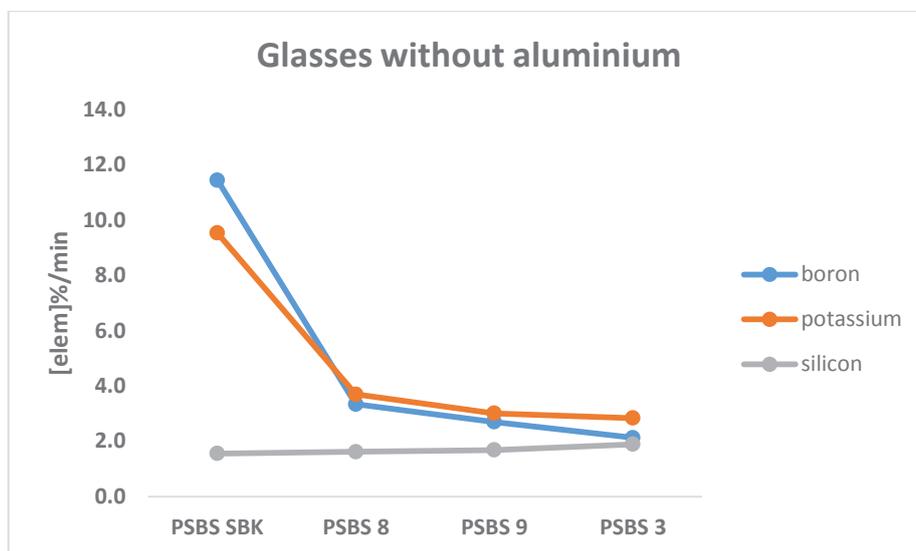


Figure 56 Release of boron, potassium and silicon from samples without aluminium.

As mentioned before, trends in different glass properties can be correlated to the concentration of the elements in compositions. The effect of depolymerisation induced by the addition of calcium is confirmed in these studies by the silicon release curve. It shows how the concentration in solution slightly increases in accordance with the increment of CaO in the glass composition. Silicate glasses have been studied for decades and experimental evidence exists to show that the basic structural unit is an SiO₄ tetrahedron, called a Q unit (where Q stands for quaternary) (Karlsson H. 1987). The tetrahedra in silicate glass have a well-defined geometry, as already stated in the first chapter of this thesis. The tetrahedra are the same units present in crystalline SiO₂ (quartz etc.) were these units are connected between each other by their corners (Geodeon O. 2008). However, there is less geometric order to the way the tetrahedral are linked via Si-O-Si binding. Adding Ca²⁺ ions, the structure is modified, allowing a faster degradation of the silica network (due to a lower number of M-O-M connections). One quantification employed for connectivity is the distribution of the so called Qⁿ units where the n is for the number of bridging oxygens (BO). As Ca²⁺ ions are added to silicate glasses, Q⁴ units are diminishing in respect to the other structural units Q³, Q² and Q¹ (fig. 58) creating more non-bridging oxygen (NBO) as a result of ion bonding between oxygen and alkali species. Borosilicate glasses contain also B₂O₃ as a network former in their compositions. Borate is usually present as a planar three coordinate structure of boron atoms; in this case, the glass network is almost completely built with these units called boroxol groups that are randomly connected between each other. But, if

alkali oxides are added some of these units are transformed into four coordinated tetrahedral units. At higher alkali oxides concentration the formation of NBO are amplified providing boroxol rings incorporating boron atoms (fig. 59). The mechanism associated with increased alkaline earth addition is different from what happens in purely silicate glasses and this could be the reason why in the acid solution the boron is found to decrease with the addition of CaO.

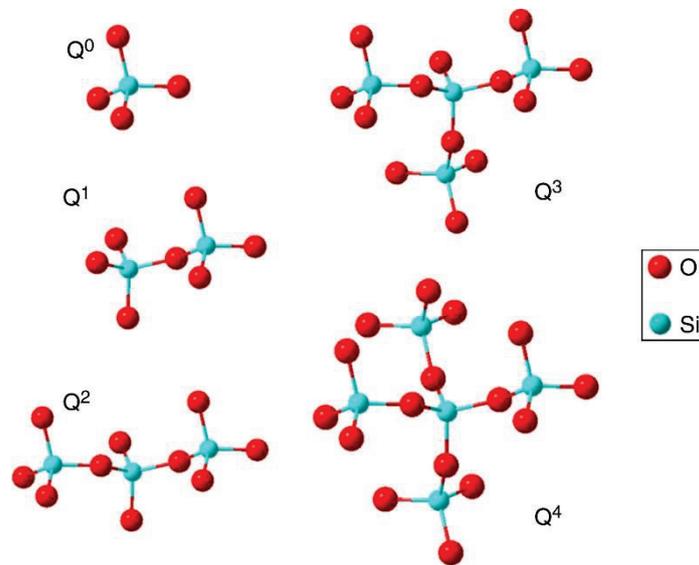


Figure 57 Different structural units of the silica glass network.

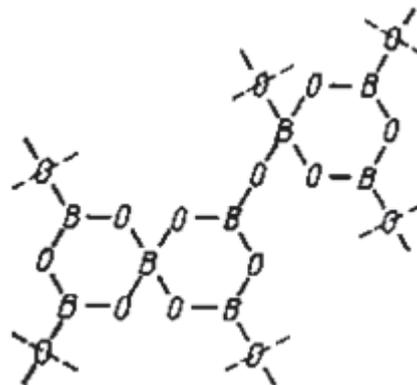


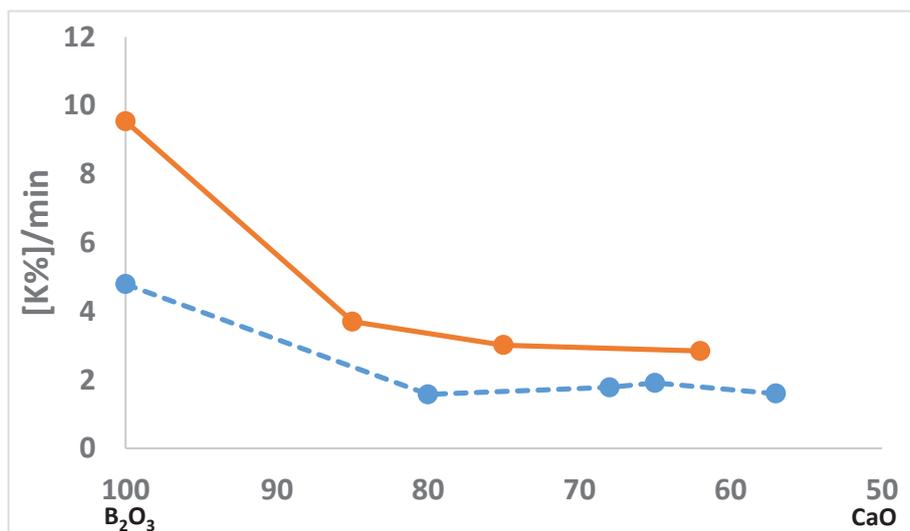
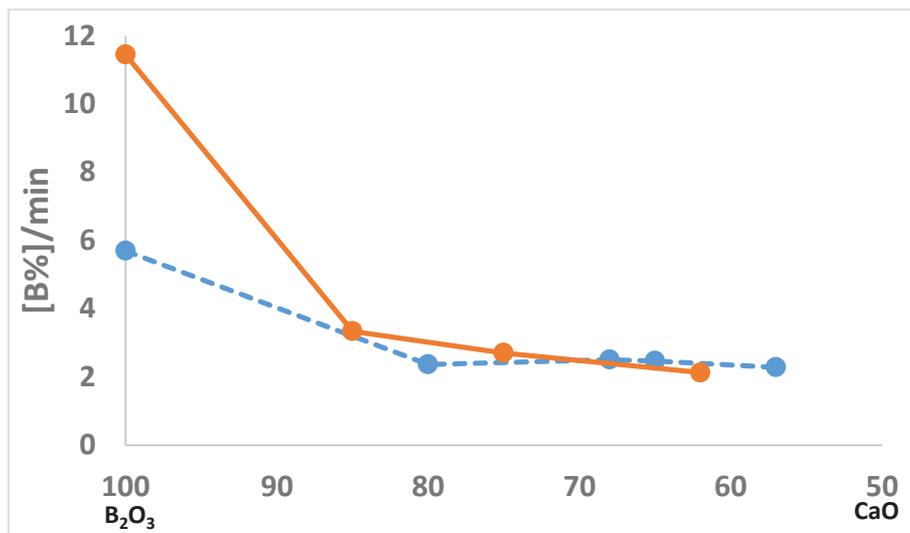
Figure 58 Boroxol rings in pure borate glasses.

Effect of the ratio B_2O_3/CaO

In figure 60 are shown the boron, potassium, calcium and silicon release values for glasses belonging to two isopleths with constant amounts of aluminium. Within these glass series,

examples of two main heat-treated glass types (transparent and opalescent) are found. In the X axis are reported the weight percentage amounts related to the B_2O_3/CaO ratio of the glasses analysed in order to make an easier comparison.

The ion release profiles suggest that the opalescent glasses release calcium with a faster kinetic rate than the transparent ones (average comparison of the kinetic rate is 4 and 2.65 Ca%/min respectively). Potassium is released faster in the transparent samples (average rate: 3.18 K%/min) compared to the opalescent ones (average kinetic rate: 2.38 K%/min). The dissolution mechanism for the opalescent glasses is found to be dependent from the B_2O_3/CaO ratio. Low values of the ratio induce a slower release of potassium, calcium and boron.



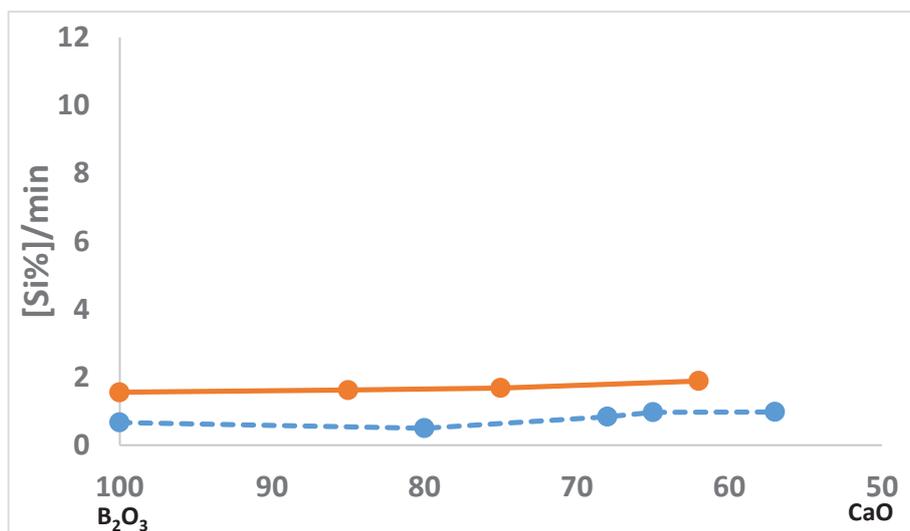
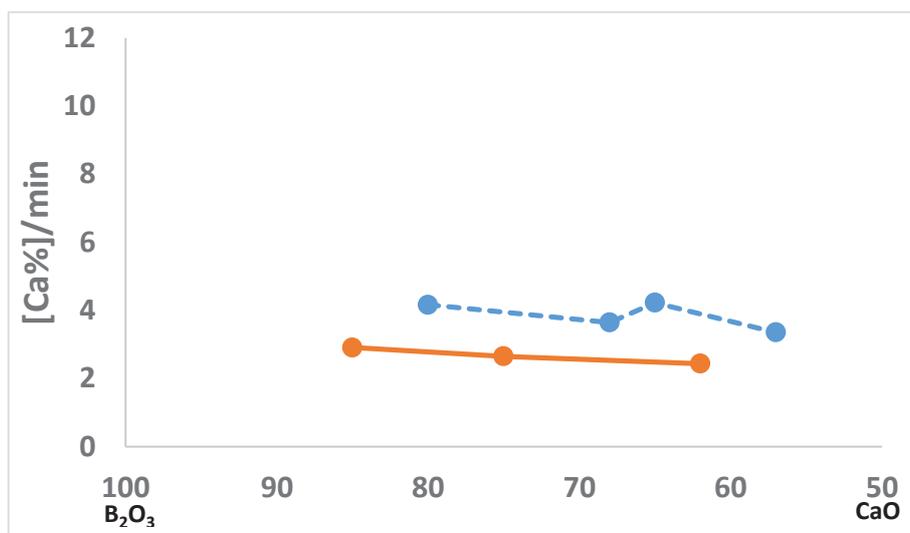


Figure 59 Kinetic study of the ion release: comparison between opalescent (dotted line) and transparent (solid line) glasses. The kinetic release of the ions is calculated as the percentage of the single element dissolved per minute and it is related to the B₂O₃/CaO ratio (in w%) of the PSBS samples.

Resuming the data showed, the opalescent glasses tend to release faster calcium ions into the acid solution, while in transparent glasses the potassium and the silicon are leached out at higher rates. The dissolution process thus depends on solution conditions (pH, temperature, etc.) as well as on composition and microstructure of the glass. In this study the experimental conditions are maintained constant (thermostatic 37°C and solution pH=2.5).

The results from this experiments not only evidences that an high B_2O_3/CaO ratio slightly increase the leaching of calcium and potassium but also reveals a clear difference in the dissolution behaviour between opalescent and transparent glasses.

4.3.2.4 Conclusion related to the ions leaching experiments

The release of ions into acid solution provides an indication of what may happen in the glass ionomer cement during the setting reaction. Cement setting and subsequent mechanical properties are influenced by a range of factors. Ion release from the glass is the most important influence on cement setting; working and initial setting times are likely to depend on how fast ions are actually released from the glass. At the end of the hardening process the material will continue leaching ions but at a slower rate probably for the formation of a silica gel layer that retards further ions release (Khoroushi M. 2013). This stage is called maturation and enhances the mechanical properties due to further stabilization of the inter-molecular forces. As modifiers are incorporated into the glass network, leaching of modifying ions out of the glass occurs. In the borosilicate phase-separated systems glass leaching occurs in the early stages of reaction with nitric acid. Ion-exchange occurs faster than network hydrolysis, as confirmed by the kinetic study, which leads to selective leaching of the modifier ions out of the glass and hydrogen ions into the glass. The dissolution depends on solution conditions (pH, temperature, etc.) as well as the composition and microstructure of the glass. In this study the experimental conditions are maintained constant (thermostatic $37^\circ C$ and solution $pH=2.5$). The only variables are the compositional changes directly influencing the glass microstructure. The leaching rate depends on the type and weight amount of modifier. In alkali silicate glasses, chemical durability increases in the order of $K^+ < Na^+ < Li^+$, suggesting that ionic field strength and free energy affect glass leaching rates. The borosilicate system in the Vycor glasses is composed of Si-Na-B. Replacing sodium (Ionic radius: 102pm) for potassium (Ionic radius: 138pm), the packing (compactness) of the glass network is changed due to the larger dimensions of the ionic radius. Increasing alkali size induces a glass network expansion, with weaker binding forces between alkali and NBO and a change in the network polymerization. This would also influence the ion release behaviour, with a less densely packed glass structure resulting in an enhanced or faster ion release (Tylkowsky 2013). Alkaline earth cations decrease the glass durability, despite their high field strength (charge divided by radius) and greater connectivity to oxygen atoms. If compared to alkali ions this will results in a lower mobility of the ions when modifying the network structure. The phenomena involved in the chemical attack of glass are very complex. These phenomena are mainly due to the effect of the chemical composition of glass.

A slow increase in the dissolution kinetic rate of the silicon ions related to the amount of CaO in the composition can be observed, especially for the transparent glasses. For that reason it is possible to affirm that the disruption of the silica network is maximized for the glasses without Al_2O_3 . In contrast, the addition of Al_2O_3 affects the glass resistance, reducing the number of NBO, increasing the network connectivity and increasing durability due to a decrease in the hydrolysis rate (Bunker 1994). Al_2O_3 is also promoting the leaching of Ca^{2+}

ions, as observed for the opalescent samples, the viscosity increased during the glassification assure the formation of one soluble phase rich of calcium. Aluminium in that case has a double role, it influences the opacity of the glasses after the heat-treatment probably encouraging the formation of two distinct phases and assists the leaching of calcium ions. The latter is caused by its network former nature that induces the increase of the glass connectivity by replacing NBO's with cross-linking Al-O-Si bonds.

The different leaching performance is proved to be related to the composition of the PSBS samples and also related to a different phase separation similar to that observed in the Vycor type glasses. Surface studies with SEM and EDX will better show the differences in the structure of the opalescent and the transparent glasses.

4.3.3 Surface analysis

Surface studies with different microscopy techniques can possibly show differences in the structure of the opalescent and the transparent glasses. The formation of different phases can be hypothesized for the glass samples analysed, but the phase separation is still not clearly defined.

4.3.3.1 BET analysis

The superficial area (SA), the pore size and the pore volume were evaluated for the first 10 heat-treated glasses before and after an acid dissolution. The results are showed in table 29.

Before the acid dissolution the surface areas are very similar, with values between 1.73 and 3.6 m²/g. The pore sizes have to be considered only after the acid dissolution because the glass is completely dense once is heat-treated. The acid dissolution dramatically increases the differences in the particle properties measured with BET. Opalescent glasses present an higher increase in the SA while the pore size varied between 9 and 17 nm (fig 61 and 63). This could bring to a logical conclusion based on the fact that smaller pores will result in high surface area of the particles. The transparent glasses in contrast show lower values for SA while the pore volume increase to 20 nm except for PSBS8 (fig 62 and 64). The results demonstrate initial differences between opalescent and transparent glasses treated with acid solution. The first result that will assume a different structure between the glass particles after the heat treatment probably due to a different phase separation.

Table 29 BET analysis results for the PSBS samples before (HT) and after (7d) the HNO₃ dissolution.

SAMPLES	SA (m ² *g ⁻¹)	Pore size (nm)	Pore volume (cm ³ *g ⁻¹)
PSBS1 HT	3.39	11.37	0.0096
PSBS1 7d	16.98	9.57	0.0406
PSBS2 HT	1.73	11.09	0.0046
PSBS2 7d	41.64	4.24	0.0441
PSBS3 HT	1.84	13.70	0.0063
PSBS3 7d	5.47	19.04	0.0260
PSBS4 HT	3.60	14.14	0.0127
PSBS4 7d	6.42	18.89	0.0303
PSBS5 HT	2.48	13.02	0.0081
PSBS5 7d	6.20	17.81	0.0276
PSBS6 HT	1.93	11.56	0.0056
PSBS6 7d	60.65	3.70	0.0562
PSBS 7 HT	3.31	11.94	0.0099
PSBS7 7d	9.94	9.99	0.0248
PSBS8 HT	1.74	12.51	0.0054
PSBS8 7d	11.78	12.34	0.0363
PSBS9 HT	2.00	14.20	0.0071
PSBS9 7d	5.62	20.55	0.0289
PSBS 10 HT	2.04	14.72	0.0074
PSBS 10 7d	11.90	9.24	0.0275

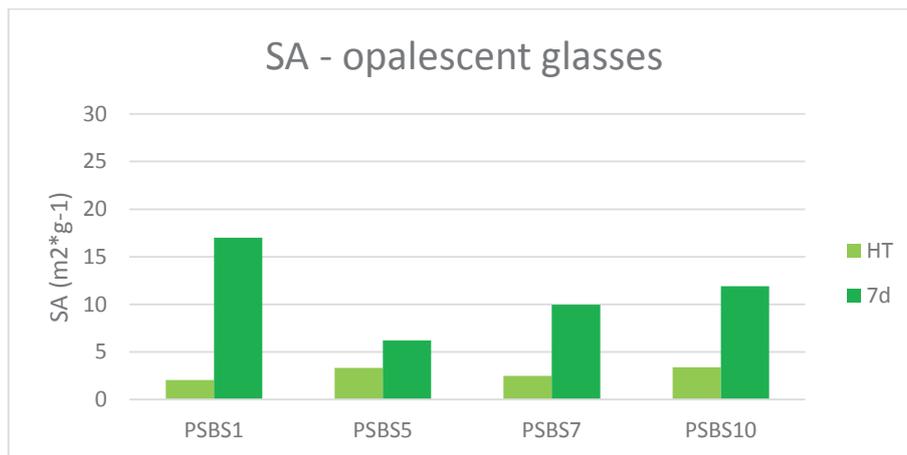


Figure 60 Superficial area for opalescent glasses before (HT) and after (7d) the acid dissolution.

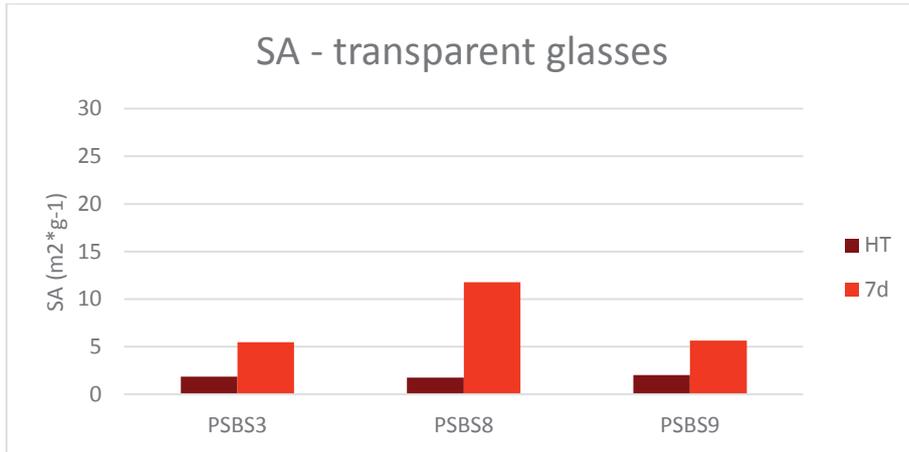


Figure 61 Superficial area for transparent glasses before (HT) and after (7d) the acid dissolution.

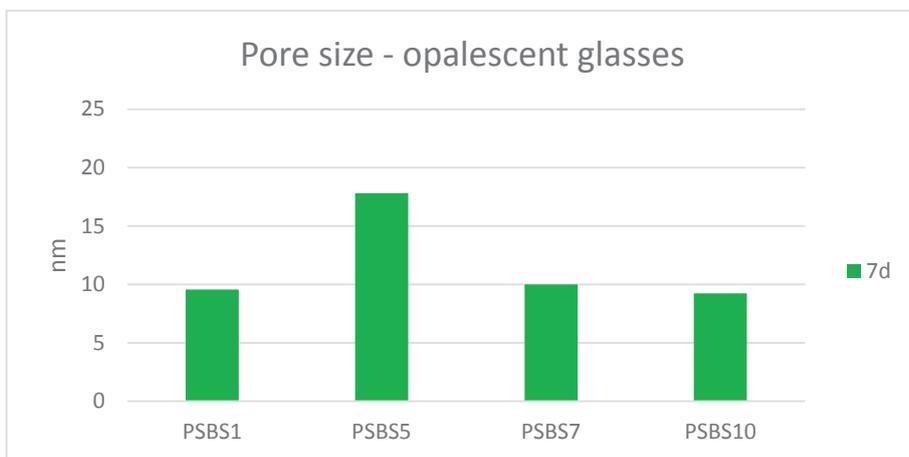


Figure 62 Pore size for opalescent glasses before (HT) and after (7d) the acid dissolution.

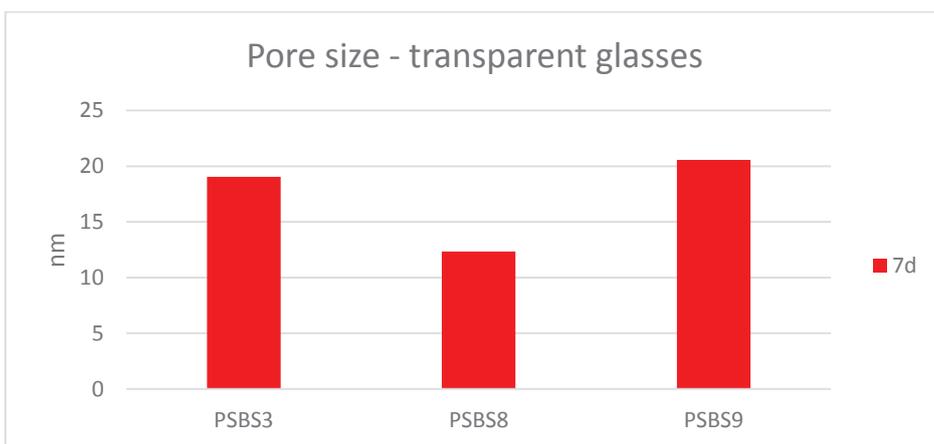
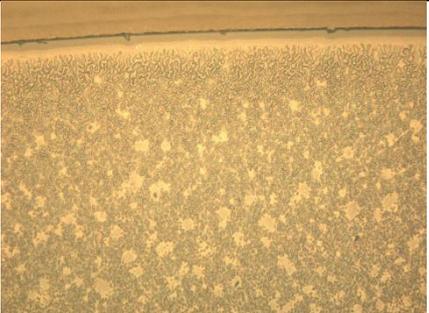
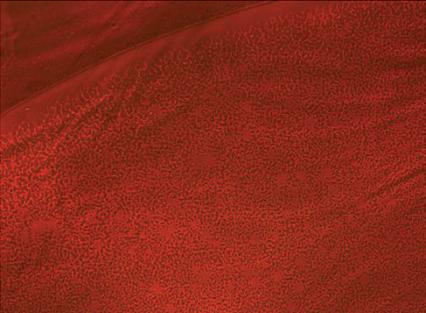
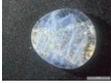
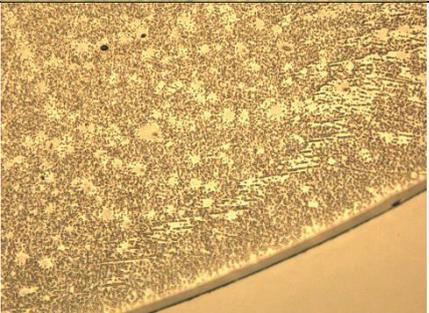
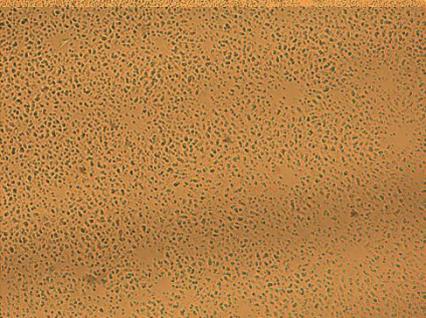


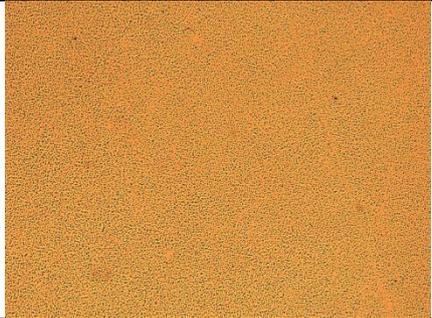
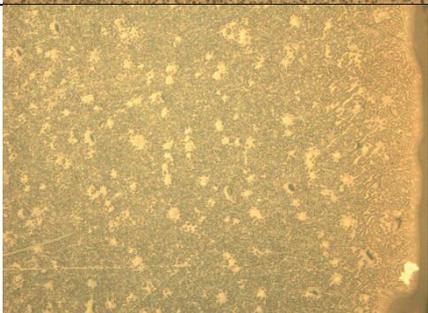
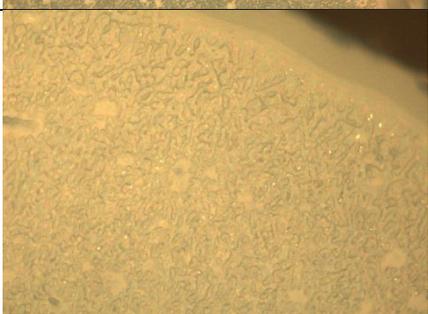
Figure 63 Pore size for opalescent glasses before (HT) and after (7d) the acid dissolution.

4.3.3.2 Optic microscopy studies

The optic microscope is a useful technique for an initial scanning of samples surfaces (tab. 30). PSBS1, PSBS2, PSBS3 and PSBS 7 surfaces were studied with reflected and transmitted light after being polished with the procedure described in the materials and method section.

Table 30 Images of the polished glass samples obtained with the optic microscope using transmitted and reflected light.

SAMPLE	Magnification	Reflected light	Transmitted light
PSBS1 	20x		
	50x		
PSBS2 	20x		
	50x		

PSBS3 	20x		
	50x		
PSBS7 	20x		
	50x		

No relevant differences were detected for the samples in reflected light mode. At higher magnifications the surfaces show approximately the same roughness and because of the absence of any elemental determination it is difficult to reach any conclusions. The analysis with transmitted light reveal more details; especially an interconnected structure linked the presence of a phase separation is observed. No differences have been observed between the opalescent and the transparent samples except for the fact that the transmitted light logically diffuses better in the transparent glasses. The conclusion is that a powerful tool has to be used for the investigation of the microstructure and the composition of the surface of the PSBS glasses.

In order to bring to light the structure of the hypothesised phase separation a final attempt has been made for PSBS1. The surface of the polished sample was etched with HCl 1M for different time-points and then analysed with the optic microscope. The images obtained are showed in figure 63 and 64.

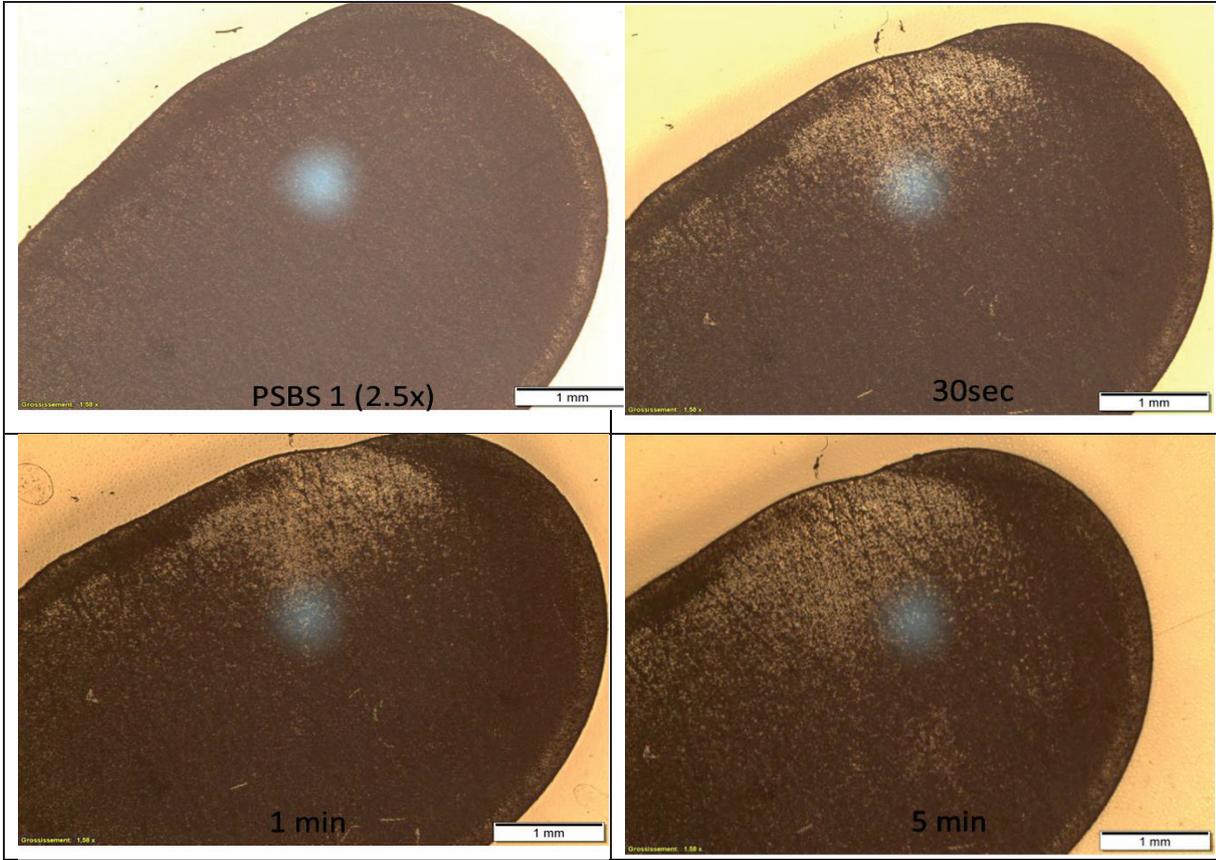
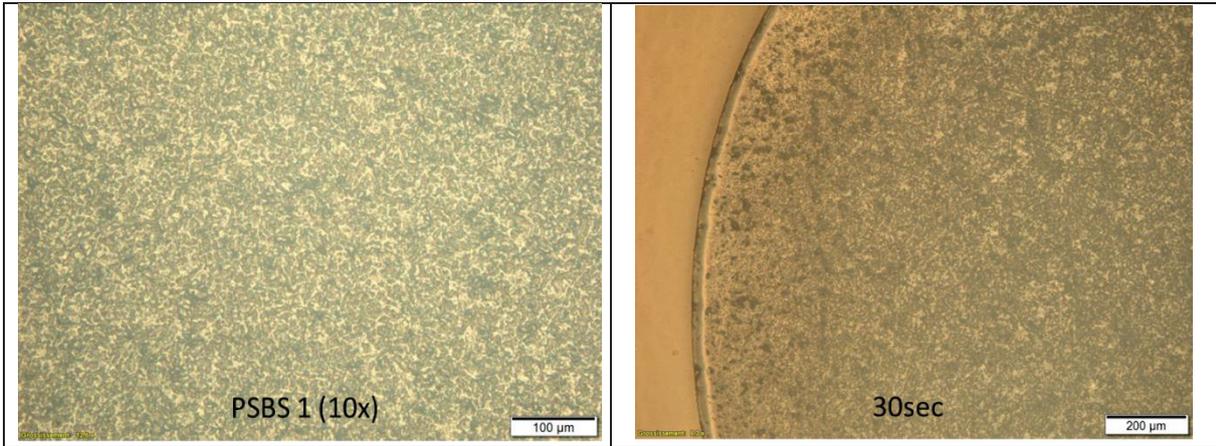


Figure 64 Surface of PSBS1 sample etched out with HCl at different time-points: 30 seconds, 1 minute and 5 minutes. Magnification: 2.5x.



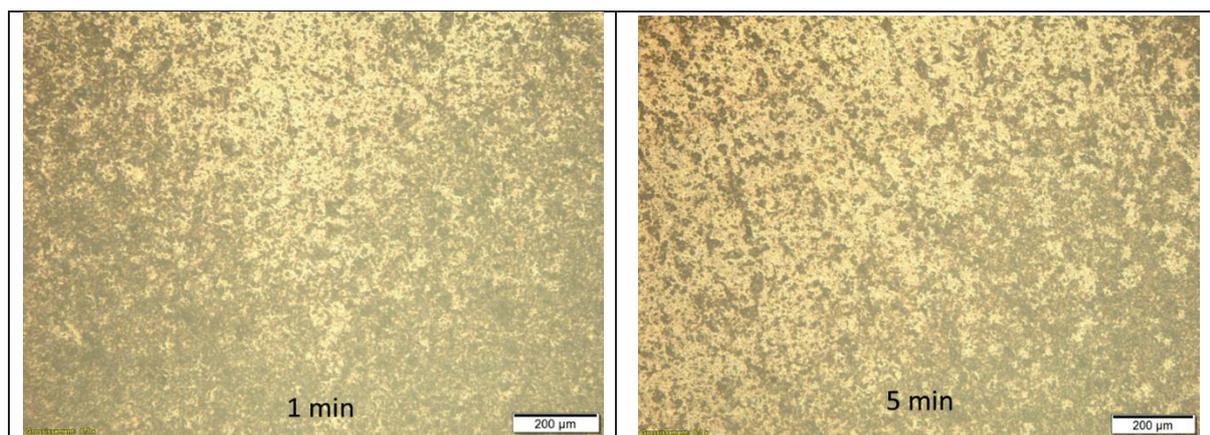


Figure 65 PSBS1 surface etched with HCl 1 M at different time-points. Magnification: 10x.

The images again show the effect of the acid corrosion in the surface of the glass, but they don't reveal details regarding the structure morphology. The phase separation is presenting changes detectable only at nanometer level. For this reason studies with SEM have been performed at this point.

4.3.3.3 SEM and EDX analysis

SEM analysis of the PSBS 16 and PSBS 8 glass samples were conducted to find evidence for two different amorphous phases and so prove that phase separation was occurring. It was hoped to find similarity with the sodium boro-silicate system of the Vycor glasses. The figures represent the analysis of the glass surface with the back-scatter detector and show two different structures for the opalescent and the transparent surfaces. The left-hand images (opalescent sample) present a number of sparse droplets ranging in size between 20 and 30 nm whilst the right-hand images suggest narrow veins surrounded by a homogeneous phase. It is unclear why this is so and which relate to some type of phase separated structure, for this reason, leaching experiments were conducted to obtain more information.

The etching process in normal conditions it is found to be effective for revealing new details of the glass surface. A porous structure is detected for the opalescent sample PSBS 16 while a homogeneous surface is observed in the transparent glass PSBS 8 (see lower images in figure 67), proving that the surfaces are different and a distinct reaction with the nitric acid is going on.

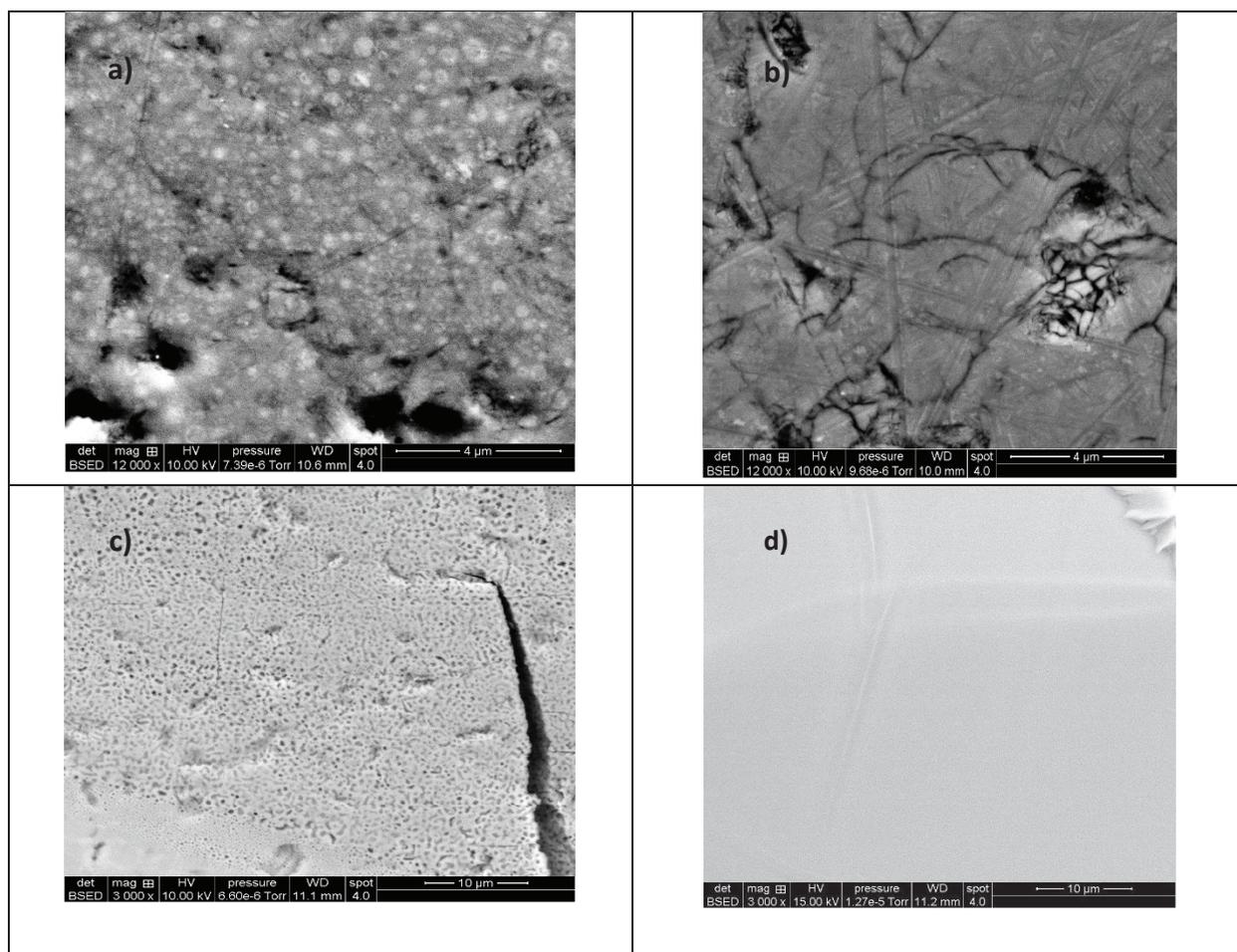


Figure 66 Glass microstructures before (a,b) and after (c,d) the dissolution test in normal condition. PSBS16 is represented in the left while PSBS 8 is showed in the right.

The harsh conditions reveal more details for the interpretation of the glass structures. The opalescent glass shows a porous structure of almost pure silica. The transparent glass structure collapses, dissolving in the solution and leaving at the bottom of the reaction beaker a white unknown powder. XRD and XRF analysis demonstrate that the powder is still completely amorphous and it is composed of pure silica (fig. 68).

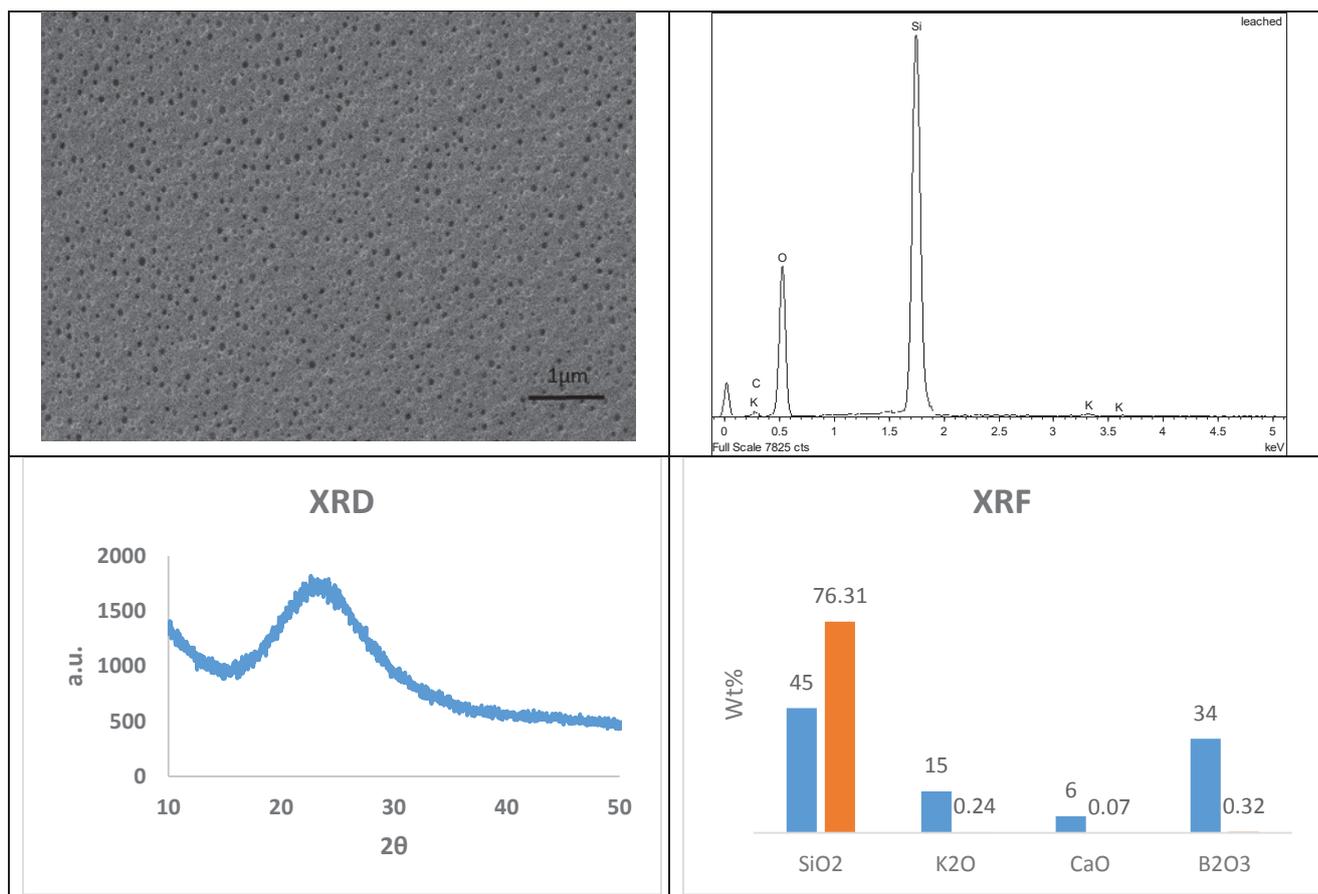


Figure 67 Hard condition dissolution studies of the remained glass structure for PSBS 16 (first line) and for the remained glass powder for PSBS 8 (second line).

The leaching experiment undertaken with harsh conditions (at higher temperature) suggests that the phase separation occurs because only one phase completely dissolves. This phase is the boron rich phase where it might be expected that the network former, boron, shouldn't be etched out as soon as the network modifiers. The process involved in the phase separation should be for both samples (transparent and opaque) the nucleation and growth mechanism (fig. 69). In the opalescent glass the nuclei are the boron-rich phases that are etched out leaving a porous structure, while in the transparent glass the nuclei are composed of silica, collapsing apart as a powder after which the surrounding boron-rich phase gets dissolved in the nitric acid solution. The pattern of the ion release implies that one of the phases is more reactive and susceptible to acid attack and will be leached out from the glass earlier (Rafferty A. 2003). The other phase will remain in the cement improving the mechanical properties of the dental restorative material. For instance, the existence of a nanoscale phase separation varying from that of Vycor glasses and could result in the different ionic dissolution/leaching rate of the constituting elements of the glass system. Changing the composition the level of leached ions and the microstructure could be altered as show in this case.

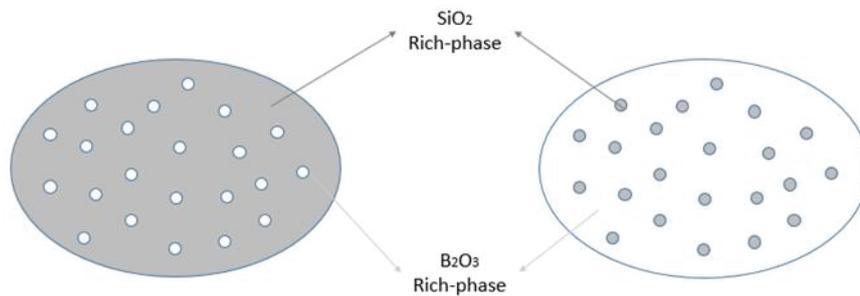


Figure 68 Hypothesis of the mechanism of phase separation

4.4 Results of the Design Of Experiments (DOE)

The data reported in the following table are the responses obtained from the dissolution experiments. The ions leaching were measured with ICP and the data converted from ppm into dissolution rate as [elem]%/min calculated on the first 15 minutes of dissolution to represent the data as the percentage of the single element detected dissolved per minute (tab. 31).

Table 31 Dissolution rate of boron, potassium, calcium and silicon detected for each glass sample.

SAMPLES	Dissolution rate ([elem]%/min) of:			
	Boron	Potassium	Calcium	Silicon
PSBS 1	2,51	1,78	3,64	0,84
PSBS 2	1,97	2,25	1,89	0,87
PSBS 3	2,13	2,84	2,43	1,89
PSBS 4	2,39	0,99	4,70	0,36
PSBS 5	2,28	1,59	3,35	0,98
PSBS 6	2,39	2,55	2,22	0,98
PSBS 7	2,38	1,77	4,06	0,50
PSBS 8	3,34	3,70	2,91	1,62
PSBS 9	2,70	3,01	2,65	1,69
PSBS10	2,35	1,38	4,27	0,37
PSBS 11	7,28	5,49	n.o.	1,16
PSBS 12	5,70	4,79	n.o.	0,67
PSBS 13	2,11	1,50	2,48	0,16
PSBS 14	1,85	1,89	1,88	0,56
PSBS 15	2,77	3,12	1,99	0,79
PSBS 16	2,47	1,91	4,22	0,97
PSBSSBK	11,45	9,54	n.o.	1,56

As a first approach the DOE allows evaluation of which factors statistically influence the response(s) studied. The data obtained should be verified for the precision and a key objective is to test if the predicted values are similar to the experimental ones. The ANOVA (analysis of variance) is a useful tool that allows an assessment of the importance of one or more factors by comparing the response variable means at the different factor levels. The idea behind the ANOVA is that the variation in the response variable (total variation) is divided in 2 parts: a part that indicates how much of the variation is explained by the regression model and a part that shows how much of the variation is left unexplained by the regression model (“residual”). The total variation is the variation explained by the regression model plus the error. To summarize the total variation for all data points with one number, the sum of squares is done for each source of variation. These have to be done for all the responses and so in this instance for: boron, potassium and calcium leaching. The values for each response are listed in the following tables and are calculated by the software (NEMRODW software, LPRAI, Marseille) once all the compositions had been prepared and the associated leaching responses were obtained. Finally the significance is calculated by a statistical test called “Fisher’s test”. This test is used for comparing the two statistical models that have been fitted to a data set, in order to identify the model that best fits the population from which the data were sampled. In this case the models compared are the response values calculated and the response values obtained experimentally.

$$F = \frac{\text{explained variance}}{\text{unexplained variance}} \text{ or } \frac{\text{between – group variability}}{\text{within – group variability}}$$

The first term correspond to:

$$\sum_{i=1}^K n_i \frac{(\bar{Y}_i - \bar{Y})^2}{K - 1}$$

Where:

\bar{Y}_i = samples mean in the i-th group

n_i = number of observations

\bar{Y} = overall mean of the data

K = number of groups.

And the second term:

$$\sum_{i=1}^K \sum_{j=1}^{n_i} \frac{(Y_{ij} - \bar{Y}_i)^2}{N - K}$$

Where:

\bar{Y}_{ij} = is the j-th observation in the i-th out of K group

N= overall sample size.

Table 32 Analysis of variance for the response: boron.

Variation	Sum of squares	Degree of freedom	Mean square	Ratio	Signif (F)
Regression	1.5740	6	0.623	8.3477	0.654**
Residuals	0.2200	7	0.0314		
Total	1.7940	13			

Table 33 Analysis of variance for the response: potassium.

Variation	Sum of squares	Degree of freedom	Mean square	Ratio	Signif (F)
Regression	6.8695	6	1.1449	8.7001	0.580**
Residuals	0.9212	7	0.1316		
Total	7.7907	13			

Table 34 Analysis of variance for the response: calcium.

Variation	Sum of squares	Degree of freedom	Mean square	Ratio	Signif (F)
Regression	7.8646	6	1.3108	1.9950	19.4
Residuals	4.5992	7	0.6570		
Total	12.4639	13			

The data points unfortunately are not well balanced comparing the F test obtained especially for the particular approach that was done from the beginning of the experimentations, with the addition of new experimental points.

Another possibility with DOE is to represent response surfaces for each in order to generate a 2-D figure which will give contour lines indicating the variation of the response. From this it is possible to better understand the impact of the three different factors of the study.

Three different figures have been obtained setting the response in order to maximize the leaching of each single element at the time. In figure 70 the boron leaching is maximized for the composition that introduces higher amounts of B₂O₃. In figure 71 the contour lines show that for maximum leaching of potassium the tendency is very similar to the previous one, i.e. higher B₂O₃ contents will also enhance the release of potassium. Finally in figure 72 is

represented the trends for calcium that are the opposite for boron and potassium. During the planning of the experiment a target composition was to be expected at this point delivering a compromised maximum release for all three elements. From the results obtained so far it is impossible to choose one single composition to characterize with further experiments and for that reason for the next experiences have been selected two sample with opposed leaching trends. This choice was made to obtain further information on the biological and mechanical properties of borosilicate glasses that release the ions of their composition with a different kinetic rate.

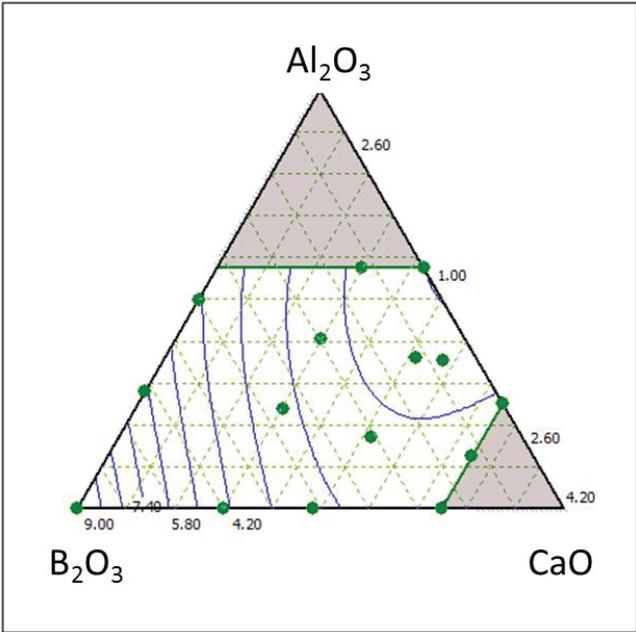


Figure 69 Boron leaching maximization

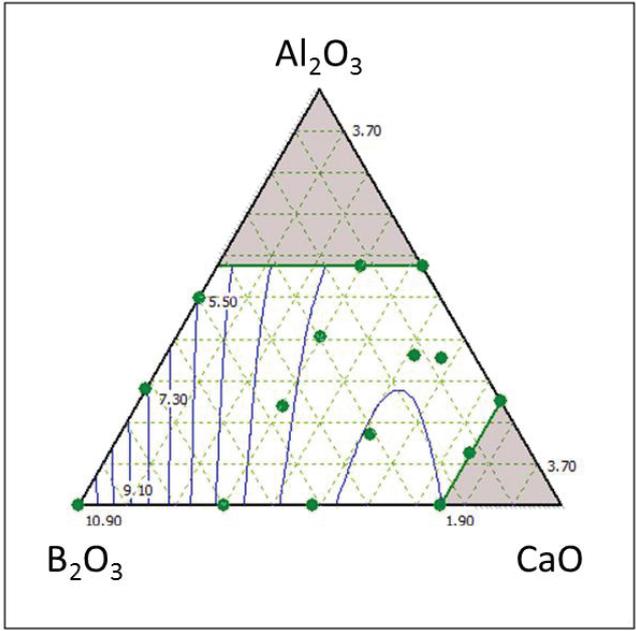


Figure 70 Potassium leaching maximization

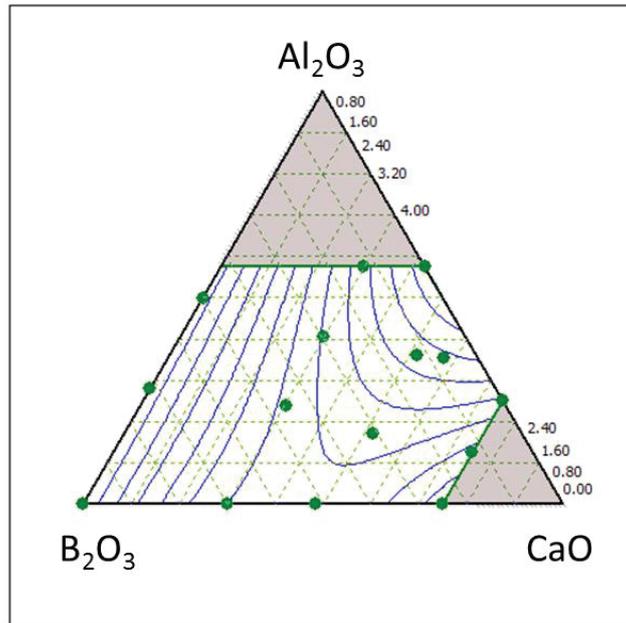


Figure 71 Calcium leaching maximization.

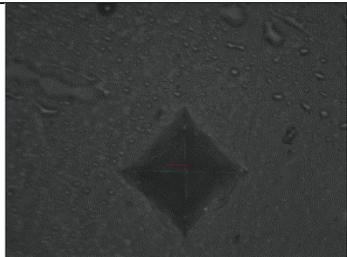
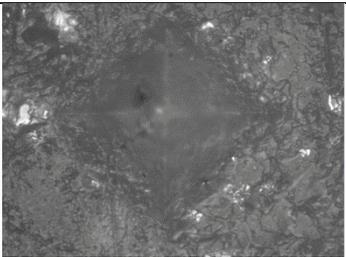
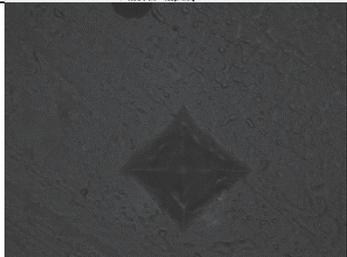
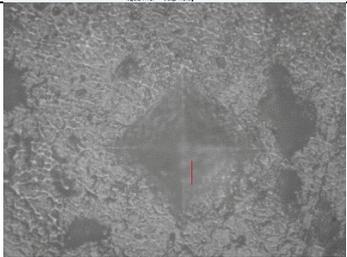
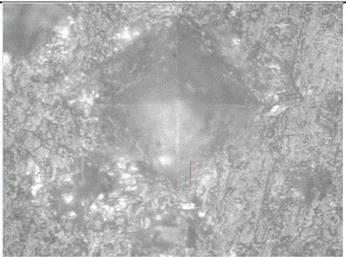
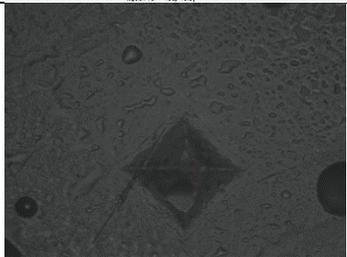
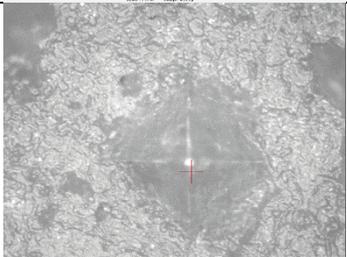
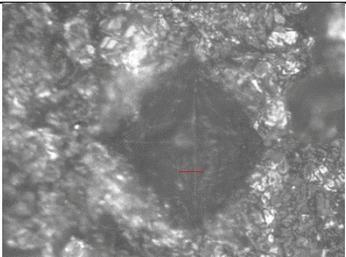
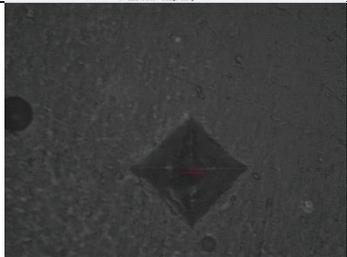
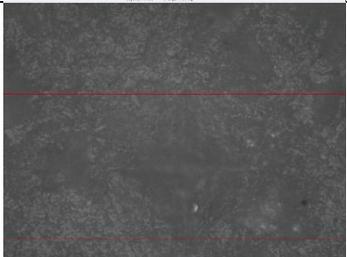
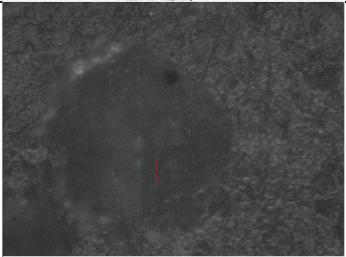
5. MECHANICAL AND BIOACTIVE PROPERTIES ANALYSIS

5.1 Mechanical properties

5.1.1 Vicker's microhardness test

The microhardness measurements are reported in the following table 35 and figure 73. The analysis were realized with the method used for the bone structural unit (BSU) system. At least 15 indents for each sample were measured and the average values are repoted in the table that show the evolution of HVN during 42 days.

Table 35 Vicker's indentation on PSBS cements samples

Day	GIC (FUJI IX)	PSBS8	PSBS16
8			
14			
21			
28			

42

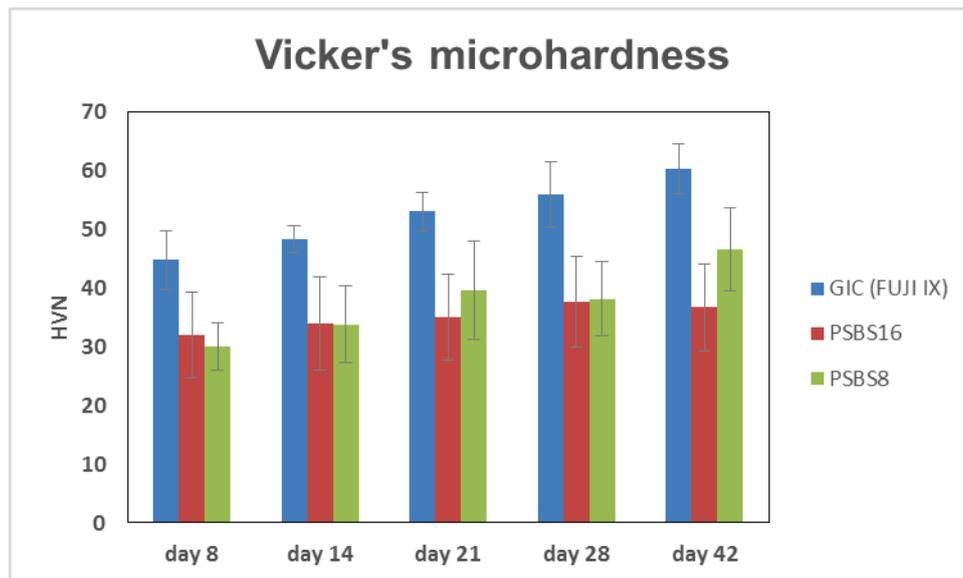
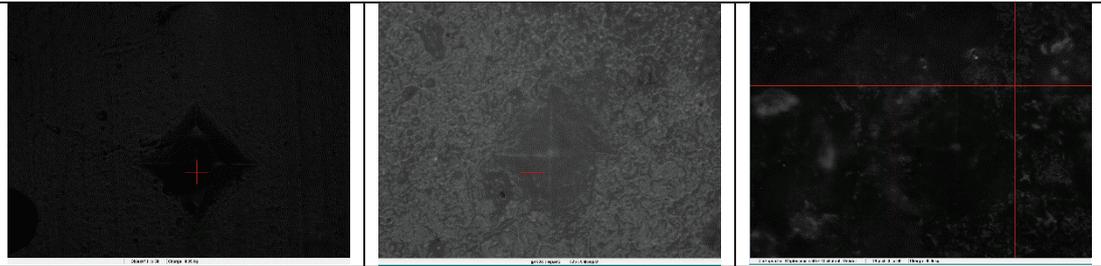


Figure 72 Vicker's microhardness comparison between two PSBS samples and FUJI IX.

All tested PSBS cements showed high surface roughness probably due to a rapid setting reaction that did not allow the glass particles to mix properly in contact with the poly(acrylic acid).

The surface hardness of the FUJI IX[®] GICs were found to be higher than those of PSBS samples. During all time-points the Vicker's microhardness is significantly higher in the commercial GIC (FUJI IX[®]) when compared with PSBS16 and PSBS8 singularly, with a p value of <0,001 in average. The standard deviation (represented by the error bars in the graph) shows how there are no significant differences between the PSBS samples studied, but it is possible to expect different behaviour in long term data-points. PSBS16 seems to stabilize its hardness values between 21 and 42 days while PSBS8 shows a continuous increase along the time-points. In this respect, PSBS8 mirrors the FUJI IX microhardness values). The changes in surface hardness that were found in this study are most probably related to the reactivity of the PSBS glasses. It appears that that no significant differences exist in values as a function of the aluminium concentration in the borosilicate glasses. Aluminum is considered very important in the setting mechanism due to its 3+ charge and its ability to efficiently crosslink the polyacrylic acid. Interestingly the absence of aluminium does not

affect the microhardness in PSBS8 that do not contain this element in its composition. This confirms the hypothesis that the boron released as boric acid acts as a crosslinker providing a rapid setting reaction and substituting the effect of the aluminium of the conventional glass ionomer cements.

A longer setting period and an assay with the interaction in a body fluid can provide a most complete scenario with the closer environment as the one in the mouth.

5.1.2 Flexural strength

Flexural strength analysis was performed for PSBS8, PSBS16 and a FUJI IX control after 24 hours and after 7 days of maturation at ambient conditions. The results are shown in the figure 74.

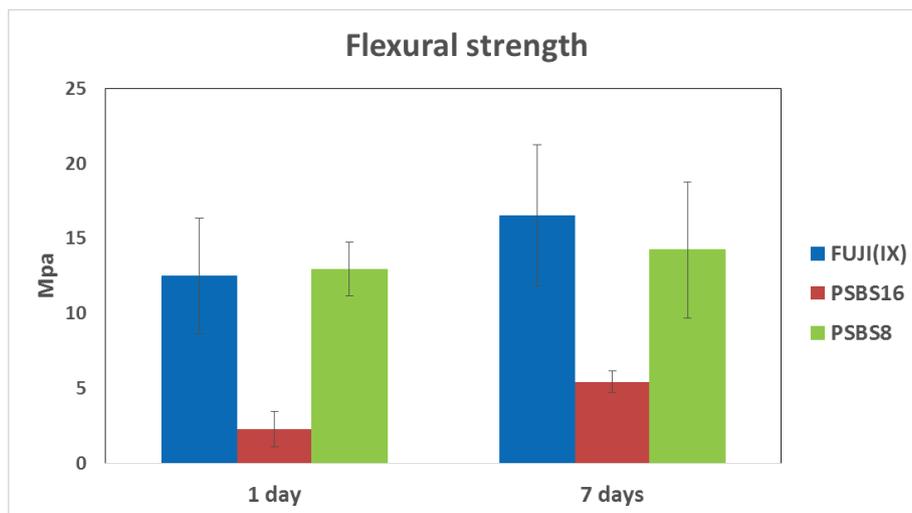


Figure 73 Flexural strength values at after 1 and 7 days of maturation.

It can be seen that after 24 hours of maturation, the PSBS8 cement exhibits higher mean flexural strength compared to the Fuji IX control. In contrast after 1 week there was only a small further increase in strength for the PSBS8 cement whilst a high increase was encountered for the conventional FUJI IX cement. PSBS16 didn't show any comparable flexural strength after 24 hours or one week. In the study of Xie et al. (D. Xie 2000) the flexural strength after 7 days of immersion in distilled water for other commercial products revealed values between 11 and 82 MPa. The improvements in mechanical properties of the experimental GIC made with the PSBS8 may indicate increased homogeneity and polysalt bridge formation in the final set material due to the leaching of boron ions as boric acid. Undoubtedly, stronger bonds between the organic and inorganic networks caused the increase in flexural strength of the final set cement. The results of the analysis suggest that some important general relationships exist between the compositions, microstructures and mechanical properties of commercial GICs. Advances in the product improvement should arise via a deeper study that considers the rapid setting of the cements immersed in a

solution (distilled water, SBF or simulated saliva) because improvements in strength are important in providing resistance to attack by moisture and thanks to the remineralization the PSBS samples could give superior strength value compared to a conventional ionomer glass.

5.2 Bioactivity

5.2.1 Bioactivity test ISO 23317:2014. In vitro evaluation for apatite-forming ability of implant materials.

The formation of an HA layer is generally considered as an indication of the bioactivity. It facilitates the attachment of the osteoblast (via protein) on bioactive glasses and so enabling the bonding with the bone. The ability of the bioactive glasses to form HA in body fluid is also used in toothpaste for treating dentine hypersensitivity (B.J. Tai 2006). The deposition of HA in dentinal tubules of root dentin allows to block the pulp to be in contact with the external environment thus reducing the sensitivity.

The objective of this experiment is to comprehensively characterize the conversion of five PSBS glasses and compare the results with the control 45S5K. Weight loss and pH changes accompanying the conversion were measured to provide additional insight into the kinetics and mechanisms of the conversion of borosilicate phase separated glasses.

In the following table (tab. 36) are shown the values of the glass disk's measurements and the calculation to obtain the volume of SBF necessary for the experiment.

Table 36 Glass disk dimensions and calculation for the volume of SBF (d=diameter, h=high, SA=superficial area, V=volume).

		d (cm)	h (cm)	SA(cm ²)	SA(mm ²)	V _{SBF} (mm ³)	V _{SBF} (mL)
PSBS2	7days	0.84	0.20	1.64	163.53	16353.12	16.35
	21days	0.87	0.20	1.73	173.47	17346.93	17.35
PSBS3	7days	0.81	0.20	1.54	153.88	15387.57	15.39
	21days	0.80	0.20	1.51	150.72	15072.00	15.07
PSBS8	7days	0.73	0.20	1.30	129.51	12950.93	12.95
	21days	0.80	0.20	1.51	150.72	15072.00	15.07
PSBS9	7days	0.73	0.20	1.30	129.51	12950.93	12.95
	21days	0.78	0.20	1.45	144.50	14450.28	14.45
PSBS16	7days	1.02	0.20	2.27	227.40	22739.88	22.74
	21days	0.95	0.20	2.01	201.35	20135.25	20.14
45S5K	7days	1.03	0.20	2.31	231.25	23124.53	23.12
	21days	1.04	0.20	2.33	233.18	23318.03	23.32

Table 37 HA formation in the surface of a 45S5K glass disk after 1 week and 3 week of immersion in SBF

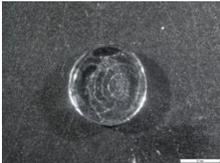
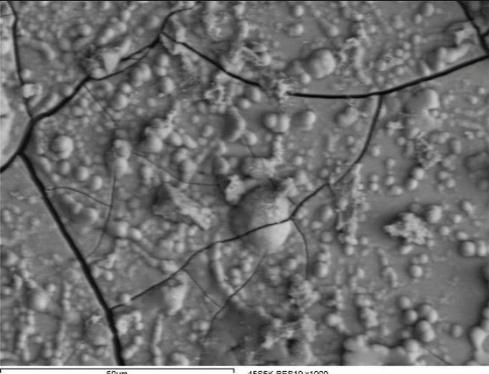
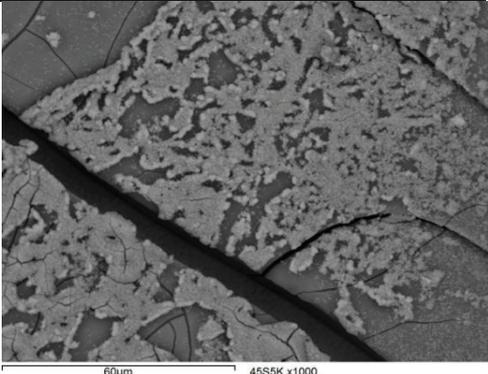
45S5K	1 week	3 weeks																											
																													
	<table border="1"> <thead> <tr> <th>EDX (w%)</th> <th>Ca</th> <th>P</th> <th>Si</th> <th>O</th> <th>K</th> <th>Na</th> <th>Mg</th> <th>Cl</th> </tr> </thead> <tbody> <tr> <td>1 week</td> <td>44.9</td> <td>17.4</td> <td>2.4</td> <td>33.1</td> <td>0.3</td> <td>0.6</td> <td>0.6</td> <td>0.8</td> </tr> <tr> <td>3 weeks</td> <td>15.04</td> <td>7.2</td> <td>27.97</td> <td>48.4</td> <td>0.98</td> <td>0.6</td> <td>0.11</td> <td>0.68</td> </tr> </tbody> </table>	EDX (w%)	Ca	P	Si	O	K	Na	Mg	Cl	1 week	44.9	17.4	2.4	33.1	0.3	0.6	0.6	0.8	3 weeks	15.04	7.2	27.97	48.4	0.98	0.6	0.11	0.68	
EDX (w%)	Ca	P	Si	O	K	Na	Mg	Cl																					
1 week	44.9	17.4	2.4	33.1	0.3	0.6	0.6	0.8																					
3 weeks	15.04	7.2	27.97	48.4	0.98	0.6	0.11	0.68																					

Table 38 HA formation in the surface of a PSBS2 glass disk after 1 week and 3 week of immersion in SBF

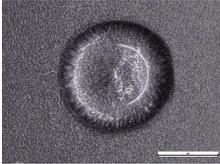
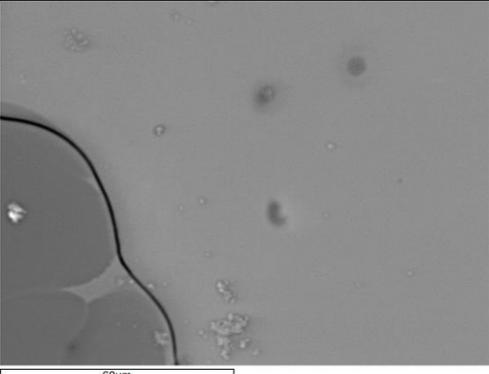
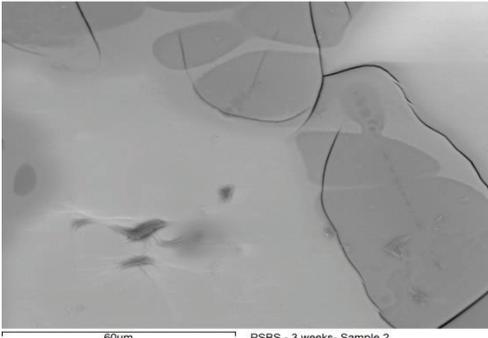
PSBS2	1 weeks	3 weeks																											
																													
	<table border="1"> <thead> <tr> <th>EDX (w%)</th> <th>Ca</th> <th>P</th> <th>Si</th> <th>O</th> <th>K</th> <th>Na</th> <th>Cl</th> <th>Al</th> </tr> </thead> <tbody> <tr> <td>1 week</td> <td>7.21</td> <td>0</td> <td>29.99</td> <td>48.86</td> <td>11.39</td> <td>0</td> <td>0</td> <td>2.54</td> </tr> <tr> <td>3 weeks</td> <td>7.13</td> <td>0</td> <td>32.95</td> <td>45.37</td> <td>10.97</td> <td>0.56</td> <td>0.16</td> <td>2.85</td> </tr> </tbody> </table>	EDX (w%)	Ca	P	Si	O	K	Na	Cl	Al	1 week	7.21	0	29.99	48.86	11.39	0	0	2.54	3 weeks	7.13	0	32.95	45.37	10.97	0.56	0.16	2.85	
EDX (w%)	Ca	P	Si	O	K	Na	Cl	Al																					
1 week	7.21	0	29.99	48.86	11.39	0	0	2.54																					
3 weeks	7.13	0	32.95	45.37	10.97	0.56	0.16	2.85																					

Table 39 HA formation in the surface of a PSBS3 glass disk after 1 week and 3 week of immersion in SBF

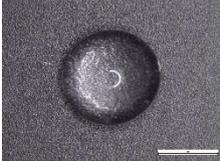
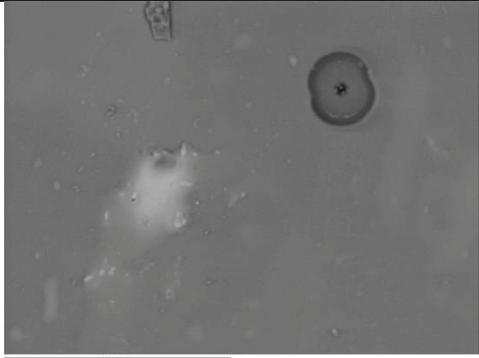
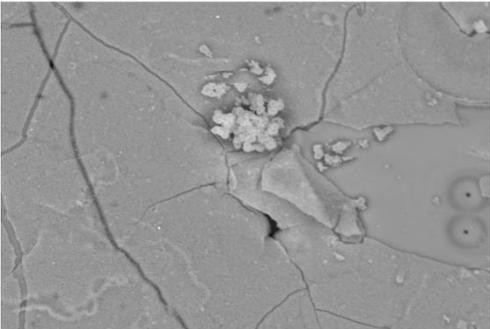
PSBS3	1 week					3 weeks		
								
	EDX (w%)	Ca	P	Si	O	K	Na	Cl
	1 week	10.44	0	28.67	51.06	8.71	0	1.12
3 weeks	27.58	13.49	12.96	43.58	0.22	0.98	1.18	

Table 40 HA formation in the surface of a PSBS3 glass disk after 1 week and 3 week of immersion in SBF

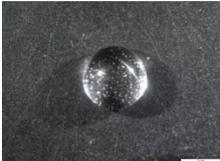
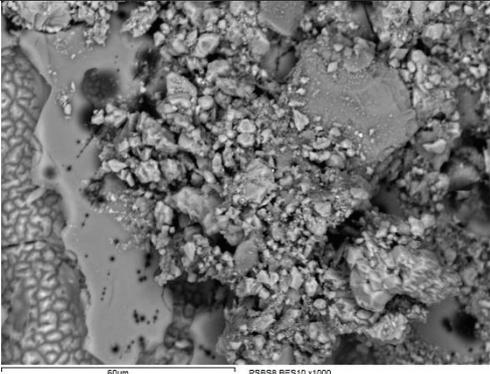
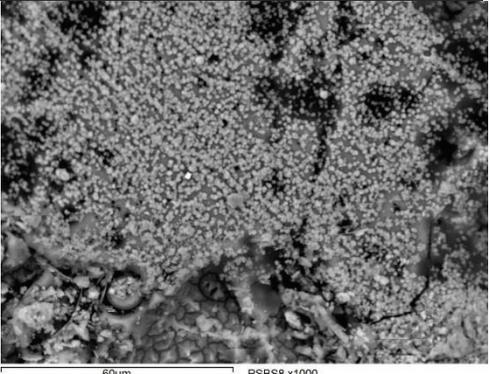
PSBS8	1 week					3 weeks			
									
	EDX (w%)	Ca	P	Si	O	K	Na	Mg	Cl
	1 week	23.8	0.1	26.6	44.9	0.4	0.2	3.7	0.3
3 weeks	20.8	9.8	21.82	46.16	0.46	0.43	0.14	0.38	

Table 41 HA formation in the surface of a PSBS9 glass disk after 1 week and 3 week of immersion in SBF

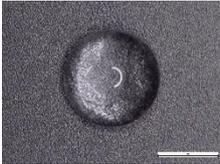
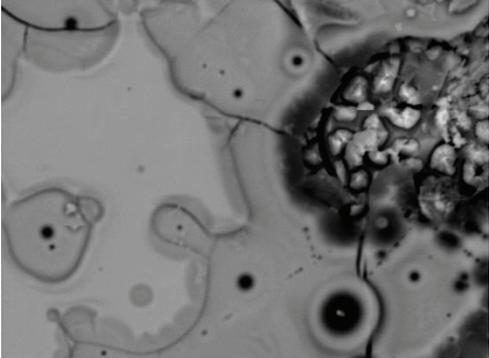
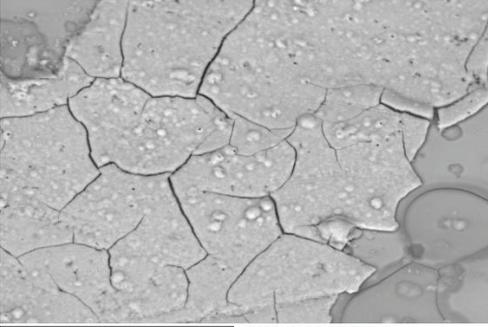
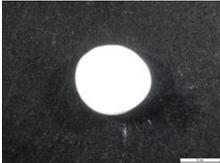
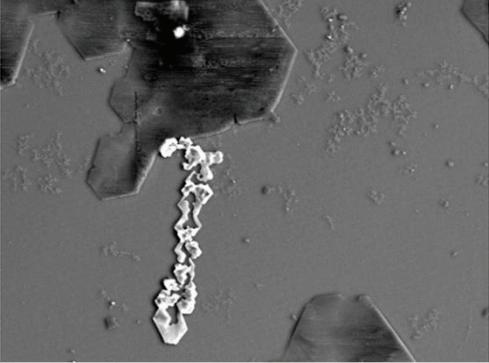
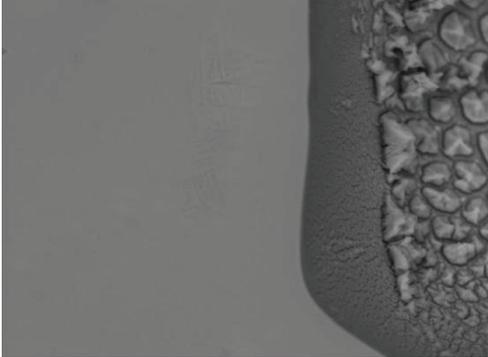
PSBS9	1 week					3 weeks				
										
	EDX (w%)	Ca	P	Si	O	K	Na	Mg	Cl	
	1 week	4.79	0	34.42	51.09	9.05	0.39	0	0.26	
	3 weeks	25.51	12.32	12.61	41.94	3.52	1.34	0.46	2.31	

Table 42 HA formation in the surface of a PSBS16 glass disk after 1 week and 3 week of immersion in SBF

PSBS16	1 week					3 weeks				
										
	EDX (w%)	Ca	P	Si	O	K	Na	Mg	Cl	Al
	1 week	7.1	0.1	30.1	38.1	15.5	0.2	0.1	3.9	4.9
	3 weeks	10.14	10.77	12.75	42.32	4.97	5.17	0.77	5.8	7.33

Tests for HA formation are very controversial. As said before it is known and accepted that the HA layer formed between the hard tissue and the biomaterial facilitate the attachment of osteoblast/odontoblast and enhance the bonding between the two surfaces. The results obtained from previous experiments in scientific literature sometimes lead to false positive or false negative conclusions and for this reason the interpretations should be read carefully.

In this study the immersion of glass disks in SBF is seen as an indication of the behaviour of the PSBS powder in a solution that is very similar to the one found in the human body. Moreover the formation of HA has been interpreted as a positive effect and of special interest for dental applications as confirmed by current toothpaste products: Novamin® and BiominF® [REF]. Finally, a comparison with the pH evolution and the associated mass loss gives a complete insight of the glass behaviour in SBF.

HA is a naturally occurring mineral and the principal component of vertebrate bone and tooth enamel. The ideal atomic ratio for the crystalline apatite $\text{Ca}_{10}(\text{PO}_4)(\text{OH})_2$ is equal to $\text{Ca}/\text{P}=1,67$. Actually the composition of bone mineral is significantly different due to carbonate ions incorporation and is more accurately represented by $\text{Ca}_{8.3}(\text{PO}_4)_{4.3}(\text{HPO}_4, \text{CO}_3)_{1.7}(\text{OH}, \text{CO}_3)_{0.3}$. In some studies has been identified as “hydroxyapatite” a composition that present a Ca/P ratios ranging around 2. This can be identified as non-stoichiometric biological apatite (Ca/P molar ratio $\neq 1.67$) and in some cases provides an excellent connection with living tissue (G. Stanciu 2007).

It is also confirmed by the studies of Kim et al. (H.M. Kim 2000, H.M. Kim 2001) that the HA layer formed with the SBF solution presents some dissimilarity with the bone apatite found in the human body. This could explain the stoichiometric values encountered in the EDX experiments of this work. For example the lower CO_3^- ion concentration and higher Cl^- of the SBF solution in comparison to human plasma could influence the doping in new HA formation.

The SEM images in this work demonstrate how for PSBS8 (after one week of immersion in SBF) the process of HA formation is starting with deposition of a calcium-silicate layer that is rich in calcium but poor in phosphate. This is probably corresponding to step 3 of the HA formation reaction as illustrated in fig.22. In this stage the formation of a SiO_2^- rich gel on the glass surface by ion exchange reactions occurs. The following step is the further dissolution of ions through the SiO_2^- rich gel layer in the glass followed by the reaction between Ca^{2+} provided by the glass and PO_4^{3-} ions coming from the surrounding SBF, finally leading to the growth of the HA on the gel layer.

Comparing SEM images for PSBS8 and the 45S5K control, it is noticed that the HA formation process in PSBS8 is slower. In fact the control already presents the formation of an initial HA structure confirmed by the shape encountered in the SEM images and by the observation of a Ca/P ratio of 2.58 (higher in respect to crystalline apatite, but it becomes 2,08 after the third week). After the third week of immersion the sample PSBS8 showed a well developed HA structure. The surface deposition featured almost spherical shapes, which were reflection images of Ca-P amorphous aggregates. Similarities with the control were found in terms of shape of the layer and in terms of Ca/P ratio, in this case corresponding to 2.12.

PSBS16, the opalescent glass, did not show any formation of HA even after 3 weeks of immersion. A flat surface characteristic of the glass it is encountered during the analysis, except for salt depositions detected with the help of the EDX apparatus. NaCl, KCl and other possible hydrates are present but also Ca and P are detected also if the HA structure is missing. Salts deposition are very probably coming from SBF solution precipitation. Interesting is the presence of aluminium that is confirming to precipitate as a salt as soon as it is released from the glass. It is believe that immersed in poly(acrylic acid) it should cross-links with the carboxil group of the polymer chains. A parallel ICP study (fig. 75) conducted

with the same glass, PSBS16, demonstrated how the aluminium (if present in the initial glass composition; in this case $\text{Al}_2\text{O}_3 = 7,1 \text{ w\%}$) is not found in large amounts the solution. Detecting this element with the EDX in the surface of the glass demonstrates that it is highly insoluble in aqueous solution from $\text{pH}=2$ to 10. Due to its amphoteric nature Al_2O_3 can react with both bases and acid. According to pH of the medium, it precipitates as aluminum hydroxide or alkaline aluminate as it is released into the solution.

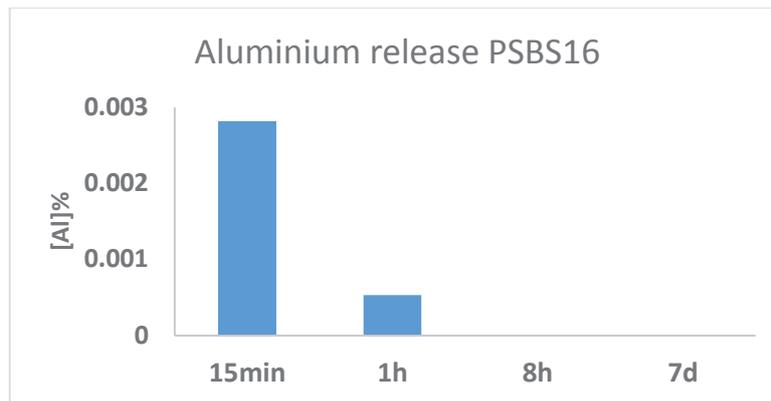


Figure 74 Aluminium release detected with ICP at different time-points. The glass powder is immersed in an acid solution $[\text{HNO}_3] = 0,003\text{M}$.

Amongst the PSBS samples analysed, HA is found to be developed only in the glasses without aluminium. The enhanced durability due to the presence of aluminium is not allowing fast leaching of the glass avoiding subsequent formation of hydroxapatite on the surface. PSBS8, PSBS9 and PSBS3 are in this same order (8>9>3) able to develop a layer of HA on the surface. The surface of the samples exhibit signs of dissolution as well as deposition of bright contrasting particles of regular shapes, corresponding to the HA shape found in the control. In the same sample order (8>9>3) the conversion of the glass to HA is faster. The commercial glass ionomer cements contain both calcium and phosphate, but they do not show any bioactivity. It would be definitely an advantage for this material if it could possess bioactivity because currently, there is a trend for the development of biomaterials that have therapeutic or bioactive functions, in addition to their inherent properties.

The conversion of the borosilicate glasses to HA in a SBF solution is accompanied by a decrease in the mass of the glass, for this reason weight loss measurements could provide a useful parameter for monitoring the kinetics of the conversion reaction.



Figure 75 Weight loss of glass disks immersed in SBF at different time-points: 7, 14 and 21 days.

All the samples analyzed except from PSBS2 and PSBS16 present an high mass loss after the first week of dissolution in SBF. The higher values are encountered for PSBS8 and PSBS3. In the second week the mass losses decrease and the reason could be the initial deposition of HA on the surfaces that provoke a mass gain to the samples. Again, PSBS16 and PSBS2 present different trends, the results show a small mass loss for these samples at the second week that could be explained in a delay of the dissolution to the higher durability of the samples that contains aluminium as in this case. Finally, after 3 week of immersion all the samples gain mass due to the deposition of HA or other salts coming from the SBF solution. The water absorbed should not count in the mass gain experiments because the samples were dried before the measurement.

The behaviour observed is an indication of the ions release properties due to the differences in the composition of the glasses. In fact, samples without aluminium dissolve faster in SBF and present an important HA formation with values comparable with the control glass made in the 45S5K system. Comparing the data with the pH variation it is noted that higher pH change can be associated to a higher mass change. PSBS2 and PSBS16 exhibit a minimal pH variation and at the same time their mass do not change significantly.

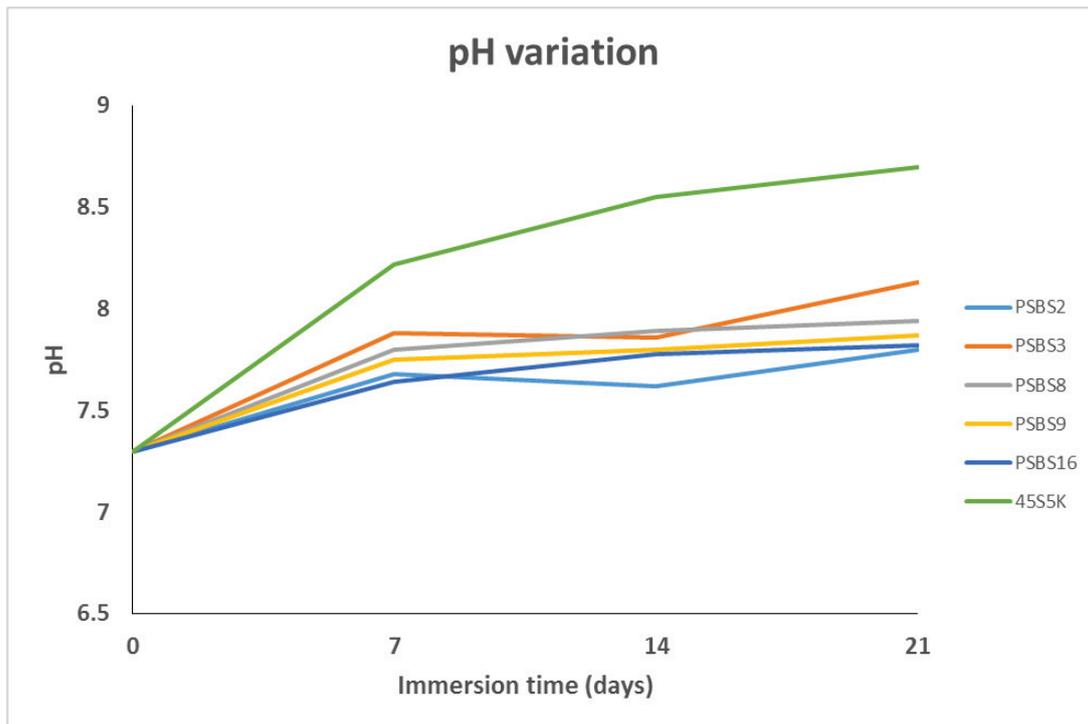


Figure 76 pH variation of SBF solution in contact with glass disks after 7, 14 and 21 days.

The increase in pH results in differences in the acidity and basicity of the ionic species involved in the solution precipitation reactions that occur during the conversion process. Components in the glass such as K_2O , SiO_2 , and B_2O_3 dissolve into the solution to form K^+ , BO_3^{3-} (becoming boric acid), and SiO_4^{4-} ions, whereas Ca^{2+} ions from the glass react with PO_4^{3-} from the solution to precipitate HA. The rate of increase of the solution pH is controlled by the rate of the solution-precipitation reactions during the conversion to HA. It is confirmed that a higher B_2O_3 content gives a glass with lower chemical durability which reacts faster with the solution, so the pH increases faster. Apparently the faster the HA formation, the faster is the pH increase. 45S5K used as control shows better HA formation and a faster pH increase and it is followed by the glasses that are transparent and contain more boron in the composition.

6. CONCLUSIONS AND FUTURE PROSPECTIVES

Biomaterials have revolutionized areas of medical therapies, transforming or extending the lives of many patients. This is proved observing the actual situation, where millions of people are dependent on the polymers of intraocular lenses, pacemakers, coronary stents that help them in improving and extend their life expectancy. Bioceramic and inorganic materials are used to build scaffolds which support cells performing the regeneration process, providing mechanical support and biocompatibility. They are currently used for joint replacement allowing people to walk again and are applied in dentistry helping to get rid of the pain. Indeed, with most type of implantable materials, the performance is controlled first by the quality of the surgery, second by the type of patient and finally by the nature of the biomaterial itself. The ability to achieve better control and performances, especially in newer technologies, it is strongly dependent on a better understanding of the mechanism of dissolution and resistance in biological environment. Properties of this material such as microstructure and surface chemistry play a primary role in obtaining a consistent answer to what may happen once they are implanted in living organisms. The degradation rate is essential in promoting the cell and tissue proliferation and additional properties like antibacterial effect.

In this work has been realized the preliminary study of different types of borosilicate glasses with same elements in the composition but with different weight amount. Between them, new formulations revealed interesting mechanical and biological properties in the perspective of the application in advanced glass ionomer cements.

The result has been achieved thanks to a complete characterization of all the glasses produced, from the point of view of the ions release and from the analysis of the microstructure. The first aspect is a key-point for the comprehension of the glass properties. Knowledge of ionic mobility in glasses is of great importance for a proper understanding of these materials and for their applications. The ions released from the PSBS samples are known for their beneficial effect especially for the biological environment. The second point concerning the microstructure analysis introduces the effect caused by the heat treatment after the glass synthesis for melt-quenching. Although also this last part is well known in scientific literature for its several applications, the innovation of the work presented in this thesis is about combining the two points. The microstructure is modified by the thermal treatment inducing the phase separation.

To asses a compositional area of interest for the study, a series of samples were produced and characterized. Analysis technique like XRD, DSC and BET were used as first approach to the material produced. All of that was made with attentive correlation to the characteristics of the glasses already used in the commercial product. Along this line of investigation the parameters of amorphousity and particle size are studied after the thermal treatment. This was done especially to avoid any crystallization that will generate a less bioactive material. The concept of bioactivity has always been one of the basement of the project so far and finally one of the main objectives until the last test performed on which was measured the HA formation on the surface of the glasses.

The different appearance of the glasses following the thermal treatment and the studies on the porosity resulting after the acid dissolution have begun to arise the first differences inside the samples constituting the subject of the study. Between the opalescent and transparent glasses the chemical and physical properties are changing, this is the reason why the subsequent experiments were focusing on highlighting the structural and compositional reasons of the changing properties, trying to formulate a general rule that could possibly optimize the manufacturing of the final product.

The study of the ions leaching has revealed how the thermal treatment enhance the leaching of the ions in acid environment, confirming the idea that a phase with high affinity for the acid was formed thanks to the phase separation. This founding could be considered an innovative prospective for the glass manufacturing of new bioactive glass systems. Finally a thermal process, carried out under the crystallization temperature, bring to maximization of the ions release.

Understanding the structure was playing a very important part in refining the properties of the PSBS glasses.

After a long history of glass-ionomer cement (GIC) evolution, researchers focused their thoughts to the development of entirely new materials which eventually evolved in new products. The most important requirement for an ideal restorative material include good adhesion to tooth structure without excluding aesthetic properties and avoiding the use of any toxic components. This study offered an overview of the process of projecting and developing a new inorganic powder for GICs. Some of the borosilicates glass samples produced where selected because of their valuable characteristics in terms of mechanical properties in comparison with an already existent commercial product. Especially flexural strength is high and comparable with a commercial GIC during the first period of the hardening reaction. Vicker's microhardness is also measured with values closed to the FUJI IX® GIC. These founding were attributed to the boron leached from the glass present in the weaker phase. It should be the element releases causing the fast cross-linking between the polyacid chains provoking the hardening of the cement at the place of aluminium. No toxicity should be found in the material because of the absence of alumina in the composition. Finally the hydroxyapatite growth during the biocompatibility test demonstrates the potentiality for the formation of a strong bond with the tooth tissue.

The problem encountered so far is related to the aesthetic features. Once mixed with the poly(acid) of the FUJI IX® the cement produced assumes a greyish colouring. At this point have to be considered that further studies should be done in terms of the liquid part of the cement. The FUJI IX® liquid contains different elements in its mixture like distilled water, other polycarboxylic acids, tartaric acid and all of them are present in unknown concentrations. The investigation done so far is approaching the subject from the fabrication of new glass powders which is one of the two components of the glass ionomer cement. After all the work done seems reductive to say that the research is half way to the final

product because the results obtained fulfil the initial purpose of the study. In fact, a new powder has been characterized presenting bioactivity (not found until very recent application in GIC) and better mechanical properties than the ones feasible with the 45S5 bioactive glass.

The phrase “more research is needed” is here not an empty cliché, but a motivation to continue the study investigating different aspects of the material to have a complete understating on controlling and regulating the final properties. The addition of fluoride as CaF_2 could be a potential route for future investigation because the cement with better mechanical and bioactivity properties can gain the caries-inhibitory effect due to the long-term fluoride release. Stronger adhesion and fluoride release could really bring to an optimal restoration that can be used also for high load-bearing situations. In this case the colour will be not so determining because the restoration will be placed in posterior teeth. In any case caution may be adopted and a parallel study on the poly(acid) use for the mix should be considered. The need for new restorative materials is day by day more demanding and glass ionomer cements may be very useful as they provide minimal intervention and a lot of margins in their development could be explored.

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8. APPENDIX

APPENDIX 1

Composition expressed in weight %, molar % and Seger formula of each elaborated glasses.

PSBS2/6	w%	mol%	Seger formula
SiO ₂	45	50,1	2,18
K ₂ O	15	10,7	0,46
B ₂ O ₃	26,2	24,9	1,096
Al ₂ O ₃	3,4	2,2	0,1
CaO	10,4	12,1	0,54

PSBS3	w%	mol%	Seger formula
SiO ₂	45	49,3	1,79
K ₂ O	15	10,5	0,38
B ₂ O ₃	25	23,1	0,84
Al ₂ O ₃	0	0	0
CaO	15	17,1	0,62

PSBS4	w%	mol%	Seger formula
SiO ₂	45	51,4	2,42
K ₂ O	15	10,9	0,52
B ₂ O ₃	20	19,7	0,93
Al ₂ O ₃	11,5	7,7	0,37
CaO	8,5	10,3	0,48

PSBS5	w%	mol%	Seger formula
SiO ₂	45	49,6	1,76
K ₂ O	15	10,5	0,37
B ₂ O ₃	20	18,99	0,67
Al ₂ O ₃	5	3,24	0,12
CaO	15	17,67	0,63

PSBS7/10	w%	mol%	Sejer formula
SiO ₂	45	51,13	2,82
K ₂ O	15	10,85	0,6
B ₂ O ₃	25,9	25,26	1,39
Al ₂ O ₃	8,1	5,43	0,3
CaO	6	7,33	0,4

PSBS8	w%	mol%	Sejer formula
SiO ₂	45	49,34	2,8
K ₂ O	15	10,47	0,6
B ₂ O ₃	34	33,14	1,88
Al ₂ O ₃	0	0	0
CaO	6	7,05	0,4

PSBS9	w%	mol%	Sejer formula
SiO ₂	45	49,41	2,26
K ₂ O	15	10,49	0,48
B ₂ O ₃	30,3	28,69	1,3
Al ₂ O ₃	0	0	0
CaO	9,7	11,41	0,52

PSBS11	w%	mol%	Sejer formula
SiO ₂	45	51,39	4,72
K ₂ O	15	10,93	1
B ₂ O ₃	34,4	33,91	3,11
Al ₂ O ₃	5,6	3,77	0,35
CaO	0	0	0

PSBS12	w%	mol%	Sejer formula
SiO ₂	45	52,11	4,69
K ₂ O	15	11,08	1
B ₂ O ₃	30	29,99	2,7
Al ₂ O ₃	10	6,82	0,6
CaO	0	0	0

PSBS13	w%	mol%	Sejer formula
SiO ₂	45	51,62	2,82
K ₂ O	15	10,97	0,6
B ₂ O ₃	22,5	22,27	1,22
Al ₂ O ₃	11,5	7,77	0,43
CaO	6	7,37	0,4

PSBS14	w%	mol%	Sejer formula
SiO ₂	45	49,16	1,76
K ₂ O	15	10,45	0,37
B ₂ O ₃	22,5	21,22	0,757
Al ₂ O ₃	2,5	1,61	0,06
CaO	15	17,56	0,63

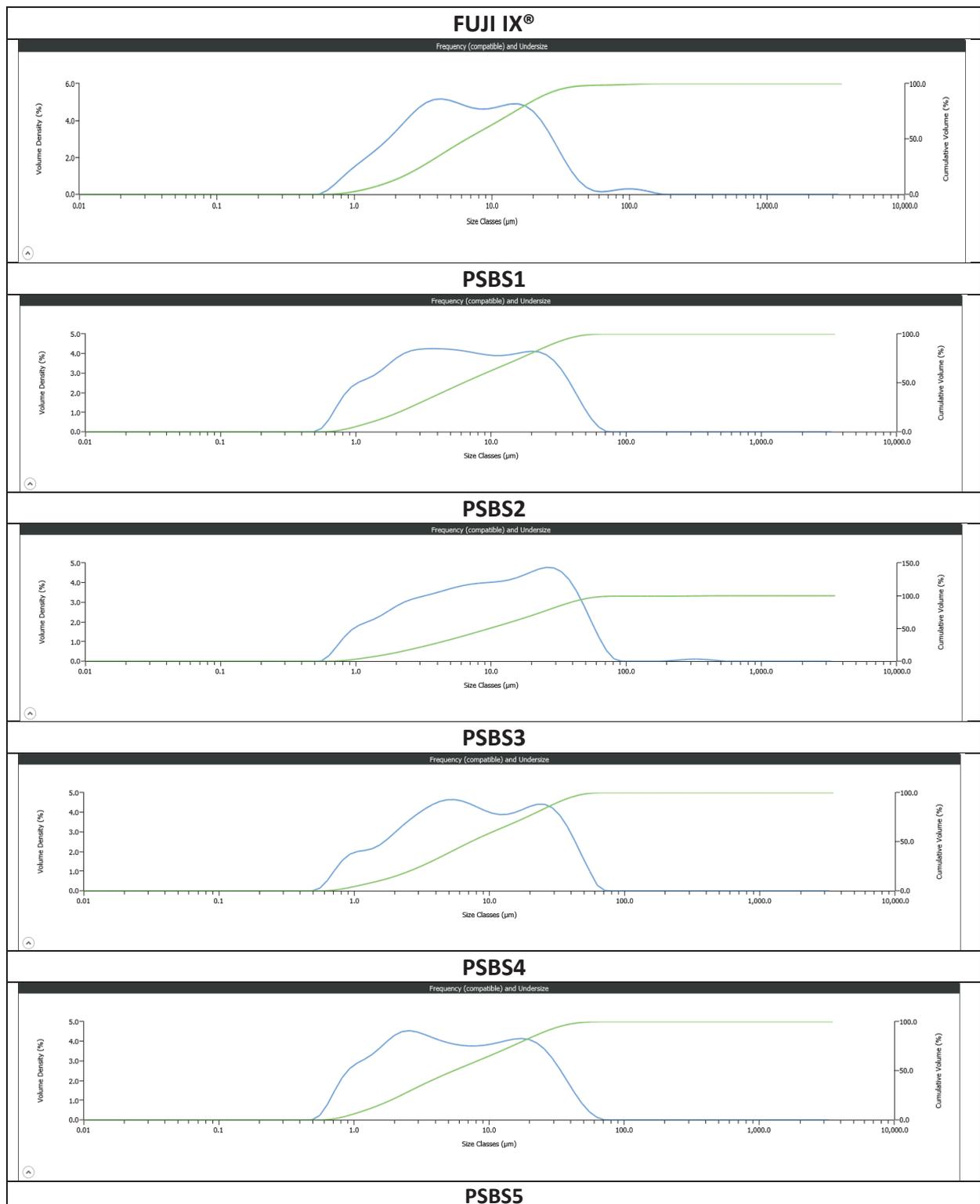
PSBS15	w%	mol%	Sejer formula
SiO ₂	45	50,53	2,79
K ₂ O	15	10,75	0,59
B ₂ O ₃	29,1	28,2	1,56
Al ₂ O ₃	4,8	3,18	0,18
CaO	6,1	7,34	0,41

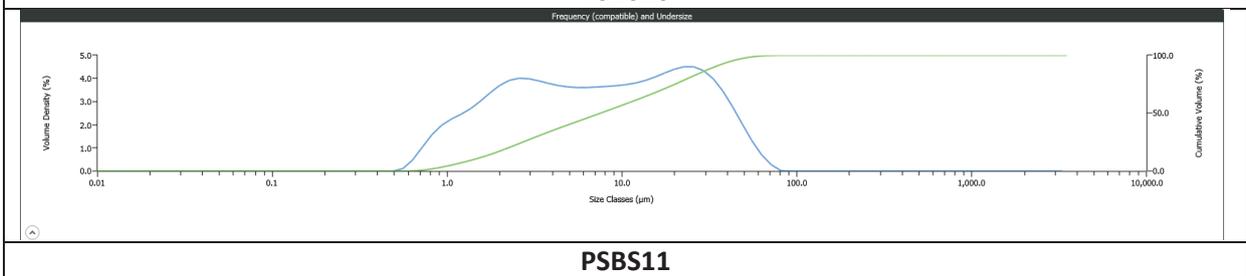
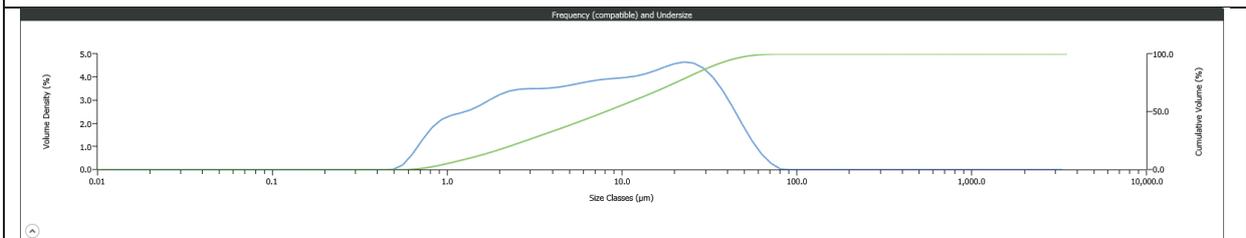
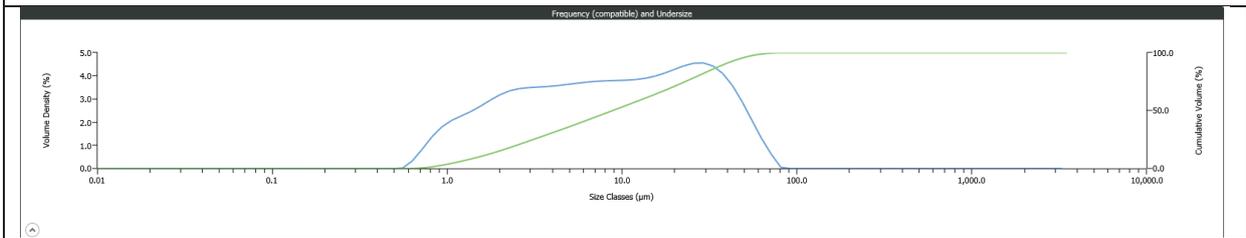
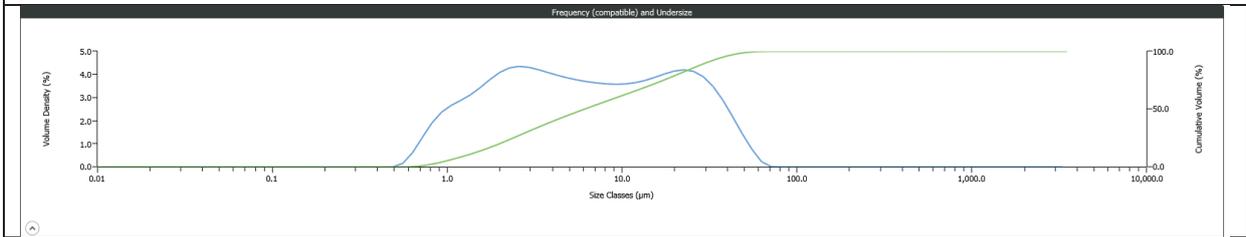
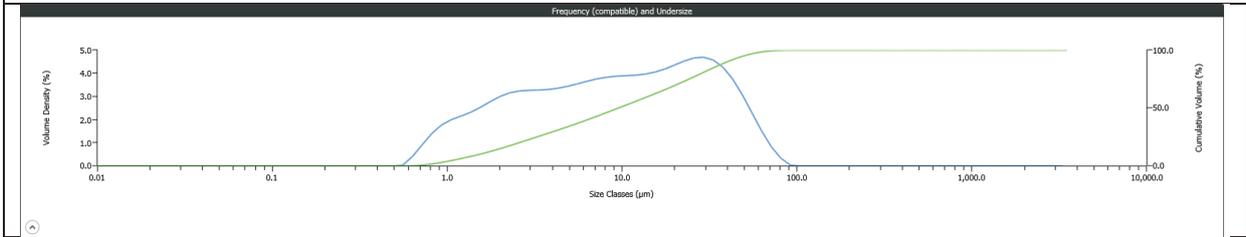
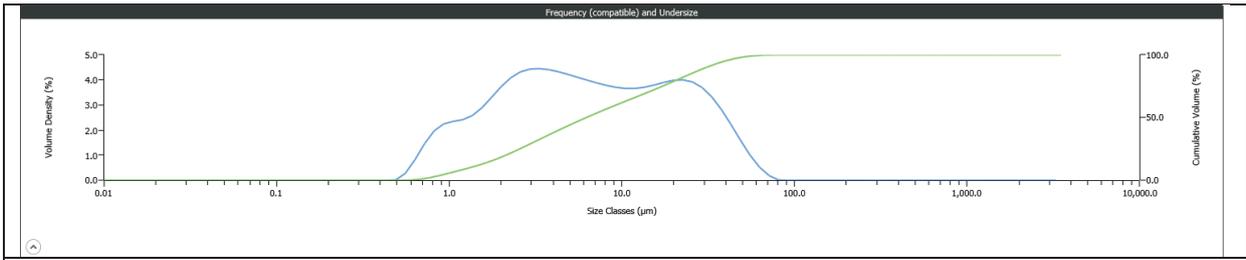
PSBS16	w%	mol%	Sejer formula
SiO ₂	45	50,25	2,06
K ₂ O	15	10,69	0,43
B ₂ O ₃	21,4	20,63	0,83
Al ₂ O ₃	7,1	4,67	0,19
CaO	11,5	13,76	0,57

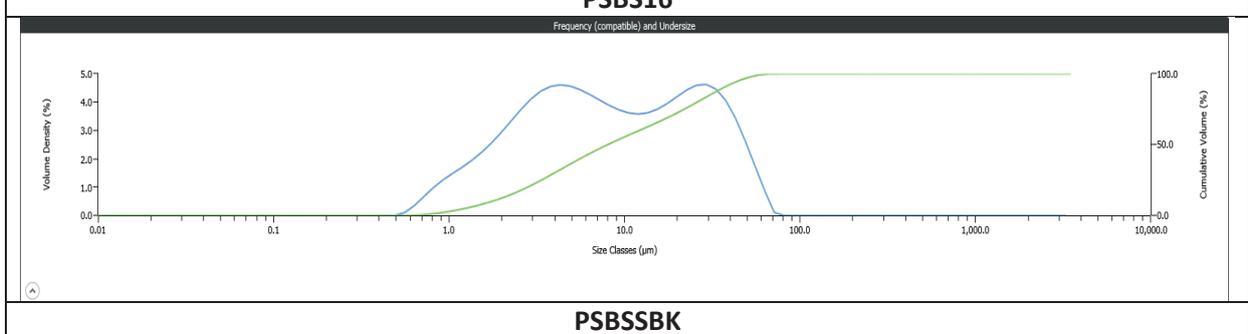
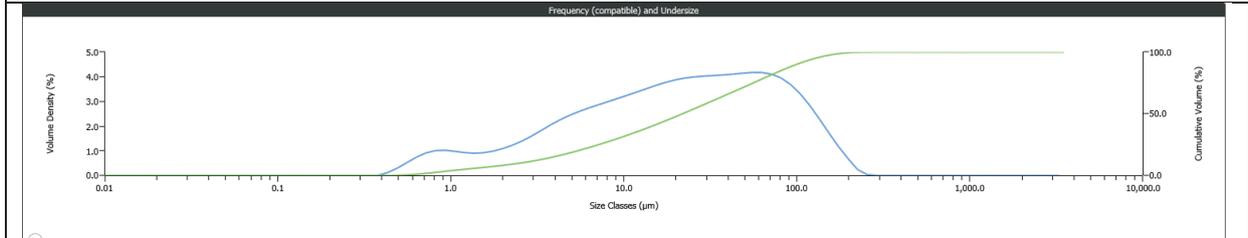
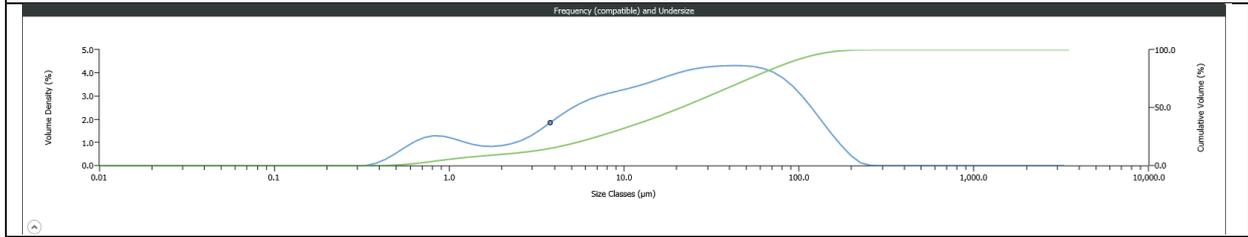
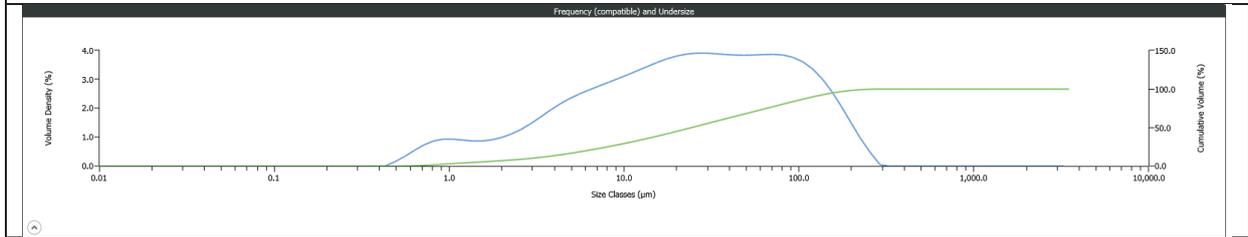
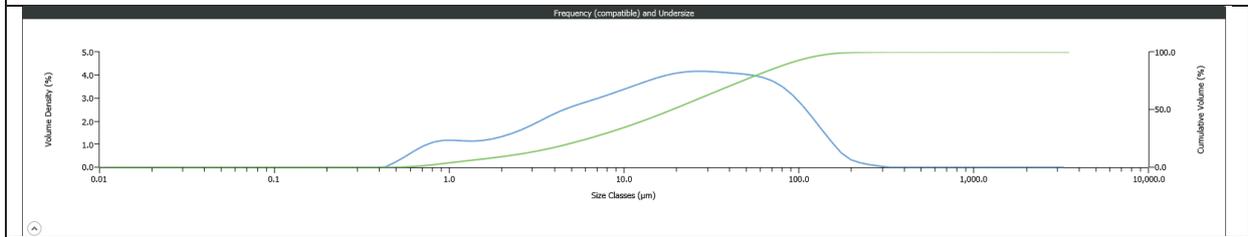
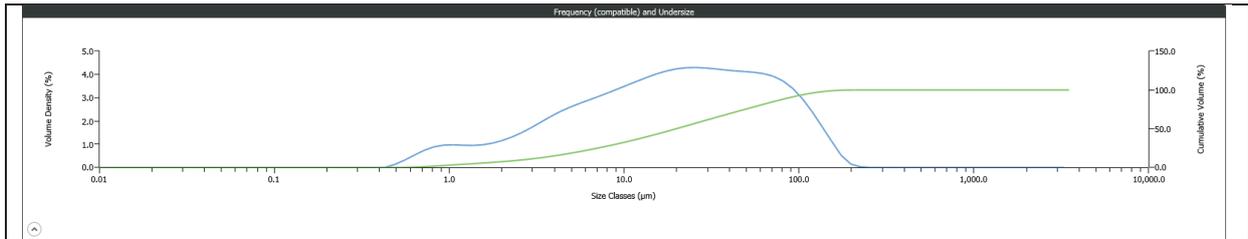
PSBSSBK	w%	mol%	Sejer formula
SiO ₂	45	50,5	4,72
K ₂ O	15	10,74	1
B ₂ O ₃	40	38,76	3,63
Al ₂ O ₃	0	0	0
CaO	0	0	0

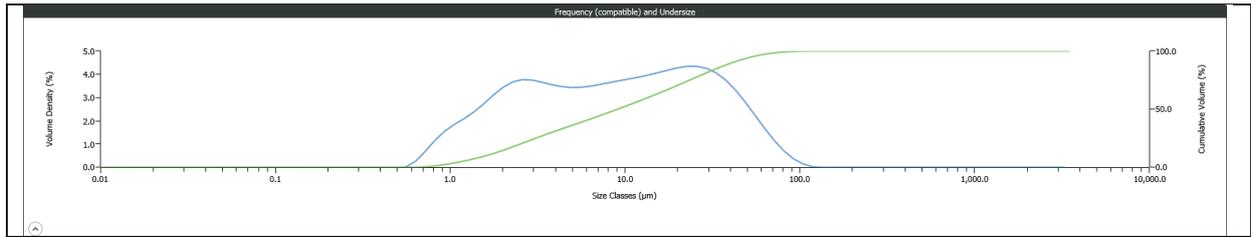
APPENDIX 2

Particle size distribution after milling the glass samples.



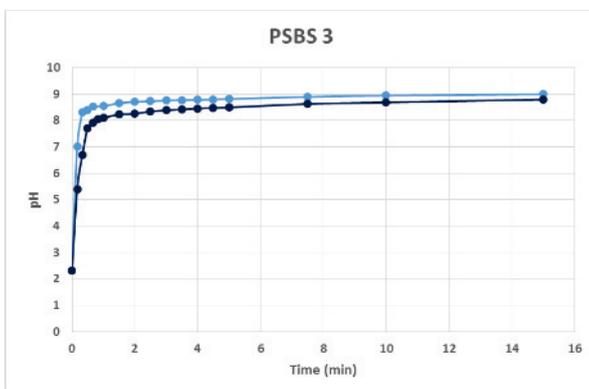
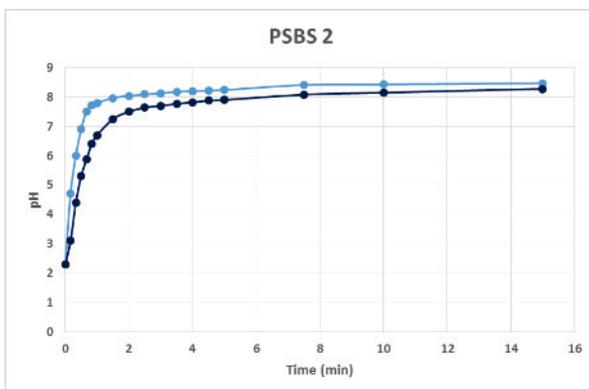
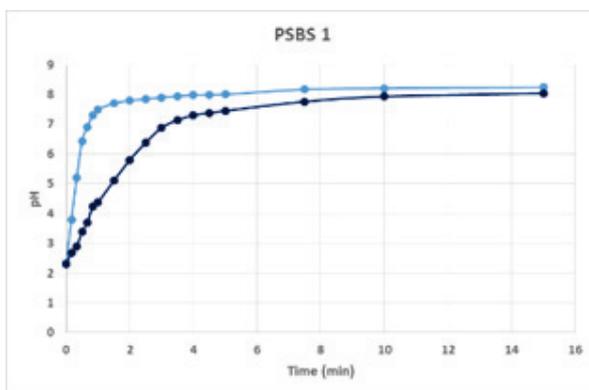


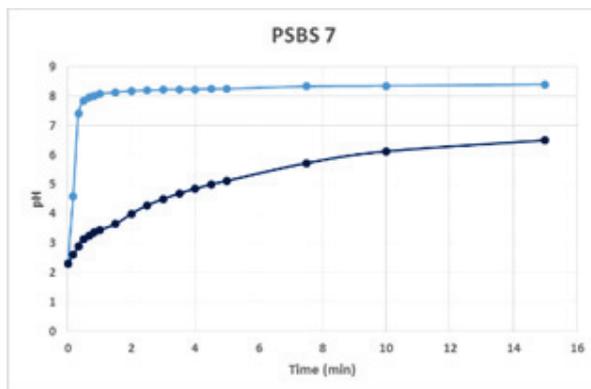
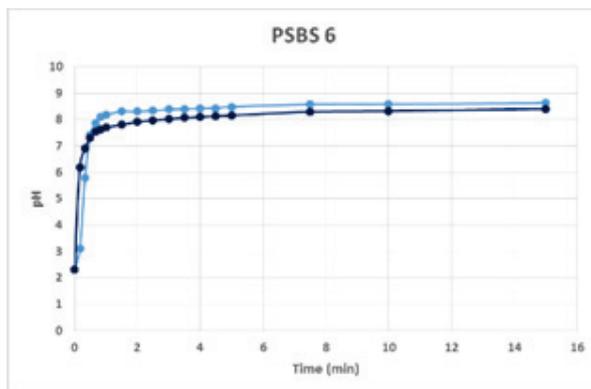
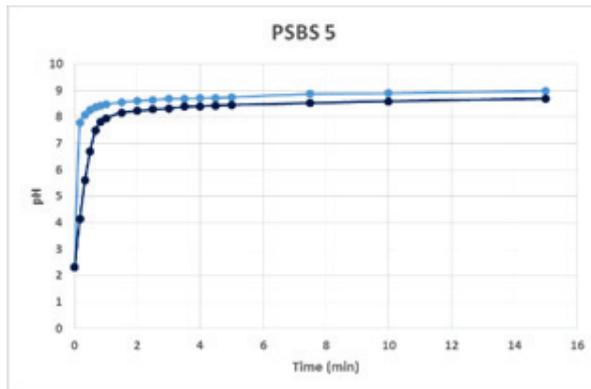
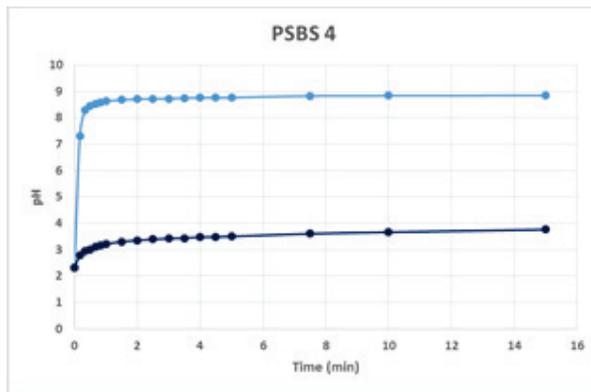


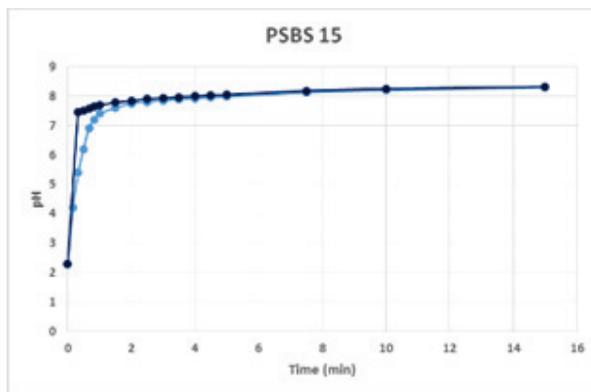
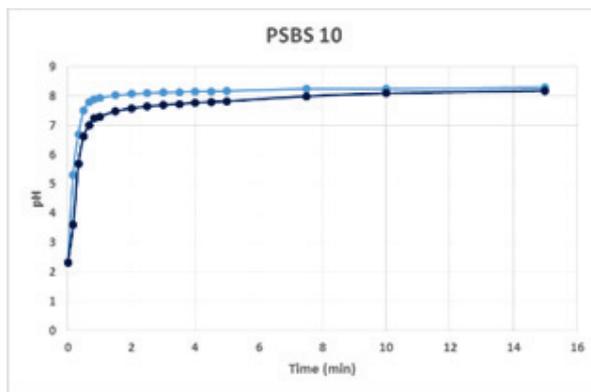
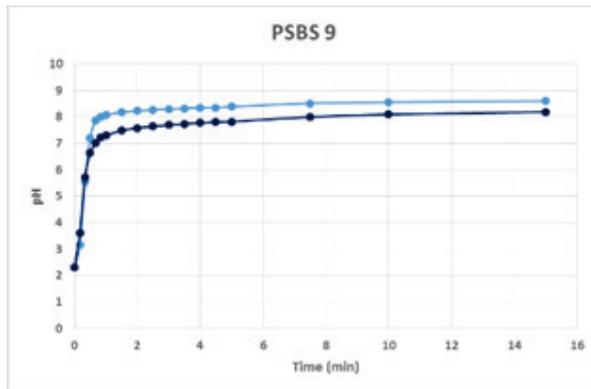
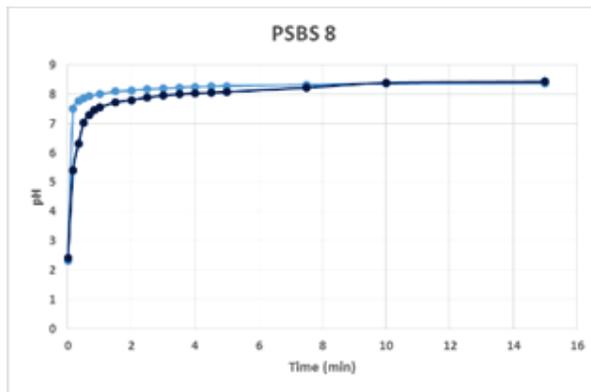


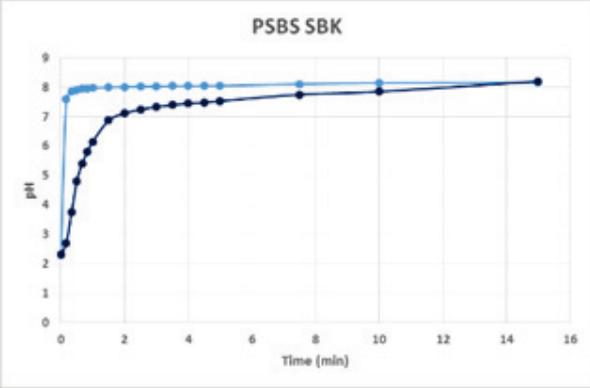
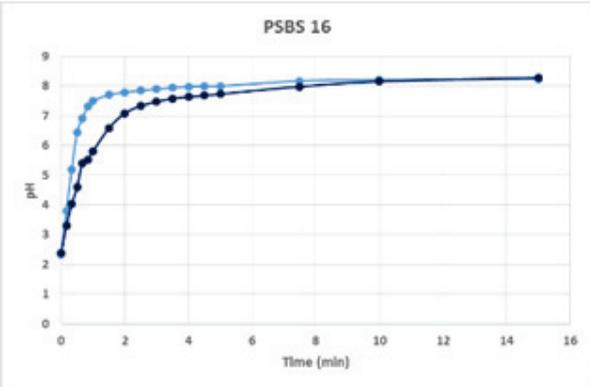
APPENDIX 3

pH evolution curves of the PSBS samples before (dark blue) and after the heat-treatment (light blue).



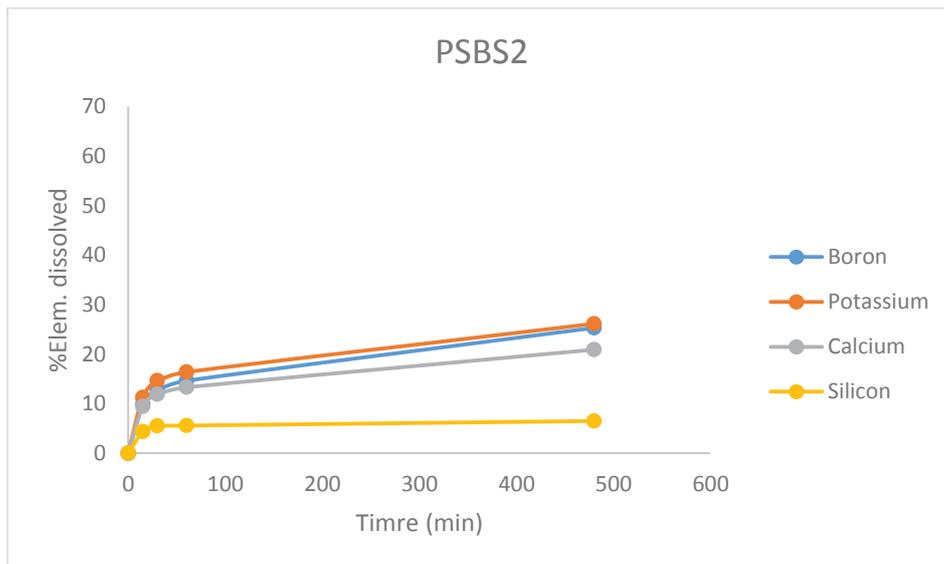
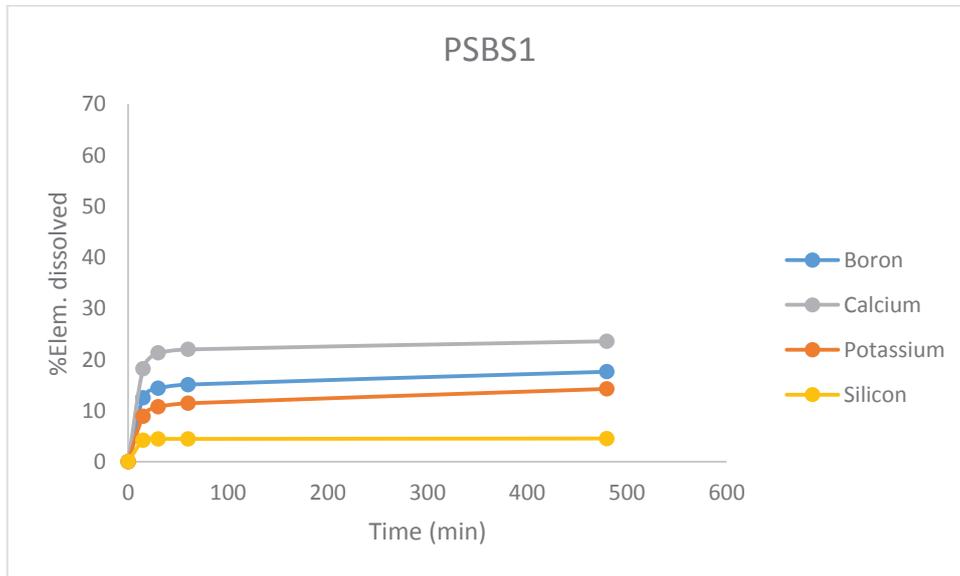


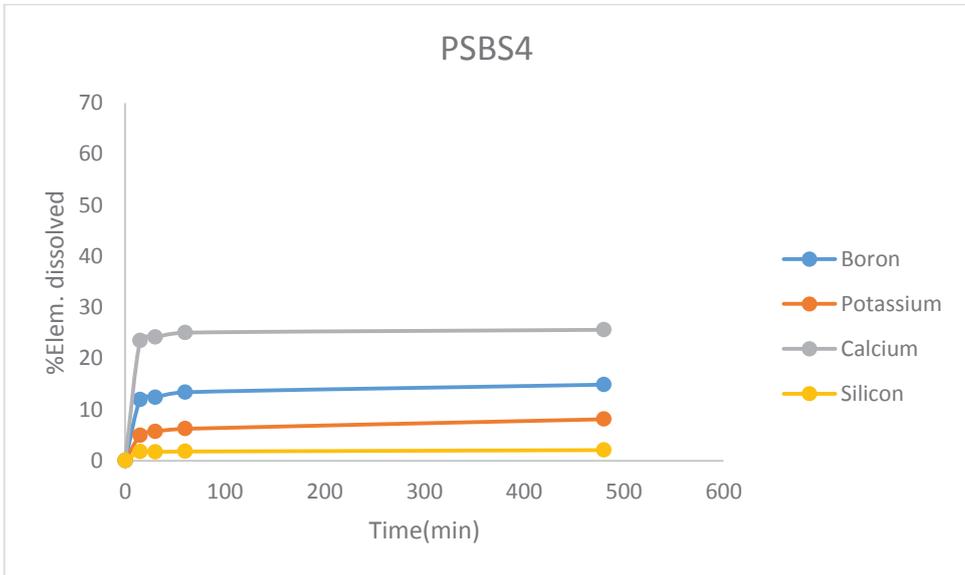
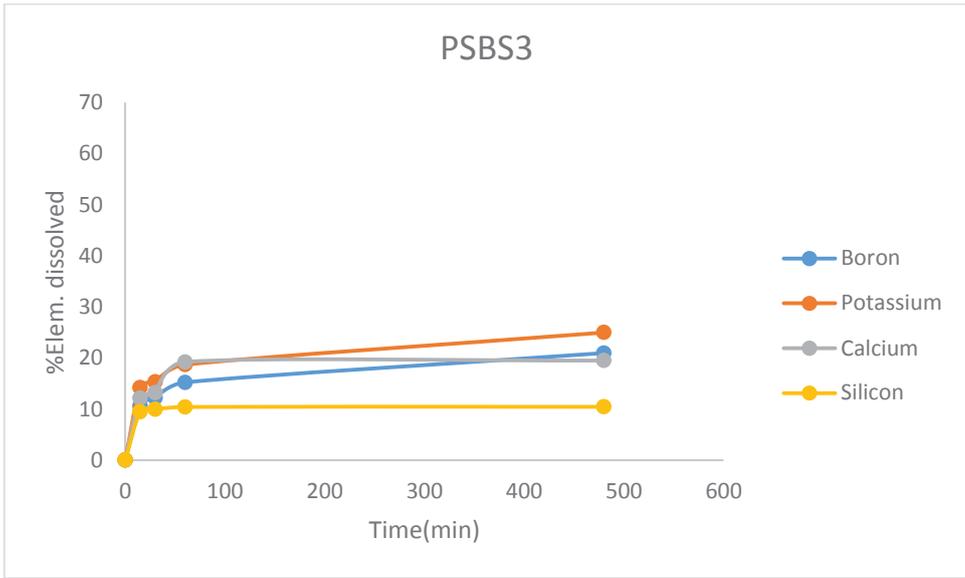


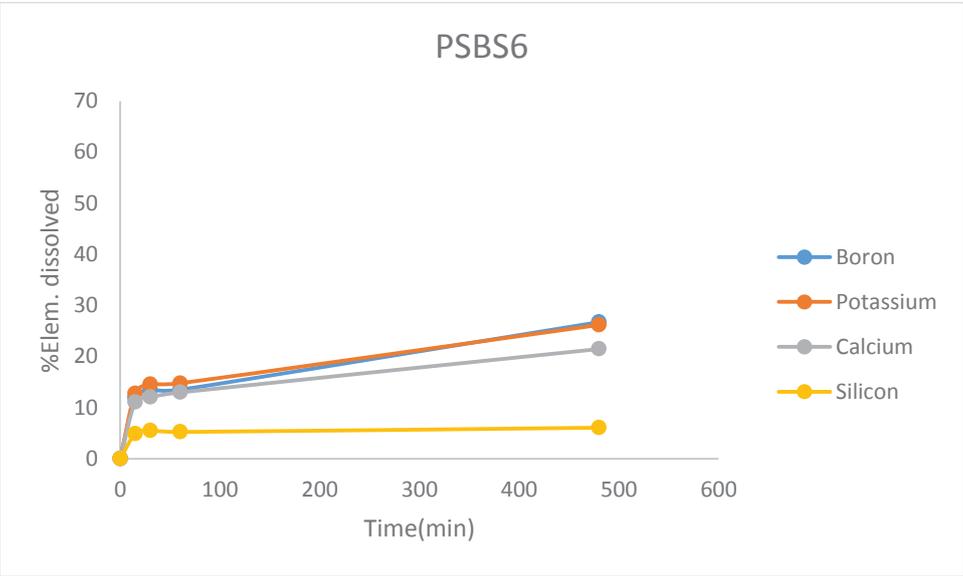
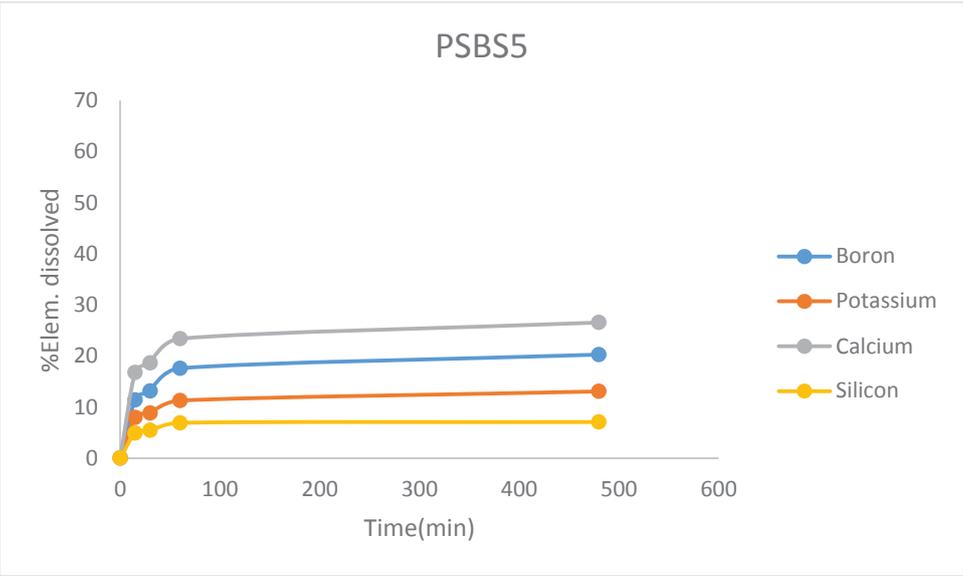


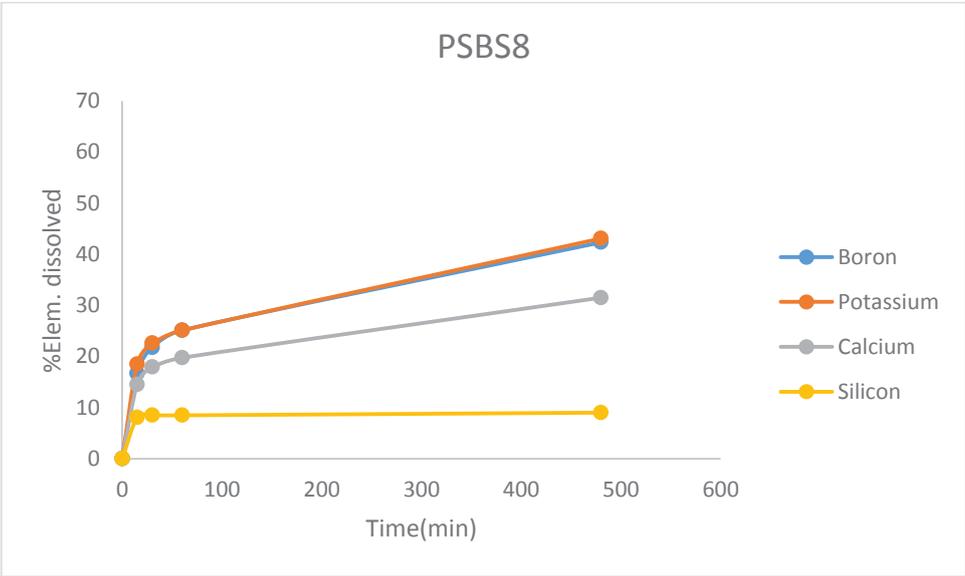
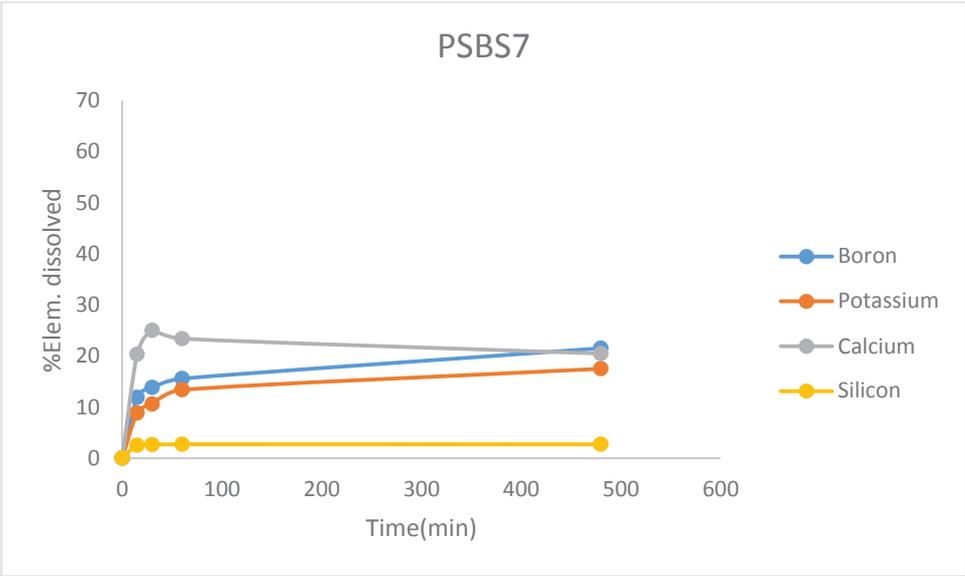
APPENDIX 4

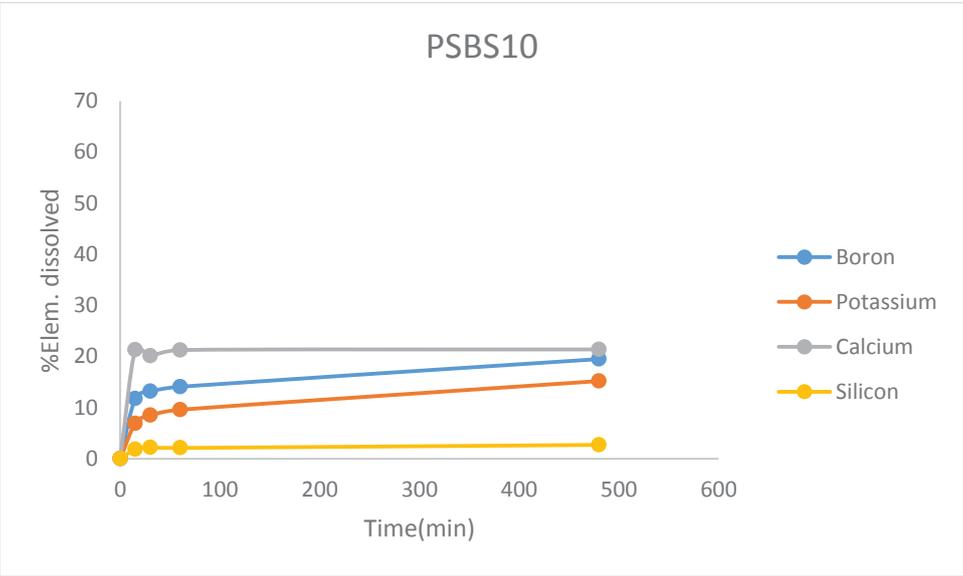
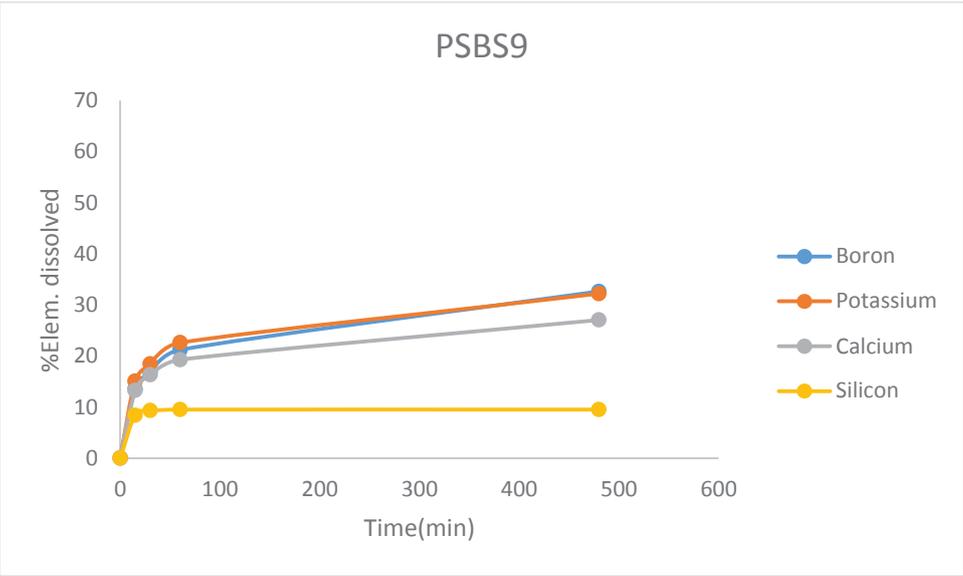
Fraction of dissolved elements ([element]%) measured with ICP after the acid immersion of the glasses and values obtained for each time-points.

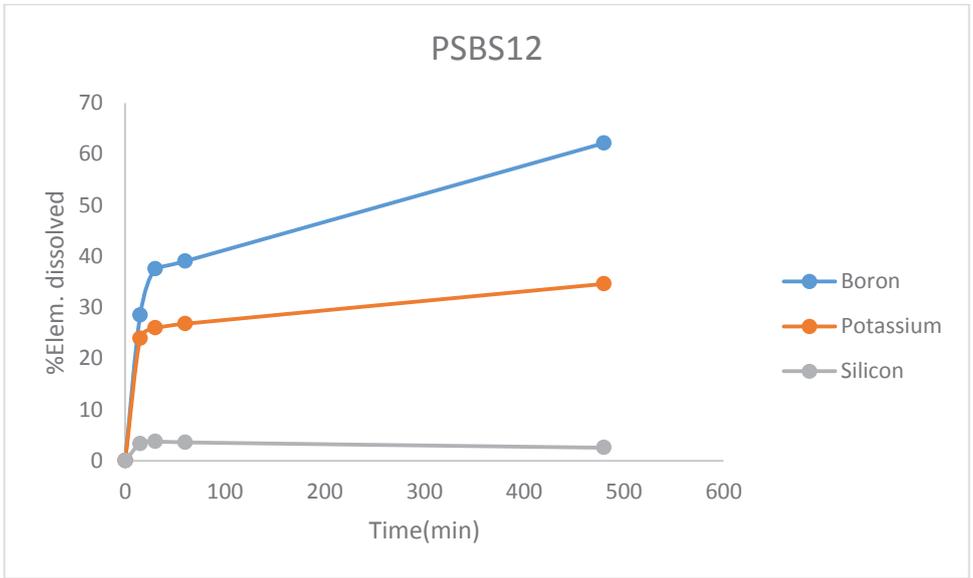
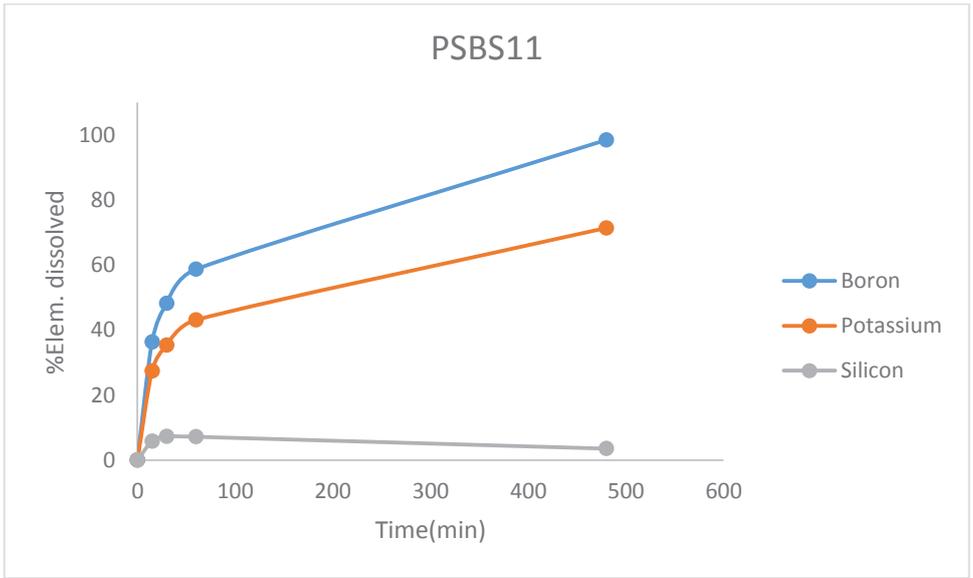


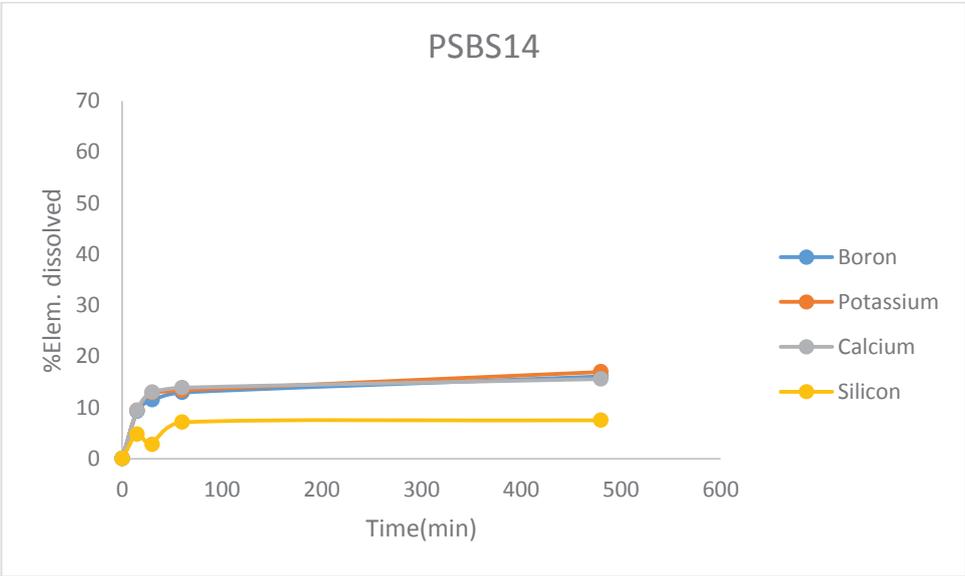
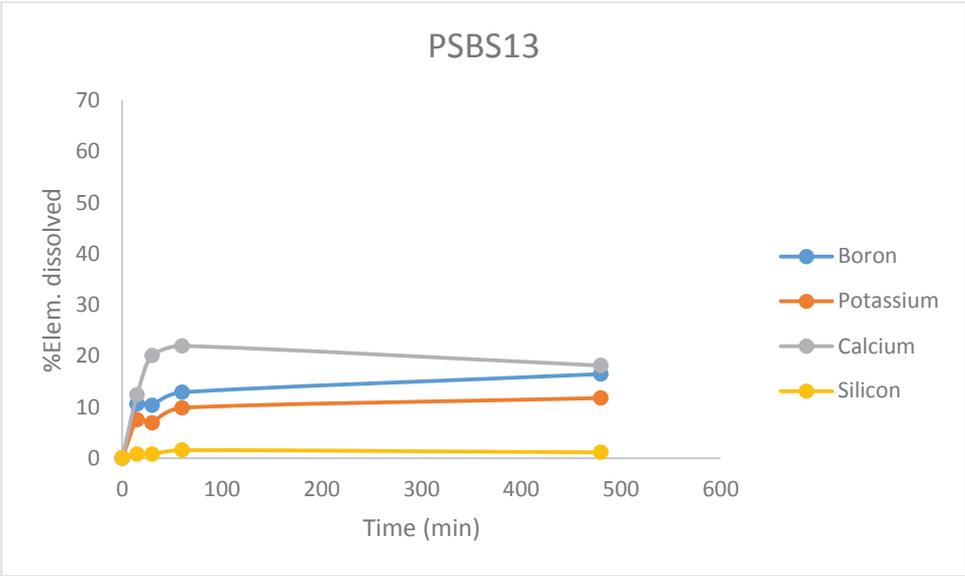


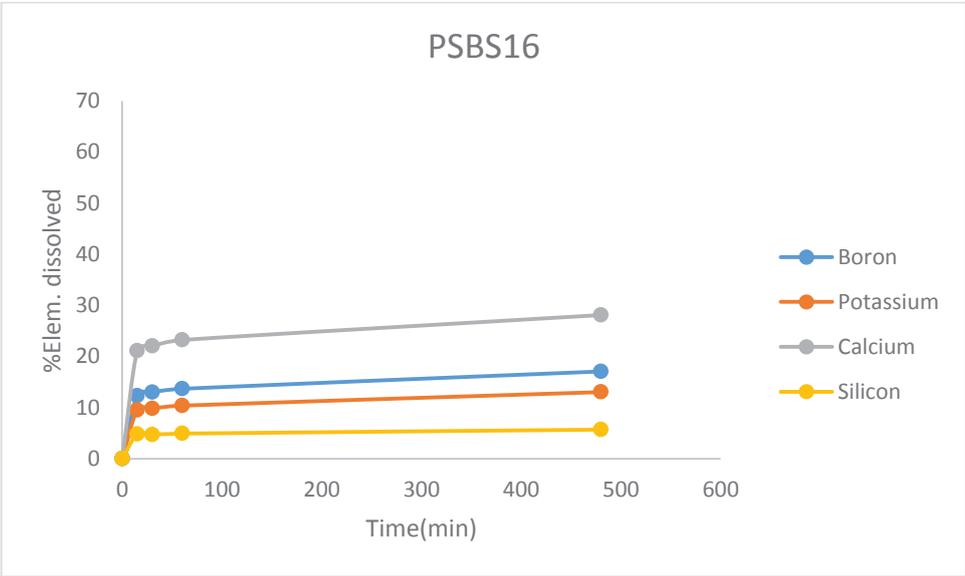
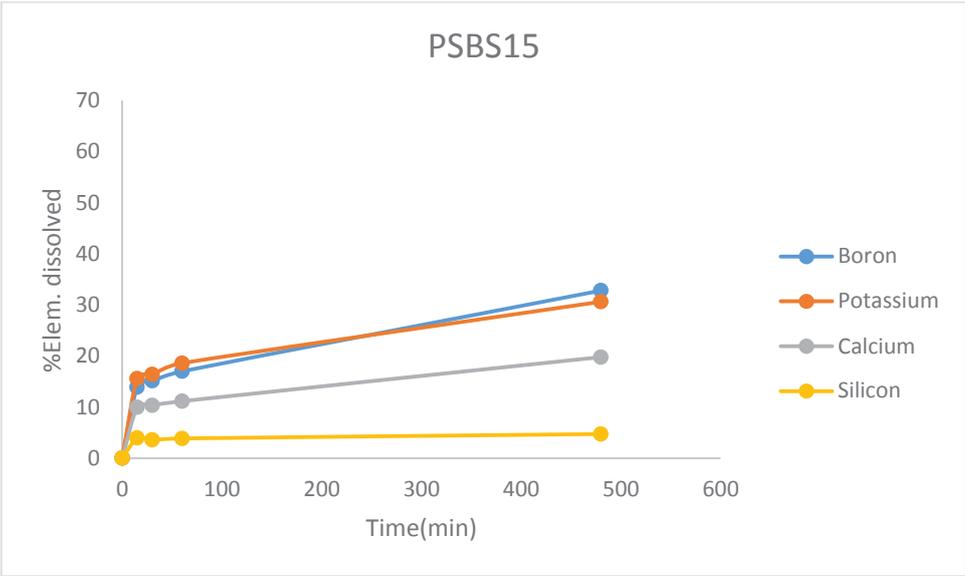


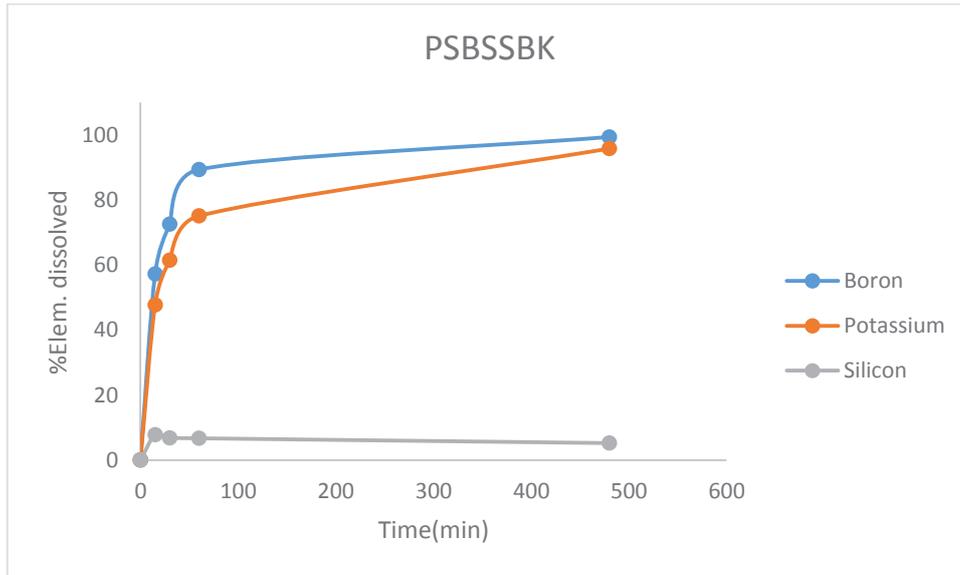












PSBS1	Boron	Potassium	Calcium	Silicon
15min	12,54	8,90	18,22	4,20
30min	14,41	10,76	21,31	4,48
1h	15,11	11,44	21,98	4,48
8h	17,61	14,25	23,57	4,55

PSBS2	Boron	Potassium	Calcium	Silicon
15min	9,83	11,24	9,45	4,34
30min	12,79	14,66	11,92	5,53
1h	14,65	16,41	13,32	5,60
8h	25,33	26,16	20,93	6,51

PSBS3	Boron	Potassium	Calcium	Silicon
15min	10,65	14,19	12,17	9,46
30min	12,23	15,34	13,20	9,96
1h	15,23	18,69	19,23	10,42
8h	20,96	24,98	19,50	10,44

PSBS4	Boron	Potassium	Calcium	Silicon
15min	11,94	4,95	23,50	1,79
30min	12,41	5,74	24,18	1,75
1h	13,42	6,26	25,08	1,81
8h	14,90	8,13	25,64	2,09

PSBS5	Boron	Potassium	Calcium	Silicon
15min	11,42	7,97	16,77	4,90
30min	13,15	8,86	18,63	5,43
1h	17,60	11,28	23,40	6,92
8h	20,28	13,07	26,55	7,08

PSBS6	Boron	Potassium	Calcium	Silicon
15min	11,93	12,76	11,08	4,91
30min	13,44	14,59	12,09	5,51
1h	13,51	14,79	12,99	5,25
8h	26,74	26,19	21,48	6,08

PSBS7	Boron	Potassium	Calcium	Silicon
15min	11,88	8,84	20,30	2,52
30min	13,86	10,58	24,99	2,64
1h	15,58	13,38	23,40	2,72
8h	21,52	17,51	20,47	2,71

PSBS8	Boron	Potassium	Calcium	Silicon
15min	16,70	18,49	14,53	8,11
30min	21,79	22,61	17,96	8,50
1h	25,16	25,12	19,77	8,50
8h	42,35	43,11	31,53	9,03

PSBS9	Boron	Potassium	Calcium	Silicon
15min	13,52	15,05	13,26	8,43
30min	16,91	18,44	16,33	9,31
1h	21,21	22,61	19,29	9,51
8h	32,64	32,20	27,05	9,52

PSBS10	Boron	Potassium	Calcium	Silicon
15min	11,77	6,90	21,33	1,87
30min	13,20	8,54	20,11	2,19
1h	14,10	9,61	21,29	2,12
8h	19,51	15,22	21,40	2,72

PSBS11	Boron	Potassium	Calcium	Silicon
15min	36,39	27,44	/	5,82
30min	48,22	35,37	/	7,34
1h	58,73	43,15	/	7,19
8h	98,50	71,44	/	3,55

PSBS12	Boron	Potassium	Calcium	Silicon
15min	28,48	23,94	/	3,36
30min	37,54	25,99	/	3,81
1h	39,06	26,82	/	3,60
8h	62,16	34,62	/	2,56

PSBS13	Boron	Potassium	Calcium	Silicon
15min	10,56	7,51	12,41	0,81
30min	10,35	6,90	20,07	0,78
1h	12,89	9,86	21,97	1,57
8h	16,46	11,77	18,13	1,13

PSBS14	Boron	Potassium	Calcium	Silicon
15min	9,25	9,43	9,41	4,77
30min	11,49	12,89	13,04	2,79
1h	12,97	13,41	13,92	7,12
8h	16,11	16,99	15,61	7,53

PSBS15	Boron	Potassium	Calcium	Silicon
15min	13,86	15,58	9,93	3,95
30min	15,12	16,38	10,36	3,59
1h	17,00	18,61	11,17	3,86
8h	32,80	30,62	19,78	4,72

PSBS16	Boron	Potassium	Calcium	Silicon
15min	12,34	9,53	21,11	4,86
30min	13,04	9,86	22,07	4,72
1h	13,70	10,41	23,25	4,94
8h	17,09	13,05	28,13	5,71

PSBS SBK	Boron	Potassium	Calcium	Silicon
15min	57,23	47,69	/	7,79
30min	72,57	61,50	/	6,82
1h	89,40	75,14	/	6,74
8h	99,41	95,82	/	5,25

9. GENERAL OVERVIEW OF THE THESIS (IN FRENCH)

THESE de DOCTORAT DE L'UNIVERSITE DE LYON

Verres borosilicatés à séparation de phase pour applications dentaires. Formulation de la composition en relation avec la dissolution des ions en milieu acide et la microstructure.

Introduction

BIODENSOL est un projet de recherche et mobilité, financé par la Commission Européen et géré par l'Université de Lyon. BIODENSOL se concentre sur le développement de nouveaux matériaux bioactifs pour la restauration et la régénération dentaires. Ce projet propose trois études de doctorat pour aborder les problèmes de caries et d'érosion de l'émail par les aliments acides. Les recherches ont évolué indépendamment en trois voies parallèles, examinant trois matériaux différents qui pourraient apporter des solutions potentielles. Ces matériaux sont i)des verres phosphatés comme filler dans les échafaudages dentaires, ii)des verres bioactifs mésoporeux (MBG) comme filler dans les adhésifs dentaires et enfin iii)l'objet de cette thèse qui est les poudres de verre borosilicaté a séparation de phases pour les GICs. Le projet Biodensol est conçu pour aider à établir des relations entre la recherche académique et industrielle et pour engendrer des innovations médicales. Pour cette raison, les études ont été réalisées entre ces deux endroits : Université de Lyon et Lucideon à Stoke-On-Trent (Royaume-Uni), avec 6 mois dans chaque site. L'avantage était de travailler sur le projet de thèse dans deux environnements différents: l'université et l'industriel. Il était également pertinent pour l'expérience d'être impliqué dans des activités externes liées à l'industrie (travailler sur des projets secondaires avec des matériaux céramiques dans ce cas) et être familier avec les emplois commerciaux.

State of the art

L'état de l'art commence par une introduction générale sur les verres en parlant de leur structure et de leurs propriétés et apportera le lecteur plus proche du sujet principal de la thèse en abordant d'autres sujets qui décrivent comment l'une des propriétés vitreuses est spécialement étudiée pour améliorer la propriété d'un matériau de restauration dentaire. Les sujets racontés sont de plus en plus restreints pour décrire en détail quel matériau de restauration dentaire a été choisi pour l'étude et comment la formulation de nouvelles compositions de verre, en l'occurrence des verres bioactifs, pourrait être si importante pour les améliorations futures du matériau final. Enfin, il sera présenté l'effet de la libération d'ions dans différents systèmes de verre et en relation avec la propriété du verre dans laquelle la thèse est mise au point, la séparation de phase amorphe. Le système de verre choisi était le borosilicate et le matériau de restauration dentaire est le ciment verre ionomère. Les verres sont des matériaux importants qui sont essentiels à notre mode de vie, nous les utilisons pour créer des outils et parce qu'ils sont transparents forts, très utiles pour

les conteneurs et les fenêtres. Les verres ont été découverts il y a environ 4000 ans, probablement sous la forme d'un mélange de cendres de sable et d'os fondus dans un feu et refroidis rapidement. Dans le XIXème siècle le développement des techniques, fournissent une évolution rapide des applications des verres qui ont été introduits dans d'autres domaines: verres de fenêtre pour le rayonnement et / ou de résistance à la chaleur, des verres d'éclairage pour ampoules et des fibres optiques. Cette amélioration est due à la recherche qui améliore la compréhension de nombreuses propriétés. Les verres sont des solides non cristallins avec une composition variable définie par un mélange de composants. La structure est composée de formateurs de réseau, modificateurs et intermédiaires (figure 1). Les premières forment le réseau de verre interconnecté, les secondes sont présentées comme des ions qui modifient le réseau vitreux compensé par l'oxygène non-pontant (NBO) dans des verres d'oxyde et enfin les modificateurs peuvent agir comme formateurs de réseau ou modificateurs selon la composition du verre.

1 H Hydrogène 1,00794																	2 He Hélium 4,003
3 Li Lithium 6,941	4 Be Béryllium 9,0121832											5 B Bore 10,811	6 C Carbone 12,0107	7 N Azote 14,00674	8 O Oxygène 15,9994	9 F Fluore 18,9984032	10 Ne Neon 20,1797
11 Na Sodium 22,989770	12 Mg Magnésium 24,3050											13 Al Aluminium 26,981538	14 Si Silicium 28,0855	15 P Phosphore 30,973761	16 S Sulfure 32,066	17 Cl Chlore 35,4527	18 Ar Argon 39,948
19 K Potassium 39,0983	20 Ca Calcium 40,078	21 Sc Scandium 44,955910	22 Ti Titane 47,867	23 V Vanadium 50,9415	24 Cr Chromium 51,9961	25 Mn Manganèse 54,938049	26 Fe Fer 55,845	27 Co Cobalt 58,933200	28 Ni Nickel 58,6934	29 Cu Cuivre 63,546	30 Zn Zinc 65,39	31 Ga Gallium 69,723	32 Ge Germanium 72,61	33 As Arsenic 74,92160	34 Se Sélénium 78,96	35 Br Bromine 79,904	36 Kr Krypton 83,80
37 Rb Rubidium 85,4678	38 Sr Strontium 87,62	39 Y Yttrium 88,90585	40 Zr Zirconium 91,224	41 Nb Niobium 92,90638	42 Mo Molybdène 95,94	43 Tc Technetium (98)	44 Ru Ruthénium 101,07	45 Rh Rhodium 102,90550	46 Pd Palladium 106,42	47 Ag Argent 107,8682	48 Cd Cadmium 112,411	49 In Indium 114,818	50 Sn Étain 118,710	51 Sb Antimoine 121,760	52 Te Tellure 127,60	53 I Iode 126,90447	54 Xe Xénon 131,29
55 Cs Césium 132,90545	56 Ba Baryum 137,327	57 La Lanthane 138,9055	72 Hf Hafnium 178,49	73 Ta Tantale 180,9479	74 W Wolfram 183,84	75 Re Rhenium 186,207	76 Os Osmium 190,23	77 Ir Iridium 192,217	78 Pt Platine 195,078	79 Au Or 196,96655	80 Hg Mercure 200,59	81 Tl Thallium 204,3833	82 Pb Plomb 207,2	83 Bi Bismuth 208,98038	84 Po Polonium (209)	85 At Astatine (210)	86 Rn Radon (222)
87 Fr Francium (223)	88 Ra Radium (226)	89 Ac Actinium (227)	104 Rf Rutherfordium (261)	105 Db Dubnium (262)	106 Sg Seaborgium (263)	107 Bh Bohrium (262)	108 Hs Hassium (265)	109 Mt Meitnerium (266)	110 (269)	111 (272)	112 (277)	113	114				

Figure 77 Tableau des éléments dans lesquels sont surlignés en bleu les formateurs de réseau, en rouge les modificateurs de réseau et en vert les éléments intermédiaires.

Les verres manquent d'ordre périodique à longue distance des cristaux, ils ont une cellule unitaire infinie (il n'y a pas de répétition de structures à grande échelle), leur réseau 3D manque de symétrie et de périodicité qui amène à différents atomes et donc à différentes propriétés. Pour introduire la séparation de phase amorphe, il faut dire que les verres ont parfois été considérés comme un mélange liquide. Un liquide différent a des propriétés différentes et ainsi, lorsqu'ils sont combinés, ils peuvent former un mélange homogène (miscible) ou un mélange hétérogène (non miscible ou séparé en phase comme par exemple l'huile et l'eau). Dans les oxydes fondus, un processus similaire se produit et certaines combinaisons d'oxydes sont miscibles, certaines ne sont pas miscibles. Cette propriété est

fondamentale pour la compréhension du sujet de cette thèse et sera expliquée plus loin sur ce résumé, en relation avec le matériel dentaire étudié. Dans la seconde partie du siècle dernier, la compréhension de leur structure et de leurs propriétés permet d'utiliser les verres pour des applications plus spécifiques comme la vitrification des déchets nucléaires, l'énergie solaire et enfin la découverte de verres bioactifs pour la thérapie osseuse. A ce stade de la thèse, un sous-chapitre est consacré au concept des verres bioactifs à partir de la découverte de Larry Hench en 1969, les différents types de verres bioactifs et leurs applications potentielles (1). Une attention particulière a été utilisée pour décrire la liaison au mécanisme osseux et la formation de l'hydroxyapatite dans l'intercouche entre le bioverre et l'os.

Ensuite, les ciments de verre ionomère sont finalement introduits. Depuis leur développement à la fin des années 1960, les ciments au verre ionomère (CVI) ont été d'un grand intérêt en tant que matériau de restauration dentaire (2). Plusieurs caractéristiques telles qu'une bonne adhérence à la dentine, une bonne biocompatibilité et un temps de travail et de prise adéquat font des GIC le matériau idéal pour les restaurations dentaires.

Les CVI sont fixés par une réaction acide-base entre une phase minérale et un polymère soluble plus faible (figure 2). Typiquement, un poly (acide) aqueux, tel qu'un poly (acide acrylique), réagit avec un verre ionomère. La composition de verre est conçue pour être dégradée par l'acide relativement faible provoquant la formation de chaînes polymères acides réticulées ioniquement entre les contre-ions multivalents lessivés du verre et le groupe carboxylique du poly (acide). Le résultat est un processus d'auto-durcissement qui se termine par un ciment dentaire dur (3).

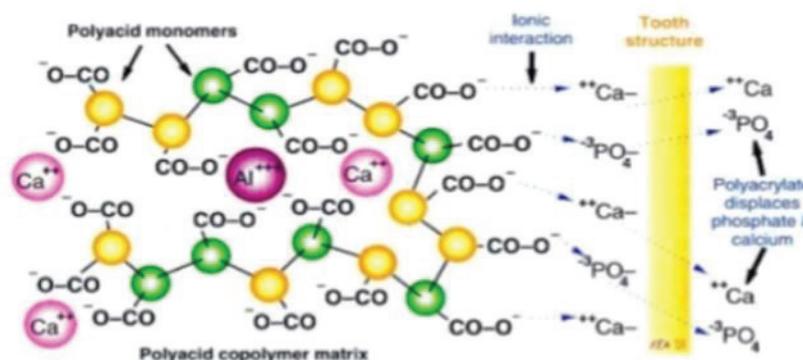


Figure 78 Chimie de prise des ciments verre ionomère

Leur chimie et propriétés sont profondément étudiées avec une recherche bibliographique complète qui guide de l'invention et à travers les innovations les plus récentes de ce matériau dentaire. L'ajout de bioverre semble être une voie intéressante pour l'étude des futures (4).

Les verres ionomères commerciaux, y compris le verre classique G338 (aluminosilicate), sont connus pour subir une séparation de phase au moins partielle lorsqu'ils refroidissent. Cela

conduit à des régions de composition variable et à l'apparition d'une phase plus sensible aux attaques acides que les autres (5). En principe, on peut s'attendre à ce que cela modifie les propriétés optiques du verre (et par la suite du ciment), mais aucune étude n'a été rapportée jusqu'à présent sur ce point.

La tendance à la séparation de phases dans les verres de borosilicate a été étudiée de manière approfondie. La première application pratique de la séparation de phases a été celle de Hood et Nordberg qui ont développé le procédé Vycor (SiO_2 , B_2O_3 , système de verre Na_2O) pour la production de produits à haute teneur en silice par voie vitreuse poreuse (6). La séparation de phases amorphe (APS) provoque la séparation d'une phase unique initialement homogène en deux phases ou plus de compositions différentes. L'énergie libre de Gibbs du système avec deux ou plusieurs phases distinctes doit être inférieure à celle du système avec une seule phase homogène. Le degré d'interconnexion des deux phases de verre dépend de la nature du mécanisme de séparation de phases. Le processus peut se produire par un processus de nucléation et de croissance qui donne des particules sphériques isolées ou par décomposition spinodale où une structure interconnectée est trouvée (7).

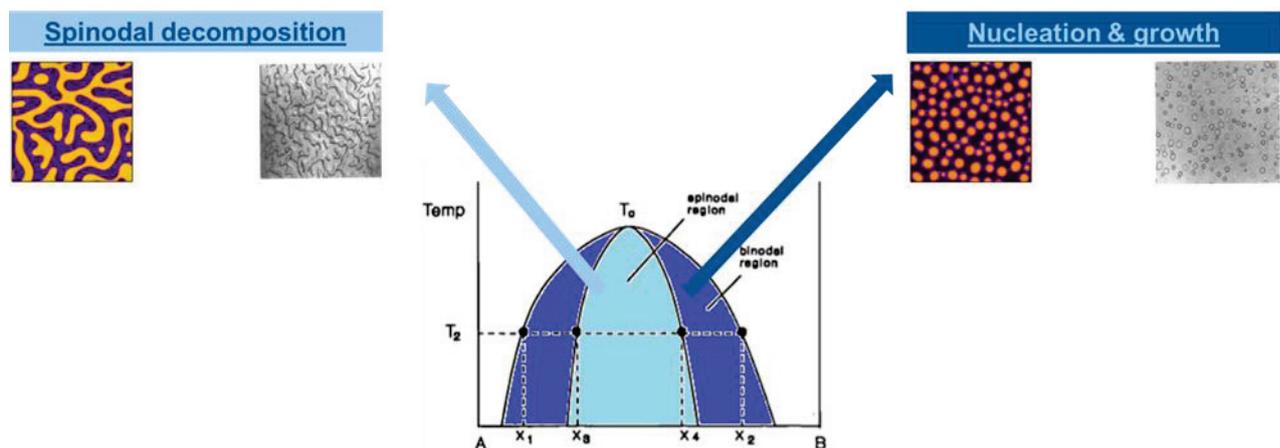


Figure 79 Les deux mécanismes de la séparation des phases: la décomposition spinodale et la nucléation et la croissance dans un système binaire. (8)

Le nouveau système de verre rapporté ici est un borosilicate avec une forte tendance à la séparation de phases. Il est composé de: SiO_2 - K_2O - B_2O_3 - CaO - Al_2O_3 . Il est significatif qu'avant un traitement thermique les verres soient optiquement clairs, mais deviennent opalescents à différents degrés suite à un traitement thermique dépendant du % en poids des éléments de la composition. Le schéma de la libération d'ions implique que l'une des phases est plus réactive et sensible aux attaques acides et sera lessivée plus tôt du verre (9). L'autre phase restera dans le ciment améliorant les propriétés mécaniques du matériau de restauration dentaire (figure 4).

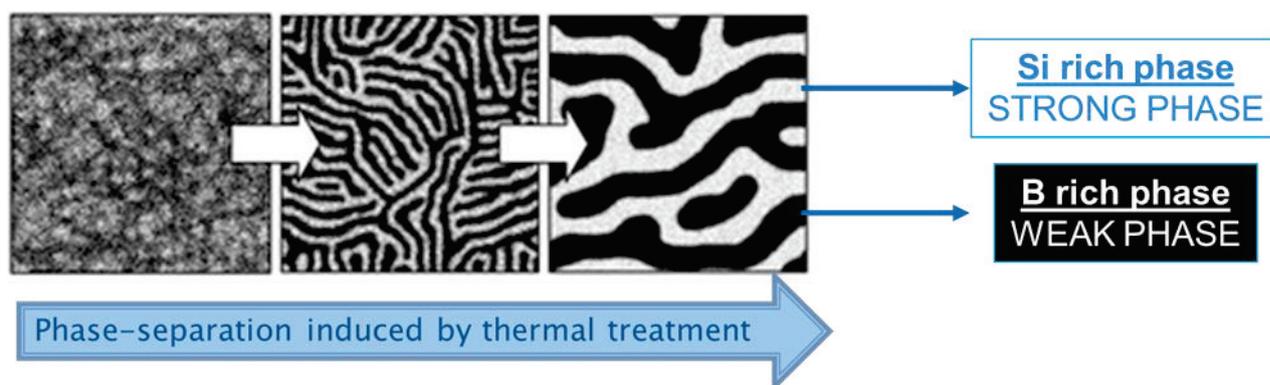


Figure 80 Représentation schématique de l'idée originale basée sur la séparation des phases par le mécanisme spinodal.

L'hypothèse est que le degré de séparation de phase influencerait directement le comportement de libération d'ions. SiO_2 et K_2O sont fixés respectivement à 45 et 15 % en poids dans les verres. Le premier pourcentage en poids concerne le verre classique 45S5 étudié par Hench (10). Ce dernier pourcentage en poids est basé sur les études d'immiscibilité menées sur les verres Vycor avec une composition choisie proche du centre du dôme d'immiscibilité pour créer une microstructure séparée (11). Les autres éléments du verre borosilicaté sont variés afin d'étudier l'influence sur les propriétés du système. Tous les éléments présentent plusieurs caractéristiques clés, bénéfiques pour le matériau de restauration. Compte tenu de sa composition, le bore joue un rôle régulateur sur d'autres éléments tels que le calcium (également présent dans le système vitreux étudié) et joue donc un rôle dans le métabolisme osseux (12). Le bore a été associé à la prévention de la perte de calcium et à la déminéralisation osseuse chez les femmes post-ménopausées et il a été démontré que la supplémentation en bore réduit les effets de la carence en vitamine D chez les poussins (13). Le bore influence aussi clairement les hormones de régulation des os impliquées dans la croissance osseuse et le remodelage osseux (14). Le calcium est crucial pour la restauration du tissu dentaire endommagé, car il est un composant primaire de l'hydroxyapatite dans l'émail et la dentine. L'hydroxyde de calcium est utilisé depuis de nombreuses années dans les agents de coiffage de la pulpe en raison de sa fonction de reminéralisation du tissu dentaire environnant. Le potassium est un agent désensibilisant utile (15). L'aluminium a été considéré comme un élément important pour fournir des ions Al^{3+} utiles pour le processus d'auto-durcissement du ciment (16). Cependant, l'aluminium est reconnu comme une neurotoxine et la cause de certaines maladies neurologiques, telles que la maladie d'Alzheimer et la maladie de Parkinson (17); par conséquent, une diminution du pourcentage en poids de cet élément dans la composition de verre par rapport aux verres ionomères conventionnels est à la fois difficile mais nécessaire. Les propriétés structurales des verres, les caractéristiques physico-chimiques et morphologiques liées au degré de séparation de phase sont étudiées dans cette thèse et corrélées à la libération d'ions.

Le chapitre sur l'état de l'art est clôturé par un rapport de la revue systématique sur les propriétés mécaniques et biologiques des verres bioactifs publiée dans "Dental Materials" le 29 mars 2017 (figure 5). Diverses bases de données électroniques (PubMed, Science Direct) ont été utilisées pour la collecte d'articles sur ce sujet. Cette recherche comprend des articles de janvier 2011 à mars 2016. Les directives PRISMA pour l'examen systématique et la méta-analyse ont été utilisées. 109 résumés ont été recueillis et examinés, 68 articles ont été lus comme articles pertinents et un total de 22 articles ont finalement été sélectionnés pour cette étude. La plupart des études ont mis en évidence l'amélioration des propriétés mécaniques et biologiques; cependant, il n'existe toujours pas de solution définitive et satisfaisante pour les matériaux porteurs hautement biocompatibles en médecine et en dentisterie. L'étude de nouvelles compositions de bioverre et des composites à base de bioverre offre un domaine très prometteur pour de futures recherches, qui devraient être menées avec des méthodes plus comparables et des résultats significatifs.



Figure 81 Page de titre de la revue systématique publiée sur Dental Materials en 2017.

Matériels et méthodes

L'approche utilisée pour établir une méthode de recherche appropriée est illustrée sur la figure 6. Elle montre l'étape unique qui amène du développement du verre à l'étude des propriétés et finalement à la compréhension de l'effet dans le matériau dentaire final. L'idée est que la formulation du verre est une étape clé qui fournira différentes propriétés au matériau appliqué et cette thèse veut contribuer à élucider la relation entre ces aspects.

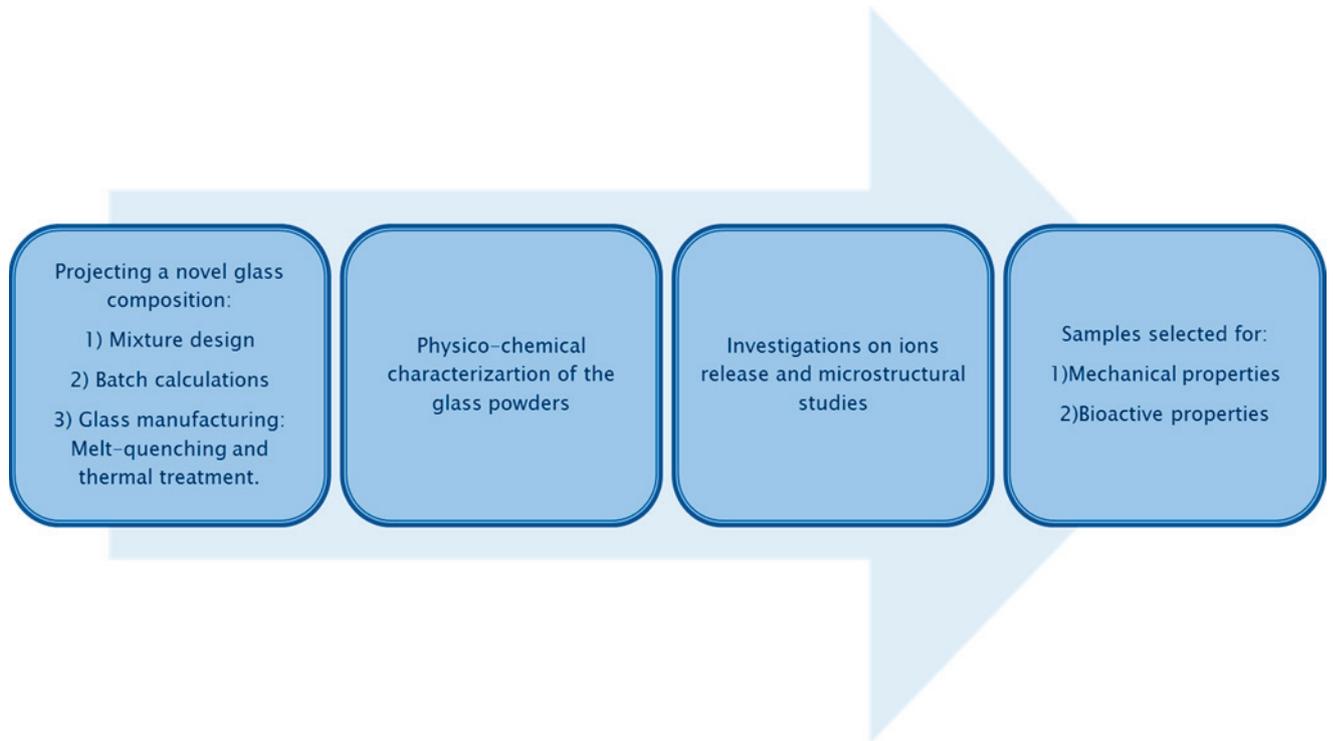


Figure 82 Chemin d'investigation qui guide à travers la section différente du chapitre sur les matériaux et les méthodes.

Les verres étudiés dans cette thèse sont basés sur le système quinaire $\text{SiO}_2\text{-B}_2\text{O}_3\text{-K}_2\text{O-Al}_2\text{O}_3\text{-CaO}$ et ont été obtenus par une technique de trempe-fusion. Dans ce chapitre, un plan expérimental factoriel présentera comment l'ensemble des échantillons a été construit. Le plan d'expériences est un outil utile qui permet une organisation efficace des expériences nécessaires à la recherche scientifique ou à l'optimisation des processus industriels. Plus en détail à cet effet était l'utilisation d'une expérience de mélange, dans lequel les facteurs indépendants sont des proportions de différents composants d'un mélange. B_2O_3 , CaO et Al_2O_3 sont les trois facteurs indépendants qui ont été choisis pour varier afin d'observer leur influence sur la séparation des phases. SiO_2 et K_2O sont fixés avec les valeurs respectivement de: 45 et 15% en poids. La réponse obtenue après le test de dissolution permet à l'expérimentateur de choisir une ou plusieurs compositions de borosilicate avec la cinétique de libération d'ions désirée. La zone de composition étudiée est représentée sur l'image (figure 7).

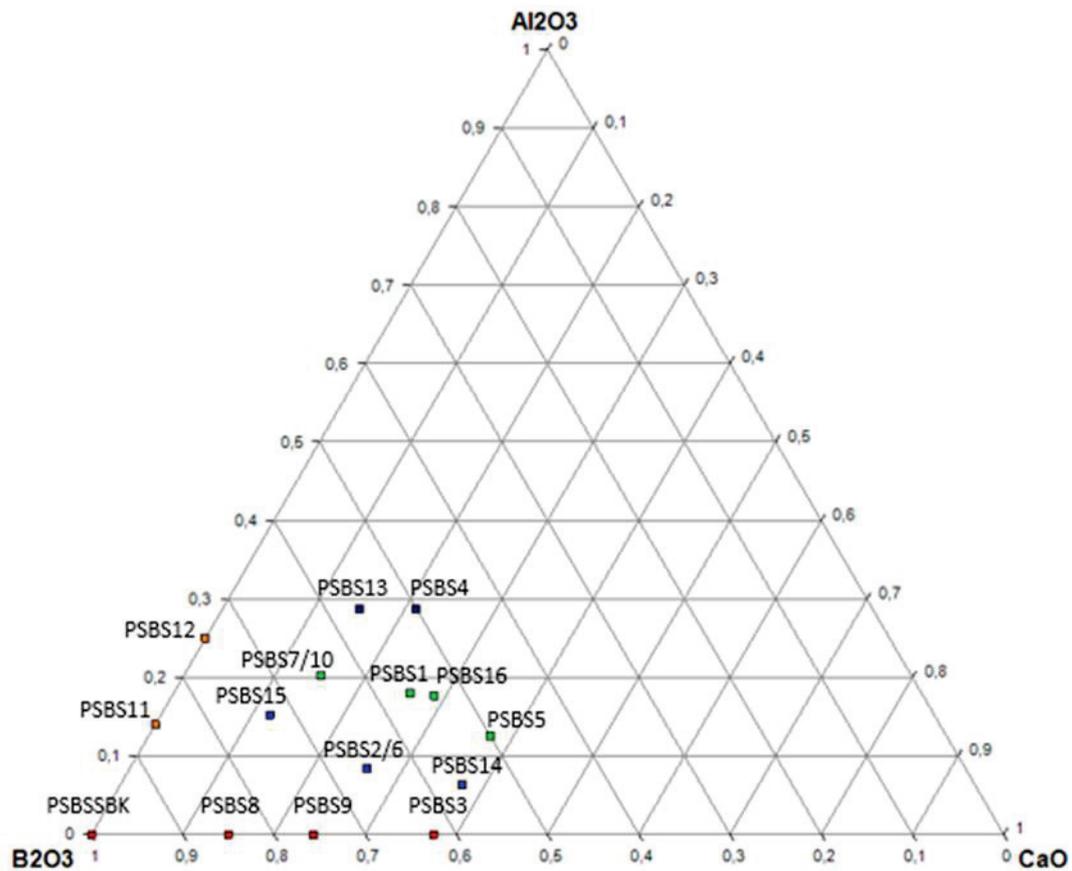


Figure 83 Diagramme triaxial avec la représentation des échantillons PSBS.

Une première partie est consacrée à la description des méthodes utilisées pour la production de la poudre de verre borosilicate avec la technique de trempe-fusion et le traitement thermique (figures 8-9), avec un aperçu des matières premières et de leurs comportements.



Figure 84 Procédés de fabrication des échantillons de verre borosilicaté: creuset à quartz, technique de trempe à l'état fondu.

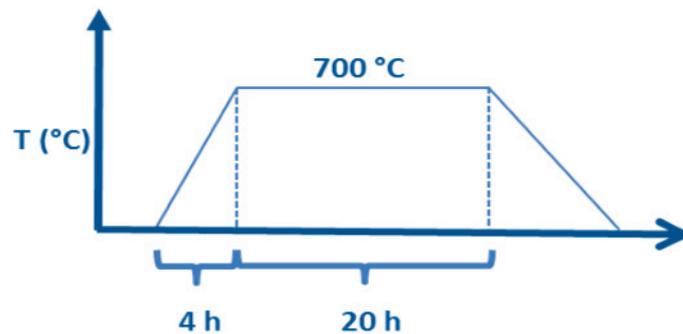


Figure 85 Heat-treatment performed to the entire set of glass samples after the melting.

Une deuxième partie expliquera les concepts qui sous-tendent les techniques utilisées pour la caractérisation préliminaire des poudres de verre obtenues et comment elles sont appliquées à l'étude des borosilicates. Le calcul de l'indice de réfraction, la diffraction des poudres aux rayons X et l'analyse thermique différentielle ont été les techniques utilisées à cet effet.

Une troisième partie est consacrée aux études de surface pour l'étude des différentes microstructures trouvées dans les verres induits par le traitement thermique. La microscopie optique et la microscopie électronique à balayage (MEB) sont les techniques les plus importantes utilisées. Dans cette section, il y aura la description de la façon dont les échantillons ont été préparés pour l'analyse.

Les deux dernières parties sont destinées à des tests développés pour l'analyse de la libération d'ions des verres dans des conditions acides et aux propriétés mécaniques et bioactives liées au matériau final mélangé à un acide polyacrylique commercial. La lixiviation des ions, mesurée avec la spectrométrie d'émission atomique à plasma induit de manière inductive (ICP), a été très utile pour déterminer le comportement des compositions de verre borosilicaté dans différents milieux acides (HNO_3 et acide polyacrylique). La technique a été évaluée pour l'analyse en préparant des solutions étalons à éléments multiples et en vérifiant la quantité d'ions libérés à différents moments.

Les échantillons ont été comparés entre eux en termes de composition et par rapport au traitement thermique. Ces études de dissolution réalisées avec l'analyse ICP sont utilisées pour élucider le mécanisme de libération des ions et comment cela est corrélé avec la composition du verre et la microstructure formée après le traitement thermique et la lixiviation. Les propriétés bioactives sont évaluées avec le test ISO 23317: 2014 dans lequel la formation d'hydroxyapatite est vérifiée par microscopie électronique à balayage (MEB) et spectroscopie à rayons X à dispersion d'énergie (EDX) sur la surface des disques de verre borosilicaté (figure 10), après l'immersion pour différents moments dans une solution de

fluide corporel simulé (SBF). La formation d'une couche de HA est généralement considérée comme une indication de la bioactivité. L'objectif de cette expérience est de caractériser globalement la conversion de cinq verres PSBS et de comparer les résultats avec le témoin 45S5K. Les changements de perte de poids et de pH accompagnant la conversion ont été mesurés pour fournir un aperçu supplémentaire de la cinétique et des mécanismes de la conversion des verres séparés en phase borosilicate.

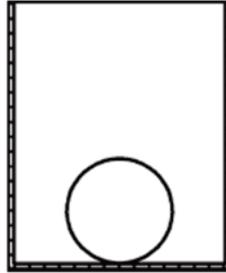


Figure 86 Disque de verre inséré dans un tube et immergé dans SBF pour le test de bioactivité.

Parmi les verres analysés, l'objectif était de ne donner la priorité à quelques-uns d'entre eux pour une analyse ultérieure dans laquelle les propriétés mécaniques (résistance à la flexion, dureté de Vicker) devaient être évaluées (figures 11-12). Pour cette raison, la réponse du mélange d'expériences a été utile pour déterminer la tendance à la libération d'ions. Il n'y avait pas une seule composition capable de libérer tous les ions à la même vitesse, donc deux verres de borosilicate ont été sélectionnés avec une tendance opposée à étudier leur effet sur les propriétés mécaniques une fois mélangé avec le poly (acide acrylique) FUJI IX®GP, GC Europe NV, Louvain, Belgique).

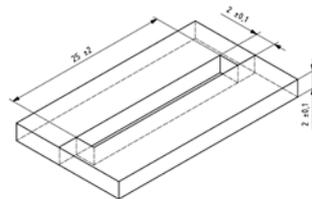


Figure 87 Forme et dimension du moule utilisées pour les essais mécaniques

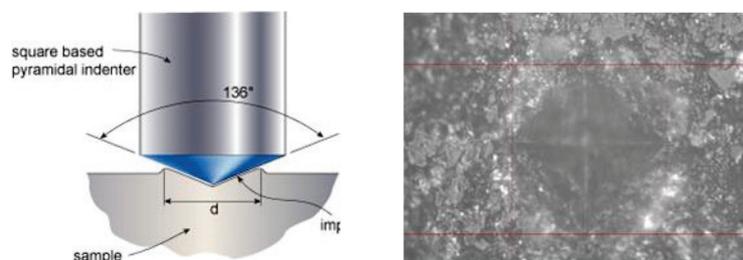


Figure 88 Pénétrateur de dureté Vickers et indentation sur la surface de ciment

Elaboration et caractérisation des verres

Ce chapitre rendra compte des résultats d'analyse menés durant toute l'étude. La succession des opérations peut être résumée comme suit :

- Préparation de verre séparée par une phase de borosilicate
- Analyse de criblage des poudres de verre pour la caractérisation structurale
- Expériences de lixiviation d'ions
- Etudes de surface et de microstructure
- Test de bioactivité
- Propriétés mécaniques (résistance à la flexion et microdureté Vicker)

Tous les verres ont été fondus et traités thermiquement selon la procédure décrite dans le chapitre précédent de la thèse (Matériaux et Méthodes). Les verres obtenus se sont révélés complètement amorphes avant et après le traitement thermique par la technique de diffraction des rayons X sur poudre (XRD). Les premières différences en termes de couleur du verre ont été mises en évidence après le traitement thermique. En effet, les échantillons étaient transparents après la fusion mais certains d'entre eux prennent une coloration opalescente après le traitement thermique prouvant que la composition pourrait influencer le degré ou le type de séparation de phases. En comparant à l'intérieur du diagramme triangulaire donné par les coordonnées de Janecke, il apparaît que différentes régions sont détectées (figure 13).

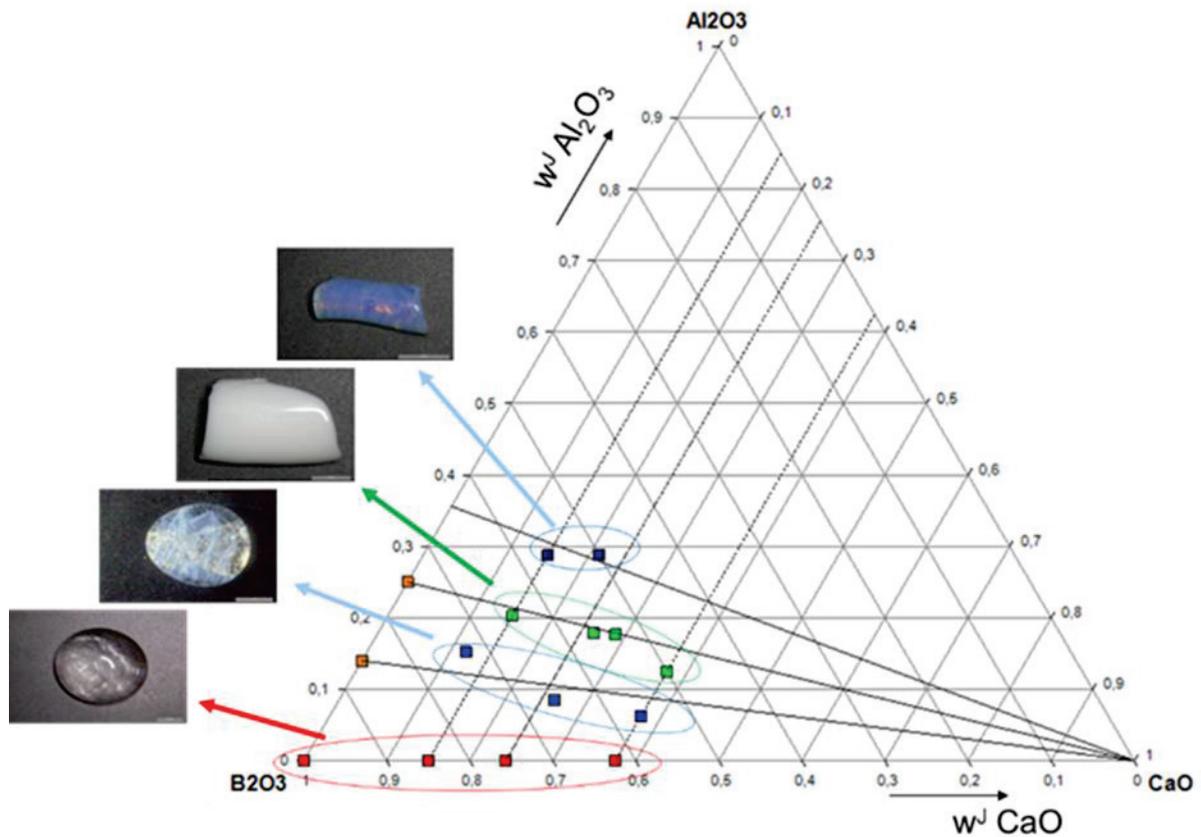


Figure 89 Diagramme triaxial montrant la composition des échantillons dans le système quinaire (SiO_2 et K_2O sont fixes). La composition influence l'opacité des échantillons.

Les échantillons surlignés avec des points rouges appartenant à l'isopleth sans aluminium semblent être transparents. Les échantillons mis en évidence avec des points verts appartenant à la même isopleth deviennent opalescents après le traitement thermique sauf pour l'échantillon sans calcium dans la composition (taches orange). Les échantillons trouvés dans la zone entre et au-dessus des deux isoplethes et mis en évidence avec des taches bleues prennent une couleur opalescente partielle.

Les verres à ce stade ont été broyés avec un broyeur Tema® à une taille de particule inférieure à $50 \mu\text{m}$ en fonction de la taille de particule mesurée pour la poudre de GIC commerciale appelée GC FUJI IX®.

Les techniques utilisées pour la caractérisation structurale des poudres de verre obtenues étaient: XRD, Calorimétrie différentielle à balayage et calculs d'indice de réfraction. La superposition des spectres XRD résultants (figure 14) montre un décalage d'angle de 2θ du halo amorphe typique. Il a été trouvé qu'il était lié à l'addition de CaO dans les compositions d'échantillons. Les cations de calcium ne forment pas la partie principale de la structure de verre, et l'introduction de ces oxydes appelés « modificateurs » (figure 15) entraîne une altération de la structure de verre (18). L'analyse DSC a révélé que l'absence d'aluminium

dans la composition amène les échantillons à cristalliser plus facilement et qu'un pic de cristallisation a été identifié et étudié en détail pour l'un des échantillons de verre (PSBS3).

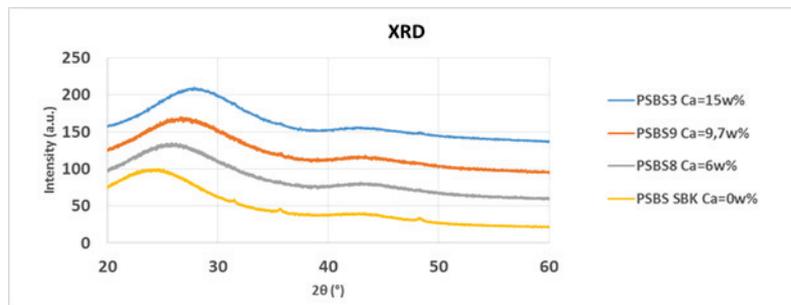


Figure 90 Diffraction des rayons X des échantillons sans Al_2O_3 .

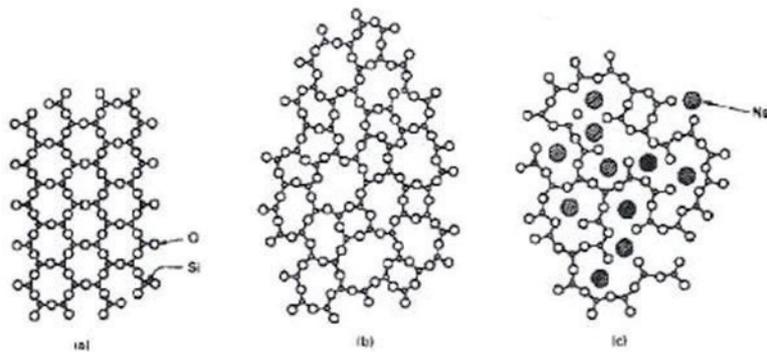


Figure 91 a) Structure cristalline de la silice, b) Structure amorphe, c) Structure amorphe avec addition d'oxydes modificateurs.

Il est difficile de modifier la composition du verre et de contrôler sa réactivité une fois immergé dans la solution, mais des études peuvent fournir des informations précieuses sur les espèces lessivées et quand. Dans ce chapitre, des études de dissolution à la suite d'une analyse ICP sont utilisées pour élucider le mécanisme de libération des ions et comment cela est corrélé avec la composition du verre et la microstructure formée après le traitement thermique et la lixiviation. En ce qui concerne les expériences de lixiviation des ions, on a d'abord révélé l'effet positif du traitement thermique (figure 16) sur la vitesse de libération des ions pour les verres transparents et opalescents (mesures ICP-OES).

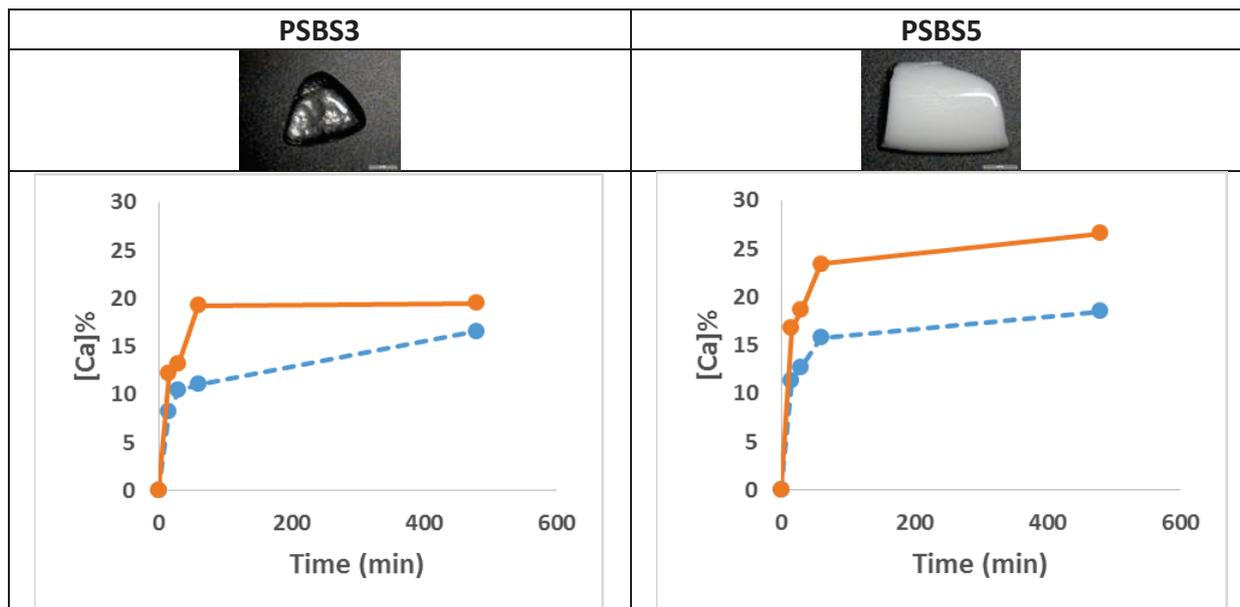


Figure 92 Exemple comparant la dissolution du calcium d'un échantillon transparent et opalescent. La comparaison a été faite dans un échantillon traité thermiquement (ligne continue) et un échantillon trempé à l'état fondu (ligne pointillée)

La comparaison des échantillons PSBS16 et PSBS8 respectivement avec et sans Al_2O_3 révèle un comportement différent de lixiviation d'ions au cours du temps (figure 17). Le PSBS 16 (verre opalescent, 15% en poids d' Al_2O_3) libère du calcium à une vitesse de dissolution plus élevée ($\Delta Ca/\Delta t = 2.982 \text{ Ca\%/min}$). Pour PSBS8 (verre transparent, sans Al_2O_3) la libération de potassium et de bore est congruente et se produit à une vitesse de dissolution plus rapide que le calcium ($\Delta K/\Delta t = 2,744 \text{ K\%/min}$; $\Delta B/\Delta t = 2,66 \text{ B\%/min}$). La libération de silicium est très faible pour les deux échantillons de verre.

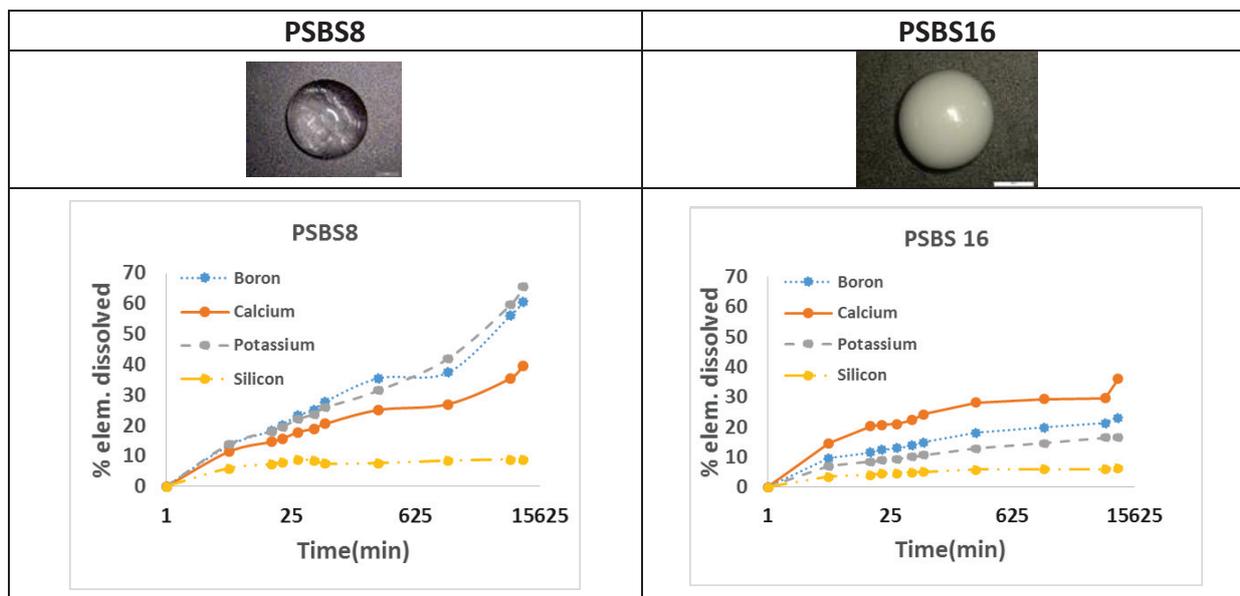


Figure 93 Etude de dissolution de la libération d'ions: comparaison entre verres transparents (PSBS8) et opalescents (PSBS16). Les données sont présentées dans une échelle de temps logarithmique. La libération d'ions bore, calcium, potassium et silicium est détectée à différents moments.

L'ensemble des verres a ensuite été analysé dans les mêmes conditions et avec les mêmes temps. Les échantillons traités thermiquement ont été utilisés pour étudier l'effet de composition sur la lixiviation des ions. L'effet de la composition est décrit à travers différents aspects dans la section consacrée dans la thèse qui couvre: les verres sans CaO ($W^J=0\%$ Ca), les verres sans Al_2O_3 ($W^J = 0\%$ Al) et l'effet du rapport B_2O_3/CaO . Une discussion à la fin du chapitre donnera une description globale des données obtenues.

Les expériences menées sur le comportement de lixiviation des ions pourrait être expliquée en se référant aux changements microstructuraux se produisant lorsque les échantillons sont chauffés pendant longtemps sous la température liquide. Le phénomène connu sous le nom de séparation de phases, largement décrit dans la littérature pour les systèmes de borosilicate, est l'hypothèse la plus valable pour décrire les résultats obtenus. Le mécanisme proposé pour la formation des deux phases provient d'autres études menées avec différentes techniques: SEM, EDX, XRD et la spectroscopie fluorescente aux rayons X (XRF).

La lixiviation des verres en vrac dans de l'acide nitrique dans des conditions dures (HNO_3 1M, $90^\circ C$, 48h) a été poursuivie pour accélérer le processus de dissolution qui devrait décaper la phase la plus soluble (riche en bore) des échantillons.

Ce qui était obtenu était différent des verres opalescents et transparents. Le premier montre une structure poreuse de silice presque pure. La structure de verre en vrac en verre transparent s'effondre laissant au fond du béccher une poudre blanche inconnue. Les analyses XRD et XRF démontrent que la poudre est encore complètement amorphe et qu'elle est composée de silice presque pure.

Les expériences suggèrent que la séparation de phase se produit parce qu'une seule phase se dissout complètement. Cette phase est la phase riche en bore qui, en tant que structure d'ancien réseau, ne doit pas être gravée dès que le réseau est modifié. Le processus impliqué dans la séparation des phases doit être pour les deux échantillons le mécanisme de nucléation et de croissance (figure 18). Dans le verre opalescent, les noyaux sont la phase riche en bore qui est gravée en laissant une structure poreuse, tandis que dans le verre transparent, les noyaux sont composés de silice, qui s'effondre sous forme de poudre après que la phase riche en bore se dissout dans la solution d'acide nitrique. Cette séparation de phase influence directement la microstructure qui affecte les comportements de lixiviation des ions.

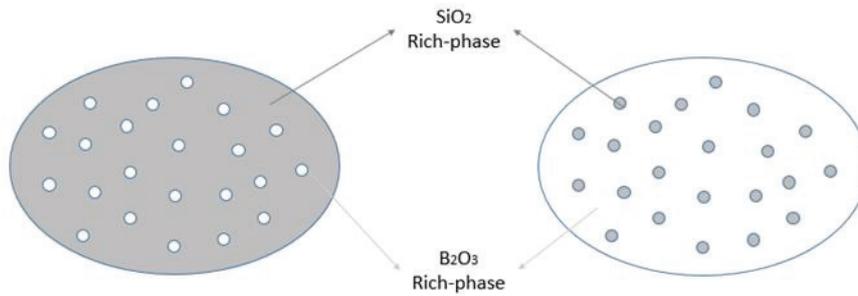


Figure 94 Hypothèse du mécanisme de la séparation de phase.

Des résultats intéressants ont également été obtenus en comparant les compositions des verres et leur influence sur la lixiviation des ions. Plus précisément, on a comparé des verres appartenant à des isoplèthes spécifiques dans lesquels sont détectées les deux séries de verre principales (transparentes et opalescentes). Les mêmes isoplèthes identifient des compositions avec une quantité constante d'aluminium. Ceci parce que l'aluminium est considéré comme l'élément prédominant pour provoquer la séparation de phase différente (comme aspect de couleur) et sa présence a été prouvée pour améliorer la durabilité du verre.

Les profils de libération d'ions suggèrent que les verres opalescents libèrent du calcium avec une vitesse cinétique plus rapide que les verres transparents (la comparaison moyenne de la vitesse cinétique est respectivement de 4 et 2,65 Ca%/min). Le potassium est libéré plus rapidement dans les échantillons transparents (taux moyen: 3,18 K%/min) par rapport aux opalescents (taux cinétique moyen: 2,38 K%/min). Le mécanisme de dissolution des verres opalescents dépend du rapport B_2O_3/CaO .

Les tests de bioactivité ont été lancés pour la détection de l'hydroxyapatite sur les surfaces des verres en vrac immergés dans le SBF (suivant la méthode ISO/FDIS 23317). Un verre de sodium 45S5K remplacé par du potassium a été utilisé comme référence et un verre opalescent et un verre transparent ont été étudiés avec SEM après une semaine et trois semaines d'immersion (figures 19-20). Un échantillon de PSBS augmente la couverture de HA sur la surface à la troisième semaine, démontrant une bonne bioactivité par rapport au témoin. Les verres sans aluminium dans la composition entraînent une formation de HA élevée. Cela est dû à la plus grande durabilité des verres qui contiennent de l'aluminium.

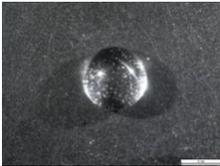
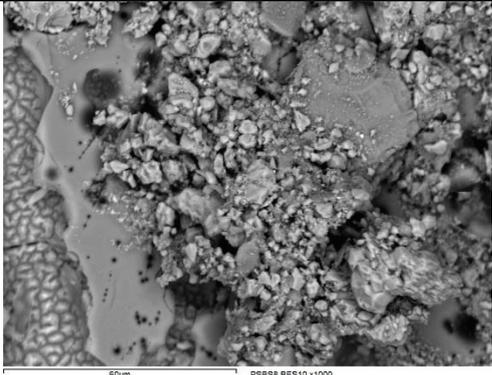
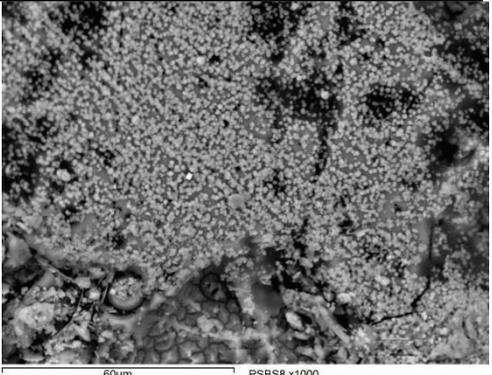
PSBS8	1 week	3 weeks																																	
																																			
	<table border="1"> <thead> <tr> <th>EDX (w%)</th> <th>Ca</th> <th>P</th> <th>Si</th> <th>O</th> <th>K</th> <th>Na</th> <th>Mg</th> <th>Cl</th> </tr> </thead> <tbody> <tr> <td>1 week</td> <td>23.8</td> <td>0.1</td> <td>26.6</td> <td>44.9</td> <td>0.4</td> <td>0.2</td> <td>3.7</td> <td>0.3</td> </tr> <tr> <td>3 weeks</td> <td>20.8</td> <td>9.8</td> <td>21.82</td> <td>46.16</td> <td>0.46</td> <td>0.43</td> <td>0.14</td> <td>0.38</td> </tr> </tbody> </table>								EDX (w%)	Ca	P	Si	O	K	Na	Mg	Cl	1 week	23.8	0.1	26.6	44.9	0.4	0.2	3.7	0.3	3 weeks	20.8	9.8	21.82	46.16	0.46	0.43	0.14	0.38
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Figure 95 Formation de HA à la surface d'un disque de verre PSBS8 après 1 semaine et 3 semaines d'immersion dans SBF.

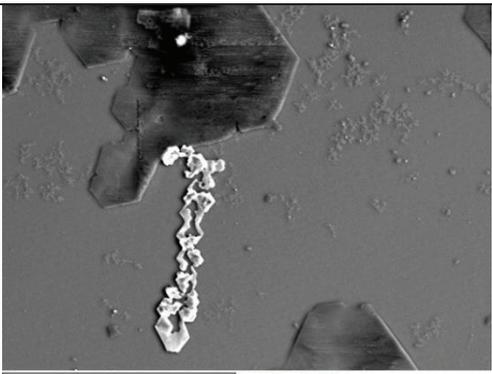
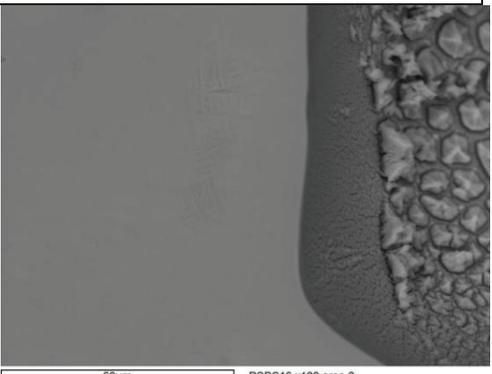
PSBS16	1 week	3 weeks																																				
																																						
	<table border="1"> <thead> <tr> <th>EDX (w%)</th> <th>Ca</th> <th>P</th> <th>Si</th> <th>O</th> <th>K</th> <th>Na</th> <th>Mg</th> <th>Cl</th> <th>Al</th> </tr> </thead> <tbody> <tr> <td>1 week</td> <td>7.1</td> <td>0.1</td> <td>30.1</td> <td>38.1</td> <td>15.5</td> <td>0.2</td> <td>0.1</td> <td>3.9</td> <td>4.9</td> </tr> <tr> <td>3 weeks</td> <td>10.14</td> <td>10.77</td> <td>12.75</td> <td>42.32</td> <td>4.97</td> <td>5.17</td> <td>0.77</td> <td>5.8</td> <td>7.33</td> </tr> </tbody> </table>								EDX (w%)	Ca	P	Si	O	K	Na	Mg	Cl	Al	1 week	7.1	0.1	30.1	38.1	15.5	0.2	0.1	3.9	4.9	3 weeks	10.14	10.77	12.75	42.32	4.97	5.17	0.77	5.8	7.33
EDX (w%)	Ca	P	Si	O	K	Na	Mg	Cl	Al																													
1 week	7.1	0.1	30.1	38.1	15.5	0.2	0.1	3.9	4.9																													
3 weeks	10.14	10.77	12.75	42.32	4.97	5.17	0.77	5.8	7.33																													

Figure 96 Formation de HA à la surface d'un disque de verre PSBS16 après 1 semaine et 3 semaines d'immersion dans SBF

Deux des poudres de PSBS ont été sélectionnées pour des tests mécaniques. Tout d'abord, ils ont été mélangés avec le liquide poly (acide) d'un ciment commercial (FUJI IX®GP, GC Europe NV, Leuven, Belgique) dans un rapport poudre/liquide de 1:2.

La résistance à la flexion a été mesurée avec l'essai de flexion à 3 points dans des échantillons de ciment. La forme a été modélisée pour ressembler à la demande de test mécanique ISO (dimensions: 2mmx2mmx20mm). Un des échantillons analysés présentait des valeurs similaires de résistance à la flexion au produit commercial qui était utilisé comme témoin à la fois après un et sept jours de maturation (figure 21).

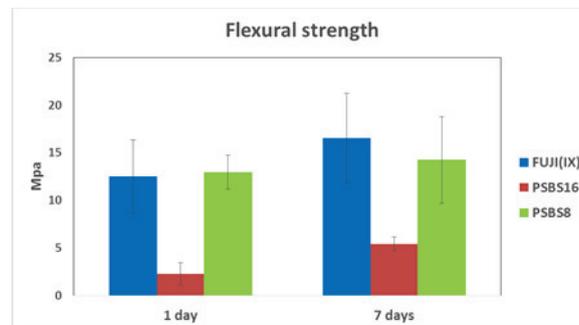


Figure 97 Valeurs de résistance à la flexion après 1 et 7 jours de maturation.

L'échantillon cité libérait une plus grande quantité de bore au cours des essais cinétiques. Il est bien connu que le bore dans l'acide borique et les borates forment un réseau de liaison hydrogène avec un composé contenant des groupes poly-hydroxy (figure 22). C'est la base de son importance dans les adhésifs. L'utilisation de Boron dans les adhésifs montre qu'il joue un rôle structurel de réticulation. Dans les produits adhésifs réticulés, les ions borates sont tous liés à la matrice adhésive. La présence de ce composé libéré du verre pourrait également être pertinente pour améliorer le processus de durcissement dans les ciments de verre ionomère a démontré par les résultats dans la résistance à la flexion. Il vaudra la peine de comparer tous les échantillons libérant une grande quantité de bore sur les propriétés mécaniques.

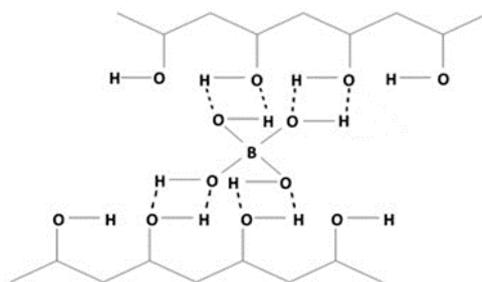


Figure 98 Réticulation des ions borate avec le groupe hydroxyle des chaînes d'un polymère. La formation de liaison hydrogène est mise en évidence.

La dureté de surface des CVI FUJI IX® s'est révélée supérieure à celle des échantillons de PSBS. A tous les points temporels, la microdureté du Vicker est significativement plus élevée dans le GIC commercial (FUJI IX®) que dans le PSBS16 et le PSBS8 (figure 23).

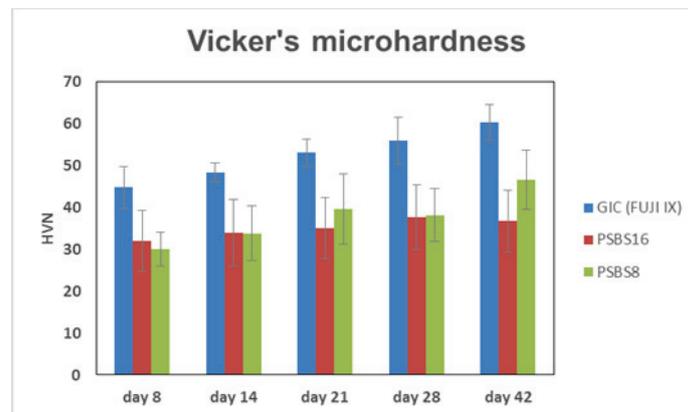


Figure 99 Comparaison de la microdureté de Vicker entre deux échantillons de PSBS et FUJI IX.

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