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Behaviour of nanocrystalline tricalcium silicate-based cements at early stages of hydration

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Abstract

Tricalcium silicate-based cements (TCS-BC) have reached great relevance in modern dentistry, where they are used in root-end filling or perforation repair treatments, and also for restoring deep and/or voluminous coronary carious lesions, among other applications. In some TCS-BC such as BiodentineTM, the physical and chemical properties, e.g. high compressive strength, have led to consider this cement as a dentine substitute. One of the drawbacks of BiodentineTM is its very high washout; which refers to the tendency of disintegrate at initial stage of hydration upon early contact with blood or other fluids, just at the moment after it is still as a freshly prepared paste. The present research was focused to compare properties related to workability and hardening at early stages of hydration of experimentally synthetized nano-sized tricalcium silicate (nTCS) and BiodentineTM (BIOD). Both pastes were tested for hardening at 0, 1, 2 and 3 h of hydration, using a penetration test adapted from standards D3441-79 and D1558-84. The hydrated products were evaluated by X-ray diffraction (XRD), thermogravimetry (TG) and 29Si NMR spectroscopy; pH measurements were taken along 28 days of hydration. The set cement pastes were evaluated for sealing ability and microleakage. Following literature reports, the CSH phase as a hydration product, was identified and modeled in both cements as defective clinotobernorite Ca11Si9O28(OH)2·8.5H2O. The CSH phases obtained differ from each other by its microstructural arrangement and packing densities, which depend on the w/c ratio of nTCS and BIOD pastes, which were 0.5 and 0.36 respectively. The hardening of BIOD is significantly influenced by its w/c ratio with a hydration fluid that presumably contains about 0.05% of polycarboxylate-based superplasticizer, which was identified by Raman spectroscopy. The washout and hardening properties were picted by and scheme for flocculation, w/c ratio, and packing density of the CSH particles characterizing the cement pastes.

1. Introduction

Among the hydraulic cements of the Portland type, those based on tricalcium silicate offer very interesting properties in the field of dentistry. One of them is BiodentineTM (Septodont) which has been developed as dentin replacement material. It can be used in wet areas without deteriorating, allowing it to be indicated for root-end filling and perforation repair treatments [1]. It is not adversely affected by fluids like saliva and blood [2]. Biodentine powder is mainly composed by 80.1 wt% of tricalcium silicate, 14.9 wt% of calcium carbonate, and 5
wt% of zirconium oxide for ensure adequate radiopacity. The powder is supplied with an ampoule containing a liquid that is composed by water with some additions of calcium chloride (for decreasing the setting time) and a water-soluble polymer (as water reducing agent) for keep a good flowability with a low water/cement (w/c) ratio [1].

Interesting information about tricalcium silicate cements and Biodentine can be found in the literature; specifically, that concerned with the way in which the pastes progress over time after their preparation. Some of this information refers to events that occur at very early stages, which are important for understanding the behavior of the paste when it is prepared and handled for use in a cavity of a few mm³ in volume; as is normally required during root-end filling or perforation repair treatments in dentistry.

When hydraulic cement is mixed with water, initial solubilization occurs in such a way that dispersed grains come into contact forming a connected structure in the first tens of seconds [3]. The formation of any of the solid hydration products are governed by nucleation processes that quickly leads to the formation of a thin layer of intermediate amorphous calcium silicate hydrate (CSH) covering the tricalcium silicate anhydrous grains. Since not all the calcium ions are destined to form CSH, the concentrations of calcium and hydroxide ions in solution increase (promoting high pH) and eventually lead to a supersaturation of the liquid phase with respect to calcium hydroxide, which then precipitates into portlandite [3]. The inclusion of calcium carbonate has been performed in Biodentine, where acts as a nucleation site - with particle sizes around 2–3 μm during the initial stage. It enhances the hydration reactions helping the subsequent production of a denser microstructure [4], which eventually help to obtain a better compressive strength in the subsequent stages of hydration. On its part, when CaCl₂ is added to tricalcium silicate, it accelerates the rate of nucleation of early intermediate CSH with low-density microstructure within the original boundary of the hydrating cement grains [5]. This can be associated by the increase of pore volume that has been reported to occur in the cement pastes as consequence of the inclusion of CaCl₂ [6]. At the end of the initial stage, it is generally supposed that the cement particles in the paste become fully coated with a layer of intermediate metastable amorphous CSH. It hinders the diffusion of the reacting species and in consequence, the rate of the hydration reactions is reduced. At this time, a second stage known as induction or dormant stage starts. It is characterized by slow dissolution and slow precipitation reactions. During this period, the paste is in a plastic state which allows the paste to be manipulated and placed without any major difficulty. Rheologically, the material shows a reversible behavior in its evolution. At microscopic level, the explanations available in the literature focus on a physical phenomenon such as reversible colloidal flocculation and de-flocculation [7]. The induction stage in Biodentine at 37 °C temperature, occurs around 17 min after mixing and is short, owing to the small particle size and the presence of CaCl₂ accelerator [8]. The induction period appears to be shortened or inexisten tricalcium silicate when it is obtained by fast cooling after burning, or it is fine ground or it is mixed with an excess of water [9]. It has been shown that the hydrate phase prevailing during the induction stage, is structurally distinct to the CSH phase typically recognized as final product of hydration; the former contains only isolated (monomeric) SiO₄ tetrahedra whereas in the final CSH phase, the SiO₄ tetrahedra are condensed to more or less long chains [10]. The end of the induction period for tricalcium silicate is around 2 h 30 min [10]. The induction stage in Biodentine ends much lower and is followed by the initial setting and subsequently the hardening of tricalcium silicate paste, around 12 min after mixing [1, 8, 11]. The stage after induction is known as the acceleration period, in which the main hydration reactions take place, producing a notable decrease in the tricalcium silicate that is accompanied by the growth of the CSH phase. In the acceleration stage, the CSH shows condensation of silicate tetrahedra in end members in chains, and middle groups in chains of SiO₄ units. These two types of condensation can be respectively noticed by the presence of the Q₁ and Q₂ signals by ²⁹Si magic-angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopy [10]. The setting reactions in Biodentine are rapid with a maximum heat evolution at 42 min after mixing [8] which approximately marks the end of the acceleration stage. Around this time, the indenter used for Vicat test, fails to leave a mark on the set cement surface and is the final setting time; for Biodentine, it is 45 min while for tricalcium silicate is 3 h [12]; and for tricalcium silicate with 20% of ZrO₂ increases to 4 h 50 min [13].

The deceleration stage that follows, marks a decline in the hydration rate as the reactions become diffusion-controlled [8]. Large time after the beginning of the deceleration stage (28 days after mixing), the compressive strength and Vickers microhardness in Biodentine reach 67 MPa and 48.4 respectively [13]. In contrast, tricalcium silicate with 20% of ZrO₂, shows a compressive stress and Vickers micro hardness of 23.6 MPa and 8.2 respectively [13].

The stiffening is not due to larger forces but to the multiplication of contacts between CSH particles [3]. Since CSH is denser than the spaces between cement particles, the process of hardening is accompanied by a process of densification. Lower content of water implies higher densification. The higher strength and micro-hardness have been corroborated with the very low w/c ratio needed for good workability of Biodentine [13]. This was possible with the addition of a water-soluble polymer which diminishes the surface tension between the cement particles and the liquid phase [14].
The hardening and setting of cement materials have been extensively tested using various empirical techniques. One of these is the Vicat test, which is based on measuring the depth of penetration for an imposed load; while others (using penetrometers) are based on measuring the penetration resistance force under an imposed speed [15]. An inconvenience of the sharp Vicat needle is that it does not give information about the mechanical properties of the paste but only determines a time for solidification [15]. On the other hand, the case of penetrometers offers interesting alternatives as explained below. When the cement paste is manipulated, the capacity of this to flow, can be achieved as soon as the stress applied to the paste overcomes the so-called yield stress; the yield stress is a dominant intrinsic parameter of what is called in practice workability of the paste [7]. The yield stress represents a critical value that occurs when the stress applied to the paste overcomes what can be supported by the network of particles in interaction [7]. Additionally, CSH is the decisive hydrate phase which ‘bridges’ the particles and leads to flocculation before the initial setting point [16]. By using penetrometers, it has been found that under quasi-static conditions, the force measured scales with the yield stress of the tested material [15]. A suitable experimental geometry to do the test is by measuring the force required to push a hemispherical tip (wider than the rod on which it is mounted) through the paste at a very slow constant rate (−1 μm s⁻¹) [15, 17]. This kind of measurements could be useful to understand the washout phenomena reported to occur for Biodentine and not for tricalcium silicate; which is of practical relevance.

In dental practice, Biodentine usually hardens in a cavity of a few mm³ volume after being mechanically mixed with the supplied liquid. The way the paste hardens in such cavity, led us to intend a comparative study under this circumstances, between experimentally synthesized nano-sized tricalcium silicate and Biodentine. For this purpose, experiments for measuring the penetration resistance under an imposed speed and under quasi-static conditions were performed for this investigation. With the aim of discuss and understand the properties of the pastes in terms of composition and microstructure of both the solid reacting powders, as well as the hydrated products according to reported models in the literature; techniques such as X-ray diffraction (XRD), thermogravimetry (TG) and 29Si NMR spectroscopy were used for characterize the samples. The nature of the water-soluble polymer included in the liquid formulation of Biodentine was conveniently analyzed by means of Raman spectroscopy. The evolution of the pH over time, as well as the sealing ability and microleakage of the set cement pastes, were performed in order to collect useful information of practical relevance.

2. Materials and methods

2.1. Tricalcium silicate synthesis

The synthetic tricalcium silicate was obtained using CaCO₃ (J.T. Baker) and SiO₂ (Sigma-Aldrich) as reagents applying a multi-stage methodology which can be summarized as follows [18]: (i) preparation of a mix of finely sieved CaCO₃ and SiO₂ powders in appropriate stoichiometric proportions; (ii) homogeneous paste elaboration by incorporating ethanol into the powder mixture; (iii) drying of the paste by heating at 100 °C for 24 h; (iv) calcination at 1000 °C for 2 h 40 min for decarbonation of CaCO₃, followed by sample-grinding at the end of the treatment; (v) preparation of the sample as a pellet; (vi) sintering at 1600 °C for 4 h 40 min. This sample will be referenced as nTCS from now on. 5 wt% of ZrO₂ (Sigma Aldrich) was added to the nTCS sample to give it radiopacity properties. In order to obtain a cement paste, one drop of distilled water (equivalent to 0.05 g of water) were added to 0.1 g of the powder (w/c = 0.5) which was then homogenized with the help of a metallic spatula on a glass plate; the paste obtained by this way will be referenced as nTCS-Z henceforth.

2.2. Reference materials

Biodentine™ (Septodont, Saint-Maur-des-Fosses Cedex, France) and MTA-Angelus™ (Angelus, Londrina, PR, Brazil) were used as reference materials for the analyzes. Pastes of these materials were prepared following the instructions of each manufacturer. All pastes were manually mixed with its corresponding liquid. Biodentine™ and MTA-Angelus™ will be respectively referenced as BIOD and ANG henceforth.

2.3. Penetration resistance measurements

nTCS and BIOD pastes were tested for hardening at 0, 1, 2 and 3 h after mixing with their corresponding liquids, using a penetration test adapted from standards D3441-79 and D1558-84. Brass containers were performed with cylindric cavities of 2 mm diameter and 3 mm high with the purpose of resemble the geometry and dimensions of the dental root. The cavity of each container was filled with a respective cement paste for a test. The measurements were carried out in a Shimadzu Autograph AG-100 kNG Mechanical Probe Test Machine (Shimadzu Corporation, Japan) using a 1.54 mm diameter tip (wider than the rod on which it was mounted). In the measurement, the paste was penetrated using a very slow constant speed of 16.7 μm s⁻¹ in order to approximately obtain a measurement under quasi-static conditions.
2.4. Raman spectroscopy

The water-soluble polymer of BIOD was characterized by Raman Spectroscopy using a μ-one equipment (Raman, Thermo Scientific DXR Raman Microscope). For the analysis, a liquid drop from the liquid container supplied in the Biodentine kit, was placed on a slide glass sample holder and analyzed with a 563 nm Argon laser.

2.5. X-ray diffraction (XRD) measurements

This technique was selected for performing qualitative and quantitative phase analysis of the nTCS and BIOD powders before their mixing with the respective liquids. The crystallite-size for each one of the phases composing the powders of both nTCS and BIOD was characterized. The hydration reaction products at 28 d of hydration were analyzed for nTCS and BIOD. The samples were measured using a Rigaku Ultima IV diffractometer with a parallel beam geometry (Cu Kα radiation) and nickel filter. The measurements were taken from 2θ range of 4°–70° with 20-step-angle of 0.02° and a speed of 0.5° min⁻¹. For the measurement of hydrated samples, the paste was prepared on a glass tile with the help of a metallic spatula; after that, the paste was carefully placed in the sample-holder where the hydration reactions started. The mounted sample was kept isolated inside of a sealed plastic container in order to prevent carbonation of the sample until its measurement.

2.6. Thermogravimetric analysis (TG)

TG was used to follow the hydration reactions in the nTCS and BIOD samples by means of a SDT Q600 calorimeter (TA Instruments, USA). The measurements were made after 28 days of hydration using platinum crucibles containing few milligrams of paste sample. Each measurement was taken from room temperature to 1000 °C, with a heating rate of 10 °C min⁻¹ under air atmosphere.

2.7. 29Si MAS NMR spectroscopy

This technique was used to determine the local environment and extent of polymerization of silicate species. In particular, the type and degree of condensation showed by SiO₄ in end members in chains, and middle groups in chains of SiO₄ units in the CSH phase. The 29Si MAS NMR spectra were recorded at room temperature on a Bruker Avance 400 spectrometer, operating at frequencies of 79.5 MHz. A 4 mm double tuned (1 H-X) MAS probe (Bruker Biospin) was used to perform MAS NMR (HPDEC sequence) measurements. Pulse excitation consisting of 4 μs pulses (τ/2 pulses) and recycle delays of 90 s were used to exclude saturation effects.

2.8. Qualitative and quantitative phase analysis

Phase identification was achieved consulting the Powder Diffraction File of the ICDD [19]. Since portlandite, Ca(OH)₂, is one of the products of the hydration reactions; the decomposition chemical reaction Ca(OH)₂ → CaO + H₂O occurring within the range of 425 °C–510 °C [20], was carefully considered for the TG analysis of the samples. The loss of water from portlandite decomposition, allowed to determine the proportion in weight of portlandite in the tested sample. A similar procedure was performed for quantitie calcite, which decomposes following the reaction CaCO₃ → CaO + CO₂ within the temperature range of 600 °C–750 °C. This information was combined with XRD data for perform the qualitative phase analysis of the samples.

2.9. XRD rietveld refinement analysis

Qualitative and quantitative phase analysis was performed, extracting the structural information of each one of the identified phases from the ICSD databank [21]. Rietveld refinements were carried out using the GSAS-II [22] software package. The qualitative analysis of amorphous or poorly crystalline phases - such as CSH- can be performed if a crystalline structure adequately models the positions and relative intensities of the ‘humps’ attributable to an amorphous component in the diffraction pattern [23]. By hypotesis, we have considered this to be the case for the poorly crystalline CSH phase generated in the cement pastes nTCS and BIOD.

2.10. pH characterization

For pH measurements, pH measuring strips were used to characterize pastes of nTCS, BIOD and ANG. Once the pastes were prepared, each sample was stored inside an Eppendorf tube waiting for a measurement of pH, which was taken at 4 h; and 1, 7, 14, 21 and 28 d of hydration.

2.11. Dental roots for evaluating microleakage

In order to preliminarily evaluate microleakage and sealing properties, extracted human mandibular premolar teeth with one root canal, were selected. The specimens were divided into three groups according to the cement paste used: Group I (nTCS-Z); Group II (BIOD); and group III (ANG). Each group consisted of teeth that were previously cleaned by removing the dental pulp. The roots were filled with the corresponding cement paste.
2.12. Microleakage and sealing ability by optical microscopy

Six measurements for microleakage and sealing ability were performed for each one of the groups (I, II and III) cited in the previous section. Root-end-filled dental pieces were placed individually in methylene blue for 24 h and then left for 30 min under water stream in order to remove the excess of dye. A sagittal cut was practiced to each tooth root for observing the sample in Olympus SZX16 stereoscopic microscope in order to register how much dye each tooth had filtered.

3. Results

3.1. Characterization by XRD before hydration

Experimentally synthesized nTCS (figure 1) is completely composed by 100% of tricalcium silicate (ICSD-64759); while BIOD sample is composed by 77.9(8)% of Ca$_3$SiO$_5$ (ICSD-64759); 16.6(4)% of CaCO$_3$ (ICSD-16710); and 5.5(1)% of ZrO$_2$ (ICSD-80050). These results coincide well with the values reported by Camilleri et al for Biodentine [1]. The crystallite size for each one of the phases present in the nTCS and BIOD samples, was modeled considering crystallites with axial symmetry in all cases. The crystallite size for the Ca$_3$SiO$_5$ phase in the nTCS sample have dimensions of 22 $\times$ 8 nm respectively; while for BIOD, the equatorial and axial sizes for each phase are respectively 115 $\times$ 33 nm for Ca$_3$SiO$_5$; 73 $\times$ 102 nm for CaCO$_3$; and 76 $\times$ 133 nm for ZrO$_2$. Smaller crystallite size was observed for the tricalcium silicate phase in the nTCS powder than that of the BIOD sample.

3.2. Penetration resistance measurements

Figure 2 shows the penetration force over time, measured at the start of hydration, and at 1, 2 and 3 h after the elaboration of the paste. At the start of hydration (figure 2(a)), the nTCS-Z and BIOD samples have passed the stage of initial solubilization, where the formation of interconnected structure is expected in the first tens of seconds. The curve corresponding to the BIOD sample in figure 2(a), is not uniform, and an abrupt change can be seen that can be interpreted as evidence of structural in-homogeneity in the sample. This behavior is not observed in the nTCS-Z sample, where when the tip penetrates the sample, a smooth curve that tends to a constant value slightly above 0.25 N is displayed. The same situation can be observed at 1 and 2 h of hydration (figures 2(b) and (c)) where the involved penetration forces have increased with the time of hydration. At 1 and 2 h of hydration, the nTCS-Z sample offers higher resistance to penetration than BIOD. However, when the time of hydration reaches 3h, both samples show clear signals of hardening. Above 0.5 min of penetration, a linear behavior is observed. At this time, the BIOD sample offers higher resistance to penetration than nTCS-Z.

3.3. Analysis of the liquid phase by Raman spectroscopy

The liquid phase for Biodentine™ show bands in its Raman spectrum (figure 3(a)) that were assigned according to the data reported for a comb-like structure of a polycarboxylate [24]. According to this, the bands at 3410 and 3265 cm$^{-1}$ are due to $\nu$(OH) stretching; 2975 and 2910 cm$^{-1}$ correspond to $\nu$(CH$_3$), $\nu$(CH$_2$) asymmetric
stretching; 1700 cm$^{-1}$ is due to $\nu$(C=O)$_{acid}$ stretching; 1470 cm$^{-1}$ is attributed to both $\delta$(CH$_3$) asymmetric in-plane bending and $\delta$(CH$_3$) scissoring; 1410 cm$^{-1}$ corresponds to $\nu$(CO$_2$) symmetric stretching, $\delta$(CH$_2$) wagging and $\delta$(CH$_3$) asymmetric stretching; finally, 1290 cm$^{-1}$ is due to $\delta$(CH$_2$) twisting and $\delta$(CH$_3$) rocking. In the simple case, the comb-like structure of the polycarboxylate presents a backbone chain constituted by polymethacrylate, with grafted polyethylene oxide side-chains (figure 3(b)).

Adsorption of the polymer on cement via the carboxylic acid group has been proposed, while the polyoxyethylene side chains disperse the cement particles (figure 3(b)) by means of its essential structure, which is represented in figure 3(c) [25].

3.4. Analysis of hydration products

Figures 4(a) and (b) show the crystalline phases identified at 28 d of hydration in the nTCS and BIOD samples respectively. Portlandite, Ca(OH)$_2$ (ICSD-34240) and tricalcium silicate, Ca$_3$SiO$_5$ (ICSD-64759) are identified.
in both samples; while CaCO₃ (ICSD-16710) and ZrO₂ (ICSD-80050) are only identified in the BIOD sample. A defective clinotobermorite \( \text{Ca}_11\text{Si}_9\text{O}_{28}(\text{OH})_2 \cdot 8.5\text{H}_2\text{O} \) appears identified as nanocrystalline CSH phase in the diffractograms of both nTCS and BIOD samples [26] (figures 4(a) and (b)).

The data for the crystal structure represented in figure 4(c), was used for defective clinotobermorite reported in reference [26], and was obtained from the hypothetical model T3\_14sc reported by Richardson [27]. This structural model was taken for the simulation of the curves showed in figures 4(a) and (b), and labeled as CSH, T3\_14sc. The annotation of ‘fwhm = 8 deg’ after ‘T3\_14sc’ in figure 4(a), stands for the full-width at half maximum value (FWHM) used for simulate the peaks of the diffraction pattern (which implies very small crystal size, e.g. of the order of few nm). For the case of the nTCS sample, showed in figure 4(a), the model fits very good with the humps observed in the diffractogram. The structural model is then suitable for displaying the contribution of the poorly crystalline CHS phase, according to Kern et al [23]. For the BIOD sample (figure 4(b)), a good fit was obtained when the effect of preferred orientation planes (207) and (3–33) is taken into account (figure 4(d)). In this case, the model also fits very good with the humps observed in the diffractogram (figure 4(b)).

The crystal structure of defective clinotobermorite (figure 4(c)) presents \( \text{Si}_2\text{O}_7 \) diorthogroups that give rise to a \( Q_1 \) signal observed in the \( ^{29}\text{Si} \) MAS-NMR spectrum for the BIOD sample (figure 4(f)). The \( Q_1 \) signal is also produced by \( \text{Si}_4\text{O}_8(\text{Q}_2) \) in end members in chains of \( \text{Si}_4\text{O}_8 \) tetrahedra in the same structure (figure 4(d)). The \( \text{Si}_4\text{O}_8(\text{Q}_2) \) in middle groups in chains (figure 4(d)) give rise to a \( Q_2 \) signal observed in the \( ^{29}\text{Si} \) MAS-NMR spectrum. This local environment and kind of condensation is in agreement with the XRD interpretation considering the clinotobermorite \( \text{Ca}_11\text{Si}_9\text{O}_{28}(\text{OH})_2 \cdot 8.5\text{H}_2\text{O} \) as the CSH phase.

From TG analysis, the weight proportion of Ca(\( \text{OH} \))₂ in the nTCS sample is 21.34% while for the BIOD sample, this proportion is 13.17%. CaCO₃ is only present in the BIOD sample with a proportion of 13.99%.

3.5. pH results
The pH for the BIOD, nTCS-Z and ANG pastes are shown in figure 5. The behavior of the pH is very similar in the nTCS-Z and BIOD samples, starting with a value of 12 that rises and remains at 13 until 21 days. The change
from 12 to 13 is recorded at 24 h in the nTCS-Z sample, while in the BIOD sample the change is observed up to 7
days. At 28 days, the pH returns to a value of 12. These results are in agreement with those reported by Grech et al
for Biodentine [4].

3.6. Microleakage and sealing ability
The six measurements made for each group of samples gave the next results. Group I (nTCS-Z): 0.59 ± 0.77
mm; Group II (BIOD): 0.73 ± 0.49 mm; and Group III (ANG): 2.36 ± 0.77 mm. These results indicate great
similarity in the test for microleakage and sealing ability between the Groups I and II; while the Group III, is
clearly differenced from the other groups.

4. Discussion
The way the nTCS-Z and BIOD samples hardens during hydration reactions shows remarkable differences.
Quickly, at the beginning of hydration, the BIOD sample shows evidence of structural inhomogeneity,
according to the results of the penetration resistance tests. This structural inhomogeneity can be explained
considering that large aggregates have been formed at this stage in the BIOD sample, just in diferent way that the
nTCS-Z sample, which mechanically, shows structural homogeneity. The liquid of the BIOD sample is
formulated by a polycarboxylate superplasticizer; in consequence, it is illustrative to take into account the facts
reported by Qian et al [28]. They find that, when a polycarboxylate ether superplasticizer is used as water
reducing agent to enhance flowability of cement pastes, it promotes the formation of higher percentages of
bigger particles and lower percentages of smaller particles when the cement paste is added with 0.05% of the
superplasticizer; basically, small particles or agglomerates bond and form bigger agglomerates. According to this
scheme, without the addition of the superplasticizer the small particles or agglomerates are not bonded and
under this environment, the surface tension in the cement pore solution is around 69 mN m
−1 as consequence
of salt chemicals in solution (i.e. a little less than 72.75 mN m
−1, which is the water-air surface tension at 20 °C).
With the addition of 0.1% superplasticizer, the surfactant effect of this makes the surface tension decrease to
reaching a maximum lower limit of approximately 43 mN m
−1. Above this concentration (the Critical Micelle
Concentration, CMC) the superplasticizer form micelles in the cement pore solution. It is considered that for
concentration values around CMC, the cement particles are covered by a single layer of superplasticizer [28].
The superplasticizer is a comb-shaped molecule with an adsorbing linear anionic backbone and non-adsorbing
side chains [29]. The backbone is known to adsorb on cement particles and by means of side chains, induce steric
hindrance, thus reducing attractive interparticle forces [29]. Below the CMC, some part of the cement particles
will be exposed to the action of CaCl
2 admixture (present as component in BIOD sample). Hence, the hydration
reactions will be accelerated in this sample. On the other hand, if the polycarboxylate superplasticizer in the
BIOD sample had been added in a proportion lower than 0.1%, then it would show signs of flocculation showing
large agglomerates [28]. This explain the results of the penetration resistance measured in the BIOD sample
during the first two hours of hydration (figures 1(b), (c)). A fragile connection between the large agglomerates in
the BIOD sample could be the cause of this sample having a lower resistance to penetration than the nTCS-Z
sample. Although, at 45 min, the cohesion of the BIOD sample is enough to avoid the formation of a mark by an
indenter during the Vicat test (just at the final setting time). Hence, it would be assumed that the nTCS-Z sample

Figure 5. pH variation over time for the nTCS-Z, BIOD and ANG samples.
would be composed of smaller and better-connected aggregates; while by its features, it can be supposed that the BIOD sample would be composed by ~0.05% of polycarboxylate superplasticizer. Therefore, the fragile connection between large aggregates in the BIOD sample, leads to a possible interesting schematic model. In particular, for explaining the high washout resistance reported for Biodentine™ when it is compared with radiopacified tricalcium silicate, Bioaggregate™ and intermediate restorative material (IRM) [13]. Since washout refers to the tendency of freshly prepared cement pastes to disintegrate upon early contact with blood or other fluids’ [13] we point out the relevance of consider this phenomenon as related to the fragile connection between large aggregates in Biodentine™ during the early step of hydration. In this stage, the network of cement particles can be easily disrupted and quickly reformed, as was claimed by Jönsson et al [30] (1 h after disruption, the elastic modulus in the cement paste has regained the value that it would have reached otherwise).

After three hours of setting however, the resistance to penetration of the BIOD sample is greater than the nTCS-Z sample, as can be noticed by checking the figure 1(d). The sample BIOD is in a deceleration stage characterized by a decline in the hydration rate where the reactions become diffusion-controlled [8]: the contacts between the CSH particles multiply so that the paste hardens. In the BIOD sample, the process of hardening is accompanied by a process of densification favored by the lower w/c ratio achieved by the superplasticizer addition. Since lower w/c implies higher densification, the hardening reached by the BIOD sample is greater than that offered by the nTCS-Z sample. When the CSH particles grow around the cement particles, it is supposed that they form a network that fills the spaces between them [30]. By electron microscopy, it has been found that the CSH layer around the anhydrous cement grains has high density, and corresponds to the CSH formed during the first stage of hydration [3]. The CSH that develops during the deceleration stage (by means of the diffusion mechanism previously mentioned) fills the pores of the cement paste and typically shows a fibrillar aspect [3].

Considering that CSH consists of five nano-meter sized particles with different packing densities, Ulm & Vandamme have distinguished CSH nanostructures with low density (LD), high density (HD) and ultra-high density (UHD) packing [31]: the size of the CSH particles considered in the research of Ulm & Vandamme are in agreement with the simulations of the CSH phase presented in the diffractograms of figures 4(a) and (b). Each nanostructure assignment was possible to be noticed from the implementation of micromechanics-based scaling relations for a large array of nanoindentation tests done in cement pastes with different w/c ratios. The idea is that by pushing a needle onto the surface of the cement paste, its surface deforms in a way that reflects its mechanical properties [31]. The packing density distributions found for different w/c provides strong evidence of three statistically significant LD, HD and UHD phases. For the BIOD sample, the w/c ratio is 0.36 (if the paste of Biodentine is prepared mixing 700 mg of powder with 5 drops of liquid). For this w/c ratio, the volume fractions for LD, HD and UHD in the microstructure of the cement paste should be 0.47, 0.35 and 0.18 respectively [31]. As a rule, increasing the w/c ratio, the water available for hydration increases the amount of looser packed LD in detriment of HD; while the UHD remains almost constant. For the nTCS-Z sample, the w/c ratio was 0.5, so the amount of HD is lower than the BIOD sample and this would explain its lower penetration resistance after three hours of hydration. This interpretation is supported by the results reported by Bentz et al where they found that compressive strength in cement pastes decreases as the w/c ratio increases within the range 0.325–0.425 [32].

For tricalcium silicate prepared with w/c = 0.41, the formation of colloid nanoparticles globules of approximately 5 nm has been reported to exist at mesoscale length [26]. The CSH nanoparticles consist of defective clinotobermorite Ca\(_{11}\)Si\(_6\)O\(_{24}\)(OH)\(_2\)·8.5H\(_2\)O covered by a monolayer of Ca(OH)\(_2\). The presence of large Ca(OH)\(_2\)\(_2\) crystals forming part of the cement paste, but with dimensions of 15 or 20 \(\mu\)m, as well as a remaining tricalcium silicate were also reported [26]. This report is in good agreement with the results obtained for the nTCS sample analyzed by X-ray diffraction (figure 4(a)). The case of BIOD sample with w/c = 0.36 will give a more compacted CSH microstructure than the nTCS sample. Hence, a change in the microstructural arrangement would be expected, as can be noticed by the discrepancies observed in the X-ray diffractogram by effect of density packing and preferred orientation showed by the defective clinotobermorite (figure 4(b)).

The w/c ratio is a fundamental parameter, related to the porosity of the cement pastes [31], in such a way that it could be talked into account for interpreting the results obtained for the microfiltration observed in the group I (nTCS-Z) and group II (BIOD) of samples. Experimentally, great similarity was found for these two groups of samples. However, the Group III (ANG) was clearly differentiated from the other groups. This result can be interpreted as a consequence that the amount of tricalcium silicate is lower in the ANG sample compared to the BIOD and nTCS-Z samples. The lower presence of tricalcium silicate leads to a slower reaction rate and a more porous microstructure, as was explained by Camilleri et al [1].

In summary, Biodentine is an endodontic cement that has rheological and hardening properties that are significantly influenced by its ratio w/c = 0.36, and the liquid formulation for hydration that presumably contains about 0.05% of superplasticizer based on polycarboxylate. Under this picture, at the beginning of hydration, when Biodentine is manipulated it starts flocculating forming large aggregates with weak
interconnection between them, showing high washout. The paste is reinforced by hardening strongly between 2 and 3 h of hydration, giving rise to a higher content of high density-packed CSH particles. Comparatively, the interconnection between them, showing high washout. The paste is reinforced by hardening strongly between 2 and 3 h of hydration, giving rise to a higher content of high density-packed CSH particles. Comparatively, the interconnection between them, showing high washout. The paste is reinforced by hardening strongly between 2 and 3 h of hydration, giving rise to a higher content of high density-packed CSH particles. Comparatively, the interconnection between them, showing high washout. The paste is reinforced by hardening strongly between 2 and 3 h of hydration, giving rise to a higher content of high density-packed CSH particles. Comparatively, the interconnection between them, showing high washout. 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