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# Rheological properties of calcium carbonate self-setting injectable paste

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With the development of minimally invasive surgical techniques, there is growing interest in the research and development of injectable biomaterials with controlled rheological properties. In this context, the rheological properties and injectability characteristics of an original CaCO<sub>3</sub> self-setting paste have been investigated. Two complementary rheometrical procedures have been established using a controlled stress rheometer to follow the structure build-up at rest or during gentle mixing and/or handling on the one hand, and the likely shear-induced breakdown of this structure at 25 or 35 °C on the other. The data obtained clearly show the influence of temperature on the development of a cement microstructure during setting, in all cases leading to a microporous cement made of an entangled network of aragonite-CaCO<sub>3</sub> needle-like crystals. Linear viscoelastic measurements arriving from an oscillatory shear at low deformation showed a progressive increase in the viscous modulus ( $G''$ ) during paste setting, which is enhanced by an increase in temperature. In addition, steady shear measurements revealed the shear-thinning behaviour of this self-setting paste over an extended period after paste preparation and its ability to re-build through progressive paste setting at rest. The shear-thinning behaviour of this self-setting system was confirmed using the injectability system and a procedure we designed. The force needed to extrude a homogeneous and continuous column of paste decreases strongly upon injection and reaches a weight level to apply on the syringe piston around 2.5 kg, revealing the ease of injection of this CaCO<sub>3</sub> self-setting paste.

## 1. Introduction

Over the last few years, the development of minimally invasive surgical techniques, especially in the field of bone substitution and repair, has boosted the research and development of injectable calcium phosphate suspensions and cements [1]. In particular, fast-setting calcium phosphate (CaP) bone cements have developed considerably in the past few years due to their excellent biocompatibility and bioactive properties [2,3]. Although several fast-setting CaP bone cements have been used for more than 10 years, there is still a need to develop hydraulic bone cements with improved rheological properties to facilitate handling and injection by surgeons.

Mineral cement paste with improved rheological properties should limit or avoid the two main drawbacks commonly observed with such injectable systems which greatly limit their use in vivo: (i) “filter-pressing”, corresponding to a phase separation between

the particulate/powder and the liquid within the syringe used and resulting in plugging and thus partial paste extrusion; and (ii) “cement washout”, corresponding to a disintegration of the paste affecting the paste cohesion and occurring at the early contact of the paste with biological fluids [4–9]. Consequently, the presence of scattered cement particles after implantation can lead to more or less intense inflammatory response of tissue [6].

Recently, several original injectable calcium carbonate-based cement compositions have been presented as promising biocompatible and resorbable self-setting pastes for bone substitution and repair [10,11]. Their solid-phase composition included high proportions of CaCO<sub>3</sub> metastable phases. As in the case of CaP cements, they showed excellent biocompatibility and bioactive properties and also ease of use, especially as a self-setting injectable paste with adaptable shape. In addition, the high proportion of CaCO<sub>3</sub> metastable phases with higher solubility than apatite can confer better resorption properties to such cement compositions.

Interestingly, the handling of CaCO<sub>3</sub>-based cement paste during and after its preparation is perceptibly different from that of other well-known CaP-based self-setting pastes. A rapid and reversible transformation of paste consistency characterized by a drastic

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modification of its viscosity (from viscous state to liquid state) can be noticed during mixing, and thus when the level of stress applied to the paste is prolonged.

Therefore, the characterization of rheological properties and injectability of this original CaCO<sub>3</sub>-based cement composition is of interest with a view to specifying and controlling the variations of rheological behaviour and injection properties of the paste from its preparation at room temperature to its injection. The objective of this study was to set up protocols for the determination of CaCO<sub>3</sub> self-setting paste rheological properties in conditions as close as possible to a surgeon's operating conditions.

Investigations on self-setting mineral pastes are complex as they are generally fast reactive systems under continuous transformation involving the development of an internal microstructure during cement setting and hardening. Despite these difficulties, rheological measurements can help us to thoroughly characterize self-setting paste and understand setting and hardening phenomenon, and such approaches are just beginning to be explored. Different procedures to characterize the rheological properties of biomedical CaP pastes or suspensions can be found in the literature, but only a very few studies have investigated self-setting pastes using dynamic rheometry over an extended period during setting [12–14]. In the field of paper-maker applications, Kugge and Daicic [15] studied the rheological properties of non-setting calcium carbonate concentrated suspensions using a controlled strain rheometer. However, the rheometrical protocol should be adapted to take into account the occurrence or not of a setting reaction in the studied system.

One of the first studies reporting rheometrical analysis of self-setting CaP pastes is that of Sarda et al. [12]. They investigated the rheological behaviour of injectable CaP cements by studying the creep response of the paste as a function of shear stress and other parameters controlling the paste's rheological properties, such as temperature, the liquid-on-solid ratio (L/S) and the presence of additives such as citric acid. More recently, Liu et al. [13] reported a steady and dynamic rheological study on CaP cement. They performed dynamic rheological measurements involving the application of low-amplitude oscillatory strains to the paste that allowed the simultaneous investigation of the viscous and elastic properties of the paste during the development of its internal structure due to setting reaction. In addition, they used these rheometrical data to evaluate the paste-setting time based on the storage modulus ( $G'$  value) analysis. Wang et al. [14] presented steady rheological measurements to characterize the stability of a paste consisting of a mixture of amorphous calcium phosphate and dicalcium phosphate dihydrate based on a novel approach of thixotropy measurement. They also reported measurements of paste injectability based on the calculation of the volume of paste extruded compared to the initial volume of paste within the syringe.

It appears from all the previous cited studies that the determination of such complex suspension properties has to be made very carefully. A well-controlled and reproducible experimental procedure has to be determined. It has to take into account the paste history, from its preparation to its implantation in vivo. The interactions between the paste and the biological environment are another important parameter to consider. Moreover, the surgery operating conditions, such as temperature and handling stress, are determinant factors too.

It is thus necessary to understand and follow the structural build-up and breaking both before and during the injection. In the case of hydraulic cements, this structural build-up is associated with physical–chemical processes, especially a dissolution–recrystallization process involving free water that leads to the solidification of the system. During the injection, the paste undergoes high shear that is capable of inducing the structure's breaking. Under-

standing and controlling the rheological properties of the system during these two distinct but not independent phenomena will allow us to determine the parameters and time scales that control the behaviour of the system during use. Therefore, non-destructive tests, based on small-amplitude oscillatory shear using very low strain amplitude, are considered. They are appropriate to quantify the stiffness or growth of structures in unsheared flows [16], and are thus representative of the material behaviour at rest, before injection. Moreover, shear flow is also considered. This may involve the breaking down of the structures that form as the crystallization reaction progresses and are representative of the evolution of the paste structure under injection conditions. These tests, and the experimental conditions associated, are described in detail in Section 2.4.

Injectability is also a determinant property to be considered for biomedical self-setting substitutes as the paste has to be easily extruded by surgeons. Even though the injectability of CaP pastes has been investigated theoretically and experimentally by several authors, there is still no standard procedure by which to measure the injectability of a self-setting paste [14,17–22]. Bohner et al. [8,18] and Qi et al. [23] showed that improvement of injectability can be achieved in two ways: by modifying the injection system parameters, such as the length and diameter of syringe cannula and/or the injection rate; or by varying cement composition parameters, including the L/S ratio, the powder particle size and/or the presence of additives. In the former case, a compromise between cement's rheological properties and the injection system must be found, but the possibilities of variation are limited as injection parameters must fit in with surgical use.

Injectability remains an undefined property of a cement paste and several protocols can be found in the literature to evaluate this property. In all cases a syringe system is used with or without a cannula, and authors measure the amount or volume of paste extruded at a constant force [14,19–22,24], and/or the pressure/force required to extrude the paste [8,17,18]. Less attention is paid to the cement's properties (setting, cohesion) and composition after injection, although these parameters are fundamental in the evaluation of the injectability of a cement formulation.

## 2. Materials and methods

The original cement composition studied consists of a mixture of vaterite and amorphous calcium carbonates constituting the solid phase (S) with deionized water as the liquid phase (L). The preparation and characterization of the reactive powders, along with the characterization of the paste's rheological properties, its injectability and setting time, are described hereafter.

### 2.1. Reactive powders synthesis and characterization

The reactive powders vaterite and amorphous calcium carbonate, which constitute the solid phase of the cement, were synthesized by precipitation at ambient temperature, following protocols described previously [10]. The precipitates were filtered, washed with deionized water (only for vaterite CaCO<sub>3</sub>), lyophilized and stored in a freezer to avoid any evolution of these metastable powders before their use.

The as-prepared powders were characterized using Fourier transform infrared (FTIR) spectroscopy (Nicolet 5700), X-ray diffraction (INEL CPS 120) and scanning electron microscopy (SEM, LEO 435 VP, sample silver plating before observation) techniques. Laser diffraction granulometry (Malvern, Mastersizer 2000) and specific surface area analyses (Monosorb MS22, Quantachrom) were also carried out on these powders. Mean particle diameter and specific surface area are important physical characteristics of

powders to consider when evaluating the rheological properties and injectability of concentrated and reactive suspension of particles, such as the CaCO<sub>3</sub> self-setting paste studied in this paper.

## 2.2. Self-setting paste preparation

The cement paste was prepared by mixing the appropriate amount of liquid phase (deionized water) with a powder mixture of metastable calcium carbonate phases (amorphous CaCO<sub>3</sub> and vaterite with a weight ratio of 1:2), as previously published [10]. The L/S ratio was equal to 0.67. In this study, the initial time corresponds to the end of the mixing of the liquid and solid phases, up to a maximum of 1 min of mixing, i.e. when a homogeneous paste is obtained.

In order to prepare the reference hardened cement, the paste was then placed in a sealed container in an atmosphere saturated with water at 37 °C for setting and hardening.

## 2.3. Physical and chemical characterization of the cement

Cements set and hardened in reference conditions (i.e. in an oven at 37 °C) or during rheometrical measurements were characterized using FTIR spectroscopy (Nicolet 5700), X-ray diffraction and SEM (sample silver plated before observation) techniques. These complementary techniques have been used to thoroughly characterize the cement's composition (CaCO<sub>3</sub> crystalline phase identification) and microstructure after setting and hardening. A paper on the characterization of calcium carbonate-based cement compositions for bone substitution has recently been published [10].

## 2.4. Rheometrical characterization

Accurate characterization of the rheometrical behaviour of the calcium carbonate self-setting paste was difficult due to several perturbing effects induced by the apparatus and, mainly, by the chemical reaction that the system undergoes during measurement.

In this study, rotational rheometry is considered. The rheometrical tests were conducted using a controlled stress rheometer (CARRI-Med CSL<sup>2</sup> 100, TA Instruments) equipped with a parallel-plate geometry. Three main problems arise when using this geometry. First, the shear rate varies throughout the test sample (although this does offer the advantage of the adaptability of the gap size, which is important in cases of such concentrated suspension systems). Moreover, measurements could be affected by "wall slip". This was avoided by sticking an abrasive paper to the surface of the plates. Finally, to prevent water evaporation during measurement, geometries and samples were enclosed in a water trap to maintain an atmosphere saturated with water. The lower plan was equipped with a Peltier thermoelectric device that insures a controlled temperature, fixed at 25 or 35 ± 0.1 °C for this study. These values were chosen as temperatures close to the preparation of the paste at room temperature by the surgeon (25 °C) and the injection of the paste in vivo (35 °C). The set is schematically represented in Fig. 1a.

Two different rheometrical procedures were considered to follow the structure build-up at rest or during gentle mixing and handling on the one hand, and the likely shear-induced breakdown of the structure, representative of injection conditions, on the other. Linear viscoelastic measurements and steady shear measurements were carried out at 25 or 35 °C. The former consisted of an oscillatory shear at low deformation. This is representative of the behaviour of the sample at rest and allows the follow-up of the structure build-up before or after injection. Evolution of loss and elastic modulus were recorded. Schematic representations of the set and procedure are presented in Fig. 1a and b, respectively.

Briefly, the cement paste to be tested was prepared as detailed in Section 2.2. The paste was then transferred onto the rheometer top plate and the gap immediately set to 700 µm. This value allows the easy control of the sample set-up; however, it is also quite high, and may induce some fracturation of the sample during the rheometrical characterization. It was thus reduced before the measurements, as explained below.

In procedure 1, nearly 3 min after the paste had been placed between the parallel plates (the time required for the entire sample to reach the right temperature), it was sheared under a constant shear of 30 s<sup>-1</sup> for 10 s at 25 °C. This step allows the paste's initial state to be standardized with regard to the structure's development. In addition, such a level of shear can be representative of the paste injection conditions. The sample was then left to rest for about 3 min before being sheared again under low-amplitude oscillatory conditions. During the rest period the structure develops in a controlled and reproducible way, after which it is possible to start the experiment. Note that after 5 min ( $t = 5'$ ) the gap was reduced to 500 µm to minimize any possible fracturation occurring within the paste due to the high level of shear considered. This gap value was high enough to consider the sample homogeneous. It was also sufficient to ensure that the cement microstructure development during setting was not disturbed by the vicinity of the interfaces, thus allowing us to consider the setting mechanism in the sample as being representative.

At  $t = 5'45''$ , low-amplitude oscillatory shear measurements started under 0.05% deformation and at an angular frequency of 6.823 rad s<sup>-1</sup>, corresponding to 1 Hz. It was thus possible to follow the setting of the paste during a sufficiently long period of time without disturbing the setting and hardening dynamic of the sample. Experiments were performed at 25 and 35 °C.

The second rheometrical procedure considered was performed in order to follow the structural evolution of the sample under high deformations, representative of the injection conditions. Procedure 2 consisted of steady shear measurements, repeating procedure 1 three times every 20 min, as schematically represented in Fig. 1c. The shearing period allows the shear-induced breakdown of the structure, which can build-up and develop once again during the following period of rest under a low-amplitude oscillatory shear. This structure development could be followed and recorded during the oscillation period. The steady shear viscosity of the sample during the shearing period was recorded as a function of time.

Each sample was analysed in triplicate. The uncertainty on the rheometrical measurements was around 12%, but we chose not to report the statistical results on the figures to keep them more legible.

## 2.5. Injectability and setting time measurements

Injectability was measured using a TAXT2 texture analyser (Stable Micro Systems) equipped with a specific syringe system, including a 2.5 ml syringe (inner diameter of the syringe body = 9 mm and opening/exit diameter = 2 mm) without a needle. It followed a protocol designed to measure: (i) the force, expressed as a weight to be applied on the piston, needed to extrude a volume of paste corresponding to a displacement of 15 mm of the syringe piston at a constant rate of 2 mm s<sup>-1</sup> (piston surface = 64 mm<sup>2</sup>); and (ii) the weight of the volume of paste extruded.

In addition, the system permits testing commercially available syringes and fits to a surgeon's manipulation.

Briefly, each sample (1.5 g of powder mixture and 1 g of deionized water) was prepared as described in Section 2.2. Measurements were performed at room temperature and 7 min after the paste had been prepared (i.e. mixing of the L and S phases). This period corresponds to the time needed to prepare the paste, to introduce it into the syringe and to leave it at rest for about

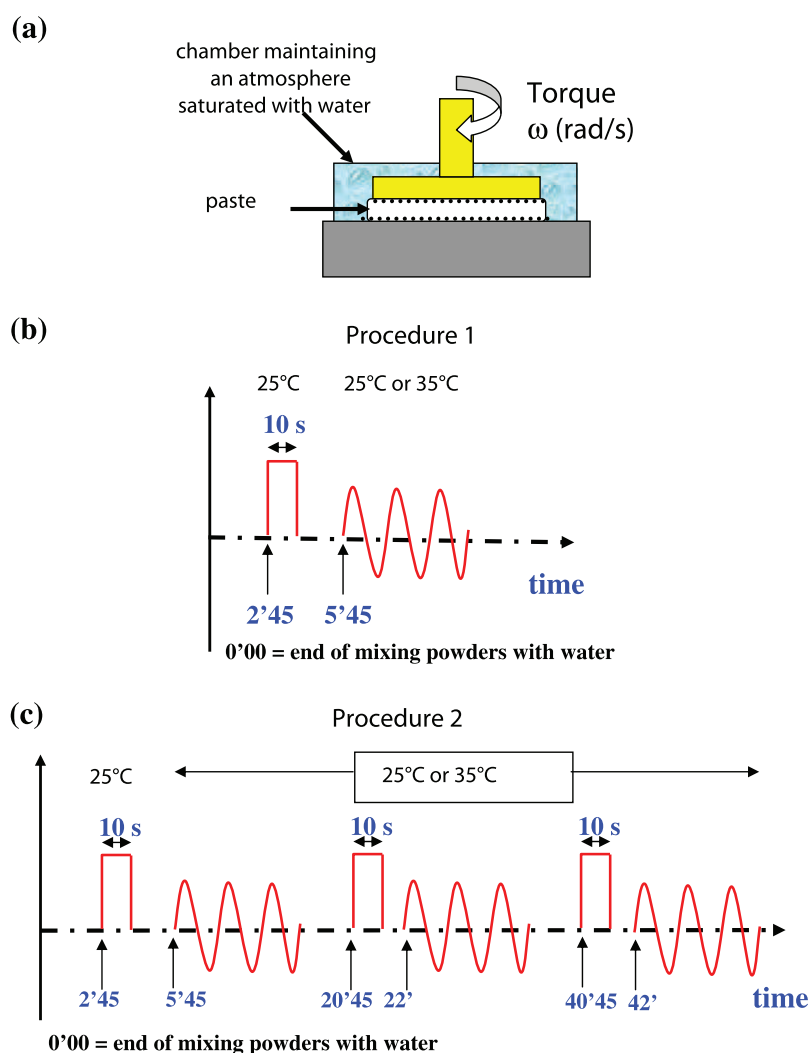


Fig. 1. Rheometrical characterization: schematic representation of (a) the experimental set; (b) procedure 1; and (c) procedure 2.

3 min (like for the resting period in rheometrical procedure detailed in Section 2.4) before starting the injectability measurements. This period could also correspond to the time a surgeon would need to prepare the cement paste and introduce it into a device for implantation by the injection technique.

In addition, a balance was placed under the support of the syringe to weigh the amount of paste extruded at the end of the injection period. This protocol was chosen as it allowed comparison between cements with largely differing rheological properties [25]. The volume extruded corresponding to a displacement of 15 mm of the syringe piston was chosen as it was sufficiently large to minimize the response of the system related to the syringe geometry that could cause small differences between samples.

The setting of the cement was followed as a function of time using a TAXT2 texture analyser (Stable Micro Systems) fitted with a cylindrical needle 1 mm of diameter (surface =  $0.785 \text{ mm}^2$ ). The setting time was considered to be reached when the paste developed a resistance to needle penetration greater than  $600 \text{ g mm}^{-2}$ .

For all measurements, each sample was analysed in triplicate.

### 3. Results

Fig. 2a illustrates the lentil-like morphology of vaterite particles. Their mean diameter and specific surface area were deter-

mined to be  $2.1 \pm 0.5 \mu\text{m}$  and  $33 \pm 11 \text{ m}^2 \text{ g}^{-1}$ , respectively. The morphology of the amorphous calcium carbonate particles is less well defined, as seen in Fig. 2b. Their mean diameter and specific surface area were determined to be  $5.1 \pm 0.8 \mu\text{m}$  and  $22 \pm 7 \text{ m}^2 \text{ g}^{-1}$ , respectively.

Fig. 3 presents the evolution of the viscous modulus ( $G''$ ) as a function of time at 25 and 35 °C following procedure 1. A progressive increase in  $G''$  can be observed during paste setting, and this phenomenon is enhanced by an increase in temperature. For example, the time needed to reach a level of  $G''$  of 3 MPa is 38 min at 35 °C and 96 min at 25 °C. An analogous evolution was obtained for the elastic modulus  $G'$  (data not presented).

The determination of the setting time at 37 °C (setting time = 90 min) and 20 °C (setting time = 370 min) using a texture analyser also revealed the drastic effect of temperature on the setting process of this calcium carbonate self-setting paste.

Physical-chemical characterization of the cement set in reference conditions or during dynamic rheological measurements following procedure 1 was performed. FTIR spectra of both cements are presented in Fig. 4a. When we zoom in on the  $\nu_2\text{CO}_3$  domain (Fig. 4b) we can clearly identify in both cases the presence of carbonate vibration bands at 855 and 713  $\text{cm}^{-1}$ , characteristic of aragonite. Formation of aragonite was also confirmed by X-ray diffraction analysis. In addition, SEM micrographs of the cement set in reference conditions (Fig. 5a and b) and under dynamic

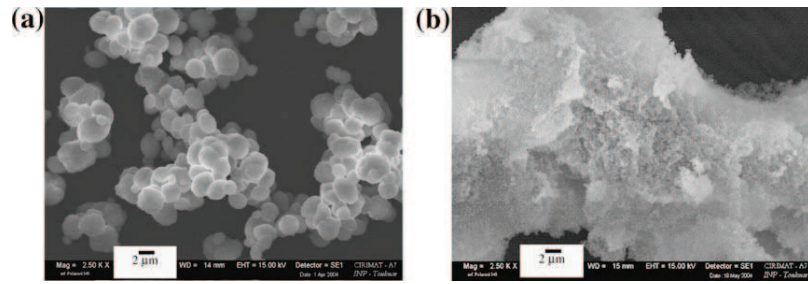


Fig. 2. Scanning electron micrographs (2500× magnification) of the reactive powders used to prepare the cement paste: (a) vaterite  $\text{CaCO}_3$ ; (b) amorphous calcium carbonate.

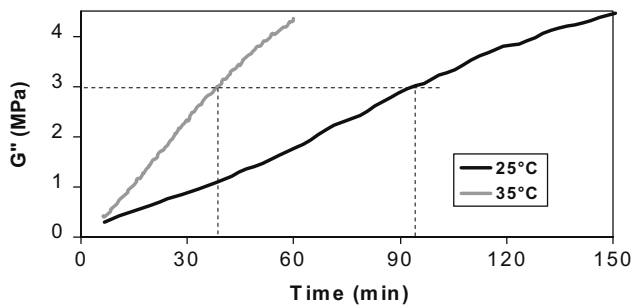


Fig. 3. Evolution of the viscous modulus  $G''$  of the paste as a function of time (procedure 1) and temperature.

rheological measurements (Fig. 5c and d) show the formation of a microporous cement made of an entangled network of aragonite needle-like crystals of about  $2 \mu\text{m}$  in length in both cases. Furthermore, we note in both cases the absence of lentil-like crystals char-

acteristic of vaterite (Fig. 2a) in the set cement (Fig. 5), confirming that the latter fully transformed into aragonite during setting and hardening.

We next examine the results obtained through rheometrical characterization using procedure 2, characterized by shearing periods followed by small-amplitude oscillatory flows.

Fig. 6 presents the evolution of the shear viscosity during the three shearing periods (about 3, 21 and 41 min after paste preparation). It shows that the viscosity of the paste strongly decreases during the applied shear and reaches a plateau after 8 s of flow, revealing the shear-thinning behaviour of this self-setting paste. This phenomenon is also observed even 41 min after paste preparation. The level of viscosity at the end of the paste flow was close to that reached at 21 min, but above that measured during the first shear. In addition, we noted that the initial viscosity level, measured after 2 s of shear, increased with time. This effect may be attributed to the progressive setting of the paste occurring during measurement.

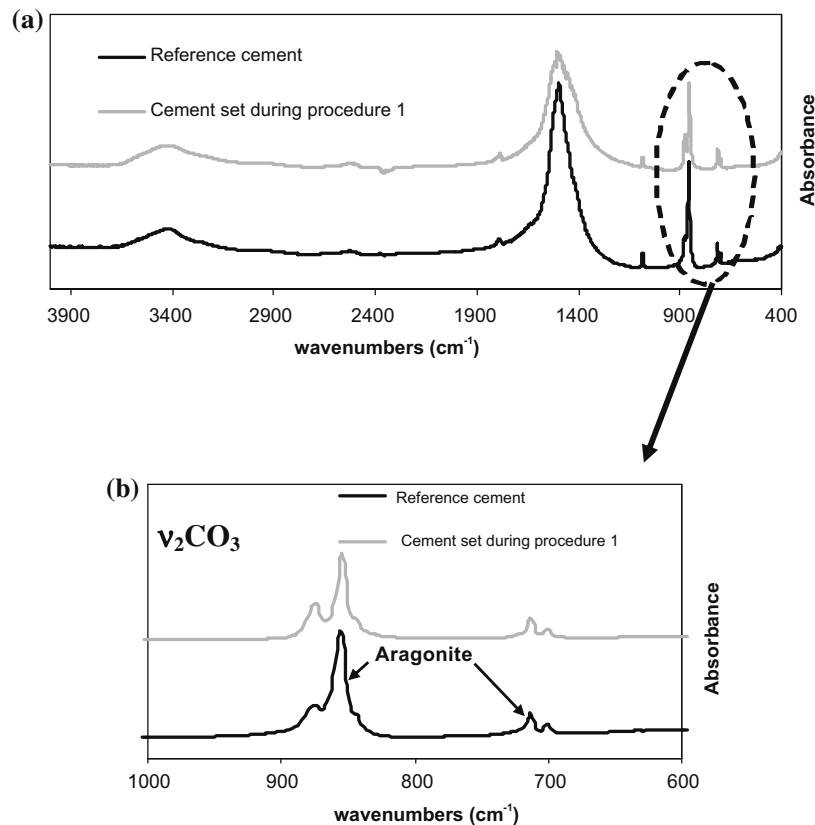


Fig. 4. FTIR spectrum of the cement set and hardened in an oven at  $37^\circ\text{C}$  (reference cement) or during procedure 1: (a)  $400\text{--}4000 \text{ cm}^{-1}$  domain; (b)  $\nu_2\text{CO}_3$  domain.

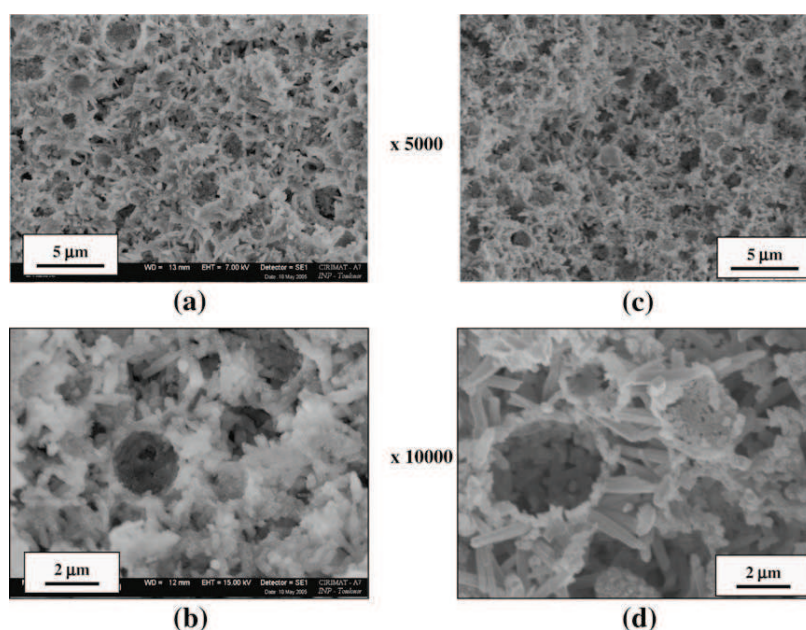


Fig. 5. Scanning electron micrographs (5000 $\times$  and 10,000 $\times$  magnifications) of the cement set in an oven at 37  $^{\circ}$ C (a and b) or during procedure 1 (c and d).

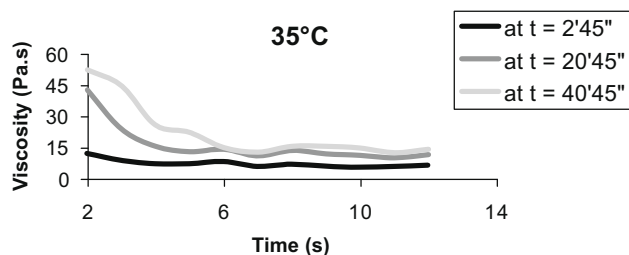


Fig. 6. Evolution of the viscosity of the paste at 35  $^{\circ}$ C and during the flows at constant shear rate (30  $s^{-1}$  during 10 s; procedure 2) imposed at about 3, 21 and 41 min after paste preparation.

The viscous modulus  $G''$  was recorded as a function of time during the small-amplitude oscillatory flows following each shearing period. The results indicate a clear increase in the viscous modulus for tests performed at both 25 and 35  $^{\circ}$ C (Fig. 7). It thus appears that after each of the three flows and following a strong decrease in paste viscosity (Fig. 6) the sample is then allowed to re-build through a progressive setting of the paste left to rest (Fig. 7).

The increase in the viscous modulus is fast and high at 35  $^{\circ}$ C, but moderate at 25  $^{\circ}$ C (Fig. 7). In addition, we note that the level of the viscous modulus  $G''$  is higher all along the setting for tests at 35  $^{\circ}$ C compared to those at 25  $^{\circ}$ C. These results reconfirmed the strong influence of temperature on the (re)development of cement microstructure during setting.

The shear-thinning behaviour revealed by the measurements presented in Fig. 6 was confirmed with the procedure used to characterize the injectability of the paste. Indeed, we note in Fig. 8a a strong decrease in the force needed to extrude a given volume of paste followed by a plateau. It is important to note that the shape of the curve, the weight level measured and the amount of paste extruded ( $1.29 \pm 0.01$  g) are repeatable. This confirmed the reliability of the specific syringe system and protocol set used to evaluate the injectability of this self-setting paste. Fig. 8b presents a photograph of the paste once extruded from the syringe. We see that a continuous and homogeneous “spaghetti” of paste is obtained after extrusion.

## 4. Discussion

### 4.1. Rheological properties

The two rheometrical procedures used to characterize the self-setting calcium carbonate paste are complementary. They both clearly show the influence of temperature on the development of cement microstructure during setting and hardening. In a previous study, we showed that the setting and hardening properties of calcium carbonate cement are due to the progressive dissolution of amorphous calcium carbonate and vaterite, and the crystallization of aragonite crystals. This dissolution–reprecipitation mechanism can be accelerated by an increase in temperature. This mechanism was confirmed in the present study, especially by the absence of the carbonate vibration band characteristic of vaterite at 745  $cm^{-1}$  in the cement set in both conditions (i.e. reference conditions and during rheometrical measurements), which revealed a vaterite dissolution–reprecipitation process during paste setting and hardening.

In addition, it is important to note that the rheometrical protocol involving low mechanical stress on the paste under dynamic measurements (procedure 1) did not prevent the cement setting reaction to occur through recrystallization of amorphous calcium carbonate and vaterite into aragonite (Figs. 4 and 5). In particular, physical–chemical characterizations revealed the development of the microporous cement microstructure resulting in an entangled network of aragonite needles whatever the conditions analysed, i.e. paste set between the parallel-plate geometry of the rheometer and during rheometrical measurements at 35  $^{\circ}$ C or in the reference conditions (Fig. 5). Such stability in the setting process is important for assessing the reliability of the rheological procedures used and should be performed for every rheological property determination on cement paste, although this is rarely done. In addition, the use of such non-destructive procedures represents a decisive advantage for the further investigation of the rheological properties of other bone cements.

The originality of procedure 2 helped evaluate the “longevity” and workability of the paste at different temperatures: (i) room temperature (25  $^{\circ}$ C) for paste preparation; and (ii) close to

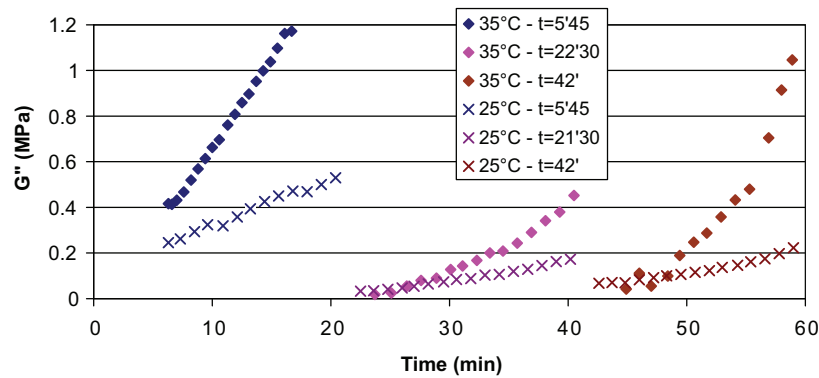


Fig. 7. Evolution of the viscous modulus  $G'$  as a function of time after each flow for the paste tested under low-amplitude oscillatory shear (procedure 2) at 25 and 35 °C.

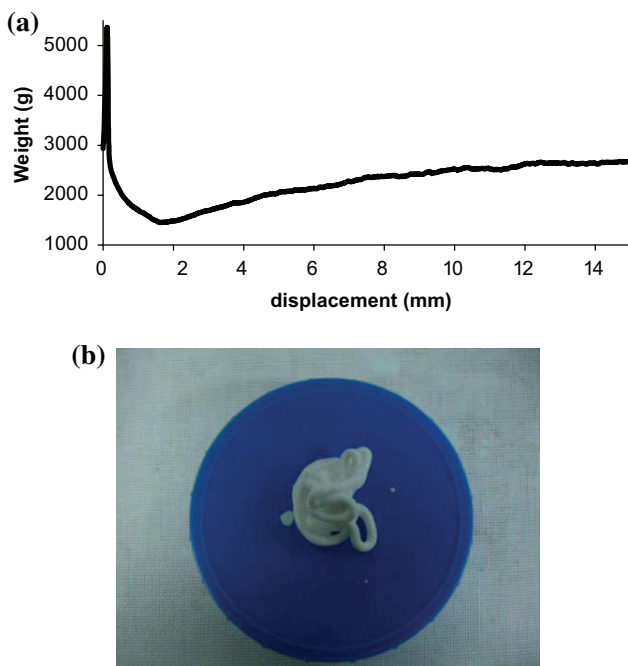


Fig. 8. Evaluation of the injectability of the paste at room temperature: (a) force measured, expressed as a weight to be applied to the piston, to extrude the paste as a function of the syringe piston displacement; (b) photograph of the paste after extrusion from the syringe.

physiological temperature (35 °C) for paste implantation through the injection technique. We showed that the viscosity of paste increased during the rest period but decreased strongly during each shear/flow and reached quite similar values, indicating a fully reversible transformation from paste to viscous liquid due to the shear-thinning properties of this self-setting paste. The shear-thinning behaviour is associated with a destructurement of the paste, corresponding to its fast transformation into a liquid suspension. At rest, under a low oscillatory shear, the progressive recovery of the structure was followed through the increase in the viscous modulus with time (Fig. 7). The capacity of this calcium carbonate self-setting paste to restructure itself after shear, namely thixotropy, depends on both temperature and the time after which paste flow behaviour is examined. One can hypothesize that several phenomena are superimposed, including the setting and hardening of the cement, which are irreversible after undergoing dissolution-precipitation during setting, and the cement thixotropy, which, by definition, is reversible. Both processes are temperature-depen-

dent. It would take further investigation to distinguish these opposite behaviours of the calcium carbonate paste, namely reversible thixotropy and irreversible setting. We are currently using several physical-chemical characterization techniques to investigate which phenomenon is involved in the shear-thinning behaviour associated with the very fast transformation of this calcium carbonate self-setting paste into viscous liquid under stress.

Interestingly, at 35 °C the cement paste rapidly recovered its consistency even if the shear stress was performed more than 41 min after paste preparation. This extended period of testing (60 min) was an overestimate with regard to the paste workability and self-setting ability expected by surgeons using such injectable paste. However, the strong decrease in paste consistency and self-setting rate observed after shearing at 25 °C suggested a risk of “cement washout”, resulting in the disintegration of the paste on contact with tissue or fluids which could limit its implantation using an injection process. To compensate for this high flowability of the paste during its injection, consideration could be given to using a slightly heated syringe to minimize this risk after injection.

#### 4.2. Injectability of the paste

One of the main challenges for injectable hydraulic cements is to better control their injectability and avoid the well-known phenomenon of “filter-pressing”, or the separation of powder particles and liquid within the syringe, which greatly limits their implantation using an injection process. In the literature there are a number of studies on the injectability of calcium phosphate cements based on the weight of paste extruded (i.e. injectability expressed as a percentage of the paste extruded) or the measurement of force or injection pressure needed to extrude a volume of paste [14,17,22]. As already pointed out by several authors, many extrinsic parameters affect injectability measurements, such as the presence of a cannula, its diameter, temperature, the time after which measurements are performed, a prolonged mixing of the paste and the injection environment [25–28]. These factors make comparison of the results presented in the literature difficult.

The protocol and the specific syringe system we designed to evaluate the injectability of this  $\text{CaCO}_3$  self-setting paste combined the measurement of two commonly used quantitative parameters: the force, expressed as a weight to apply on the syringe piston, needed to extrude a volume of paste and the extruded paste weight. It is important to note that we checked whether, once extruded, the paste was homogeneous, set and led to hardened cement with the same composition and microstructure as that obtained for the reference cement.

In addition, the technique used and protocol set led to sensitive characterization of the injectability of the paste. For example,



powder particle size and/or paste L/S variation involved significant change in the force needed for paste extrusion [29]. It is thus necessary to carefully and reproducibly prepare the reactive powders and the paste to obtain reproducible results.

The injectability measurements presented in Fig. 8 confirmed the shear-thinning behaviour of calcium carbonate cement revealed by the rheometrical study as the force needed to extrude the paste decreases strongly and then reaches a plateau corresponding to a weight level to apply to the syringe piston of around 2.5 kg. This reveals the ease of injection of this CaCO<sub>3</sub> self-setting paste. However, we noted that a very slight increase in the weight level needs to be applied to extrude the paste when the piston approaches the bottom of the syringe (see the right part of the curve in Fig. 8a), suggesting that a filter-pressing phenomenon could occur even if it is of low intensity.

On the one hand, this shear-thinning property and the ability of the calcium carbonate self-setting paste to become more liquid under shear stress improved its capacity to diffuse within the whole bone defect and keep homogeneous during injection, thus decreasing the filter-pressing. On the other hand, this property increases the risk of cement washout after injection in vivo. To prevent or minimize such an undesirable phenomenon, it would be of use to customize a system capable of controlling the temperature of the syringe and/or the cannula with a view to finding the best compromise between ease of paste injection and paste cohesion.

## 5. Conclusions

In this study, the rheological characterization of an original calcium carbonate self-setting paste has been carried out in conditions as close as possible to surgeon operating conditions: paste stress preparation and introduction into the syringe at room temperature, and injecting in a wet environment and at a temperature close to that of the body.

The shear-thinning behaviour of this calcium carbonate self-setting paste has been demonstrated both by dynamic rheological and injectability measurements using two analysis techniques: a controlled stress rheometer and a texturometer. Interestingly, this study pointed out the ease of injection of this calcium carbonate paste and demonstrated a limited filter-pressing phenomenon, a workability and a self-rebuilding ability even over an extended period after paste preparation.

Even if it appeared difficult to distinguish the cement setting, which is an irreversible process, from the cement thixotropy, which is a reversible one, the experimental procedures set up in this study, i.e. based on dynamic rheological characterization of this CaCO<sub>3</sub> self-setting paste and involving flow and low-amplitude oscillatory shears, are non-destructive. The setting and hardening processes occurred as observed in reference conditions, and led to a microporous cement made of an entangled network of aragonite-CaCO<sub>3</sub> needle-like crystals. These rheometrical procedures deliver important data with a view to improving and controlling the paste's rheological properties during preparation at room temperature and injection in vivo. They also provide a method to compensate for the lack of a standard procedure for characterizing the rheological properties and injectability of such injectable bone substitutes.

## Appendix Figures. with essential colour discrimination

Certain figures in this article, particularly Figures 1, 7 and 8, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2009.08.032.

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