

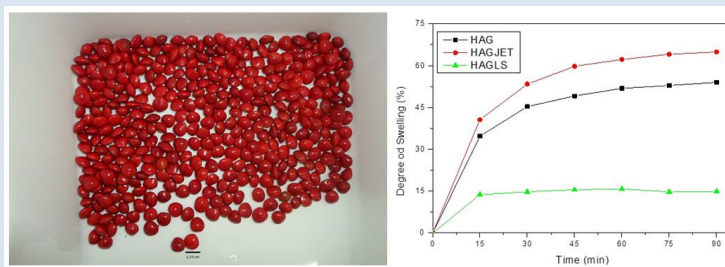
PREPARATION AND CHARACTERIZATION OF CEMENT-BASED HYDROXYAPATITE AND GALACTOMANNAN EXTRACTED FROM ADENANTHERA PAVONINA L. SEEDS

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ABSTRACT

The aim of this study was to prepare and characterize a ceramic matrix cement of hydroxyapatite (HAp) and galactomannan (Gal) extracted from *Adenanthera pavonina* L. seed. Three composites were prepared: the solid phases used were 75 wt% HAp and 25 wt% Gal (HAG) both powder adding to composite 0.30 ml distilled water. The composite HAGJET consists of 0.10 mL catalyst commercial JET® (methyl methacrylate and dimethyl-p-toluidine) and 0.20 mL distilled water (see table 1). The HAGLS composite was composed of 60 wt% HAp powder, liquid phase of 40% Gal solution and 0.10 mL of catalyst commercial LS® (phosphoric acid, zinc oxide, aluminum hydroxide and water) (see table 1). The solid phase in each material was dissolved in the liquid corresponding to each composite formed. The phases of HAp and Gal were characterized by X-ray diffraction. The setting time, flow and solubility were tested according to ISO 6876/2001. Furthermore, the amount of water that each material absorbs was determined, as well as their microhardness and morphology. The data were analyzed using 1-way ANOVA with Student's t-test ($p < 0.05$). All of the materials exhibited good setting time and flow above that allowed by the ISO ($p < 0.05$). The HAGLS composite had solubility of less than 3% ($p < 0.05$), absorbed less water ($p < 0.05$) and had the highest hardness value. The HAG and HAGLS composites showed roughened surfaces, while the HAGJET exhibited a smooth surface. These results suggest that there is potential to develop a sealer using HAp and Gal.

Keywords: Ceramic-matrix cement; Cure behaviour; Hydroxyapatite; Galactomannan.

PREPARAÇÃO E CARACTERIZAÇÃO DE CIMENTOS A BASE DE HIDROXIAPATITA E GALACTOMANANA EXTRAÍDA DAS SEMENTES DE ADENANTHERA PAVONINA L.

RESUMO

Este estudo refere-se à caracterização de um compósito a base de hidroxiapatita (Hap) e galactomanana (GAL) extraída da semente de *Adenanthera pavonina* L. Três compósitos foram preparados: o primeiro compósito é formado por 75% em massa de Hap e 25 % de GAL em fases sólidas utilizando 0,30 mL de água destilada como fase líquida (HAG). O segundo compósito é formado pela mesma proporção de Hap e Gal adicionando 0,20 mL de água destilada e 0,10 mL de catalisador comercial JET® (HAGJET) (ver tabela 1). O terceiro compósito (HAGLS) é composto de 60% em massa de Hap em fase sólida e 40 % de GAL em fase líquida e 0,10 mL de catalisador comercial LS® (ver tabela 1). A fase sólida de cada material foi dissolvida no líquido de cada compósito formado. As fases de Hap e GAL foram caracterizadas por difração de raios-X. Os compósitos foram caracterizados por técnicas de tempo de secagem ou cura, fluxo e solubilidade de acordo com a ISO 6876/2001. Além disso, a quantidade de água absorvida em cada compósito foi observada pelas técnicas de microdureza e morfologia. Os dados foram analisados estatisticamente utilizando o teste t-student com $p < 0,05$. Todos os compósitos apresentaram bons tempode de cura e fluxo. O HAGLS solubilizou abaixo de 3% com baixa absorção de água e um alto valor de microdureza. HAG e HAGLS apresentaram superfícies rugosas, enquanto o HAGJET apresentou superfície lisa. Esses resultados sugerem que os compósitos de Hap e Gal são potencialmente favoráveis a selantes.

Palavras Chaves: Compósitos de matriz cerâmica, comportamento de cura, hidroxiapatita e galactomanana.

1. INTRODUCTION

Endodontics is the field of dentistry that deals with the morphology, physiology and pathogenesis of the pulp tissues and periradicular of human teeth. Endodontic treatment aims to recover the tooth when the pulp or periradicular tissue is damaged by caries, dental wear, accidental exposure during surgical procedures or some trauma [1].

Several materials are used in endodontic treatments, such as folders that take prey or not, gutta-percha associated with sealers and plasticized gutta-percha. Endodontic cement is a material in plastic state that is of fundamental importance to the success of endodontic therapy because it optimizes the sealing process and fills the spaces between the gutta-percha and the walls of the root canal.

Many types of sealers are available in the market and are classified according to their basic constituent. Among them stand out cements based on zinc oxide and eugenol (ZOE), calcium hydroxide, resin, glass ionomer and – the latest – mineral trioxide aggregate (MTA) [2-3].

Grossman (1982) established certain requirements for a sealer: good setting time, radiopaque and insoluble to oral fluids, presenting a good flow and easy to handle. Furthermore, they must present excellent hermetic sealing, be biocompatible and not induce the growth of microorganisms [4]. However, no commercial sealer has all these characteristics.

The endodontic market has intensified its search for new materials with satisfactory results and all of the requirements for a root canal sealer. Recently, reports have shown that the use of hydroxyapatite (HAp) in combination with other materials can improve the properties of sealers because of its excellent biocompatibility and bioactivity, which benefit developing bone and forming chemical bonds with the host tissue [5].

Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is a synthetic bioceramic that has already been used in the medical field with high success rates, by virtue of its excellent biocompatibility and bioactivity, therefore benefiting bone development and the formation of chemical connections with the host tissue [6].

The high biocompatibility of HAp with the biological system occurs due to chemical similarity between HAp and the mineral phases of bone and teeth. The bioactivity of HAp promotes dentin

formation by depositing a surface layer of apatite in the presence of pulpal fluid. This deposition aids in maintaining the integrity of the pulp tissue and induces the formation of neodentina more quickly and efficiently [6].

Recently, a study was conducted on BisGMA/TEG, the combination of bisphenol A glycidyl methacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGMA) with hydroxyapatite for root canal treatment [7]. Other studies have been performed using HAp, including an evaluation of the physicochemical properties of the sealer MTA associated with hydroxyapatite and DCPD (calcium hydrogenphosphate dehydrate) for endodontic treatment [8], the effect of nano-hydroxyapatite in vivo on the remineralization of toothpaste enamel, dentin surface lesions [9] and the improvements in the mechanical properties of the adhesive layer and the bond strength of dentin by incorporating fibrous crystalline hydroxyapatite nanoparticles [10].

In this study, we used a natural biopolymer – galactomannan extracted from seeds of *Adenanthera pavonina* L. – to produce a cement in combination with HAp. This biopolymer was selected because it is easy to obtain and greatly abundant in nature, a biocompatible bonding agent and does not have any value in its acquisition, thus making it an economically viable material. Moreover, it presents biocompatibility and high stability, is non-toxic and non-carcinogenic, and acts as a binder, thus preventing the dispersion of the cement [11].

The aim of this study was to obtain and characterize cement-based hydroxyapatite (HAp) and galactomannan (Gal) extracted from *Adenanthera pavonina* L. seeds, in order to find satisfactory results and meet the requirements established for endodontic cement..

2. MATERIALS AND METHODS

2.1 Materials

Galactomannan extraction from *Adenanthera pavonina* L. seeds. Gal was extracted from *Adenanthera pavonina* L. seeds (1,25 cm of diameter in mean); see Fig. 1 (collected at the Federal University of Ceará – [UFC], Fortaleza, Ceará). The method consisted of heating the seeds in distilled water for 30 minutes to 100°C and subsequent swelling them for a period of 24 hours. Then, the seeds were washed and the endosperms

were separated manually from the embryo and integument. Afterwards, the endosperms were dehydrated and sprayed in order to prepare the cements [12-13] (Laboratory of Biomaterials – UFMA, Imperatriz, Brazil).



Figure 1. Seeds *Adenanthera pavonina* L.

Synthesis of Hydroxyapatite (HAp). Hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ was synthesized by mechanical milling with a high-energy planetary ball mill, a Fritsch Pulverisette 5. The components used were calcium hydroxide – $[\text{Ca}(\text{OH})_2]$ (Sigma/Aldrich, Brazil) and calcium monohydrogen phosphate $[\text{CaHPO}_4]$ (Sigma/Aldrich, Brazil) in stoichiometric amounts. The milling was carried out for 20 hours at 370 rpm rotation [4]. A 10-min break followed every 30 min of milling, in order to avoid excessively heating up the mill [14-16] (Laboratory of Biomaterials – UFMA, Imperatriz, Brazil).

Preparation of Cements. The cements were formulated from a solid phase and a liquid phase. The solid phase comprised hydroxyapatite and galactomannan extracted from *Adenanthera pavonina* L. seeds, while the liquid phase consisted of distilled water, liquid JET (methyl methacrylate and dimethyl-p-toluidine) and liquid LS (phosphoric acid, zinc oxide, aluminium hydroxide).

Three types of cements were developed, for which the amounts of liquid and solid varied. For the preparation of the first cement, called HAG, 75 wt% of HAp and 25 wt% of Gal were used for the solid phase, while the liquid phase comprised 0.30 mL of distilled water (see Table 1). The second cement, designated HAGJET, was produced using 75 wt% of HAp and 25% of Gal powder. For the liquid phase, 0.10 mL of catalyst commercial JET

(Clássico, Brazil) and 0.20 mL of distilled water were used (see Table 1).

Table 1. Composites Nomenclature

Material	Description
HAG	75 wt% of HAp and 25 wt% of Galactomannan (Gal) + 0.30 mL water
HAGJET	HAG + 0.10 mL of methyl methacrylate and dimethyl-p-toluidine (JET catalyst commercial) and 0.20 mL water
HAGLS	60 wt% of HAp + 40 wt% Galactomannan (Gal) solution + 0.10 mL of phosphoric acid, zinc oxide, aluminum hydroxide and water (LS catalyst commercial)

The solid phase of the third cement (HAGLS) comprised 60 wt% HAp; the liquid phase was formed with 40% Gal solution and 0.10 mL of commercial LS (Coltene, Brazil) as a catalyst. These amounts were determined using empirical methods. The samples were prepared according to the traditional method. The solid phase in each material was dissolving in the liquid corresponding to each biocement formed. The cements HAG, HAGJET, and HAGLS were developed at the Laboratory of Biomaterials – UFMA, Imperatriz, Brazil.

2.2 X-Ray Diffraction Analysis

X-ray diffractions were obtained using a Rigaku Miniflex X-ray II diffractometer, using Cu K α radiation at 40 mA and 30 kV. Intensity dates were collected using the step counting method (step 0.02° in 2 s) in the range 2 θ (10–60°) (Laboratory of X-ray Diffraction – UFMA, Imperatriz, Brazil). The Joint Committee on Powder Diffraction Standards (JCPDS) database was used to identify the crystalline phases of HAp. The crystallite size (L_c) of the HAp was calculated by Scherrer's equation [17]:

$$L_c = \frac{k\lambda}{\beta \cos \theta} \quad (\text{Eq. 1})$$

Where k is the shape coefficient (value between 0.9 and 1.0), assuming coefficient $k = 1$; λ is the wavelength ($\lambda = 0.15418$ nm for radiation CuK α); β is the full width at the half maximum (FWHM) of each phase; and θ is the diffraction angle. We used the LaB $_6$ (SRM 660 – National Institute of Standard Technology) powder standard pattern to determine the instrumental width ($w_{\text{inst}} = 0.087^\circ$) [17]. The β

parameter has to be correct using the following equation, in which w_{exp} corresponds to the half width of each phase:

$$\beta = \sqrt{w_{exp}^2 - w_{ins}^2} \quad (\text{Eq. 2})$$

2.3 Setting Time

The setting times of the HAG, HAGJET and HAGLS cements were recorded according the International Organization for Standardization's – ISO 6876/2011 [18]. The prepared biocement samples were then placed in a mold with 10 mm diameter and 3.5 mm height.

A Gilmore needle with a weight of 100 g and an active tip of 2.0 mm diameter was used to determine the setting time. The needle was lowered vertically onto the horizontal surface of the cements, and the setting time was identified as the point when the inserted needle failed to make an indentation. The materials were tested every 10 min until cured.

The setting time was determined as the time elapsed between the time of preparation for the cement until the moment when the needle did not mark the surface of the samples. Three determinations for each cement sample were taken (Laboratory of Biomaterials – UFMA, Imperatriz, Brazil).

2.4 Flow

The flows of the sealers were tested according to ISO 6876/2001 [18]; volumes of 0.05 ± 0.005 mL mixed sealers were placed on the center of a glass plate by using a graduated, disposable 1 mL syringe. Three min later, a second glass plate weighing 20 g and a 100 g weight were placed centrally on top of the cements. After 10 min from the start of homogenization, the load was removed and the minimum and maximum diameters of the sample disks were measured by a digital caliper with a resolution of 0.01 mm. If the disks were not uniformly circular (the maximum and minimum diameters were not within 1 mm), the test was repeated. Three tests were taken for each cement, and the mean, expressed in millimeters, was considered to be the flow of the material (Laboratory of Biomaterials – UFMA, Imperatriz, Brazil).

2.5 Solubility

The solubility of HAG, HAGJET, and HAGLS was determined in accordance with ISO standard

6876/2001 [18]. After the homogenization of the cements, they were placed in Teflon rings with 20 mm diameters and 1.5 mm thick, up until completely filled. The molds were placed in an incubator (37 °C, > 95% relative humidity) for a period of time that was 50% longer than the setting time. The cements were removed from the molds and weighed with an accuracy of 0.0001 g (SHIMADZU AVY 220 analytical balance, Brazil). The samples of each cement were put in a Petri dish, which was weighed before use and contained 50 mL distilled water. After 24 hours in the incubator (37 °C, > 95% relative humidity), the samples were rinsed with 2–3 mL distilled water, and the washings were allowed to drain back into the Petri dish. The samples were then discarded, and the Petri dishes were dried in an oven at 110 °C, cooled in the desiccator to room temperature and reweighed. The amounts of cement removed from each specimen were calculated as the difference between the initial mass and the final mass of the Petri dish. Three tests were taken for each cement (Laboratory de Biomaterials – UFMA, Imperatriz, Brazil).

2.6 Degree of Swelling

To determine the degree of swelling, the cements were first weighed with an accuracy of 0.0001 g (SHIMADZU AVY 220 analytical balance, Brazil). Afterwards, they were placed in a Petri dish with 50 ml of distilled water and kept for different periods of time at room temperature. After the pre-established time intervals, the samples were removed with the aid of metal tweezers, carefully blotted with filter paper sheets to remove the excess water and reweighed. The period corresponding to the immersion time was 90 min, with 15-min intervals. The degree of swelling was determined by the swollen mass (m') difference of the dry mass sample (m) divided by the dry weight. The result was expressed in percentage. Three determinations were carried out for cement. (Laboratory of Biomaterials – UFMA, Imperatriz, Brazil).

$$GI = \frac{m' - m}{m} \quad (\text{Eq. 3})$$

2.7 Vickers Microhardness

To analyze microhardness, a Shimatzu microdurometer was used with a Vickers diamond indenter, using a 50 gf load for 30 seconds. Three

determinations were carried out to sample each cement (Physics Department of Aveiro University – UA, Portugal).

2.8 Morphology

The cement samples were fixed in a metal holder on a carbon tape and carbon with a metallic layer. The analyses were carried out using a TESCAN VEGA3 scanning electron microscope at room temperature and 30 kV. (Physics Department of Aveiro University – UA, Portugal).

2.9 Statistical analysis

For each test, the data were statistically tabulated, described through graphs and percentage frequency tables and analyzed using analysis of variance (ANOVA) and Student's t-tests ($p < 0.05$).

3. RESULTS AND DISCUSSION

In dentistry, it is important for the constituents of cement to have nanometric crystal sizes and adequate morphology, in order to facilitate their penetration into the dental tubules [19], as well as to control the interaction of the material with proteins (adsorption, configuration and bioactivity), modulate a perfect adhesion with osteoblasts and have long functionality [20]. Therefore, the crystallinity of HAp and the crystallite size were determined by X-ray diffraction.

Fig. 2 shows the characteristic peaks of the hexagonal crystalline lattice of HAp according to the data sheet for the JCPDS standard n°. 203027. The bioceramic showed an average crystallite size of $23.61 \text{ nm} \pm 0.70$, confirming that HAp is nanocrystalline as single phase formed. The amorphous nature of the polysaccharide has been confirmed by the presence of broad peaks that were not delimited and of low intensity (Fig. 3). Figure 2 shows that the XRD pattern of the HAp synthesis produces a material with a high crystalline content without the need for heat treatment. Fig. 3 shows the XRD pattern of Gal with broad, unconfined peaks and an amorphous structure.

Teeth are essentially formed by enamel, dentin, cementum and pulp. The enamel and dentin contain inorganic (hydroxyapatite) and organic (natural polymers and water) material, and presents dentin tubules designated as intercanalicular dentin, rich phase in the organic matrix and peritubular dentin [5]. The diffractogram of Gal (Fig. 3) confirms that

the material is a polymer; thus, teeth are also formed by natural polymers. Galactomannan is a natural polymer that subtends the use of this material and provides a better acceptance for the interaction of cement with dental walls.

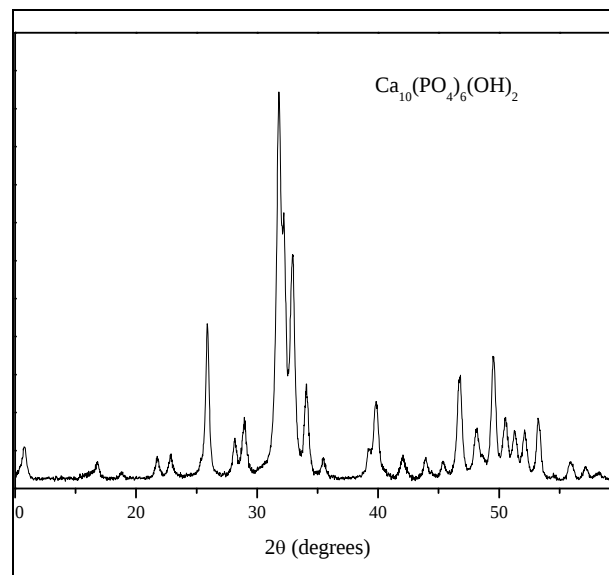


Figure 2. XRD pattern of hydroxyapatite.

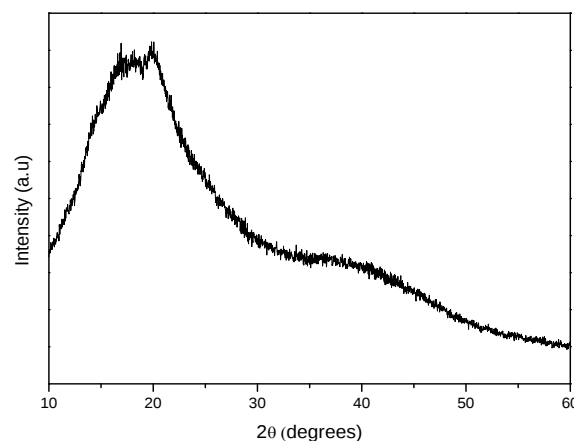


Figure 3. XRD pattern of galactomannan.

Amorphous apatites are also present in tooth formation, comprising about 20 to 30% of teeth. The remaining amorphous material is very important because it can be absorbed quickly when there is the need for additional calcium in physiological fluid [21]. This feature also helps in use the Gal, because the XRD pattern (Fig. 3) showed that Gal is amorphous, thus helping in the resorption of the material, if necessary [22].

A swelling test was performed to verify the amount of solvent that the cements absorbed. Fig. 4 shows that the HAGJET cement absorbed the largest amount of water, while the HAGLS cement absorbed the least amount of solvent. Statistically, there was no significant difference in water absorption between the HAG and HAGJET cements. Additionally, there were no differences in absorption between the HAG and HAGLS cements and the HAGJET and HAGLS cements.

The means and standard deviations of the setting time, flow and solubility for the HAG, HAGJET, and HAGLS cements are shown in Table 2. The setting time of each material showed significant differences ($p < 0.05$). Among the cements, HAG had the highest setting time due to the absence of an activator when preparing the material. The flow of cement was greater than 20 mm, which is consistent with the recommendation of ISO 6876/2001 [18], with no significant difference between the flow values ($p < 0.05$). Of the materials tested, only HAGLS presented solubility within the limits allowed by ISO (mass fraction less than 3%). Statistically significant differences were found between the HAG and HAGLS and HAGJET and HAGLS cements ($p < 0.05$), while the HAG and HAGJET had no significant difference.

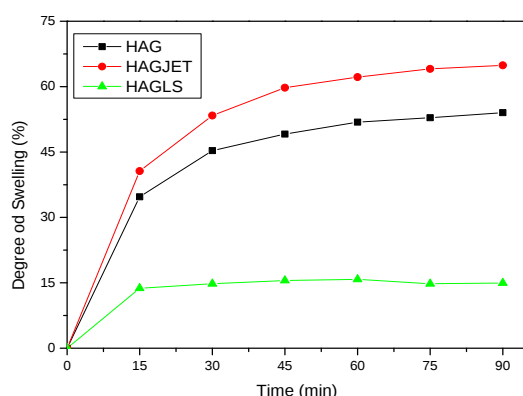


Figure 4. Swelling cement HAG, HAGJET and HAGLS in immersion time function in water.

The setting time of sealers is clinically important because it suggests the time available to carry out the treatment after the cement is prepared. This time can neither be too long nor too short, since an extended setting time can damage the clinical behavior and favor the deterioration of the cement, while a short setting time may not be sufficient for

the complete root canal treatment [22].

The activators reduced the setting time when added because they accelerated the setting reaction of the material. However, when comparing the setting times between the cements in which the chemical activators were used (HAGJET and HAGLS), HAG exhibited the highest setting time, due to the presence of the distilled water used to prepare the cement.

When comparing the setting times of the cements (Table 2) with some commercial sealers, such as Endosequence BC®: 162 min; MTA Fillapex®: 150 min; AH PLUS®: 690 min; ThermaSeal®: 1380 min; PCS®: and 1578 min, as analyzed by Zhou et al. (2013) [23], that the studied cements had shorter setting times.

The flow capacity of the root canal system sealers depends on the particle size, setting time, temperature, rate insertion and internal diameter of the channels [24]. Thus, the cement with the highest flow showed the greatest setting time (Table 2). Although there small statistically significant differences between the flux values, the HAGJET cement demonstrated the lowest flow due to the presence of JET liquid, which provides a contraction as the material cures.

The HAG and HAGJET cements exhibited higher solubility than that permitted by the ISO standard [18], which may be attributed to the volume of water used in cement preparation, because the higher the water content of the material, the greater its greater solubility. The HAGJET provided greater solubility than the other cements because it has a larger amount of polymers during preparation (the Gal and methyl methacrylate present in the liquid JET).

The presence of the polymer can also influence the mass loss, as they have the ability to generate processes of adsorption and dispersion in the water/cement system, favoring the exposure of the material to react with water [25]. Although the HAG and HAGJET cements have showed solubility above that permitted by the standard, some commercial sealers analyzed by Borges et al. (2012) [26] also exhibited high solubility rates: iRoot SP® (20.64%), MTA Fillapex® (14.89%) and Sealapex® (5.65%).

HAGJET has higher water absorption due to the properties of the its material constituents, as well as the presence of galactomannan, which displays

structure glycosidic units – usually three hydroxyl groups forming hydrogen bonds with water molecules trapping these molecules, thus benefiting the hydration of the polysaccharide. Other polymers are contained in the cement, such as methyl

methacrylate monomer, whose hydrophilic characteristics are attributed to the presence of polar units in its polymer chain [27], which helps to increase the material's water absorption.

Tabla 2: Setting time, Confidence intervals, flow, solubility and microhardness (mean and standard deviation, n =3 for each material). Data with different superscript letters are statistically different ($p < 0.05$).

Material	Setting time (min)	Confidence Intervals (%)	Flow (mm)	Solubility (%)	Microhardness (MPa)
HAG	183.33 (5.77) ^a	3.14	26.00 (0.71) ^a	4.28 (0.96) ^a	325.6 (0.21) ^b
HAGJET	143.33 (5.77) ^b	4.03	24.13 (0.54) ^a	4.77 (0.50) ^a	203.8 (0.36) ^c
HAGLS	116.66 (5.77) ^c	4.94	25.35 (0.43) ^a	2.82 (0.015) ^b	470.7 (0.45) ^a

Water absorption can promote the expansion of the material, thus improving the sealing ability of the cements studied [28]. However, high water absorption may decrease the cement's mechanical properties, as can be seen in Fig. 4, which shows that HAGJET absorbed more water and demonstrated low hardness (Table 2). The presence of hydrophilic compounds decreases the mechanical properties of the cements. The HAGLS cement showed the highest hardness value, probably due to its low quantity in hydrophilic compounds.

The microhardness data of the cements (Table 2) show that the material hardness (HAGJET < HAG < HAGLS) decreased as the hydrophilic structures in the cements increase. Fig. 3 shows that a greater amount of water was absorbed by HAGJET, which consequently showed the lowest hardness value. By contrast, HAGLS exhibited the highest hardness value due to its low water absorption. The hardness value of each material showed significant differences ($p < 0.05$).

Figs. 5 (a, b and c) show the HAG, HAGJET and HAGLS samples, respectively, with a 20,000X (5a and 5b) and 10,000X (5c) amplification factor. Fig. 5a shows the micrograph of the HAG cement, which reveals a rough surface with faceted and needle aspects. The microphotograph of HAGJET showed a smoother surface with less surface roughness, suggesting a change in the average particle size (Fig. 5b). Fig. 5c shows that the HAGLS cement has a rough surface with plate-shaped particles, forming aggregate. The SEM images (Figs. 5a and 5c) showed that the HAG and HAGLS cements have

rough surfaces. The roughness may favor better adhesion of the material with the dental walls. However, an excessive increase may lead to greater surface area and can thus contribute to the retention of dyes and plaque buildup [29]. This surface roughness is characterized by micro-roughness on the surface of the cement due to the production process or by the difference in the sizes of the particles present in the sample.

4. CONCLUSIONS

On the basis of the results obtained, all of the cements were formed in viable way, which confirmed the possibility of cement use in endodontics as an innovative material. The main innovation is the presence of hydroxyapatite associated with a natural polymer: galactomannan extracted from *Adenanthera pavonina* L. seeds. This polymer had the function of acting as a binder and prevent the dispersion of the material in the presence of fluids.

5. ACKNOWLEDGEMENT

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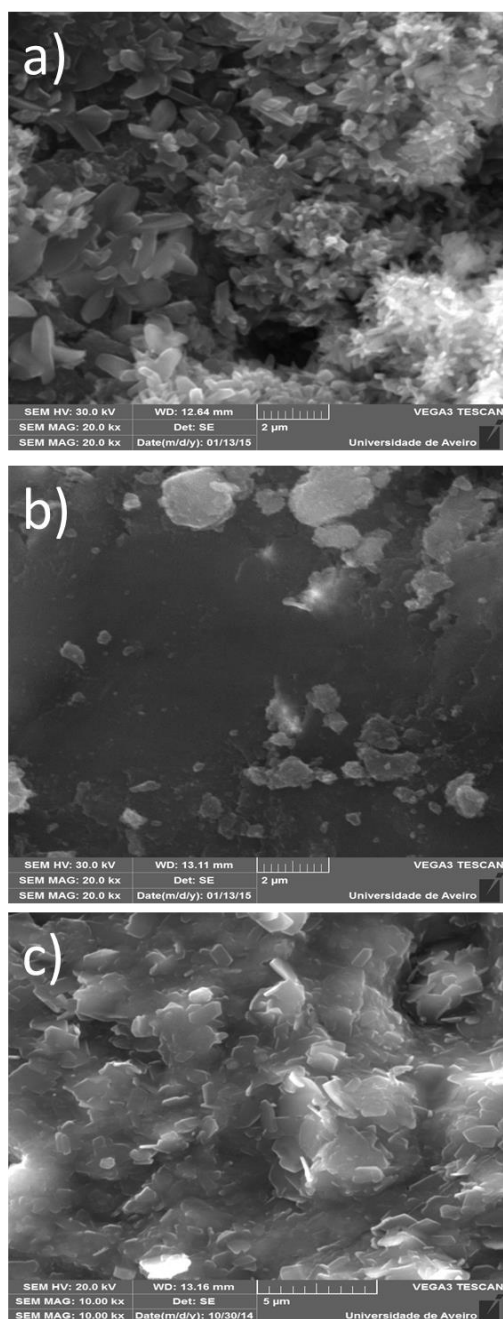


Figure 5. (a)-(b) Micrographs cement HAG and HAGJET with 20000X. (c) Micrograph cement HAGLS with 10000X.

6. REFERENCES

- [1]. R.V. Noort, Introduction to Dental Materials. Elsevier, Rio de Janeiro, Brasil (2010)
- [2]. M.A. Blanco, Incorporation of evaluation chitosan nanoparticles to cement endodontic AH PLUSTM. Rio de Janeiro, Brasil (2011)
- [3]. R. Bernardes, A.A. Campelo, D.S.S. Junior, L.O. Pereira, M.A.H. Duarte, I.G. Moraes, C.M. Bramante, Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics **109** (2010), 47-49.
- [4]. G.T.M. Candeiro, F.C. Correia, M.A.H. Duarte, S.D.C. Ribeiro, G. Gavini, J. Endod. **38**, (2012), 842-47.
- [5]. M.B. Mansur, I.M. Oliveira, V. Seshadri, Journal of Chemical Engineering **82** (2004). 956-967.
- [6]. C.S. Delfino, C. Ribeiro, G.F. Vieira, A.H.A. Bressian, M.L. Turbino, Ceramics **56** (2010), 381-388.
- [7]. L. Chen, Q. Yu, Y. Wang, H.A.O. Li, Dental Materials **27** (2011), 1187-1195.
- [8]. R.P. Vitti, C. Prati, M.A.C. Sinhoreti, C.H. Zach, M.G.S. Silva, F.A. Ogliari, E. Piva, M.G. Gandolfi, Dental Materials **29** (2013), 1287-1294.
- [9]. P. Tschoppe, D.L. Zandim, P. Martus, A.M. Kielbassa, Journal of Dentistry **39** (2011), 430-437.
- [10]. M.S. Shojai, M. Atai, A. Nodehi, L.N. Khanlar, Dental Materials **26** (2010), 471-482.
- [11]. S. Sumathi, A.R. Ray, Journal of Pharmacy & Pharmaceutical Sciences **5** (2002), 12-18.
- [12]. A.A.M. Macedo, A.S.B. Sombra, S.E. Mazzetto, C.C. Silva, Composites **44** (2013), 95-99.
- [13]. C.E.A. Soares. Structural Characterization and Potential of galactomannan *Adenanthera pavonina* L. as the raw material for production of bioactive edible films, 2009.
- [14]. C.C. Silva, A.G. Thomazine, A.G. Pinheiro, J.F.R. Lanciotti, J.M. Sasaki, J.C. Goes, A.S.B. Sombra, Journal of Physics and Chemistry of Solids **63** (2002) 1745-1757.
- [15]. C.C. Silva, A.G. Pinheiro, M.A. Miranda, J.C. Goes, A.S.B., Solid State Sciences **5** (2003), 553-558.
- [16]. C.C. Silva, M.P.F. Graça, A.S.B. Sombra, M.A. Valente, Physica B **404** (2009), 1503-1508.
- [17]. C.C. Silva, H.H.B. Rocha, F.B.A. Freire, M.R.P. Santos, K.D.A. Saboia, J.C. Goês, A.S.B. Sombra, Materials Chemistry and Physics **92**(2005), 260-268.
- [18]. International Organization for Standardization, ISO-6876. Dental root canal sealing materials. Geneva: ISO; 2001.
- [19]. E. Assmann, Bond strength to dentin of the evaluation of MTA-based cements and based on epoxy resin, through the micro push-out test. Porto Alegre: 2010.
- [20]. R.K. Scarparo, F.S. Grecca, E.F.V. Fachin, J.

Endod **35** (2009), 229-232.

- [21]. D.S. Gouveia, A.C.S. Coutinho, J.C., Key Engineering Materials **41** (2008), 2049-2056.
- [22]. N.A. Allan, R.E. Walton, M. Shaffer, J Endod. **27** (2001), 421-423 and Le Geros, R. Z., Calcium phosphate in oral biology and medicine. Monographs in oral Science **15** (1991) 1-20.
- [23]. H. Zhou, Y. Shen, W. Zheng, L.I. Li, Y. Zheng, M. Haapasalo, JOE **39** (2013), 1281-1286.
- [24]. D. Ørstavik, Endod Topics **12** (2005), 25-38.
- [25]. W. Zhang, Z. Li, B. Peng, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod **107** (2009), 79-82.
- [26]. R.P. Borges, M.D. Sousa-Neto, M.A. Versiani, Int Endod J **45** (2012), 419-428.
- [27]. I. Küçük, A. Kuyulu, O. Okay, Polymer Bulletin **4** (1995), 511-516.
- [28]. R.P. Vitti, C. Prati, M.A.C. Sinhoreti, C.H. Zanchi, M.G.S. Silva, F.A. Ogliari, E. Piva, M.G. Gandolfi, Dental Materials **29** (2013), 1287-1294.
- [29]. P. Mourouzis, E.A. Koulaouzidou, L. Vassiliadis, M. Helvatjoglu-Antoniades, J Or Sci **51** (2009), 607-14.