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Synthesis and characterization of a new methacrylate monomer derived from the cashew nut shell liquid (CNSL) and its effect on dentinal tubular occlusion

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ABSTRACT

Objective. The aim of this study was to synthesize, to characterize and to evaluate the effects on tubular occlusion of new monomer derived from cashew nut shell liquid (CNSL), also studying the effects of acid challenge (AC) on dentin surfaces treated with desensitizers.

Methods. The intermediary cardanol-epoxy (CNE) was synthesized through epoxidation of CNSL, followed by synthesis of cardanol-methacrylate-epoxy (CNME) through methacryloyl chloride esterification. Products were purified through chromatography column and characterized by Fourier transform infrared spectrometry and nuclear magnetic resonance. Resinous dentin desensitizers were formulated containing either unsaturated cardanol (CNU), CNE or CNME. Dentin disks were divided into seven groups: SL — Smear-layer, EDTA — EDTA-treated only, GLUMA — Gluma Desensitizer, OCB — One Coat Bond, CNU — CNU desensitizer, CNE — CNE desensitizer and CNME — CNME desensitizer. Dentinal fluid rate (DFF) was obtained using a Flodec equipment and tubular occlusion employing a scanning electron microscope (SEM), before and after AC. Data of DFF were submitted to two-way ANOVA and Tukey's test ($p < 0.05$).

Results. GLUMA showed the lower reduction in DFF when compared to the other products, which were statistically similar. Even after AC, CNME presented the most homogenous and occluded surface, while CNE and CNU were partially removed, GLUMA was completely removed and OCB keep an occluded, but irregular surface.

Significance. CNME showed a great reduction of DFF and a homogenous occluded surface, suggesting that it may be a suitable and acid-resistant treatment option for dentine hypersensitivity.

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1. Introduction

Hypersensitive dentin (DH) is a common oral health issue in the adult population, associated with exposed cervical dentin and characterized by short and sharp pain in response to several external stimuli [1,2]. This significant increase in the dentin sensitivity is explained by the most widely accepted “hydrodynamic theory” proposed by Brännström et al. [3]. Such explanation advocates that movements of fluid within the dentinal tubules stimulate pulpal mechanoreceptors, which are interpreted as pain. This clinical condition affects directly the quality of life, thereby motivating the investigations of mechanisms leading to DH and the development of a wide range of treatment methods to prevent, reduce or eliminate this condition [4].

Several desensitizing agents have been assessed for the treatment of DH, relying on two approaches: nerve desensitization or occlusion of patent dentinal tubules [5]. However, in agreement with the hydrodynamic theory [3], most of the currently employed agents are used to occlude the dentinal tubules, once this strategy may suppress dentinal fluid movement regardless the stimuli evoked, thereby decreasing DH. Although many studies have reported the initial efficacy of these agents, most of them have their therapeutic effects readily arrested or diminished over time due to daily tooth brushing or intermittent consuming of acidic beverages [6].

Gluma Desensitizer (Hereaus Kulzer, Hanau, Germany) is one of the desensitizing agents with the longest history of use in dental clinics. Its mechanism of action relies on precipitation of plasma proteins within dentinal tubules thanks to the high capacity of glutaraldehyde in promoting protein crosslinking. Nevertheless, such compound is highly detrimental to the metabolism of odontoblast-like pulp cells (MDPC-23) [7] and possesses high dissolution capacity in acidic solutions [8], what might cause several cytotoxic issues. Recently, several more biocompatible plant-derived products were investigated in order to replace glutaraldehyde as dentin collagen crosslinking agent [9]. In this regard, new monomers could be created from these plant-derived compounds yielding improved desensitizing agents.

Cashew nut shell liquid (CNSL) emerges as an abundant renewable resource of naturally occurring phenols, also highlighting affinity with dentin collagen and potential to form crosslinking [10]. After the industrial thermal treatment, CNSL is mainly composed by cardanol (approximately 70%), a long carbon chain phenol (Fig. 1), which grants this compound high hydrophobicity and many reactive sites for organic synthesis [11]. Besides, it does not depict cytotoxic or mutagenic effects [12]. Advances in the development of long-lasting dentin sealing technologies should achieve acid-resistant desensitizing agents. Therefore, the synthesis of a methacrylate epoxy cardanol monomer could obtain biocompatible, hydrophobic and acid-resistant agent, once the oxirane ring can open in acid environments [13,14] and act as a latent polymerization improving the material over time.

The aim of this manuscript was to synthesize, to characterize and to evaluate the effects on dentin desensitization (tubular occlusion) of new methacrylate monomer derived from the CNSL, also studying the effects of acid chal-

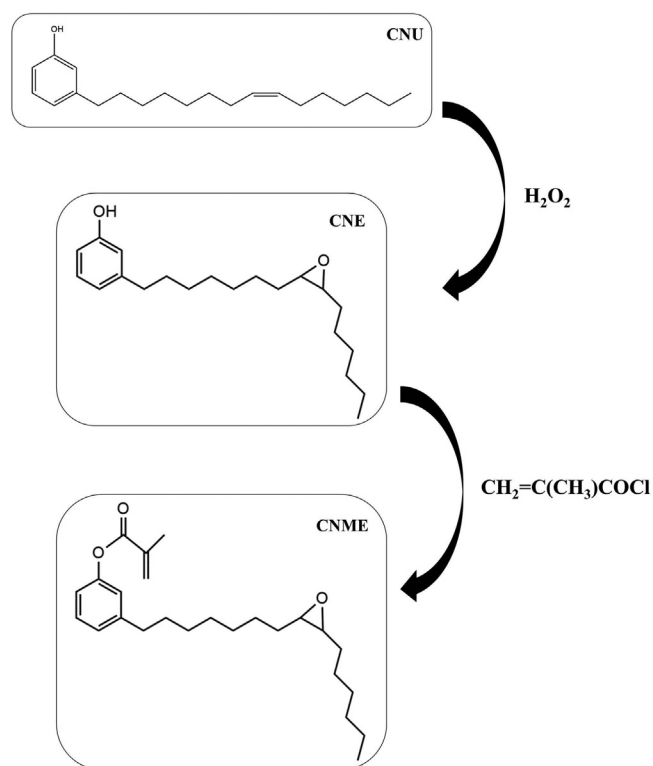


Fig. 1 – Synthetic route: unsaturated cardanol (CNU) was epoxidized with hydrogen peroxide, using formic acid as a catalyst, in order to incorporate an oxirane ring where the unsaturation was located and form cardanol-epoxy (CNE). CNE was esterified with methacryloyl chloride, in the presence of triethylamine as a catalyst, to incorporate one methacrylate functionality where the hydroxyl was located and to obtain the final monomer cardanol-methacrylate-epoxy (CNME).

lenge on dentin surfaces treated with desensitizers. The study hypotheses under investigation were that (1) there are differences in the dentinal tubule occlusion among the desensitizing agents, and (2) the new monomer can promote acid resistance.

2. Materials and methods

2.1. Reagents

CNSL (technical grade) was kindly supplied by Amêndoas do Brasil LTDA (Fortaleza, Brazil). Formic acid, hydrogen peroxide, triethylamine, 3,5-Di-tert-4-butylhydroxytoluene (BHT), methacryloyl chloride, anhydrous sodium sulfate and solvents were purchased from Sigma-Aldrich (St. Louis, USA) and used as received. Silica gel (Merck) was employed in the chromatographic separations.

2.2. Synthesis of cardanol-epoxy (CNE)

Briefly, the cardanol-epoxy (CNE) synthesis was accomplished with epoxidation reaction of unsaturations of the CNSL, using hydrogen peroxide and formic acid with a molar ratio of 1:3:1,

respectively. First, CNSL was placed in a round bottom flask and then formic acid was added, followed by the slow addition of hydrogen peroxide under magnetic stirring at room temperature (25 °C). The resulting solution was vigorously stirred at 65 °C for 2 h and 30 min and monitored by thin layer chromatography. Next, the product was isolated by solvent extraction from the aqueous solution and the organic phase was dried using anhydrous sodium sulfate. Finally, the product was filtered followed by the evaporation of the solvent in a rotary evaporator, and the crude product was passed through a silica chromatography column to afford the purified CNE monomer (Fig. 1).

2.3. Synthesis of cardanol-methacrylate-epoxy (CNME)

In a generic way, cardanol-methacrylate-epoxy (CNME) synthesis was accomplished with an esterification reaction of the aromatic hydroxyl of CNE, using triethylamine and methacryloyl chloride with the molar ratio of 1:1:1, respectively. Overall, CNE was placed into a round bottom flask and dissolved in toluene at room temperature (25 °C) under vigorous stirring. Triethylamine was added as catalyst to the mixture, followed by BHT, as polymerization inhibitor. Thereafter, methacryloyl chloride was slowly added and the slurry was stirred at 60 °C for 4 h, being monitored by thin layer chromatography. Then, the product was isolated by simple solvent extraction from the aqueous solution and the organic phase was dried using anhydrous sodium sulfate. Finally, the product was filtered followed by the evaporation of the solvent in a rotary evaporator, and the crude product was passed through a silica chromatography column to afford the purified CNME monomer (Fig. 1). Due to the presence of the methacrylate group, the product was kept in a fridge (4 °C) and protected by aluminum foil after the addition of BHT to avoid spontaneous polymerization.

2.4. Fourier transform infrared spectroscopy (FTIR)

Samples of the synthesized and isolated products were characterized by a Fourier transform infrared spectrophotometer (Spectrum Frontier, Perkin-Elmer Corp., Norwalk, USA) equipped with a crystal to perform attenuated total reflectance (ATR-FTIR) analysis. Samples were individually dispensed onto the crystal and spectra were obtained in a spectral range of 4000–550 cm⁻¹ with 4 cm⁻¹ resolution in transmittance mode. FTIR spectra were obtained in triplicate for each product, using the unsaturated cardanol as reference, and then processed for baseline correction and normalization.

2.5. Nuclear magnetic resonance (NMR)

The nuclear magnetic resonance (NMR) spectra of carbon and hydrogen were obtained in a Bruker spectrometer instrument, model DPX 300 (Rheinstetten, Germany) operating at 75 MHz for NMR 13C and 300 MHz for NMR 1H. Deuterated chloroform was used as a solvent at room temperature.

2.6. Resin-based dentin desensitizer formulation

A resinous dentin desensitizer was formulated containing 20 wt% CNME, 50 wt% triethyleneglycoldimethacrylate

(TEGDMA), 28.5 wt% urethane dimethacrylate (UDMA), 0.5 wt% camphorquinone (CQ) and 1 wt% ethyl-4-dimethylamine benzoate (EDAB). Similar resin-based desensitizers were also formulated with 20 wt% of unsaturated cardanol (CNU) or 20 wt% CNE instead of CNME. As commercial controls, Gluma Desensitizer (Heraeus Kulzer, Hanau, Germany) was used, which is based on glutaraldehyde and hydroxyethyl methacrylate (HEMA), and One Coat Bond SL (Coltène/Whaledent Inc., Mahwah, USA) was employed as resin-based adhesive for desensitization.

2.7. Preparation of specimens

Forty nine extracted sound human third molars were used after approval by the Research Ethics Committee of Federal University of Ceará (protocol 1482602). They were stored in 0.1% thymol solution at 4 °C for no longer than one month. Occlusal enamel and roots were removed using a slow-speed water-cooled diamond saw (Isomet 4000; Buehler, Lake Bluff, USA) in order to obtain a disk (0.9 ± 0.2 mm in thickness) from middle coronal dentin from each tooth as described by Sauro et al. [15]. These specimens were wet-abraded using a 600-grit silicon carbide paper (30 s) to create a standard smear layer, that was subsequently removed using 17% EDTA (pH 7.4) for 2 minutes followed by an ultrasonic bath containing distilled water for 5 min. The dentin disks were randomly divided into the groups (n = 7). Group SL comprised the reference specimens, which were untreated with retained smear-layer. Group EDTA comprised control EDTA-treated specimens with opened dentin tubules (100% permeability) and that no desensitizing treatment was applied. Specimens in further groups were stored individually with moist paper to keep them hydrated. They were hydrated in distilled water for 30 min [16] before treatment as follows: group GLUMA was treated with Gluma Desensitizer; group OCB was treated with One Coat Bond SL; group CNU was treated with the resin-based desensitizer containing unsaturated cardanol; group CNE was treated with the resin-based desensitizer containing cardanol-epoxy and group CNME was treated with the resin-based desensitizer containing cardanol-methacrylate-epoxy. After treatment and initial investigation, two specimens of each group were exposed to acid challenge with 6% citric acid for further evaluation [15].

2.8. Dentin desensitizing treatment

GLUMA and OCB were applied according to manufacturer's instructions as described in Table 1. Specimens treated with CNSL compounds were pretreated with absolute ethanol for 30 s and the experimental resin-based desensitizers with CNU, CNE or CNME were applied with microbrush for 20 s, gently air-dried, applied again for 20 s and light-cured for 40 s using the LEDBiolux (Bio Art, São Carlos, São Paulo, Brazil). Afterwards, specimens of each group were immersed individually for 1 min in a 12-well plate, with 6% citric acid. After the immersion, they were thoroughly rinsed with deionized water.

Table 1 – Commercial desensitizing agents and application procedures.

Desensitizing agent	Components	Procedure	Manufacturer
Gluma Desensitizer (GLUMA)	Aqueous solution containing 5% glutaraldehyde and 35% HEMA	Apply and leave for 60 s, dry, rinse	Hereaus Kulzer, Hanau, Germany
One Coat Bond SL (OCB)	Methacrylate ^a , polyalkenoat methacryliert ^a , water ^a	Apply (dwell for 20 s), gentle air, light-cure ^b (30 s)	Coltène/Whaledent Inc., Mahwah, USA
HEMA: 2-hydroxyethyl methacrylate.			
^a Not provided by the manufacturer.			
^b LED Biolux (BioArt, São Carlos, São Paulo, Brazil).			

2.9. Dentine permeability evaluation

The rate of fluid flow was obtained using Flodec equipment (Flodec-System, Demarco Engineering, Switzerland) by recording measurements of hydraulic conductance (Lp). Specimens were attached to a plexiglass plate, penetrated with a stainless steel tube, with an exposed standard dentin surface, which were individually connected to the hydraulic pressure device. Fluid passed under a constant deionized water pressure of 15 cm H₂O (1.47 kPa). The movement of an air bubble inside a capillary tube in the hydraulic system was recorded during 5 min; the last 3 min records were used to obtain the Lp for each experimental condition [17].

After the immersion of all specimens into the EDTA solution, the initial Lp of each specimen was calculated, which were submitted to statistical analysis. Following, the dentin disks were grouped into the experimental groups (n = 3) in order to obtain normal data (p > 0.05) and no statistical difference among baseline permeability values. Subsequently, the specimens were wet-abraded on the occlusal side with 600-grit SiC paper for 30 s to create standardized smear layer, then Lp was measured to evaluate the minimum permeability. To obtain the maximum permeability (Lp max = 100% was arbitrarily assigned), that allows an evaluation of the changes in dentin permeability after the application of treatments, all specimens were etched for 15 s with 37% phosphoric acid solution, followed by rinsing with deionized water.

Moreover, the application of experimental materials on the dentin specimens was carried out as aforementioned. After treatments, hydraulic conductance was measured again and treatment permeability was expressed as a percentage (Lp %) of the maximum Lp value (100%). Finally, each treated dentin disk was immersed in 6% citric acid solution for 60 s in order to evaluate the acid resistance of desensitizing treatments and the last Lp was performed after washing the acid. The means (% reduction) and standard deviations for each group were calculated and the statistical analysis was performed with two-way ANOVA and Tukey's test (p < 0.05).

2.10. Scanning electron microscopy (SEM)

Four specimens per group were analyzed by scanning electron microscopy (SEM). They were prepared as cited in Subsections 2.7 and 2.8 and used two specimens after treatment and two after acid challenge. Specimens were dehydrated in silica gel, mounted on stubs, gold-sputter coated and observed in SEM (Inspect S50, FEI Company, Amsterdam, Netherlands) operated at 20 kV. Representative scanning electron micrographs were taken at different magnifications and were chosen by

two evaluators based on the frequently observed appearance of the dentin surface.

3. Results

3.1. Characterization of CNU, CNE and CNME

Fig. 2 illustrates the infrared spectra (FTIR) of CNU, CNE and CNME. The stretch of the C–O–C (oxirane ring) bond at 826 cm⁻¹ is presented in CNE and CNME spectra. The characteristic signal of the vibrational stretch of the O–H bond of phenolic rings present in the spectra of CNU and CNE at 3388 cm⁻¹, disappeared in CNME, indicating the substitution of this group. Absorption bands at 1740 cm⁻¹ and 1238 cm⁻¹ are referred to the bonds C=O and C–CO–O, respectively, are due the presence of the methacrylic group of CNME.

The 1H and 13C NMR spectra of the reactant (CNU) and products (CNE and CNME) are displayed in Fig. 3. In the 1H NMR spectra (Fig. 3a) were observed common signals for all these compounds in the regions between 0.8 to 1.7 ppm and 6.5 to 7.5 ppm, attributed to the aliphatic hydrogens from the side chain and the aromatic ring hydrogens, respectively. The respective carbon atoms attached to these hydrogens were observed in the 13C NMR spectra (Fig. 3b), where signals from 10 to 40 ppm were attributed to aliphatic carbon atoms of the side-chain, while the signals between 110–160 ppm were originated from both the aromatic ring as also from the unsaturated carbons from the methacrylic group. Specific changes were observed in the spectra of CNE and CNME when compared to the starting material (CNU). Signals at δ 2.75–3.00, attributed to the oxirane ring of CNE and CNME (Fig. 3a, 3), was confirmed by the signal at the range of 55–60 ppm in the 13C NMR (Fig. 3b, 5). The successful esterification of the aromatic hydroxyl was confirmed by the signals at δ 5.5–6.5, referred to the vinylic hydrogens (Fig. 3a, 1) of CNME, and the sharp signal at 2.2 ppm (Fig. 3a, 4), attributed to the terminal methyl group. In the 13C NMR spectra, was also observed a signal at 166 ppm, characteristic of the carbonylic carbon (Fig. 3b, 4) of the methacrylic group.

3.2. Dentin permeability evaluation

The results of percentage reduction in dentinal fluid flow achieved by the desensitizing agents when compared to the baseline flow rate are illustrated in Fig. 4. CNME showed the highest reduction in flow rate after treatment (87.1%). The application of OCB, CNU, CNE and CNME onto the dentin disks reduced dentine permeability with no statistical difference

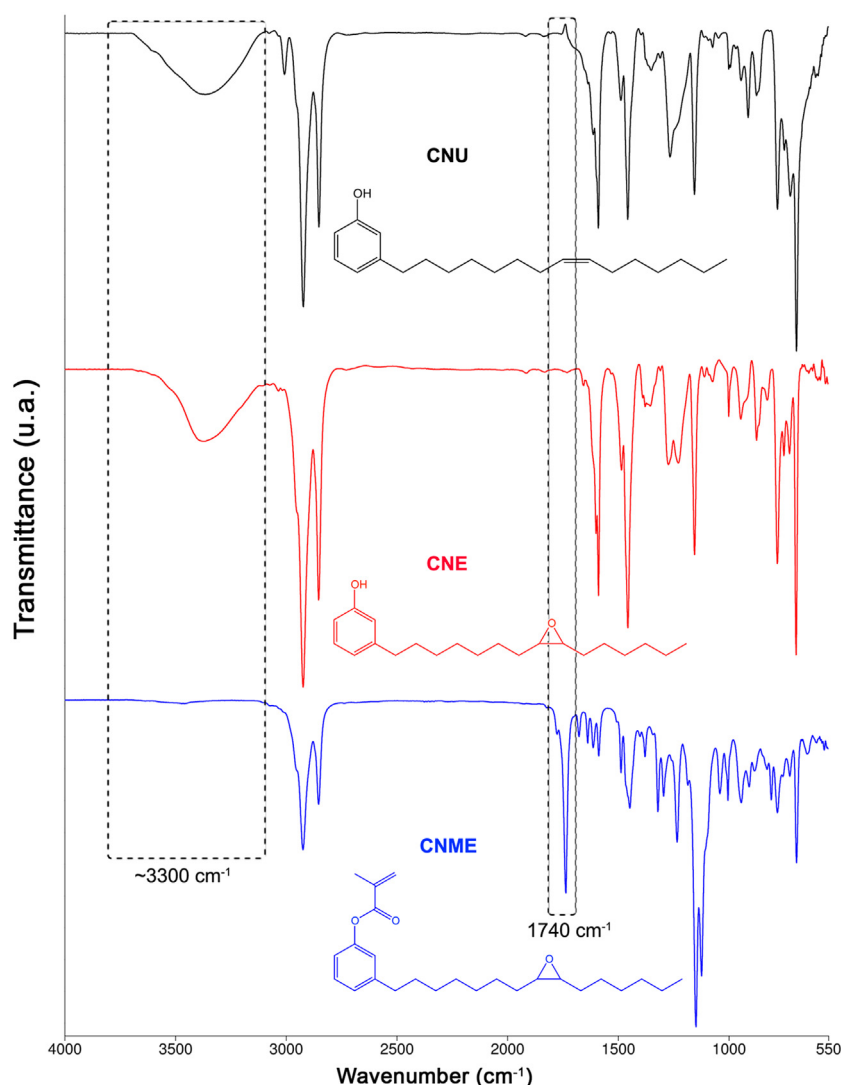


Fig. 2 – FTIR spectra obtained from the monomers unsaturated cardanol (GNU — top spectrum), cardanol-epoxy (CNE — middle spectrum) and cardanol-methacrylate-epoxy (CNME — bottom spectrum). The stretch of the C–H sp^2 (aliphatic) bond at 3009 cm^{-1} , is only observed in GNU. The stretch of the C–O–C (oxirane ring) bond at 826 cm^{-1} is presented in CNE and CNME spectra (first dotted line in the right), demonstrating the successful epoxidation. The spectra of GNU and CNE shows the absorption band at 3388 cm^{-1} , characteristic of vibrational stretch of the O–H bond of the phenol, that has disappeared in CNME. The absorptions bands at 1740 cm^{-1} and at 1238 cm^{-1} referring to the bonds C=O (carbonyl from methacrylate) and C–CO–O, respectively, occurred only in CNME.

between them, whilst GLUMA induced the lowest reduction of the dentinal flow ($p < 0.05$). There was no statistical difference between before and after acid challenge within each desensitizing treatment.

3.3. SEM evaluation of the occlusion of dentinal tubules

Characteristic SEM images of the dentin surfaces on which the desensitizing materials were applied are shown in Fig. 5. Different changes of the morphologies of the dentin surfaces were observed according to the groups. GLUMA presented partially occluded tubules (Fig. 5c), which were virtually all exposed after acid challenge (Fig. 5d). OCB showed occluded

surface with some water droplets (Fig. 5e), and some porosities after citric acid (Fig. 5f). GNU and CNE presented occluded dentin tubules after treatment (Fig. 5g and i, respectively), but they were partially removed after acid, showing some patent tubules (Fig. 5h and j, respectively). CNME produced an homogenous and occluded surface (Fig. 5k), even after acid challenge (Fig. 5l).

4. Discussion

The present study evaluated the effectiveness of dentinal tubule occlusion among desensitizing agents, which depicted different degrees of occlusion. Therefore, hypothesis (1) needs to be accepted. Besides, the acid resistance of these agents

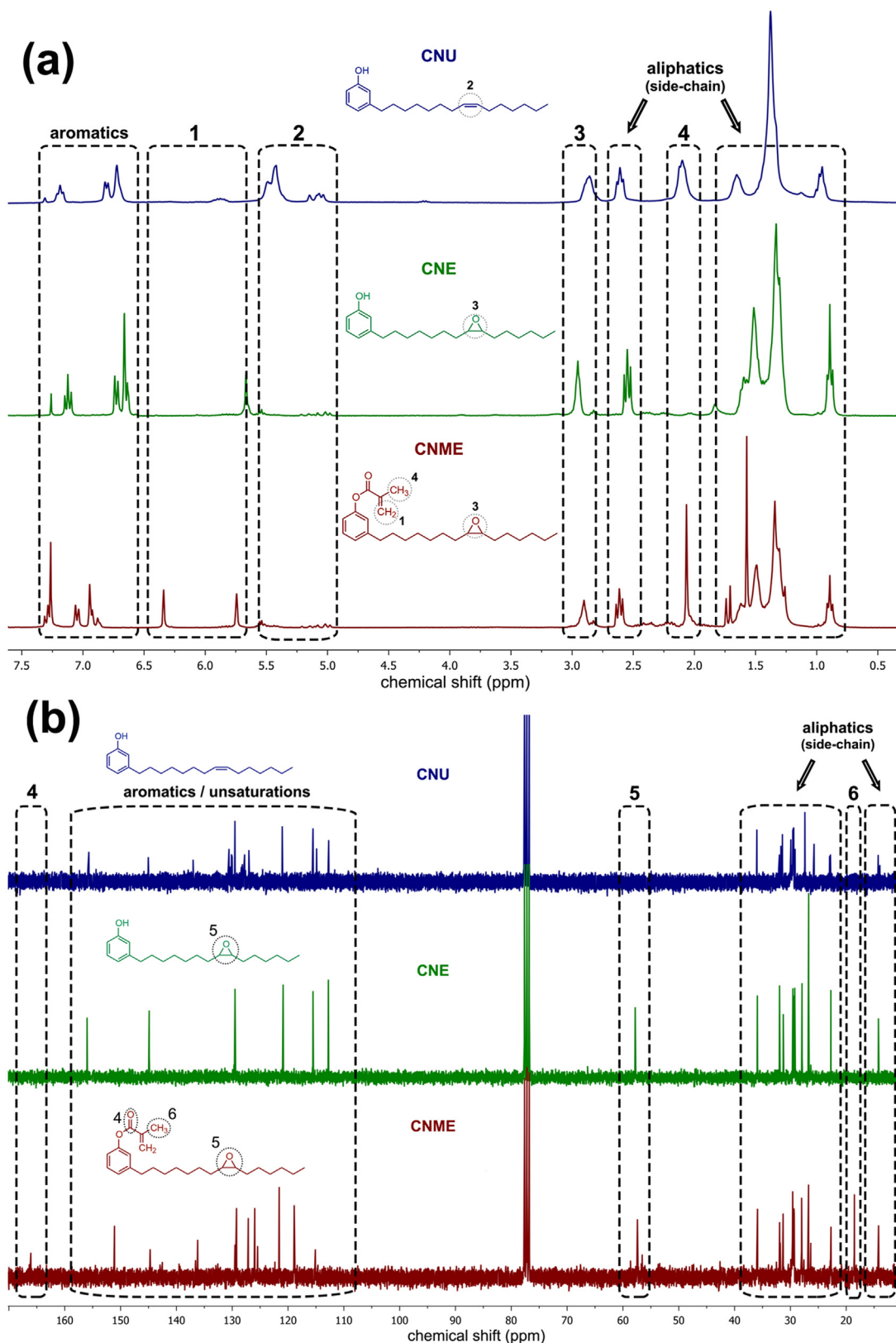


Fig. 3 – ^1H NMR (a) and ^{13}C NMR (b) spectra of the monomers cardanol unsaturated (CNU — top spectrum), cardanol-epoxy (CNE — middle spectrum) and cardanol-methacrylate-epoxy (CNME — bottom spectrum). CNU showed a signal at the range of δ 5–6 (Fig. 3a — dotted rectangle 2) relative to the olefinic hydrogens of the lateral chain, that was absent in CNE and CNME. The signal at the range of δ 2.75–3 (Fig. 3a — dotted rectangle 3) is attributed to the oxirane ring of CNE and CNME, which was confirmed by the signal at the range of 55–60 ppm in ^{13}C NMR (Fig. 3b — dotted rectangle 5). Between δ 5.5–6.5 (Fig. 3a — dotted rectangle 1) the signals referring to the vinylic hydrogens of methacrylate group of CNME may be observed.

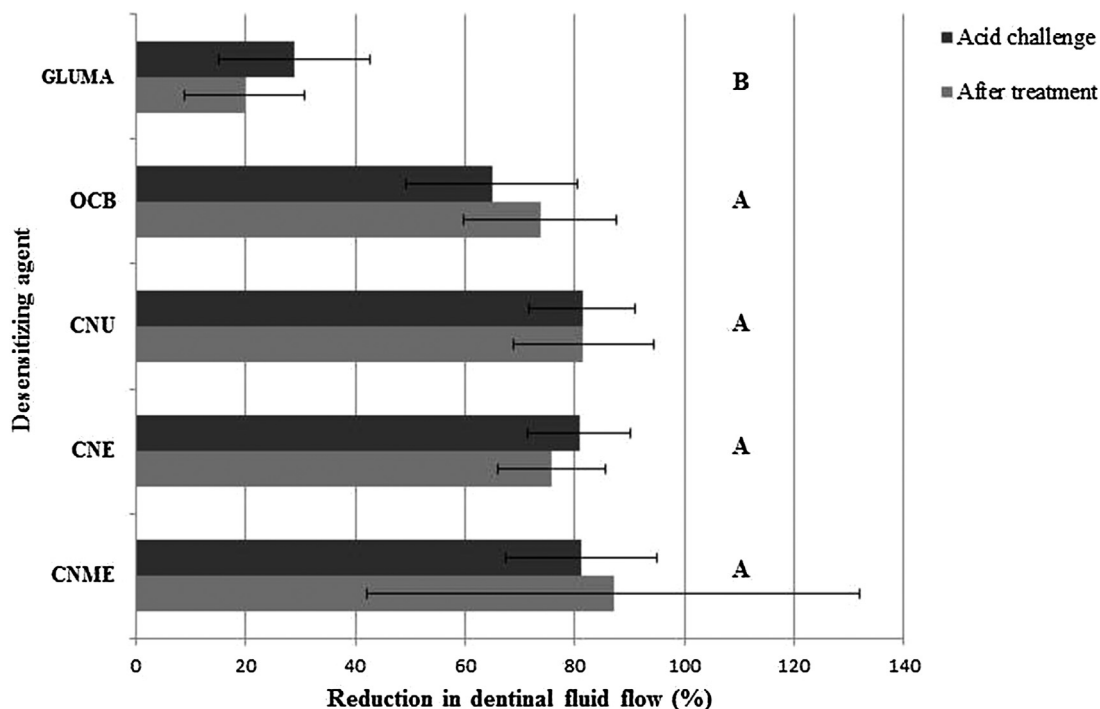


Fig. 4 – Means and standard deviations of the reduction in dentinal fluid flow (%) values after treatment and after acid challenge. Different capital letters indicate significant differences between the five desensitizing agents ($p < 0.05$). No statistical difference was found after treatment and after acid challenge within each desensitizer ($p > 0.05$). Numerically, CNME showed the highest reduction in dentinal flow rate after treatment (87.1%). GLUMA induced the lowest reduction of the dentinal flow when compared to further products ($p < 0.05$), which were statistically similar. GLUMA — Gluma Desensitizer, OCB — One Coat Bond SL, CNU — unsaturated cardanol, CNE — cardanol-epoxy, CNME — cardanol-methacrylate-epoxy.

was evaluated, which fully accomplished only by the new monomer. Consequently, the hypothesis (2) also requires acceptance.

The CNME monomer was synthesized via a two-step procedure (Fig. 1). In the first step, the intermediary CNE was successfully achieved, by means of an adaptation of a previously published protocol [18]. The second step was the esterification of the phenolic hydroxyl in CNE with methacryloyl chloride, in order to afford the final desired CNME in high yields (>75%). The monomers were isolated and characterized by FTIR, ^1H and ^{13}C NMR. All spectra clearly show the functionalization of unsaturation with an oxirane ring (CNE and CNME) and of the phenol moiety with a methacrylate pendant (CNME) (Figs. 2 and 3). By the author's knowledge, this two-step reaction to synthesize dental monomers has not been reported in the literature so far. Furthermore, this is the first study investigating a dental monomer synthesized from CNSL compounds.

The influence of desensitizing agents on dentin permeability was tested with a Flodec computerized device. After introducing an air bubble in the system, the linear displacement of the bubble within the glass capillary was measured. This movement occurs by water pressure through the discs at simulated pulpal pressure, once only a low, physiological

pressure is suggested to be suitable for measuring hydraulic conductance in dentin [19]. This information was transmitted by microflow sensors to a computer connected to the equipment, which converts by software this displacement to rate of fluid flow.

GLUMA revealed significant lower reduction of the dentinal flow when compared to the further agents (Fig. 4). This result was also found in other study [20], which found that photocurable resin desensitizers (Seal & Protect; Dentsply Caulk, Milford, DE, USA) demonstrates greater reduction on permeability than Gluma Desensitizer. The investigation of Camps et al. [21] may provide an explanation for this result, as they found that when tubules were filled with phosphate-buffered saline (PBS), it was observed an decrease in hydraulic conductance in dentin disks treated with Gluma desensitizing agent. Contrariwise, other study [22], even using PBS, revealed the same amount of reduction in permeability by Gluma and Seal & Protect, but they showed the highest hydraulic conductance (less than 40% of permeability reduction) among the tested agents. The present study did not use PBS or simulated body fluid. Therefore, the mechanism of precipitation of proteins by glutaraldehyde may have been negatively affected by the use of deionized water. Yet, it is important to remember that besides plasma proteins, there are many other sources of

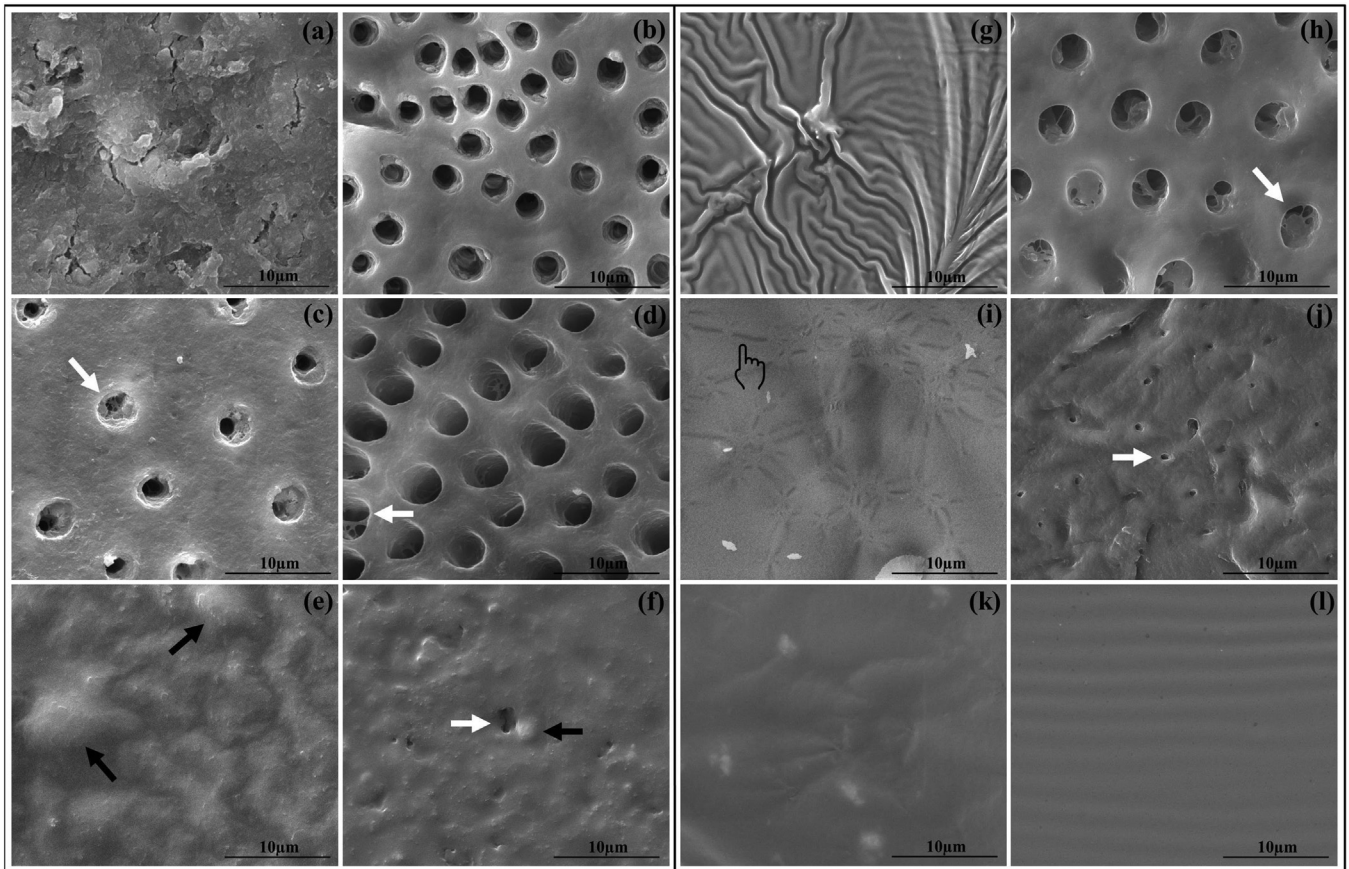


Fig. 5 – SEM micrographs depicting the most common features of dentin surfaces after treatment and after acid challenge with 6% citric acid. White arrows indicate partially occluded dentinal tubules, while black arrows are designated for water droplets. The pointer is indicating phase separation. The different groups are divided as follows: (a) retained smear-layer, (b) EDTA-treated only, with opened dentin tubules, (c) dentin surface treated with Gluma Desensitizer (GLUMA), (d) GLUMA-treated dentin after acid challenge, (e) surface treated with One Coat Bond SL (OCB), (f) OCB-treated dentin after acid, (g) dentin surface treated with desensitizer containing unsaturated cardanol (CNU), (h) CNU-treated specimens after acid, (i) surface treated with desensitizer containing cardanol-epoxy (CNE), (j) CNE-treated specimens after acid, (k) surface treated with desensitizer containing cardanol-methacrylate-epoxy (CNME) and (l) CNME-treated specimens after acid. SEM demonstrated that initially GLUMA only partially occluded the tubules (c) but showed almost complete dentin exposure after acid challenge (d). OCB created an occluded surface, but irregular due to a great amount of water droplets (observed in e and f). Besides, an initial degradation and exposure of tubules were noted (f). CNU covered the dentin surface with a frilled film thanks to its incomplete polymerization (g), the reason why it was partially removed after acid (h). CNE showed phase separation on occluded surface (pointer in i), which facilitated the opening of tubules entrance after acid (j). CNME achieved a complete obliteration of the dentinal tubules, even after acid challenge, presenting a homogenous film (k, l).

proteins such as collagen and non-collagenous proteins from dentin. Nevertheless, the use of PBS or simulated body fluid as a dentinal tubular fluid could result in sedimentation in the glass capillary what would impair consistency of the results. This is the reason why deionized water was chosen for this study.

The SEM images after the application of GLUMA (Fig. 5c) revealed only minor occlusion of tubules demonstrated by the decrease in tubule diameter, which became wide-open after acid exposure (Fig. 5d). GLUMA possesses low acid dissolution resistance [8], which may be explained by its hydrophilic components that can be easily removed or degraded in acidic environment. It is proposed that during the reaction between amino group-containing substances in dentin and glutaralde-

hyde, the polymerization of HEMA take place and start the formation of a HEMA polymer [23]. Nevertheless, the leachability of HEMA is well-known due to its small molecular weight and hydrophilicity, which indeed may contribute to higher dissolution in acid solutions. Clinically this implicates in many returns of patients to re-apply the desensitizing agent, thereby increasing the costs and reducing the satisfaction with treatment.

The further desensitizers obtained statistically similar reduction on dentinal fluid flow. However, CNME monomer achieved a numerically higher reduction on permeability than the others, especially when compared to OCB, which presented an intermediary reduction (Fig. 4). OCB is a water-based two-step etch-and-rinse adhesive chosen for this study due

to great bonding performance and resistance to thermocycling degradation [24]. Moreover, OCB contains HEMA in its composition (similar to GLUMA) and water as a solvent, thus, it is interesting to investigate the resistance of a relatively hydrophilic adhesive to acid challenge. The presence of these hydrophilic compounds may lead to higher permeability due to leaching of HEMA or even a hydrolytic degradation of the polymer [25]. On the other hand, CNME is a hydrophobic monomer that can create a regular layer thereby preventing these types of degradation.

The survey of Brunton et al. [8] revealed a level of acid resistance of OCB. On the other hand, in this study OCB exhibited great amount of water droplets before and after acid challenge (Fig. 5e and f) on SEM, also depicting signs of adhesive layer degradation (Fig. 5f). The water permeability of simplified hydrophilic dental adhesives resembles permeable membranes, which allows higher fluid diffusion [26]. Indeed, in a clinical point of view, the longevity of desensitizing treatment may be jeopardized by such imperfections [27].

The desensitizing containing unsaturated cardanol depicted a corrugated layer (Fig. 5g) due to an incomplete polymerization. It is expected that only methacrylate monomers have been light-cured and unsaturated cardanol remained unchanged within the polymer formed. The effective antioxidant role of phenols of the CNSL has been evidenced [28] and may play a role on such incomplete polymerization of desensitizer. This defective and superficial layer was removed after acid, although some tubules remained partially occluded (Fig. 5h). The desensitizing containing the intermediary CNE exhibited a layer with clear phase separation (Fig. 5i). This could be due to the presence of the hydroxyl functionality of cardanol-epoxy pendant to aromatic ring that can act as a hydrophilic site permeating the hydrophobic UDMA-polymer. After acid challenge, the entrance of the tubules was also exposed, most likely caused by hydrolytic degradation of CNE (Fig. 5j), which does not participate on methacrylate co-polymerization due to the absence of such polymerizable functionality. Nevertheless, CNE showed higher occlusion after acid than CNU, thanks likely to an increase of crosslinking obtained by the acid-catalyzed hydrolysis of epoxides [13,14].

CNU and CNE showed a great reduction of permeability, even after acid challenge, which seems contradictory with the SEM results. This discrepancy between dentinal fluid flow and SEM observations might be explained because SEM observation is able to assess only superficial characteristics whilst dentinal fluid flow might be affected by internal tubular occlusion. Kolker et al. also demonstrated that there is not a correlation between these two experiments [22].

The desensitizing containing the final monomer CNME attained uniform and occluded layer before and after acid (Fig. 5k and l). CNME methacrylate functionality ensured the regular photo-polymerization and formation of a homogenous polymer. Furthermore, methacrylate removed the hydrophilic hydroxyl and, consequently, generated a more hydrophobic monomer. This hydrophobicity yields a polymer with lower wettability, water sorption, water droplets and hydrolytic degradation than the hydrophilic ones [25]. Also, the opening of the oxirane ring in acid environment and the resulting increased crosslinking might create a smart material [29],

which becomes more resistant in contact with many different acids from the diet of patients over the years.

CNME monomer was synthesized through a simplified procedure and emerges as a potential dental monomer that can add value to a natural by-product from the cashew nut industrial processing, which is produced in large scale (1,000,000 tonnes in 2013) [11]. Besides, it is a HEMA-free desensitizer, which might be a safer option to reduce dentin sensitivity [7]. Furthermore, CNME confirmed that its hydrophobicity and acid-resistance developed a potential long-lasting desensitizing agent, improving the material over time.

5. Conclusion

CNME showed a significant reduction of dentin permeability and a uniform occluded surface even after acid exposure, suggesting that it may be a suitable acid-resistant treatment option for dentin hypersensitivity.

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