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Effect of Calcium Hydroxide Nanoparticles Incorporation on the Physical Properties of Root Canal Sealer (In Vitro Study)

Rana A. Shafei[1], Abeer H. Mahran[2], Mohamed Nabil[3]

ABSTRACT

Introduction: Obturation of the root canal system is an integral part of endodontic treatment. The main objective of obturation is to three dimensionally fill the root canal, to provide adequate seal and prevent leakage from periapical tissues. For root canal sealers to achieve these objectives, they must exhibit adequate physical properties ***Aim:*** the present study evaluated the effect of adding different percentages of calcium hydroxide nano particles (CHNPs) to a calcium hydroxide-based sealer (Sealapex) on its physical properties and comparing it to the unmodified formula of the sealer. ***Materials and methods:*** Calcium hydroxide nanoparticles were added to (Sealapex) root canal sealer in concentrations of 3% and 8% by weight respectively. Samples were divided into three main groups: Group I (Sealapex+3%CHNP), Group II (Sealapex+8%CHNP), and Group III (Sealapex) in the conventional formula. Setting time and flow were assessed according to ISO standardization 6878:2012 specification. Wettability was assessed using Contact angle goniometer. Data showed parametric distribution and were analyzed using one-way ANOVA followed by Tukey's post hoc test for intergroup comparisons and repeated measures ANOVA followed by Bonferroni post hoc test for intragroup comparisons. ***Results:*** There was a significant increase in setting time for Group I (Sealapex+3%CHNP), on the other hand, Group II (sealapex+8%CHNP) showed significant decrease in setting time. Regarding the flow of sealers, there was a significant increase in flow for both Group I (Sealapex+3%CHNP) and Group II (Sealapex+8%CHNP) in comparison to the conventional formula. ***Conclusion:*** Addition of calcium hydroxide nanoparticles (CHNP) enhanced physical properties of root canal sealer Sealapex.

Keywords: Calcium hydroxide, Nano- calcium hydroxide, Endodontic sealers.

INTRODUCTION

Root canal sealers have an important role in root canal obturation. Although they are employed in obturation as additional materials, research has shown that they have

1.Postgraduate Master Student, Conservative Dentistry Department (Endodontic Division), Faculty of Oral and Dental Medicine, Misr International University, Cairo, Egypt.
2.Professor of Endodontics, Endodontic Department, Faculty of Dentistry, Ain Shams University, Cairo, Egypt.
3.Lecturer of Endodontics, Conservative Dentistry Department (Endodontic Division), Faculty of Oral and Dental Medicine, Misr International University, Cairo, Egypt.

an impact on the success of root canal therapy.[1] They are used to overcome the physical limitation of the rigid core, seal inaccessible accessory canals and ramifications, fill voids between the core material and dentinal walls, and act as lubricant to facilitate the placement of core material into the canal.[1]

Grossman (1982) outlined the properties of an ideal root canal sealer. When set, a sealer should form and maintain a hermetic seal, have adequate working and setting time, have appropriate adherence to canal walls, produce no tooth discoloration, be radiopaque, hydrophobic to tissue fluids but dissolve in solvents during retreatments, bacteriostatic, and biocompatible. None of the commercially available sealers has met all these requirements yet.[2]

One of the first commercially available endodontic sealers containing calcium hydroxide was Sealapex. Since the early 1980s, Sealapex has been used as a calcium hydroxide type sealer. It is a polymeric calcium oxide-based sealer that forms calcium hydroxide when it comes in contact with tissue fluid through hydration. It is also known for its biocompatibility and ability to induce the deposition of mineralized tissue, resulting in apical seal following endodontic treatment.[3,4]

It was reported in the literature that Sealapex has a prolonged setting time.[5,6,7] It takes up to 3 weeks in 100% relative humidity and will not set in a dry environment.[8,9] Slow or incomplete setting time can cause tissue irritation and higher solubility, which can contribute to sealing failures. Setting time in particular is clinically important for endodontic treatment, as depending on how fast or slow a sealer sets, the capability of proper penetration of root canal morphology is determined.[10]

The flow is a key property of endodontic sealers as it allows for deeper penetration into the irregularities of the root canal system, which contributes to the interlocking between sealer and dentine, which is one of the crucial physical features to a proper obturation.[11] Particle size, temperature, film thickness, shear rate, and the internal diameter of the canal are all factors that influence the sealer's flow rate.

Another important feature of endodontic sealers is having a proper wetting ability, as sealers with hydrophilic surface may have a facilitated penetration in root canal system irregularities and ramifications, which may indirectly enhance the elimination of residual bacteria in such areas and may impact the overall success rate of endodontic treatment. If the contact angle is smaller than 90 degrees, the material is considered hydrophilic, while if the contact angle is larger than 90 degrees, the material is considered hydrophobic.[12,13]

Nanosizing a material affects its chemical reactivity; a material may show different properties than its larger in scale counterparts as stated by Roduner.[14] In the recent years, there has been modifications of root canal sealers to enhance their physicochemical properties and enhance the overall drawbacks of sealers through using different nanoparticles and herbal extracts, either added to endodontic sealers or even used to create new sealers.[15] Wong et al. stated that compared to their traditional counterparts, calcium hydroxide nanoparticles may have better penetration depth, a larger surface area to interact with pathogens, higher anti-microbial activity, and superior properties in general.[16] The goal of the current study is to evaluate the effect of adding different percentages of nano calcium hydroxide particles (3% and 8%) on the setting time, flow, and wettability of calcium hydroxide-based sealer Sealapex and compared it to the conventional formula.

MATERIALS AND METHODS

I-Preparation and identification of calcium hydroxide nanoparticles (CHNP):

a. CHNPs were prepared following Daniele's method using aqueous solutions of $CaCl_2$ and NaOH with added surfactant Triton X-100. The particle size identification was done using dynamic laser scattering.[17,18,19]

b. Calcium hydroxide content determination of the sealer's base paste was done using inductively coupled plasma optical emission spectrometer (ICP-OES), for accurate modification of the original formula with CHNPs with the assigned percentages of (3% and 8%) by weight.

II-Manipulation of the sealers:

The amounts of CHNPs added to the sealer is based on how much calcium hydroxide there is in the base paste, which is known through the analysis of the base paste sample by (ICP-OES). The concentration was found to be 30% which the following calculations are based on:

> Total weight of the base paste tube x concentration percentage of calcium hydroxide in the base paste
> **X** percentage of CHNP to be added

a. The percentage of CHNPs to be added to the base paste is calculated using the following formulas:

- 12 x0.3 x0.03= 0.1080 g which is the amount for the 3% addition of CHNP. **(Figure 1, a)**

- 12 x0.3x 0.08= 0.2880g which is the amount for the 8% addition of CHNP. **(Figure 1, b)**

Group III (conventional formula): Calcium hydroxide-based sealer in the conventional formula.

Figure (1): Using analytical balance for weighing (a) 3% of CHNPs. (b) 8% of CHNPs.

b. CHNPs powder is weighed using analytical balance and added to the sealer Sealapex™ (Sybron-Kerr, Romulus, MI, USA) Base paste(12g) and mixed until homogenous. The final product is then mixed with the catalyst paste(18g).[20]

III-Sample Grouping:

Samples were divided into three main groups according to the material:

Group I (Sealapex+3%CHNP): Calcium hydroxide-based sealer modified with the addition of 3% by weight of Nano calcium hydroxide particles.

Group II (Sealapex+8%CHNP): Calcium hydroxide-based sealer modified with the addition of 8% by weight of Nano calcium hydroxide particles.

IV-Methods of Evaluation:

A. Evaluation of setting time:

For setting time Teflon molds with internal diameter of 10 mm and height of 2 mm were fabricated following ISO standardization 6876/2012[21] **(Figure 2)**.

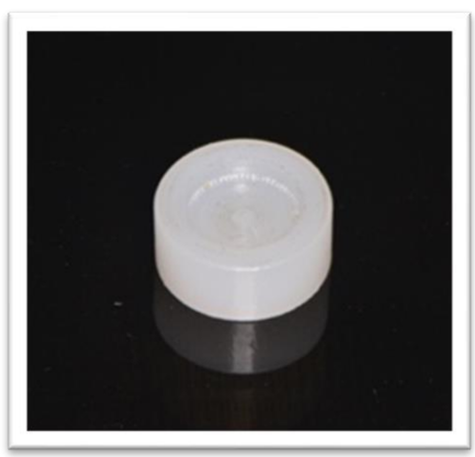

Figure (2): Teflon mold for setting time measurement, fabricated according to ISO standardization 6876:2012.

For each group of sealer six samples were prepared. After mixing, sealers are packed in Teflon molds, then stored in 95% relative humidity and temperature of 37°C,

after 24 hours from the time the mixing procedure began, setting time is determined using Gillmor type indenter with a mass of (100 ± 0.5) g and a flat end of (1,5 ± 0.1) in diameter. Indenter is lowered vertically on the horizontal surface of the sealer, needle tip is raised, cleaned and operation is repeated until indentations cease to be visible. The setting time is recorded from ending of the mixing time.[22,23]

B. Evaluation of flow

Following the recommendation of ISO standardization 6876/20129[21] using a graduated disposable 1ml syringe, 0.05ml of mixed sealers is placed on a glass slab (40x40x5mm and a mass of approximately 20g±3g), six samples for each sealer group were prepared at (180 ± 5s) from beginning of mixing. A second glass slab is applied centrally on the material and an additional mass to total 120g. Ten minutes from the beginning of mixing the load are removed, maximum and minimum diameter of the compressed plates is measured using a digital caliper and the mean of the two readings is recorded. This procedure is repeated 6 times for each sealer. If the difference is more than 1 mm, the experiment is repeated.[24]

C. Evaluation of wettability:

For each sealer group, five samples were prepared. On a glass slides, sealers are spread evenly, and until setting, they are kept in 100% relative humidity at 37°C, on each surface of the set samples pumping not less than 10 droplets (4μL) of ultrapure water then sessile drop contact angle is measured, determined using contact angel measurement.[12-23]

V-Statistical analysis:

Numerical data were presented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution, and using Shapiro-Wilk test. Data showed parametric distribution and were analyzed using one-way ANOVA, followed by Tukey's post hoc test for intergroup comparisons and repeated measures ANOVA, followed by Bonferroni post hoc test for intragroup comparisons. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows.

RESULTS

I- Evaluation of setting time:

Impact of addition of CHNP on the setting time of all groups presented in Table (1) and Figure (3).

There was a statistically significant difference between different groups ($p<0.001$). Group III (conventional formula)

Table (1): Mean and standard deviation (SD) values of setting time for different Groups.

Time	Setting time (mean±SD)			p-value
	Group I (Sealapex+3% CHNP)	Group II (Sealapex+8% CHNP)	Group III (Conventional sealer)	
Hours	194.05 ± 0.85^{A}	162.00 ± 0.46^{C}	168.09 ± 0.03^{B}	**<0.001***

**; significant ($p \le 0.05$).*
Different superscript letters indicate a statistically significant difference within the same row.

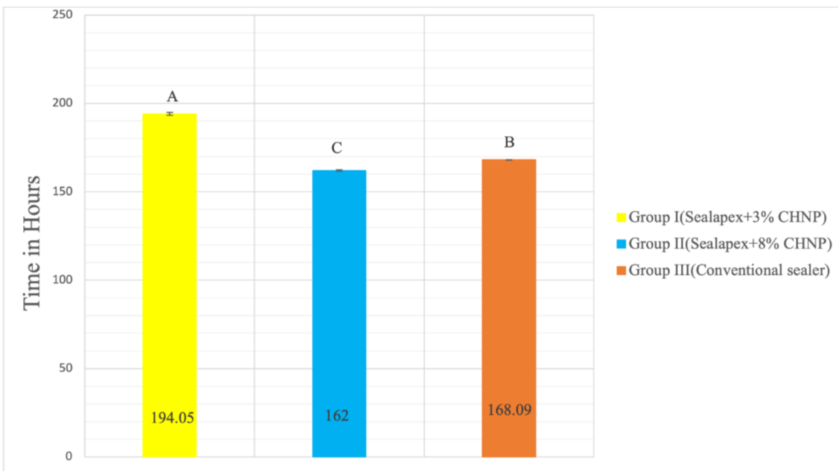

Figure (3): Bar chart showing average setting time (hours) for different Groups. Error bars denotes standard deviation. Different letters on top of the bar denote statistically significant differences between groups ($P < 0.05$).

There was a statistically significant difference between different groups ($p<0.001$). The highest value was found in Group I (Sealapex+3% CHNP), followed by Group III (conventional Formula), while the lowest value was found in Group II (Sealapex+8% CHNP).

II-Evaluation of flow presented in Table (2) and Figure (4).

had a significantly lower flow value than both Group I (Sealapex+3%CHNP) and Group II (Sealapex+8%CHNP).

III-Evaluation of wettability (contact angle) presented in Table (3) and Figures (5,6).

There was no statistically significant difference between all groups ($p=0.120$). The highest contact angle value was found in

Table (2): Mean and standard deviation (SD) values of flow (mm) for different Groups.

Flow (mm) (mean±SD)			p-value
Group I (Sealapex+3% CHNP)	**Group II (Sealapex+8% CHNP)**	**Group III (Conventional sealer)**	
22.79±1.16[A]	22.90±0.64[A]	19.86±0.70[B]	**<0.001***

*; significant ($p \leq 0.05$).
Different superscript letters indicate a statistically significant difference within the same row.

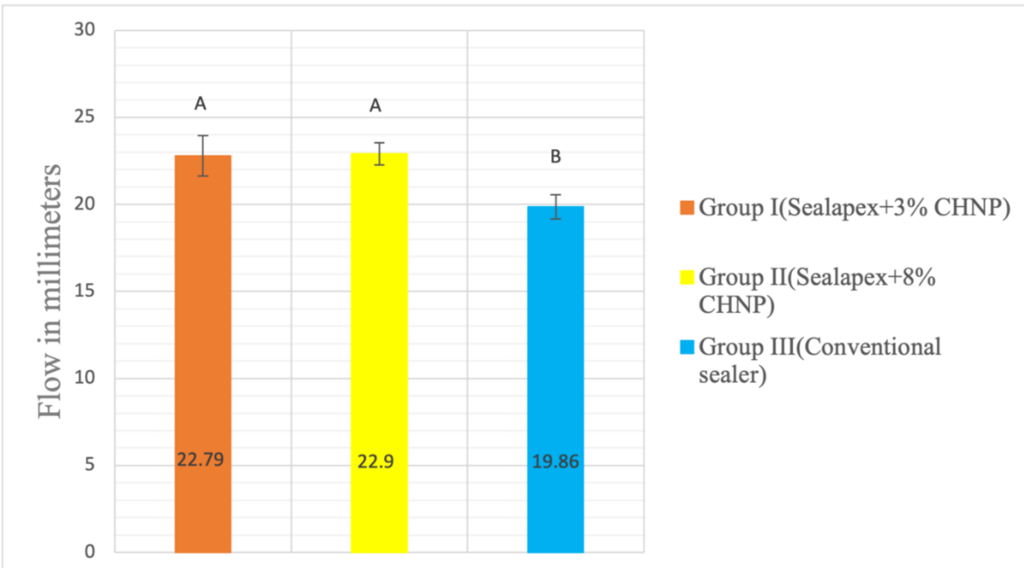

Figure (4): Bar chart representing average flow (mm) of different Groups. Error bars denotes standard deviation. Different letters on top of the bar denote statistically significant differences between groups ($p \leq 0.05$).

Group I (sealapex+3%CHNP) followed by Group III (conventional formula), while the lowest contact angle value was found in Group II (sealapex+8%CHNP).

Table (3): Mean and standard deviation (SD) values of contact angle (°) for different Groups.

Contact angle (°) (mean±SD)			p-value
Group I (Sealapex+3% CHNP)	**Group II (Sealapex+8% CHNP)**	**Group III (Conventional Formula)**	
89±9.8	**77.3±7.3**	**80.3±8.4**	**0.120 ns**

- significant ($p \leq 0.05$).
- ns; non-significant ($p>0.05$).

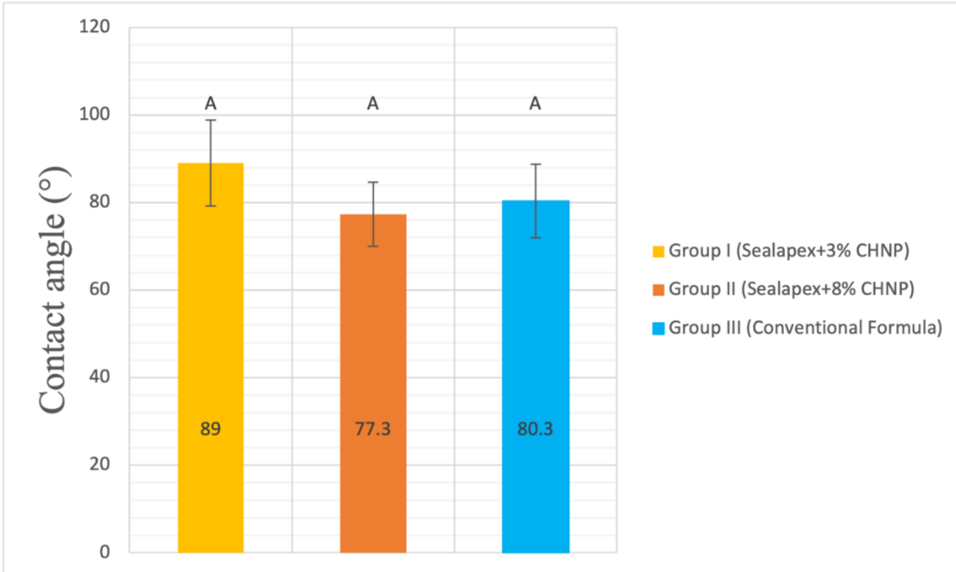

Figure (5): Bar chart showing average contact angle (°) for different Groups. Error bars denotes standard deviation. similar letters on top of the bar indicates no statistically significant differences between groups(p>0.05).

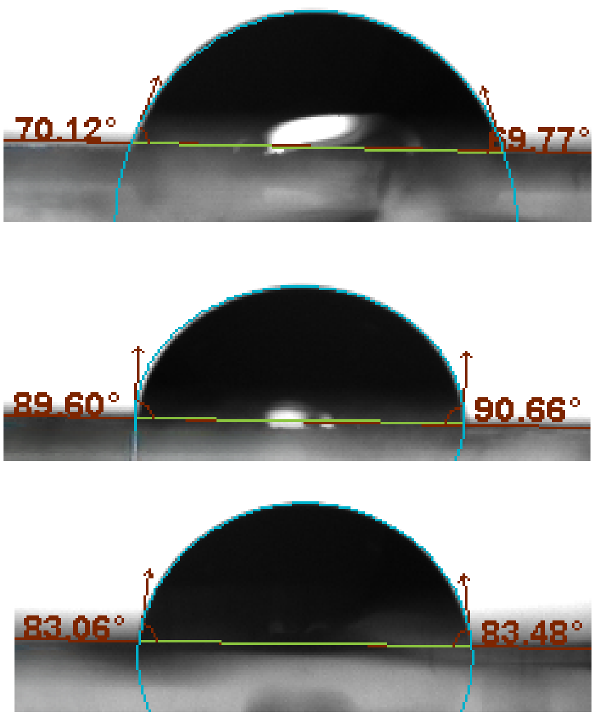

Figure (6): Picture representing Contact angle measurement of different sealers. a) Group I(Sealapex+3%CHNP), b) Group II (Sealapex+8%CHNP), c) Group III (Sealapex in conventional Formula).

DISCUSSION

There are a variety of root canal sealers commercially available with various formulations and components. One of the most important aspects influencing the choice of root canal sealer material is its physical and chemical qualities. Up till now no ideal sealer exists, fulfilling all requirements.

Calcium hydroxide-based sealers are one of the available sealers that are biocompatible[3], promote healing due to their high alkalinity, have the capability of mineralized tissue formation[25,26] and possess an antibacterial effect.[27,28] For these sealers to exert their therapeutic effect on the surrounding tissues dissociation of hydroxyl ions is essential. Sealapex (Sybron Endo, CA, USA) is one of several calcium hydroxide-based root canal filling products that shows biocompatibility.[3] It contains calcium hydroxide in a polymeric matrix[29] and induces apical sealing following root canal therapy by stimulating the deposition of calcified material.[4] However, due to its extended setting time,[5,6,7] this sealer presents high degree of solubility,[30] leading to non-homogeneous setting reaction and poorly formed matrix.[31-32]

Recently, there has been modification of root canal sealers to enhance their physicochemical properties,[15] bioactivity,[33] increase their antibacterial efficiency and fix the overall drawbacks of sealers,[22,34-35] through using different nanoparticles and herbal extracts either added to endodontic sealers or even used to create new sealers. The aim is to improve some properties of the calcium hydroxide-based sealer Sealapex.

Methodology used and specimen molds created for assessment of setting time followed ISO Standardization 6878/2012 recommendation. The time required for setting stated by the ISO standardization is from 30 min to 72 hours.[21]

The setting time of sealer depends on the experimental method used. In root canal specimen, it takes longer than in molds or on glass slabs,[36,37] where in molds a larger surface area of the sealer is exposed to air and humidity. In the current study sealers are placed in molds and the degree of surface set is tested at different time intervals with a pre-weighted needle of a Gilmore needle apparatus. The needles are approximated towards the sealer surface if it causes indentation in the material, it considered unset and vice versa if it leaves no indentation, it indicates a set material.

This experimental model cannot be correlated to the clinical situation as there are a lot of factors not taken into consideration, such as the amount of moisture present naturally in root canals from dentinal tubules which could affect the setting reaction by speeding or retarding it [36,37,38]. Also, the presence of smear plugs, or tubular sclerosis may affect setting time.[39] Most of root canal sealers produce some degree of toxicity until completely set [40] and a longer setting time could be related to increased solubility.[41]

In the present study, the sealer did not meet the time required for the sealer to set for the ISO specification, nor as recommended by the manufacturer which is 24 hours for final setting in 100% relative humidity. Group III which is the conventional formula took 168 hours to set, while group I (Sealapex+3% CHNP) took the longest time to set of 194 hours,[11] for Group II

(Sealapex+8% CHNP) there was a decrease in setting time at 162 hours.

Group II showing the least setting time recorded is probably due to the decrease in particle size, which increased the reactivity of the materials allowing the material to set in a shorter period of time.[14-15]

Caicedo and von Fraunhofer (1988)[6] stated that sealapex presented prolonged setting time which maybe attributed to the continuous reaction during setting in high humidity, it was accompanied by expansion and caused the material to be fragile.

On the other hand, Marín-Bauza et al. (2012),[7] Allan et al. (2001),[36] silva et al. (2021),[37] Vapiana et al. (2014),[42] and Andolfatto et al. (2017)[43] revealed that Sealapex did not set at all whether in vivo or in vitro testing conditions and that it did not set in dry environment as moisture is a requirement for Sealapex setting reaction.

There are conflicting results in the literature regarding setting time which may be attributed to the subjectivity of interpretation of the results as it depends on visual inspection only.[6,7,42] Also, variation of temperature and humidity of experimental setting, different devices and types of molds used.[36]

Flow is a key quality that permits the sealer to access difficult to reach areas in the root canal system including dentin abnormalities, isthmuses, accessory canals, and fill voids between canal wall and gutta percha.[44] Particle size, temperature, film thickness, shear rate, and the internal diameter of the canal are all factors that influence the sealer's flow rate. In the present study, group I (sealapex+3%) and group II (sealapex+8%) showed significant increase in flow rate in comparison to group III the original sealer formulation

The size of the sealer particles has an impact on the flowability of the sealer. A sealer can flow more readily when the particles are smaller. Root canal sealer should have a moderate flow rate. Excessive flow rate increases the likelihood of extravasation and inadequate flow reduces penetration to accessory canals.

In accordance with ISO 6876/2012, the recommended flow value of a root canal sealer should be at least 17 mm.[21] In the present study, addition of CHNP enhanced the flow of Sealapex specially in group II (sealapex+8%CHNP) with the highest flow rate followed by Group I of (sealapex+3%CHNP) in comparison to the conventional formula, all groups coincide with ISO standardization.[21] Siqueira et al., (2000)[45] Stated that sealers with superior flow characteristics and antibacterial qualities could, in theory, aid in the demise of microorganisms in confined areas of the root canal.

Teixeira et al., (2017)[46] tested incorporations of different percentages of nanostructured silver vendate particles to Sealapex and they concluded that it did not affect the flow, but it still was within the acceptable range.

The contact angle is a useful indicator of wettability or spread ability. This angle is formed at the intersection of liquid, gas and solid. If the contact angle is smaller than 90° degrees, the material is considered hydrophilic as it is more likely to spread as the contact angle decreases, and if the contact angle is larger than 90° degrees, the material is considered hydrophobic.[12-47]

In the clinical situation, wettability influences the adaptability to root canal dentin, and it is affected by multiple factors such as presence or absence of smear layer, the irrigants used during preparation of root canal treatment, which in return affects the surface energy of dentin and finally the surface tension of the sealer itself. [48,49]

The present study followed previous method by Barros et al. (2014)[23]using contact angle measurement for determination of Sealer wettability. All sealers showed a hydrophilic behavior the lowest contact angle value was presented by Group II (sealapex+8%) which indicates that the addition of 8%CHNP to Sealapex enhanced the wettability of sealer.

Barros et al. (2014)[23] stated that the addition of quaternary ammonium polyethylenimine nanoparticles to different root canal sealers enhanced their wetting ability which may indirectly enhance the effectiveness of the antibacterial properties of the sealer.

CONCLUSION

Within the limitation of this study, it could be concluded that:

(1) Addition of 3% CHNP to Sealapex enhanced the flow and wettability of the sealer but had a negative effect on setting time.

(2) Addition of 8% CHNP to Sealapex root canal sealer enhanced setting time, flow, and wettability.

(3) Conventional formula had superior physical properties in comparison to the 3% CHNP group but when compared to the 8% CHNP group, the latter showed superior physical properties.

(4) Based on findings of this study the null hypothesis was rejected.

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Non-Funded Research.

CONFLICT OF INTEREST

The authors deny any conflict of interest related to this study.

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