

Evaluation Of The Physical Properties Of Sealapex Incorporated With Calcium Hydroxide Nanoparticles (An In-Vitro Study)

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ABSTRACT

Background: Nanotechnology is used in enhancing some existing materials. Nano-calcium hydroxide sealers are introduced to eliminate the short-comings of the conventional one. **Objectives:** To evaluate the physical properties of Sealapex incorporated with calcium hydroxide nanoparticles (CHNP). **Methods:** Calcium hydroxide nanoparticles were incorporated to Sealapex root canal sealer in concentrations of 3% and 8% by weight respectively. Three groups were established: Group I (Sealapex + 3% CHNP), Group II (Sealapex + 8% CHNP) and Group III (Sealapex in the conventional formula). Solubility was evaluated according to ISO standardization 6878:2012 specification at time intervals of (24 hours and 14 days). For pH evaluation, specimens were immersed in deionized water and measured using a pH meter at time intervals of (1, 3, 24 hours and 14 days). **Results:** Group II (Sealapex + 8% CHNP) showed a significantly lower solubility value at 24 hours and the highest pH value after 3 hours. **Conclusions:** The addition of 3% CHNP to Sealapex resulted in a negative effect on the solubility of the sealer but enhanced its alkalinity. On other hand, the addition of 8% CHNP enhanced the physical properties of the sealer. **Clinical Significance:** The addition of nanoparticles to currently existing sealer formulas may enhance its physical properties and eliminate some of its drawbacks.

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

INTRODUCTION

Different root canal sealers have been introduced to the market as an attempt to fulfill the ideal requirements of sealers used in the root canal system, among them are calcium hydroxide-based sealers. Calcium hydroxide as a material showed proper

antibacterial effect,¹ tissue repair properties by hard tissue formation² and tissue dissolving ability.³⁻⁵ In the late 1970s Goldberg and Gurfinke used the combination of Dycal (Densply-Caulk, Milford, DE) and gutta-percha as root canal filling.⁶

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One of the first commercially available calcium hydroxide-based root canal sealers was Sealapex which had been used since the early 1980s. When Sealapex is placed in contact with tissue fluids, it forms calcium hydroxide through hydration. Hydroxyl ions dissociation is essential for calcium hydroxide to exert its effect on the surrounding tissues. This renders the long-term sealing ability and therapeutic effect of the sealer questionable.

Recently, Nanotechnology is used in enhancing some existing materials⁷ and the construction of new materials⁸ with unique physical and chemical properties. Nanoparticles are added to endodontic sealers to enhance penetration and antimicrobial properties. Incorporation of such particles increases the chemical reactivity and the surface area/ mass ratio in comparison with micro/ macrostructures and nanosizing contributes to the distinctly different properties of nanomaterials.^{9,10}

Nano-calcium hydroxide sealers were introduced to eliminate the short-comings of the conventional calcium hydroxide-based sealers. It provides better physical and chemical properties and better penetration into the dentinal tubules due to its smaller particle size, as decreasing the size increases the efficacy of the material in lesser doses,

which in return decreases its side effects.¹¹ Therefore, the aim of the present study was to evaluate the physical properties of Sealapex incorporated with calcium hydroxide nanoparticles.

MATERIALS AND METHODS

1. Setting and design

This in-vitro study was carried out at the National Research Center (Dokki, Giza, Egypt). Modification of root canal sealer Sealapex™ (Sybron-Kerr, Romulus, MI, USA) was performed by addition of CHNP in 3% and 8% by weight. CHNPs were prepared at the nanomaterial investigation Lab at, National Research Center. Solubility and pH of the sealers were measured according to International Organization for Standardization.¹²

2. CHNPs Preparation and particle size identification

CHNPs were prepared using aqueous solutions of CaCl₂ and NaOH with added surfactant Triton X-100.¹³ The particle size identification was performed using dynamic laser scattering^{14,15} (**Figure 1**).

The content of calcium hydroxide of the base paste of the sealer was determined using Inductively Coupled Plasma- Optical Emission Spectrometer (ICP-OES), for accurate modification of the original formula by CHNPs with the assigned percentages of -

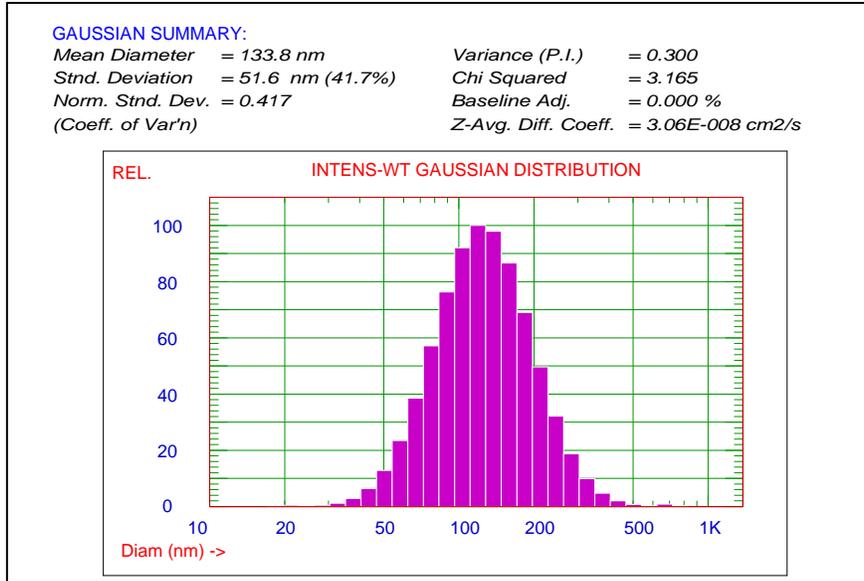


Figure (1): Histogram representing intensity weighted size distribution.

(3% and 8%).⁷

3. Modification and manipulation of the sealer

ICP-OES is used for the determination of the concentration of calcium hydroxide available in the base paste of the sealer to situate the percentage of addition of nanoparticles calculations on it for accuracy. The concentration was found to be 30% by weight based on the following calculations:

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

$$\text{Total weight of the base paste tube} \times \text{concentration percentage of calcium hydroxide in the base paste} \times \text{percentage of CHNPs to be added.}$$

▪ $12 \times 0.3 \times 0.03 = 0.1080$ g which is the amount for the 3% addition of CHNP.

▪ $12 \times 0.3 \times 0.08 = 0.2880$ g which is the amount for the 8% addition of CHNP.

3.2. CHNPs powder is weighed using an analytical balance (± 0.0001 g) and added to Sealapex base paste (12g) and mixed until it became homogenous. The final product is then mixed with the catalyst paste (18g).

4. Classification of samples

Three groups were established according to the sealers used:

Group I (Sealapex + 3% CHNP): Calcium hydroxide-based sealer modified with added 3% calcium hydroxide nanoparticles.

Group II (Sealapex + 8% CHNP): Calcium hydroxide-based sealer modified with added 8% calcium hydroxide nanoparticles.

Group III (Conventional formula): Calcium hydroxide-based sealer Sealapex in its conventional form.

5. Evaluation of Solubility

For each group of sealers, twelve Teflon molds (n=36) that measure 1.5 mm in thickness and 20 mm in diameter were fabricated following the ISO standardization 6876/2012 and used for sample preparation¹⁶ (Figure 2).

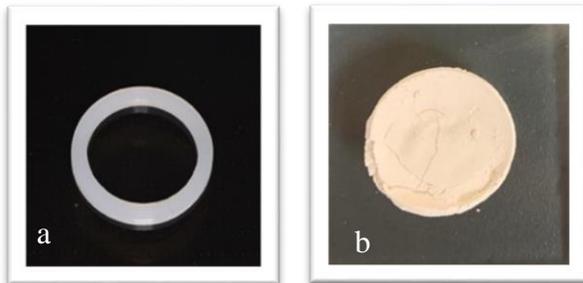


Figure (2): a) Teflon mold used for solubility samples.
b) Sample after removal from the mold.

The molds were placed on a glass plate and slightly over-filled with the pre-mixed sealers, then the assembly is left to set in a cabinet at 37 °C and not less than 95% relative humidity. Using analytical balance (AS 220.R2, RADWAG, Poland), the samples were weighed and the weight was recorded as W1, then immersed in a beaker that contains 10 mm of deionized water. W2 is recorded at time intervals of 1 and 14 days. Samples were removed from the beaker and dried using absorbent paper and then placed

in a desiccator to a constant weight ($\pm 0.001\text{g}$). Solubility is then calculated according to the following formula:

$$S = (W1 - W2) / W1 \times 100.$$

6. Evaluation of pH

For each group of sealers, six Teflon molds (n=18), that measure 10 mm in diameter and 2 mm in height were fabricated (Figure 3).

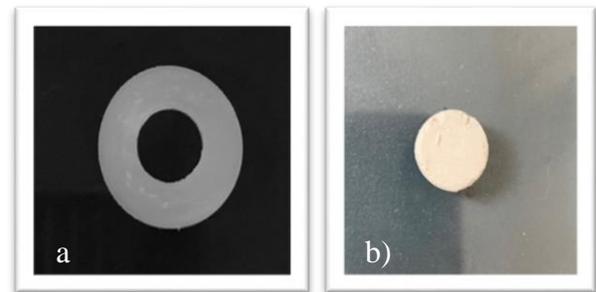


Figure (3): a) Teflon mold used for pH samples.
b) Sample after removal from the mold.

The Teflon molds were filled with sealer and then transferred to a cabinet until setting. Each sample is placed in a flat bottom container filled with 10 ml deionized water at 37 °C. This deionized water in which the samples were immersed is then checked after pre-determined periods of (1, 3, 24 hours and 14 days). A digital pH meter (Orion Star A111, Thermoscientific, USA) was used to measure the pH values, pre-calibrated with buffer solutions (pH=4,00, pH=7,00, pH=10,00) before each evaluation.

RESULTS

1. Evaluation of Solubility The values of the percentage of solubility of the tested samples were recorded (**Table 1, Figure 4**).

significant difference between the tested groups ($p=0.045$). At 14 days, Group I (Sealapex + 3% CHNP) showed the highest solubility percentage value, followed by

Table (1): Descriptive statistics for the values of solubility (%) and results of Two-way ANOVA test of comparison between the three groups at different time intervals.

Time	Solubility (%) (mean \pm SD)						p-Value
	Group I (Sealapex+3% CHNP)		Group II (Sealapex+8% CHNP)		Group III (Conventional formula)		
	Mean	SD	Mean	SD	Mean	SD	
24 hours	4.97	2.12 ^A	2.24	0.34 ^B	2.83	2.25 ^{AB}	0.045*
14 days	5.13	1.52 ^A	3.42	0.24 ^A	3.36	2.02 ^A	0.089ns
p- Value	0.882ns		<0.001*		0.680ns		

Different superscript letters indicate a statistically significant difference within the same row.

*; significant ($p \leq 0.05$).

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

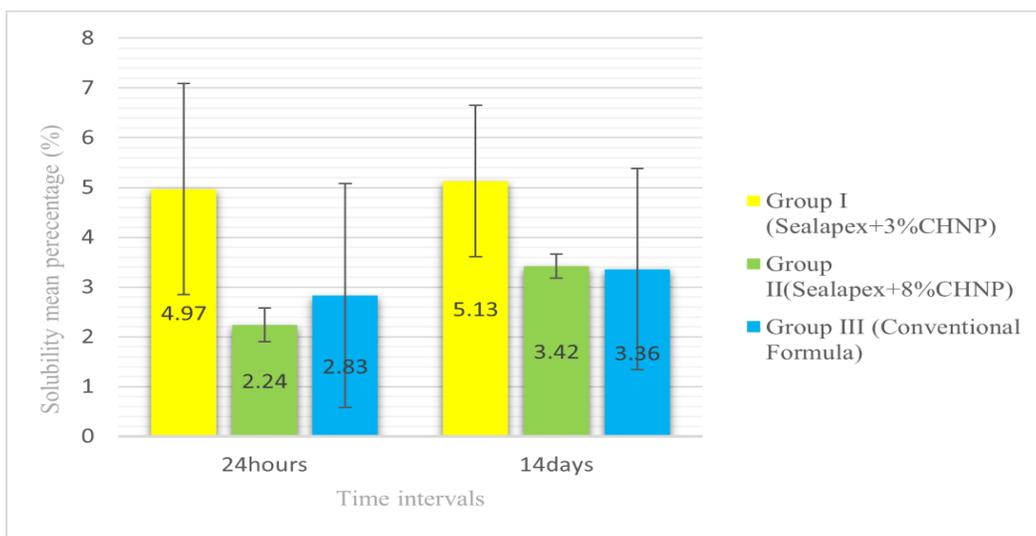


Figure (4): Mean percentage of solubility over time, error bars denote Standard deviation.

At 24 hours, Group I (Sealapex + 3% CHNP) showed the highest solubility percentage value, followed by group III (conventional formula), while group II (Sealapex + 8% CHNP) showed the least solubility percentage value. Statistically, there was a

group II (Sealapex + 8% CHNP), while group III (conventional formula) showed the least solubility percentage value. Statistically, there was no significant difference between the tested groups ($p=0.089$).

2. Evaluation of pH

The pH changes of the tested materials at different immersion times (1, 3, 24 hours and 14 days) was recorded (**Table 2, Figure5**).

modified with 3% CHNP and the least pH value was recorded for Sealapex modified with 8% CHNP. At the 24 hours interval,

Table (2): Descriptive statistics for the values of pH of the sealers and results of Two-way ANOVA test of comparison between the three groups at different time intervals.

Time	pH (mean ± SD)						p-value
	Group I		Group II		Group III		
	(Sealapex+3% CHNP)		(Sealapex+8% CHNP)		(Conventional Formula)		
	Mean	SD	Mean	SD	Mean	SD	
After 1 hour	9.47	0.26 ^B	8.85	0.15 ^C	10.09	0.16 ^A	<0.001*
After 3 hours	9.71	0.19 ^B	9.29	0.15 ^B	10.72	0.44 ^A	<0.001*
After 24 hours	11.91	0.07 ^{AB}	12.28	0.26 ^A	11.62	0.55 ^B	0.021*
After 14 days	11.57	0.28 ^A	11.04	0.55 ^A	9.89	0.17 ^B	<0.001*
P-value	<0.001*		<0.001*		<0.001*		

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

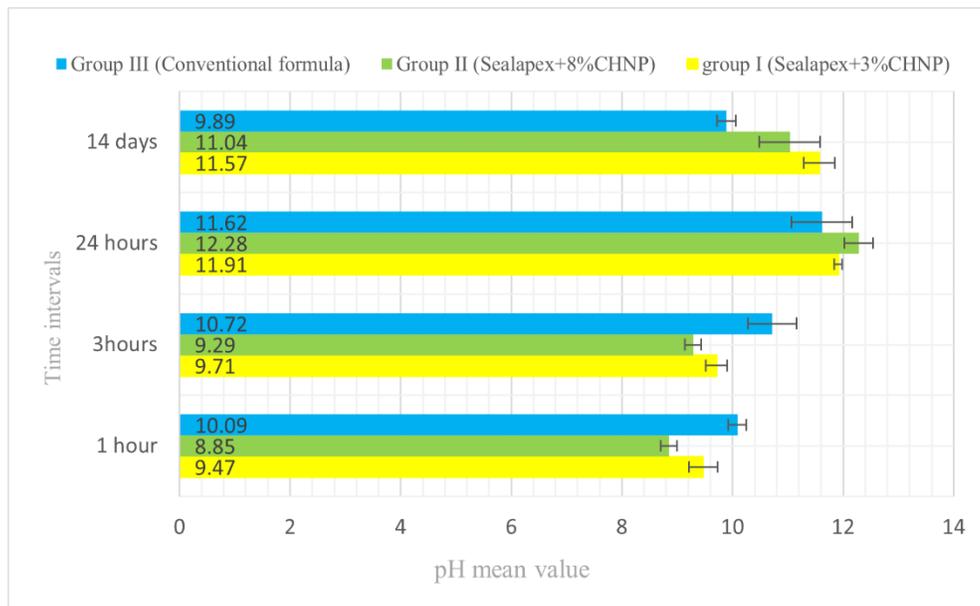


Figure (5): Mean pH value over time, error bars denote Standard deviation.

For the first hour and the three-hour time intervals, conventional formula showed the highest pH value followed by Sealapex

Sealapex modified with 8% CHNP showed the highest pH value followed by 3% CHNP and the lowest value was for the conventional

formula. After 14 days both Sealapex modified with 3% CHNP. Sealapex modified with 8% CHNP showed a slight drop in pH values but continued to have a higher pH value than the conventional formula. The highest value at 14 days was recorded for Sealapex modified with 3% CHNP. Statistically, there was a significance difference between the tested groups.

DISCUSSION

According to ANSI/ ADA specification 57¹⁷ and ISO Standards 6876,¹⁶ solubility of a root canal sealer was defined as the mass loss of a material after a period of immersion in water. Solubility of a root canal sealer should not exceed 3% of its initial total mass after immersion in water for 24 hours. Solubility of root canal sealers is desirable to a certain extent, as it may contribute to the release of the antibacterial components and increase its pH. On the other hand, a highly soluble sealer will lead to leakage and gap formation between root dentin and the sealer and within the sealer itself.¹⁸

In the current study, evaluation of the sealer's solubility followed the ISO Standards 6876/2012 for root canal sealing materials.¹⁶ pH was evaluated following similar methodologies used in previous studies.^{19,20} According to each methodological requirement, either Teflon/g-

ypsum molds were fabricated.²¹

The experimental setup used in the present study is recommended by the ISO standards, which is weighing specimens before and after immersion in water. It must be taken into consideration that this kind of testing is a measurement of elution of the water-soluble components of the sealer such as filler particles and not necessarily an indication of solubility nor the actual clinical behavior of the material. In clinical situations, the amount of sealer used is much less than in experimental setting.²² Root canals are dried using paper points prior to sealer insertion and further flow of fluids is prevented by coronal restoration.²¹ Also, the water uptake may compensate for the disintegrated components which in-turn influence the weight of the material.²³

Sealapex presents a high degree of solubility²⁴, due to its extended setting time,²⁵⁻²⁸ leading to non-homogeneous setting reaction and poorly formed matrix.^{29,30} Another explanation for its high solubility is the continuous ingress of water as it prolongs the reaction between powder and binder,²⁵ there-by liberating additional Ca and OH ions and further increasing the porosity. Some studies suggested that this continued reaction during setting under humidity results in volumetric expansion and

decrease in leakage³¹ which may compensate for the diluted material providing seal theoretically.

In the current study, in a span of 24 hours, Sealapex modified with 8% CHNP had the lowest solubility percentage of all groups followed by the conventional formula. Both percentages didn't exceed the recommendation of the ISO Standards 6876.¹⁶ At the 14 days period, both Sealapex modified with 8% CHNP and the conventional formula slightly exceeded the recommended maximum percentage. On the other hand, Sealapex modified with 3% CHNP violated this recommendation having the highest solubility value at all exposure times.

The results of the present study were in agreement with previous studies' reports.^{25,26,31,32} Schäfer and Zandbiglari²³ tested the solubility of different root canal sealers in water and artificial saliva, their results showed that Sealapex presented a high solubility percentage ranged from 5.55% to 10.14% over the span of 28 days depending on the immersion liquid. Also, their results revealed that Sealapex was more soluble in water than in saliva³³ and that Sealapex presented the highest solubility values among all sealers tested at all time intervals. Other studies showed conflicting results that

Sealapex has low solubility. Those studies claimed that the low solubility of Sealapex is due to the presence of bismuth trioxide in its composition, which was formulated to provide greater radiopacity, better shelf life and less solubility without losing its biological properties.³⁴

It was well documented in the literature that calcium hydroxide-based sealers had a higher solubility than other sealers as part of their mechanism.^{29,30,33} Despite its high solubility, multiple reports stated that it has a long-term sealing ability compared to other sealers.³⁴⁻³⁹ Desouky et al.³⁷ found that nano-calcium hydroxide-based sealer had a proper sealing ability in comparison to the resin-based sealer and claimed that this may be due to the greater contact surface area and charge density provided by the smaller size of the nanoparticles material in comparison to its conventionally micro-sized counter-parts.

In the current study, the addition of 3% CHNP further increased the solubility percentage of Sealapex. This may be attributed to the fact that the availability of hydroxyl ions was increased as a result of the smaller particle size which in-turn increases the reactivity of the particles.¹¹ The results also showed that the addition of 8% CHNP revealed a lesser solubility percentage than the 3% CHNP. It also formed a better

specimen with lesser cracking and not as fragile as in the conventional formula.³² This also was revealed even after immersion and drying the specimens, they remain intact as posed to the other groups where the specimens were cracked due to solubility and water sorption (**Figure 6**).



Figure (6): Specimens after immersion.

In general, increased solubility of the sealer is an unfavorable effect. This is not the case with calcium hydroxide-based sealers theoretically in which the more solubility leads to more release of calcium and hydroxyl ions. This action in turn favors alkalinity and higher pH⁴⁰ with sustained effect. This also activates calcium-dependent ATPase⁴¹ which reacts with carbon dioxide from tissues forming calcium carbonate promoting mineralization and providing a biological seal of the root apex,^{19,20} and indirectly limiting the nutrition source of the anaerobic bacteria,⁴² leading to rapid healing and a better antibacterial effect.⁴³

The results of the present study also revealed that Sealapex modified with 8% CHNP showed a decrease in solubility. This could be attributed to the difference in mixing

ratios, as in the addition of CHNP in powder form to the sealer in a paste form could have affected the physical properties of the sealer.⁴⁴ Another possible explanation is the higher reactivity of nanoparticles which causes them to agglomerate to satisfy their bonds and stabilize their surface energy which may reinforce the sealers microstructure providing a stable crystalline matrix of the sealer, thus reducing its solubility and porosity.^{32,45}

pH of root canal sealers plays an important role in the healing process through stimulation of mineralized tissue deposition and neutralization of osteoclastic activity preventing dissolution of mineralized tooth components,^{46,47} It also provides an antibacterial effect and alteration of the ecology of periapical inflammatory tissue.⁴⁸

The results of the present study revealed that Sealapex as a sealer demonstrated higher pH in comparison to different sealers. The results were in accordance with previous studies' reports.^{38,48}

Calcium hydroxide in contact with aqueous fluids dissociates into calcium and hydroxyl ions.¹ This might explain its solubility and its high alkalinity. This promotes activation of the tissue enzyme alkaline phosphatase. Alkaline phosphatase is a hydrolytic enzyme that has the capability

of liberating free phosphate ions that react with calcium ions from the blood stream forming calcium phosphate precipitate which is the forming unit of hydroxyapatite and activates calcium-dependent adenosine triphosphatase reaction that plays a vital role in hard tissue formation. Mineralized tissue formation is observed from about the 7th to 10th day of tissue contact.⁴² Calcium hydroxide also has the capability to control inflammatory root resorption by deactivating the osteoclastic activity through neutralization of lactic acid preventing the dissolution of tooth mineralized components and accelerating the healing process⁸ as it diffuses through dentinal tubules communicating with periodontal ligament space⁴⁹.

CONCLUSIONS

1. 3% CHNP added to Sealapex root canal sealer significantly increased solubility. This impacted the pH value to be higher and more sustainable at 14 days.

2. The addition of 8% CHNP to Sealapex root canal sealer showed significantly lower solubility values than the 3% CHNP. Regardless of its lower solubility, it sustained its pH value for up to 14 days.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no competing interests.

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None.

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