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**Original Article** 

## SETTING TIME, FLOWABILITY, AND SOLUBILITY OF EPOXY RESIN-BASED SEALER MIXED WITH CHITOSAN NANOPARTICLES

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### ABSTRACT

**Objective:** The purpose of this study was to investigate the effect of the incorporation of chitosan nanoparticles into epoxy resin-based sealer on its setting time, flowability and solubility.

**Methods:** This study was divided into three evaluations: setting time, flowability, and solubility of sealers. Each study used 20 samples, which were divided into two groups. Group 1, epoxy resin-based sealers, and group 2, epoxy resin-based sealers mixed with chitosan nanoparticles. The Gilmore needle was used to observe the setting time, a simple press method based on ISO 6876 was employed to evaluate the flowability of the sealer, and the solubility test, which was according to ISO 6876 standard, was used to determine the solubility of sealers. The surface structure of the sealers before and after the solubility test was observed under Scanning Electron Microscopy (SEM). The data obtained from each study were analyzed by an unpaired t-test with a degree of significance of 95%.

**Results:** The results showed that the setting time of epoxy resin and epoxy resin incorporated with chitosan were  $567\pm30.20$  and  $572.5\pm27.91$  min, flowability was  $25.06\pm0.89$  and  $23.18\pm1.06$  mm, and solubility was  $0.0051\%\pm0.0016$  and  $0.0045\%\pm0.0018$ , respectively. No significant difference occurred between epoxy resin-based sealer and epoxy resin-based sealer mixed with chitosan nanoparticles in setting time, flowability, and solubility of sealers (\*P>0.05).

**Conclusion:** The incorporation of chitosan nanoparticles produced a similar effect in setting time, flowability, and solubility as the epoxy resinbased sealer. Thus, epoxy resin-based sealer mixed with chitosan nanoparticles had acceptable properties in setting time, flowability, and solubility based on ISO 6876 standards.

Keywords: Setting time, Flowability, Solubility, Epoxy resin-based sealer, Chitosan nanoparticle

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## INTRODUCTION

Root canal sealers are crucial in the obturation of the root canal and are used in association with gutta-percha. The purpose of using a root canal sealer is to assurance the closure of the root canal system of the teeth, thereby eliminating residual microorganisms and avoiding ingress of any bacteria from the oral environment to periapical tissues [1, 2]. Sealers also assist the positioning of the master gutta-percha cone and perform as a linking agent between the gutta-percha and the canal wall, filling remaining spaces. Therefore, sealers must be capable of providing a fluid-tight seal [3]. Although root canal sealers used only as additional materials in the root canal system's obturation have been revealed to affect the result of root canal treatment [4]. The ideal root canal sealer must have biocompatibility, antibacterial, good apical sealing ability, adequate flowability, insoluble in tissue fluid, and no coloring to teeth [5, 6].

The epoxy resin-based sealer has recently been popular as root canal obturation material because it has several beneficial properties such as good apical closure, adequate flowability, low setting time, and solubility [4, 7]. However, according to previous studies, the epoxy resin-based sealer has low antibacterial properties; hence the possibility of adding a high antibacterial material to the epoxy resin-based sealer might enhance its antibacterial property [8-10]. Besides, the addition of nano-sized fillers into the resin-based sealer could increase the bond strength between the sealer and the root canal dentin, leading to enhance apical sealing ability [11].

Chitosan has been widely used in dentistry due to its beneficial properties, namely bio-degradation, biocompatibility, bio-adhesion, and non-toxic to human cells. Chitosan is a non-toxic natural polysaccharide generated from the deacetylation of chitin, which is attained from the shells of crustaceans and shrimp [12]. Previous studies have elucidated that chitosan has antibacterial properties against *E faecalis* and *C albicans* [13, 14].

Incorporating chitosan nanoparticles into an epoxy resin-based sealer is interesting to be studied to increase its antibacterial property, especially against E faecalis, and enhancing its adhesion to the root canal dentin wall. However, the addition of chitosan nanoparticles should not affect the physical properties of root canal sealers, and it must follow the requirements in the International Standards Organization (ISO) 6876 standard for root canal sealing materials [6, 15]. Nowadays, no study has evaluated the incorporation of chitosan nanoparticles into epoxy resin-based sealers on its physical properties, such as setting time, flowability, and solubility. These physical properties of the sealer are important parameters and needed to be investigated because setting time indicates adequate working time and sufficient consistency to fulfill the root canal sealer property [16]. The flowability of the sealer determines the ability of the sealer to penetrate the root canal, especially to the accessory canal; hence it can fill the voids between the main gutta-percha and gutta-percha accessories [17]. The solubility of sealers needs to be evaluated because it could release materials that are able to irritate periapical tissues. The solubility could also create a gap between the root canal wall and obturation material, resulting in apical leakage [18]. Therefore, the aim of this study was to evaluate the effect of the incorporation of chitosan nanoparticles into epoxy resin-based sealer on its setting time, flowability and solubility.

## MATERIALS AND METHODS

The protocol of this study was approved by the Institutional Ethics Committee, no. 00109/KKEP/FKG-UGM/EC/2019. Chitosan was synthesis from shrimp shells (degree of deacetylation>75%) using the ionic glass method and Polyanion Tripolyphosphate (TPP) as a crosslinker [19]. This study was divided into three evaluations, the first study evaluated the setting time of sealer, the second study evaluated flowability of sealer, and the third study evaluated solubility of sealers. Each study used 20 samples randomly assigned into two groups of 10 each. Group 1 used epoxy resin-based sealer (AH 26, De Trey, Dentsply, Konstanz, Germany), and group 2 used epoxy resin-based sealer (AH 26, De Trey, Dentsply) mixed with chitosan nanoparticle. The epoxy resin-based sealer (Group 1) was mixed by the same operator on a glass plate according to the manufacturer's instruction, namely two units of powder (100 mg) with one resin unit (50 mg) using a spatula until the mixture evenly mixed and could be pulled up to 1.5-2.5 cm. Group 2 used an epoxy resin-based sealer mixed with chitosan nanoparticles. Chitosan nanoparticles were added to epoxy resin-based sealer powder by comparison between chitosan nanoparticles (15 mg) with epoxy resin-based sealer powder (100 mg) [15]. Both ingredients were mixed thoroughly using a spatula. The mixture was mixed with oneunit resin (57.7 mg) using a spatula until it was thoroughly mixed and could be pulled up to 1.5-2.5 cm.

## Setting time evaluation

The setting time of the sealers was determined according to the ISO 6876 specification [15]. This study used 20 samples were randomly assigned into two groups of 10 each. Group 1: epoxy resin-based sealer (AH 26, De Trey, Dentsply), and group 2: epoxy resin-based sealer (AH 26, De Trey, Dentsply) mixed with chitosan nanoparticle. All sealers were mixed and manipulated in the same manner above. The sealer mixture was inserted into the metallic molds (10 mm in diameter and 2 mm in height), then transferred to a chamber with 95% relative humidity and temperature of 37 °C. After the initial setting time, the Gilmore needle with a weight of 110 g and a tip diameter of 2 mm was lowered vertically onto the horizontal surface of each sample. The setting times were determined as the time elapsed from the commencing of mixing to the time at which no indentation was noticed on the surface of the specimens. Three measurements were done for each sealer.

## **Flowability evaluation**

This evaluation also used 20 samples, which were randomly divided into two groups of 10 each. Group 1: epoxy resin-based sealer (AH 26, De Trey, Dentsply), and group 2: epoxy resin-based sealer (AH 26, De Trey, Dentsply) mixed with chitosan nanoparticle. All sealers were mixed and manipulated in the same manner above. The mixed sealers were then placed in the middle of the glass plate. After 180 s following the start of mixing, another glass plate with a mass of  $20\pm 2$ g and a load of 100 N was placed in the middle on the top of the plate; therefore, the total weight was 120 g. Ten minutes after beginning the mixing, the load was removed, and the average of the maximum and minimum diameters of the compressed disc was measured by the digital calipers (Mintuyo MTI Corporation, Tokyo, Japan). If the difference between both diameters was not more than 1.0 mm, the results were recorded.

#### Solubility evaluation

This study used 20 samples were randomly assigned into two groups of 10 each. Group 1: epoxy resin-based sealer (AH 26, De Trey, Dentsply), and group 2: epoxy resin-based sealer ((AH 26, De Trey, Dentsply) mixed with chitosan nanoparticles. All sealers were mixed and manipulated in the same manner above. The solubility of sealer was carried out in accordance with ISO 6876 standards [15]. Teflon ring molds with an inner diameter of 7.5 mm and a thickness of 1.5 mm were used for sample preparation. The molds were placed on a glass plate and covered with a cellophane sheet. A nylon thread

was located inside the sealer material, and another glass plate, which was also covered with cellophane film, was positioned on the mold and pressed manually; hence the plates touched the entire mold in a uniform manner. All samples were stored in an incubator (37 °C, with 95% relative humidity) and left to stand for a period corresponding to three times of setting time.

After setting, the specimens were removed from the molds, and any loose material was removed from the surface. Each sample was weighed three times using an analytical balance with 0.0001 g (Mettler Toledo, Jiangsu, China). The samples were suspended by nylon thread and placed inside the glassware containing deionized distilled water. Special attention was taken to keep the specimens hung in the water, not touching the walls of glassware. The containers were stored for 7 d in an incubator ((37 °C, with 95% relative humidity). After this period, the samples were then removed from the container and washed with distilled water, dried with absorbent paper, placed in the oven for 24 h and then weighed again. The experiment was repeated three times for each sealer. Solubility was determined by calculating the weight loss (initial mass-final mass), expressed as the percentage of the original mass. This value indicated the solubility of the sealer.

All samples before and after the solubility test were observed under SEM. All samples were secured on metal stubs, desiccated, and sputter-coated with gold, and the morphologies of the external surface of the samples were qualitatively analyzed using SEM (JEOL JSM-5510, Tokyo, Japan) at 1000x magnification.

#### Statistical analysis

Data of setting time, flowability and solubility were presented as mean and standard deviation (SD). The data obtained from each study were analyzed by an unpaired t-test with a degree of significance of 95%.

## RESULTS

The results of this setting time study (table 1) for both sealers were in the range of ISO 6876 standard, which was more than 30 min and less than 72 h. This study also showed a longer setting time of epoxy resin-based sealer mixed with chitosan nanoparticles, although both sealers were not significantly different (\*P>0.05) (table 2).

Even though an insignificant difference occurred between both sealers (\*P>0.05) (table 2), epoxy resin-based sealer mixed with chitosan nanoparticles produced greater flowability than epoxy resin-based sealer (table 1). According to ISO 6876 standard, both sealers fulfilled the criteria as a sealer since the diameter was greater than 20 mm.

Regarding solubility, it can be seen that both sealers met the criteria of ISO 6876 standard, which should not exceed 3% by mass when the solubility of the set material is tested. Both sealers revealed no significant differences occurred in solubility (\*P>0.05) (table 2).

Representative SEM micrograph of samples before and after water storage for 7 d is presented in fig. 1 and 2. It was noted that all surfaces of the sealers had micromorphological changes after the solubility test. The epoxy resin-based sealer had an external surface that appeared to be mostly homogenous rough surface and uniformly scattered globular-like particles (fig. 1 A). The loss of matrix was apparent, and the filler particles were more noticeable (fig. 1 B). The chitosan nanoparticles were distinguishable on the surface of the epoxy resinbased sealer mixed with chitosan nanoparticles before the solubility test (fig. 2 A). After the solubility test, the surface appeared to be similar to before; although some matrix dissolved, chitosan nanoparticles remained obvious embedded in the sealer (fig. 2 B).

 Table 1: Mean and standard deviation of incorporation of chitosan nanoparticles into epoxy resin-based sealer on setting time, flowability, and solubility

Sealers	Setting time (min)	Flowability (mm)	Solubility (%)	
Epoxy resin	567±30.20	25.06±0.89	0.0051±0.0016	
Epoxy resin+chitosan nanoparticles	572.5 <b>±</b> 27.91	23.18±1.06	$0.0045 \pm 0.0018$	

n=10 in each study and data are given in mean±SD

## Table 2: Analysis using t-test of incorporation of chitosan nanoparticles to epoxy resin-based sealer on setting time, flowability, and solubility

Treatment	Т	Р	
Setting time	0.007	0.937	
Flowability	0.918	0.351	
Solubility	0.774	0.390	

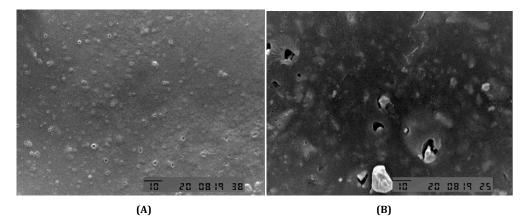
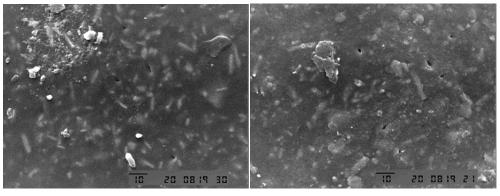


Fig. 1: Epoxy resin-based sealer before solubility test demonstrated the external surface was observed homogeneously rough (A); Epoxy resin-based sealer after solubility test exhibited the external surface was observed the loss of some matrix and the detachment of filler particles were more noticeable (B). Original magnification X1000



(A)

(B)

Fig. 2: Epoxy resin-based sealer mixed with chitosan nanoparticles before solubility test revealed the external surface was observed homogeneously rough and chitosan nanoparticles were distinguishable (A); Epoxy resin-based sealer mixed with chitosan nanoparticles before solubility test revealed the external surface was observed less matrix loss and chitosan nanoparticles remained obvious embedded in the sealer (B). Original magnification X1000

## DISCUSSION

It is essential to generate standardized procedures to evaluate the physicochemical properties of a new and gold standard of materials, such as root canal sealers. The standardizations were established after the publication of ISO 6876 [20]. Although there is no specific standard for the setting time of root canal sealers, the dentist needs adequate working time for material placement in the root canal. Setting time is principally a control assessment on the stable behavior of a product, and this parameter relies on several factors, for example, the constituent of components, particle size, the ambient temperature, and relative humidity [21]. In the present study, the setting time of both sealers was in accordance with ISO 6876. Both sealers produced almost a similar setting time and were appropriate in the range of the setting time stated in the manufacturer's instruction, which is 24 to 36 h. In this study, humidity and temperature were controlled for both sealers.

The setting of a sealer occurs due to the reaction between the two components of the material (powder with liquid). The long setting time of epoxy resin-based sealer may be due to the that sealer consists of powder and liquid, namely the bisphenol-epoxy resin and hexamethylenetetramine, which needs a slow polymerization reaction of epoxy resin amines with high molecular weight. Therefore, the conversion of polymers from monomers occurs slowly [22]. The incorporation of chitosan nanoparticles into epoxy resin-based sealer produced a longer setting time compared to epoxy resin-based sealer because the addition of particles that do not contribute to the reaction during setting or polymerization may enhance the setting time [23].

The long setting time might generate advantages of both sealers since it leads to no cracking or rapid separation from dentinal walls resulting in good adhesion to the root canal wall. However, this setting time of the sealer cannot be very long because the contact of the sealer with the periapical tissues may cause irritation and influence the biocompatibility. Additionally, a long time to harden may increase solubility, creating gaps that could be occupied by microorganisms resulting in reinfection [24].

Among the root canal sealers available in the market, epoxy resinbased sealers have been widely used for obturation material due to their resorption resistance and dimensional stability [16]. Besides, the epoxy resin-based sealer has antibacterial property, good flow, low toxicity, and well tolerated by periapical tissue, biocompatibility, low solubility, and shrinkage, sealing ability, adequate working time and penetration into dentinal tubules, as well as good handling characteristic [25].

The crucial consideration for the use of sealers in the root canal is flowability. The greater the penetrating capability of sealer into dentinal tubules, the greater the ability to trap microorganisms and prevent their growth. The flow and penetration ability of sealers between gutta-percha cones and into the accessory canals are also essential [17]. According to ISO 6876 [15], the sealers should have a diameter of not less than 20 mm at the flowability test. In the present study, the flow of epoxy resin-based sealer and epoxy resinbased sealer mixed with chitosan nanoparticles were measured at 25.06±0.89 mm and 23.18±1.06 mm, respectively. Therefore, both sealers fulfilled the ISO 6876 specifications. The flowability of sealers affected by several factors such as film thickness, particle size, temperature, rate of insertion, the internal diameter of the canal, powder/liquid, and shear rate. In this study, the incorporation of chitosan nanoparticle, which has nanoparticle size, could influence the flowability of sealers [1, 21].

According to previous investigators, an endodontic sealer should have a moderate flow rate since undue flow enhances the risk of extravasation and deficient flow decreases the sealer penetration in accessory canals. Additionally, flowability is an essential factor in achieving hermetic seal of root canal obturation because low flow within the working time may induce endodontic sealer to reach seal the apical foramen and lateral dentinal wall irregularities [9]. Conversely, high flowability might result in extrusion of sealer through the apical foramen to the periapical tissues, thereby compromise healing [26, 27].

As previously stated, the flowability is affected by the size of sealer particles. This statement is consistent with this present study that revealed the incorporation of chitosan even though in nano-size particles produced a lower flowability compared to the epoxy sealer-based sealer. Preferably, an endodontic sealer must have reasonable flowability since excessive flow increases the risk of extravasation while inadequate flow diminishes the penetration of resin into the accessory canals [17, 28].

The solubility of sealers is one of the requirements of using sealers in the root canal system. Solubility is directly related to the detachment of material components by interaction with fluids, creating gaps that might be populated by microorganisms leading to reinfection [24]. In this study, the solubility of epoxy resin-based sealer and sealer mixed with chitosan nanoparticles was 0.0051±0.0016% and 0.0045± 0.0018%, respectively. Thus, the results fulfilled the criteria of the requirements of ISO 6876, which stated that the loss of mass must not surpass 3% of the total weight. The solubility of both sealers was also low, and these results were in accordance with other previous studies [5, 18]. The polymerization of the epoxy resin-based sealer is achieved through the reaction between hexamethylene tetraamine and bisphenol resin monomer to form a covalent bond; hence the polymer is heavily cross-linked, rigid, and durable resulting in low solubility [29]. This phenomenon may explain the low solubility of epoxy resin-based sealer and, as shown in SEM observation, seem homogenous and rough on the external surface.

This study also showed that the addition of chitosan in nanoparticle size, which acts as a filler in the epoxy resin-based sealer, leads to induce less solubility than an epoxy resin-based sealer. However, no significant difference occurred between both sealers (\*P>0.05), and both sealers had low solubility, according to ISO 6876. The solubility is of primary importance for a root canal sealer because successful results of endodontic treatment rely the majority on the sealer constituent integrity [18, 30]. Degradation of the sealer may create voids and gaps in the interface of the sealer/dentin or the sealer/gutta-percha, leading to provide a pathway for microorganisms and their toxic products into periapical tissues [31]. Thus, the insolubility of root canal sealers might indeed influence the success of root canal treatment. Observation using SEM exhibited that both sealers surface presented micromorphological

changes following the solubility test, and the epoxy resin-based sealer showed more obvious in micromorphological alteration.

#### CONCLUSION

The incorporation of chitosan nanoparticles produced a similar effect in setting time, flowability, and solubility as the epoxy resinbased sealer. Thus, epoxy resin-based sealer mixed with chitosan nanoparticles can be potential as a sealer in the future since it had acceptable properties in setting time, flowability, and solubility based on ISO 6876 standards.

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## **AUTHORS CONTRIBUTIONS**

All the authors have contributed equally.

## **CONFLICT OF INTERESTS**

Authors declare no conflict of interest.

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