Zinc Oxide-Based Endodontic Sealer with Proanthocyanidin-PLGA Nanoparticles: Synthesis and Physical Characterization

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ABSTRACT
This study developed a zinc oxide-based endodontic sealer incorporating proanthocyanidin nanoparticles (PAC-NPs) encapsulated in poly(lactic-co-glycolic acid) (PLGA), utilizing PAC's proven ability to enhance dentine collagen stability. The formulation was designed to provide both antimicrobial and collagen-stabilizing benefits, aiming to improve dentine-sealer interface integrity and prolong the functional lifespan of root canal treatments. Chemical characterization was conducted using Attenuated Total Reflectance - Fourier Transform Infrared (ATR-FTIR) spectroscopy, confirming the presence of Zn-O and C=O bonds, indicative of the successful integration of zinc oxide and encapsulated PAC. Physical and chemical properties, including flow, setting time, solubility, dimensional stability and pH were evaluated according to ISO 6876:2012 and ANSI/ADA standards 57, with the sealer meeting all clinical performance criteria. This preliminary characterization of the physical and chemical properties establishes a basis for further in vitro studies on the mechanical and biological performance of the sealer, aimed at validating its potential to enhance dentine collagen stabilization and material durability in endodontic applications.

1. Introduction

Endodontic sealers play a pivotal role in the success of root canal therapy by sealing the spaces between the obturating material and the dentinal walls. A reliable sealer not only prevents microbial re-infiltration but also reinforces the mechanical stability of the root canal filling [1]. The choice of sealer is therefore crucial in achieving long-term clinical success in endodontics. Among the various types of sealers available—zinc oxide-eugenol, calcium hydroxide, epoxy resin-based, and bioceramic—zinc oxide-based sealers are widely used due to their established antimicrobial properties and ease of manipulation [2,3].

Despite their widespread usage, conventional zinc oxide-based sealers have inherent limitations, particularly in their interaction with dentine. The adhesion of these sealers to the dentine walls is often weak, primarily because they fail to adequately interact with the dentine collagen matrix. Dentine consists of approximately 20% organic material, mainly collagen, which forms a critical component in the structural integrity of the root canal [4]. Over time, enzymatic degradation of this collagen by endogenous proteolytic enzymes such as matrix

metalloproteinases (MMPs) and cysteine cathepsins can lead to a compromised dentine-sealer interface, resulting in microleakage, secondary infections, and ultimately, the failure of the root canal therapy [5,6].

Moreover, zinc oxide sealers, while being antimicrobial, do not contribute significantly to the bioactivity of the surrounding dentine, and their mechanical properties can degrade over time due to solubility in tissue fluids [7]. These challenges have necessitated the development of bioactive sealers that not only possess antimicrobial properties but also promote the stabilization and preservation of the dentine-collagen matrix [8].

Recent advances in nanotechnology have paved the way for the incorporation of nanoparticles into endodontic materials to improve their properties. Zinc oxide nanoparticles (ZnO-NPs), in particular, have gained attention due to their potent antimicrobial activity against a broad spectrum of pathogens, including those involved in endodontic infections such as *Enterococcus faecalis* [9]. The smaller size and higher surface area of ZnO-NPs allow for enhanced penetration into dentinal tubules and better interaction with bacterial cell membranes, resulting in superior antimicrobial efficacy compared to their micron-sized counterparts [10].

In addition to their antimicrobial properties, ZnO-NPs have been shown to contribute to the mechanical properties of endodontic sealers. The incorporation of ZnO-NPs into the sealer matrix enhances its compressive strength and reduces its solubility in aqueous environments, thereby improving its long-term stability within the root canal system [11]. This is particularly significant in reducing microleakage and preventing post-treatment infections. However, ZnO-NPs alone do not address the issue of collagen degradation, which remains a critical factor in the long-term success of root canal treatments [5].

The degradation of dentine collagen is a major concern in endodontics, as the integrity of the dentine-sealer interface is compromised when collagen fibres degrade [4]. The degradation is primarily mediated by MMPs, which are activated during the demineralization process, such as during acid etching or bacterial colonization [6]. The activation of these enzymes leads to the breakdown of collagen fibrils, weakening the structure of the dentine and reducing its bonding strength with the sealer [12]. Consequently, this enzymatic degradation results in microgaps between the sealer and dentine, allowing for bacterial ingress and subsequent root canal failure.

To address this issue, recent research has focused on the development of materials that can stabilize the dentine-collagen matrix by cross-linking the collagen fibrils and protecting them from enzymatic degradation. Among the various agents studied, proanthocyanidins (PACs) have emerged as promising natural cross-linkers due to their ability to interact with collagen and form stable, cross-linked structures that are resistant to proteolytic degradation [13].

Proanthocyanidins (PAC), polyphenolic compounds found in grape seeds, cranberries, and other plant sources, have been widely studied for their role in stabilizing collagen by forming non-covalent cross-links between collagen molecules [14]. This cross-linking strengthens the collagen matrix, making it more resistant to enzymatic degradation. In the context of endodontics, the incorporation of proanthocyanidin nanoparticles (PAC-NPs) into a sealer matrix represents a novel approach to enhancing the longevity of root canal treatments by

preventing collagen breakdown and maintaining the integrity of the dentine-sealer interface [15].

The encapsulation of PACs in biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA) allows for the controlled release of these bioactive molecules, ensuring sustained collagen stabilization over time [16]. PLGA is a biocompatible, FDA-approved polymer that degrades into lactic and glycolic acid, both of which are naturally metabolized by the body [17]. This controlled release system ensures that PACs are delivered in a consistent manner, providing long-term protection to the collagen matrix without requiring repeated applications.

By incorporating PAC-NPs into a zinc oxide-based sealer, this study aims to create a bioactive endodontic material that not only seals the root canal but also actively preserves the dentine structure by preventing collagen degradation [18]. This novel formulation offers dual benefits: the antimicrobial and mechanical properties of ZnO-NPs, coupled with the collagen-stabilizing effects of PAC-NPs, represent a significant advancement in the design of endodontic sealers.

The aim of the study was to synthesize and characterize a zinc oxide-based endodontic sealer incorporating proanthocyanidin nanoparticles encapsulated in PLGA for enhanced collagen stabilization. This study evaluated the physical and chemical properties of the sealer, including flow, setting time, solubility, dimensional stability and pH, in accordance with ISO 6876:2012 and ANSI/ADA specification 57. Additionally, Attenuated Total Reflectance - Fourier Transform Infrared (ATR-FTIR) spectroscopy was used to confirm the presence of ZnO and PAC nanoparticles.

2. Materials and Methods

2.1 Synthesis of zinc oxide-based sealer

The sealer was synthesized by preparing a base paste and an accelerator paste, (Table 1) followed by mixing the two components in a 1:1 ratio to prepare the final set cement.

Table 1: Composition of base paste and accelerator paste along with the role of each ingredient

Base paste	Accelerator paste
• Mineral oil: 5% - Carrier, plasticizer, viscosity (Sigma-Aldrich) • Barium sulphate: 20% - Radiopacity (Sigma-Aldrich) • Zinc oxide nanoparticle: 25% - Main active ingredient, antimicrobial property, sealing capability, strength (Sigma-Aldrich) • Magnesium oxide: 10% - Setting agent (Sigma-Aldrich) • Calcium phosphate: 10% - Biocompatibility, promotes hard tissue formation (Sigma-Aldrich) • Bismuth subcarbonate: 10% - Radiopacity, sealing property (Sigma-Aldrich) • Sodium borate: 5% - Buffer, stabilizer, controls pH, stability (Sigma-Aldrich) • Lecithin: 2% - Emulsifier, dispersing agent (Sigma-Aldrich) • Proanthocyanidin nanoparticle with PLGA: 13% - Dentine collagen stabilization	• Vinyl polysiloxane: 70% - Flow, handling, base for accelerator paste (Gelest, Inc.) • Hydrogenated resin: 20% - Strength, stability (Arakawa Chemical Inc.) • Silica: 10% - Filler, thickening agent (Sigma-Aldrich)

2.1.1 Preparation of the Base Paste

The following components were weighed and mixed: zinc oxide nanoparticles (25 g), barium sulphate (20 g), PAC-PLGA nanoparticles (13 g), magnesium oxide (10 g), calcium phosphate (10 g), bismuth subcarbonate (10 g), and sodium borate (5 g). These powders were mixed using a planetary mixer for 2 hours to ensure homogeneity. Lecithin (2 g) was dissolved in mineral oil (5 g) to form an emulsifying solution, which was gradually added to the powder mixture under mechanical stirring to ensure uniform dispersion. The base paste mixture was subjected to ultrasonication for 30 minutes to break down any agglomerates and ensure an even distribution of nanoparticles. The base paste was degassed under vacuum for 30 minutes to remove entrapped air bubbles.

2.1.2 Preparation of Accelerator Paste

Vinyl polysiloxane (70 g), hydrogenated resin (20 g), and silica (10 g) were weighed and mixed using a planetary mixer for 2 hours to create a uniform paste. The gelation reaction between Si-H groups and vinyl double bonds in the silicone matrix was facilitated by a platinum-based catalyst. This catalyst promotes efficient hydrosilylation, ensuring a consistent cross-linking process that enhances the uniformity and mechanical stability of the sealer. The accelerator paste was subjected to 30 minutes of ultrasonication to ensure a homogeneous mixture. Both resins were combined in the accelerator paste to create a uniform polymer network, reducing potential inconsistencies in polymer distribution and supporting mechanical stability. The accelerator paste was degassed under vacuum for 30 minutes to eliminate any trapped air.

2.1.3 Preparation of Final Set Cement

Equal amounts (1:1 ratio) of the synthesized base paste and accelerator paste were mixed using a spatula on a glass slab until a homogenous mixture was achieved.

2.2 ATR-FTIR analysis

ATR-FTIR analysis was performed on the base paste, accelerator paste, and final set cement to identify the functional groups present in each phase and to confirm proper chemical interactions between the components. Samples of the base paste, accelerator paste, and final set cement were placed directly on the ATR crystal. FTIR spectra were collected over the wavenumber range of 4000–400 cm^{-1}. Each spectrum was obtained by averaging 32 scans at a resolution of 4 cm^{-1}.

2.3 Physical and Chemical Property Analysis:

The following analyses were conducted on the prepared sealer according to ISO 6876:2012 and ANSI/ADA Specification No. 57.

2.3.1 Setting time

Setting time was determined according to the ISO 6876:2012 standard: The final cement was placed into a cylindrical stainless-steel mould with an internal diameter of 10 mm and a height of 2 mm, ensuring the sample was level with the mould's surface. A Gilmore needle (50 g) with a flat end (2.0 mm diameter) was gently lowered onto the surface of the sample. The indentation left on the surface was examined at regular intervals. The time taken for no visible indentation to occur was recorded as the initial setting time. The process was repeated until no indentation was visible when using a heavier Gilmore needle (100 g), marking the final setting time. The samples were stored at 37°C with 95% relative humidity during the setting process.

2.3.2 Solubility

Solubility was measured following ISO 6876:2012 guidelines: Sealer samples were prepared in a cylindrical mould with a diameter of 20 mm and a height of 1.5 mm. Excess material was removed, and the samples were left to set for three times the recorded setting time. The samples were weighed after setting (W_0). The samples were placed in distilled water at 37°C for 24 hours. After water immersion, the samples were removed, rinsed, and dried in a dehumidifier for 48 hours. The final weight (Wf) was recorded. The difference between the final mass and the initial mass was divided by the initial dry weight of the sample x 100 corresponding to the loss of mass of each specimen expressed as a percentage of solubility.

2.3.3 Dimensional Stability

Dimensional stability was assessed by measuring the change in the volume of the sealer over time: Cylindrical samples (6 mm diameter, 12 mm height) were prepared and stored in distilled water at 37°C after setting. Dimensional measurements were taken at 6, 24, and 72 hours, and 7, 14, and 30 days. A digital calliper was used to measure the initial (L_0) and final (L_1) lengths. As per ISO 6876:2012 requirement, the sealer should not expand or contract more than 1.0% after setting.

2.3.4 Flow Test

The flow was measured as per ISO 6876:2012. A volume of 0.05 ml of mixed sealer was placed on a glass plate (40 mm × 40 mm) using a calibrated syringe. Another glass plate (40 mm × 40 mm) was placed on top of the sample, followed by a mass of 120 g. After 10 minutes, the mass was removed, and the diameters of the compressed sealer disc were measured using a digital calliper. The mean diameter of the disc was recorded, and the sealer's flow was expressed in millimetres (mm).

2.3.5 pH Measurement

The pH of both fresh and set samples was measured at various time intervals. Freshly mixed sealer was placed in a mould with a diameter of 5 mm and a thickness of 1 mm. Set samples were prepared and stored for their corresponding setting times. Both fresh and set samples were immersed in 50 ml of deionized water at 37°C. The pH was measured using a digital pH meter at predetermined intervals: 3 minutes, 30 minutes, 60 minutes, 2 hours, 12 hours, and 24 hours for fresh samples; 12 hours, 24 hours, 3 days, 7 days, 2 weeks, and 4 weeks for set samples.

3. Results

3.1 ATR-FTIR

ATR-FTIR analysis was conducted on the base paste, accelerator paste, and final set cement to confirm the presence of functional groups and chemical interactions. The key observations are outlined in the table below. (Table 2)

Table 2: Key observations as per ATR-FTIR analysis

Component	Peak (cm^{-1})	Functional group	Significance
Base paste	2950-2850 cm^{-1}	C-H Stretching	From mineral oil, improves flow and handling properties
	3200-3600 cm^{-1}	O-H Stretching	From proanthocyanidin, lecithin, sodium borate

	1750 cm^{-1}	C=O Stretching	From PLGA, encapsulating proanthocyanidin
	450-600 cm^{-1}	Zn-O Stretching	From zinc oxide nanoparticles
Accelerator	950-2850 cm^{-1}	C-H Stretching	From vinyl polysiloxane and hydrogenated resin
	1000-1130 cm^{-1}	Si-O-Si Stretching	From vinyl polysiloxane and silica
Final set cement	3200-3600 cm^{-1}	O-H Stretching	From residual proanthocyanidin, lecithin, sodium borate
	1750 cm^{-1}	C=O Stretching	Retained from PLGA encapsulating proanthocyanidin
	450-600 cm^{-1}	Zn-O Stretching	Confirming zinc oxide nanoparticles in the final cement

3.1.1 Base paste (Figure 1)

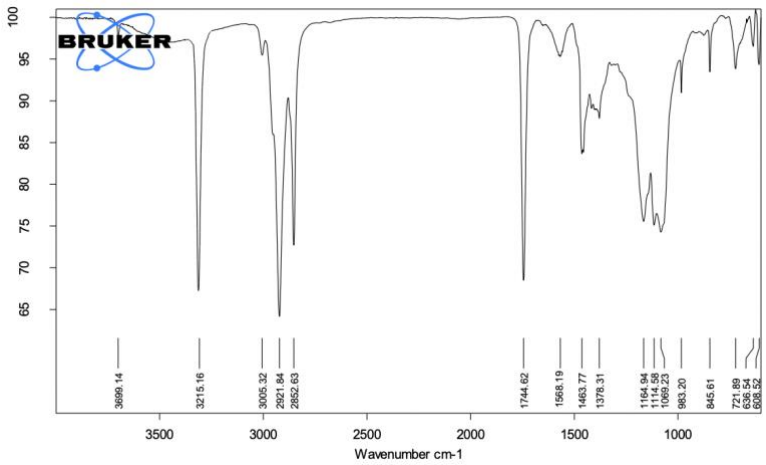

Figure 1: ATR-FTIR spectra of base paste

2950-2850 cm^{-1} (C-H Stretching): These peaks are attributed to the C-H stretching vibrations from the mineral oil used as a carrier and plasticizer. This oil enhances the flowability and viscosity of the base paste, allowing better handling during application. C-H stretching from the mineral oil component.

3200-3600 cm^{-1} (O-H Stretching): The broad peak here corresponds to O-H stretching vibrations, likely from proanthocyanidin nanoparticles, lecithin, and sodium borate. These hydroxyl groups are indicative of water interactions, antioxidant properties from PAC, and the buffering capacity provided by sodium borate, which controls the pH of the sealer. O-H groups from PAC (antioxidant), lecithin (emulsifier), and sodium borate (pH buffer).

1750 cm^{-1} (C=O Stretching): This peak is attributed to carbonyl stretching vibrations from PLGA encapsulating the proanthocyanidin nanoparticles. This encapsulation ensures the controlled release of PAC, which enhances the bioactivity of the sealer by stabilizing dentine collagen. C=O groups from PLGA encapsulating PAC.

1100-1000 cm^{-1} (P-O Stretching): The P-O stretching vibrations indicate the presence of calcium phosphate nanoparticles, which promote biocompatibility and encourage hard tissue formation. This contributes to the sealer's role in aiding the healing of dental tissues after endodontic procedures. P-O bonds from calcium phosphate, supporting biocompatibility and tissue regeneration.

450-600 cm^{-1} (Zn-O Stretching): This region corresponds to Zn-O stretching, confirming the presence of zinc oxide nanoparticles. Zinc oxide is the primary antimicrobial and sealing agent in the paste, providing both strength and a robust barrier against bacterial infiltration. Zn-O bonds from zinc oxide nanoparticles, contribute to antimicrobial and sealing properties.

3.1.2 Accelerator paste (Figure 2)

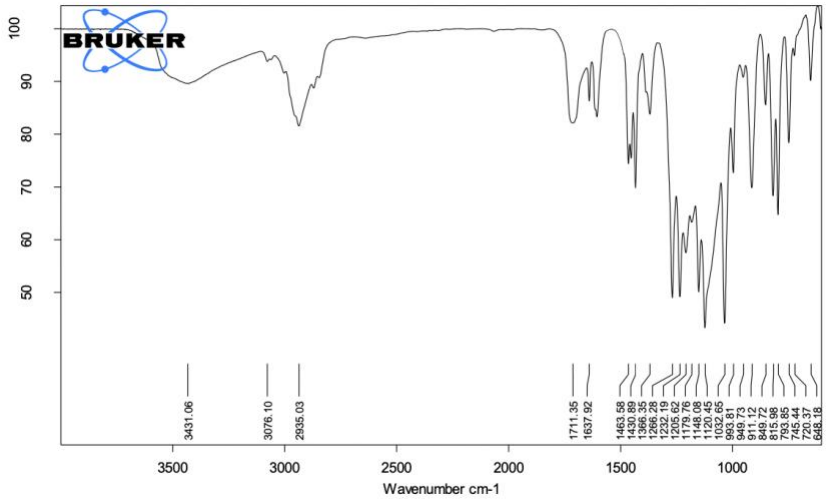

Figure 2: ATR-FTIR spectra of accelerator paste

950-2850 cm^{-1} (C-H Stretching): These peaks arise from the C-H stretching in vinyl polysiloxane and hydrogenated resin, which form the bulk of the accelerator paste. Vinyl polysiloxane provides handling properties and flow, while the hydrogenated resin enhances strength and stability. C-H bonds from vinyl polysiloxane and hydrogenated resin.

1000-1130 cm^{-1} (Si-O-Si Stretching): This peak corresponds to the Si-O-Si stretching vibrations in vinyl polysiloxane and silica. These bonds are essential for maintaining the paste's structural integrity and providing thickening and reinforcing effects, ensuring proper flow and handling during clinical application. Si-O-Si bonds from vinyl polysiloxane and silica, providing structural strength and viscosity.

1250 cm^{-1} (Si-CH_3 Stretching): This peak is indicative of Si-CH_3 stretching in the vinyl polysiloxane structure. These groups provide hydrophobic properties to the accelerator paste,

ensuring that it is resistant to moisture and degradation during application. Si-CH_3 bonds from vinyl polysiloxane, contributing to moisture resistance and durability.

3200-3700 cm^{-1} (Si-OH Stretching): The Si-OH stretching peak may come from residual silica in the paste, indicating interactions between the silica and other components. This peak suggests that the silica acts as a filler and contributes to the paste's thickening properties. Si-OH stretching from silica, acting as a filler.

1600 cm^{-1} (C=C Stretching): This peak suggests the presence of C=C bonds, likely from any residual unsaturation in the hydrogenated resin. These residual bonds indicate possible leftover double bonds that could contribute to cross-linking and stability of the resin over time. C=C bonds from hydrogenated resin, indicating possible cross-linking properties for stability.

3.1.3 Final set cement (Figure 3)

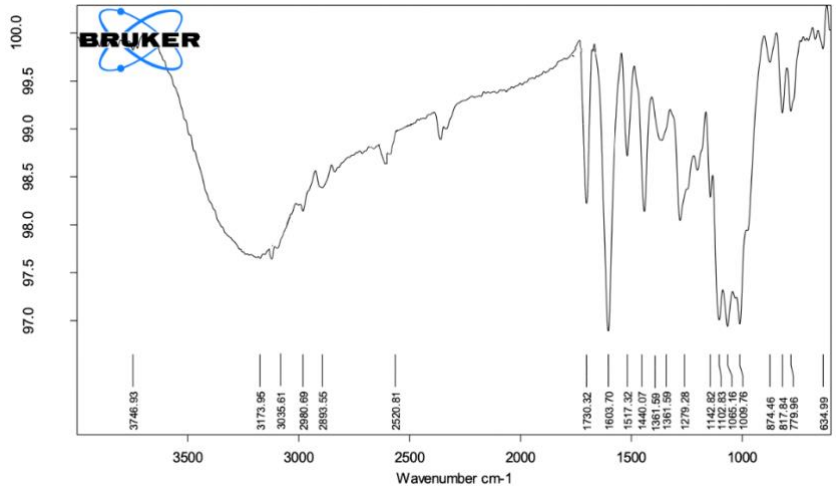

Figure 3: ATR-FTIR image of final set cement

3200-3600 cm^{-1} (O-H Stretching): The broad O-H stretching peak indicates the presence of hydroxyl groups from residual moisture, PAC, and other components such as lecithin and sodium borate. These hydroxyl groups contribute to the cement's continued antioxidant properties and pH stability, aiding in biocompatibility and antibacterial effects. O-H groups from PAC, lecithin, and sodium borate, contribute to the biological properties of the cement, including collagen stabilization and antibacterial action.

1750 cm^{-1} (C=O Stretching): This peak reflects the continued presence of PLGA encapsulating PAC, ensuring that the proanthocyanidin remains bioactive in the set cement. This controlled release helps maintain collagen stabilization over a prolonged period, making the cement effective in long-term dental restorations. C=O groups from PLGA, encapsule PAC to sustain bioactivity.

1000-1200 cm^{-1} (Si-O-Si and P-O Stretching): This region combines the Si-O-Si stretching from silica and polysiloxane with the P-O stretching from calcium phosphate. These peaks indicate the structural reinforcement provided by these fillers, contributing to the strength and biocompatibility of the set cement. Si-O-Si bonds from vinyl polysiloxane and P-O bonds from calcium phosphate, both reinforce the cement's mechanical and regenerative properties.

450-600 cm^{-1} (Zn-O Stretching): Zn-O stretching in the final cement confirms the presence of zinc oxide nanoparticles, which provide antimicrobial properties and enhance the sealer's ability to prevent bacterial penetration. Zinc oxide remains a key component for the sealer's functionality. Zn-O bonds from zinc oxide nanoparticles, ensuring the antimicrobial effectiveness of the cement.

3.2 Physical and Chemical Property Analysis

3.2.1 Setting time

The initial setting time of the sealer was 80 minutes, which exceeded the minimum required setting time of 30 minutes. The final setting time of 1400 minutes (23.3 hours) was within the 24-hour limit specified by ISO standards (Table 3).

Table 3: Initial and final setting time of the sealer

Property	Measured value	ISO 6876:2012 Standard requirement
Initial setting time	80 minutes	≥ 30 minutes
Final setting time	1400 minutes	≤ 1440 minutes (24 hours)

3.2.2 Solubility

Solubility was determined by calculating the percentage weight loss of the sealer samples after immersion in distilled water for 24 hours. The solubility of the sealer was measured as 1.921%, which was within the acceptable limit of 3% according to ISO 6876:2012 standards, demonstrating low solubility and stability when exposed to aqueous environments (Table 4).

Table 4: Percentage of solubility of sealer

Sample	Initial weight (W_o)	Final weight (Wf)	Solubility (%)	ISO requirement (≤ 3%)
Zinc oxide-based sealer	0.125 g	0.1226 g	1.921%	Compliant

3.2.3 Dimensional stability (Table 5)

Table 5: Stability percentage of sealer

Time interval	Initial length (L_o)	Final length (L_1)	Dimensional change (%)	ISO 6876:2012 standard (≤ 1%)
6 hours	12.00 mm	11.98 mm	0.167%	Compliant
24 hours	12.00 mm	11.95 mm	0.417%	Compliant
7 days	12.00 mm	11.94 mm	0.500%	Compliant
30 days	12.00 mm	11.94 mm	0.500%	Compliant

The dimensional stability was assessed by measuring the change in sample dimensions after immersion in distilled water for up to 30 days.

The dimensional change was consistently below 1%, meeting the ISO 6876:2012 standard requirement for dimensional stability. This indicates that the zinc oxide-based sealer exhibited minimal shrinkage over time.

3.2.4 Flow

The flow property of the zinc oxide-based sealer was measured by the diameter of the compressed disc formed by the sealer after 10 minutes of compression under 120 g of weight. The flow of the sealer was measured at 26.4 mm, which was greater than the minimum ISO 6876:2012 requirement of 20 mm, demonstrating excellent flow characteristics suitable for clinical application (Table 6).

Table 6: Flow of sealer

Property	Measured value	ISO requirement (≥ 20 mm)
Flow	26.40 mm	Compliant

3.2.5 pH measurement

The pH of the zinc oxide-based sealer was measured at various time intervals for both fresh and set samples. The results are summarized in the table below. The pH remained neutral to slightly alkaline for both fresh and set samples. The presence of sodium borate as a buffer helped maintain stable pH levels, which is beneficial for antimicrobial activity and biocompatibility (Table 7).

Table 7: pH of sealer

Time interval	Fresh sample pH	Set sample pH
3 minutes	7.2	-
30 minutes	7.1	-
60 minutes	7.3	-
2 hours	7.5	-
12 hours	7.8	7.2
24 hours	7.7	7.3
3 days	-	7.6
7 days	-	7.7
2 weeks	-	7.5
4 weeks	-	7.5

4. Discussion

The results of this study demonstrate the successful synthesis of a zinc oxide-based endodontic sealer containing proanthocyanidin nanoparticles (PAC-NPs) encapsulated in PLGA. The novelty of this sealer lies in its dual functionality—combining the antimicrobial properties of ZnO-NPs with the collagen-stabilizing effects of PAC-NPs. This is a significant advancement over conventional sealers, which do not offer active protection against collagen degradation [10].

Zinc oxide nanoparticles are known for their potent antimicrobial activity, which is enhanced by their nanoscale size and high surface area [9]. In the context of endodontic sealers, the antimicrobial properties of ZnO-NPs are critical in preventing post-treatment infections, particularly from resistant bacteria such as *Enterococcus faecalis* [19]. The ATR-FTIR spectra in this study confirmed the presence of ZnO-NPs in the sealer matrix, as evidenced by the characteristic Zn-O stretching at 450-600 cm^{-1} [20]. This confirms the successful incorporation of ZnO-NPs into the sealer, which is essential for its antimicrobial functionality.

The incorporation of ZnO-NPs also contributes to the mechanical properties of the sealer. Nanoparticles are known to enhance the compressive strength and reduce the solubility of dental materials, making them more stable over time [11]. The results of this study showed that the sealer's flow rate (26.4 mm) and dimensional stability (0.485%) were within acceptable limits as per ISO and ANSI/ADA standards, indicating that the addition of ZnO-NPs did not compromise the handling properties of the sealer [21].

The inclusion of PAC NPs encapsulated in PLGA represents a significant innovation in the field of endodontic materials. PACs are known for their ability to cross-link collagen fibrils, thereby preventing enzymatic degradation and improving the mechanical properties of dentine [13]. The controlled release of PACs from the PLGA matrix should ensure that the dentine-collagen matrix is stabilized over an extended period, offering long-term protection against collagen degradation [15].

This study demonstrated that the presence of PAC-NPs in the sealer matrix was confirmed by FTIR, with a characteristic C=O stretching vibration at 1750 cm^{-1}, indicative of the PLGA encapsulation of PACs [14]. The controlled release mechanism provided by PLGA ensures a sustained release of PACs, allowing for continuous collagen stabilization. This is particularly important in endodontic applications, where the longevity of the dentine-sealer interface is critical for the success of the treatment [16].

The degradation of dentine collagen is a well-documented cause of failure in endodontic treatments. The activation of MMPs during acid etching or bacterial colonization leads to the breakdown of the collagen fibrils, compromising the integrity of the sealer-dentine interface. The incorporation of PAC-NPs into the zinc oxide-based sealer provides a novel approach to address this critical issue. By cross-linking with collagen fibrils, PACs not only stabilize the collagen matrix but also enhance its resistance to enzymatic degradation, thus preserving the strength and stability of the dentine-sealer interface over time [13, 14]. The stabilization effect of PAC on dentine collagen can be attributed to molecular interactions, including hydrogen bonding and van der Waals forces. The hydroxyl groups in PAC are capable of forming hydrogen bonds with the carbonyl and amide groups of collagen fibrils, strengthening the collagen network and making it more resistant to enzymatic degradation. Additionally, van der Waals interactions may contribute to further stabilization by promoting close packing of collagen fibrils [15]. These molecular-level interactions not only improve the mechanical resilience of the collagen matrix but also extend the longevity of the sealer's bond with dentine, which is critical for clinical success. Traditional sealers often lack dedicated collagen cross-linking agents, which are crucial for sustaining dentine stability and preventing enzymatic degradation. The encapsulation of PAC NPs in PLGA will provide a controlled release, overcoming limitations in flow, setting time, and biocompatibility typically associated with free cross-linking agents [16].

The encapsulation of PAC NPs within a PLGA matrix ensures their controlled release over an extended period. This sustained release is essential for maintaining continuous protection of the dentine-collagen matrix against degradation. Traditional collagen stabilizers, when applied in a free form, may lose their efficacy over time due to rapid degradation or diffusion away from the target site [17]. In contrast, the PLGA-based delivery system used in this study will allow for a gradual and sustained release of PAC, thereby ensuring long-term collagen stabilization and improving the durability of the root canal treatment [22].

The advantage of using PLGA is twofold: (1) it is biocompatible and degrades into non-toxic by-products, and (2) it enables the controlled release of PACs, extending their therapeutic window. Given PLGA's established biocompatibility, its degradation products—lactic and glycolic acid—are naturally metabolized by the body, minimizing cytotoxic risk. This localized application within the root canal confines any potential release of by-products to the dentine-canal interface, reducing exposure to surrounding tissues and ensuring a safe degradation profile [23]. Studies have shown that PLGA supports the controlled release of bioactive agents, which sustains collagen stabilization over time without adverse effects on surrounding tissues [24]. The release kinetics of PACs from the PLGA matrix can be further optimized to match the clinical needs of root canal treatments, ensuring that the sealer provides continuous protection throughout the critical early stages of healing [25]. The ability to sustain collagen stabilization over time is particularly important in preventing the reactivation of latent bacterial infections and ensuring the long-term success of the treatment.

The findings of this study underscore the potential of this novel zinc oxide-based sealer for clinical applications. Its dual functionality—offering both antimicrobial protection through ZnO-NPs and long-term collagen stabilization through PAC-NPs—positions it as a superior alternative to conventional sealers. By addressing two critical challenges in root canal therapy—bacterial reinfection and collagen degradation—this material offers significant improvements in the longevity and effectiveness of endodontic treatments [9].

Future research should focus on the in vivo performance of this sealer in clinical trials, evaluating its long-term effects on periapical healing and root canal success rates. Additionally, optimizing the release kinetics of proanthocyanidins from the PLGA matrix could further enhance the material's performance. Exploring the synergistic effects of other bioactive agents, such as bioactive glass or calcium phosphate nanoparticles, in conjunction with proanthocyanidins, may also yield further improvements in the biological and mechanical properties of the sealer [11].

5. Conclusions

This study introduces a novel zinc oxide-based endodontic sealer enhanced with proanthocyanidin nanoparticles encapsulated in PLGA, designed to harness PAC's proven collagen-stabilizing effects alongside zinc oxide's antimicrobial properties. The sealer demonstrated compliance with ISO 6876:2012 and ANSI/ADA 57 standards, exhibiting ideal flow rate, setting time, low solubility, dimensional stability, and pH all indicative of its robustness and suitability for clinical application. ATR-FTIR analysis confirmed the effective integration of PAC and zinc oxide within the matrix, supporting the formulation's potential to maintain dentine-collagen integrity, a crucial factor for extending the longevity of root canal treatments. By achieving both physical stability and bioactivity, this innovative sealer sets a new standard for endodontic materials. This foundational characterization establishes a strong

platform for further in vitro studies to validate its mechanical strength and biological efficacy, paving the way for improved clinical outcomes in endodontics.

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References

1. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J. 1997;30(5):297-306. Published correction appears in: Int Endod J. 1998;31(2):148.
2. Waltimo TM, Boiesen J, Eriksen HM, Ørstavik D. Clinical performance of 3 endodontic sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;92(1):89-92.
3. Grossman LI. Physical properties of root canal cements. J Endod. 1976;2(5):166-75.
4. Pashley DH. Dentin: a dynamic substrate--a review. Scanning Microsc. 1989;3(1):161-74.
5. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, et al. Strategies to prevent hydrolytic degradation of the hybrid layer—A review. Dent Mater. 2013;29(10):999-1011.
6. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, et al. Adhesion to enamel and dentin: current status and future challenges. Oper Dent. 2003;28(3):215-35.
7. Eldeniz AU, Erdemir A, Belli S. Shear bond strength of three resin-based sealers to dentin with and without smear layer removal. J Endod. 2005;31(4):293-6.
8. Zhang W, Li Z, Peng B. Ex vivo cytotoxicity of a new calcium silicate-based canal filling material. Int Endod J. 2010;43(9):769-74.
9. Shrestha A, Kishen A. Antibacterial efficacy of photosensitizer-functionalized bioactive nanoparticles on multispecies biofilm. J Endod. 2014;40(10):1604-10.
10. Shrestha A, Kishen A. Antibiofilm efficacy of photosensitizer-functionalized bioactive nanoparticles on multispecies biofilm. J Endod. 2014;40(10):1604-1610
11. Sodagar A, Bahador A, Khalil S, Shahroudi AS, Kassaee MZ. The effect of TiO2 and SiO2 nanoparticles on flexural strength of poly (methyl methacrylate) acrylic resins. J Prosthodont Res. 2013;57(1):15-9.
12. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater. 2008;24(1):90-101.
13. Bedran-Russo AK, Pereira PN, Duarte WR, Drummond JL, Yamauchi M. Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. J Biomed Mater Res B Appl Biomater. 2007;80(1):268-72.
14. Castellan CS, Pereira PN, Grande RH, Bedran-Russo AK. Mechanical characterization of proanthocyanidin-dentin matrix interaction. Dent Mater. 2010;26(10):968-73.
15. Liu Y, Dusevich V, Wang Y, Proanthocyanidins rapidly stabilize the demineralized dentin layer. J Dent Res. 2013;92(8):746-52.
16. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel). 2011;3(3):1377-97.
17. Acharya G, Shin CS, McDermott M, Mishra H, Park H, Kwon IC, et al. The hydrogel template method for fabrication of homogeneous nano/microparticles. J Control Release. 2010;141(3):314-9.
18. Liu Y, Wang Y. Proanthocyanidins: a novel agent to crosslink dentin collagen. Dent Mater. 2012;28(3):304-11.
19. Love RM. Enterococcus faecalis–a mechanism for its role in endodontic failure. Int Endod J. 2001;34(5):399-405.
20. Khashaba RM, Moussa MM, El-Bassiouny AM, El-Sheikh MM. Fatigue and microhardness of a nanohybrid composite: effect of different bleaching protocols. J Prosthodont. 2011;20(1):21-32.
21. International Organization for Standardization. ISO 6876:2012 Dentistry—Root canal sealing materials. Geneva: ISO; 2012.
22. Hechler B, Yao X, Wang Y. Proanthocyanidins alter adhesive/dentin bonding strengths when included in a bonding system. Am J Dent. 2012 Oct;25(5):276-80.

23. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv Drug Deliv Rev. 2003;55(3):329-47.
24. Mota FL, Queimada AJ, Pinho SP, Macedo EA. Aqueous solubility of some natural phenolic compounds. Ind Eng Chem Res. 2008;47(15):5182-9.
25. Mano JF, Sousa RA, Boesel LF, Neves NM, Reis RL. Bioinert, biodegradable and injectable polymeric matrix composites for hard tissue replacement: state of the art and recent developments. Compos Sci Technol. 2004;64(6):789-817.