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Setting Time and Solubility of Thymol Incorporated Zinc Oxide-Guaiacol Root Canal Sealer: An in Vitro Study

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Abstract

Aims: This study aimed to establish whether changes in setting time and solubility of the locally prepared zinc oxide-guaiacol endodontic sealer when incorporated with thymol crystals as an antibacterial agent. **Materials and Methods**: zinc oxide-guaiacol endodontic sealer with thymol crystals incorporated at three different concentrations (2%, 5%, and10%) were tested, For setting time test similar specimens were prepared using ring molds with an internal diameter of (10 mm) and a height of (2 mm), nearly (120±10) seconds once mixing, the sample was located on a metal block in an incubator. A Gilmore needle with a mass of (100±0.5 g) which had (2±0.1 mm measurement) was used; the period of "no indent" was noted from the start of mixing. For the solubility test, similar specimens were prepared using ring molds with an internal diameter of (20 ± 0.1 mm) and a height of (1.5 ± 0.1 mm) and digitally weighted to register the mass of each specimen before and after immersion in distilled water. Solubility was determined after 24 hours and statistically analyzed using descriptive statistics, a One-Way ANOVA test, and post-hoc Duncan's Multiple Range Test was used. **Results**: The means of setting time of three experimental sealers were decreased as thymol crystal concentrations increased and the means of solubility for three experimental sealers were increased as the concentrations of thymol crystal increased. **Conclusions**: This study concluded that the prepared sealers with three concentrations were still adapted within the requirements of ANSI\ADA specifications NO.57\2008 for root canal sealer materials.

الخلاصة

الأهداف: تهدف الدراسة إلى تحديد التغييرات في وقت التصلب وقابلية الذوبان لسدادة قناة الجذر (أكسيد الزنك – غواياكول) المحضر محليًا عند دمجه مع بلورات الثايمول كعامل مضاد للبكتيريا. **المواد والطرائق العمل**: تم اختبار سدادة قناة الجذر (أكسيد الزنك – غواياكول) و دمجه بثلاث تركيزات مختلفة (٢٪ ، ٥٪ ، ١٠٪) من بلورات الثايمول ، لاختبار وقت التصلب ، تم تحضير عينات مماثلة باستخدام قوالب حلقية بقطر داخلي (١٠ مم) وبارتفاع (٢ مم) ، ما يقرب من (١٢٠ ± ١٠) ثانية بمجرد الخلط ، تم وضع العينة على قالب معدني في الحاضنة. تم استخدام إبرة جيلمور كتلتها (١٠٠ ± ٠,٥ جم) قطرها (٢ ± ٠,١ مم)؛ يتم تسجيل الوقت من بداية الخلط الى عدم حصول اي (تضريس سطحي) إشارة على السطح. لاختبار قابلية الذوبان، تم تحضير عينات مماثلة باستخدام قوالب حلقية بقطر داخلي ٢٠ ± ٠,١ مم و ارتفاع ١,٥ ± ٠,١ مم ويتم وزنها رقمياً لتسجيل كتلة كل عينة قبل وبعد الغمر في الماء المقطر. تم تحديد قابلية الذوبان بعد ٢٤ ساعة وتم تحليلها إحصائيًا باستخدام الإحصاء الوصفي واختبار ANOVA أحادي الاتجاه واختبار Duncan. **النتائج**: انخفض متوسط وقت التصلب للسدادات تجريبية مع زيادة تركيزات بلورات الثايمول وزاد متوسط قابلية الذوبان للسدادات تجريبية مع زيادة تراكيز بلورات الثايمول. **الاستنتاجات**: خلصت هذه الدراسة إلى أن السدادات المحضرة بادخال التراكيز الثلاثة بقيت ضمن متطلبات ومواصفات (ANSI \ ADA) رقم ٥٧ \٢٠٠٨ (لمواد سدادة قناة الجذر.

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INTRODUCTION

The usage of endodontic sealers to achieve root canal fillings in obturation procedures is an established basis in endodontic and plays an important role in the success of the management. Therefore, these materials should display a set of features that allow effective root canal filling with the healing of periapical inflammatory and/or infectious developments and stop additional microbial contamination (1).

The new locally prepared zinc oxide-guaiacol endodontic sealer prepared to overcome the disadvantages and toxicity of eugenol that detected in researches, its powder ingredients consist of zinc oxide 62%, natural rosin 20%, locally made egg-shell hydroxyapatite 2%, bismuth sub-carbonate 15%, zinc acetate 1%, while the liquid part consists of: guaiacol 85%, and olive oil 15%. This root canal sealer fulfilled the requirement of ANSI/ADA specification No.57/2008 for dental root canal sealer materials (2).

The adding of antibacterial agents into root canal sealers can be achieved only if it does not disturb the features, harm the root filling's reliability, and does not pose a health hazard to the patient(3). One of the therapeutic plants broadly utilized in pharmacology is thyme (Thymus vulgaris L., Lamiaceae); that have obtained care among investigators owing to their safety and risk-free; thymol displays the possible to restrain dental infection (4).

Setting time and solubility consider having importance according to ANSI/ADA specifications in controlling all actions of root canal sealer materials during and after manipulation (5).

The setting time of zinc oxide- guaiacol sealer allow sufficient working time for better obturation, radiographical assessment, and perform any adjustment if needed (6).

Perfectly, the endodontic sealer materials must have low solubility, appropriate flow rate, and film thickness that efficiently obturate the auxiliary canals and holes between the master and accessory cones (7).

Based on this important standard (ADA specification No. 57), the locally prepared zinc oxide-guaiacol root canal sealer with low solubility (ADA did not exceed 3%) related to the presence of natural resin content and bismuth subcarbonate (6).

The present study aimed to compare and evaluate the effect of (2%, 5%, and 10%) thymol crystals incorporation on the setting time and solubility of the locally prepared zinc oxide- guaiacol endodontic sealer.

MATERIALS AND METHODS

1. Thymol incorporation

The thymol crystals (BDH, England) crushed with an electrical grinder (Geepas,China) and sieved to standard the particles size, and incorporated in the locally prepared zinc oxide- guaiacol root canal sealer in three formulas, Table (1).

Table (1): The Thymol Crystals Incorporated in Zinc Oxide- Guaiacol Root Canal Sealer in Three Formulas.

First formula		Second formula		Third formula	
Powder	%	Powder	%	Powder	%
Zinc oxide	60%	Zinc oxide	57%	Zinc oxide	52%
Thymol crystals	2%	Thymol crystals	5%	Thymol crystals	10%
Natural rosin	20%	Natural rosin	20%	Natural rosin	20%
Hydroxyapatite	2%	Hydroxyapatite	2%	Hydroxyapatite	2%
Bismuth sub-carbonate	15%	Bismuth sub-carbonate	15%	Bismuth sub-carbonate	15%
Zinc acetate	1%	Zinc acetate	1%	Zinc acetate	1%
Liquid	%	Liquid	%	Liquid	%
Guaiacol oil	85%	Guaiacol oil	85%	Guaiacol oil	85%
Olive oil	15%	Olive oil	15%	Olive oil	15%

All test specimens preparation was prepared at 23± $2C^0$ and 50±5% relative humidity. The powder/liquid ratio is (2:1) by volume, the period required for mixing was (1minute) according to the pilot study.

2. Setting time test

Regarding ANSI/ADA specification No.57, Three samples were organized as follows: using a mold of stainless steel ring form with an inner distance of (10 mm) with (2 mm) height, placed on the glass plate (slide of the microscope), Figure (1). One hour at least earlier than the start of mix, a metal block with (8 × 20 × 10 mm measurements) positioned in an incubator with 100% humidity and 37±1˚C temperature. The trial material was mixed and adapted to the level surface of the mold. Nearly (120±10) seconds once mixing, the sample was located on a metal block in an incubator.

A Gilmore needle with a mass of (100±0.5 g) which had (2±0.1 mm measurement) a plane cylinder-shaped tip, was let fall vertically 5 mm over the plane surface of the material and stay 5 seconds, Figure (2). The tip is cleansed and the process is done every 5 minutes. As the setting phenomena in progress, the indentation is done after every 30 seconds till the indenter be unsuccessful to make a whole circular notch in the material. The period of “no indent” is noted from the start of mixing. The average rate is identified as the setting time [8].

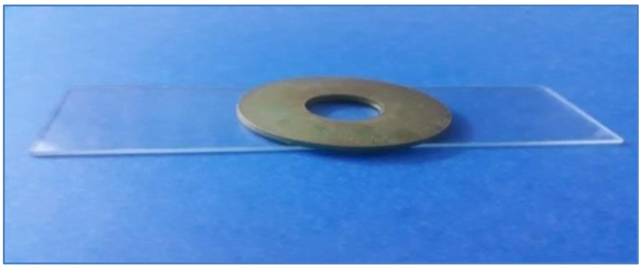


Figure (1): Ring of stainless steel mold located on a slide of microscope.

Figure (2): Gilmore needle device with the sample.

3. Water solubility test

Ten samples for trial material prepared as follows: the mold with an inner distance of (20±0.1) mm and a height of (1.5±0.1) mm were used for sample planning, Figure (3).

The mold was located on a microscope slide and packed with slightly overfilled material after mixing. An additional glass plate was pushed with a plastic sheet on topmost of the material and wisely detached to leave a flat, identical surface. In an incubator, all samples were left to set for 24 hours at 37˚ C and 100% qualified moisture. The samples were assessed 3 times and the typical evaluation was noted using an investigative balance to an accurateness of ± 0.001 g [9]. Then, each sample was suspended and fixed by nylon filament inside a plastic bottle having (50 ± 1) ml of distilled water at (37 ± 1) ºC for 24 hrs., Figure (4). the sample was then detached and splashed with 2-3 ml of fresh distilled water[10]. Permit the samples to dehydrate in the desiccator (Marienfeld W., China) having phosphorus pentoxide or other appropriate desiccants at room temperature, Figure (5) before each evaluating (nearest to 0.001).

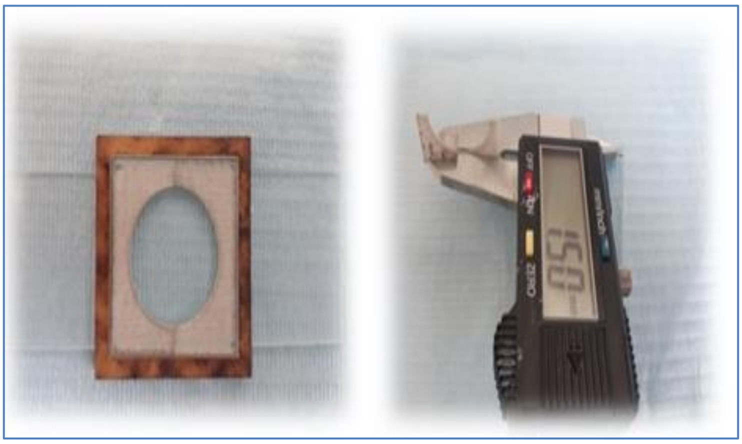


Figure (3): Gauging the thinness of the ring mold used for sample planning.

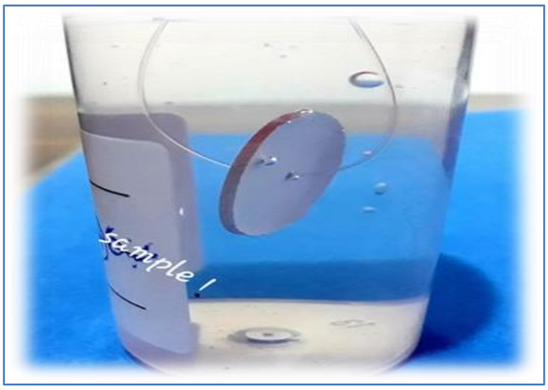


Figure (4): The model was suspended and fixed by nylon fiber.

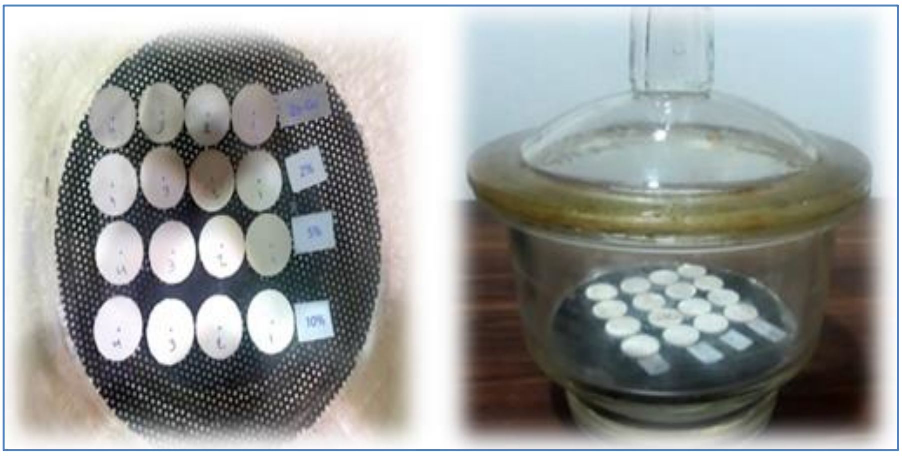


Figure (5): The samples in the desiccator.

The mass loss was identified as a percentage of the original mass. The ratio of solubility was measured as follows: **(IM-FM)/IM*100** where IM is the initial mass and FM is the final mass of the sample [(10)].

RESULTS

Setting time test

The mean of the net setting time (minutes) was showed in, Table (2).

Analysis of variance (ANOVA) test and Duncan's Multiple Range Test for zinc oxide-guaiacol sealer before and after thymol crystals incorporation was listed in, Table (3). The result in general showed a significant difference between the (Zn-Gu) sealer before and after thymol powder incorporation. While there are no significant differences among all powder percentages incorporation (2%, 5%, and 10%). However, the thymol incorporation in different concentrations, not disturb the setting reaction of the main formula.

Table (2): Setting Time Descriptive Statistics.

Materials	N	Minimum	Maximum	Mean	Std. Deviation
ZN-GU sealer	3	52.07	55.64	53.5967	1.84023
Thymol 2%	3	40.14	42.36	41.2933	1.11253
Thymol 5%	3	40.58	41.31	40.9833	.37099
Thymol 10%	3	40.00	40.26	40.1233	.13051

Table (3): Analysis of Variance (ANOVA) and Duncan's Multiple Range Test for Setting Time of Tested Endodontic Sealers.

	Df	Mean Square	F	Materials	Duncan Groups**
Between Groups	3	23.551	103.415**	Zn-Gu sealer	B
				Thymol 2%	A
				Thymol 5%	A
Within Groups	8	1.195		Thymol 10%	A
Total	11				

** **Different letters mean highly significant different at p≤0.01.

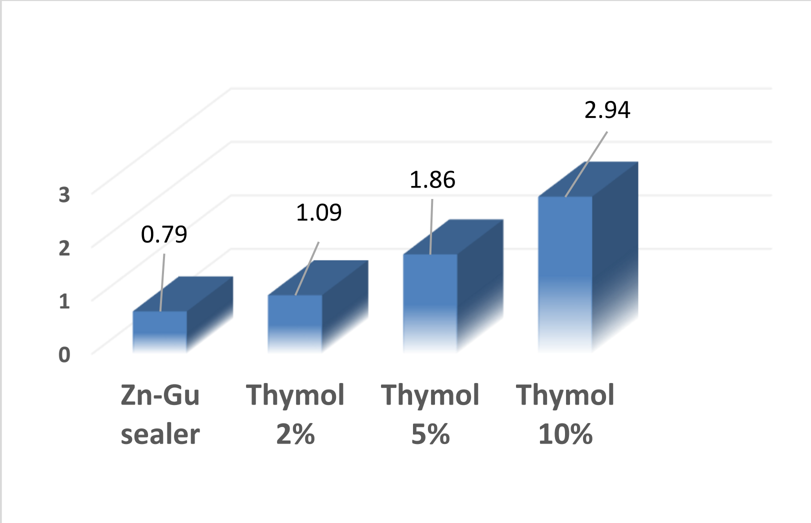
Water solubility test

The mean of the solubility value and standard deviation of the experimental endodontic sealer are presented in, Table (4).

Table (4): Solubility descriptive statistics.

Materials	N	Minimum%	Maximum%	Mean%	Std. Deviation
Zn-Gu sealer	10	.735	.883	.794020	.081063
Thymol 2%	10	1.040	1.183	1.09720	.078324
Thymol 5%	10	1.499	1.951	1.86060	.202141
Thymol 10%	10	2.678	3.268	2.94200	.217093

The mean values of solubility for the experimental sealer groups before and after thymol incorporation in studied percentages are still within the requirements of ANSI/ ADA specifications No. 57 that demonstrating a solubility of less than 3%, except for some samples of 10% thymol incorporation. In general, the solubility increased as the thymol incorporation percentage increased, Figure (6).



Figure(6): The effect of thymol incorporation (2%, 5%, and 10%) in the solubility of zinc oxide- guaiacol endodontic sealer.

Analysis of variance (ANOVA) test and Duncan's Multiple Range Test for zinc oxide-guaiacol sealer before and after thymol crystals incorporation are listed in, Table (5). The results revealed that there are significant differences among all sealer groups at p≤0.01 and there is a statistically significant increase in solubility as the incorporation percentages of thymol powder increases.

Table (5): Analysis of Variance (ANOVA) and Duncan's Multiple Range Test for Solubility of Tested Endodontic Sealers.

	Df	**Mean Square**	**F**	**Materials**	**Duncan Groups****
Between Groups	3	4.582	182.025**	Zn-Gu sealer	A
Within Groups	16	.025		Thymol 2%	B
Total	19			Thymol 5%	C
				Thymol 10%	D

**Different letters mean highly significant different at p≤0.01.

DISCUSSION

Setting time

The perfect root canal sealer setting time should allow sufficient working time. While, a slow setting time can end in tissue irritation, with most endodontic root canal sealers generating a certain degree of toxicity till being totally set [(11)].

Setting time is the time essential for the endodontic sealer to accomplish its definitive actions. It rests on the ingredients, size of particles, ambient temperature, and proportional humidity. There is no specified standard setting time for endodontic sealers, but clinical benefit loads that it must be long enough to permit the application and modification of core root filling when required [(12)].

American National Standard Institution/ American Dental Association does not display particular time for materials that exceed 30 minutes in their setting times, so the only requirement is that the setting time should be assessed and stated by the manufacturer.

The setting time of zinc oxide-guaiacol sealer was 53.5967 minutes, after the addition of thymol powder in three different concentrations, the setting time was decreased but with no significant differences as the concentration of thymol crystals was increased, so the setting time of sealer with thymol of (2% was 41.2933 minutes), the setting time of sealer with thymol of (5% was 40.9833 minutes), and the setting time of sealer of (10% was 40.1233 minutes).

The challenge in the present study used thymol crystals not thyme oil as many of the previous studies of incorporation in dental materials, so cannot comparison of results with similar studies, which led to citing the effect of the addition of thymol in food industries or mentioning the effect of incorporation of thymol oil in other situations.

The integration of antimicrobials such as (QPEI, benzalkonium chloride, and iodoformium nanoparticles) into the endodontic root canal sealers presented a rise in setting time. On the other hand, another study also observed a decline in the setting time of the AH Plus incorporated with amoxicillin. Even with the decrease in time for the set, this time is sufficient for the conditions of clinical usage [(8)]. The setting of root canal sealer happens due to the reaction between the two constituents of the material (powder with liquid or base paste with catalyst paste), therefore, the adding of particles that do not share in the reaction of set or polymerization may rise the setting time [(13)].

Water solubility

Solubility is the mass deprivation of material throughout an interval of immersion in water. According to ANSI/ADA Specification 57, the solubility of a root canal sealer should not exceed 3% by mass. An extremely soluble root canal endodontic sealer would habitually allow the creation of gaps within and between the root dentin and the material, thereby given paths for leakage from the periapical tissues and the oral cavity [11].

According to the result of this study zinc oxide-guaiacol sealer has solubility (0.73%) which is conformed to ANSI/ADA specifications No. 57, with the integration of thymol crystals in three different concentrations, water solubility was increased as the concentration of thymol crystal was increased, so the result of water solubility of sealer with (2% was 0.88%), the result of water solubility of sealer with (5% was 1.56%), and the result of water solubility of sealer with (10% was 2.94%) although this increase, the solubility is conformed to ANSI/ADA Specifications No. 57.

The water solubility is clearly based on the molecular weight as it declined; the solubility rise at nearly pH 7; Thymol theorizes with low molecular weight [14].

The result of the present study agreement with Nunez-Flores [15] indicated that the incorporation of thymol especially at higher concentrations caused a significant increase ($P \leq 0.01$) in solubility. The addition of thymol in vigorous fish gelatin films may obstruct polymer chain-to-chain interactions and decrease cross-linking and subsequently, raise the solubility of the films. Commonly, the actions of the additives on the solubility of films rest on the kind of compounds and concentrations and their hydrophilicity and hydrophobicity index.

According to Nostro[16], thymol is as much hydrophilic as hydrophobic, which can support the diffusion of this compound. The low solubility of the cross-linked gelatin films; is raised slightly but not very large in the existence of thymol [17].

CONCLUSION

The setting time and water solubility of (2%, 5%, and 10%) thymol incorporated zinc oxide - guaiacol root canal sealer fulfilled the requirement of ANSI/ADA specification No.57 for the root canal sealer materials.

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