

# EVALUATION OF PHYSICAL STABILITY AND DRUG CONTENT OF ZOLEDRONATE BISPHOSPHONATE GEL

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## ABSTRACT

**INTRODUCTION:** The gel emulsion form of zoledronate bisphosphonate has been proven to increase the number of osteoclasts via apoptosis when applied topically. The gel form is commonly used for topical application in the oral cavity.

**OBJECTIVES:** We aimed to evaluate the physical stability of zoledronate bisphosphonate in its gel form to assess its suitability for clinical application.

**MATERIAL AND METHODS:** The gel formulation was prepared using carboxymethylcellulose, glycerine, and sodium benzoate in a dosage form of 40 µg of zoledronate per 25 mg of the gel. The prepared gels were stored at 25°C and 40°C for 28 days and evaluated for viscosity, pH value, spreadability, adhesive strength, and drug content on days 1, 7, 14, and 28 of incubation.

**RESULTS:** Throughout the study, at 40°C of storage, the gel demonstrated significantly increasing viscosity and adhesive strength, but decreasing spreadability. However, the pH and drug content decreased at 25°C and 40°C.

**CONCLUSION:** Zoledronate bisphosphonate gel showed stability in physical characteristics and drug content when stored at 25°C for 28 days.

**KEY WORDS:** zoledronate, drug stability, orthodontic tooth movement.

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## INTRODUCTION

In orthodontic treatment, undesirable tooth movement of the anchorage unit and the relapse of moved teeth to their original positions are important problems [1, 2]. Various mechanical appliances have been used to reinforce the anchorage in such cases, such as the transpalatal arch, Nance holding arch, temporary anchorage device, and headgear. However, anchorage loss and other side-effects continue to occur, thereby

compromising the treatment outcomes [3-5]. Besides the mechanical appliances, studies have shown that undesirable tooth movement could be prevented by blocking the bone resorption with a pharmacological agent [6].

Bisphosphonates are widely used as drugs to treat osteoporosis and other bone diseases. Past studies have shown that the administration of bisphosphonate can increase the amount of osteoclast apoptosis and thereby inhibit tooth movement [6, 7]. There are two types

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of bisphosphonates, which are distinguished by the nature of the side chains attached to the carbon atom. Bisphosphonates that contain a nitrogen atom (*N*-containing bisphosphonates) possess a higher potency than those without a nitrogen atom (non-*N*-containing bisphosphonates). The *N*-containing bisphosphonates inhibit the farnesyl pyrophosphate synthase enzyme, which in turn inhibits the prenylation of a small protein called guanosine triphosphate in osteoclasts. This event eventually leads to osteoclast apoptosis [8, 9]. Zoledronate is an *N*-containing bisphosphonate and the most potent inhibitor of the hydroxyapatite growth rate [8].

Past studies have shown that the administration of bisphosphonates can inhibit orthodontic tooth movement in animal models [10-12]. It has been suggested that the administration of zoledronate bisphosphonate in gel emulsion can also increase the amount of osteoclast apoptosis. Topical application is less painful than that through injection [13]. Topical application in the gel form has been widely used in the oral cavity, such as in the treatment of periodontitis, ulceration, and oral candidiasis [14-16]. In comparison with ointments and cream bases, gels possess a higher water content, thereby permitting greater dissolution of drugs [17].

Stability testing of drug products is performed at various stages of product development. The purpose of pharmaceutical stability testing is to ensure that the products retain acceptable quality for a certain period of time to maintain the efficacy and safety of the drugs [18]. The present study aimed to evaluate the physical stability and drug content of zoledronate bisphosphonate in the gel form to evaluate one of the prerequisites in developing a new drug product.

## MATERIAL AND METHODS

Zoledronate powder was purchased from Sigma-Aldrich (Singapore). Carboxymethylcellulose (CMC) and sodium benzoate were purchased from Harum Kimia CV (Jakarta, Indonesia). Glycerine was purchased from Indogen Ltd. (Jakarta, Indonesia). Distilled water was used throughout the study.

### GEL PREPARATION

Zoledronate powder was dissolved in phosphate-buffered saline in a 1 : 1 ratio (w/v). CMC, glycerine, sodium benzoate, and distilled water were mixed with constant stirring in a glass beaker until a homogeneous solution was formed. The resultant gel was mixed with zoledronate solvent using a mixer in a dosage form of 40 µg zoledronate per 25 mg of the gel.

## GEL EVALUATION

The following parameters were used to evaluate the gel properties:

1. Organoleptic test: The prepared zoledronate bisphosphonate gel formulations were inspected visually for color, odor, taste, consistency, and homogeneity.
2. Viscosity: Viscosity measurement was performed using a viscometer (Brookfield, USA) with spindle 7. The corresponding dial reading was noted at each speed.
3. pH value: The pH value of the gel was measured using a digital pH meter (Eutech Instruments, USA) by dissolving 1 g of gel in 100 ml of distilled water.
4. Spreadability: The spreadability was evaluated by placing the gel between 2 glass plates, followed by putting a stone weighing 100 g on top of the glass plate for 1 min. The diameter of the spread gel was then measured.
5. Adhesive strength: Adhesive strength was determined by placing the gel between 2 glass plates, followed by placing a stone weighing 250 g on top of the glass plate for 5 min. The glass plates were placed in the apparatus, and then a weight of 80 g was added. The time required to detach the glass plates from each other was recorded.
6. Drug content: The drug content of the gel was measured with an ultraviolet-visible spectrophotometer (Spectroquant Pharo 300, Germany). The gel (100 mg) was diluted to 5 ml with distilled water. A volume of 1 ml of this solution was further diluted to make an amount of 5 ml with distilled water. Then, the solution was placed in a cuvette and absorbance was measured at 210 nm.
7. Stability studies: The prepared gels were packed in Eppendorf tubes and subjected to stability studies at 25°C and 40°C for 28 days. The samples were evaluated on days 1, 7, 14, and 28 for viscosity, pH value, spreadability, adhesive strength, and drug content.

## STATISTICAL ANALYSIS

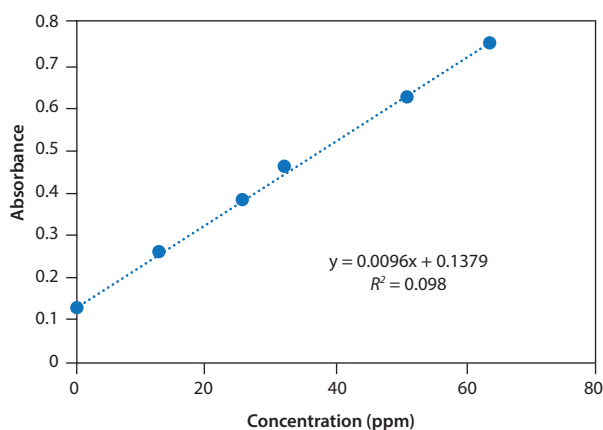
The data were analyzed using the independent *t*-test to determine the differences in the outcomes between storage at 25°C and 40°C. Repeated measure ANOVA was used to calculate the significant differences among the outcomes on days 1, 7, 14, and 28. Statistical calculations were performed using SPSS 23.0 version.

## RESULTS

Zoledronate bisphosphonate gel was evaluated for physical appearance, viscosity, spreadability, adhesive strength, pH, and drug content at 25°C and 40°C after 28 days of storage. The results of the physical appearance test are given in Table 1. The standard calibration curve of zoledronate for the spectrophotometry test is shown in Figure 1.

**TABLE 1.** Physical appearance of zoledronate bisphosphonate gel

	Baseline		Day 7		Day 14		Day 28	
	25°C	40°C	25°C	40°C	25°C	40°C	25°C	40°C
Organoleptic								
Color	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Odor	Odorless	Odorless	Odorless	Odorless	Odorless	Odorless	Odorless	Odorless
Taste	Bland	Bland	Bland	Bland	Bland	Bland	Bland	Bland
Consistency	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Hard
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous



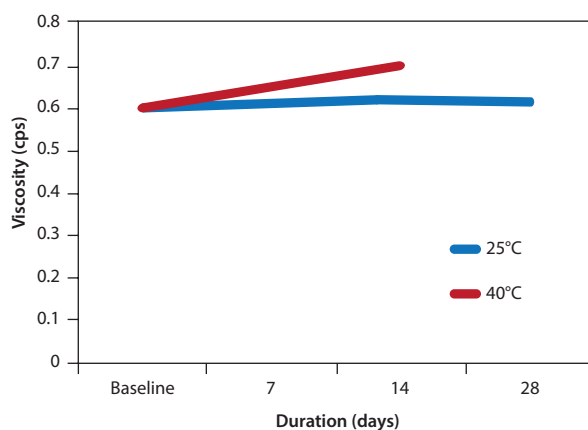
**FIGURE 1.** Standard calibration curve of zoledronate

The changes in viscosity of the gel at 25°C and 40°C are illustrated in Figure 2. The viscosity of the gel increased significantly during storage at 40°C over 14 days. Figure 3 represents the mean spreadability of the gel at 25°C and 40°C throughout the study. Statistical analysis revealed a significant difference in the spreadability values before and after storage at 40°C. Figure 4 shows the mean adhesive strength of the gel at 25°C and 40°C for different periods of time. Changes in the adhesive strength were found to significantly increase at storage temperature of 40°C.

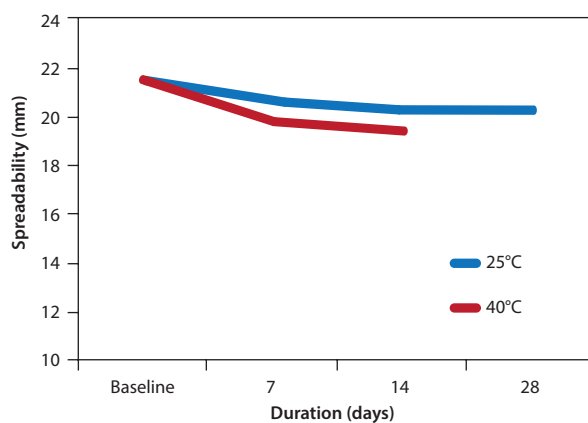
The mean pH values of all prepared gels are shown in Figure 5. From the statistical analysis, the pH value significantly decreased at 25°C and 40°C. The drug content also showed significant changes at 25°C and 40°C throughout the study, as shown in Figure 6.

## DISCUSSION

In the present study, we evaluated the physical stability and drug content of zoledronate bisphosphonate gel as one of the prerequisites for developing a new drug product. The physical appearances of the prepared gels during storage at 25°C and 40°C for 28 days were as follows: clear in color, bland in taste, odorless, and homogeneous. However, the consistency of the gel hardened



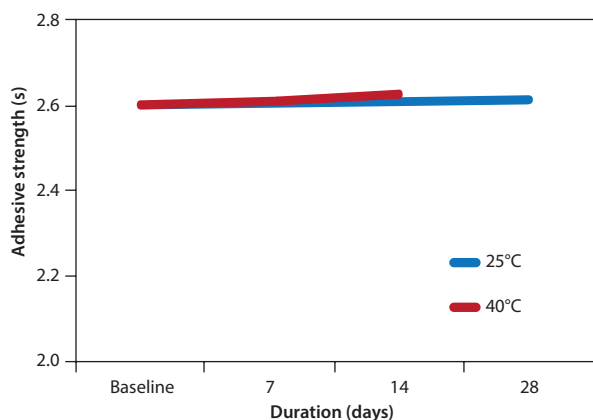
**FIGURE 2.** Mean viscosity of zoledronate bisphosphonate gel at 25°C and 40°C for different time periods. Changes in viscosity over 14 days at 40°C were statistically significant ( $p < 0.05$ )



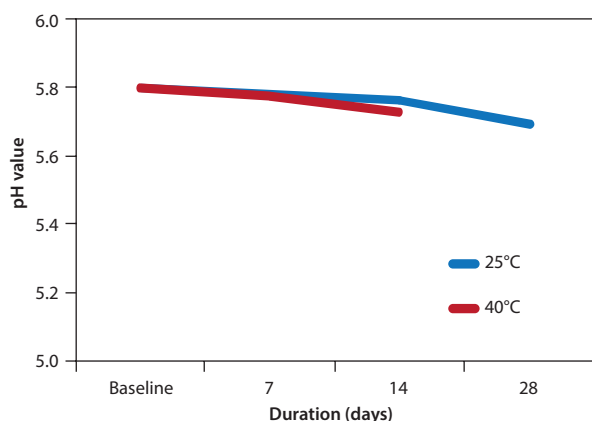
**FIGURE 3.** Mean spreadability of zoledronate bisphosphonate gel at 25°C and 40°C for different time periods. Changes in the spreadability value over 14 days at 40°C were statistically significant ( $p < 0.05$ )

on day 28 at 40°C; therefore, the stability test could not be performed.

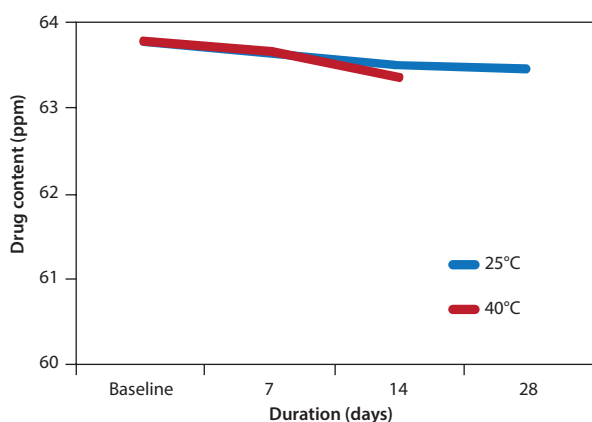
This study showed that the viscosity value increased throughout the study period, but it was statistically



**FIGURE 4.** Mean adhesive strength of zoledronate bisphosphonate gel at 25°C and 40°C for different time periods. Changes in the adhesive strength over 14 days at 40°C were statistically significant ( $p < 0.05$ )



**FIGURE 5.** Mean pH value of zoledronate bisphosphonate gel at 25°C and 40°C for different time periods. Changes in the pH value at 25°C over 28 days and at 40°C over 14 days were statistically significant ( $p < 0.05$ )



**FIGURE 6.** Mean drug content of zoledronate bisphosphonate gel at 25°C and 40°C for different time periods. Changes in the drug content at 25°C over 28 days and at 40°C over 14 days were statistically significant ( $p < 0.05$ )

significant at 40°C. This finding indicates that an increase in the temperature increased the viscosity. The rise in temperature decreases the moisture content. Gels with lower moisture contents were firmer than those with higher moisture contents [19]. The influence of temperature on the cellulose-based gel viscosity in this study was also similar to that reported by other studies. Ghannam *et al.* reported that the higher the CMC concentration in the solution was, the lower was the flow behavior index [20]. Owen *et al.* also reported an increase in viscosity of cellulose-based gel with increasing temperature [21].

In this study, the gel's spreadability and adhesive strength at 25°C were stable over 28 days. The spreadability was found to decrease significantly at 40°C over a period of 14 days. However, the adhesive strength was found to increase significantly at 40°C over a period of 14 days. This finding is consistent with that of the study by Riski *et al.* showing that spreadability has a nonlinear relationship with viscosity, whereas adhesive strength has a linear relationship with viscosity. The higher the viscosity value is, the lower is the spreadability but the higher is the adhesive strength [22]. In this study, the range of the spreadability value was 19.5–21.5 cm and that of the adhesive strength value was 2.60–2.63 s. These values were below the standard values for topical application, which are approximately 5–7 cm for spreadability and  $\geq 4$  s for adhesive strength [23]. However, our findings are still acceptable for topical drug application in the oral cavity, which does not need a large area and a long time for application to prevent being carried away and removed by the saliva. CMC possesses a good adhesive property with biological substrates, such as the oral cavity [24].

Zoledronate monohydrate is designated chemically as (1-hydroxy-2-imidazole-1-Cyl-phosphonoethyl) phosphonic acid, and the pH of a 0.7% solution of zoledronic acid in water is approximately 2.0 [25]. In the present study, CMC, glycerine, and distilled water, which have more basic pH, were added and mixed with zoledronate to make the final pH of the gel to approximately 5.8. Throughout the study, the pH value decreased at 25°C and 40°C, with the final pH of 5.69 at 25°C. The oral cavity uses the salivary system as a buffer, which maintains the pH of 6.0–7.5. Therefore, this value was close to that of the oral mucosal pH and hence acceptable. The decrease in the pH value was similar to that in other studies and could have been caused by hydrolysis, oxidation, light, microorganisms, and chemical reaction between CO<sub>2</sub> and the water phase in the gel during storage [26].

The zoledronate dosage in this study was based on the previous research by Anggraini, which was 40 µg of zoledronate per 25 mg of gel or 0.16% of zoledronate in the gel [13]. When converted to the unit parts per million (ppm), the drug content of zoledronate was 16,000 ppm. The zoledronate bisphosphonate gel was diluted 25-fold to fit the range of the zoledronate standard calibration curve, achieving the final concentra-

tion of approximately 64 ppm. Our results revealed that the concentration of zoledronate in the gel on day 1 was 63.8 ppm as per the spectrophotometry test. The drug content decreased at 25°C and at 40°C throughout the study, with a more significant decrease at 40°C. The decreasing value was in accordance with the bisphosphonate drug half-life, which is up to 10 years. The rising temperature can also accelerate the chemical degradation of a substance [6].

In this study, the gels were packed in polypropylene Eppendorf tubes. The hardened consistency of gel at 40°C on day 28 indicates that the tubes could not protect the gels from the rising temperature. Further studies are needed on drug packaging along with long-term drug stability testing for better clarification.

Zoledronate is a third-generation bisphosphonate that possesses the potential to inhibit bone resorption. Zoledronate bisphosphonate in the gel emulsion form can increase the amount of osteoclast apoptosis and hence facilitate bone remodeling to modify orthodontic tooth movement [13]. Osteoclast apoptosis can inhibit bone resorption and decrease the rate of alveolar bone remodeling during orthodontic tooth movement. Bisphosphonate has been recommended as a potential pharmacological agent for orthodontic anchorage reinforcement [6]. Our findings confirm that zoledronate bisphosphonate in the gel form possesses good stability at 25°C and therefore has potential in topical drug application for the oral cavity.

## CONCLUSIONS

In conclusion, zoledronate bisphosphonate gel is stable in its physical appearance, viscosity, spreadability, adhesive strength, pH, and drug content at 25°C after 28 days of storage, suggesting its potential for use in topical drug preparation.

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## CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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