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Chemical and physical stability investigations of captopril extemporaneous suspension for oral administration

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Abstract

Captopril is frequently used for hypertension and heart failure in pediatrics. In Thailand, no captopril liquid formulation is available because of its instability. The aim of our study was to develop the physically and chemically stable captopril extemporaneous suspension. The captopril suspension was prepared by grinding captopril tablets and triturating with syrup 80% w/v to the desired volume resulting in 1 mg/mL captopril suspension. For the formulations containing stabilizers, citric acid 2% w/v, vitamin C tablets 4 and 5 mg/mL were triturated with the ground captopril tablet before mixing with the vehicle. All formulations were kept in amber bottles at 2-8°C for 90 days and 30°C for 60 days. At each time point, the appearance, pH and number of redispersibility were evaluated for the physical stability. For the chemical stability, the amounts of captopril and dimer were quantified. The addition of vitamin C increased the number of redispersibility and minimally lowered the pH of formulations. Citric acid decreased the pH of formulation from 4.53-4.67 to 2.50-2.80. As the storage time increased, the number of redispersibility and the dimer content increased while the captopril amount reduced. The increased concentration of vitamin C had no effect on the stability. Obviously, the increasing storage temperature decreased the chemical stability. At 2-8°C, the addition of all stabilizers could preserve the amount of captopril over 90% for 90 days whereas the drug in the formulation without stabilizer remained over 90% for 74 days. At 30°C, the drug content was less than 90% after 28 days.

Keyword: Captopril, Extemporaneous preparation, Stabilizer, Suspension, Syrup

1. INTRODUCTION

Captopril is a sulfhydryl-containing angiotensin converting enzyme inhibitors (ACEIs) frequently used to treat arterial hypertension and congestive heart failure in pediatrics[1-3]. It is a drug of choice for infants during the first month of age. In Thailand, captopril is available only as a tablet dosage form with the strengths of captopril at 12.5 mg, 25 mg and 50 mg per tablet. Although the captopril tablet gains more advantages over a liquid formulation such as good compliance for adult patient and good chemical stability, however it is inconvenient and incompliant for infants and children less than 6 years of age. The hospital pharmacies have compounded captopril solutions or suspensions for the administration of this drug in pediatrics. The liquid formulations provided the flexibility of dosage adjustment for individual patients by adjusting the volume of formulation and the easiness of administration. Owing to poor taste of sulfur-containing moiety, most compounded formulations consisted of syrup as a sweetener and a vehicle which improved the palatability of formulation and aided the compliance of patients.

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The significant pharmaceutical problem of liquid dosage form is that the captopril is prone to be oxidized at sulfhydryl group[4] in aqueous system yielding captopril disulfide or dimer and resulting in the chemical instability of extemporaneous product[5] (Figure 1). The reaction is catalyzed by metal ions and the rate of degradation depends on pH and oxygen concentration[3,6]. However, if the pH of solution is around 4.0, the degradation of captopril can be retarded. Furthermore, it has been reported that the hydrolysis at an amide linkage of captopril was apparent under the forced condition. Many strategies have been used to increase the stability of captopril in liquid formulations such as controlling the pH of formulation lower than 4.0, adding a chelating agent[7] or an antioxidant[8], preparing the high concentration of captopril suspension[9] and filling an inert gas at the head space of bottle[5]. Although many studies have investigated the stability of captopril extemporaneous preparations, the differences in sources of drug products and vehicles may affect the stability of captopril in the formulations even under the similar experimental conditions[8, 10, 11]. Because the extemporaneous preparations are produced and used only in the hospital, the finished preparations are always different among countries or even hospitals.

Therefore, the objective of this study was to develop the physically and chemically stable extemporaneous captopril suspension from generic captopril tablets available in Thailand for oral administration to newborns and young children. To improve the stability of the extemporaneous suspension, citric acid (CA) and vitamin C were employed as a chelating agent and an antioxidant, respectively. The concentration of vitamin C used was varied at 4 and 5 mg/mL.

2. MATERIALS AND METHODS

2.1. Materials

The commercial captopril tablets (containing captopril 12.5 mg per tablet, Boryung Pharmaceutical Co., Ltd, Seoul, Korea) were kindly provided by Pharmacy Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The captopril powder (Changzhou Xinhua Industry General Co., Ltd., Jiangsu, China) was gifted by the Pharmasant Laboratories Co., Ltd., Nonthaburi, Thailand. Sucrose (refined sugard, MitrPhol Group, Bangkok, Thailand and Thai Roong Ruang Industry Co., Ltd., Phetchabun, Thailand), CA (Carlo Erba Reagenti, Milan, Italy), vitamin C tablet (100 mg/tablet, Interthai Pharmaceutical Manufacturing Ltd, Bangkok, Thailand), sodium benzoate (Carlo Erba Reagenti, Milan, Italy), methanol (high performance liquid chromatography (HPLC) grade, Honeywell Burdick & Jackson, Ulsan, Korea) and absolute ethanol (Honeywell Burdick & Jackson, Ulsan, Korea) were used as received. Sterile water for irrigation was purchased from General Hospital Products Public Co.,Ltd., Pathum Thani, Thailand.

2.2. Preparation of vehicle

Syrup (80% w/v) was used as a vehicle for captopril suspension. Briefly, 800 g of sucrose was weighed and added to the previously calibrated beaker. The deionized (DI) water was added and mixed with an aid of heating by a hot plate to dissolve the sugar. After completely dissolved, the syrup was left at room temperature for cooling down. Subsequently, 1 g of sodium benzoate was added and dissolved in the syrup. Finally, the volume of syrup was adjusted to the required volume using DI water. The DI water was boiled for 30 min prior to use.

2.3. Extemporaneous preparation of captopril suspension

The extemporaneous captopril suspension was prepared by the porcelain mortar and pestle in a horizontal laminar air flow cabinet. Four formulations of captopril suspension were used in this study namely the formulations without stabilizer, with CA (2% w/v), with vitamin C tablet (4 mg/mL, V4) and with vitamin C tablet (5 mg/mL, V5). Six captopril tablets were ground to a fine uniform powder in the mortar. The two-third of required volume of vehicle was triturated with the ground captopril tablets until obtaining the uniform suspension. The

volume of suspension was adjusted to 75 mL using the vehicle and mixed thoroughly. Eventually, each 20 mL of the obtained captopril suspension (1 mg/mL) was dispensed to two 30-mL amber polyethylene terephthalate (PET) bottles and one 30-mL clear glass bottle. In case of the formulations containing stabilizers, 1.5 g CA, three vitamin C tablets or finely ground vitamin C tablet equivalent to 375 mg was triturated with the ground captopril tablets before mixing with the vehicle. The suspension was prepared as previously described.

2.4. Stability testing

All formulations were kept in the refrigerator (2-8°C) for 90 days and an incubator (30°C) for 60 days. The physical and chemical stability of all formulations was tested at initial time, day 7, 14, 28, 45, 60 and 90. The samples kept in the clear glass bottle were protected from light by wrapping with aluminum foil.

2.4.1. Physical stability evaluation

The physical stability of captopril suspension was evaluated in terms of appearance, color, number of redispersibility and pH. The appearance and color of all formulations in the clear glass bottle was visually observed. The number of redispersibility was counted by turning the bottle upside down until no precipitate was observed at the bottom of the bottle. The pH of all formulations was measured by using a calibrated pH meter (Cyberscan PC 300, Eutech Instruments, Rajah Crescent, Singapore).

2.4.2. Chemical stability

The remaining captopril content and the forming dimer content were analyzed by HPLC method. The extraction of captopril and dimer was performed according to the previously published method[12]. Shortly at each time point, 0.5 mL of sample was withdrawn by 1-mL syringe and added into 10-mL volumetric flask. Then 90% v/v ethanol was added to the volumetric flask, mixed by vortex mixer and sonicated by sonicator bath for 15 min. The mixture was transferred into the centrifuge tube and centrifuged at 4,500 rpm for 15 min. One milliliter of supernatant was pipetted into 5-mL volumetric flask and diluted to the final volume using a HPLC mobile phase. The solution was filtered through 0.45 µm nylon syringe filter prior to HPLC analysis. The percentages of remaining captopril and forming dimer contents were calculated by equations 1 and 2, respectively. The %remaining captopril content of formulation in the range of 90-110% was accepted and considered as chemically stable formulation.[13]

$$\% \text{ Remaining captopril content} = \frac{\text{Analyzed amount of captopril at each time point}}{\text{Analyzed amount of captopril at initial time}} \times 100 \quad (1)$$

$$\% \text{ Forming dimer content} = \frac{\text{Analyzed amount of dimer at each time point}}{\text{Analyzed amount of captopril at initial time}} \times 100 \quad (2)$$

2.5. HPLC analysis

The captopril and dimer contents were analyzed according to the reported HPLC method[12]. In brief, the sample was eluted by the mixture of methanol and 0.1% v/v phosphoric acid in water (47:53, v/v) at a flow rate of 1.0 mL/min through a reverse phase C18 Phenomenex® Gemini-NX column (110 Å, 5 µm 250×4.60 mm, Phenomenex Inc., Macclesfield, UK) with a guard column (Inertsil® ODS-3, 5 µm, 4.0×10 mm, GL Sciences Inc., Tokyo, Japan). The captopril and dimer were detected at a wavelength of 220 nm by Shimadzu HPLC machine (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with SPD-20A UV/VIS detector. The captopril and dimer contents were calculated from the calibration curve of captopril and dimer standards over the concentration range of 0.75-20 µg/mL. The calibration curve was considered as linear

with r^2 over 0.9995 and the inter- and intra-day precisions were less than 2%.

2.6. Statistical analysis

The results are expressed as the mean ± standard deviation from at least three measurements. The statistical comparison was performed by Student's t-test or one-way ANOVA with the Scheffe test applied post hoc to determine the significant difference between 2 groups or multiple groups, respectively. The results are considered to be significant different at *p*-value < 0.05 (95% confidence interval).

3. RESULTS AND DISCUSSION

It has been established that the degradation of captopril proceeded through the oxidation reaction forming captopril disulfide (Figure 1)[4]. In solution, the oxidation of captopril is a combination of metal ion-catalyzed oxidation and autooxidation and depends on the ionization of thiol group. To delay the oxidation of captopril in the formulation, the chelating agent, the antioxidant and the acid modifying agent can be added into the formulation. In this study, CA and vitamin C were used as stabilizers. The CA was used at a concentration of 2% w/v to serve as a chelating agent which can prevent the metal ion-catalyzed oxidation and to reduce the pH of the formulation[14]. The optimum pH of solution for captopril stability was reported to be 3.5 and the captopril was more stable at pH lower than 3.5. The vitamin C is a reducing agent which can retard the oxidation by the self-oxidation. The studies reported that the use of vitamin C 5 mg/mL could increase the stability of captopril in liquid formulations. However, the addition of vitamin C 5 mg/mL by vitamin C tablet was not convenient in our study due to the need of grinding vitamin C tablets and weighing the ground powder. Since the batch size of captopril suspension was 75 mL, the required amount of vitamin C (5 mg/mL) was equivalent to 375 mg of vitamin C. To conveniently use the whole vitamin C tablets, three tablets equivalent to 300 mg of vitamin C were used resulting in the final vitamin C concentration of 4 mg/mL. Therefore, the formulations containing 4 and 5 mg/mL vitamin C were included in this study.

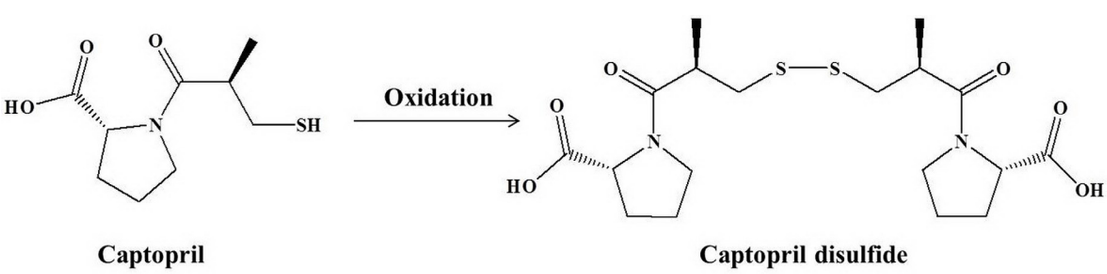

Figure 1. Oxidation pathway of captopril forming captopril disulfide (dimer).

3.1. Freshly prepared captopril suspension

After fresh preparation, the suspensions without stabilizer and with CA were white turbid while the color of those with vitamin C turned to yellow (Figure 2). The pH of formulation without stabilizer was 4.63 ± 0.06. The addition of CA dramatically reduced the pH of suspension to 2.66 ± 0.10 while the vitamin C 4 and 5 mg/mL significantly decreased the pH of formulations to 4.44 ± 0.02 and 4.42 ± 0.04, respectively (*p*-value < 0.05). The increasing amount of vitamin C did not affect the pH of formulation. The captopril content after fresh preparation was in the range of 90-100%. Additionally, the captopril dimer was also detected and quantified in all freshly prepared suspensions. The %dimer content was around 3.22-3.58% which was consistent with the presence amount of dimer in the captopril tablet.

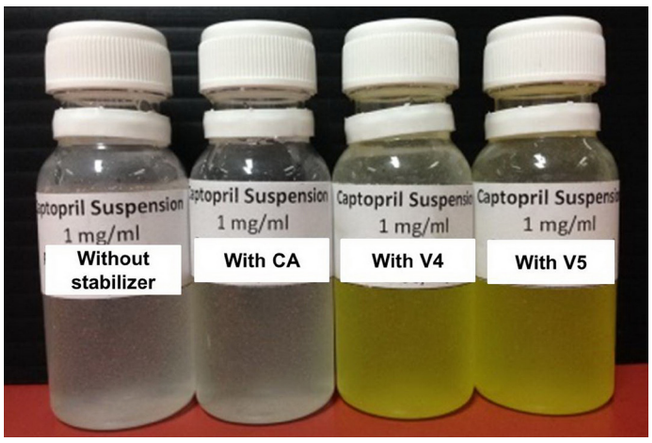

Figure 2. Appearance of freshly prepared captopril suspensions without stabilizer, with citric acid (CA 2% w/v), with vitamin C 4 mg/mL (V4) and with vitamin C 5 mg/mL (V5).

3.2. Physical stability

After storage at 2-8°C, the appearance of all captopril suspensions was not changed from the beginning (data not shown). As the time passed, the number of redispersibility of all formulations varied from time to time and no correlation between the number of redispersibility and time was observed when storing the formulations under both conditions. The storage temperature did not affect the number of redispersibility of the formulations. The formulations with vitamin C 4 and 5 mg/mL had the higher number of redispersibility than the others (p-value < 0.05) due to the excipients of vitamin C tablets added in the formulation (Figure 3). The different amount of vitamin C in the formulations did not have an impact on the redispersibility. Upon storage at 2-8°C over 90 days, the pH of formulations tended to decrease by less than 0.15 pH unit (Figure 4). Meanwhile at 30°C, the decrease of pH of the formulations without stabilizer and with CA was as much as 0.26 and 0.21 pH units, respectively, whereas the pH of the formulations with vitamin C reduced by less than 0.10 pH unit. The results suggested that vitamin C could prevent the reduction of pH of formulation possibly due to either the buffering capacity of ascorbic acid/sodium ascorbate or other excipients in the vitamin C tablets.

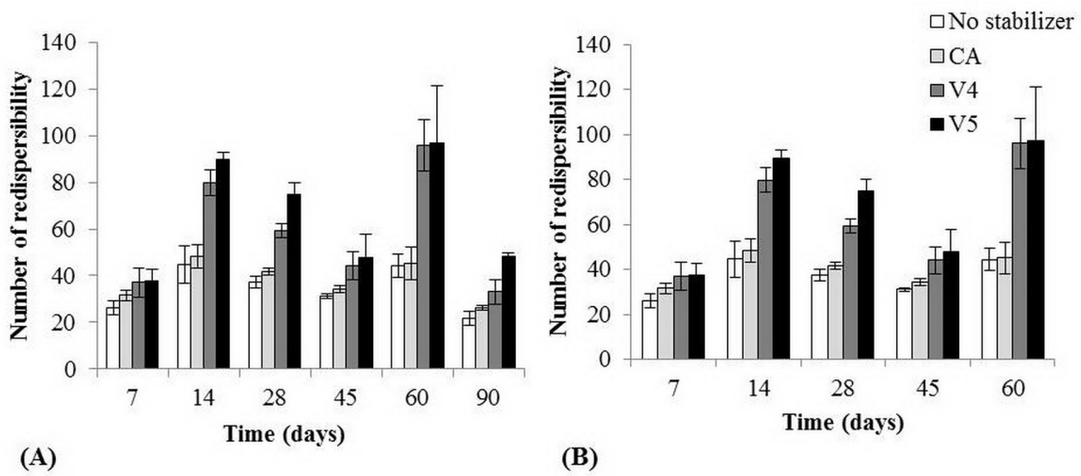

Figure 3. The number of redispersibility of formulations without stabilizer (no stabilizer), with citric acid (CA), with vitamin C 4 mg/mL (V4) and with vitamin C 5 mg/mL (V5) when storing at 2-8°C for 90 days (A) and 30°C for 60 days (B) (n=3).

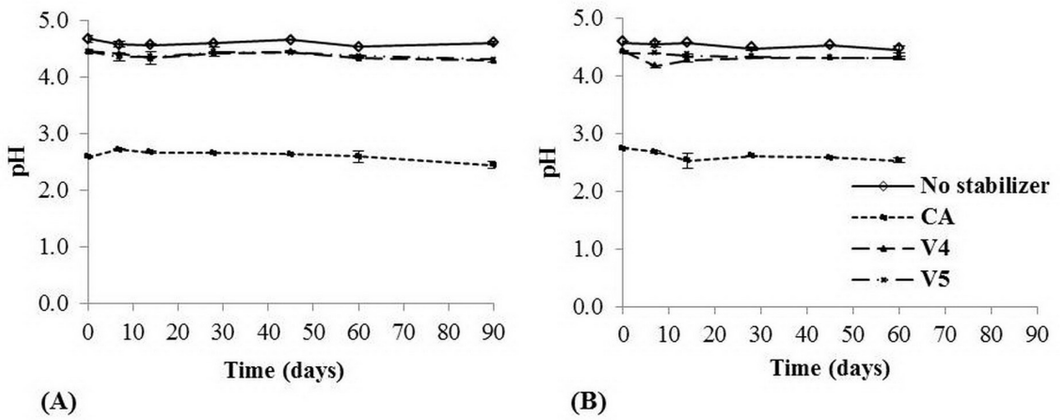

Figure 4. The pH of formulations without stabilizer (no stabilizer), with citric acid (CA), with vitamin C 4 mg/mL (V4) and with vitamin C 5 mg/mL (V5) when storing at 2-8°C for 90 days (A) and 30°C for 60 days (B) (n=3).

3.3. Chemical stability: Captopril remaining and dimer content

Considering the amount of remaining captopril and forming dimer in the formulations (Figure 5), the captopril content of all suspensions gradually declined with an increase of dimer content under both conditions. The decrease of drug remaining was more pronounced when stored at 30°C. The increasing dimer content was associated with the decrease of captopril content. It has been established that the major degradation of captopril is captopril dimer. Therefore, the degradation of captopril in these formulations proportionally resulted in captopril dimer except for the formulation with CA. However, the remaining amount of captopril of all formulations stored at 2-8°C was considerably higher than those stored at 30°C. This result was in consistent with the study of Pereira and Tam.[15] They found that the degradation of captopril in 1 mg/mL captopril suspension in tap water directly depended on the storage temperature and the captopril content was over 90% for 28 and 12 days when storing at 5°C and 25°C, respectively. In our study, all formulations with stabilizers at 2-8°C could preserve the amount of captopril over 90% for 90 days while the formulation without stabilizer remained the %captopril over 90% for only 74 days. At 30°C, the captopril content of all formulations was higher than 90% for 28 days except for the formulation with CA.

For the formulation without stabilizer, the captopril content decreased gradually with increasing storage time. In addition, the dimer content was obviously found and directly proportioned to the decrease of captopril content. The degradation of captopril was more profoundly at 30°C than 2-8°C. The captopril content remained over 90% for 74 and 28 days when storing at 2-8°C and 30°C, respectively.

Considering the use of CA as a stabilizer, it was found that CA accelerated the degradation of captopril at 30°C. In addition, the occurrence of dimer content was not in proportion to the degraded captopril. Indeed, the addition of CA in the formulation could prevent the degradation of captopril by chelating effect of CA as reported by Chen et al[16]. They discovered that the lower rate of captopril degradation was found in citrate buffer pH 6.0 at 80°C in comparison with acetate and phosphate buffers at the same pH and temperature. Our finding was probably due to the acidic pH of this formulation (around 2.50) causing the degradation of captopril through the combination of acid hydrolysis and oxidation. The acid hydrolysis of captopril was confirmed by our previously published result on the stress test of captopril in acidic medium[12]. The degradation of captopril in syrup was catalyzed by acidic medium resulting in the hydrolyzed product without an observation of captopril dimer in the HPLC chromatogram. Therefore, the use

of CA as a chelating agent in the formulation should be optimized and the pH of the formulation should be taken into account for the enhancement of stability of captopril stored at 30°C. However, the storage at 4°C did not cause the acceleration of captopril degradation in the formulation with CA. The remaining amount of captopril was over 90% for 90 days.

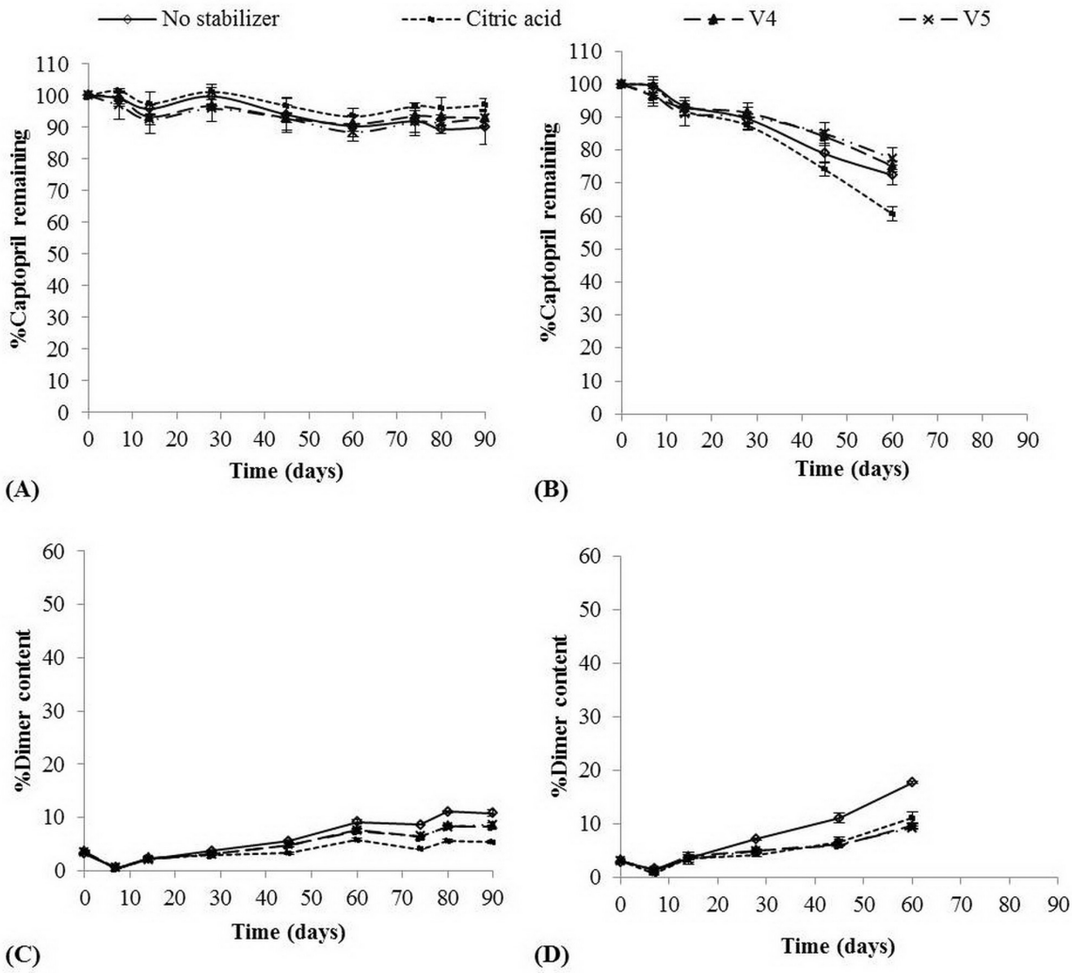

Figure 5. The remaining content of captopril (A and B) and the content of dimer (C and D) in formulations without stabilizer, with citric acid (CA), with vitamin C 4 mg/mL (V4) and with vitamin C 5 mg/mL (V5) when storing at 2-8°C for 90 days (left column) and 30°C for 60 days (right column) (n = 3).

Regarding the addition of vitamin C in the formulation, the result revealed that vitamin C retarded the degradation of captopril under both storage conditions. The captopril in these formulations degraded slower than the formulations without stabilizer and with CA. This finding was in agreement with the previous reports[8, 17]. They reported the higher stability of captopril at 1 mg/mL in distilled water mixed with 5 mg/mL of either sodium ascorbate injection or crushed ascorbic acid tablet than syrup and distilled water. The use of sodium ascorbate injection or ascorbic acid tablet extended the shelf-life of captopril for

56 days at 4°C. However, the ascorbic acid tablet prolonged the remaining captopril over 90% at 22°C longer than the sodium ascorbate injection for 28 and 14 days, respectively[17]. As aforementioned that the major degradation pathway of captopril is oxidation, vitamin C served as an antioxidant in the formulation and retarded the degradation of captopril. Our result discovered that vitamin C tablets added in the formulations at 4 and 5 mg/mL could last the %captopril remaining over 90% for 90 days at 2-8°C and for 28 days at 30°C. The dimer content in these formulations was much lower than the formulation without stabilizer by half after storing at 30°C. Both concentrations of vitamin C tablets did not significantly affect the amounts of captopril remaining and captopril dimer in the formulations.

From our results, it can be concluded that the addition of vitamin C and CA could retard the degradation of captopril and the formation of captopril dimer. The remaining captopril content was over 90% for 90 days at 2-8°C and 28 days at 30°C for the formulations with these stabilizers.

4. CONCLUSION

The captopril suspension (1 mg/mL) was successfully prepared using syrup 80% w/v as a vehicle. Various stabilizers were added in the captopril suspensions namely CA 2% w/v, ground vitamin C tablets 4 and 5 mg/mL. The results indicated that all formulations were more physically and chemically stable when stored at 2-8°C as compared to 30°C. As compared to the formulation without stabilizer, the addition of vitamin C increased the number of redispersibility and slightly reduced the pH of formulations. CA dramatically decreased the pH of formulation as compared to the formulation without stabilizer and the pH was further decreased upon storing especially at 30°C. Regarding the chemical stability, the captopril amount decreased while the dimer content increased with increasing storage time at both temperatures. The increasing concentration of vitamin C had no effect on the stability. At 2-8°C, all added stabilizers could retain the amount of captopril over 90% for 90 days whereas the formulation without stabilizer had the remaining captopril over 90% for 74 days. At 30°C, the drug content was less than 90% after 28 days.

5. ACKNOWLEDGEMENT

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