In urolithiasis, stones are formed in the urethra, kidney, or bladder. Kidney stones cause severe pain in the abdomen and flank [1]. Supersaturation of urine leads to the formation of kidney stones, and their formation is dependent on the pH, ionic strength, specific gravity, and solute concentration of urine [2]. Five types of urinary stones are primarily encountered: calcium oxalate, calcium phosphate, urates, cysteines, and magnesium ammonium phosphate hexahydrate (MgNH₄PO₄·6H₂O, struvite) [3,4]. Of these types of stone, struvite is unique owing to its association with the presence of infection in the urinary tract and thus struvite stones are also called infection stones [1,4]. The main cause of the infection is urease-producing organisms, such as Proteus spp., Klebsiella pneumoniae spp., and Providencia spp [5,6]. Urease is a characteristic bacterial enzyme that hydrolyzes the urea (H₂N-CO-NH₂) in urine to form ammonia [1,5]. As a result of this hydrolysis reaction, the pH of the urine and then the concentration of ammonia (NH₄⁺) and phosphate (PO₄³⁻) in the urine increases [5]. These urea ions combine with the Mg²⁺ that is normally present in urine under alkaline conditions, leading to the formation of struvite [7,8]. Naturally formed struvite crystals usually exhibit coffin-like morphology, but when they grow rapidly dendrites can also occur [7,9]. The dendrite form of struvite crystals is particularly dangerous because they damage the epithelium of the urinary tract as they move through it [7]. If the problems related to struvite stones are not treated appropriately, kidney loss may be seen. Moreover, the high rate (up to 50%) of recurrence of infection stones leads to serious medical problems [9]. Thus, the precipitation of struvite is an important medical research topic that needs to be addressed. Currently, urinary stones are usually treated using drug therapy for pain relief and inflammation reduction, whilst surgery is applied in extreme cases. Acetohydroxamic acid is prevalently used for treatment of patient suffering from struvite stones. However, the potential side effect of acetohydroxamic acid cannot be ignored. Thus, alternative ways to
eliminate health problem related to struvite stones have gained a great importance [1]. For these reasons, many studies have been conducted on the struvite precipitation in urine to eliminate these problems with an alternative way. Li et al. proved the inhibitory effects of polyaspartic acid with different concentrations on the struvite crystallization [10]. Olszynski et al. revealed that nanosilver particles having different size and shape had a distinctive impact on the growth process of struvite in artificial urine in the presence of Proteus mirabilis [11]. They showed that nanosilver particles had an inhibitory effect on struvite formation depending on their size and shape and also observed that smaller particles exhibited greater negative effect on the growth of struvite. Moreover, Manzoor et al. performed a study to understand the role of vitamin C on struvite precipitation. They revealed that vitamin C or ascorbic acid modulated the formation of struvite crystals in the presence of uropathogenic bacteria [12]. The growth rate of the struvite crystals decreased depending on the increasing vitamin C concentration. Sayan et al. [13] researched the influence of different amino acids such as lysine, proline, alanine and tryptophane with different concentrations on struvite crystals at 37 °C and pH 8. Adding these additives in studied concentrations did not lead to any changes in crystal morphology and structure. Although several studies are reported in the literature regarding struvite urinary stone formation, more research on struvite crystallization is still needed to find a viable method of removing struvite stones to give a suitable treatment alternative. Thus, studying the influence of crystal modifiers, especially biocompatible modifiers, on the precipitation of struvite is a highly attractive and promising research area to solve this problem. Proline, a type of nonessential amino acid, was studied in vitro as a crystal modifier to struvite crystallization media and found to be a potent inhibitor of struvite crystallization.

**MATERIAL AND METHODS**

Magnesium chloride hexahydrate (MgCl₂·6H₂O), ammonium dihydrogen phosphate (NH₄H₂PO₄), L-proline (C₅H₉NO₂), and sodium hydroxide (NaOH) were of reagent grade and purchased from Merck Company. Distilled water was utilized when preparing the solutions. All experiments were performed at least in triplicate.

The crystallization experiment was conducted in batch mode at 37 °C in a double-jacketed crystallizer. MgCl₂·6H₂O and NH₄H₂PO₄ were used as the reactants for struvite crystallization. The procedure was as follow: Firstly, 1 M MgCl₂·6H₂O and 1 M NH₄H₂PO₄ solutions were prepared. 300 ml of MgCl₂·6H₂O solution was put into the crystallizer. The solution was maintained at a constant temperature of 37 ± 0.5 °C using a thermostat. The stirring rate was 500 rpm. When the solution reached its target temperature and equilibrium, the NH₄H₂PO₄ solution was placed into the crystallizer via a peristaltic pump at a flow rate of 5 ml/min. During the crystallization process, the pH of the solution was continuously followed and kept at pH 7.4 using dilute sodium hydroxide.

The effect of proline and its concentration on the crystallization of struvite was examined in this study. Proline solution was added to the crystallizer via an infusion line solution was added to the crystallizer via an infusion pump to provide the desired concentrations of 25 and 100 ppm in the crystallizer. At the end of the experiments, all suspension was taken from the crystallizer, filtered and collected the solid and liquid separately. Finally, the upper solid phase washed thoroughly with distilled water. The prepared crystals were dried at room temperature and the samples are kept for further analysis.

X-ray diffraction (XRD; Bruker D2 Phaser benchtop) was used to identify the phase structure of the struvite crystals. The functional groups of samples were confirmed by ATR method on a Fourier Transform-Infrared Spectrometer (FTIR; Shimadzu). The crystal size and morphology of the struvite were investigated by particle size analyzer (Malvern) and scanning electron microscopy (SEM, Zeiss EVO LS 10), respectively. The length and width of the struvite crystals were determined using Data Translation Image-Pro Plus image analysis software. BET analysis was performed by a Quantachrome Autosorb SI instrument to investigate the surface area of the products. Moreover, zeta potential of the samples was analyzed using a Malvern Zetasizer Nano ZS to detect the surface charge of the struvite. The thermal property of the struvite formed was determined using a Setaram LABSYS Evo thermogravimetric analyzer in a N₂ atmosphere between 30 °C and 500 °C with a heating rate of 10 °C/min.

**RESULTS AND DISCUSSION**

**XRD Analysis**

The structure analysis of the struvite crystals formed in the absence and presence of proline was performed and the XRD diffraction patterns are shown in Fig. 1. In pure media, the main diffraction peaks were distributed at 14.9°, 20.8°, 27.0°, and 33.2°, corresponding to crystal lattice planes (101), (111), (103), and (022) of struvite, indicative of high crystallinity of struvite. The relevant results were consistent with the literature reports [14,15]. The crystal obtained belonged to the orthorhombic Pmn2₁ space group. The diffraction peaks of the (101), (111), (103), and (022) crystal planes were observed among all the samples prepared under different conditions, which confirmed that the prepared crystals with and without proline were in the struvite form, indicating that no intermediate phase was occurred.
The addition of proline caused the intensity of the diffraction peaks to change. The a, b, and c unit cell parameters were 6.957 Å, 6.138 Å, and 11.220 Å for pure media, and these parameters were 6.970 Å, 6.147 Å, and 11.224 Å; 6.967 Å, 6.146 Å, and 11.225 Å for the struvite crystals formed in the presence of 25 and 100 ppm media, respectively.

**FTIR Analysis**

The struvite samples prepared were characterized by FTIR spectrometer to identify the functional groups on the surface of the samples. FTIR was also used to analyze the adsorption of the proline modifier on the surface of struvite. Fig. 2 illustrates the spectra of struvite obtained in the absence and presence of proline. The FTIR result of the crystal obtained in pure media showed the specific peaks of struvite at ~2900 cm⁻¹ (N-H stretching), ~2350 cm⁻¹ (O-H stretching), ~1430 cm⁻¹ (N-H stretching), and ~980 cm⁻¹ (PO₄³⁻ stretching) which was consistent with the literature [16-18]. Furthermore, the peaks at ~880 cm⁻¹ and ~760 cm⁻¹ were associated with the hydrogen bond in the absorption peaks of weak water-water and ammonium-water bonds, respectively.

**Zeta Potential Analysis**

To further reveal the adsorption characteristics of proline on struvite crystals, the surface charges of the crystals were measured, and the zeta potential analysis results are given in Fig. 3.
The surface charge of the struvite crystals formed in pure media was –12.70 mV. It can be seen in Fig. 3, the zeta potential value reached –4.00 mV at 100 ppm crystal modifier concentration, compared with the value of –6.10 mV at 25 ppm concentration. These results clearly illustrated that less negative surface charge value was obtained at higher proline concentration, which might result in more electrostatic repulsion between struvite surface and the positively charged proline species. Thus, crystal modifier concentration increase can strengthen the proline adsorption, and finally increase in the inhibitory effect.

**Morphology Analysis**

To clarify the size and morphological changes of the crystals obtained under different proline concentrations, the SEM analysis were performed. The SEM images taken at different magnifications and the particle size distributions are shown in Fig. 4 and 5, respectively.

![Figure 4](image-url)
The rod-like struvite crystals with a mean size of 18.0 ± 1.8 µm were synthesized in pure media. The struvite obtained in pure media had homogenous-looking and regular form. Meanwhile, they had the tendency of growing on each other and the surface of the rod-like crystals were porous. The BET surface area of struvite obtained in pure media was determined to be 241.93 m²/g. The length, width and aspect ratio of products are displayed in Fig. 6. The length and width of struvite crystals varied with different concentrations of proline. The rod-like struvite crystals had an aspect ratio of 0.363 ± 0.19 and a mean length of 40.10 ± 2.1 μm. According to SEM images, the crystal morphology of the struvite can be significantly changed with addition of the proline to crystallization media. Therefore, it is inferred that proline has ability to control the crystal morphology of struvite. The surface properties and morphology of struvite crystals were connected to the concentration of proline. By increasing the proline concentration to 25 ppm, the mean width reached to 20.0 ± 2.9 µm and the mean length dramatically decreased to 23.15 ± 2.4 µm. The particle size of the samples obtained in the solutions with proline were reduced due to the surface adsorption of proline. As seen in Fig. 5, the mean particle size of the struvite crystals obtained in media supplemented with 25 and 100 ppm proline were 14.6 ± 2.2 µm and 10.4 ± 1.4 µm, respectively. The morphology results for the crystals obtained with varying concentrations of modifier are consistent with the particle size results.

At 100 ppm proline concentration, the surface of the struvite crystals was smooth, non-porous and smaller crystals were precipitated. The BET surface area of the struvite crystals decreased 0.034 m²/g at 100 ppm, compared with the value of 9.30 m²/g at 25 ppm modifier concentration. For specific surface area aspect, a higher proline concentration value led to a lower BET surface area. Fig. 6 shows that mean width and mean length of struvite crystals obtained with proline were determined to be 12.67 ± 1.8 µm and 16.75 ± 1.9 µm, respectively. The quantitative variations in crystal morphology showed that with the increase of the proline concentration, the struvite crystals were shortened in length and enlarged in width, resulting in the increase of aspect ratio. The morphology results indicated that the presence of proline can effectively hindered the crystal nucleation and growth of struvite, and the magnitude of the effect depended on the proline concentration. Thus, it can be concluded that proline is an effective crystal modifier to control the crystal habit of struvite. The morphological transformation can be performed, short rod crystals with smaller particle size can be obtained in the presence of proline. This change could be explained by an adsorption mechanism. The adsorption of proline accounted for its interaction with struvite. Proline can bound on the active growth surface by chemical, physical forces and electrostatic interaction between proline and struvite; it could slow the growth rate on the crystal faces and thus lead to a modification of the habit of crystals grown in its presence of the crystal modifier.

**Thermal Analysis**

Thermogravimetric analysis was utilized for the evaluation of the thermal degradation behavior of struvite crystals obtained with and without crystal modifier. The TG and DTG curves are illustrated in Fig. 7. As observed in Fig. 7, the thermal degradation of struvite crystals obtained with and without proline included one main step, which corresponded to the ammonium degradation and the loss of water simultaneously in the temperature range of approximately 70–200 °C [19]. In accordance with the literature [20], the total weight loss of the struvite obtained without crystal modifier was ~51.0%. Unlike the crystals from the pure media, the increment of 1.8% was observed for the struvite obtained in media supplemented with 100 ppm proline. Moreover, the addition of
the crystal modifier was found to shift the degradation temperature higher. The increased weight loss and shifting behavior of the peak temperature indicated that the proline adsorbed onto and interacted with the surface of the struvite.

In order to determine the activation energy during the thermal degradation of struvite, the Horowitz–Metzger equation was used for a first-order (n=1) kinetic process as shown in Eq. (1) [21].

$$\ln \left( \frac{\ln \left( \frac{w_f}{w_i} \right)}{2.303RT_{\text{peak}}^2} \right) = \frac{E_a \theta}{2.303RT_{\text{peak}}^2} - \ln 2.303$$ (1)

Where $w_f = w_i - w$, $\theta = T - T_{\text{peak}}$, $E_a$ is the activation energy (kJ/mol), $T_{\text{peak}}$ is the peak temperature of DTG curve, $R$ is the ideal gas constant (8.314 J/mol K), $w_i$ is the final sample weight, and $w$ is the sample weight at time $t$. According to the Eq. (1), when plotting of $\ln \left( \frac{\ln \left( \frac{w_f}{w_i} \right)}{2.303RT_{\text{peak}}^2} \right)$ versus $\theta$, the value of $E_a/2.303RT_{\text{peak}}^2$ is obtained from the slope of the graph and the activation energy is calculated by using this relation. Moreover, the thermodynamic parameters such as the enthalpy ($\Delta H$), entropy ($\Delta S$), and Gibbs free energy ($\Delta G$) were also calculated.

The thermodynamic parameters such as enthalpy change ($\Delta H$), Gibbs free energy change ($\Delta G$), and entropy change ($\Delta S$), were calculated using Eyring equations [22].

$$\Delta H = E_a - RT$$ (2)

$$\Delta G = E_a + RT_{\text{peak}} \ln \left( \frac{K_B T_{\text{peak}}}{hA} \right)$$ (3)

$$\Delta S = \frac{\Delta H - T \Delta G}{T_{\text{peak}}}$$ (4)

Where $K_B$ is the Boltzmann constant, and $h$ is the Planck constant.

The activation energy was 95.6 kJ/mol for the struvite crystals obtained without crystal modifier. The values of $\Delta H$, $\Delta G$ and $\Delta S$ were determined to be 92.17 kJ/mol, 197.85 kJ/mol, and -254.63 J/mol.K, respectively. The positive value of $\Delta H$ calculated for the crystals attributed to the endothermic nature of thermal degradation process. The negative $\Delta S$ entropy value revealed the decrease in randomness during the degradation process. The positive $\Delta G$ showed that the degradation process of the struvite was nonspontaneous.

CONCLUSION

In this study, the proline used as the crystal modifier with different concentration was studied to modify the crystal size and morphology of the struvite. XRD results showed that the crystals obtained were in orthorhombic structure. SEM images showed that proline had a significant modification effect on struvite morphology. With the increase of the proline concentration, the struvite crystals were shortened in length and enlarged in width, resulting in the increase of aspect ratio. In the presence of 100 ppm proline, the aspect ratio of struvite was 0.756 ± 0.15 μm with the mean length of 16.75 ± 1.9 μm. Zeta potential measurements showed that proline played a significant role in controlling the crystal surface charge during the crystal growth process. The value of zeta potential decreased along with the increase of crystal modifier concentration. The surface of the crystals became less negative in the presence of proline. From the characterization results, it is possible to conclude that proline has the ability to change the crystal size and morphology and it can be used a potential crystal modifier for the inhibition of struvite stones. In addition to characterization analysis, thermal degradation of the struvite crystals was investigated in this study. According to the kinetic analysis performed by Horowitz–Metzger model, the calculated activation energy value was 95.6 kJ/mol.
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