INHIBITION OF STRUVITE CRYSTAL GROWTH IN THE PRESENCE OF HERBAL EXTRACT ORTHOSIPHON ARISTATUS BL.MIQ

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ABSTRACT

The formation of urinary stones is a serious clinical problem worldwide. Struvite (MgNH4PO4•6H2O) is known as one of the main components of the stones. Traditionally, herbal extracts treatment is recommended. This paper discusses the effects of Orthosiphon aristatus Bl.Miq leaves extract (OA) on struvite crystals grown by diffusion gel method. After three weeks the crystals were separated from the gel, air dried and stored for characterisation. The crystals exhibit different morphologies depending upon the location of growth. It was found that OA caused the crystals to have noticeable surface defects, discolorisation and fragmentation. Also, OA (up to 5.0%) decreased the growth rate. XRD analysis confirms struvite crystallinity. Pure crystals and those grown with OA showed identical XRD pattern, indicating that the crystalline nature may be unaffected. For crystals with OA, FTIR spectrum shifted to higher wave numbers, implying that the various organic compounds in OA might be absorbed on the crystal surface. TG-DTA studies showed similar thermal behaviour for both pure crystals and those with OA. However, the later revealed lower endothermic temperature, indicating lower thermal stability. Fragmentation and lower thermal stability of struvite indicated that OA was a potent inhibitor of struvite and could be effective for urinary stone treatment.

Keywords: crystal growth, Orthosiphon aristatus, SEM, struvite, XRD

INTRODUCTION

The formation of urinary calculi is a serious clinical problem worldwide (Suguna et al., 2012). Phosphate minerals, such as magnesium ammonium phosphate hexahydrate (MgNH4PO4•6H2O), mineralogically known as struvite, is one of the main components of the calculi. It was also reported that recurrence after treatment is relatively high (ElMahdy and Persad, 2014; Suguna et al., 2012; Chauhan, 2011; Miyaoka and Monga, 2009; Grohe et al., 2006).

The urinary calculi (= urinary stones, renal stones or kidney stones) may arise due to chemical activities within the body (Suguna et al., 2012; Chauhan, 2011). The condition favourable for the formation of the stones includes high concentrations of calcium salts (Ca-oxalate and Ca-phosphate), infections of urinary tracts, pH, and a decrease in the body's natural ability to inhibit the formation of the crystals (Suguna et al., 2012; Rajendran and Keefe, 2010).

Traditionally, urolithiasis or kidney stone disease can be prevented or treated with herbs (herbal medicines or herbal extracts). These herbs include Origanum vulgare, Moringa oleifera, Asparagus racemosus, Rotula aquatica, Mimosa pudica, Trigonella foenum graecum, Nigella sativa, and Punica granatum (Khan et al., 2011; Butterweck and Khan, 2009). These studies indicate that herbal extracts could inhibit the growth of the crystals forming the kidney stones. As opposed to treatment using antibiotics, these herbs were claimed to cause no bacterial resistance and other adverse effects (Miyaoka and Monga, 2009). As such, the herbal medicines have gained significant popularity.

A number of research has also been done to explore the effects of the leave extract of Orthosiphon aristatus (OA) – commonly known as cat whiskers in Malay, or Java tea, on urolithiasis (Yam et al., 2013; Abdelwahab et al., 2011; Adam et al., 2009; Yam et al., 2007). These studies focused on the medicinal properties of OA.

However, only a few studies have been conducted on the effects of herbs on the crystal growth of struvite as one of the main components of renal calculi. These herbs include Boerhaavia diffusa Linn, Commiphora wightii, Rotula aquatica Lour, and Citrus medica Linn (Chauhan, 2011). As far as the present authors know, the effects of OA leaves extract on the crystal growth and properties of struvite has not been examined. Reported in this paper are the results of such studies discussed in terms of morphology, crystallinity, elemental composition, thermal and mechanical properties.

METHODOLOGY

The crystallisation experiment was done using a single diffusion gel method, which is a standard method as a substitute for an in vivo model. This method enables a continuous observation of the crystal growth (Chauhan and Joshi, 2013; Chauhan, 2011; Rajendran and Keefe, 2010).

Preparation of the gel

The gel selected for the growth medium was silica gel, since it has a number of favourable characteristics: it requires no other chemicals, irreversible, elastic, has a stable form, and does not react with the growing crystals (Chauhan, 2011).
To grow the struvite crystals within the gel, two solutions containing the crystal-forming components were mixed. The two solutions would diffuse through the gel, so that ions and molecules of the crystal-forming components would react slowly and in a controlled mode. This contact would yield an insoluble phase in the form of crystals. The crystallisation process can be easily conducted using ordinary lab test tubes.

The silica gel (Na₂SiO₃) was diluted with distilled water as necessary and would react as follows (Chauhan, 2011):

\[ \text{Na}_2\text{SiO}_3 + 3 \text{H}_2\text{O} \rightarrow \text{H}_4\text{SiO}_4 + 2 \text{NaOH} \]

Further, H₄SiO₄ would polymerise and release the water to form covalent bonds. The bonds repeat continuously to form a three-dimensional structure, which can be a perfect medium for crystal growth (Suguna et al., 2012).

**Preparation of the leaves extract of the Orthosiphon aristatus BL.Miq (OA)**

The leaves were purchased locally, washed using flowing tap water and rinsed with distilled water. Then, the cleaned leaves were sun-dried, crushed into powder using a household blender, and stored in a tight container. The powder was subsequently mixed with distilled water and boiled (50 g powder/500 mL water) in a beaker until the volume reduced to half the initial volume. The liquid obtained was then vacuum-evaporated using a modified Soxhlet extractor until the liquid turned into a paste. The paste, which is the OA extract, was stored in a refrigerated place and ready for the experiment.

**Crystallisation of struvite**

1.0 M NH₄H₂PO₄ solution (reactant 1) was mixed with Na₂SiO₃ solution in a test tube until a gel was formed. Then, 1.0 M MgCl₂ solution (reactant 2) was poured on top of the gel. Next, the test tube was sealed with a silver film and placed in a closed cupboard to prevent contamination. The reaction occurred in the gel as follows:

\[ \text{NH}_4\text{H}_2\text{PO}_4.2\text{H}_2\text{O} + \text{Mg(CH}_3\text{COO)}_2.4\text{H}_2\text{O} \rightarrow \text{MgNH}_4\text{PO}_4.6\text{H}_2\text{O} + 2 \text{CH}_3\text{COOH} \]

In the case of OA trials, the magnesium acetate solution was mixed with the extract (0.1% to 5.0%) prior to the experiment. Crystals started appearing at the interface between the gel and the supernatant one day after the initiation of the run. After three weeks the crystals were separated from the gel, air dried and stored for subsequent analysis. The crystals were washed cautiously with distilled water and subsequently dried in an oven (40°C) for 24 hours. The dry crystals were then stored for subsequent analysis.

**Characterisation of the struvite crystals**

Characterisation of the crystals included morphology, crystallinity, elemental composition and thermal properties. Thus, it required scanning electron microscope (SEM-EDX, XRD), Fourier transform infra red (FTIR), and TG-DTA.

**RESULTS AND DISCUSSION**

**Crystal Growth**

Single gel diffusion method was used to grow struvite crystals because the method imitates the gel-like medium in human body wherein the urinary stones develop (Suguna et al., 2012).

![Figure-1](image1). Dendritic crystals appeared near the gel-liquid interface: pure (left); with OA (right)

In the current work, struvite crystals were grown in the absence and in the presence of OA at room temperature. Tiny crystals, which were predominantly dendritic, appear at the gel-liquid interface one day after the experimental run (Fig 1). Other forms, e.g. needle-like and plate-like can also be seen. It is also seen that there is a change in morphology of the crystals along the test tubes from top to bottom. It can be postulated that the change was due to the availability of the struvite-forming components as well as their diffusivity in the gel (Suguna et al., 2012).

OA was found to affect the morphology and size of crystals. It can be argued that the organic components of OA could have been affecting the growth rate of the crystals by adsorption on the crystal surface and blocking the active growth sites resulting in the inhibition of the growth. This current work indicates that OA may affect the nucleation and growth of struvite resulting in poor yield.
Morphological Analysis

Figure 2 compares the morphological characteristics of the struvite crystals obtained. In the absence of OA the surface of the crystal appears smooth (left). On the other hand, in the presence of OA (5.0%), the surface of the crystals are rougher and fragmented.

This findings is similar to that of Suguna et al. (2012) on the crystallisation of struvite doped with fluoride. It is postulated that the change in surface characteristics of the crystals with OA could be caused by the adsorption of OA on the crystal surface.

X-Ray Diffraction Analysis

X-ray diffraction (XRD) analysis is believed to be a reliable method for analysis of crystalline materials, including the crystalline phases of struvite. X-ray identification was performed using Cu-Kα monochromated radiation in a conventional Bragg-Brentano (BB) parafocusing geometry. Results of the XRD analysis (the peak positions and peak heights of the spectrum) were checked against the data for the spectrum of struvite standard (JCPDS – #015762).

Figure 3 depicts the XRD analysis for the crystals. The diffraction peak positions and intensities of the crystals obtained mostly match up with the standard data. Overall, it can be stated that the crystals produced were struvite.

FTIR Analysis

The FTIR analysis was carried out to examine the chemical bondings which subsequently provide the information of the molecular structure of the compounds. Figure 4 depicts the recorded spectrum obtained for the struvite crystals.

The spectrum clearly shows the four absorption regions as expected for the struvite: metal-oxygen bonds, NH₄⁺ units, PO₄⁻³ units, and water of crystallization.
A broad asymmetric band between 2100 and 3600 cm\(^{-1}\) can be attributed to O-H and N-H stretching vibrations. A medium broad band at 2889.25 cm\(^{-1}\) indicates the \(\nu_1\) symmetric stretching vibration of N-H in NH\(_4^+\). Bending modes of water of crystallisation (H-O-H) is seen at absorption of around 1700 cm\(^{-1}\). There is a strong vibration taking place at 1432.45 cm\(^{-1}\) which suggests \(\nu_4\) asymmetric bending vibration of N-H in NH\(_4^+\). Another strong vibration at 1164.26 cm\(^{-1}\) indicates \(\nu_4\) asymmetric bending vibration of N-H. A very sharp absorption peak at 991.98 cm\(^{-1}\) indicates the NH\(_4^+\) rocking. The metal-oxygen bond is shown as the weak absorption band at 683.97 cm\(^{-1}\). The weak bands between 500 and 600 cm\(^{-1}\) can be attributed to PO\(_4^{3-}\) units. Overall, the FTIR spectrum recorded confirms the presence of the four compound species of struvite: metal-oxygen bond (due to Mg), NH\(_4^+\), PO\(_4^-=\) and water of crystallisation. As can be seen in Figure 4, for the experiments with OA (bottom) all absorption peaks shifted to higher wave numbers which indicate the influence of OA.

**TG-DTA Analysis**

For the crystals without OA, there is a major weight loss of about 75\% between 80\(^{\circ}\)C and 150\(^{\circ}\)C. This loss denotes the elimination of six water molecules and one ammonia molecule (Suguna et al., 2012). In addition, this coincides well with the DTA curve which shows a strong endothermic peak at 140\(^{\circ}\)C. In the higher temperature region between 150 and 710\(^{\circ}\)C, two molecules of MgHPO\(_4\) combine to form magnesium pyrophosphate (Mg₂P₂O₇) with the loss of one water molecule, and resulting in nearly 53\% of the sample remains stable. The following reactions were postulated to occur during the heating of the crystals (Suguna et al., 2012).

\[
\begin{align*}
\text{MgNH}_4\text{PO}_4\cdot6\text{H}_2\text{O} & \rightarrow \text{MgH}_4\text{PO}_4 + \text{NH}_3 + 6 \text{ H}_2\text{O} \\
\text{MgH}_4\text{PO}_4 & \rightarrow \text{Mg}_2\text{P}_2\text{O}_7 + \text{H}_2\text{O}
\end{align*}
\]

The thermal behaviour of struvite with OA is similar in terms of weight loss (Fig 5 – bottom). However, there is a reduction in endothermal peak, which was recorded at as low as 110\(^{\circ}\)C. This reduction indicates the lowering of the thermal stability of the crystals formed under the influence of OA. Thermal properties of the crystals obtained were examined at temperature range between 30\(^{\circ}\) and 1000\(^{\circ}\)C at the rate of 20\(^{\circ}\)C per minute under nitrogen atmosphere (Fig. 5). The exothermic peak of the struvite without OA is slightly higher than 700\(^{\circ}\)C, whereas for the crystals with OA is slightly lower than 700\(^{\circ}\)C. This condition further confirms the reduced thermal stability of the crystals with OA.

**CONCLUSIONS**

Struvite (MgNH\(_4\)PO\(_4\).6H\(_2\)O) is known as one of the main components of the urinary stones. Traditionally, herbal extracts are recommended for the urinary treatment. This paper presents an investigation on the effects of *Orthosiphon aristatus* Bl.Miq leaves extract (OA), commonly known as Java tea, on struvite crystals grown by a single diffusion gel method.

Under SEM the crystals exhibit different morphologies: dendritic, prismatic, platelet and needle-like, depending upon the location of growth. It was also found that for the crystals with OA, the crystal surface was rough which could indicate the adsorption of OA on the surface.
The XRD of the crystals confirms the crystalline phase of struvite. Both pure crystals and those grown with OA showed identical XRD pattern, indicating that the crystalline nature seems to be unaffected by OA.

The FTIR spectrum proves four absorption peaks: water of crystallisation, NH\textsuperscript{+4}, PO\textsubscript{4}\textsuperscript{3-}, and Mg-oxygen bonds, which agrees with previous studies. For the crystals with OA the spectrum indicates shifting to higher wave numbers, implying that components of OA might be absorbed on the crystal surface.

TG-DTA studies (30\textdegree C to 1000\textdegree C; 20\textdegree C/min; nitrogen atmosphere) showed similar thermal behaviour for both pure crystals and those with OA. However, the later revealed a decrease in endothermic peak temperature, indicating lower thermal stability.

Vickers microhardness test showed that crystals with OA showed lower hardness.

Properties of the struvite crystals grown with OA seemed to be affected by the various organic compounds contained in OA. Fragmentation, lower thermal stability and reduced hardness of the crystals proved that OA was a potent inhibitor of struvite and could be effective for urinary stone treatment.

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REFERENCES


