CHEMICAL ENGINEERING TRANSACTIONS
VOL. 56, 2017
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Jiří Jaromír Klemeš, Peng Yen Liew, Wai Shin Ho, Jeng Shiun Lim

ISBN 978-88-95608-47-1; **ISSN** 2283-9216
DOI: 10.3303/CET1756273

Effect of Blumea Balsamifera Extract in the Kinetics of Calcium Oxalate Crystallisation

Charlimagne M. Montealegre*, Allieson C. Ilao, Raphael Victor T. Mendoza, Ray Paulo M. Carpio, Rizalinda L. de Leon

Department of Chemical Engineering, University of the Philippines Diliman, Quezon City Philippines 1101
cmmontealegre@up.edu.ph

Calcium oxalate stones comprise a large number of kidney stone cases. Kidney stone treatment with medicinal herbs such as Blumea balsamifera (B. balsamifera) had been practiced traditionally. In this study, the effect of B. balsamifera extract on the crystallisation of calcium oxalate was determined by modelling Ca^{2+} concentration in solutions. Crystallisation was performed by mixing equal concentrations of Ca^{2+} and $C_2O_4^{2-}$ at 200 rpm and supersaturations of 20, 25, 30, 35 and 40. Ca^{2+} concentration was measured using potentiometry. A second order kinetic model best fitted the concentration-time data with an average correlation coefficient of 0.9759 ± 0.0155. The rate constant is increased by the presence of the extract with a maximum increase of 6,460 % from 0.0152 ± 0.0079 ppm^{-1} s^{-1} to 0.9993 ± 0.5524 ppm^{-1} s^{-1} at a supersaturation of 30 and 0.5 mg/mL of extract. The minimum increase in rate constant was observed at supersaturation of 25 and 1.0 mg/mL of extract from 0.0088 ± 0.0058 ppm^{-1} s^{-1} to 0.0120 ± 0.0020 ppm^{-1} s^{-1} corresponding to a 36.71 % change. The extract increased the rate of crystallisation, consistent with the findings from other studies using different approaches. An increased crystallisation rate would favour the formation of smaller crystals that are easily eliminated from the urinary system.

1. Introduction

Calcium oxalate is an inorganic salt found in almost all types of kidney stones (Kirejczyk et al., 2014) and in 68 % of documented cases, identified calcium oxalate as the main component of the stone (Hughes, 2007). Amongst the treatments for kidney stones are extracorporeal shock-wave lithotripsy, ureteroscopic extraction, and percutaneous nephrolithomy (Bushinsky, 2016). Though some of these are non-invasive, treatment cost is high. Nowadays, the herbal remedies for renal stones are gaining popularity. Several studies explored the effect of herbal extracts on the crystallisation process of calcium oxalate. The average size particles of precipitates significantly decreased due to the addition of extracts from Desmodium styracifolium, Orthosiphon stamineus and Cystone during in vitro crystallisation (Rodgers et al., 2014). Extracts of Achyranthes indica Linn (Pareta et al., 2011) and Bergenia ciliata (Saha and Verma, 2013) showed inhibition of nucleation and aggregation of calcium oxalate monohydrate (COM) crystals and favoured the formation of COD crystals.
In the Philippines, Blumea balsamifera (B. balsamifera), locally known as Sambong, is promoted by the Philippine Department of Health as a diuretic and medicine for dissolution of kidney stones (Oyson, 2013). There are researches confirming its effectiveness as a diuretic and in the dissolution. One of these claimed that B. balsamifera extract has maximum chemolytic effects at 40 mg/d dose (Rico, 1992). However, the effect of B. balsamifera in the kinetics of kidney stones formation is unknown.
The rate of a reaction limited system is determined by the reaction between Ca^{2+} and $C_2O_4^{2-}$. For equimolar concentrations of calcium and oxalate ion, the rate law is written as Eq(1), here the order is n = 2 for an elementary reaction and n ≠ 2 for non-elementary reactions.

$$-\frac{dCa}{dt} = k[Ca^{2+}][C_2O_4^{2-}] = k\,[Ca^{2+}]^n \quad (1)$$

Please cite this article as: Montealegre C.M., Ilao A.C., Mendoza R.V.T., Carpio R.P.M., Leon R.L.D., 2017, Effect of blumea balsamifera extract in the kinetics of calcium oxalate crystallization, Chemical Engineering Transactions, 56, 1633-1638 DOI:10.3303/CET1756273

The crystallisation of calcium oxalate was well studied in the literature. Among the earliest proponents of kinetic studies were Nancollas and Gardner (1974) who established the second order dependence of the kinetic equation on concentration. Various studies confirmed the early observations by Nancollas and Gardner in systems with and without inhibitors (Grases et al., 1989) and in surface controlled systems (Öner et al., 2015). Even recent studies confirmed this but with limitations. Kassemi et al. (2011) used a combined transport-kinetics model to study the crystallisation of renal calculi. They reported that surface reaction kinetics is limiting at low supersaturation and low Damköhler number but for higher supersaturations and large Damköhler number, and as the crystal size increases, transport limitations are more likely.

The effect of different compounds on the kinetics of crystallisation was also studied. Ions of magnesium, zinc, copper, and manganese as well as copper complexes of citrate and EDTA reported inhibition of crystallisation (Grases et al., 1989). Biomolecules such as transferrin, bovine serum albumin, chondroitin sulfate (Farmanesh et al., 2014), phosphate-containing biological compounds (Grases et al., 1990), and various plant extracts (Rodgers et al., 2014) also reported inhibition on calcium oxalate crystallisation kinetics.

This study aims to model the crystallisation kinetics of calcium oxalate in equimolar Ca^{2+} and $C_2O_4^{2-}$ solutions based on the Ca^{2+} concentration profile and to determine the effect of B. balsamifera extract on the kinetics of crystallisation.

2. Methodology

2.1 Design of Experiment

To investigate the crystallisation kinetics of and to determine the effect of B. balsamifera extract on the crystallisation, two factors were varied: initial supersaturation and extract concentration in the solution. The initial supersaturation was varied from 20, 25, 30, 35, and 40. These initial supersaturations were based on the solubility product of calcium oxalate monohydrate at 25 °C and calculated from Eq(2):

$$S = \sqrt{\frac{[Ca^{2+}]_i [C_2O_4{}^{2-}]_i}{K_{sp,25\,°C}}} \tag{2}$$

The concentrations of calcium and oxalate ions were maintained as 1 : 1 ratio to avoid immediate formation of calcium oxalate dihydrate crystals. The solubility product used in a study (Jung et al., 2005), $K_{sp} = 2 \times 10^{-9}$ M^2, was adapted. The extraction concentration was varied from 0 mg/mL, 0.5 mg/mL, and 1.0 mg/mL. These concentrations refer to the mass of dried leaves over the final volume of the calcium oxalate solution.

2.2 Extract Preparation

B. balsamifera leaves were dried at 40 °C for 6 d. The dried leaves were crushed using mortar and pestle, and load in a Soxhlet extractor with 99.5 vol% ethanol. The extraction was run for 12 h. After the extraction, ethanol was evaporated and deionised water was added. The stock solution was then filtered using Watman filter paper to remove unnecessary solids.

2.3 Potentiometry

Eutech 700 (pH/mV/°C/°F Bench meter) and Cole-Parmer™ Calcium combination electrode were used for obtaining potential reading. The voltage reading was transmuted to concentration by a calibration curve constructed by plotting the potential readings of Ca^{2+} solutions having different concentrations. The relationship between Ca^{2+} concentration and mV reading is expressed in Eq(3) (Grases et al., 1989):

$$mV = a \ln(Ca^{2+}) + b \tag{3}$$

Required amounts of $Na_2C_2O_4$ and $CaCl_2$ solution were prepared to have a 200 mL calcium oxalate solution. Then, the $CaCl_2$ solution and 1 mL of extract were put in a 250 mL beaker. The solution was constantly mixed at 200 rpm. After the millivolt reading was constant, the $Na_2C_2O_4$ solution was added. The millivolt readings were recorded in 10-second intervals for 30 min. The equation obtained from the calibration was used to convert the millivolt readings into calcium concentrations.

3. Results and Discussion

The potential readings from the Ca^{2+} selective electrode is converted into Ca^{2+} concentration. These are then used to determine the kinetic model which best describes the concentration profile of calcium oxalate crystallisation. A typical Ca^{2+} concentration profile is shown in Figure 1.

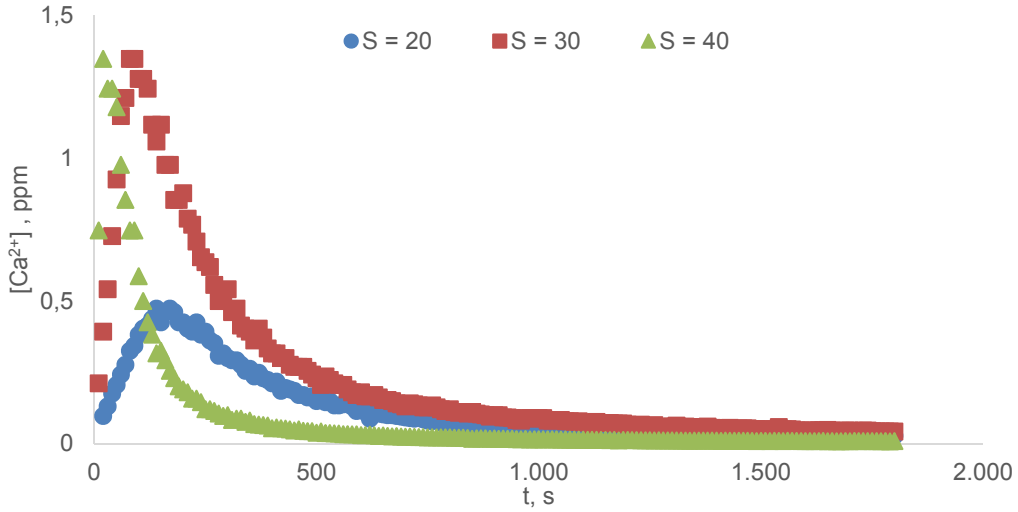

Figure 1: Calcium ion concentration profile for an initial supersaturation of 20, 30 and 40

The start of the concentration profile shows an increasing calcium concentration. This is counter intuitive since for a reacting system, the concentration should decrease. This increase in concentration reflects the time delay of the calcium electrode used. The time delay in measurement is a limitation of the potentiometric method. This is minimised by adjusting the start of curve fitting at t = 30 s.
The fit of the n^{th} order kinetic model was assessed at varying reaction orders from 1 to 2.1. The correlation coefficients are summarised in Figure 2 which showed a satisfactory fit from 1.8 until 2.1. This suggests a second order reaction i.e. an elementary reaction between calcium and oxalate ions. This is consistent with various literature data on calcium oxalate crystallisation where the reported reaction order is 2 (Öner et al., 2015).

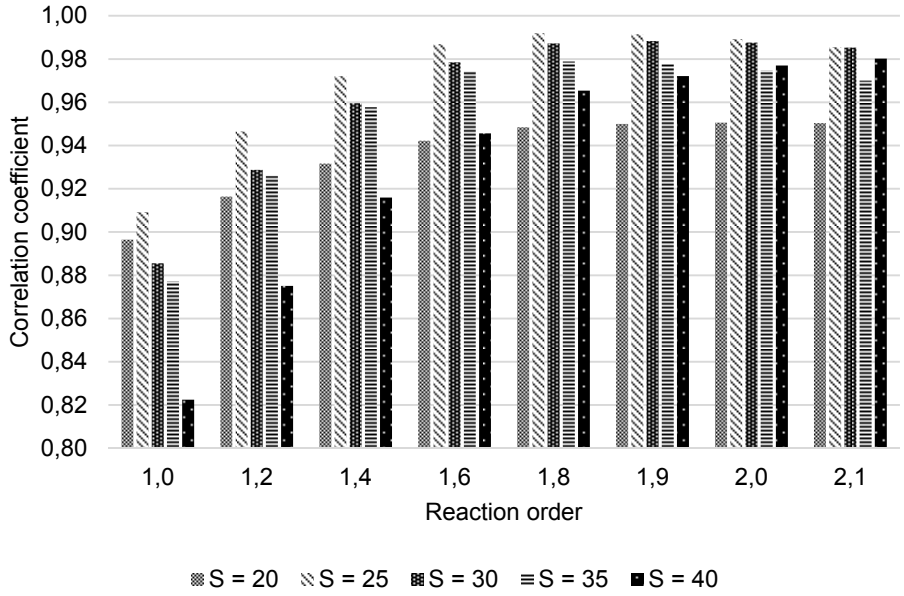

Figure 2: The correlation coefficient increased as the reaction order approaches 2 suggesting a second order reaction mechanism that is consistent with previous literature findings

The second order reaction fitted the data satisfactorily with an average correlation coefficient of 0.9759 ± 0.0155 across all degrees of supersaturation. The rate constants were calculated from the fit of the model and are summarised in Figure 3. The rate constant shows a strong dependence on supersaturation. Theoretically, reaction rate constants are only functions of temperature. However, in writing rate laws in terms of

concentration, the rate constant is grouped with activity coefficients. In changing the supersaturation from 20 to 40, the ionic strength of the solution also changes and this will affect the activity of the ions.

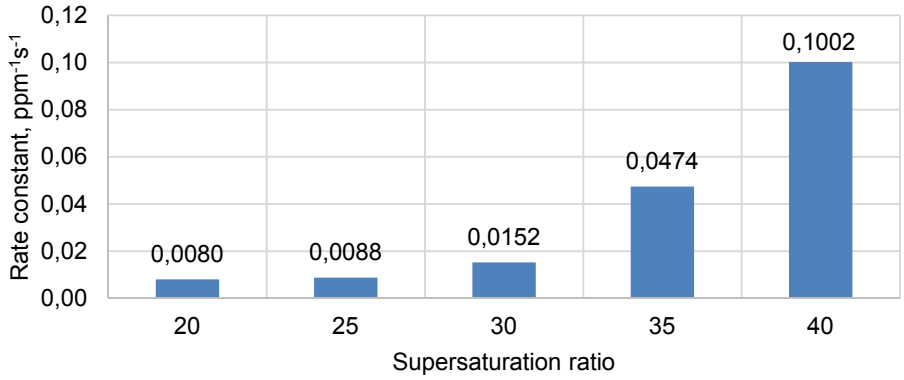

Figure 3: The second order reaction rate constant increase with supersaturation which shows the dependence of the rate constant to the activity coefficient of the reactants

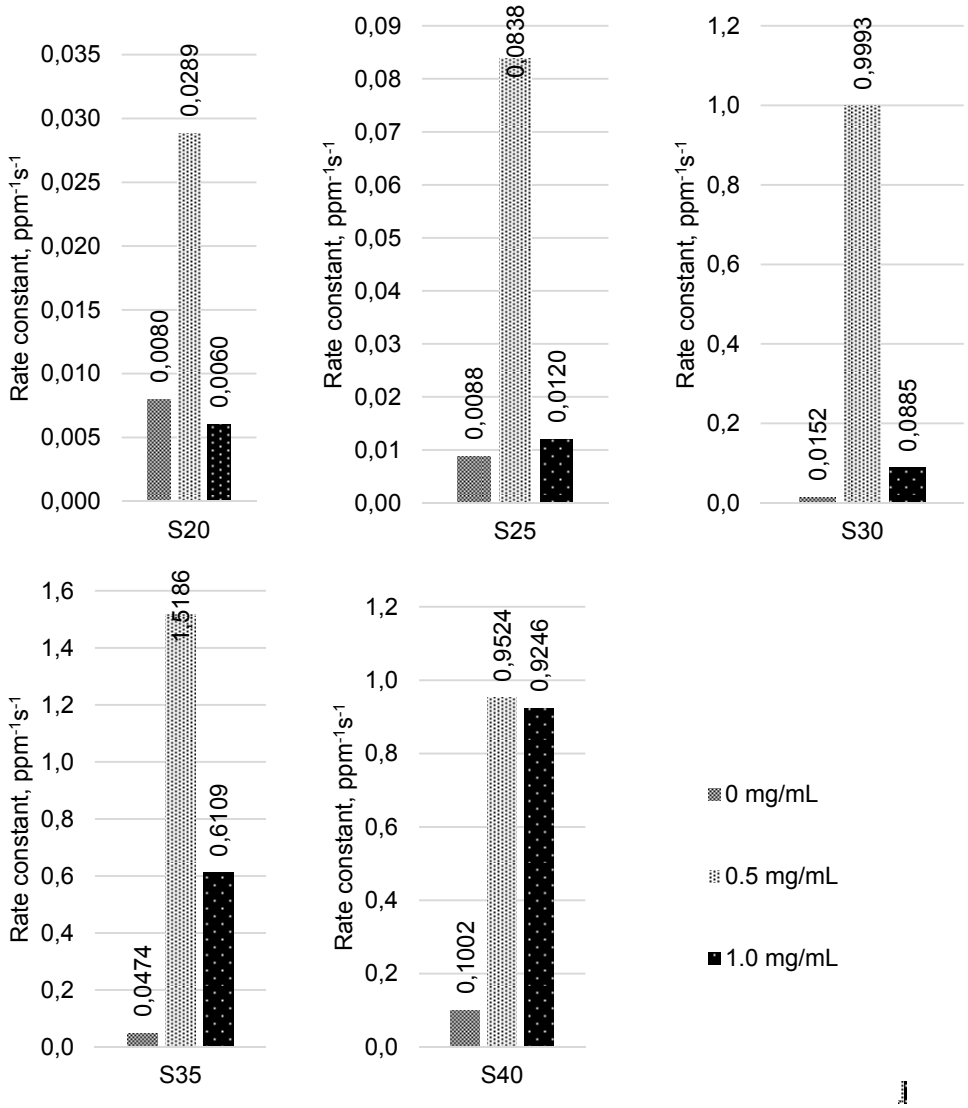

Figure 4: The effect of the extract on the rate constant had different extents depending on supersaturation.

The effect of the presence of the extract was studied using a similar approach. The fit of the second order reaction was satisfactory with a minimum value of 0.9256 ± 0.0093, a maximum of 0.9935 ± 0.5524 and an average of 0.9731 ± 0.0193. The rate constants are summarised in Figure 4. The rate constant generally increased especially at 0.5 mg/mL of extract.

In the presence of the extract, the rate constants generally increased except at a supersaturation of 20 and 1.0 mg/mL of extract. At 0.5 mg/mL of extract, the rate constant increased remarkably with a minimum percentage increase of 260 % at S = 20 and a maximum increase of 6,460 % at S = 30. The changes are smaller at 1.0 mg/mL of extract, with a minimum increase of 36.7 % at S = 25 and a maximum of 1,188 % at S = 35.

The kinetic model resulted into a reaction order of 2 that is elementary and consistent with the literature data from studies using chemical activity (Nancollas and Gardner, 1974), systems with other inhibitors (Grases et al., 1989), and in surface controlled systems (Öner et al., 2015). The rate constants increased with increasing supersaturation. This is possibly an effect of the increased activity coefficient due to increased ion concentration. The model showed that in the presence of B. balsamifera extract, the rate constant of the reaction increased and crystallisation is promoted. Metal ions, citrate and EDTA complex (Grases et al., 1989), phosphate-containing compounds (Grases et al. 1990), aspartic acid (Grases et al., 1988), chondroitin sulfate, serum albumin, and transferrin (Farmanesh et al., 2014), and other plant extracts (Rodgers et al., 2014) reported an inhibition of crystal nucleation and growth, contrary to the results of this study. However, the observed increase in the crystallisation rate constant is consistent with a thermodynamic study on the effect of B. balsamifera extract on nucleation of crystals (Montealegre and De Leon, 2016) which also reported an increase in nucleation rate, and a decrease in the size of the crystals (Montealegre and De Leon, 2016).

4. Conclusions

The crystallisation of calcium oxalate from equal concentrations of Ca^{2+} and $C_2O_4^{2-}$ at degrees of supersaturations ranging from 20 to 40 was described by a second order kinetic model. The rate constant showed a dependence on the degree of supersaturation. B. balsamifera extract increased the rate constant by up to 6,460 %. This kinetic study supports the conclusion of a thermodynamic study which reported that the extract increased the crystallisation of calcium oxalate by an increased nucleation rate. Increasing the crystallisation rate is beneficial to kidney stone forming individuals since a large amount of small crystals that is easily eliminated is favoured instead of formation of large and pathological crystals.

Acknowledgments

The authors would like to thank Engineering Research and Development for Technology (ERDT) for the financial support of this study.

Reference

Bushinsky D.A., 2016, Nephrolithiasis. Eds. Shelmo M., Polonsky K.S., Larsen P.R., Kronenberg H.M., Williams Textbook of Endocrinology, 13th Ed., Philadelphia, Elsevier, Armstedam, The Netherlands, 811–816.

Farmanesh S., Ramamoorthy S., Chung J., Asplin J.R., Karande P., Rimer J.D., 2014, Specificity of growth inhibitors and their cooperative effects in calcium oxalate monohydrate crystallization, Journal of the American Chemical Society 136 (1), 367–76.

Grases F., Genestar C., Millán A., 1989, The influence of some metallic ions and their complexes on the kinetics of crystal growth of calcium oxalate, Journal of Crystal Growth 94 (2), 507–512.

Grases F., Genestar C., Palou J., 1990, Study of the effects of several related phosphorus derivatives of biological interest in calcium oxalate crystal growth, Colloids and Surfaces 44, 29–34.

Grases F., March J.G., Costa-Bauza A., 1989, The crystallization of calcium oxalate at different pH values and in the presence of various adenosine phosphates, Journal of Colloid and Interface Science 128 (2), 382–387.

Grases F., March J.G., Bibiloni F., Amat E., 1988, The crystallization of calcium oxalate in the presence of aminoacids, Journal of Crystal Growth 87 (2), 299–304.

Hughes P., 2007, The CARI guidelines, Kidney stones epidemiology, Nephrology (Carlton) 12 Suppl 1, S26-30.

Jung T., Kim W.S., Kyun Choi C., 2005, Crystal structure and morphology control of calcium oxalate using biopolymeric additives in crystallization, Journal of Crystal Growth 279 (1–2), 154–162.

Kassemi M., Brock R., Nemeth N., 2011, A combined transport-kinetics model for the growth of renal calculi. Journal of Crystal Growth 332 (1), 48–57.

Kirejczyk J.K., Porowski T., Filonowicz R., Kazberuk A., Stefanowicz M., Wasilewska A., Debek W., 2014, An association between kidney stone composition and urinary metabolic disturbances in children, Journal of Pediatric Urology 10 (1), 130–135.

Montealegre C.M., De Leon R.L., 2016a, Blumea Balsamifera (Sambong) Tea as a Therapeutic Drink for Calcium Oxalate Stones, MATEC Web of Conferences, 62, 2002, DOI: 10.1051/matecconf/20166202002.

Montealegre C.M., De Leon, R.L., 2016b, Effect of Blumea balsamifera extract on the phase and morphology of calcium oxalate crystals, Asian Journal of Urology, DOI: 10.1016/j.ajur.2016.08.009.

Nancollas G.H., Gardner G.L., 1974, Kinetics of crystal growth of calcium oxalate monohydrate, Journal of Crystal Growth 21 (2), 267–276.

Öner M., Khan A., Khan S.R., 2015, Importance of Calcium-Based Scales in Kidney Stone, In Mineral Scales and Deposits, 1st Ed., Elsevier, Armsterdam, The Netherlands.

Oyson, K., 2013 August, Health benefits of sambong tea, The Freeman, Cebu City <www.pressreader.com/philippines/the-freeman/20130829> accessed 10 October 2016.

Pareta S., Chandra Patra K., Harwansh R., 2011, In-Vitro Calcium Oxalate Crystallization Inhibition by Achyranthes Indica Linn . Hydroalcoholic Extract : An Approach to Antilithiasis, International Journal of Pharma and Bio Sciences 2 (1), 432–437.

Rico F., 1992, Sambong (*Blumea Balsamifera*): Its Effect on Calcium Stone, Philippine Journal of Urology 2 (1), 9–13.

Rodgers A.L., Webber D., Ramsout R., Gohel M.D.I., 2014, Herbal preparations affect the kinetic factors of calcium oxalate crystallization in synthetic urine: implications for kidney stone therapy, Urolithiasis 42 (3), 221–225.

Saha S., Verma R.J., 2013, Inhibition of calcium oxalate crystallisation in vitro by an extract of Bergenia ciliata, Arab Journal of Urology 11 (2), 187–192.