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A kinetic study for calcium oxalate crystallization in the presence of *Viburnum opulus* extract

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ABSTRACT

In this study, the effects of *Viburnum opulus* (VP) extract on crystal kinetics and morphology of calcium oxalate were investigated in vitro in an attempt to elucidate the mechanisms of formation of calcium oxalate calculi and further help in seeking inhibitors for preventing the crystallization. The results showed that *Viburnum opulus* extract affected the crystal growth of calcium oxalate. It was found that VP tested in this study was effective on crystallization rate and behaved as an inhibitor for calcium oxalate crystallization. The presence of VP inhibited the crystal growth of calcium oxalate possibly through adsorption onto the active growth sites for crystal growth.

Keywords: *Crystal growth inhibition, calcium oxalate crystallization, Viburnum opulus extract (VP).*

1. INTRODUCTION

The crystallization of calcium oxalate has been the subject of an increasing number of investigations since it is the most common component of pathological deposits in the urinary tract. The majority of kidney stones (80%) contain calcium oxalate (CaOx) as their primary mineral phase. The urinary stones are comprised of the crystals not resolved or disposed in the urine. An increase in urinary supersaturation, the crystallization of CaOx starts and solid crystalline particles can be observed. Then, nucleation occurs and stone-forming salts in supersaturated urinary solution join, so the size of the particles increases [1-6]. Nucleation and the growth of calcium oxalate crystals in urine affect the formation of kidney stones. The level of supersaturation of Ca^{2+} and $C_2O_4^{2-}$, urinary components such as proteins and citrate and interactions with the kidney epithelium are thought essential factors for the formation of kidney stones [7]. Calcium oxalate monohydrate (COM) and dihyrate (COD) can be formed in urine or at epithelial cell surface. Thermodynamically stable COM has a bigger tendency to form stones than COD. COD crystals are considered as less urolithic than COM, so COD is easily expelled out from body [8].

The urinary proteins have important role at several stages of the nucleation, growth and aggregation of COM [7-9]. Citrate [10], amino acids [11], acid-rich protiens [12], the Tamm-Horsfall protein [13] and osteopontin [14] can be used to inhibit CaOx crystallization. Moreover, glutamic and aspartic acids [11], poly(glutamic acid) and poly(aspartic acid) [15, 16], carboxylate-modified biopolymers [6], polyelectrolytic and graft copolymers [1, 9, 17] have also been studied in the crystallization of calcium oxalate.

In the treatment of kidney stones, there are some treatment types such as extracorporeal sound wave lithotripsy, surgical treatments, oral supplements and herbal remedy. The surgical and medical treatments for urolithiasis are highly associated with risk factors. Therefore, it is needed to find some better alternative to these conventional methods. Hence there has been an increasing interest in growing for using natural ingredients as alternative or complementary medicines in pharmaceutical industry. To develop the potential therapeutic drugs, in the form of either extracts alone or in combination with other herbs can be provide many opportunities for traditional herbal medicines [18, 19]. Polyasparatic acid, polyglutamic acid and heparin were used for the inhibition of CaOx [20]. Plants can be used as a traditional healthcare system to treat kidney stones [21, 22]. The effects of plant Herniaria hirsute [22, 23], Phyllanthusniruri [24, 25], Cystone (a polyherbal formulation) [26], lemon and orange juice [27], Crataevanurvala [28], Chinese herbal medicines [29], Dolichosbiflorus L. [30] and Khella extract [31] on CaOx were studied. Still, many drug companies in the world have been working about traditional folk medicines to identify their active substances and degree of effect. *In vitro* crystallization systems were widely studied to understand the crystallization mechanism. Data from *in vitro*, *in vivo* and clinical trials reveal that phytotherapeutic agents could be useful as either an alternative or an adjunctive therapy in the management of urolithiasis [21]. In the light of these facts, we studied the spontaneous crystallization of calcium oxalate in the presence of and absence of Viburnum Opulus (VP) extract.

2. EXPERIMENTAL SECTION

In this study, the effects of VP on the calcium oxalate crystallization have been investigated with spontaneous crystallization method. Crystal growth experiments were carried out in a water-jacketed Pyrex glass vessel of 1 L capacity at 37 °C. Supersaturated solutions for crystal growth experiments were prepared by slow mixing of 300 mL volume of calcium chloride ($CaCl_2$) and 300 mL volume of sodium oxalate ($Na_2C_2O_4$) solutions. In the experiments where VP extract was used a similar procedure was followed. The freshly prepared VP extract solutions were added to the oxalate solution. The amount of the fruit juice of VP was varied from 0.1 mL to 2 mL. The fruit juice of VP was used after filtered through filter paper. The effect of VP on the precipitation rate of calcium oxalate was evaluated by recording the decrease in [Ca^{2+}] as a function of time in a solution containing 3.25×10^{-4} M $CaCl_2$ and 3.25×10^{-4} M $Na_2C_2O_4$. The precipitation process, which is accompanied by a decrease in the calcium activity as a function of time, was monitored and quantified by means of a Radiometer Impulsomat (PHM290) using the Ca-ISE electrode (Radiometer, ISE-K-CA). This technique is used to measure soluble calcium ions. At the same time, during the course of some selected experiments reaction aliquots were removed at various times and quickly filtered through Millipore filters of 0.22 µm pore size. The aqueous phase was analyzed for calcium by atomic absorption spectroscopy (Perkin Elmer AAnalyst 200). The atomic absorption results are consistent with the Ca-ISE electrode results. This means that the reduction in calcium concentration (drop in soluble calcium ion) is accompanied by crystal formation. The effect of an additive can be quantified as the ratio of the rate of crystallization of the pure solution (R_0, mol/L.min) to the rate of crystallization in the presence of additive (R_i, mol/L.min) at the same concentration and temperature. The rates reported were the initial rapid growth rate calculated from slope of the calcium ion concentration versus time plots for each experiment. The rates were determined from at least three separate experiments and only the average values were reported. The reproducibility of this approach is 4-5%. The crystals removed by filtration were examined by scanning electron microscopy (SEM) (JEOL JSM-SEM) and XRD (PanalyticalX'pertProPW3040/60).

3. RESULTS SECTION

3.1. Effect of VP on calcium oxalate crystallization rate and crystal morphology.

The crystallization of calcium oxalate has been investigated in the presence of VP extract at 37 °C. Table 1 summarizes the effect of VP extract on CaOx crystallization rate. The effect of extract on crystal growth rate was evaluated by comparing growth inhibition of calcium oxalate crystallization in the absence (R_0) and presence of the VP extract (R_i). The ability of the VP extract to act as inhibitor was evaluated by R_0/R_i ratios. The higher R_0/R_i values correspond to a better inhibition.

The inhibitory efficiency of VP was defined by Equation 1:

$$Inhibition\ (\%) = \frac{R_0 - R_i}{R_0} \times 100 \qquad (1)$$

The presence of additives or foreign ions and molecules can influence crystal nucleation. The active sides of crystal surface can be blocked by additives. Adsorption of additives on the surface of pre-nuclear clusters or embryos reduces the rate at which they pass through the critical size barrier, thereby decreasing the nucleation rate [3].

As shown in Table 1, VP is an effective inhibitor in the crystallization kinetics of calcium oxalate. In this study, the crystallization begins immediately; there is no induction effect both in the presence of VP and in control experiments. The efficiency of additive can be described according to its ability to inhibit crystallization. Table 1 shows that the degree of inhibition was proportional to amount of VP. The experimental results show that R_0/R_i ratio increases with increasing amount of VP. The growth rate reduced to 89.7% in the presence of 1 mL VP. VP effectively blocked all the active growth sites and hence brought the calcium oxalate rate to a complete stop over a 6 hr at 2 mL. Hence, VP is an effective inhibitor in calcium oxalate crystallization.

Table 1. Effect of VP on calcium oxalate crystallization at 37 °C.

Amount of additive, mL	R_0/R_i	Inh. (%)
0.1	1.76	43.0
0.2	1.86	46.4
0.3	2.22	54.9
0.4	5.09	80.4
0.5	6.48	84.6
1.0	9.67	89.7
2.0	n.c*	-

*:no-crystallization

The chemical composition of VP is given in Table 2. As can be seen in Table 2 the major constituents in VP are malic, oxalic, ascorbic and citric acid.

Table 2. The chemical composition of Viburnum Opulus extract [33, 34].

Minerals	Mineral Composition (mg/kg)
Calcium (Ca)	60.35
Magnesium (Mg)	60.78
Iron (Fe)	3.42
Copper (Cu)	0.86
Zinc (Zn)	5.00
Sodium (Na)	402.62
Potassium (K)	2473.80
Organic Acids	**Organic Acid Composition (mg/100g)**
Oxalic	80.5±2.4
Malic	1082.6±10.5
Ascorbic	52.7±1.1
Citric	38.6±0.9

It is known that acidic molecules inhibit calcium oxalate nucleation, growth, aggregation. A number of studies have demonstrated that citric acid modifies the calcium oxalate stone formation by affecting crystal nucleation, crystal growth and

prevent the formation of large crystals or aggregates [8, 32]. It can be said that the inhibition of CaOx precipitation involves the adsorption and/or interactions of the inhibitor –COOH groups with Ca^{2+} of crystallites [1, 3, 11]. Therefore its inhibitory effect on calcium oxalate crystallization may be explained by the acidic molecules adsorption of the active growth sites on crystal surfaces.

It is known that the calculation of the solution supersaturation with respect to any calcium oxalate hydrate requires knowledge of the activities of all ions involved. The distribution and the activities of the ionic species in the supersaturated solutions were computed by taking into consideration the possible equilibria which can occur in calcium oxalate solutions are given by Equations 2-6 [9].

$$H^+ + C_2O_4^{2-} \leftrightarrow HC_2O_4^- \quad (K_1) \quad (2)$$

$$Ca^{2+} + C_2O_4^{2-} \leftrightarrow CaC_2O_4 \quad (K_2) \quad (3)$$

$$Na^{2+} + C_2O_4^{2-} \leftrightarrow NaC_2O_4^- \quad (K_3) \quad (4)$$

$$Ca^{2+} + CaC_2O_4 \leftrightarrow Ca_2C_2O_4 \quad (K_4) \quad (5)$$

$$CaC_2O_4 + C_2O_4^{2-} \leftrightarrow Ca(C_2O_4)_2^{2-} \quad (K_5) \quad (6)$$

The relative solution supersaturation with respect to COM, σ, is defined as [1, 9].

$$\sigma = \frac{IP^{1/2} - K_{sp}^{1/2}}{K_{sp}^{1/2}} = S^{1/2} - 1 \quad (7)$$

in which IP is the ionic activity product and K_{sp} represented the thermodynamic solubility product was taken as 2.57×10^{-9} mol^2/L^2 for COM at 37 °C [1]. The supersaturation, S, is defined IP/K_{sp}. The solution speciation was computed by using MINEQL + chemical equilibrium modeling software [35]. It is a free energy minimization program taking into account all equilibria in the solution, mass balance and electroneutrality conditions and the degree of supersaturation was computed by this program.

The protonation reaction, Equation 2, could be ignored, because the experimental pH was greater than 5 (pH = 6.0–6.2) [36, 37]. The values of the association constant of ions (K_1, K_2, K_3) were obtained from the literature [38].

Crystallization rate varies with the relative supersaturation (σ). The crystallization rate can be expressed in terms of semiempirical kinetics equation;

$$R_{crys} = k_r \sigma^n \quad (8)$$

in which R_{crys} is the crystallization rate, k_r *is* rate constant for crystal growth and n is the apparent order of the crystal growth.

The equilibrium of different concentrations of calcium oxalate solutions was investigated with using MINEQL + program. Logarithmic plots of the rates of calcium oxalate formation as a function of relative solution supersaturation yielded a straight line as shown in Figure 1. The value of n, the effective order of the reaction, is calculated from the slope of the line. It was found as 2.10 calcium oxalate growth in the presence of 0.5 mL VP. The order of the crystallization, n = 2, is indicative of a surface nucleation controlled mechanism. This value is in agreement with earlier reports on the crystallization of calcium oxalate [1, 39].

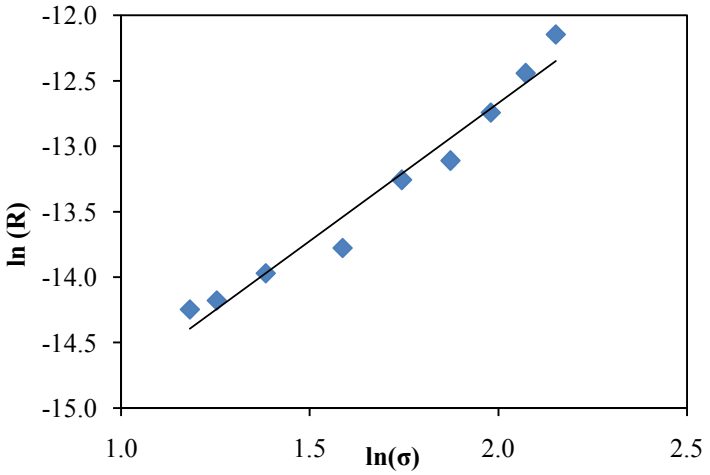

Figure 1. Logarithmic plot of rate of precipitation of COM versus the relative solution supersaturation.

Scanning Electron Microscopy (SEM) was used to observe the effect of VP on particle size and crystal morphology. The presence of additives in supersaturated solutions affects not only the kinetics of crystal growth but crystal size as well, as shown in Table 3 and Figure 2. In all experiments calcium oxalate monohydrate (COM) was the dominant phase in all concentrations as identified by X-ray diffraction (XRD). The dimensions of minimum of 50 crystals in each sample were measured from SEM photomicrographs. The average value of the dimensions was given in Table 3. The individual crystals of platelets and flower-like agglomerates were observed during crystallization of CaOx. The crystals with an average width of 3.12 µm and a length of 7.92 µm were obtained in the absence of additive. The data given in Table 3 show that an average particle size of the crystals reduced in the presence of VP. The plate like particles with an average width of 2.01 µm and a length of 4.07 µm were grown at 0.5 mL.

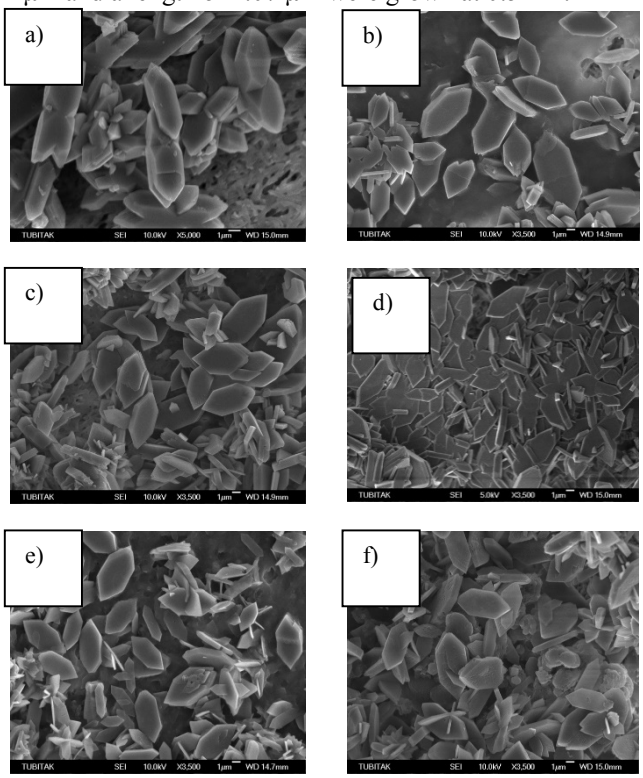

Figure 2. SEM of calcium oxalate crystals grown at 37 °C (a) without additive, (b) with 0.1 mL VP, (c) with 0.2 mL VP, (d) with 0.3 mL VP, (e) with 0.4 mL VP and (f) with 0.5 mL VP.

Table 3. Comparison of calcium oxalate crystal characteristics based on SEM results in the presence of VP.

Additive, (mL)	L (µm)	W (µm)	L/W
Control	7.92 (±1.48)	3.12 (±0.69)	2.54
0.1	6.27 (±0.99)	2.86 (±0.52)	2.19
0.2	5.12 (±1.28)	2.35 (±0.68)	2.18
0.3	4.91 (±1.69)	2.24 (±0.83)	2.19
0.4	4.70 (±1.30)	2.08 (±0.83)	2.26
0.5	4.07 (±1.68)	2.01 (±0.91)	2.03

W: width; L: length

The X-ray powder diffraction patterns of calcium oxalate crystal samples in the presence of and in the absence of VP are shown in Figure 3. XRD spectra of all samples have shown peaks characteristic for COM. In the presence of VP, the grown prismatic crystals were identified as COM by XRD and compared with that of the powder diffraction File 9-432 JCPDS 2000. The principal diffraction peaks of COM appear at 2θ values of 14.931 for reflection (101), at 15.291 for reflection (110), at 24.371 for reflection (020) and at 30.111 for reflection (202). All intensity peaks of the XRD patterns of the COM powders produced were exactly matched with the structural data of the COM described in standards.

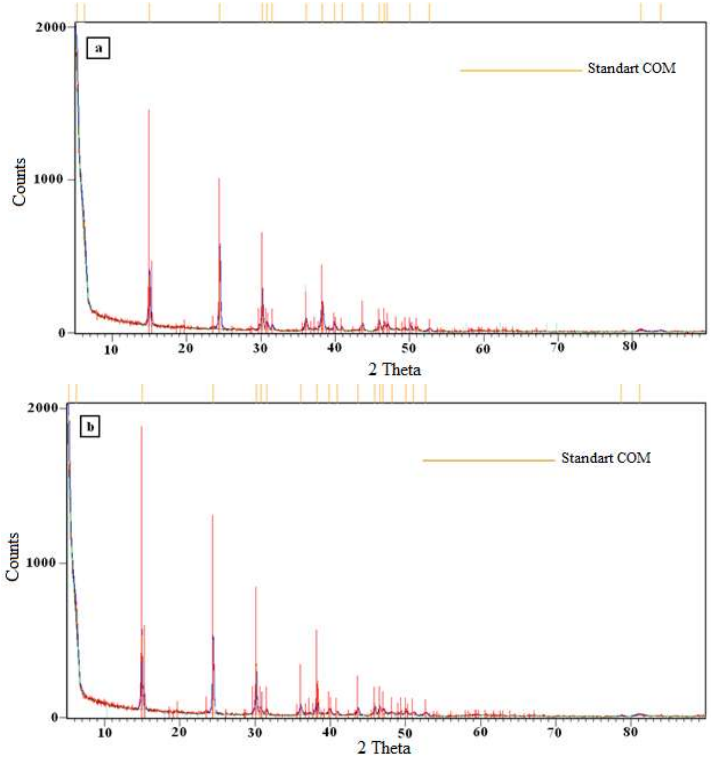

Figure 3. XRD powder patterns of crystals (a) in the absence of VP (b) in the presence of 0.5 mL VP.

3.2. Adsorption mechanism.

There have been many papers related to the effect of additive and/or impurities on growth rates and morphology of crystals [40-42]. Th kinks in ledges and at the terrace of surface. According to the model, inhibition is assumed to be accomplished through a fence of adsorbed additives ions upon the crystal surface [3]. Simple linear adsorption isotherms are appropriate to evaluate the experimental data on growth kinetics [44, 45]. Crystal faces can be restricted with the additives on the crystal surface. The growth of the crystallites is reduced or blocked by adsorption of additive, so they never reach critical size [3, 46].

According to the Vermilyea [38], the inhibitor will stop a step moving across the crystal surface. Only a few proportion of available crystal surface are covered with inhibitor, so the growth of mineral will be blocked. This phenomenon can be explained by preferential adsorption. In recent years, a new kinetic model describing the adsorption of an impurity along steps was developed by Kubota and Mullin [45]. As pointed by kinetic model, there is a direct proportion between step velocity and surface coverage. Impurities are adsorbed on the growing crystal. An impurity effectiveness factor α for the adsorption is described.

In the case of a spiral growth mechanism, the relationship between the relative growth rate R_i/R_0 and the fraction coverage, θ_i of the surface in the presence of impurity may be given by Equation 9 [40],

$$\left(\frac{R_0 - R_i}{R_0}\right)^n = \alpha^n \theta_i \tag{9}$$

where α is impurity effectiveness parameter and θ_i is the coverage of adsorption-active sites. While the exponent n = 1 represents the case at which impurity adsorption occurs at kinks in step edges as in Kubota-Mullin model, n = 2 represents adsorption on surface terrace as in Cabrera-Vermilyea model. Following equations described θ_i from the Langmuir and Temkin adsorption isotherms;

$$\theta_i = \frac{KC_i}{1 + KC_i} \qquad \text{Langmuir isotherm} \tag{10}$$

$$\theta_i = Z\,ln\,C_0 + Z\,ln\,C_i \qquad \text{Temkin isotherm} \tag{11}$$

where K, C_0, Z are constants and C_i is the amount of additive in solution. In Eq. 10, K is the Langmuir constant given by,

$$K = exp\,Q_{diff} / RT \tag{12}$$

where Q_{diff} (kJ/mol) is the differential heat of adsorption corresponding to impurity coverage θ_i of the available adsorption sites. The constant C_0 is expressed as,

$$C_0 = exp\,Q^0_{diff} / RT \tag{13}$$

where Q^0_{diff} (kJ/mol) is the initial heat of adsorption corresponding to θ_i.

Using Equation 9 in combination with the Langmuir and Temkin isotherm (Equations 10-11) we can write the following equations, linear in $(1/C_i)$ and $\ln(C_i)$ for n = 1(kink site).

$$\frac{R_0}{R_0 - R_i} = \alpha^{-1}\left(1 + \frac{1}{KC_i}\right) \qquad \text{Langmuir Kink} \tag{14}$$

$$\frac{R_0 - R_i}{R_0} = z\alpha(\,lnC_0 + lnC_i\,) \qquad \text{Temkin Kink} \tag{15}$$

When the adsorption site is the surface terrace (n = 2)

$$\left(\frac{R_0}{R_0 - R_i}\right)^2 = \alpha^{-2}\left(1 + \frac{1}{KC_i}\right) \qquad \text{Langmuir Terrace} \tag{16}$$

$$\left(\frac{R_0 - R_i}{R_0}\right)^2 = z\alpha^2(\ln C_0 + \ln C_i) \qquad \text{Temkin Terrace} \qquad (17)$$

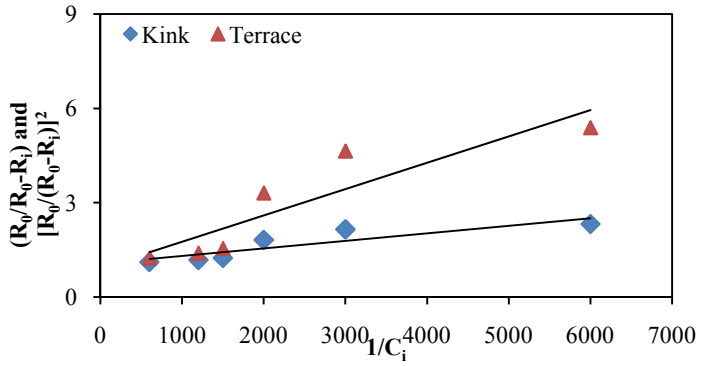

Figure 4. Plots of $R_0/(R_0 - R_i)$ and $[R_0/(R_0 - R_i)]^2$ against $(1/C_i)$.

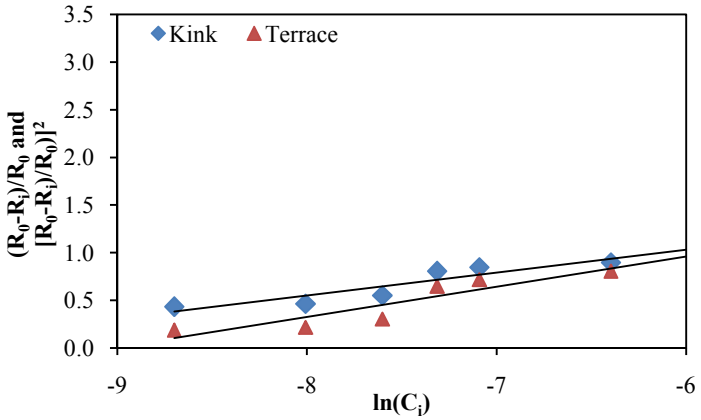

Figure 5. Plots of $(R_0 - R_i)/R_0$ and $[(R_0 - R_i)/R_0]^2$ against $\ln(C_i)$.

Equations 14 to 17 were used to control the availability of our kinetic data. Figure 4 illustrates the plots of $R_0/(R_0-R_i)$ and $[R_0/(R_0-R_i)]^2$against C_i^{-1} while Figure 5, presents the plots of $(R_0-R_i)/R_0$and $[(R_0-R_i)/R_0]^2$ against $\ln(C_i)$ according to the models involving impurity adsorption of kinks (n = 1) and the surface terrace (n = 2) respectively. Experimental data evaluation was based on regression coefficients of equations, due to linearity of equations. The best values of the constants for all equations to fit a given set of experimental data were determined using a least-squares criterion.

Table 4. Estimated values of K, Q_{diff} and $Q^0_{diff}$for crystal growth of calcium oxalate in the presence of VP.

Langmuir isotherm			
	K	Q_{diff}	R^2
Kink	5.35×10^3	22.13	0.7743
Terrace	1.16×10^3	18.19	0.8154
Temkin isotherm			
	C_0	Q^0_{diff}	R^2
Kink	2.92×10^5	32.43	0.8407
Terrace	8.32×10^4	29.20	0.8336

The value of Q_{diff} for kinks and terrace are similar predicted from Langmuir adsorption model (Table 4). The value of Q^0_{diff} predicted from Temkin model were higher during adsorption at kinks than those involved in adsorption at the surface terraces. Therefore, it is concluded that the mechanism of the inhibitory effect of VP best follows the Temkin kink model. Liu et.al found that the presence of BSA (bovine serum albumin) inhibits crystallization by absorbing at the kink sites [47]. Kirboga and Oner [3] obtained similar results in the presence of homopolymer of vinyl sulfonic acid.

4. CONCLUSIONS

It was found that the VP tested in this study is an effective inhibitor for the formation of calcium oxalate crystals and could therefore play a potential role in the prevention of calcium oxalate crystals, the main crystalline components of the stones. The presence of VP inhibited the crystal growth of calcium oxalate possibly through adsorption onto the active growth sites for crystal growth. The amount of VP was found to be an important parameter for the controlling of crystallization of calcium oxalate. Our results indicate that natural compounds can be beneficial in treating renal stone formation by inhibiting the growth of crystals.

5. REFERENCES

[1] Akyol E., Oner M., Inhibition of calcium oxalate monohydrate crystal growth using polyelectrolytes, *Journal of Crystal Growth*, 307, 1, 137-144, **2007.**

[2] Grohe B., Rogers K.A., Goldberg H.A., Hunter G.K., Crystallization kinetics of calcium oxalate hydrates studied by scanning confocal interference microscopy, *Journal of Crystal Growth*, 295, 2, 148-157, **2006.**

[3] Kirboga S., Oner M., The role of vinyl sulfonic acid homopolymer in calcium oxalate crystallization, *Colloids and Surfaces B-Biointerfaces*, 78, 2, 357-362, **2010.**

[4] Opalko F.J., Adair J.H., Khan S.R., Heterogeneous nucleation of calcium oxalate trihydrate in artificial urine by constant composition, *Journal of Crystal Growth*, 181, 4, 410-417, **1997.**

[5] Ouyang J.M., Wang M., Lu P., Tan J., Degradation of sulfated polysaccharide extracted from algal Laminaria japonica and its modulation on calcium oxalate crystallization, *Materials Science & Engineering C-Materials for Biological Applications*, 30, 7, 1022-1029, **2010.**

[6] Akin B., Oner M., Bayram Y., Demadis K.D., Effects of carboxylate-modified, "Green" inulin Biopolymers on the crystal growth of calcium oxalate, *Crystal Growth & Design*, 8, 6, 1997-2005, **2008.**

[7] Akyol E., Bozkurt A., Oner M., The effects of polyelectrolytes on the inhibition and aggregation of calcium oxalate crystallization, *Polymers for Advanced Technologies*, 17, 1, 58-65, **2006.**

[8] Wang L.J., Zhang W., Qiu S.R., Zachowicz W.J., Guan X.Y., Tang R.K., Hoyer J.R., De Yoreo J.J., Nancollas G.H., Inhibition of calcium oxalate monohydrate crystallization by the combination of citrate and osteopontin, *Journal of Crystal Growth*, 291, 1, 160-165, **2006.**

[9] Kirboga S., Oner M., Inhibition of Calcium Oxalate Crystallization by Graft Copolymers, *Crystal Growth & Design*, 9, 5, 2159-2167, **2009.**

[10] BekJensen H., Fornander A.M., Nilsson M.A., Tiselius H.G., Is citrate an inhibitor of calcium oxalate crystal growth in high concentrations of urine?, *Urological Research*, 24, 2, 67-71, **1996.**

[11] Brecevic L., Kralj D., The influence of some amino-acids on calcium-oxalate dihydrate transformation, *Journal of Crystal Growth*, 79, 1-3, 178-184, **1986.**

[12] Fellstrom B., Danielson B.G., Ljunghall S., Wikstrom B., Crystal inhibition - the effects of polyanions on calcium-oxalate crystal-growth, *Clinica Chimica Acta*, 158, 3, 229-235, **1986.**

[13] Shirane Y., Kurokawa Y., Miyashita S., Komatsu H., Kagawa S., Study of inhibition mechanisms of glycosaminoglycans on calcium

oxalate monohydrate crystals by atomic force microscopy, *Urological Research*, 27, 6, 426-431, **1999.**
[14] Taller A., Grohe B., Rogers K.A., Goldberg H.A., Hunter G.K., Specific adsorption of osteopontin and synthetic polypeptides to calcium oxalate monohydrate crystals, *Biophysical Journal*, 93, 5, 1768-1777, **2007.**
[15] Wesson J.A., Worcester E.M., Kleinman J.G., Role of anionic proteins in kidney stone formation: Interaction between model anionic polypeptides and calcium oxalate crystals, *Journal of Urology*, 163, 4, 1343-1348, **2000.**
[16] Guo S.W., Ward M.D., Wesson J.A., Direct visualization of calcium oxalate monohydrate crystallization and dissolution with atomic force microscopy and the role of polymeric additives, *Langmuir*, 18, 11, 4284-4291, **2002.**
[17] Zhang D.B., Qi L.M., Ma J.M., Cheng H.M., Morphological control of calcium oxalate dihydrate by a double-hydrophilic block copolymer, *Chemistry of Materials*, 14, 6, 2450-2457, **2002.**
[18] Yasir F.,Waqar M.A., Effect of indigenous plant extracts on calcium oxalate crystallization having a role in urolithiasis, *Urological Research*, 39, 5, 345-350, **2011.**
[19] Das I., Gupta S.K., Ansari S.A., Pandey V.N., Rastogi R.P., In vitro inhibition and dissolution of calcium oxalate by edible plant Trianthema monogyna and pulse Macrotyloma uniflorum extracts, *Journal of Crystal Growth*, 273, 3-4, 546-554, **2005.**
[20] Tomazic B.B., Sheehan M.E., Nancollas G.H., Influence of natural and synthetic inhibitors on the crystallization of calcium-oxalate hydrates, *World Journal of Urology*, 10, 4, 216-225, **1992.**
[21] Butterweck V., Khan S.R., Herbal medicines in the management of urolithiasis: alternative orcomplementary?, *Planta Medica*, 75, 10, 1095-1103, **2009.**
[22] Atmani F., Khan S.R., Effects of an extract from Herniaria hirsuta on calcium oxalate crystallization in vitro, *Bju International*, 85, 6, 621-625, **2000.**
[23] Grases F., Ramis M., Costabauza A., March J.G., Effect of herniaria-hirsuta and agropyron-repens on calcium-oxalate urolithiasis risk in rats, *Journal of Ethnopharmacology*, 45, 3, 211-214, **1995.**
[24] Freitas A.M., Schor N., Boim M., The effect of Phyllanthus niruri on urinary inhibitors of calcium oxalate crystallization and other factors associated with renal stone formation, *Bju International*, 89, 9, 829-834, **2002.**
[25] Barros M.E., Schor N., Boim M.A., Effects of an aqueous extract from Phyllanthus niruri on calcium oxalate crystallization in vitro, *Urological Research*, 30, 6, 374-379, **2003.**
[26] Mitra S.K., Gopumadhavan S., Venkataranganna M.V., Sundaram R.., Effect of cystone, a herbal formulation, on glycolic acid-induced urolithiasis in rats, *Phytotherapy Research*, 12, 5, 372-374, **1998.**
[27] Kulaksizoglu S., Sofikerim M., Cevik C., In vitro effect of lemon and orange juices on calcium oxalate crystallization, *International Urology and Nephrology*, 40, 3, 589-594, **2008.**
[28] Varalakshmi P., Shamila Y., Latha E., Effect of crataeva-nurvala in experimental urolithiasis, *Journal of Ethnopharmacology*, 28, 3, 313-321, **1990.**
[29] Gohel M.D.I., Wong S.P., Chinese herbal medicines and their efficacy in treating renal stones, *Urological Research*, 34, 6, 365-372, **2006.**
[30] Garimella T.S., Jolly C.I., Narayanan S., In vitro studies on antilithiatic activity of seeds of Dolichos biflorus Linn. and rhizomes of Bergenia ligulata Wall, *Phytotherapy Research*, 15, 4, 351-355, **2001.**
[31]Abdel-Aal E.A., Daosukho S., El-Shall H., Effect of supersaturation ratio and Khella extract on nucleation and morphology of kidney stones, *Journal of Crystal Growth*, 311, 9, 2673-2681, **2009.**
[32] Weaver M.L., Qiu S.R., Hoyer J.R., Casey W.H., Nancollas G.H., De Yoreo J.J., Inhibition of calcium oxalate monohydrate growth by citrate and the effect of the background electrolyte, *Journal of Crystal Growth*, 306, 1, 135-145, **2007.**
[33] Cam M., Hisil Y., Kuscu A., Organic acid, phenolic content, and antioxidant capacity of fruit flesh and seed of Viburnum opulus, *Chemistry of Natural Compounds*, 43, 4, 460-461, **2007.**
[34] Bolat S., Ozcan M., The morphological, phenological and pomological properties and chemical composition of Viburnum Opulus extract fruit (Vibirnum opulus L.), *2nd National Turkish Horticulture Congress*, Adana, TURKEY, OCT3-6, **1995.**
[35] MINEQL + Chemical Equilibrium Modeling System, Hallowell, ME, **1998.**
[36] Grases F., Gil J.J., Conte A., Glycosaminoglycans- inhibition of calcium-oxalate crystalline growth and promotion of crystal aggregation, *Colloids and Surfaces*, 36, 1, 29-38, **1989.**
[37] Grases F., March J.G., Costabauza A., The crystallization of calcium-oxalate at different Ph values and in the presence ofvarious adenosine phosphates, *Journal of Colloid and Interface Science*, 128, 2, 382-387, **1989.**
[38] Cabrera N., Vermilyea D.A., *Growth and Perfection of Crystals*, 1st ed., Wiley, **1958.**
[39] Millan A., Sohnel O., Grases F., The influence of crystal morphology on the kinetics of growth of calcium oxalate monohydrate, *Journal of Crystal Growth*, 179, 1-2, 231-239, **1997.**
[40] Sangwal K., Kinetic effects of impurities on the growth of single crystals from solutions, *Journal of Crystal Growth*, 203, 1-2, 197-212, **1999.**
[41] Simon B., Boistelle R., Crystal-growth from low-temperature solutions, *Journal of Crystal Growth*, 52, 779-788, **1981.**
[42] Davey R.J., *Industrial Crystallization*, 1st ed., North-Holland Publishing Company, **1979.**
[43] Bliznakov G., Die kristalltracht und adsorption fremder beimischungen, *Fortschr. Min.*, 36, 1, 149-191, **1958.**
[44] Davey R., Fila W., Garside J., The influence of biuret on the growth-kinetics of urea crystals from aqueous-solutions, *Journal of Crystal Growth*, 79, 1-3, 607-613, **1986.**
[45] Kubota N., Mullin J.W., A kinetic model for crystal-growth from aqueous-solution in the presence of impurity, *Journal of Crystal Growth*, 152, 3, 203-208, **1995.**
[46] Mullin J.W., *Crystallization*, 4th ed., Butterworth-Heinemann, **2001.**
[47] Liu J.F., Jiang H.D., Liu X.Y., How does bovine serum albumin prevent the formation of kidney stone? A kinetics study, *Journal of Physical Chemistry B*, 110, 18, 9085-9089, **2006.**

6. ACKNOWLEDGEMENTS

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