Effect of Unitary, Binary and Ternary Carboxylates on Crystallization Kinetics of Calcium Oxalate in Artificial Urine

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The effect of three types of carboxylates on the crystallization kinetics of calcium oxalate (CaC_2O_4) were studied by detecting the change of free Ca^{2+} ions concentration, size of CaC_2O_4 crystallites, zeta potential, autocorrelation curve and decay time with reaction time (t) in artificial urine. Sodium glycinate (NaGly ; a monocarboxylate), sodium tartrate (Na_2Tart ; a dicarboxylate) and sodium citrate (Na_3Cit ; a tricarboxylate) were the three carboxylates investigated in this study. The crystallization kinetics equation of CaC_2O_4 was $r = kc^{3.3}$ and the average reaction rate constant ($\bar{k}$) was 0.99×10^9 . All the three carboxylates could inhibit the kinetics process of nucleation, growth and aggregation of CaC_2O_4 crystallites. In the presence of various carboxylate, the value is arranged from smallest to largest in the following order Na_3Cit (2.29×10^5) < Na_2Tart (9.76×10^5) < NaGly (1.68×10^7) < Blank (0.99×10^9). The changes in size, zeta potential and decay time of the crystallites can also be arranged in the following order: Na_3Cit < Na_2Tart < NaGly < blank.

Keywords: Calcium oxalate, Crystallization kinetics, Carboxylate, Reaction order, Autocorrelation decay time.

INTRODUCTION

Urolithiasis is a chronic disease with an incidence increasing annually, may relapse after treatment¹. Calcium oxalate (CaC_2O_4) is the main component of urinary stones. Stone formation involves nucleation, growth, aggregation and adhesion process of urinary crystallites.

The complexity of the internal environment in the human body, observing the formation processes of stones *in situ* is difficult. Therefore, we usually use the simulative method *in vitro* to study the nucleation, growth and aggregation of CaC_2O_4 and the influence of various inhibitors on these processes¹. These simulative systems contain water, urine, artificial urine, membrane mimetic system, surface of renal epithelial cells, *etc*²⁻⁵.

Nishio *et al.*⁶ studied the inhibition effects of prothrombin fragment 1 and osteopontin on CaC_2O_4 crystallization in artificial urine, indicating that the inhibition effects of the two proteins on the growth rate of CaC_2O_4 were 27 and 32 %, respectively, when their concentration were 2 $\mu\text{g/mL}$. Wang *et al.*⁷ studied the inhibition effect of phosphorylated osteopontin segment on nucleation and growth of calcium oxalate monohydrate (COM) crystals under near-physiological pH, temperature and ionic strength. When the concentration of phosphorylated OPN was 43.9 nm, the growth rate of $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$

crystals was only 60 % of the control group. When the concentration of phosphorylated OPN reached 108.0 nm, the growth inhibition of $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ crystals was 93 %. By contrast, the inhibition of un-phosphorylated OPN on the growth of $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ crystals was not significant. Even when the concentration reached 1450 nm, the inhibition of un-phosphorylated OPN did not show a significant effect. The reason for this phenomenon is that the doubly phosphorylated peptide binds strongly to both ($\bar{1}01$) and (010) faces of $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$, thus inhibiting the growth of $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ crystals. Carvalho *et al.*⁸ studied the effect of CaC_2O_4 relative saturation (RS) on CaC_2O_4 crystallization in urine. At $\text{RS} < 10$, no crystal was formed in the urine; at RS of 10, small crystals formed into COD; at RS of 30, the number of crystals with the same size increased; at RS greater than 50, the crystal size increased and a large amount of crystals aggregated. These patterns indicate that the RS of CaC_2O_4 and calcium to oxalate ratio (Ca/Ox) in urine are important factors in determining the structure and morphology of CaC_2O_4 crystals.

Although studying the calculus formation mechanism in real urine environment is ideal, but storing real urine is difficult. In addition, real urine does not meet the dosage test requirement. Therefore, artificial urine is widely used. For example, Beghalia *et al.*⁹ studied the effect of ajuga and atriplex, which are two types of herbal medicine, on CaC_2O_4 crystallization in

artificial urine. They denoted that ajuga and atriplex could inhibit both nucleation and growth of CaC_2O_4 . Patel *et al.*¹⁰ studied the effect of lactic acid and citric acid on the solubility of CaC_2O_4 crystals in artificial urine. With increasing concentration, the soluble calcium ion concentration in the solution increased. When the concentration of two substances is equal, the ability of lactic acid to dissolve CaC_2O_4 crystals is greater than that of citric acid and the maximum dissolved amount could reach 70 % of the CaC_2O_4 crystals.

The inhibitors in urine could affect the growth kinetics process of CaC_2O_4 crystallization because the inhibitors not only could reduce the RS of CaC_2O_4 but also could be adsorbed on the surface of CaC_2O_4 crystals and close the active growth sites of crystals. The inhibitor could also change the charges and energy distribution on the crystal surface.

For carboxylic acid inhibitors, the number of carboxyl groups (COOH) of the acid will affect its coordination ability¹¹ with Ca^{2+} , thus affecting the crystallization kinetics of CaC_2O_4 . Therefore, the effect of sodium mono-carboxylate (NaGly), di-carboxylate (Na_2Tart) and tri-carboxylate (Na_3Cit) on the kinetics of CaC_2O_4 crystallization was studied by using the electrode method by detecting the change in Ca^{2+} ions concentration, size, zeta potential, autocorrelation curves and decay time of CaC_2O_4 crystallites with reaction time. The reaction order (α) and reaction rate constant (k) were calculated and the mechanism of different carboxylates in inhibiting CaC_2O_4 crystallization was discussed.

EXPERIMENTAL

Sodium glycine (NaGly), sodium tartrate (Na_2Tart), sodium citrate (Na_3Cit) and other reagents were all in analytical purity. Double distilled water was used. Artificial urine was prepared by NaCl (0.106 mol/L), KCl (0.064 mol/L), NaH_2PO_4 (0.0032 mol/L), Na_2SO_4 (0.017 mol/L), MgSO_4 (0.0038 mol/L) and Na_3Cit (0.0032 mol/L) [12]. Its pH was adjusted 6 with 0.5 mol/L *Tris* solution.

Saturated KCl calomel reference electrode (232-type), calcium ion selective electrode (PCa-1 type) and precision pH meter (PHS-3C) were purchased from Shanghai Precision Scientific Instrument Co. Ltd. The Zetasizer Nano-ZS nanoparticle size analyzer was from Malvern.

Battery, electrode potential and free Ca^{2+} ion concentration measurement: Calcium ion electrode and calomel electrode were used to compose the measurement battery: $\text{Hg}, \text{HgCl}_2 | \text{KCl} (\text{saturated}) | \text{test solution} | \text{sensitive film} | 1.0 \text{ mM calcium standard solution} | \text{Ag}, \text{AgCl}$.

The sensitive film was PVC membrane with di(*p*-isooctyl) phenyl phosphate as an active material. Before use, calcium ion selective electrode was soaked in 1 mmol/L calcium standard solution to be activated for 24 h and then cleaned by deionized water to a blank potential value of -65 mV.

Calcium standard curve preparation: After obtaining the E value corresponding to different concentration of calcium standard solution ($[\text{Ca}^{2+}]$) in the pH meter, a standard curve was obtained by using $\log [\text{Ca}^{2+}]$ as abscissa and E as longitudinal. The linear regression equation was $E = 42.5 + 27.5 \log [\text{Ca}^{2+}]$ and the correlation coefficient was $R = 0.9992$.

Preparation of CaC_2O_4 supersaturation solution: Urine calcium exists in two forms: complexed calcium (*e.g.*, the calcium bound with proteins and the calcium combined with organic acids) and free calcium. However, only free calcium, which is an important factor in stone formation, has physiological activity. According to the literature^{13,14}, ionized calcium in the male and female urine were 48.2 ± 25.5 and 42.2 ± 21.1 mg/L (1.21 ± 0.64 and 1.06 ± 0.53 mmol/L) and the concentration of $\text{C}_2\text{O}_4^{2-}$ was 0.53 ± 0.20 mmol/L. Therefore, the RS of CaC_2O_4 in the male and female urine were 15.25 ± 10.81 and 14.21 ± 9.75 , respectively. On the basis of the above RS range, a CaC_2O_4 supersaturated solution of $[\text{Ca}^{2+}] = [\text{C}_2\text{O}_4^{2-}] = 0.8$ mmol/L in artificial urine was prepared. According to $K_{\text{sp}}(\text{CaC}_2\text{O}_4) = 2.32 \times 10^{-9}$, the RS of these solution was 15.61^{7,14}.

Crystallization kinetics of CaC_2O_4 : About 4.8 mL of 10 mmol/L CaCl_2 solution was added to 50.4 mL of pH 6 artificial urinary-*Tris* solution and 4.8 mL of 10 mmol/L $\text{K}_2\text{C}_2\text{O}_4$ solution was quickly added into the mixture. At this time, the supersaturated degree of CaC_2O_4 was $\text{RS} = 15.61$. After the E value of free Ca^{2+} in the solution at different reaction times ($t = 1, 5, 10, 20, 40, 60$ min) was detected, the average concentration of free Ca^{2+} in the solution was calculated according to the linear regression equation.

The effect of different inhibitors on crystallization kinetics of CaC_2O_4 was studied next. NaGly , Na_2Tart , or Na_3Cit was added into the above CaC_2O_4 supersaturated solution and the final concentration of carboxylate is 2.0 mmol/L. At different reaction times ($t = 1, 5, 10, 20, 40, 60$ min), 1.0 mL of the reaction mixture was aspirated to detect the particle size, zeta potential, autocorrelation function and decay time of CaC_2O_4 crystallites by nanoparticle size analyzer. Diluted hydrochloric acid and diluted NaOH solution were used to maintain the system pH (6.0) in the reaction process.

All experiments were repeated three times. The experimental temperature was $(37.0 \pm 0.1)^\circ\text{C}$.

RESULTS AND DISCUSSION

Establishment of kinetic equation of CaC_2O_4 crystallization: When CaC_2O_4 crystals were precipitated from the system, the free calcium ion concentration $[\text{c}(\text{Ca}^{2+})]$ in the solution decreased. Therefore, the crystallization rate could be studied by detecting the decrease in the rate of $\text{c}(\text{Ca}^{2+})$ in the system.

To establish the kinetic equation of CaC_2O_4 crystallization according to the equimolar ratio of calcium and oxalate in the supersaturated solution, we assumed that Ca^{2+} reacted with $\text{C}_2\text{O}_4^{2-}$ equivalently and the reaction rate (r) equation is as follows:

$$r = k[\text{Ca}^{2+}]^p[\text{Ox}^{2-}]^q \quad (1)$$

Assume that x is the consumption of Ca^{2+} or $\text{C}_2\text{O}_4^{2-}$ at time t and $p + q = \alpha$ because $[\text{Ca}^{2+}] = [\text{C}_2\text{O}_4^{2-}] = c$. According to the equal-ratio reaction, the arranged eqn. 1 is expressed as follows:

$$r = kc^\alpha = k(a - x)^\alpha \quad (2)$$

$$\text{Because: } r = -\frac{d[\text{Ca}^{2+}]}{dt} = -\frac{d[a - x]}{dt} = \frac{dx}{dt} \quad (3)$$

By combining (1), (2) and (3) and by taking the logarithmic on both sides of eqn. 3, we can obtain the following equation:

$$\log r = \log \frac{dx}{dt} = \log k + \alpha \log(a-x) \quad (4)$$

where a and c in the formula are free $[\text{Ca}^{2+}]$ (mmol/L) at the initial time ($t=0$) and at time t in the solution; x is the consumption amount of Ca^{2+} (mmol/L) in the solution at time t ; α is the reaction order; k is the reaction rate constant. Eqn. 4 shows a linear relationship between $\log r$ and $\log(a-x)$. The slope of the line is the reaction order (α) and the intercept is $\log k$, which could be used to obtain the reaction rate constant k .

Effect of carboxylates on reaction order and reaction rate constant of CaC_2O_4 crystallization: We studied the effect of different sodium carboxylate salts on the crystallization kinetics of CaC_2O_4 , including mono-carboxylate, di-carboxylate and tri-carboxylate (Fig. 1). The $\log [\text{Ca}^{2+}]$ of the system decreased rapidly with the reaction time ranging from 0-20 min (Fig. 1A), which could be caused by the relative supersaturation of the solution being larger at the beginning of the reaction and CaC_2O_4 being nucleated abundantly, which led to the rapid decrease in $[\text{Ca}^{2+}]$. After 20 min, $[\text{Ca}^{2+}]$ decreased slowly, indicating that the CaC_2O_4 of the system was close to precipitation-dissolution equilibrium. After adding NaGly (Fig. 1A(b)), the amount of free $[\text{Ca}^{2+}]$ is not significantly different compared with that of the control group. This finding shows that NaGly has little influence on the crystallization kinetics of CaC_2O_4 .

Fig. 1. $\log [\text{Ca}^{2+}]$ -time curve (A) and $\log r - \log(a-x)$ curve (B) in $\text{RS} = 15.61$ CaC_2O_4 a supersaturated solution in presence of different carboxylic acid inhibitor of 2.0 mmol/L. (a) Blank, (b) NaGly, (c) Na_2Tart , (d) Na_3Cit

After adding Na_2Tart (Fig. 1A(c)), free $[\text{Ca}^{2+}]$ became smaller than that of the control group and the NaGly group because Na_2Tart is di-carboxylate and can coordinate with Ca^{2+} . When $\text{C}_2\text{O}_4^{2-}$ chelates with Ca^{2+} to form 1:1 complexes, the stability constant (K_s) is $10^{3.0}$. When Tart^{2-} chelates with Ca^{2+} to form 2:1 complexes, the K_s is $10^{9.01}$ ¹⁵. The necessary condition to generate CaC_2O_4 crystallites in solution is $[\text{Ca}^{2+}][\text{C}_2\text{O}_4^{2-}] \geq K_{sp}$. Adding 2 mmol/L Na_2Tart into the solution results not only in shifting of the balance of $\text{Ca}^{2+} + \text{C}_2\text{O}_4^{2-} \rightarrow \text{CaC}_2\text{O}_4$ to the left, *i.e.*, the CaC_2O_4 dissolves, but also in the significant decrease in the free $[\text{Ca}^{2+}]$ of the system from 1 mmol/L to 0.165 mmol/L at $t = 20$ min.

The free $[\text{Ca}^{2+}]$ further decreased when Na_3Cit was added. Na_3Cit is a tri-carboxylate and contains three carboxyl and one hydroxyl group, Na_3Cit can form a ring complex with $\text{Ca}^{2+}[\text{Ca}(\text{C}_6\text{H}_5\text{O}_3)_2 \cdot 4\text{H}_2\text{O}]$ with a five-membered and a six-membered rings. Na_3Cit has $K_s = 10^{4.68}$, which is greater than the K_s of CaC_2O_4 ($10^{3.0}$)¹⁵. The solubility of $\text{Ca}(\text{C}_6\text{H}_5\text{O}_3)_2 \cdot 4\text{H}_2\text{O}$ is larger in water. Therefore, after 20 min in the system of Na_3Cit , the free $[\text{Ca}^{2+}]$ of the system decreases to 0.096 mmol/L, which is less than 0.165 mmol/L in the presence of Na_2Tart and 0.180 in the presence of NaGly. After adding 2.0 mmol/L Na_3Cit , we still do not observed any precipitation in the solution even when the reaction time reached 22 h.

According to classical nucleation theory¹⁶, the nucleation free energy ΔG is inversely proportional to the square of $\ln \text{RS}$. With the prolongation of the reaction time, the consumption of $[\text{Ca}^{2+}]$ in the solution increased rapidly and the relative supersaturation (RS) of CaC_2O_4 was reduced. Thus, the nucleation energy barrier ΔG of CaC_2O_4 gradually increased, which results in difficulty of CaC_2O_4 to form nucleation.

By using the differential method, we plotted the reaction time (t) to Ca^{2+} consumption $[x(\text{Ca}^{2+})]$. The slope of any point of the curve (derived from the origin data analysis software) was the rate of the reaction r . We then plotted with $\log r$ to $\log(a-x)$ (Fig. 1B), in which the slope of the straight line was the reaction order (α); the reaction rate constant k was obtained through the intercept ($\log k$). The results are shown in Table-1.

TABLE-1
AVERAGE REACTION ORDERS ($\bar{\alpha}$) AND RATE CONSTANTS ($\bar{k}$) OF CaC_2O_4 A CRYSTALLIZATION IN PRESENCE OF THREE DIFFERENT CARBOXYLATES*

Inhibitor	In artificial urine		In saline system	
	$\bar{k}$	$\bar{\alpha}$	$\bar{k}$	$\bar{\alpha}$
Blank	0.99×10^9	3.3	3.1×10^9	3.3
NaGly	1.68×10^7	3.4	2.0×10^7	3.3
Na_2Tart	9.76×10^5	3.4	2.0×10^6	3.1
Na_3cit	2.29×10^5	3.4	1.0×10^6	3.1

*RS of CaC_2O_4 a solution is 15.61. The concentration of carboxylates is 2.0 mmol/L.

Fig. 1B is the relational graph of $\log r - \log(a-x)$ in the CaC_2O_4 supra-saturated solution in presence of 2 mmol/L NaGly, Na_2Tart , or Na_3Cit and $\text{RS} = 15.61$. $\bar{k}$ reduced from 0.99×10^9 - 1.68×10^7 , 9.76×10^5 and 2.29×10^5 after adding NaGly, Na_2Tart , or Na_3Cit , respectively, in the artificial urinary system (Table-1). However, the addition of these solutions had

little effect on the reaction order (α) and average reaction order $\bar{\alpha} = 3.4$. This finding shows that NaGly, Na₂Tart and Na₃Cit participate in the crystallization kinetics of CaC₂O₄ in a certain extent and has an influence on this process. $\bar{k}$ value could directly reflect the speed of reaction rate and is related with the reaction process. When two CaC₂O₄ molecules collide with each other, the CaC₂O₄ molecules win an energy. However, time is still needed to transfer energy internal molecules to make the weakest bond fractured. If the CaC₂O₄ molecule collides with other molecule in the system (for example, the inhibitor molecules), the CaC₂O₄ will lose the activation energy and an invalid collision will occur. Thus, the crystallization process of CaC₂O₄ is inhibited in the presence of carboxylate inhibitors.

Effect of carboxylates on particle size of CaC₂O₄ in crystallization process: Fig. 2 shows the change of particle size, zeta and autocorrelation decay time of CaC₂O₄ crystallites in presence of 2 mmol/L of NaGly, Na₂Tart and Na₃Cit, respectively, in the artificial urine. The figure shows that the crystallite size increased gradually within 1 min to 20 min and reached the maximum at 20 min (1070, 949 and 567 nm, respectively). The crystallite size then decreased slightly (Fig. 2A).

The RS of the system decreased from 15.61 to 7.28, 6.54 and 5.27 in $t = 20$ min after adding NaGly, Na₂Tart, or Na₃Cit, respectively. By using the Weimarn experience formula, the dispersion degree (R) of crystallites can be expressed as follows:

$$R = K \times \frac{(Q-s)}{s} \quad (5)$$

where Q is the concentration of the precipitated substance at the moment of adding the precipitator, s is the solubility of the precipitated substance at the moment of precipitation, (Q-s) is the instantaneous supersaturation at the beginning of precipitation, (Q-s)/s is the relative saturation (RS) at the moment of precipitation and K is a constant.

The formula (5) shows that the greater the relative saturation of the solution (RS) and the larger its dispersion degree (R), the smaller the particles of precipitation and *vice versa*. Given the decrease in RS in the solutions, the dispersion (R) of crystallites also decreased. Therefore, the crystallite size of CaC₂O₄ in the solution increased, which made the particle size increase from 846, 619 and 324 nm to 1070, 949 and 567 nm, respectively.

The formation and dissolution of crystals in the solution is a dynamic equilibrium process. The crystallite size slightly decreased after 20 min, which could be caused by the fact that artificial urine contains much electrolytes (NaCl, KCl, etc.). These electrolytes could increase the solubility of CaC₂O₄ crystallites and make CaC₂O₄ crystallite partially dissolved. The Cit³⁻ and Tart²⁻ in the system could also inhibit the growth of CaC₂O₄ crystallites. In addition, the zeta potential of CaC₂O₄ crystallites becomes more negative after 20 min (Fig. 2B). The electrostatic repulsion force of the crystallites also increased, inhibiting the aggregation of crystallites and decrease in the size of crystallites.

At $t = 5, 10, 20, 40, 60$ min, the size of the formed CaC₂O₄ crystallites in presence of different inhibitors can be arranged in the following order: Na₃Cit < Na₂Tart < NaGly $\approx$ Blank.

Fig. 2. Property changes of CaC₂O₄ crystallites with reaction time in presence of 2.0 mmol/L of NaGly, Na₂Tart and Na₃Cit in artificial urine: (A) crystallite size, (B) zeta potential and (C) autocorrelation decay time. (a) Blank, (b) NaGly, (c) Na₂Tart, (d) Na₃Cit. (RS = 15.61)

This order shows that Na₃Cit has a greater ability than Na₂Tart and NaGly in the inhibition of nucleation, growth and aggregation of CaC₂O₄ crystallites. Cit³⁻ complexed with Ca²⁺ to form complex Ca(C₆H₅O₃)₂·4H₂O and the solubility of the complex was 0.096 g, greater than that of CaC₂O₄ (0.004 g). $K_s = 10^{9.01}$ when tart²⁻ and Ca²⁺ formed a 2:1 complex¹⁵, thus making the balance of $\text{Ca}^{2+} + \text{C}_2\text{O}_4^{2-} \rightleftharpoons \text{CaC}_2\text{O}_4$ shift to the left, *i.e.*, CaC₂O₄ crystallite will be dissolved. However, NaGly is monocarboxylate and its complexing capacity with Ca²⁺ is smaller than that of Na₂Tart and Na₃Cit. Therefore, the size of CaC₂O₄ crystallites formed in the presence of NaGly has no significant difference compared with that of the control group. This finding also shows that NaGly has less effect on the crystallization kinetics of CaC₂O₄.

The growth of urine crystallite is a slow process and urine crystallite is difficult to grow into pathological size in a short time when urine flows through the tubular. According to Reid and Finlayson¹⁷, the separated CaC_2O_4 crystals are excreted from the body through urine before the crystals to become large enough to become fixed on the tubule. Therefore, by calculating the required time of nucleation and growth of an independent crystal, they denoted that CaC_2O_4 crystals are impossible to form urinary stones. However, urinary crystallite can agglomerate quickly in a short time. Thus, its size could increase rapidly in a short time⁹. Therefore, the ability of affecting the formation of urinary stone in a descending order is as follows: agglomeration > nucleation > growth.

Effect of carboxylates on zeta potential of CaC_2O_4 crystallites: Two types of interaction force exist between two separated particles: electrostatic repulsion force (W_R) and Van der Waals' force (W_A)¹⁸. Given that W_R is a repulsive force and W_A is an attractive force, the total potential energy (W_T) in the interaction between two particles is as follows:

$$W_T = W_A - W_R \quad (6)$$

when $W_A > W_R$, the total potential energy $W_T > 0$ and crystals tend to agglomerate; when $W_T < 0$, the crystals will disperse instead of agglomerating. W_R is closely related to the surface charges of particles. According to the colloidal theory established by Nernst-Stern-Gouy, the electric double layer can be formed on the charged particle surface between liquid and solid phases and the electric potential difference can be expressed by the zeta potential. A higher concentration of anions in the solution results in a stronger absorption force of the crystallites and the more anions are adsorbed onto the particle surface. The charge density also increases and the zeta potential becomes more negative. The repulsive force W_R also increases.

Fig. 2B shows that the absolute value of the zeta potential of CaC_2O_4 crystallites increased first in different systems and then decreased. The zeta potential reached maximum value at 20 min, which were -10.5, -12.8 and -14.9 mV in the presence of 2.0 mmol/L of NaGly, Na_2Tart and Na_3Cit respectively. These values are all smaller than that of the control group (-9.7 mV). This finding indicates that the repulsive force of CaC_2O_4 crystallites in presence of inhibitors is larger than that of the control and the CaC_2O_4 crystallites formed in the presence of inhibitors are more dispersed than those of the controls. At the same reaction time, the absolute value of Zeta potential of CaC_2O_4 crystallites in the presence of various inhibitor follows the order: $\text{Na}_3\text{Cit} > \text{Na}_2\text{Tart} > \text{NaGly} > \text{controls}$.

Comparison of system stability after adding different carboxylates: In a stable colloidal system, the light intensity autocorrelation curve is a smooth exponential decay curve at an autocorrelation decay time (Γ_a)¹⁹. The decay rate of the light intensity autocorrelation function is inversely proportional to the particle diameter (d), that is, the smaller the particle diameter is, the quicker the decay of autocorrelation curve is and the shorter the Γ_a is. On the contrary, the larger the particle diameter is, the slower the decay of autocorrelation curve is and the longer the Γ_a is.

Fig. 2C shows the variation of Γ_a in the presence of 2.0 mmol/L of NaGly, Na_2Tart and Na_3Cit . Γ_a gradually increased

within 1-20 min and reached the maximum at 20 min, in which $\Gamma_a = 35.5$, 24 and 18.5 ms, respectively. This result shows that the crystallite size continuously increased. The decrease slightly in Γ_a after 20 min indicated a decrease of the crystallite size. This result is consistent with the variation of the size of the crystallites in Fig. 2(A).

Fig. 3 shows the changes of the autocorrelation decay curves of CaC_2O_4 crystallites after adding 2.0 mmol/L Na_2Tart into CaC_2O_4 supersaturated solution. These autocorrelation curves are un-smooth decay curves, indicating the larger change of crystallite particle sizes in the system, that is, the system is unstable. Within 1-20 min, the autocorrelation curve in the range of $\Gamma_a = 9.4$ -24 ms showed many small bumps (Fig. 3a-d). This bumps occurred because the CaC_2O_4 microcrystallites continued to increase. Fig. 2A shows that the particle size increased from 619 nm to 949 nm. After 20 min (Fig. 3e-f), although the autocorrelation curves still had small bulges, the curves were smoother compared with that before 20 min (Fig. 3a-c). Γ_a decreased from 24 ms at $t = 20$ min to 5.8 ms at $t = 60$ min. This finding denotes that the system tends to be stabilized gradually.

The effect of different inhibitors on light intensity autocorrelation curve is different. Γ_a of crystallite after adding Na_3Cit was significantly less than that after adding Na_2Tart , NaGly and the control. The particle size of CaC_2O_4 crystallite formed in the presence of 2.0 mmol/L Na_3C was smaller than that in the presence of Na_2Tart , NaGly, or the control. The inhibition ability of Na_3Cit to the growth and aggregation of CaC_2O_4 crystallite was stronger than that of Na_2Tart and NaGly. The following properties enable Na_2Tart and Na_3Cit to inhibit the growth of CaC_2O_4 : These carboxylates can attach on the active growth sites of CaC_2O_4 crystallites, thereby restraining the growth and aggregation of CaC_2O_4 crystallites. Specifically, Cit^{3-} and Tart^{2-} have more than two carboxyl groups and more than one hydroxyl group and the distance between the two carboxyl groups is equal to the length of three C-C bonds. This molecular structure makes these carboxylates strongly absorbed on the surface of CaC_2O_4 crystallites²⁰⁻²². This structure not only prevents Ox^{2-} from migrating to the crystal surface, thus inhibiting crystal growth, but also makes the negative charges on the crystallite surface and the repulsive force between crystals to increase, thus inhibiting crystallite aggregation. Given that oxalic acid is a binary weak acid, the acid effect of H_2Ox on the crystallization kinetics of CaC_2O_4 cannot be ignored. The following chemical balances exist in the supersaturated solution of CaC_2O_4 :

Considering that H^+ can combine with Ox^{2-} in the solution to form HC_2O_4^- , the $\text{C}_2\text{O}_4^{2-}$ concentration decreases and the reaction of $\text{Ca}^{2+} + \text{C}_2\text{O}_4^{2-} \rightleftharpoons \text{CaC}_2\text{O}_4$ moves to left. Therefore, CaC_2O_4 crystallites are slowly dissolved and the particle size gradually decreases. Thus, the weak acidic environment (pH 6) in this experiment is beneficial to restrain the formation of CaC_2O_4 crystallites.

Conclusion

The crystallization kinetics of CaC_2O_4 and the effects of monocarboxylate NaGly, dicarboxylate Na_2Tart and tricar-

Fig. 3. Autocorrelation decay curves of CaOxa crystallites over reaction time in RS = 15.61 CaOxa supersaturated solution in the presence of 2.0 mmol/L Na₂Tart. (a) 1; (b) 5; (c) 10; (d) 20; (e) 40; (f) 60 min

boxylate Na₃Cit in artificial urine were studied. In the absence of an inhibitor, the crystallization kinetics equation of CaC₂O₄ is $r = kc^{3.3}$, the average reaction order is $\bar{\alpha} = 3.3$ and the average reaction rate constant is $\bar{k} = 0.99 \times 10^9$. When NaGly, Na₂Tart and Na₃Cit were added, no significant effect exist on $\bar{\alpha}$, but $\bar{k}$ reduced to 1.68×10^7 , 9.76×10^5 and 2.29×10^5 , respectively, indicating that these carboxylates could reduce the reaction rate of CaC₂O₄ crystallization. The addition of different carboxylates could change the particle size, zeta potential and autocorrelation decay time of CaC₂O₄ crystallites. The more the number of carboxyl groups in carboxylates, the stronger the ability of the acid to inhibit the nucleation, growth and aggregation of CaC₂O₄ crystallites. The inhibiting ability of different sodium carboxylate follows the order Na₃Cit > Na₂Tart > NaGly > controls.

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