Influence of magnetic stirring, temperature and citrate concentration on the precipitation of calcium oxalate dihydrate

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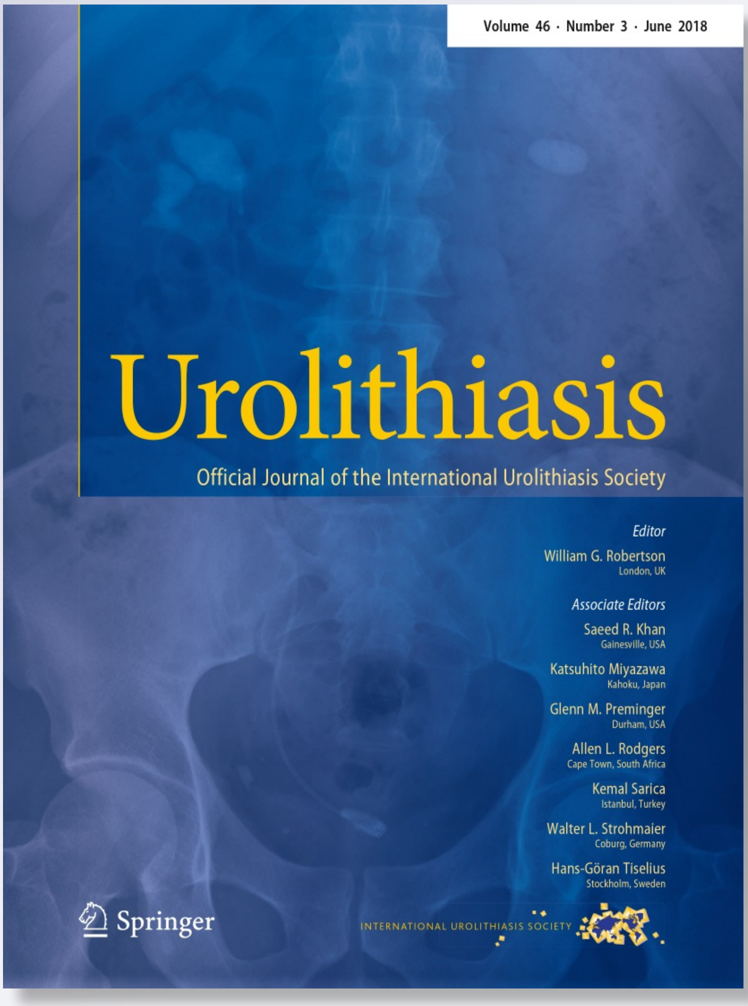

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ORIGINAL PAPER

The effect of hydrodynamic and thermodynamic factors and the addition of citric acid on the precipitation of calcium oxalate dihydrate

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Abstract This paper reports on the investigation of experimental conditions relevant for spontaneous precipitation of significant amount of pure calcium oxalate dihydrate (COD). For this purpose, the hydrodynamic and thermodynamic parameters, such as mode of agitation, temperature, supersaturation and concentration of additives (citrate ions), have been studied. The results show that in the model systems, without the citrate addition and applied mechanical stirring, calcium oxalate monohydrate (COM) was observed as dominant modification after 20 min of aging, while the magnetic stirring resulted in a formation of a mixture of COM and calcium oxalate trihydrate (COT), regardless of the temperature applied. In the mechanically stirred systems, the addition of citrate ions in the range of concentrations, 0.001 mol dm^{-3} < $c_i(Na_3C_6H_5O_7)$ < 0.012 mol dm^{-3}, caused the formation of COM and COD mixture at all temperatures. At the same conditions and in the magnetically stirred systems formation of COD, in a mixture with COT or COM, has been observed. The highest COD content in the mechanically stirred system was obtained at 45 °C and $c_i(Na_3C_6H_5O_7) = 0.001$ mol dm^{-3} ($w = 89.5\%$), while in the magnetically stirred system almost pure COD was obtained at 37 °C and $c_i(Na_3C_6H_5O_7) = 0.008$ mol dm^{-3} ($w = 96.5\%$).

Keywords Calcium oxalate monohydrate · Calcium oxalate dihydrate · Calcium oxalate trihydrate · Citrate · Temperature · Stirring

Introduction

Pathological biomineralization is a process of undesirable formation of slightly soluble inorganic salts in the soft tissues. Certainly, the most common incidence of pathological biomineralization is urolithiasis, which is a chronic disease that leads to the formation of calculi in different parts of the urinary tract [1]. Although urinary stones may be composed of calcium phosphates, magnesium ammonium phosphates, uric acid or ammonium hydrogen urate, the calcium oxalates are undoubtedly main inorganic component and their increased occurrence has been reported in industrialized countries. Calcium oxalate crystallizes in the form of hydrated salts: thermodynamically stable calcium oxalate monohydrate (COM, $CaC_2O_4 \cdot H_2O$, whewellite) [1, 2], metastable calcium oxalate dihydrate (COD, $CaC_2O_4 \cdot 2H_2O$, weddellite) [3–5] or calcium oxalate trihydrate (COT, $CaC_2O_4 \cdot 3H_2O$, caoxite) [6, 7]. It is generally accepted opinion that COM and COD are predominant hydrate modifications of oxalate stones, which can be formed, either in urine or at epithelial cell surfaces. Indeed, the COM has strong ability to bind at cell surfaces covered with arrays of different organic molecules and thus become an aggregation center for stone formation. The analyses of COM renal stones, which are actually organic–inorganic composites, showed that the organic matrix consisted from more than 60 low molecular or macromolecular constituents [8]. It should also be emphasized that at physical chemical conditions prevailing in the urine, the nucleation of calcium oxalates is heterogeneous process, so the predictability of

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their formation is doubtful and slightly reproducible. On the contrary to COM and COD, the incidence of COT occurrence, as well as its role in the urinary stone formation, is disputable. Although COT occurrence in urinary sediments is common, just few occurrences in stones are reported. On the other hand, it was demonstrated in the literature that significant number of predominantly COM calculi are covered with thin layer of COT crystals thus pointing to its possible role in formation of specific class of calculi. Indeed, this type of less dense COM calculi is probably formed by transformation of COT and COD into COM [9, 10].

Formation of the calculi in the urinary tract involves the processes of nucleation, followed by the growth, the aggregation and the transformation of the primary crystallites. A principle parameter that governs each precipitation process is a thermodynamic driving force for transition or specific component (salt) from solution into the solid phase, conveniently expressed as supersaturation. Therefore, the concentrations of constituent ions, calcium and oxalate, are supposed to predominantly influence the number, size distribution, morphology and formation of certain hydrate modification. However, the calcium oxalate precipitation in urinary tract usually started at conditions far from equilibrium and three solid phases may nucleate (COM, COD, COT), so the interactions of several elementary precipitation processes (nucleation, growth, dissolution and/or aging or aggregation) take place simultaneously. The additional parameter that could significantly influence the precipitation is a presence of dissolved or suspended impurities or additives. Indeed, the urine contains vast number of various low molecular weight and macromolecular constituents, which could inhibit or promote the mentioned processes and thus effect the formation of calculi. Among the low molecular weight inhibitors, the citric acid is certainly the most effective one [10]. Thus, it has been reported that citric acid inhibits the nucleation [11], growth [12] as well as the aggregation [13] of COM, which is the most common and abundant mineral component in kidney stones. Consequently, the citrate salts have been widely used to prevent nephrolithiasis [15]. At that, the inhibition of the calcium oxalate nucleation in the urine by citrate ions is related to their high complexation affinity for Ca^{2+} and consequently, the lowering of the relative supersaturation of the urine. However, citrate ions also inhibit the growth and aggregation of already formed crystals, by strong adsorption on their surfaces [16], while likely incorporation of citrate ions into the crystal cell of respective oxalate salt may increase their solubility and, in such a way, alter the supersaturation.

Although the COD is a common constituent of urinary stones, the mechanism and the conditions for its formation are still not fully understood. Hence, there is a pronounced research interest for the process of COD precipitation, as well as the efforts to clarify its role in the pathophysiology of a stone formation, particularly in the context of their interactions with aggregation inhibitors, like citrate ions, that are regularly present in the urine of healthy persons [17, 18]. At that, the spontaneous in vivo precipitation experiments of COD in the complex precipitation systems, like artificial urine, or at supersaturation conditions at which other modifications could nucleate as well, are of limited applicability. Therefore, a convenient approach to study the behavior of COD at controlled physical and chemical conditions is the inoculation of moderately supersaturated solution with well-defined crystal seed materials. Since the preparation of the pure COD, suitable for in vitro seeding precipitation experiments, is rather difficult and challenging [6, 19, 20], the principle aim of this work is to propose a simple and reproducible experimental protocol(s) for obtaining significant amount of well-defined and well-characterized seed materials, by systematically changing the hydrodynamics, temperature and the concentration of citrate ions in the precipitation systems of apparently identical supersaturation.

Experimental

Materials

All stock solutions used for precipitation experiments were prepared from analytical grade chemicals. Calcium chloride dihydrate, $CaCl_2 \cdot 2H_2O$; sodium chloride, NaCl; and sodium oxalate, $Na_2C_2O_4$ were supplied by BDH Prolabo, while trisodium citrate dihydrate, $Na_3C_6H_5O_7 \cdot 2H_2O$, was purchased from Sigma-Aldrich. Deionized water (conductivity $\leq 0.055\ \mu S/cm$) has been used for dilutions, while the stock solutions were standardized by means of respective analytical methods (e.g., oxalate was determined by titration with a permanganate and calcium by complexometric titration) or by means of the ion chromatography (Dionex ICS-1100) and using the respective standards supplied by the manufacturer.

Synthesis and calculations

The initial Ca^{2+} and $C_2O_4{}^{2-}$ concentrations in all precipitation systems were identical: $c_i(Ca^{2+}) = 0.0390\ mol\ dm^{-3}$, $c_i(C_2O_4{}^{2-}) = 0.0049\ mol\ dm^{-3}$. To obtain higher and approximately constant ionic strength ($I = 0.3\ mol\ dm^{-3}$), NaCl solution has been added into both calcium and the oxalate components while the respective concentration of citrate ions has been adjusted by the addition of trisodium citrate solution ($Na_3C_6H_5O_7$) into the oxalate component. In the systems in which the citrate has been added, its concentration varied in the range $0.001\ mol\ dm^{-3} < c_i(Na_3C_6H_5O_7) < 0.012\ mol\ dm^{-3}$. The initial pH of both solutions (pH 6.5) was adjusted using the HCl or NaOH standards.

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The solutions were thermostated at respective temperature (20, 25, 37 or 45 °C).

The calculations of the solution composition (molar concentrations and activities of the relevant ionic species) and the initial supersaturation have been performed using the algorithm developed within this laboratory and considering the initial known concentrations of reactants, as well as the respective protolithic equilibria, equilibrium constants, charge and mass balance equations. The supersaturations, expressed with respect to specific calcium oxalate hydrate modification, have been defined as supersaturation ratio, $S_\mathrm{i} = (\Pi/K^0_\mathrm{sp})^{1/2}$, where Π is the ion activity product, $\Pi = a(\mathrm{Ca}^{2+})\cdot a(\mathrm{C_2O_4}^{2-})$, and K^0_sp is the thermodynamic equilibrium constant of dissolution of the particular $\mathrm{CaC_2O_4}$ hydrate phase (COM, COD or COT). The detailed calculation procedure, which takes into account the respective protolithic equilibria and equilibrium constants, as well as the charge and mass balance equations, has been described previously [5, 18, 28]. The applied concentrations of reactants in the respective precipitation systems (P1, P2, P3, P4, P5) and the calculated initial supersaturations are summarized in Table 1. Since the increase of temperature just slightly decreases the supersaturation in the respective precipitation systems, only values at 20 °C are shown.

The precipitation of the respective calcium oxalate hydrate modification has been initiated by fast mixing of the cationic (Ca^{2+}) and anionic ($\mathrm{C_2O_4}^{2-}$) solutions. Precipitation experiments were performed in a double-walled glass reactor, at selected temperature. A combined glass electrode, connected to a pH meter (HANNA HI 5522) was used to measure the solutions pH and to follow a progress of precipitation. Three-point calibration was performed using the standard buffer solutions (pH 4.01, 7.01 and 10.01). The precipitation systems were agitated, either mechanically or magnetically: in the mechanically stirred systems (4-blade glass propeller), the speed was maintained at constant rate, $n = 240\ \mathrm{min}^{-1}$. When magnetic stirring was applied, a Teflon-coated stirring bar was used at constant stirring speed, $n = 120\ \mathrm{min}^{-1}$. The suspensions obtained after 20 min have been filtered through a 0.22-μm membrane filter, the precipitates were washed with small portions of water and dried for 1 h in vacuum.

Characterization

To determine a mineralogical composition of each precipitate, the IR spectra of dry samples were recorded by means of Shimadzu FT-IR 8400S spectrophotometer, using the DRS 8000 attachment in the 4000–400 cm^{-1} spectral region and resolution of 4 cm^{-1}. For this purpose, a small amount of the sample (approximately 1–2 mg) was thoroughly mixed with KBr (IR grade), while data analysis has been performed using the software package IR Solution 1.30, supplied by the manufacturer.

Thermogravimetric analysis (TGA) of dried samples has been performed to determine the weight fraction of each hydrate form, using a simultaneous TGA-DSC analyzer (Mettler-Toledo TGA/DSC 1). Each sample (up to 10 mg) was placed in the aluminum pan (100 μl) and heated in the oxygen up to 300 °C at a rate of 5 °C min^{-1}. The data collection and the analysis were performed using the program package STARe Software 10.0.

The particles size distribution (PSD) of crystals obtained in the respective experiments has been estimated, either using the SEM and light microscopy or using the electronic counting device (Coulter Counter, Multisizer II) fitted with 50- or 100-μm tubes, which enable the measurements of suspended precipitate in the size range from about 1.5–85.0 μm. The morphology of precipitate has been determined using high-resolution scanning electron microscopy (FE-SEM, model JEOL JSM-7000F).

Results and discussion

The literature data indicated that exclusive COD preparation in chemically complex precipitation systems, like in

Table 1 The initial concentration of reactant in the respective precipitation systems

Precipitation system	P1	P2	P3	P4	P5
$c_i(\mathrm{CaCl_2})$, mol dm^{-3}	0.0390	0.0390	0.0390	0.0390	0.0390
$c_i(\mathrm{Na_2C_2O_4})$, mol dm^{-3}	0.0049	0.0049	0.0049	0.0049	0.0049
$c_i(\mathrm{Na_3C_6H_5O_7})$, mol dm^{-3}	0	0.001	0.004	0.008	0.012
$c_i(\mathrm{Ca}^{2+})/c_i(\mathrm{Na_3C_6H_5O_7})$	0	39.00	9.75	4.87	3.25
S_i(COM)	34.7	34.6	34.0	33.2	32.2
S_i(COD)	24.1	24.0	23.6	23.0	22.3
S_i(COT)	21.2	21.1	20.7	20.2	19.6

The ionic strength was adjusted by the addition of NaCl ($c = 0.30$ mol dm^{-3}), while the initial pH by the appropriate amount of HCl or NaOH solutions (pH_i 6.5). The calculated initial supersaturations (S_i), expressed with respect to specific calcium oxalate hydrate, correspond to 20 °C

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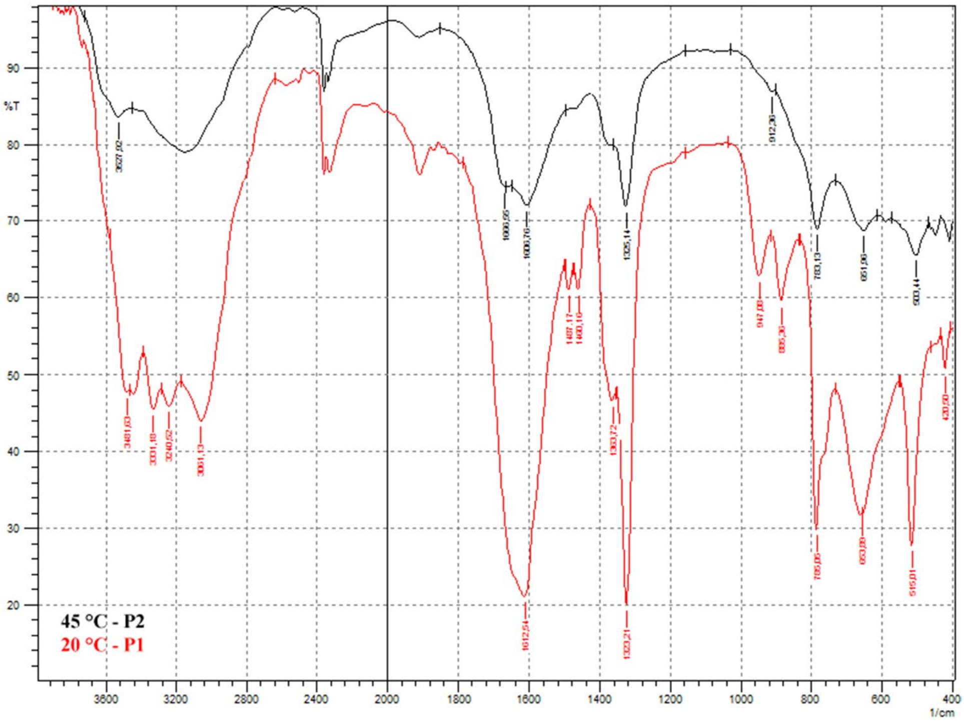

Fig. 1 FT-IR spectra of calcium oxalate samples obtained after 20 min, in the mechanically stirred systems and initial concentration of reactants. $c_i(\mathrm{CaCl_2})$ = 0.0390 mol $\mathrm{dm^{-3}}$ and $c_i(\mathrm{Na_2C_2O_4})$ = 0.0049 mol $\mathrm{dm^{-3}}$: P1 [t = 20 °C, c(citrate) = 0 mol $\mathrm{dm^{-3}}$] is a pure COM, while P2 [t = 45 °C, c(citrate) = 0.001 mol $\mathrm{dm^{-3}}$] is a mixture of COM and COD

artificial urine, might be promoted at high calcium to oxalate ratio, increased ionic strength, low temperature or presence of citrate ions [6]. In this study, relatively simple model systems have been investigated to identify the optimal experimental conditions for formation of calcium oxalate hydrates, particularly COD, and to discriminate the role of temperature, mode of stirring and the addition of additives. At that, the citrate ions have been selected as a known and effective additive that could inhibit the overall precipitation of calcium oxalates or, indirectly, promote a formation of specific hydrate modification. Thus, by applying either mechanical or magnetic agitation in the precipitation system, the temperature has been set to $t = 20$, 25, 37 or 45 °C, while the concentration of citrate was either $c = 0.001$, 0.004, 0.008 or 0.012 mol $\mathrm{dm^{-3}}$. Since it is known that citrate strongly complexes the calcium ions [12–14], its concentration is conveniently expressed as a ratio with respect to total calcium added into the system $[c_i(\mathrm{Ca^{2+}})/c_i(\mathrm{Na_3C_6H_5O_7})]$. The initial concentrations of the precipitating components have been set as $c_\mathrm{i}(\mathrm{CaCl_2})$ = 0.0390 mol $\mathrm{dm^{-3}}$, $c_i(\mathrm{Na_2C_2O_4})$ = 0.0049 mol $\mathrm{dm^{-3}}$, and c(NaCl) = 0.30 mol $\mathrm{dm^{-3}}$. However, in the referent systems, no citrate has been added. The formation of specific calcium oxalate hydrates has been confirmed by comparing the IR spectra of respective samples with the literature data [23–25] and recorded spectra of standards, while the thermogravimetric analyses have been applied for quantitative analyses: theoretical mass losses for crystalline water in pure COM is 12.33%, for COD is 21.96% and for COT is 29.67% [21, 22].

Table 2 Typical IR vibration modes of calcium oxalate monohydrate (COM) dihydrate (COD) and trihydrate (COT) [19–21]

COM ($\mathrm{cm^{-1}}$)	COD ($\mathrm{cm^{-1}}$)	COT ($\mathrm{cm^{-1}}$)	Vibrational mode
3490	3560	3530	O–H stretching
1622	1636	1630	C–O antisymmetric stretching
1316	1324	1375	C–O symmetric stretching
947	912		H–O–H bending
782	782	783	O–C–O antisymmetric bending
–	610	651	H–O–H bending
517	500	505	O–C–O antisymmetric rocking
–	–	425	O–C–O antisymmetric wagging

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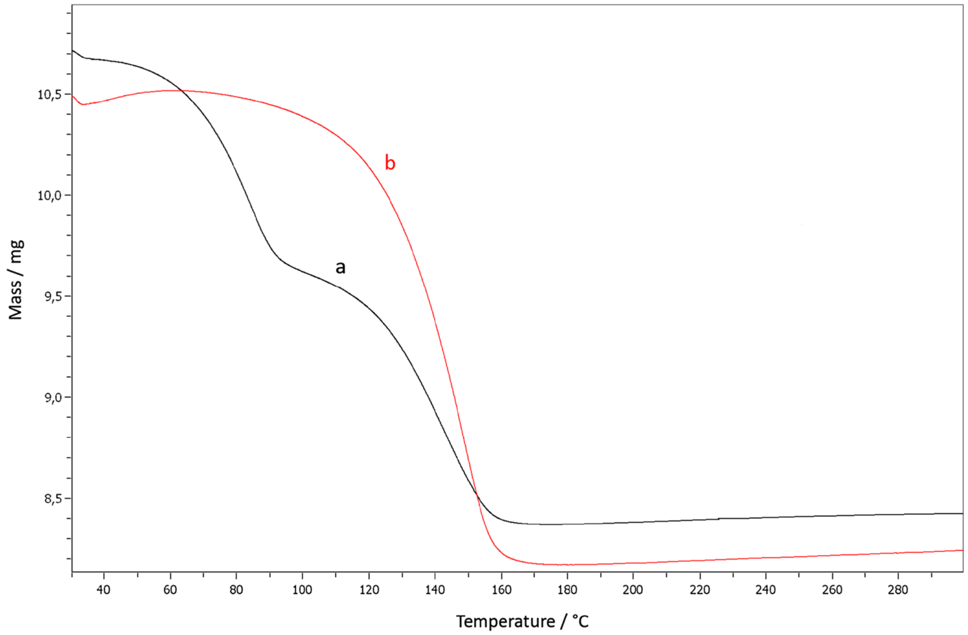
Fig. 2 Thermograms of the samples P1 (*b*) and P2 (*a*) containing COM and mixture of COM and COD, respectively. The total mass loss of P1 after heating to 300 °C is about 11.47%, which corresponds to one crystalline water molecule, while sample P2 lose about 20.95%, which corresponds to about 89.5% of COD in mixture



Mechanically stirred system

The results of structural analyses of samples obtained after 20 min in the mechanically agitated systems indicated that in the entire experimental domain, mixture of COM and COD precipitated. However, in the systems with no citrate addition and at all temperatures, only COM appeared, as confirmed with IR spectroscopy and respective standard spectra, as well as with thermogravimetric analysis. Figure 1 shows typical FT-IR spectra of dry COM samples, collected for the P1 [$t = 20$ °C, $c(\text{citrate}) = 0$ mol dm^{-3}] and P2 [$t = 45$ °C, $c(\text{citrate}) = 0.012$ mol dm^{-3}] in which the highest fraction of COD (89.5%) has been determined, while Table 2 shows the vibration modes of COM, COD and COT documented in the literature [19–21]. Thus, the existence of COD in P2 could be confirmed with a single broad absorption peak at about 3530 cm^{-1} which is characteristic of O–H vibration mode of COD, the antisymmetric and symmetric stretching vibrations of C–O group which appear at about 1605 and 1320 cm^{-1} and a characteristic COD absorption at about 913 cm^{-1}.

However, the exact mass fractions of COD in the precipitate have been determined by means of thermogravimetric analysis of dried samples: Fig. 2 shows the thermograms of the samples P1 [$t = 20$ °C, $c(\text{citrate}) = 0.0$ mol dm^{-3}] and P2 [$t = 45$ °C, $c(\text{citrate}) = 0.001$ mol dm^{-3}]. Thermogram of the P1 indicates that mass loss occurred during just one step and started at about 67 °C (inflection at 156 °C), while two distinct steps have been observed for sample P2: loss of the first water molecule of COD that started at about 40 °C (inflection at 85 °C) and for the second molecule started at about 100 °C (inflection at 145 °C). Total mass

Table 3 Content of calcium oxalate hydrates, determined by thermogravimetric analysis, and the average size of crystals precipitated after 20 min in the mechanically stirred systems at respective temperatures and citrate concentration

c(citrate), mol dm^{-3}	t, °C	w(COM), %	w(COD), %	d_{COM}, µm	d_{COD}, µm
0	20	100	0	10	0
	25	100	0	10–20	0
	37	100	0	10–20	0
	45	100	0	10–20	0
0.001	20	68.7	31.3 ± 5.0	2	10
	25	71.7	28.3 ± 5.4	3	20
	37	65.1	34.9 ± 0.4	5	20–30
	45	16.5	83.5 ± 5.9	5–10	20–30
0.004	20	69.6	30.4 ± 3.5	3	10
	25	59.9	40.1 ± 6.0	3	15
	37	34.5	65.5 ± 1.9	3	20–30
	45	60.7	39.3 ± 1.7	3	20–30
0.008	20	67.1	32.9 ± 2.4	3	10
	25	63.3	36.7 ± 4.0	3	10
	37	53.9	46.1 ± 7.1	3	20
	45	40.7	59.3 ± 4.5	3	20–30
0.012	20	56.0	44.0 ± 2.0	2	10
	25	42.9	57.1 ± 3.2	3	15
	37	51.2	48.8 ± 2.5	3	20–30
	45	52.9	47.1 ± 5.9	3	20–30

The initial concentrations of reactants were $c_i(\text{CaCl}_2) = 0.0390$ mol dm^{-3} and $c_i(\text{Na}_2\text{C}_2\text{O}_4) = 0.0049$ mol dm^{-3}, while $c(\text{NaCl}) = 0.30$ mol dm^{-3}. In all systems a mixture of COM and COD precipitated. The values of COD content are the average of three independent preparations. Distribution of data is shown

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loss of sample P1, after heating at up to 300 °C was found to be about 11.47%, which corresponds to approximately one crystalline water molecule in COM, while sample P2 lost about 20.95%, which indicates that the mixture of calcium oxalate hydrates contained about 89.5% of COD.

The results of structural and PSD analyses of calcium oxalate precipitate in the mechanically agitated systems are summarized in Table 3 and Fig. 3. It could be seen that in the model system (no citrate addition) COM was formed at all temperatures applied. However, the addition of citrate ions caused the formation of COD in mixture with COM, though the highest COD content ($w = 89.5\%$) was obtained at 45 °C and lowest citrate concentration. Since the applied supersaturation facilitates the heterogeneous process of nucleation, which is inherently less reproducible than homogeneous, the experiments have been repeated in triplicate and the average values for COD content are shown: the error bars indicate the distribution of individual data. In addition, the calculated initial supersaturations (values shown in Table 1) just slightly change with temperature and citrate concentration and, accordingly, no straightforward correlation between supersaturation and COD content

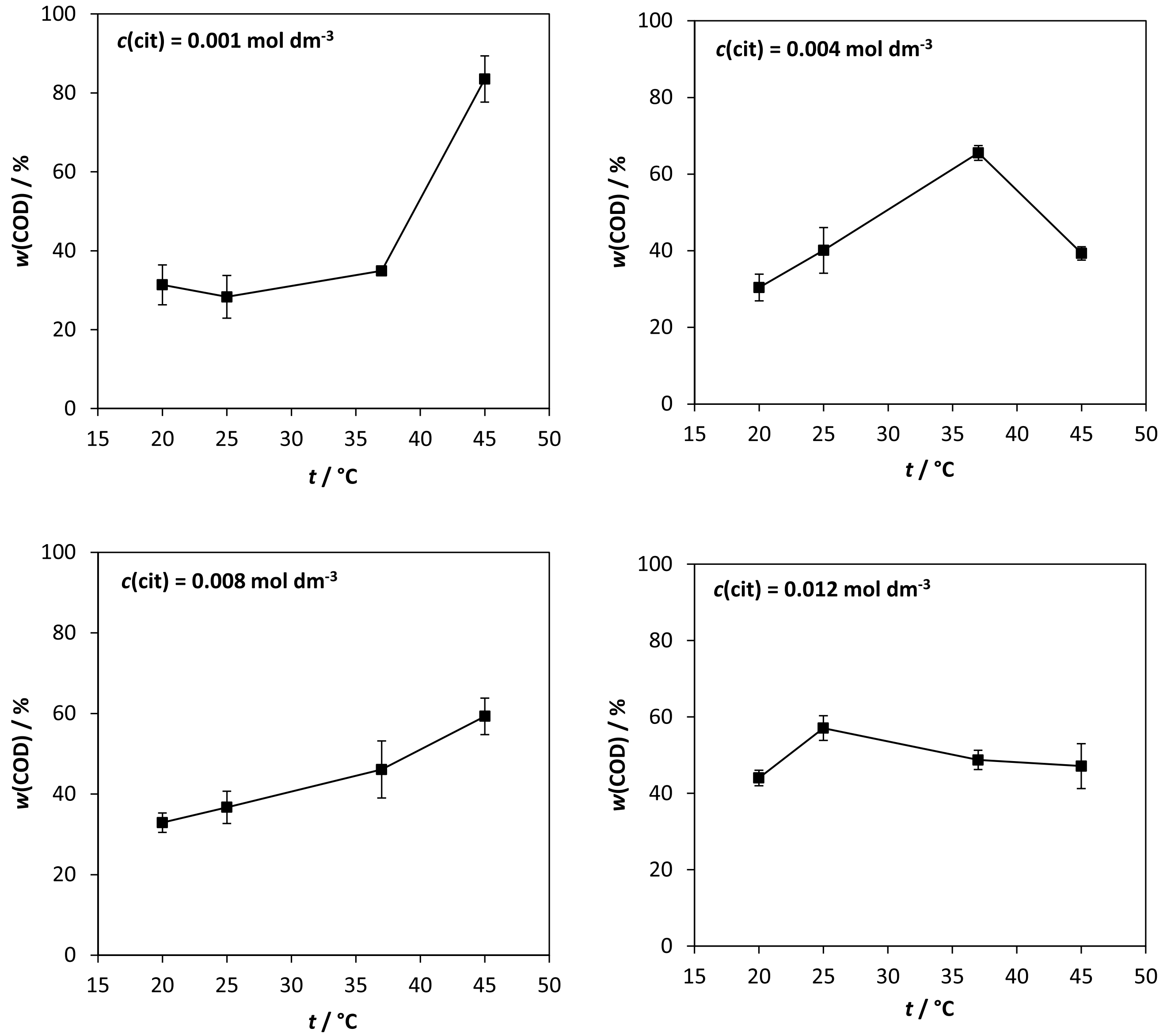

Fig. 3 Content of COD precipitated after 20 min in the mechanically stirred systems and different citrate concentrations. The initial concentrations of reactants were $c_i(\mathrm{CaCl_2}) = 0.0390$ mol dm^{-3}, $c_i(\mathrm{Na_2C_2O_4}) = 0.0049$ mol dm^{-3} and $c(\mathrm{NaCl}) = 0.30$ mol dm^{-3}. In all systems mixture of COM and COD precipitated. The values of COD content are the average of three independent preparations, while the *error bars* indicate the distribution of data

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could be set. Therefore, it could be concluded that the citrate ions do not act simply as complexing agents, which lower the supersaturation, but probably they specifically interact with particular solid phases, by either inhibiting or promoting the nucleation or crystal growth. It should be emphasized that in the precipitation systems in which solution is initially supersaturated with respect to several solid phases (polymorphs or hydrates), nucleation of less stable modification is kinetically favored. Its subsequent solution-mediated transformation into the thermodynamically stable forms assumes simultaneous nucleation and/or growth of stable and dissolution of metastable forms. Indeed, in this particular case, the highest fraction of metastable COD obtained exactly at the lowest concentration of citrate ions [c(citrate) $= 0.001$ mol dm^{-3}, $t = 45$ °C] implicate that, most probably, the nucleation and growth of stable COM are inhibited by the adsorption of citrate ions on its surfaces [11–14]. The obtained nonlinear relationship between the amount of precipitated COD and temperature, observed for lowest citrate concentrations ($c = 0.001$ mol dm^{-3} and $c = 0.004$ mol dm^{-3}), is consistent with some literature data. It was shown previously in the COM systems that the addition of strong growth and agglomeration inhibitors, such as citric acid or Mg^{2+}, exerts the maximum efficiency at certain intermediate concentration [26]. Similar “nonlinear effects” have been observed for COD precipitation in the presence of tartaric acid and different counterions [25, 27], which confirms the high complexity of interactions of thermodynamic, hydrodynamic and kinetic parameters in CaOx precipitation systems. The addition of citrate to the systems of two or three solid phases, having significantly different ability to adsorb it (COM > COT ≫ COD), additionally complicate the possible interactions and obviously inhibit the formation of COM [25, 27]. Consequently, the

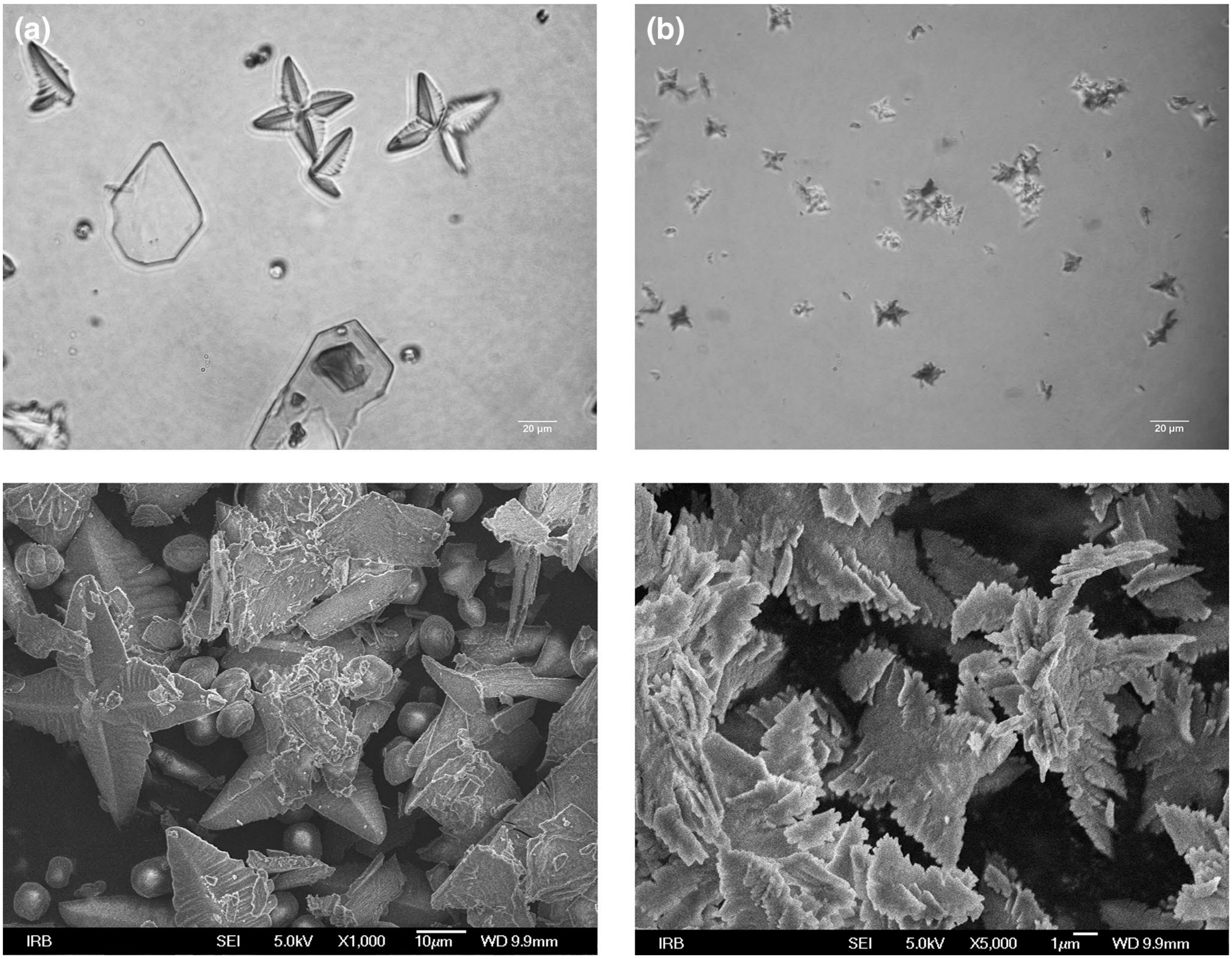

Fig. 4 **a** Typical light microscopy (*top*) and SEM images (*bottom*) of calcium oxalate hydrates precipitated in the mechanically stirred systems at $t = 45$ °C and c(citrate) $= 0.001$ mol dm^{-3}. The initial concentrations of reactants were $c_i(CaCl_2) = 0.0390$ mol dm^{-3}, $c_i(Na_2C_2O_4) = 0.0049$ mol dm^{-3} and $c(NaCl) = 0.30$ mol dm^{-3}. **b** Model system: $t = 20$ °C, no citrate

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nucleation and growth of less stable modifications, COD in this particular case, is promoted. Therefore, it may be concluded that the interactions between applied experimental parameters (temperature and concentration of citrate) are difficult to be predicted and no extrapolation of obtained data is possible, so the proposed empirical approach for optimization of COD preparation is justified.

The PSD analyses of COD crystals indicate that their average size at respective citrate content regularly increases with increasing the temperature: in all systems, the smallest COD crystals, as observed with SEM, precipitated at 20 °C ($d \approx 10$ μm), while at 45 °C they are larger ($d \approx 20$–30 μm). Similar trend of increasing the crystal size with increasing the temperature is observed for COM crystals as well.

Typical light and scanning electron micrographs of calcium oxalate hydrates precipitated in the mechanically stirred systems are shown in Fig. 4. Thus, in the system in which the highest content of COD has been obtained, $t = 45$ °C and $c(\text{citrate}) = 0.001$ mol dm^{-3}, three types of crystals are observed: small COM spherulites, medium-sized weddellite-type COD (four-bladed dendrites) and large atypical COM prisms [29]. The micrographs of the precipitate obtained in the respective model system (no citrate) show only the plate-like COM dendrites (Fig. 4b). The PSD analysis shows the calcium oxalates precipitated at $t = 45$ °C and $c(\text{citrate}) = 0.001$ mol dm^{-3}. Figure 5 shows that the average size (expressed as volume distribution) of the COM spherulites is about 8.3 μm, while diameter of weddellite-type COD crystals and COM prisms are about 16.6 and 24.8 μm, respectively. The micrographs of crystal's morphologies, assumed to correspond to certain size classes, are shown as the inserts above the respective distribution maximum. In the model system (no citrate) in which only COM precipitated, three distribution maxima could be observed at about 2.3, 4.1 and 6.3 μm. The inserted micrograph shows the fragmentation of COM dendrites, which caused the substantial widening of measured crystal size distribution. However, it should be emphasized that the apparent differences of PSDs obtained with microscopy and electronic counting device (coulter counter, CC) are caused by expressing the size of crystals as a diameter of equivalent sphere, which is a diameter of the sphere having the volume identical to the volume of specific particle. In the case of predominantly two-dimensional (plate like), or even one-dimensional (needle like) particles, the difference could be relatively high.

Magnetically stirred system

Structural analyses of samples obtained in the magnetically agitated systems showed that mixture of at least two calcium oxalate hydrated phases (COM, COD or COT) precipitated at all temperatures and citrate concentrations (Table 4; Fig. 6). Thus, a mixture of COM and COT appeared in the absence of citrate, while COD and COT precipitate at lowest concentration ($c(\text{citrate}) = 0.001$ mol dm^{-3}). On the contrary, when the highest citrate concentration has been applied ($c = 0.012$ mol dm^{-3}) COD is predominant modification which precipitated in a mixture with COM. It should also be emphasized that COD is the only modification that precipitated whenever the citrate ions have been present. Figure 7 shows typical FT-IR spectrum of the sample precipitated at $t = 20$ °C, $c(\text{citrate}) = 0$ mol dm^{-3}, (mixture of COM and COT) and a spectrum of the sample P4 [$t = 37$ °C, $c(\text{citrate}) = 0.008$ mol dm^{-3}] in which a fraction of COD was highest: $w(\text{COD}) = 96.5\%$. The spectra are compared with the vibrational modes of COM, COD and COT, given in Table 2 [23–25]. The thermograms of the sample P4 (Fig. 8, curve b) showed two distinct steps: first mass loss started at about 50 °C (inflection at about 91 °C) which corresponds to release of one water molecule and the second step, which started at about 118 °C

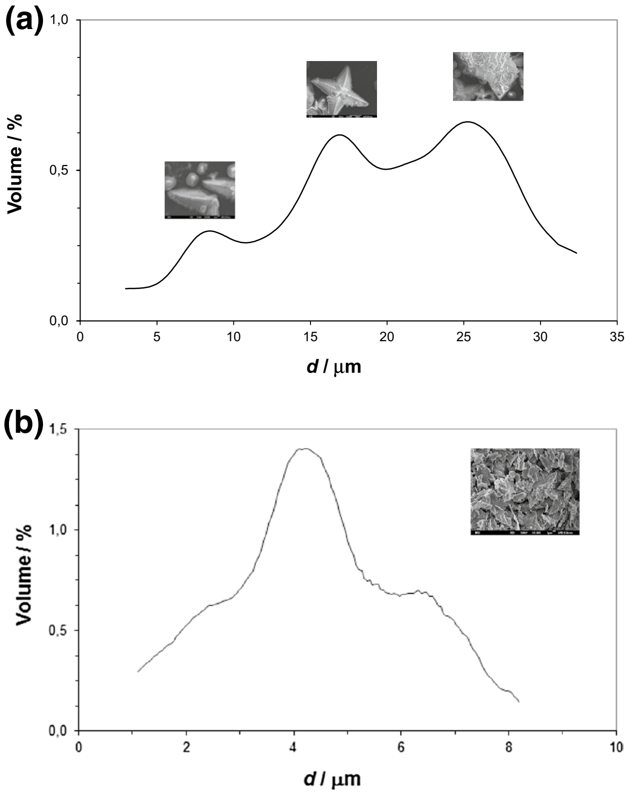

Fig. 5 **a** Particle size distribution (expressed as volume distribution) of calcium oxalate hydrate crystals, precipitated in the mechanically stirred system: $t = 45$ °C and $c(\text{citrate}) = 0.001$ mol dm^{-3}. The initial concentrations of reactants were $c_i(\text{CaCl}_2) = 0.0390$ mol dm^{-3}, $c_i(\text{Na}_2\text{C}_2\text{O}_4) = 0.0049$ mol dm^{-3} and $c(\text{NaCl}) = 0.30$ mol dm^{-3}. **b** PSD of fragmented COM plate-like dendrites precipitated in the system with no citrate addition. The crystal morphologies, assumed to correspond to specific size classes, are shown

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Table 4 Content of calcium oxalate hydrates, determined by thermogravimetric analysis, and the average size of crystals precipitated after 20 min in the magnetically stirred systems, at respective temperatures and citrate concentration

c_i(citrate), mol dm^{-3}	t, °C	w(COM), %	w(COD), %	w(COT), %	d_{COM}, μm	d_{COD}, μm	d_{COT}, μm
0	20	18.4	–	81.6	5	–	5
	25	28.1	–	71.9	5	–	5
	37	47.7	–	52.3	3	–	5
	45	45.0	–	55.0	5	–	20
0.001	20	–	5.4 ± 0.7	94.6	–	3	10–15
	25	–	45.8 ± 3.4	54.2	–	10	10
	37	–	12.2 ± 0.4	87.8	–	10	3–5
	45	–	23.5 ± 1.0	76.5	–	10–20	5
0.004	20	–	39.6 ± 7.5	60.4	–	10	10–15
	25	–	24.7 ± 3.2	75.3	–	10–20	10
	37	6.5	93.5 ± 1.9	–	3	10–20	–
	45	14.6	85.4 ± 6.3	–	3	10–20	–
0.008	20	–	40.3 ± 0.7	59.7	–	10–15	10
	25	–	41.1 ± 1.6	58.9	–	10–20	5–10
	37	8.2	91.8 ± 4.9	–	2	10–20	–
	45	33.2	66.8 ± 1.5	–	3	10–20	–
0.012	20	16.9	83.1 ± 3.8	–	3	10	–
	25	23.3	76.7 ± 0.2	–	3	10	–
	37	20.2	79.8 ± 7.1	–	3	10	–
	45	25.2	74.8 ± 0.6	–	3	10–20	–

The initial concentrations of reactants were $c_i(\mathrm{CaCl_2}) = 0.0390$ mol dm^{-3}, $c_i(\mathrm{Na_2C_2O_4}) = 0.0049$ mol dm^{-3} and $c(\mathrm{NaCl}) = 0.30$ mol dm^{-3}. The values of COD content are the average of three independent preparations. Distribution of individual data is shown

(inflection at 148 °C). Total mass loss of 21.62% is comparable to the theoretical values for pure COD (21.96%), thus indicating that predominantly COD is formed at given experimental conditions. However, the thermograms of the samples P1, which consists from a mixture of COM and COT (Fig. 8, curve a), shows the total mass loss after heating up to 300 °C of about 26.49%, which matches to about 81.6% COT.

Similar to the mechanically stirred systems, the results of structural analyses pointed to significant promotion of metastable hydrates formation (COD and COT), obviously caused by magnetically stirred suspension. Indeed, in our previous work the distribution of calcium oxalate phases, precipitated in the additive-free model systems ($c(\mathrm{Ca^{2+}}) \approx 5$ mM, $c(\mathrm{Ox^{2-}}) \approx 0.6$ mM), have been studied as a function of type and order of initial mixing of reactant solutions, type of stirring (mechanical and magnetic) and degree of grinding applied on surfaces of glass reactor [5]. It was shown that at a fixed stirring intensity, a critical parameter for COT nucleation was an abrasive (grinding) action on the base of glass crystallizer. Therefore, in this work the intensities in magnetically and mechanically stirred systems have been set just to keep suspension homogeneously distributed within the crystallizer and they were kept constant in all experiments. However, higher content of COD that is obtained in this study is obviously influenced by the addition of citrate ions, even at their lowest concentrations. The explanation of overall mechanism of formation of solid phases in systems in which three dominant parameters (supersaturation, hydrodynamics and citrate addition) influence the kinetics of basic precipitation process is rather complex: the additional difficulty is a similarity of COD and COT stability, as could be deduced from their fundamental thermodynamic properties (solubility). It should be pointed out that in all precipitation systems, the initial supersaturation was relatively high with respect to all modifications (Table 1). At such conditions, formation of new solid phases proceeded relatively fast and is observed as an abrupt formation of relatively large number of particles, usually metastable precursors.

The morphology and size distribution of calcium oxalates precipitated in the magnetically agitated system has been detected by means of light microscopy and SEM. Thus, Fig. 9a shows the typical micrographs of the sample in which almost exclusively precipitated COD [system: $c(\text{citrate}) = 0.008$ mol dm^{-3}, $t = 37$ °C, $w(\text{COD}) = 96.5\%$]. It could be seen that, unlike to mechanically stirred systems, COD appeared predominantly in a bipyramidal form,[1] while COM precipitated as spherulites. The PSD

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[1] Occasionally, COD appeared as a typical weddellite, four-bladed dendritic form.

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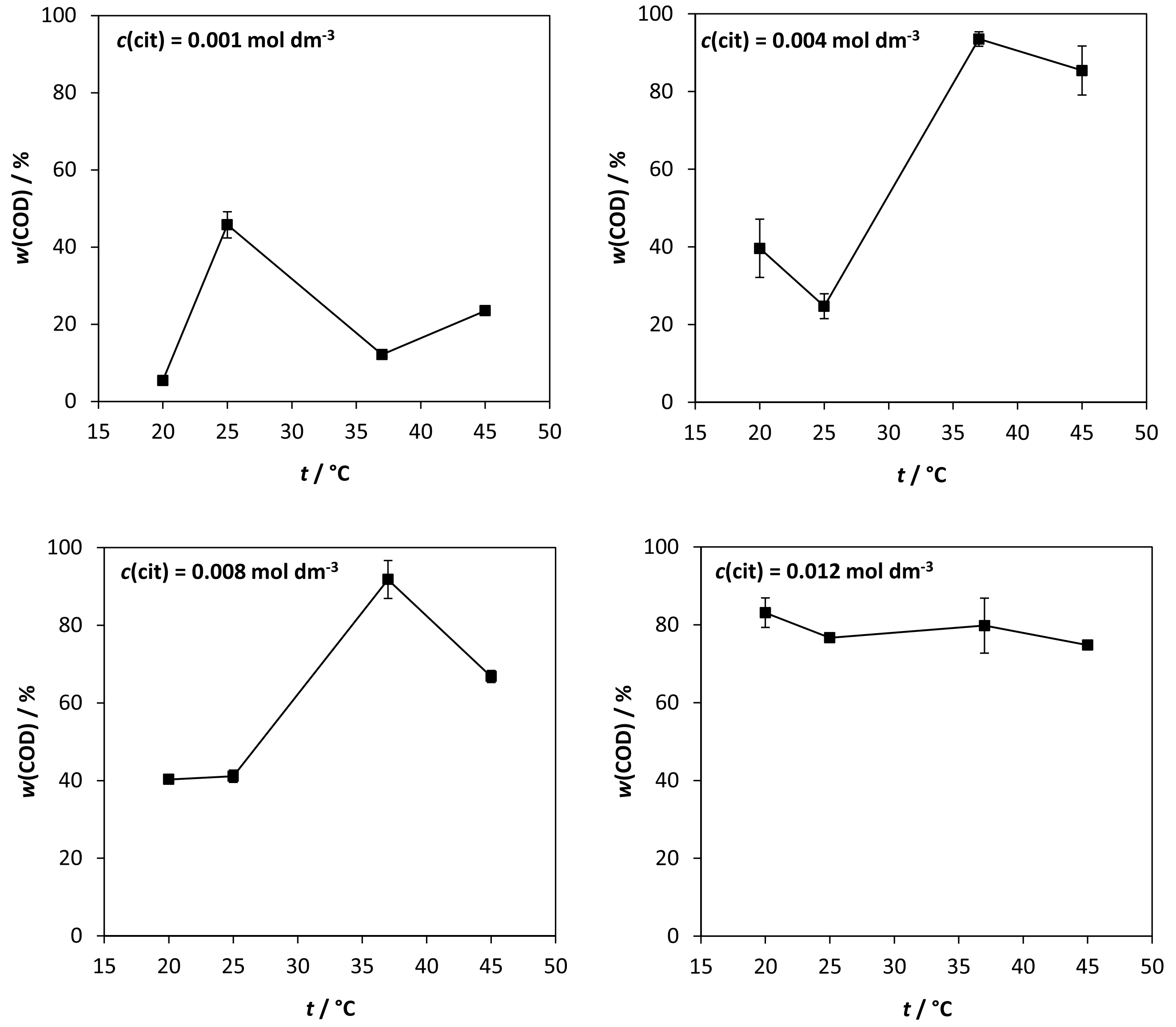

Fig. 6 Content of COD precipitated in a mixture with COM and COT after 20 min in the magnetically stirred systems and different citrate concentration. The initial concentrations of reactants were $c_i(\mathrm{CaCl_2}) = 0.0390\ \mathrm{mol\ dm^{-3}}$, $c_i(\mathrm{Na_2C_2O_4}) = 0.0049\ \mathrm{mol\ dm^{-3}}$ and $c(\mathrm{NaCl}) = 0.30\ \mathrm{mol\ dm^{-3}}$. The values of COD content are the average of three independent preparations, while the *error bars* indicate the distribution of individual data

analysis of the respective sample shows that at least three size classes could be detected (Fig. 10a). Of these, the smallest size class corresponds to COM spherulites, while larger classes correspond to COD bipyramidal crystals or agglomerates (the respective micrographs, corresponding to certain size classes, are shown as the inserts above the distribution maximum). However, a granulometric evaluation of COD crystals in all systems indicate that their average dimensions at respective citrate content regularly increase with increasing the temperature: the smallest COD crystals, as observed with light microscopy and SEM, precipitated at 20 °C ($d \approx 3$–$10\ \mu\mathrm{m}$), while at 45 °C they are larger ($d \approx 10$–$20\ \mu\mathrm{m}$). Figure 9b shows the morphology of the precipitate obtained in the respective model system (no citrate addition, $t = 20$ °C), in which mixture of COM and COT has been observed after predetermined time (w (COM) = 45%). Indeed, COM precipitated mostly in a prismatic or plate-like dendritic form, while the observed COM dendrites were predominantly broken, as well as needle-like COT crystals which is most probably a consequence of the intensive stirring and grinding with Teflon-coated magnetic bars [5]. The PSD of the same sample

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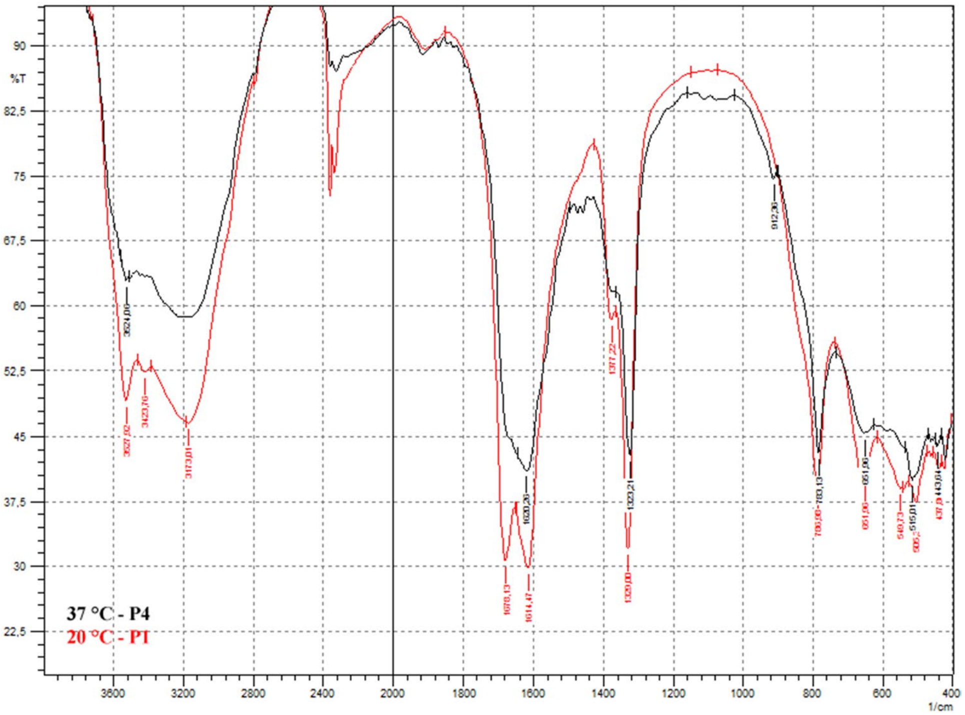
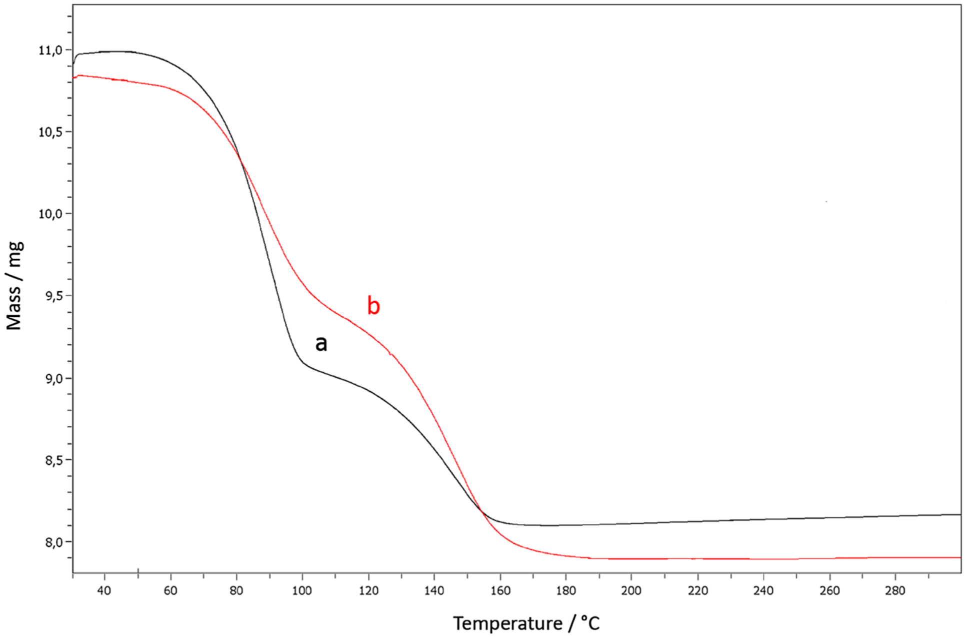


Fig. 7 FT-IR spectra of samples obtained after 20 min in the magnetically stirred systems and initial concentration of reactants, $c_i(\mathrm{CaCl_2}) = 0.0390$ mol dm^{-3} and $c_i(\mathrm{Na_2C_2O_4}) = 0.0049$ mol dm^{-3}: sample P1 ($t = 20$ °C, c(citrate) = 0 mol dm^{-3}) is a mixture of COM and COT, while P4 [$t = 37$ °C, c(citrate) = 0.008 mol dm^{-3}] is predominantly COD (w(COD) = 96.5%)

Fig. 8 Thermograms of the samples P1 (*a*) containing mixture of COM and COT and P4 (*b*) containing mixture of COM and COD. The total mass loss of P1 after heating to 300 °C is about 26.49%, while sample P4 loose about 21.62%, which corresponds to 96.5% of COD in a mixture with COM



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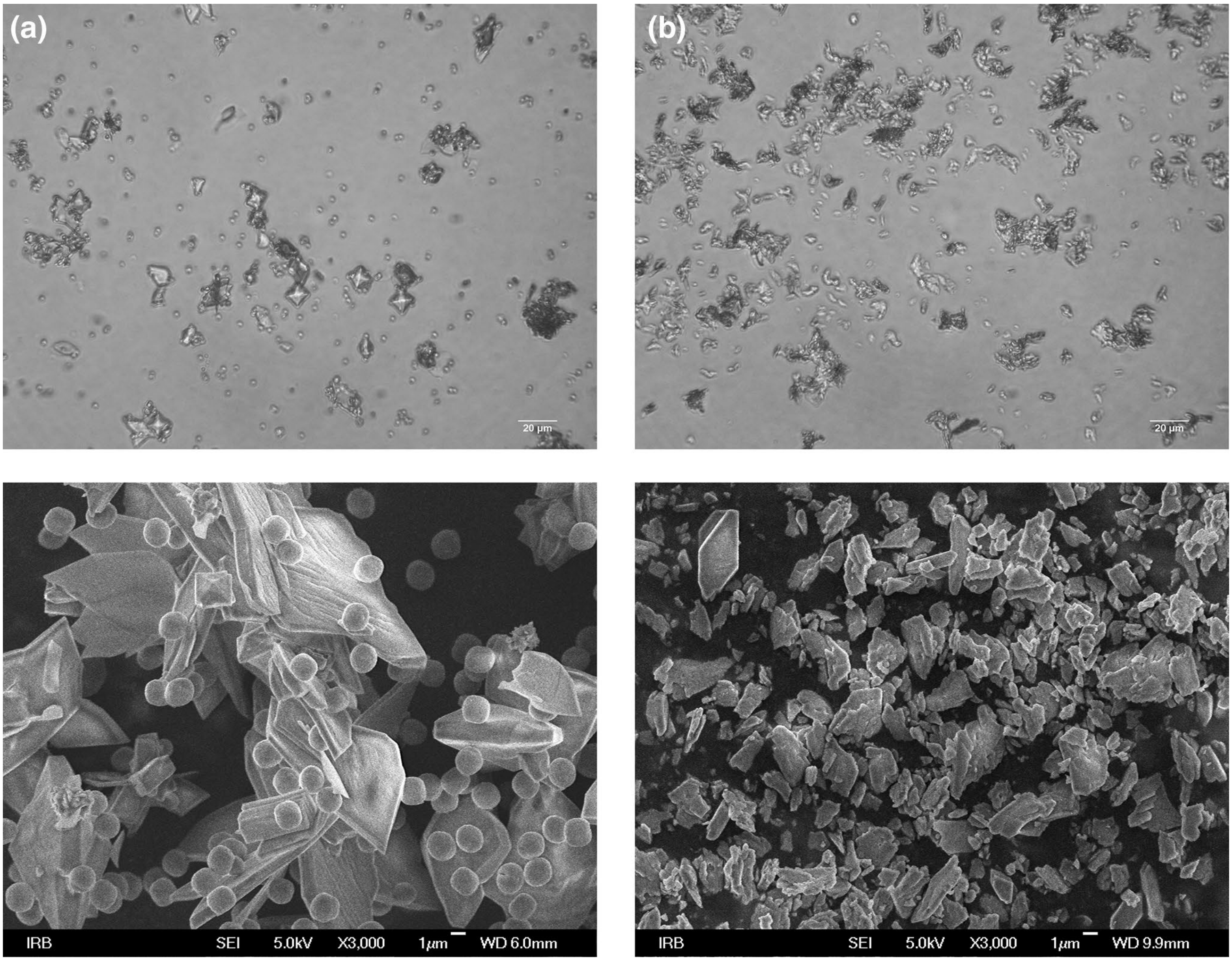

Fig. 9 **a** Typical light microscopy (*top*) and SEM images (*bottom*) of calcium oxalate hydrates precipitated in the magnetically stirred systems at, t = 37 °C and c(citrate) = 0.008 mol dm^{-3}. The initial concentrations of reactants were $c_i(\mathrm{CaCl_2}) = 0.0390$ mol dm^{-3}, $c_i(\mathrm{Na_2C_2O_4}) = 0.0049$ mol dm^{-3} and $c(\mathrm{NaCl}) = 0.30$ mol dm^{-3}. **b** Model system: t = 20 °C, no citrate

(Fig. 10b) indicates the overlapping of two size classes of the respective COM and COT crystals ($d \approx 6$ µm and shoulder at $d \approx 8$ µm).

Conclusions

Formation of calcium oxalates has been investigated in the precipitation systems in which the concentrations of $\mathrm{Ca^{2+}}$, $\mathrm{C_2O_4{}^{2-}}$ and NaCl, and consequently the initial supersaturations, were high enough to enable spontaneous nucleation of all hydrate modifications.

To optimize the experimental protocol for spontaneous precipitation of significant amount of pure COD, the hydrodynamic and thermodynamic parameters, such as mode of agitation, temperature and concentration of citrate ions, have been systematically investigated.

In the model systems, without the citrate addition and applied mechanical stirring, calcium oxalate monohydrate (COM) was observed as a dominant modification after 20 min of aging, while the magnetic stirring resulted in a formation of a mixture of COM and calcium oxalate trihydrate (COT), regardless of the temperature applied.

In the mechanically stirred systems, the addition of citrate ions at the selected range of concentrations, 0.001 mol dm^{-3} < $c_i(\mathrm{Na_3C_6H_5O_7})$ < 0.012 mol dm^{-3}, caused the formation of mixture consisting of COM and COD, at all temperatures.

In the magnetically stirred systems and at all concentrations of citrate and temperatures (25 °C < t < 45 °C)

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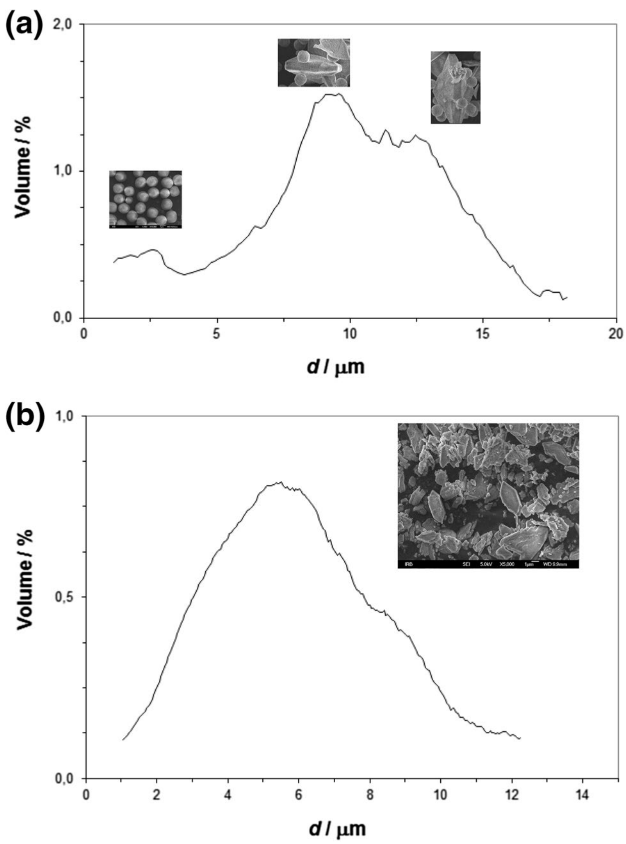

Fig. 10 **a** Particle size distribution (expressed as volume distribution) of calcium oxalate hydrate crystals, precipitated in the magnetically stirred systems at $t = 37$ °C and $c(\text{citrate}) = 0.008 \text{ mol dm}^{-3}$. The initial concentrations of reactants were $c_i(\text{CaCl}_2) = 0.0390 \text{ mol dm}^{-3}$ and $c_i(\text{Na}_2\text{C}_2\text{O}_4) = 0.0049 \text{ mol dm}^{-3}$, while $c(\text{NaCl}) = 0.30 \text{ mol dm}^{-3}$. The crystal morphologies, assumed to correspond to specific size classes, are shown. **b** PSD of COM (prisms) and COT in a mixture, precipitated in the system with no citrate addition ($t = 20$ °C)

formation of COD in a mixture, either with COM, or with COT has been observed.

The highest COD content in the mechanically stirred system was obtained at 45 °C and $c_i(\text{Na}_3\text{C}_6\text{H}_5\text{O}_7) = 0.001 \text{ mol dm}^{-3}$ ($w = 83.5\%$), while in the magnetically stirred system almost pure COD was obtained at 37 °C and $c_i(\text{Na}_3\text{C}_6\text{H}_5\text{O}_7) = 0.008 \text{ mol dm}^{-3}$ ($w = 91.8\%$).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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