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Development of a crystallization process for calcium lactate recovery from a fermentation broth

Thèse présentée en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

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List of Publications

(1) de Maere d’Aertrycke, J. B.; Robeyns, K.; Willocq, J.; Leyssens, T. Cocrystallization as a Tool to Solve Deliquescence Issues: The Case of l-Lactic Acid. *J. Cryst. Growth* **2017**, *472*, 3–10. https://doi.org/10.1016/j.jcrysgro.2017.02.025.

(2) de Maere d’Aertrycke, J. B.; Morlot, J.; Robeyns, K.; Filinchuk, Y.; Leyssens, T. Exploring the solid-state phases and thermodynamics of calcium L-lactate. *Under review*

(3) de Maere d’Aertrycke, J. B.; Payen, R.; Collard, L.; Robeyns, K.; Croker, D.; Leyssens, T. Enabling Cocrystallization of Challenging Systems: Passing through a Stable Cocrystal Solvate as a Pathway to Strenuous Cocrystal Forms. *Cryst. Growth Des.* **2020**. https://doi.org/10.1021/acs.cgd.9b01691.

Oral conferences proceedings

(1) J.B. de Maere d’Aertrycke, K. Robeyns, J. Willocq, T. Leyssens, “Cocrystals of lactic acid: how cocrystallization can avoid deliquescence of materials?”, *9th Belgian Crystallography Symposium*, October 26th, **2016**, Bruxelles, Belgium

(2) J.B. de Maere d’Aertrycke, T. Leyssens, “Stoichiometrically diverse cocrystal systems: the case of caffeine and maleic acid.”, *PhD Student’s day*, May 25th, **2016**, Louvain-la-Neuve, Belgium

(3) J.B. de Maere d’Aertrycke, T. Leyssens, “Stoichiometrically diverse cocrystal systems: the case of caffeine and maleic acid.”, *BACG annual conference,* June 20th, **2016**, Limerick, Ireland

Abstract

Calcium L-lactate is an organic salt of economic importance in many fields such as food, cosmetic or pharmaceutical industry. Indeed, this compound, generally recognized as safe, can intervene as a conservative to extend shelf life of freshly cut fruits and vegetables and is used as an excipient or to treat calcium deficiency. Since its discovery in 1780, calcium lactate has been produced by fermentation. Such process allows working in mild conditions and is economically interesting. However, at the end of the fermentation step, calcium lactate is contaminated by other metabolites of the fermentation broth and diluted in water. In this context, crystallization is the method of choice for its purification and recovery under solid form in a single step. Several attempts to crystallize calcium lactate in solution were already tried in the past and evidenced that calcium lactate crystallization kinetics are not optimal since it has a high tendency to form fines that are difficult to process afterwards. In addition, these studies are not directly applicable to the context of a crystallization in the fermentation broth at an industrial scale.

In this work, we tried to develop a crystallization process for calcium lactate recovery under a pure solid form from its fermentation broth. As this was conducted in the scope of a future industrial application, the process was designed with the objective of being easy to implement at a reduced cost and with high robustness. Indeed, the fermentation step has a strong variability in term of final lactate concentration and impurity profile, which is inherent to any biological process. The crystallization process developed was hence designed to take this variation in consideration.

For that matter, we first tried to raise a better understanding of calcium lactate thermodynamics. This aspect is crucial for the development of a crystallization process or down-line processing of the solid material to prevent uncontrolled crystallization or solid-solid transformation during storage. Up to now, the only solid forms of calcium lactate characterized were a crystalline pentahydrate and an amorphous anhydrate, while other forms were only mentioned. By combining various solid-state analysis techniques, we were able to confirm the existence of these two forms and were the first to characterize three new crystal forms of calcium lactate: an anhydrate, a monohydrate and a dihydrate.

The pentahydrated form was found to be the most interesting form both for crystallization in water and for storage. We then studied its solubility and nucleation in water. As calcium lactate solubility has a strong dependence towards temperature, a cooling crystallization process appeared to be promising. Different operation modes were considered and revealed the importance to control seeding and solid/liquid ratio of the suspension to prevent caking of the reactor. A slurry crystallization process was then designed to address these specificities. Several variables were influencing the outcome of this process and interactions between them are suspected. Therefore, the process was optimized through a full factorial design of experiment. At the end of this work, a crystallization process, robust towards the composition of the fermentation broth and economically interesting, was fully developed at laboratory scale.

List of Abbreviations

CL	Calcium l-lactate
CLA	Calcium l-lactate anhydrate
CLD	Calcium l-lactate dihydrate
CLM	Calcium l-lactate monohydrate
CLP	Calcium l-lactate pentahydrate
CNT	Classical nucleation theory
DSC	Differential scanning calorimetry
DVS	Dynamic vapor sorption
ESRF	European synchrotron radiation facility
GRAS	Generally regarded as safe
HPLC	High performance liquid chromatography
MSMPR	Mixed solution mixed product removal
MSZ	Metastable zone
PXRD	Powder X-ray diffraction
RH	Relative humidity
SNBL	Swiss-Norwegian beamline
TGA	Thermogravimetric analysis
VT-PXRD	Variable temperature - powder X-ray diffraction

Table of Content

Chapter I : Introduction

I.1. Crystallization

I.1.1. Definition

Among separation and purification processes, crystallization plays a predominant role due to its applicability to a wide variety of different compounds, its high performance and its low operational cost[1]. Indeed, recovery of a compound of interest from an initial complex matrix under a crystalline form is highly specific[2]. In pharmaceutical industry, it is considered that more than 90% of drugs available are under a crystalline form[3–5]. The control of the crystallization process is of key importance as it dictates the crystal size, shape and form of the product, which in turn will influence downstream operations as well as physico-chemical properties of the product.

Crystalline solids are defined as solid species composed of atoms, ions or molecules arranged in a periodic pattern that extends in the three dimensions[6,7]. Its constituting elements are held together by various types of interaction: stronger type of interactions such as ionic and covalent bonds, or weak interactions as Van der Waals interactions and hydrogen bonds[8]. The same compound can exist under different crystalline forms (Figure I.1)[2,9–11]. Crystals that have the exact same composition but do not share the same spatial organization are called polymorphs. It is also common to observe molecules of solvent embedded in the crystal structure in a strict molecular ratio. The new crystalline form thus obtained is called a solvate. In particular, when the solvent considered is water, the crystal form will be called a hydrate. A monohydrate contains a water molecule in a one-to-one molar ratio with the compound of interest. For higher ratios, the di-, tri-, tetra-,… -hydrate term will be used. When different molecules, solid under ambient conditions, can be combined in a given crystal structure, the terms salt and cocrystal are encountered, with a hydrogen transfer occurring only for the former.

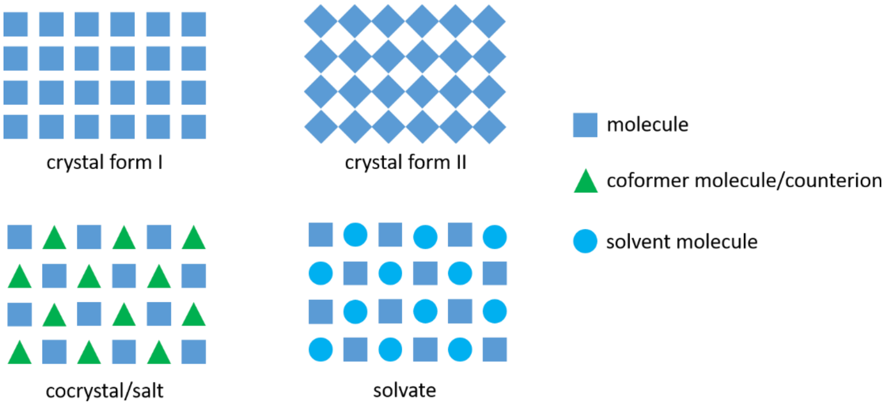

Figure I.1. Schematic representation of internal organization in polymorphs, cocrystals, salts and solvates

Crystallization is the phase transition that leads to the formation of the crystalline state from a disordered state[2]. It can take multiple forms, depending on the initial state of the considered compound[12]. Crystallization can hence happen as:

- Solution crystallization, when a solute forms crystals in its solution;
- Crystallization from the melt, when a liquid compound is cooled down below its melting temperature;
- Deposition based crystallization, when the initial state is vapor;
- Solid-solid phase transformation, when the compound is initially in the solid state (e.g. an amorphous solid).

Solution crystallization is the most often form encountered in industry. Apart from economic criteria – temperature or pressure conditions and equipment are often more expensive for the three other phenomena –, solution crystallization provides high purification performance. Indeed, it is often used to extract selectively a compound of interest from the solution. Furthermore, during filtration, impurities are washed over with the mother liquor and a pure crystalline phase of the desired product is often collected.

The driving force for solution crystallization lies in the chemical potential difference between the unstable dissolved state and the equilibrated suspension. This difference is then linked to the difference between the concentration of the compound of interest in the dissolved state and the one at equilibrium. The supersaturation, defined as the ratio between those two concentrations, is a key criterion in the study of crystallization processes. Given the way the supersaturation is applied, four different types of process can be distinguished[2,12].

- Cooling crystallization uses the fact that solubility, the concentration at which a suspension of crystals of a compound is in equilibrium, often decreases with temperature[13]. Hence, by cooling a solution, it becomes supersaturated and crystals can form.
- Evaporative crystallization consists in increasing the concentration of the solute by removing the solvent.
- In anti-solvent crystallization, the solubility of the compound of interest is decreased by adding a second miscible solvent to the solution. As the compound is less soluble in the mixture, the supersaturation increases.
- In reactive crystallization, the compound of interest is formed by a reaction between two precursors in a solvent in which it is poorly soluble. Hence, as the two precursors react with each other, the product crystallizes consecutively.

I.1.2. Thermodynamics of crystallization in solution

I.1.2.1. Solubility and solubility curve

To describe the transition of a solution, formed by a solvent and a solute, to a suspension, composed of a solution and crystals, one needs to understand the concept of solubility. This concept is defined as the composition of a solution at equilibrium in presence of a crystalline phase[13]. It represents the maximal quantity of a compound that can be dissolved at equilibrium for a given solvent at a given temperature. However, many parameters influence solubility, like temperature or the presence of other dissolved compounds[14]. Temperature dependence of solubility is often represented by a solubility curve (Figure I.2). As it is based on thermodynamic equilibrium, it is not dependent on kinetic effects.

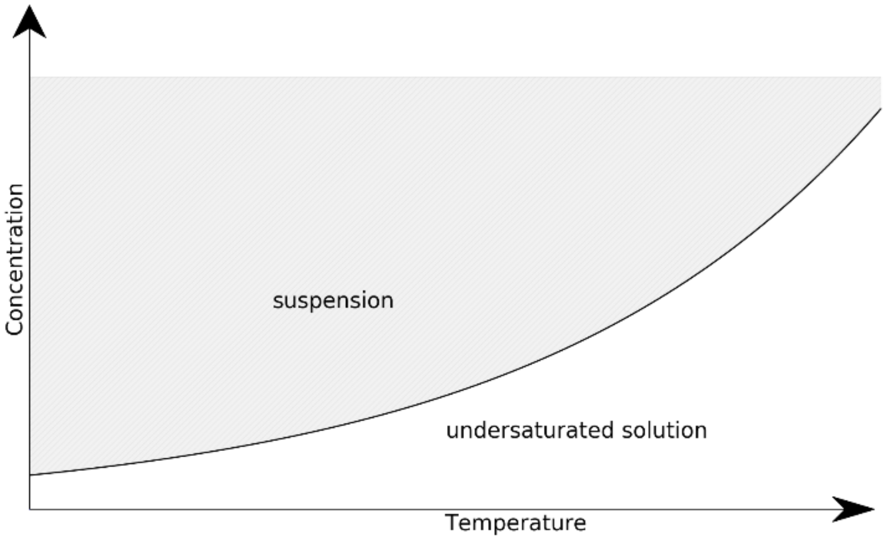

Figure I.2. Solubility curve indicates the evolution of solubility of a given compound with temperature

The force that drives the transition towards the formation of a solid phase can be represented as the chemical potential difference between the initial out-of-equilibrium state where the solution is clear and the equilibrium state where liquid and solid phases coexist[12]:

$$\Delta\mu = \mu_d - \mu_s \tag{I.1}$$

Where $\Delta\mu$ is the chemical potential difference between the initial solution and the final suspension, and μ_s and μ_c are respectively their chemical potentials.

This equation can be developed as:

$$\Delta\mu = \mu_0 + RTln(a) - [\mu_0 + RTln(a^*)] \qquad \text{(I.2)}$$

Where μ_0 is the standard chemical potential of the dissolved state, R is the universal gas constant, T the temperature, a is the activity of the solute in the solution and $a^*$is the solubility of the compound.

Considering that the activity of the compound is close to its concentration in diluted solutions, the equation can be written as:

$$\Delta\mu = RTln\left(\frac{c}{c^*}\right) \qquad \text{(I.3)}$$

Hence, at a given temperature, the chemical potential difference is function of the solubility and the concentration of the solute in the solution. It is therefore often preferred to use the concept of relative supersaturation, defined as:

$$S = \frac{c}{c^*} \qquad \text{(I.4)}$$

Supersaturation is then the key-criterion to control a solution crystallization. Indeed, a solution that is undersaturated, meaning that S is comprised between 0 and 1, will not form crystals in that state. On the other hand, a solution supersaturated, with S above 1, is not thermodynamically stable and crystals will eventually grow. With crystal growth, the concentration of the solute in the solution will decrease until it reaches its solubility value at what point the solution will be saturated and the supersaturation ratio will be equal to 1.

All solution crystallization processes are designed to increase supersaturation of the compound of interest, either by decreasing is solubility or increasing its concentration. Cooling and antisolvent crystallizations decrease the solubility of a compound by reducing temperature or adding an antisolvent. On the other hand, evaporative crystallization increases the concentration of the compound and, for reactive crystallization, the compound is created over time and crystallizes subsequently as the solubility is usually low in the reaction solvent.

I.1.2.2. Nucleation and metastable zone

Supersaturation is a thermodynamic concept that indicates whether a solution is at equilibrium or not. When a clear undersaturated solution (Figure I.3 - point A) is cooled down, its supersaturation increases. As it cools down, the solution can overpass its saturation (point B) but no crystal will spontaneously appear before a certain supersaturation is reached (point C). At that point, crystals will nucleate spontaneously and grow until the solution gets saturated (point D). The curve showing the concentration required for spontaneous crystal formation and its evolution with temperature is called the nucleation curve (Figure I.3). The zone comprised between the solubility and nucleation curve is the metastable zone (MSZ). At temperature and concentration conditions comprised in the MSZ, a suspension will be stable and crystals present will grow, but a solid phase will not form spontaneously (at least for a given timeframe) from a clear solution[13].

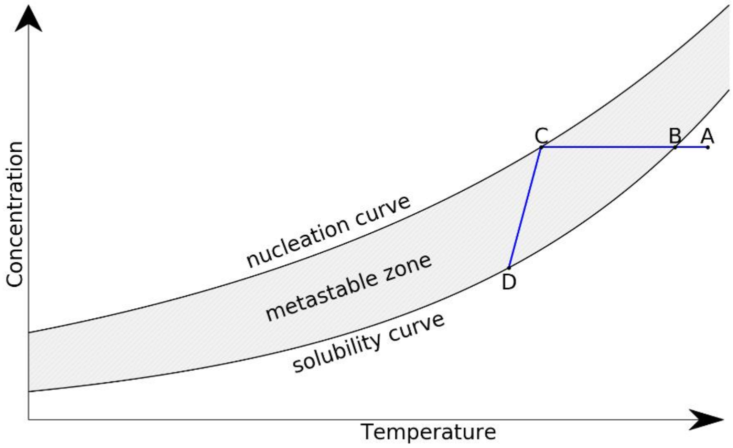

Figure I.3. Solubility curve, nucleation curve, and the MSZ (grey zone) comprised in-between

There is therefore a need to overpass the solubility by a certain range to overcome the nucleation energy barrier and for a solid phase to appear spontaneously. Between the solubility and nucleation curves, the solution is in a metastable state, and the free energy gain of crystallization is not high enough to overcome this activation energy[13,15]. In Classical Nucleation Theory (CNT), this barrier is explained by the fact that, even though the second phase has a lower free energy than the initial phase, there is also a free energy increase associated with the creation of the solid-liquid interface[16]. Hence, the free energy associated to the formation of a spherical nucleation is the sum of a negative volume term and a positive surface term, which is described according following equation:

$$\Delta G = -\frac{4}{3}\pi r^3 \Delta G_V + 4\pi r^2 \gamma \qquad \text{(I.5)}$$

Where r is the radius, ΔG_V is the bulk free energy difference per unit volume between the dissolved and solid phase, γ is the interfacial energy of the solid phase per unit area.

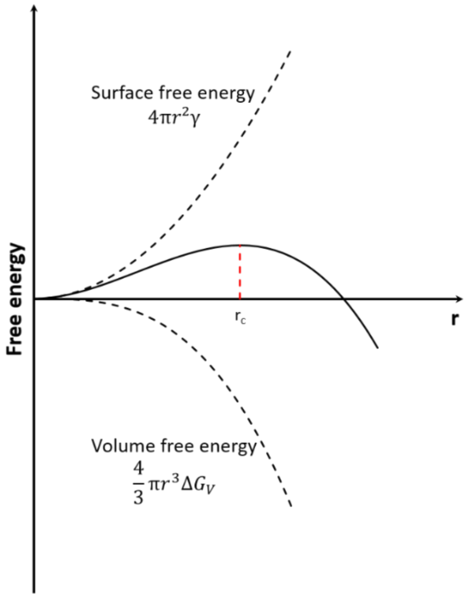

Figure I.4. Free energy diagram for nucleation

The positive surface free energy term will dominate at small cluster sizes. As the cluster size increase, total free energy goes through a maximum at a critical radius size, above which total free energy decreases, as the nucleus grows bigger (Figure I.4). If CNT is an efficient theory to understand the origin of activation energy and the forces to consider in the crystal nucleation phenomenon, it holds several limitations. Indeed, CNT considers nuclei as spherical droplets and their surfaces as infinite planes. The curvature of the surface tension is hence neglected. In addition, CNT assumes that nuclei grow by addition of one monomer at a time and that molecules in it are ordered with the same structure as the resulting crystal, which makes the size of the nucleus the only criterion to consider when investigating whether an aggregate is critical or not[17].

Because of these limitations, two-step nucleation theory is often preferred to CNT to comprehend more precisely this phenomenon. In this theory, it is considered that the apparition of a nucleus is preceded by the formation of a droplet of dense liquid called cluster that do not possess the internal arrangement of the molecules like in a crystal. The nucleus then forms in a second-step within this cluster[17,18].

Apart from the above-described phenomenon, which corresponds to the ideal case of an initial solution clear from other compounds than the solvent and the solute, other mechanisms can lead to the formation of new crystal germs. One distinguishes primary and secondary nucleation, depending on whether the formation of a new crystal occurs due to the aggregation of initially dissolved elements or its formation is imputable to the presence of another crystal. In addition, primary nucleation can be homogenous, if it was not assisted by the presence of another species, or heterogeneous if a foreign body, such as impurities or the surface of the reactor was involved in the formation of the nucleus (Figure I.5).

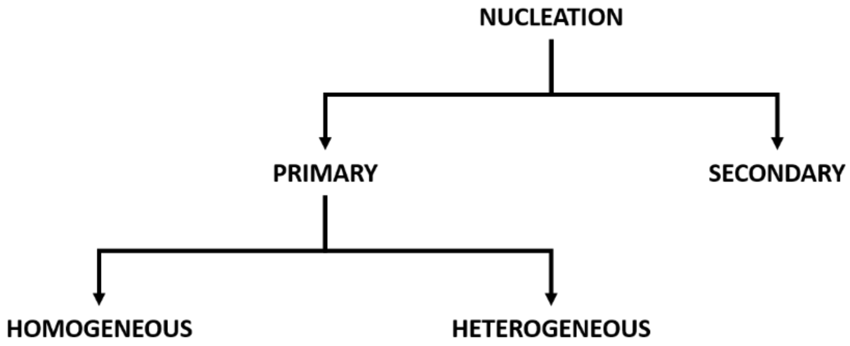

Figure I.5. Classification of the different phenomena of nucleation

Since crystal nucleation is a stochastic phenomenon, it is common in industrial processes to seed the solution with crystal germs of the same compound formed previously. If the solution is supersaturated, these crystals will grow and the supersaturation of the liquid phase will decrease. Using such procedure, the crystallization process is better controlled. In those cases, secondary nucleation plays a more important role. The apparition of a new crystal from a parent occurs essentially through attrition. During a crystallization, the repeated shocks between the crystals on the stirrer shafts or on the reactor walls lead to crystal breakage and multiplication. Secondary nucleation is therefore depending on stirring rate, crystals size and particle density of the suspension, as these parameters influence the contact frequency between crystals. Supersaturation plays also a role in this mechanism as it ensures that the fragments of attrition remain in the solid state. Indeed, as the newly generated crystals were put to constraint or were partially deformed by the collisions, their chemical potential is higher than that of the initial crystals. If the supersaturation is not high enough, these fragments having a higher interfacial energy can dissolve.

I.1.2.3. Crystal growth

Once nuclei are formed or seeds added, dissolved molecules can diffuse and get incorporated in the crystal lattice. This phenomenon is called crystal growth. It requires the presence or creation of particular sites on the surface of the crystals that stabilize solute molecules to integrate them in the crystal. Three mechanisms are classically used to describe crystal growth[13]. (i) The first one results in the combination of bidimensional nucleation events on the surface of a crystal followed by the growth in the surface plan. As a nucleation phenomenon is involved, it is highly dependent to supersaturation. (ii) In addition, the presence of defects on the crystal surface creates zones where incorporation of solute molecules is more favorable. (iii) Finally, if the supersaturation is high enough, the activation energy for molecule incorporation in the crystal is considerably lowered and is of the same order as the thermal energy of the system. Solute molecules are then easily integrated at any position of the crystal lattice.

It should be noted that the three crystal growth phenomena listed require an activation energy considerably lower than nucleation phenomena. As these energies are strongly related to supersaturation, this parameter has a strong influence on the final crystal size distribution. Indeed, the relative importance of nucleation over crystal growth will determine whether the final suspension will contain a high quantity of smaller crystal, if nucleation is predominant, or bigger crystals if crystal growth predominates.

I.1.3. Product purity

Crystallization is widely used industrially for extraction of a compound of interest from a solution containing other components. The main advantage lies in the ability of this technique to form a solid from a liquid phase, facilitating its extraction. Furthermore, the final solid purity can virtually approach 100%. Impurities in a crystalline material can be present in four different ways (Figure I.6)[2]; similar molecules to the compound of interest integrate the crystal lattice; inclusions of mother liquor inside a crystal particle; mother liquor trapped in areas formed by the aggregation of crystals ; mother liquor retention in the cake of filtration due to adsorption or capillarity. This latter is the main source responsible for impurity retention.

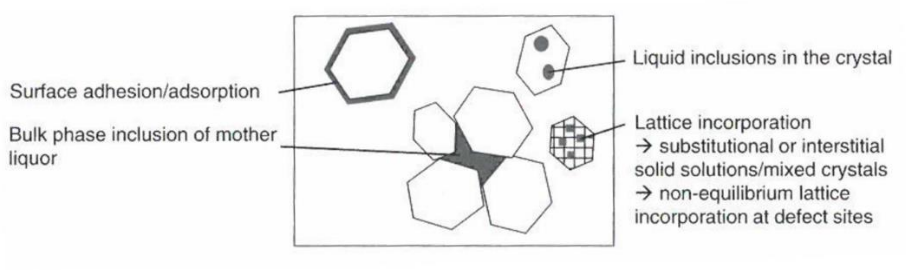

Figure I.6. Different mechanisms for impurities contamination of a crystalline product (taken from W. Beckmann[2])

It is therefore crucial to control crystal growth, as greater crystals will show a smaller specific surface area and hence a lower ability to retain mother liquor. In addition, a crystallization at a slower rate prevents the formation of inclusions inside the crystals, aggregates or lattice incorporation of impurities. Besides, crystal size distribution will also affect transport properties and filterability of the suspension[19]. Indeed, smaller crystals tend to clog the pipes of industrial installations more easily and block filter pores during the filtration step[13].

I.2. Calcium lactate

I.2.1. Objective

This thesis was realized in close partnership with the company Galactic S.A. whose core business is to produce L-lactic acid and its derivatives. Those compounds find numerous applications in food, feed, pharmaceutical or cosmetic industries. In this work, we focus on the production process of the calcium salt of L-lactic acid, calcium L-lactate (CL). Indeed, the current process is not sufficiently competitive to address various applications, mainly in feed industry where the financial margins are typically lower than in food or pharmaceutical industry.

I.4. References

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Chapter II : Exploring the solid-state phases and thermodynamics of calcium L-lactate

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Abstract: Calcium L-lactate, an organic salt derived from L-lactic acid, is used in many fields such as food, pharmaceutical or cosmetic industry. To this date, its solid-state thermodynamics are still poorly understood: a pentahydrate crystalline and anhydrate amorphous forms were already characterized, and several other hydrates are mentioned in literature. For the development of a robust crystallization process or down-line handling of this compound it is, however, important to know and understand the relationship between the different solid forms to prevent uncontrolled crystallization or solid-solid transformation during storage. In this paper, we aimed at characterizing the different solid forms of calcium L-lactate combining various techniques. The existence of the crystalline pentahydrate and amorphous anhydrate, previously described in literature, were confirmed. In addition, three other crystalline forms (crystalline anhydrate, monohydrate and dihydrate) have been successfully isolated and characterized.

II.1. Overview

A major problem encountered at the beginning of this project is the lack of information concerning CL solid-state thermodynamics. CLP is the only form characterized by PXRD, which proves its crystalline state, and an amorphous anhydrate form was also identified. In several publications, other levels of hydration are mentioned but are not identified. Furthermore, the stability range of the pentahydrate is not clearly defined. For the development of a crystallization process or the down-line handling of the solid product afterwards, it is important to know and understand the relationship between the different solid forms to prevent uncontrolled crystallization or solid-solid transformation during storage.

In this contribution, we aimed to obtain a full thermodynamic description of the different solid forms of calcium lactate. The pentahydrated form is the only one obtained by crystallization in water and, other levels of hydration were not reached by crystallization from solution. The combination of different solid-state characterizations techniques allowed finding conditions for new crystalline phases. In particular, other hydration levels were identified playing on conditions of temperature and relative humidity.

In addition to crystalline calcium lactate pentahydrate and amorphous anhydrate, three new crystalline forms were identified. Differential scanning calorimetry (DSC) determined the glass transition temperature for calcium lactate anhydrate (CLA) and the temperature of recrystallization of this form. A variable temperature powder x-ray diffraction (VT-PXRD) experiment performed in a sealed capillary provided the conditions for the formation of calcium lactate monohydrate (CLM). Finally, a calcium lactate dihydrate (CLD) form was identified by a dynamic vapor sorption (DVS) experiment performed at 60°C.

This study provides an understanding of CL solid-state thermodynamics. In particular, the relative stability of CLP is now established and storage conditions can be determined. Indeed, excessive temperature or low relative humidity can lead to partial dehydration or to amorphisation. Still, the pentahydrate form is confirmed to be the most stable crystalline form of calcium lactate.

II.2. Introduction

When dealing with a solid compound in food or pharmaceutical industry, characterizing and understanding its solid-state landscape is crucial. Regulatory instances often require commercialized compounds to be present under a single form. In addition, a lack of control of the solid form during manufacture or storage can lead to unwanted phenomena such as powder caking, deliquescence, chemical degradation or form transition[1–3]. Solid compounds typically present an amorphous or crystalline state. Amorphous forms do not show long-range order of the molecular packing[4] and are characterized by a glass transition temperature, T_g, below which the material is found in a glassy state and above which the material is said to be in a rubbery state[5]. Amorphous phases are thermodynamically unstable and conversion into a crystalline form can occur over time or when molecular mobility is increased[4,6]. Crystalline states, on the other hand, are characterized by the packing of their constituting elements under a long-range three-dimensional order[7]. For a given compound different crystalline forms can exist such as polymorphic forms, salts, cocrystals, or solvates[8].

The various solid-state forms of CL and the relation between them are, to this date, still poorly understood. Even though the existence of different hydrates has been mentioned in literature, no actual proof of the existence of most of them has been given. Calcium lactate pentahydrate (CLP) is the most frequently referred to, and, to our knowledge, the only crystalline form characterized by X-ray powder diffraction[9]. Tetra-, tri-, di- and monohydrate species have also been cited, however no experimental proof of their existence is provided[1,10–12]. Beside the pentahydrate, an amorphous anhydrate phase is the only other phase evidenced in literature[13,14]. In this paper, we propose a better understanding of CL solid-state thermodynamics, aiming at providing experimental evidence of the elusive intermediate hydrate phases and try to understand the respective stability among these forms.

II.3. Materials and method

Materials. Calcium L-lactate pentahydrate (98% purity) was provided by Galactic S.A. and used without further purification.

Crystalline CL anhydrate (crystalline CLA) was obtained by applying two successive heating ramps to samples of 14mg of pure CLP: a first heating ramp until 200°C at a 2K/min rate ensured complete dehydration and a second heating ramp until 230°C at 10K/min, where the dried sample would successively undergo glass transition and recrystallization. Those events where monitored using differential scanning calorimetry (DSC, see description below).

Differential scanning calorimetry. DSC was performed on a TA instrument D2500. Samples of approximately 6.00 mg were placed in a 40µL aluminum pan. The pan was sealed with an aluminum lid perforated with five holes to ensure the volatilization of crystalline water upon heating. Two heating ramps were applied under a constant flow of nitrogen. After an isothermal hold of 5 minutes at 30°C the temperature was increased at a rate of 2K/min up to 200°C. The sample was then cooled down to 30°C and held at this temperature for 30 minutes, after which it was heated once again to 240°C at a rate of 10K/min. The DSC experiment was repeated 9 times.

Thermo-gravimetric analysis. Thermo-gravimetric analysis (TGA) was performed on a 821e Mettler Toledo. A sample of approximately 6.00 mg was put in a 70µL aluminium oxide crucible under a nitrogen flow and gradually heated at a rate of 5K/min starting at 30°C to a final temperature of 300°C.

Dynamic vapor sorption. Dynamic vapor sorption (DVS) experiments were performed on a TA Q5000 SA instrument. Samples of approximately 6.00 mg of crystalline CLA, prepared as explained in "Materials" section, were placed in an open metalized quartz pan. Prior to the analysis, a drying step was performed at 60°C and 0% of relative humidity (RH) for 3 hours. The sample was then brought to the temperature of analysis and the RH increased by a step of 10% RH up to 90% RH. The RH was then brought down again to 0% using once more 10% RH steps. If sample mass variation was lower than 0.01% for 2 hours, the next step was subsequently started, with a maximum of 20h for each step (unless indicated otherwise). Such analysis was performed at 30°C and at 60°C.

X-ray Diffraction. Laboratory powder X-ray diffraction (PXRD) measurements were performed on a Siemens D5000 diffractometer equipped with a Cu X-ray source operating at 40 kV and 40 mA and a secondary monochromator allowing to select the $K\alpha_1$ radiation of Cu ($\lambda = 1.5418$ Å). A scanning range of 2θ values was applied from 2° to 50° at a scan rate of 0.6 min^{-1} and a step of 0.02°. For variable temperature PXRD (VT-PXRD), a sample of approximately 20 mg was placed in an open pan, without control on RH conditions. Temperature was increased from 30°C to 200°C by steps of 5°C every 10 min, after which a PXRD spectrum was acquired.

Synchrotron VT-PXRD data were measured at the Swiss-Norwegian beamline BM1A (SNBL) at the European Synchrotron Radiation Facility (ESRF) (Grenoble, France). A 0.5mm glass capillary was filled with CLP, sealed with vacuum grease, and exposed to synchrotron radiation at a wavelength of 0.78487 Å. The capillary was heated between 30°C and 230°C at 10°C/min with a nitrogen blower (Oxford Cryosystems) while accumulating PXRD data on a PILATUS 2M hybrid pixel detector. PXRD data allowed resolving the structure of the crystalline phase appearing at 99.5°C. Indexation was performed using the EXPO program, while structure was resolved by global optimization in direct space (Monte Carlo/parallel tempering algorithm) using the FOX program[15] and further refined by the Rietveld method in the Fullprof suite. Simulated diffraction patterns were calculated from their crystal structures with the Mercury 3.1 (version: February 2010) program.

II.4. Results

II.4.1. Thermal analysis

Upon heating at 5K/min under a continuous nitrogen flow, CLP shows a one-step dehydration (Figure II.1). Dehydration occurs from the start (30°C) under the 0% RH atmosphere and continues until 120°C. Dehydration corresponds to a weight loss of 4.5 equivalents of water. The remaining 0.5 equivalent of water is supposed to have been lost already prior to the data recording because of the 0% RH nitrogen flow. At 240°C degradation occurs. Such a single step dehydration process under a continuous nitrogen flow was already observed by Y. Sakata[14].

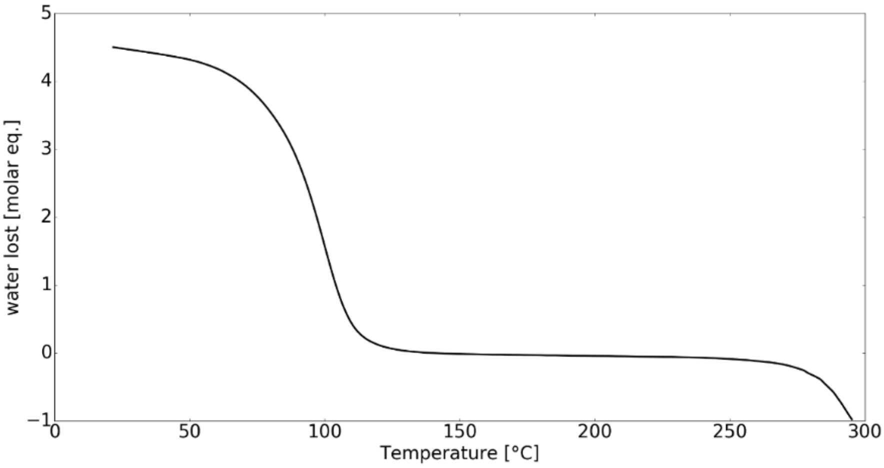

Figure II.1. One step dehydration of CLP to CLA as recorded by TGA

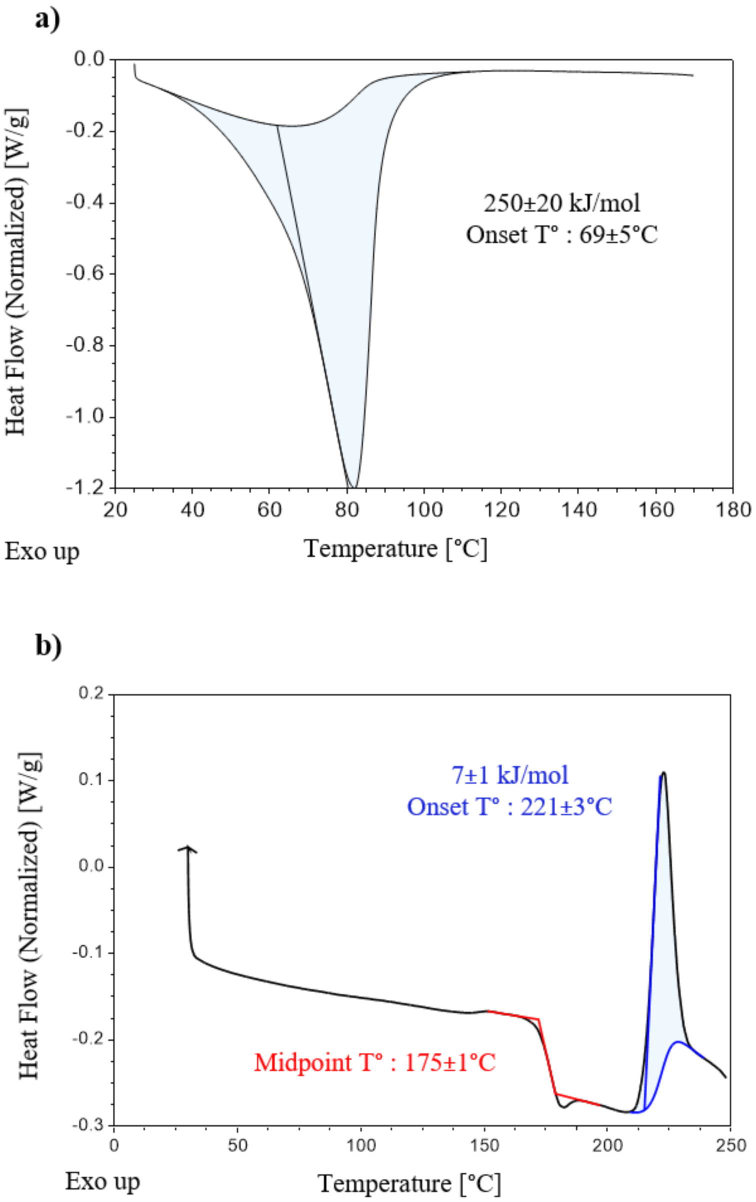

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Figure II.2: (a) Endotherm of dehydration of CLP to CLA and (b) glass transition of CLA at 175°C followed by crystallization at 215°C

A similar result is obtained when analyzing the sample under DSC analysis (1K/min). An endotherm with onset at 69 ±5°C corresponds to the single step dehydration of CLP to CLA (Figure II.2a). The heat of dehydration is estimated at ΔH= 250±20 kJ/mol. As no melting endotherm nor a recrystallization exotherm can be observed, the resulting phase is likely the amorphous dehydrated phase. Cooling this phase to 30°C and heating it at a faster rate (20 K/min) allows observing a glass transition temperature at 175±1°C, with a small enthalpy recovery signal. Further heating shows a recrystallization exotherm starting at 221±3°C (ΔH=7±1 kJ/mol) (Figure II.2b). The measured enthalpy is quite low for a recrystallization. As this event is directly followed by the degradation of the material, it is suspected that the recrystallization is only partial, probably because of the heating rate in the second ramp. VT-PXRD analysis confirms this recrystallization event to an anhydrous crystalline state as will be shown later on. In addition, the low intensity of the peak reinforces the hypothesis that the recrystallization is only partial.

DSC and TGA analyses were all performed under continuous nitrogen flow, and both showed a single dehydration step, only evidencing pentahydrate (crystalline) and anhydrate (amorphous and crystalline) phases. For hydrate phases, however, both the water activity and temperature are crucial factors impacting form stability. Therefore, DVS analysis was performed to study the effect of ambient relative humidity on the stability of CL solid forms.

Solid compounds interact with water from air humidity, following three main mechanisms: (i) adsorption on the surface of the solid, (ii) absorption of water with penetration in the bulk of the solid and (iii) liquefaction that is caused by the dissolution of the solid in the water adsorbed, a phenomenon called deliquescence[2]. Water absorption is by far more critical than adsorption, as it triggers dramatic changes in physico-chemical properties of the material. This phenomenon will occur differently for crystalline or amorphous materials. Upon water absorption, crystals will form hydrates, and their crystal structures will contain molecules of water in a strict molecular ratio. In addition, the same compound might be able to form different hydrated crystalline forms, sometimes with different levels of hydration[16]. Hydration of a crystal will depend on both temperature and relative humidity and the transition between two hydrates occurs sharply at a distinct RH, as expected for a first-order transition[2,16]. This behavior concerns stoichiometric hydrates. For some hydrates however, it can happen that the level of hydration varies in a given range. These are referred as non-stoichiometric hydrates. This phenomenon can be due to the existence of pores within a crystal structure where water molecules can be trapped. Depending on RH and temperature, a given fraction of these pores will be occupied by water molecules. Level of hydration of the crystal structure will then vary more continuously upon RH increase[17,18]. Finally, in the case of amorphous compounds, absorption of water will occur continuously with a changing RH[19]. It is often observed that the same compound absorbs significantly more water in its amorphous form than its crystalline form[3].

A first DVS experiment performed at 30°C with crystalline CLA as starting material showed a one-step hydration of five equivalents of water to form crystalline CLP (Figure II.3a), starting at 70% RH. Upon relative humidity decrease, five equivalents of water were lost at 0% RH, showing the reversibility of the hydration-dehydration process. The anhydrous material was amorphous, as confirmed by PXRD, given that the temperature (30°C) was not high enough for crystallization to occur. The remaining traces of water at 0% RH are hence probably due to water absorption by the amorphous anhydrate. Indeed, as kinetic of water absorption and desorption is particularly slow, equilibrium could not be reached after 5 days at 0% RH. However, as amorphous material is formed, the residual water is only physically adsorbed and it would eventually evaporate over time. Similarly, the value obtained at 70% RH does not correspond to equilibrium, as sample mass was not stable at the end of the plateau. Equilibrium value would have corresponded to the formation of CLP, as suggested by water absorption kinetics (see

Appendix A.1.). Finally, one should note the strong hysteresis's in both curves that evidence the stability of CLP towards dehydration.

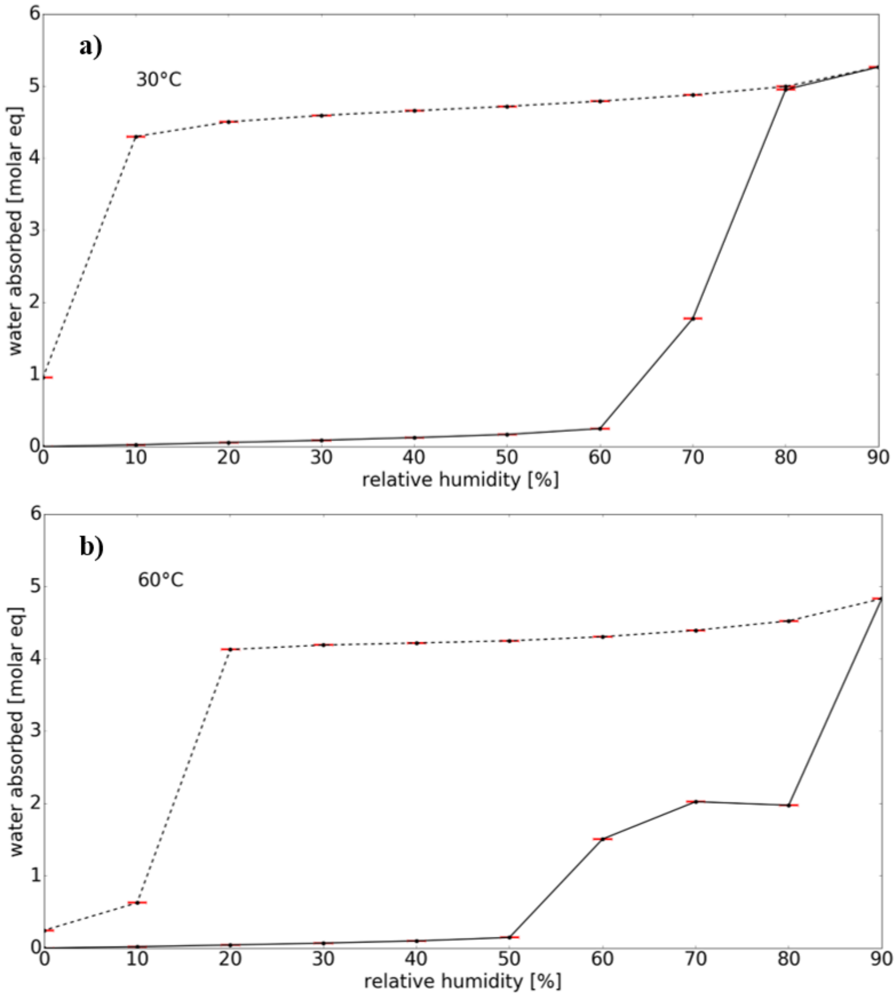

Figure II.3: sorption (solid line) and desorption (dashed line) isotherms of crystalline CLA at (a) 30°C and (b) 60°C

In a second DVS experiment, the temperature was set at 60°C (Figure II.3b). Upon increasing RH, crystalline CLA gradually absorbs water until 50% RH due to surface adsorption. At 60% RH, water absorption increases dramatically to stabilize at two equivalents of water.

Hydration kinetics (see Appendix A.2.) show a water absorption followed by a minor desorption upon RH increase, which is typical for the formation of a hydrate. This dihydrated form is stable up to 90% RH where its mass suddenly increases and CLP is formed. Upon RH lowering, the mass decrease is gradual until 10% RH and less than one equivalent of water is lost. Between 20 and 0% RH, there is a massive loss of water and amorphous CLA is formed. The remaining traces of water are due to physical adsorption at the end of the RH decreasing ramp.

This latter DVS analysis revealed the existence of a transitory CLD between 60 and 80% RH at 60°C. Formation of this dihydrate therefore requires specific conditions of temperature and relative humidity. Pure conversion from CLP to the anhydrate was not clear in the decreasing ramp where a mixture of forms is likely occurring.

II.4.2. Structural analysis

X-ray diffraction experiments were conducted at room temperature as well as variable temperature to evaluate structural changes. The CLP diffractogram is similar to the one presented by Sakata[14]. In a first VT-PXRD experiment, performed in a heated open pan on a Siemens D5000 diffractometer, a phase transition was observed at 80°C and a new crystalline phase of unknown stoichiometry was formed. Further heating led to an amorphisation of the compound, evidenced by the lack of well-defined peaks between 100 and 200°C. At 220°C, another crystalline form was identified, as new peaks appeared on the diffractogram (Figure II.4a). This crystallization event occurs at the same temperature as the one observed in DSC and it is therefore attributed to formation of crystalline CLA.

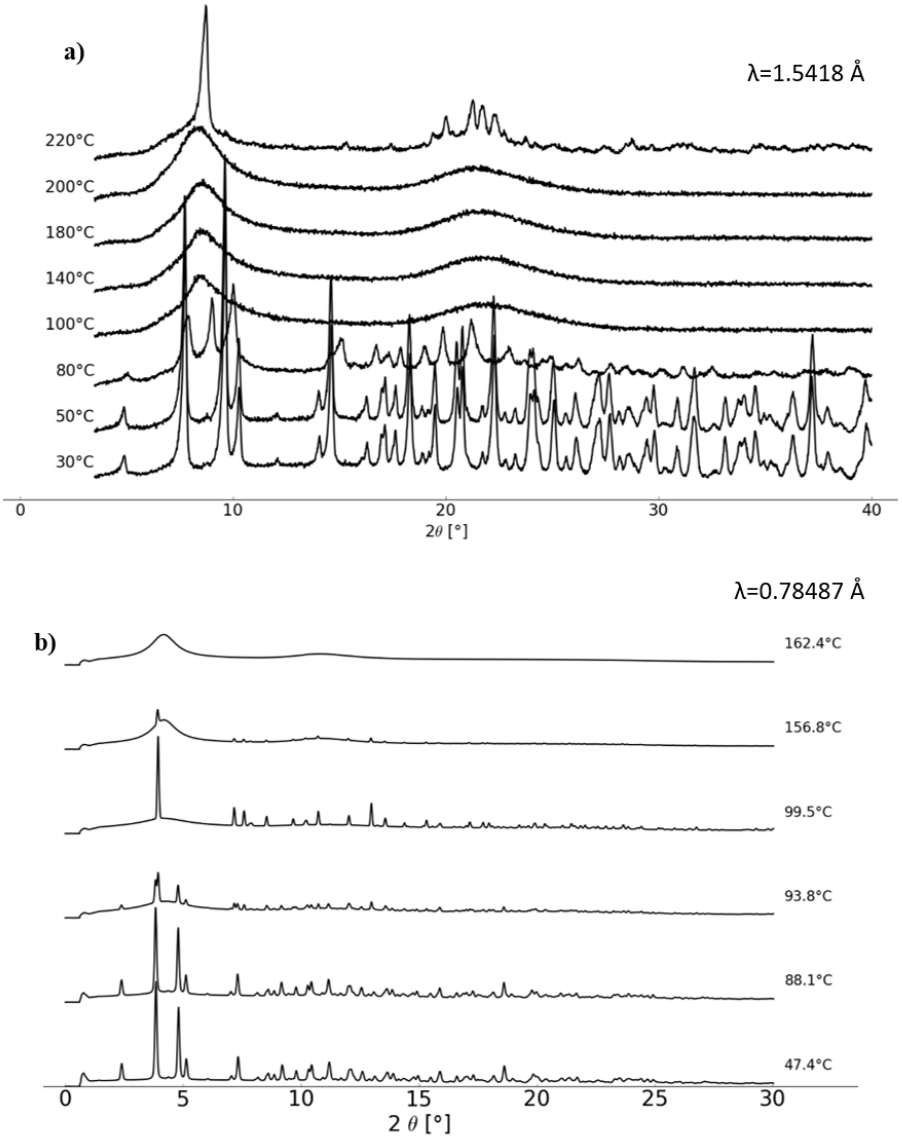

Figure II.4 : Variable Temperature PXRD of CLP performed (a) in an open pan using a Siemens D5000 diffractometer and (b) in a sealed capillary using synchrotron radiation (SNBL/ESRF, Grenoble)

A second VT-PXRD experiment was conducted using synchrotron radiation at the SNBL/ESRF, Grenoble. A temperature ramp from 40 to 230°C was applied on a sample of CLP placed in a capillary sealed with vacuum grease (Figure II.4b). Upon heating, another intermediate hydrate was identified between 90 and 150°C. Due to the fast heating rate applied, thermal degradation of CL occurred prior to the crystallization of CLA. The high-quality diffractogram obtained at 99.5°C allowed determining the crystal structure of the solid form by PXRD-structure resolution and we are the first to show experimental evidence of a monohydrate form (CLM, figure 5). Upon further heating, CLA gets completely dried and forms an amorphous compound at 157°C. Further heating led to the degradation of the compound.

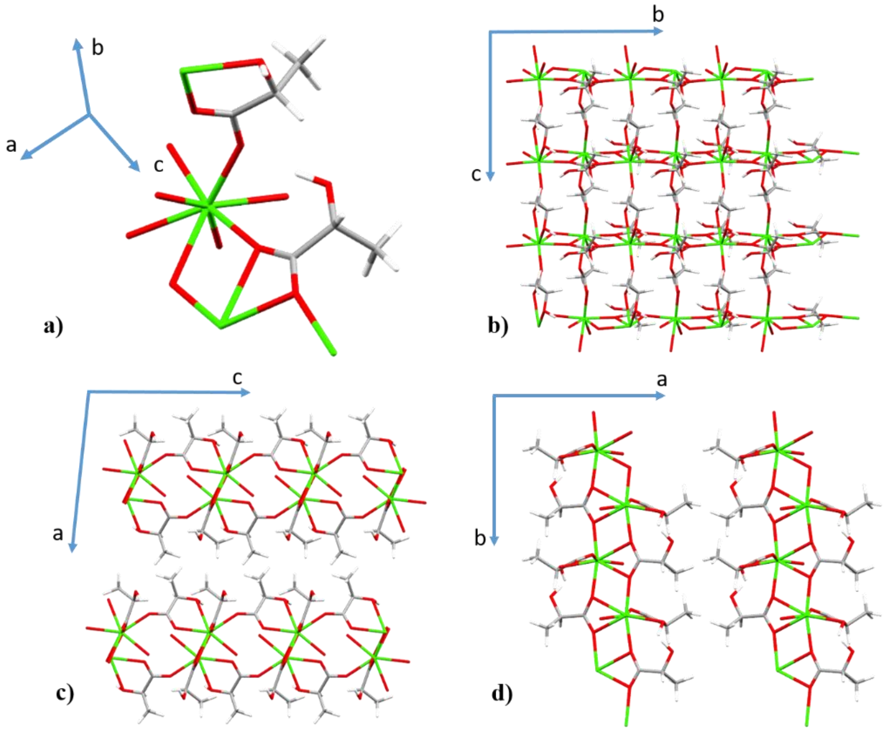

Figure II.5: Crystal structure of CLM isolated at 99.5°C in a sealed capillary: (a) extended asymmetric unit – (b,c,d) projections along the a, b and c axis, respectively

CLM crystallizes in a $P2_1$ space group, whose crystallographic data are listed in Appendix B.3. The crystal structure is essentially formed by coordination bonds that form planes normal to the a-axis. Those planes are held together by hydrophobic interactions between the methyl groups of CL. Calcium has a coordination number of eight and is coordinated with the oxygen atoms present in the carboxylic groups (6) and the alcohol functions (1) of the lactate group, as well as with the oxygen of water (1). In the crystal structure, lactate ions can have two different positions. One type of lactate is coordinated with two different calcium atoms. The second type of lactate is coordinated with three different calcium atoms, forming a hydrogen bond with the water molecule.

The combination of the different PXRD experiments, from synchrotron radiation or from Kα_1 radiation of Cu, allowed identifying five different crystal forms. The diffractogram of each form for the same wavelength can be found in Figure II.6.

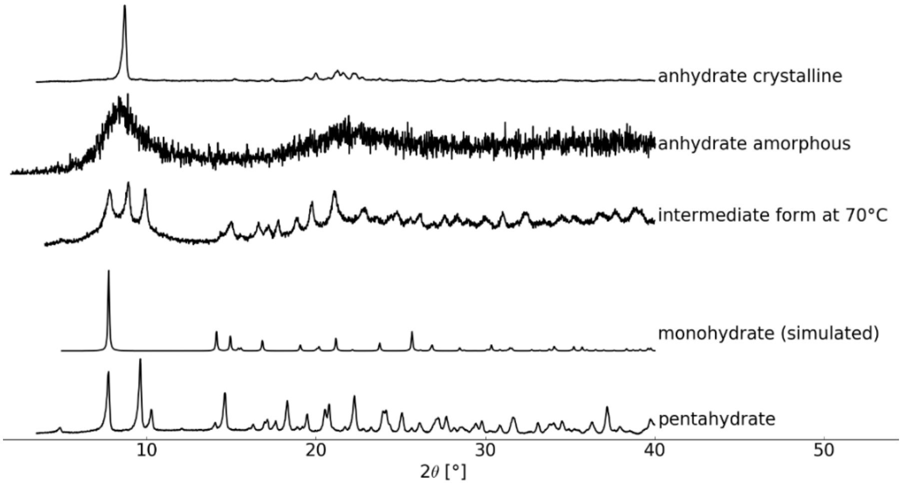

Figure II.6: Normalized PXRD diffractograms of the different CL solid forms identified (λ=1.548 Å): CLP, CLM (simulated in Mercury), intermediate hydrate (unknown stoichiometry), CLA amorphous and CLA crystalline (from bottom to top).

II.5. Conclusion

In this work, we give experimental evidence of at least five different solid-state forms of CL, three of which were hitherto not evidenced.

The pentahydrate phase is the most stable under ambient conditions, and transforms into an anhydrate amorphous phase upon heating under a dry atmosphere. This latter can recrystallize at higher temperatures into an anhydrate crystalline phase. As VT-PXRD were not performed under 0% RH nitrogen flow like in DSC or TGA, transitory hydrates were observed upon heating. During the VT-PXRD experiment in a sealed capillary, the monohydrate was identified by structure resolution based on its PXRD diffractogram. Another VT-PXRD experiment in an open pan showed another crystalline form at 70°C, that does not correspond to any of the other forms already discussed, and could potentially correspond to the dihydrated phase observed in DVS experiments. At 30°C, the DVS results indicate the anhydrate crystalline phase to absorb five molecules of water to form CLP, but at 60°C, a dihydrate is formed instead between 60 and 80% RH. To our knowledge, we are the first to give firm proof of the existence of the anhydrous crystalline form and both the mono- and dihydrate forms. With these results in mind, it would not be surprising to find other intermediate hydrates such as a trihydrate or a tetrahydrate in the future.

In addition, different experiments were performed allowing evaluating the respective stability of the different forms identified. Upon heating under dry atmosphere, CLP gets dehydrated in one step to form an anhydrated amorphous phase. This amorphous form is characterized by a glass transition occurring at 175°C, as observed in DSC. When amorphous CLA is heated above its glass transition temperature, molecular mobility suddenly increases, which allows recrystallization of the anhydrate at 220°C followed by degradation if the temperature rises above 250°C. Other hydrates were also isolated. However, as hydrate formation depends both on temperature and water activity, DSC or TGA were not able to identify them as RH is not controlled with these techniques. Therefore, VT-PXRD performed without dry nitrogen flux or DVS experiments set conditions for the formation of transitory hydrates during heating of CLP. A monohydrate was isolated at 99.5°C in VT-PXRD and its crystal structure could be resolved based on the powder diffractogram. Another hydrate was observed in VT-PXRD at 60°C but its structure could not be resolved due to the poor quality of the diffractogram. Yet, DVS experiment performed at the same temperature allowed isolating a CLD, forming at 60% RH. This dihydrate converts reversibly to the pentahydrate at 80% RH at 60°C.

Finally, these results also emphasized the importance of controlling both temperature and relative humidity to observe new crystalline forms of CL. Indeed, CLM and CLD would not have been identified if one of those parameter was not controlled. As multiple forms exists, controlling both temperature and relative humidity during transport and formulation of CL is crucial if one wants to avoid any solid-state transition related issues.

II.6. References

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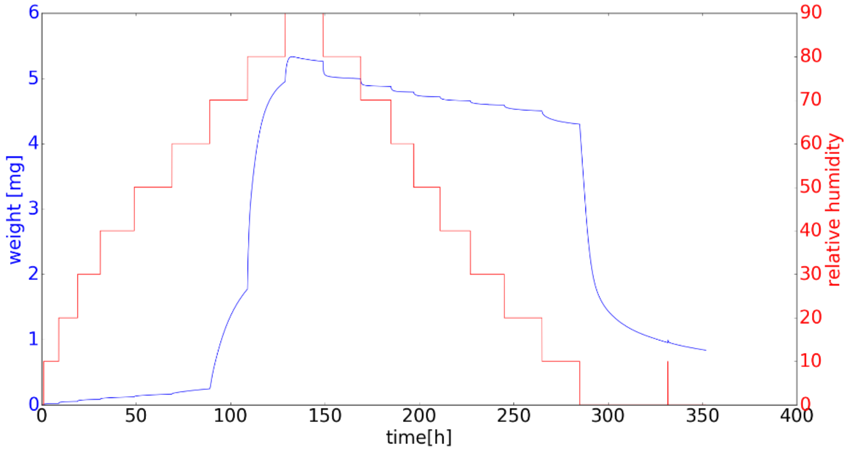
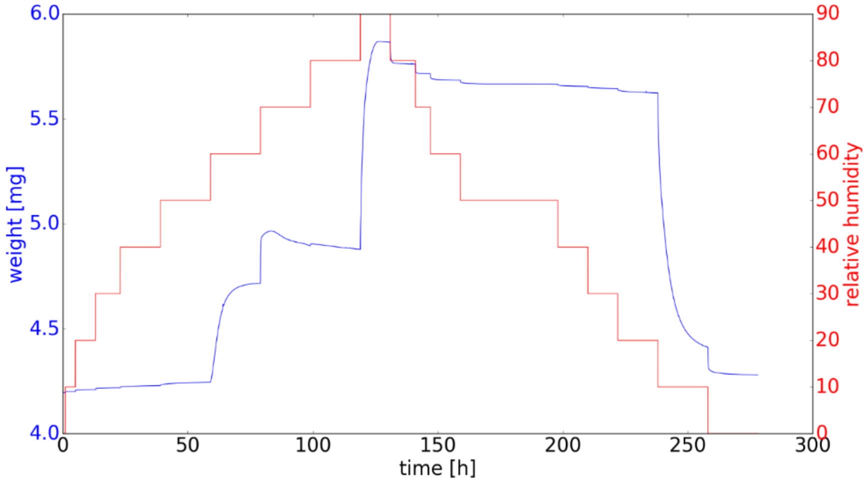
Appendices

Appendix A: Supporting information of Chapter II

A.1. Kinetics of mass absorption during DVS experiment performed at 30°C



A.2. Kinetics of mass absorption during DVS experiment performed at 60°C



A.3. Main crystallographic data for CLM

Calcium Lactate Monohydrate	
CCDC	1963439
Structural formula	$(C_3H_5O_3)_2Ca.H_2O$
Formula weight (g/mol)	236.22
Space group	*P 2₁*
a (Å)	11.4301(2)
b (Å)	6.93577(15)
c (Å)	6.29513(14)
β (deg)	95.9163(12)
V (Å^3)	496.397(19)
Z	2
ρ_{calc} (g/cm^3)	1.580
R_1 (all)	1.1640
wR_2 (all)	1.6733
N_{refl}	905
N_{parm}/N_{rest}	56/52
GOF (S)	343.1944

CCDC 1963439 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structure