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Thermodynamic Equilibrium Study of Deferoxamine Salting Out Crystallization

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The present contribution is focused on the research activity carried out in cooperation with Novartis Pharma S.p.A., finalized to the determination of the thermodynamic equilibrium conditions for the crystallization system made of the three components water (W) - acetone (A) - D, where D is the active ingredient (deferoxamine) of a commercial save-life drug (Desferal®).
The ternary system W-A-D is characterized by the complete miscibility of the system W-A, and the insolubility of D in A. The addition of acetone to the aqueous solution involves, therefore, a reduction of the solubility of D in the bi-component solution W-A with the consequent precipitation of D (formation of a solid bottom layer). The solid phase obtained at the end of the crystallization operation is typically filtered. Some batch productions are often characterized by the formation of a crystalline solid of not well-suited properties (so called "thin crystallization"), resulting in an increased filtration time of mother waters and requiring usually a crystal re-solubilization, with lengthening of the process times and reduction of the total yields.
Tests were directed to comprise the phenomena responsible for "thin crystallization", orienting the productions towards the "normal crystallization". The ternary diagram with equilibrium isotherms for the system W-A-D in the interval of composition and temperature of interest (-5°C, +5°C) was assessed.

1. Introduction

Deferoxamine mesylate USP, otherwise known as desferrioxamine or Desferal®, is a chelating agent used to remove excess iron or aluminium from the body. It acts by binding free iron or aluminium in the bloodstream and enhancing its elimination in the urine (Novartis, 2011; Lal et al., 2013). By removing excess iron or aluminium, the agent reduces the damage done to various organs and tissues, such as the liver. Deferoxamine works in treating iron toxicity by binding trivalent (ferric) iron (for which it has a strong affinity), forming ferrioxamine, a stable complex which is eliminated via the kidneys (De Pinto and Ros Barceló, 1996; Hong et al., 2006). 100 mg of deferoxamine is capable of binding approximately 8.5 mg of trivalent (ferric) iron. Moreover, deferoxamine works in treating aluminium toxicity by binding to tissue-bound aluminum to form aluminoxamine, a stable, water-soluble complex (The International Encyclopedia of Adverse Drug Reactions and Interactions, 2006).
Deferoxamine is N-[5-[3-[(5aminopentyl) hydroxycarbamoyl]propionamido] pentyl]–3-[[5-(Nhydroxyacetamido)pentyl]carbamoyl]propionohydroxamicacidmonomethanesulfonate(salt). Figure 1 shows its structural formula. Table 1 reports some properties of this compound (DrugBank, 2012). The industrial production of this compound is carried through a sequence of steps including salting out crystallization, starting from a rich solution of deferoxamine (D) in water (W), by using acetone (A). The simplified process scheme is illustrated in Figure 2.

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Table 1. Deferoxamine properties.

Property	
Synonyms	Deferoxamine methanesulfonate salt, Desferrioxamine mesylate salt, Desferal, DFOM
Molecular formula	$C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$
Molecular weight	656,79
State	white to off-white powder
Melting point	142-146 °C
Boiling point	105 °C
Density	1.34 kg/dm^3 at 20 °C
Apparent density	~ 400 kg/m^3
Solubility in water	> 200 g/l (at T=20°C)
Solubility in other solvents	Ethanol: slightly soluble 0.1% Acetone: practically insoluble 0.006% Chloroform: practically insoluble 0.007% Methanol: soluble 2.1% (20°C)

The ternary system W-A-D is characterized by the complete miscibility of the system W-A, and the insolubility of D in A. Therefore, the addition of acetone to the aqueous solution involves a reduction of the solubility of D in the bi-component solution W-A with the consequent precipitation of D (formation of a solid bottom layer). The operation is lead in modality fed-batch.

The solid phase obtained at the end of the crystallization operation has characteristics typically compatible with filtration times fixed by the process protocol. However, some batch productions are often characterized by the formation of crystalline solids of not well-suited properties (Asghari and Esmaeilzadeh, 2012). In particular the filtration time of mother waters increases meaningfully and a crystal re-solubilization is usually required, with lengthening of process times and reduction of the total yield (Prisciandaro et al., 2009).

Scope of the research was to comprise the phenomena responsible for “thin crystallization”. The proper selection of operating conditions should orient the productions towards the so called “normal crystallization”. The investigation regarded the assessment of the thermodynamic equilibrium conditions of the ternary system W-A-D.

The knowledge of the thermodynamic equilibrium conditions for the described system in the operating temperature range (-5°C, +5°C) represents a fundamental requirement. It is relevant for the understanding of the evolution of the system during industrial production. As a matter of fact the knowledge of the thermodynamic equilibrium allows to predict not only the final yield of the process but the conditions of concentration too. Then, the driving force occurring in the crystallizer during the entire crystallization process may be assessed.

The experimental activity was carried out with neglecting the presence of oligo-components (*e.g.* chlorides, etc.) that are present in the industrial process, as residual of earlier operations. Moreover, the tests were conducted under pressure and temperature constant, and free-impurity conditions. The equilibrium data so found were preliminary for the estimation on the process kinetic parameters.

The obtained result addressed the D crystallization process interpretation. In particular, a phenomenological picture of the thin crystallization processes occurring in the course of the industrial process was proposed. Moreover, phenomena that invalidate the production yields were pointed out.

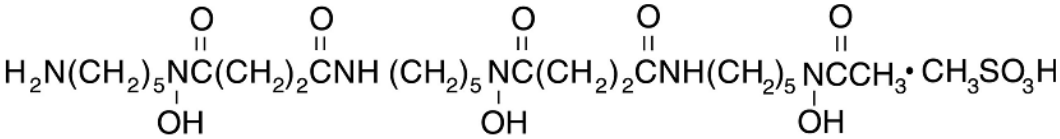


Figure 1. Structural formula of deferoxamine mesylate USP.

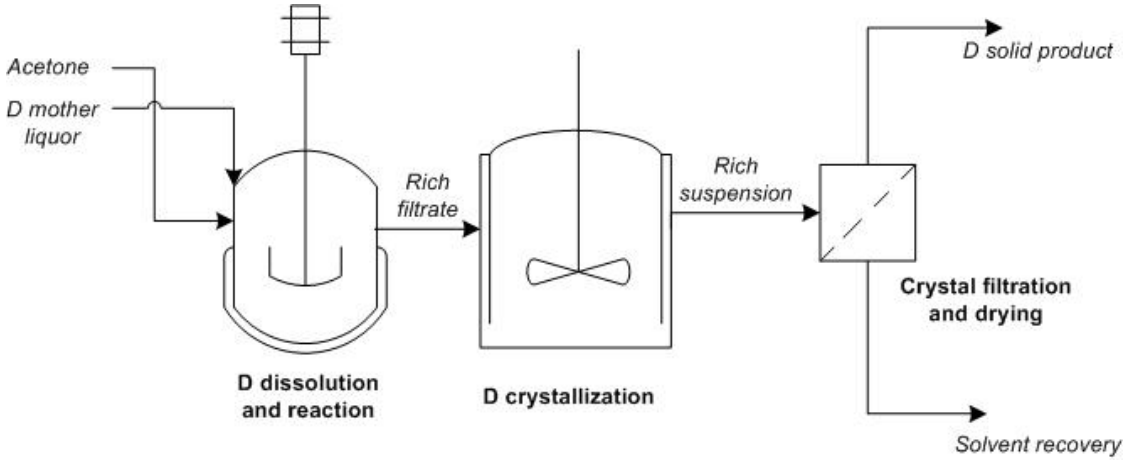


Figure 2. Deferoxamine production simplified flow-sheet.

2. Materials and Methods

Experimental tests were aimed at measuring the equilibrium concentration of D in the system water/acetone (W/A). Tests were carried out with bidistilled water, acetone 99.7% (Sigma Aldrich), and deferoxamine crystals given by Novartis Pharma S.p.A.
The solubility of component D was measured at atmospheric pressure and at temperature set at -4°C, 0°C, +4°C, and +20°C. The ratio W/A in the liquid phase was set at the beginning of each test.
Experiments were carried out under batch mode, following procedure reported hereinafter: a fixed amount of D was loaded in a graduated tube of 15 ml. At room temperature, a solution with a fixed A/W ratio was added. The tube was agitated till the complete solid dissolution. The test tube was then put in a thermostatic bath and maintained at the set temperature for 6-8 h, a time at which the system reaches equilibrium conditions. Therefore, the separation of the two phases was accomplished according to the following steps:
-sample centrifugation for 30 min at 5000 rpm at constant temperature;
-supernatant sampling;
-supernatant microfiltration (0.22 mm);
-supernatant HPLC analysis to measure D content (according to Novartis Pharma);
-solid bottom residue sampling;
-solid bottom washing with acetone;
-solid bottom drying for 48 h at room temperature;
-solid bottom weighing for gravimetric analysis.
Samples of solids were observed at scanning electronic microscope.

3. Results

Equilibrium concentrations measured during tests are reported in a ternary diagram (Figure 3). The upper vertex of the diagram - not reported to improve the graphical resolution of the figure- is pure D (100%$_W$). The reported ternary diagram was limited to solutions with D concentrations up to 30%$_W$. The diagram shows the equilibrium conditions of crystallization estimated at the temperatures of 20°C, 4°C, 0°C and -4°C.
The experimental points of the curves are representative of solutions made of the three components at equilibrium with a solid phase constituted by pure D. For a fixed temperature T, the region above each curve represents supersaturated systems at that temperature; the region below represents solutions of the three components. At room temperature (T=20°C) the saturation concentration of the solution diminishes monotonically with the A concentration. At temperatures lower than 4°C, the saturation concentration for the ternary system vs. A concentration shows a maximum for A concentration comprised between 30 and 40%$_W$. For dilute solutions of A (lower than approximately 35%$_W$) the addition of A to W increases the solubility of D; for concentrated solutions of A (higher than approximately 45%$_W$), the further A addition to the system reduces the D solubility (salting-out).

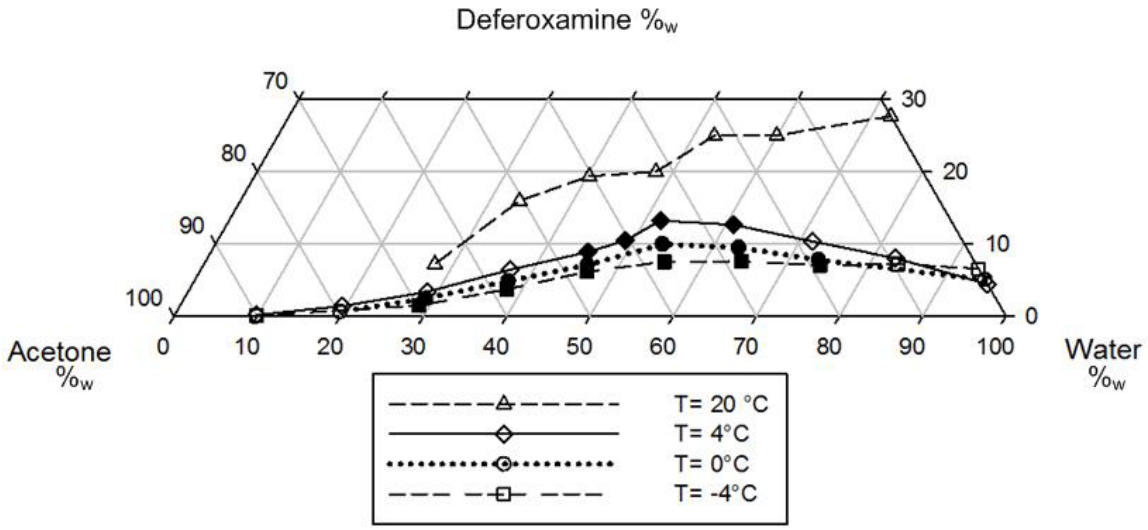


Figure 3. Experimental ternary diagram with equilibrium isotherms for the system W-A-D. Full symbols mark "gel-like" behaviour.

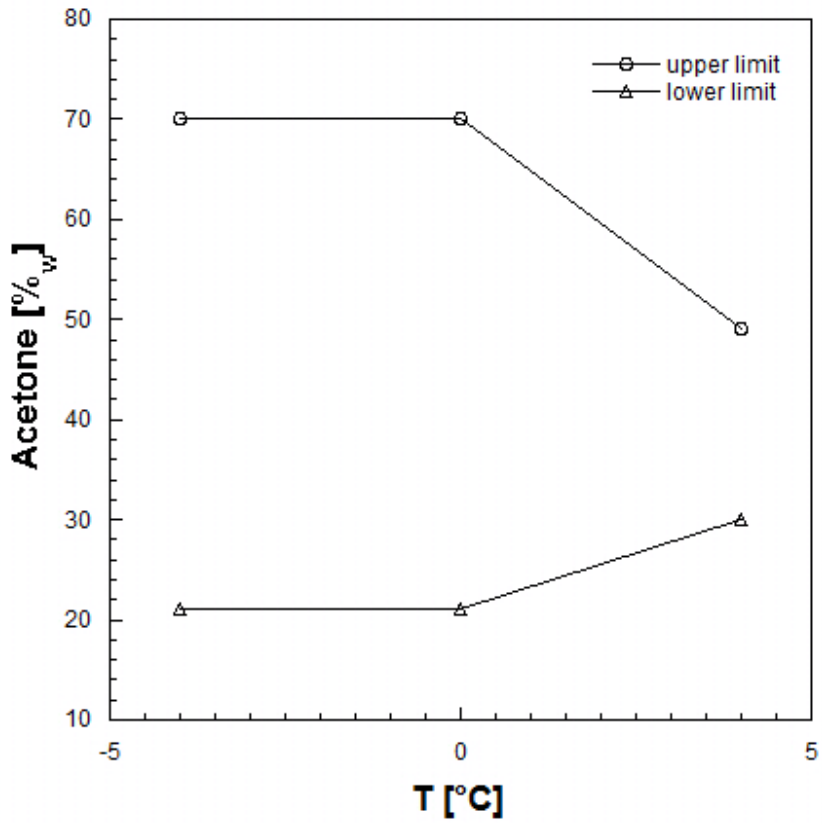


Figure 4. Interval of system instability - gel-like behaviour - of the system W-A-D vs. the temperature.

The observed thermodynamic behaviour at room temperature and at lower temperatures for acetone concentrations higher than 40%$_W$, is responsible for the precipitation of D from solutions when A is further supplemented under quasi-equilibrium conditions.

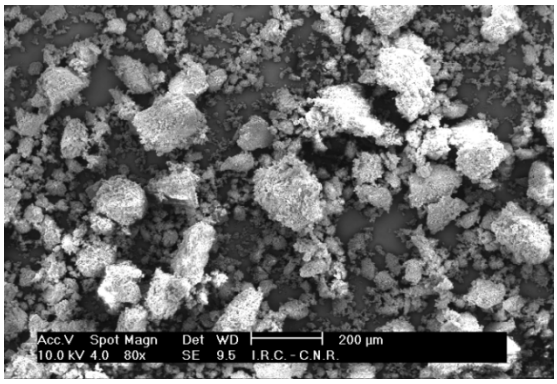
In Figure 3 the representative points of the equilibrium conditions are reported as full symbols and void symbols. The full symbols indicate a behaviour defined as “observed gel-like”, indicating an instability of the system according to the composition. The instability conditions were often recurrent for the same values of composition, once fixed the temperature. The values of superior and inferior limits for the volumetric percentage of acetone inside of which instability conditions are present vary with the temperature. In particular the instability interval decreased with the temperature. Figure 4 shows that the interval of critical composition W/A at T=4°C extends from 30 to 50%$_W$ of acetone. It amplifies between 20 and 70%$_W$ of acetone as for experiments carried out at T=0°C and -4°C. Under gel-like conditions the crystallization process is inhibited because the resistance to the mass transport phenomena from the liquid bulk to the surface of the growing nucleus increases. Obviously, the absence of stirring of the reaction volume emphasizes the phenomenon because the gel-coordinated aggregates are not destroyed and the process continues until it involves the entire volume of crystallization.

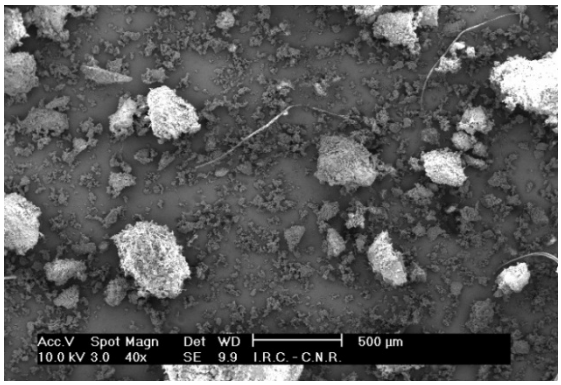
Figures 5 and 6 show typical SEM micrographs of samples collected at the end of crystallization laboratory batches. The images are relative to tests carried out without D-W-A stirring (stagnant system), at -4°C, atmospheric pressure, and at two concentrations of acetone: 88%$_W$ (Figure 5) and 35%$_W$ (Figure 6). It should be remembered that gel-like behaviour was observed at acetone low concentration (≤60 %). From the analysis of particles coming from the sample at high concentration of acetone (88%$_W$, Figure 5), the following considerations can be derived:

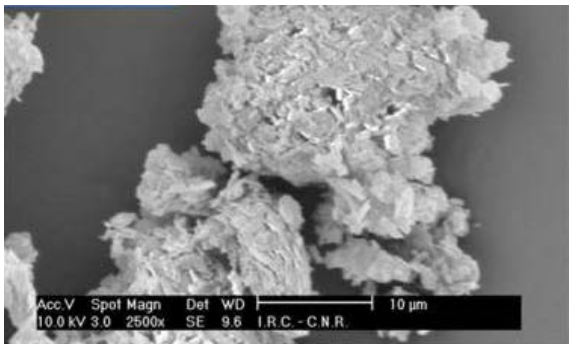
- most particles presents sizes between 100 and 200 μm;
- very small and thin particles in the range 10-30 μm are present.

The analysis of images of particles from the sample at low concentration of acetone (35 %$_w$, Figure 6) reveals the following general considerations:

-a few particles are larger than 200 μm;
- most of the sample solid consists of very variable size particles, also considerably less than 50 μm.







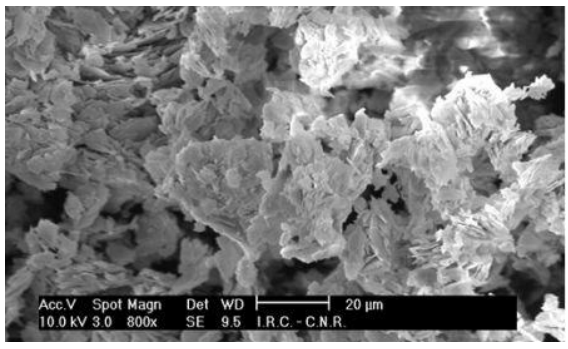


Figure 5. SEM observations of crystals: 88%w of acetone

Figure 6. SEM observations of crystals: 35%w of acetone. Crystallization under “gel-like” behaviour.

4. Conclusions

The ternary system W-A-D (water-acetone-deferoxamine) was investigated. The solubility of the deferoxamine was assessed under controlled conditions close to those adopted in industrial processes. The crystallization behaviour depended on the concentration water/acetone. A “gel-like” behaviour was observed and the W/A interval within which it was observed depended on the temperature. Under “gel-like” behaviour particle distribution was characterized by the presence of a huge amount of particles smaller than 50 μm. The presence of fines drastically increases the cake specific resistance of the powder.
In both cases, the surface detail highlights the presence of agglomeration of lamellar units, each with size of approximately 1-2 μm (see high magnification micrographs in Figures 5 and 6).

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