INVESTIGATIONS ABOUT BREAKAGE OF PROTEIN CRYSTALS DURING FILTRATION PROCESSES

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ABSTRACT

Protein crystals have a low mechanical stability in comparison to non-organic crystals. Low mechanical stability of crystals might influence the solid-liquid-separation process. This paper focuses on crystal breakage during filtration. A piston puts pressure on the filter cake, and the variation of the cake resistance by pressure is detected. Crystal sizes of the model system lysozyme are determined by image analysis after the compression of the filter cake. Aim of this work is to evaluate the influence of crystal breakage on the compressibility of the filter cake. The result shows a diminution of particle size, which is independent of the particle size of the original suspension. Hence the cake resistance is similar for all experiments.

KEYWORDS

Protein Crystals, Mechanical Stability, Breakage, Filtration

1. Introduction

Crystallization is a common technique to purify and formulate proteins in the pharmaceutical industry. In comparison to non-organic crystals, protein crystals have a lower mechanically stability due to a higher water content. Furthermore the mechanical response of protein crystals, e.g. lysozyme, is described as highly anisotropic [Zamiri 2010]. By this reason indentation tests only deliver a reference value for the mechanical stability of crystals during crystallization and separation processes.
Low mechanical stability of crystals influences solid-liquid-separation processes in two ways. Firstly attrition due to stirring in the crystallization step influences the particle size distribution and leads to worse separation [Lee 2002] [Shamlou 1996].
Secondly crystals break during the separation step itself. In centrifuges the regions of strong stress are the suspension inlet and the solid discharge [Boychyn 2001].
Crystal breakage occurs as well during the filtration process. In this case pressure causes breakage phenomena. For non-organic crystals the usually applied filtration pressures do not exceed the material strength [Wiedemann 1996]. However, protein crystals have a 10 times lower mechanical strength in comparison to e.g. non-organic sodium chloride. Therefore we assume breakage phenomena during filtration of protein crystals. There are many reasons for the compressibility of filter cakes such as rearrangement, deformation, interaction between particles and the breakage of particles. The aim of the work is to examine the influence of crystal breakage on the

compressibility of filter cakes. The compressibility is determined by experiments in a compression-permeability cell. In this apparatus pressure is applied on the filter cake by a piston. After consolidation of the cake the resistance is determined by a flow of the mother liquor through the filter cake. To get information about breakage of crystals caused by pressure, parts of the cake are carefully resuspended in the mother liquor and the particle size is analyzed. In this work the size is detected by image analysis of pictures of the crystal suspensions. Two main influencing parameters for the mechanical stability of the crystals are the water content and the crystal size and distribution. First examinations will be shown of the influence of pressure on lysozyme crystals.

2. Materials and Methods

Crystallization

Lysozyme from chicken egg white was provided from OVOBEST Eiprodukte GmbH & Co.KG. The crystallization conditions were adapted from Hekmat et al [6]. Sodium chloride is used as the precipitant. The final concentration of sodium chloride in the crystallization slurry was 40 g/l and the lysozyme concentration was 50 g/l. At first 100 g/l of lysozyme was completely dissolved in a 25 mM sodium acetate buffer at pH 4. In the next step the same amount of a solution containing 80 g/l sodium chloride was slowly added to the lysozyme solution. Thus high local concentrations of the precipitant and hence an amorphous precipitation are prevented. After 24 hours of stirring the crystallization was stopped. A floating magnetic stir bar (Figure 1) and a stir speed of 200 rpm was used. For the determination of the crystal area distribution pictures of the slurries were taken by a transmitted light microscope at a magnification of 40. Prior to image sampling the slurry was diluted with its mother liquor. The pictures were converted into binary pictures and the size distributions were determined using a public domain image processing software (ImageJ). Figure 2 shows the crystal area size distributions of

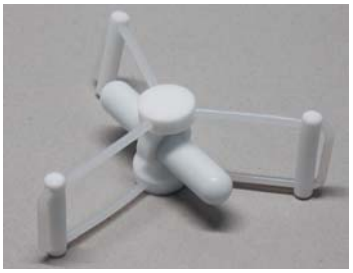

Figure 1: Floating magnetic stir bar

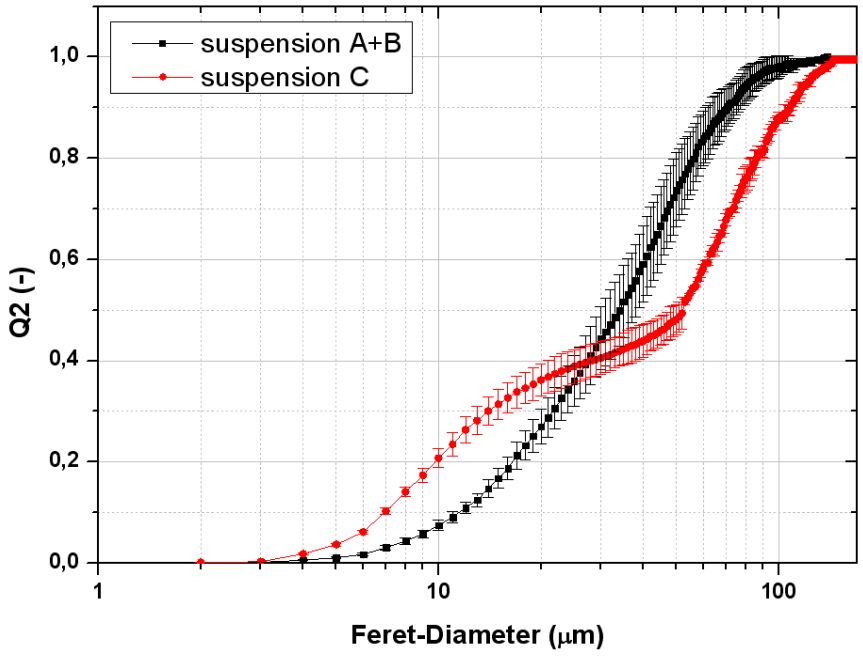

Figure 2: Size distribution of two produced lysozyme crystal slurries

the original suspensions. The error bars display the standard deviation of three taken samples of the crystal slurry.

Filtration

The filtration experiments were conducted in a CPF-Cell. Its inner diameter is 20 mm (see Figure 3). The filter medium is a polyamid membrane of PALL GmbH with a mean pore diameter of 0.1 µm.

To avoid the influence of sedimentation on the structure of the filter cake the suspension was concentrated by sedimentation. After sedimentation the supernatant was drained and filtered through a membrane of mean pore diameter of 0.1 µm to remove all remaining particles from the mother liquor. This avoids remaining particles ending up in the cake during the flow step and influencing the measured cake resistance. Before filling the concentrated crystal suspension in the cake building device the filtrate outlet was prefilled with mother liquor. By this way dead volume was avoided, so the filtrate mass was detected form the beginning. Then the concentrated suspension was filled in the device and the plunger was set carefully on the surface of the suspension. Finally the remaining dead volumina of the device were prefilled with mother liquor.

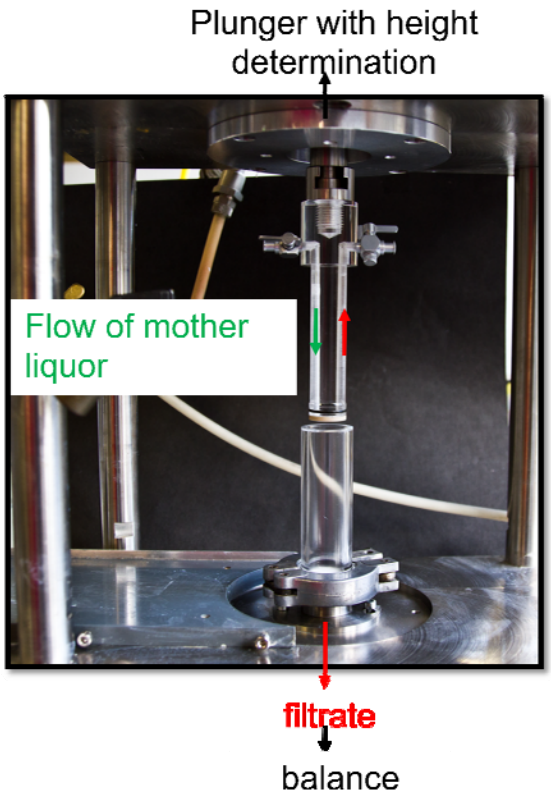

Figure 2: CPF-Cell

The test was started subsequently by applying pressure on the filter cake and flowing of mother liquor through the cake in turn. The experimental scheme is shown in figure 4. P indicates a pressure step where the plunger puts 2.5 respectively 5 bar pressure on the surface of the filter cake for 600 min during each pressure step. F indicates the flow step where the resistance of the consolidated cake is detected by flowing of mother liquor through the cake. The pressure in the receiver tank, which is filled with mother liquor, was kept constant at 0.2 bar. By keeping the flow pressure below the press power changes in the structure of the filter cake due to high liquid pressure is avoided. The cake was pressed in each pressure step two times to investigate whether the flow of mother liquor through the cake or temperature fluctuations changes the cake structure and hence the resistance. The filtrate mass is detected by a balance at the outlet and the temperature was recorded as well. A mass of 2 ml was filtered in each flow step. The filtrate mass over time was recorded.

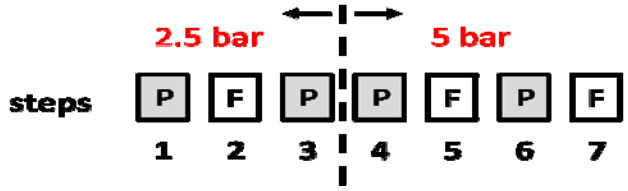

Figure 4: Experimental scheme

The filter cake resistance was calculated for every pressure step according to the following formula:

$$\alpha = \frac{\Delta p \cdot A^2}{V \cdot \eta \cdot m_s}$$

A: filter area [cm^2]
Δp: pressure difference [Pa]
α: cake resistance [m/kg]
V: Volume of filtrate [m^3]
m_s: mass of dry cake [g]

The cake porosity ε of the cake was calculated from the measured cake height and the dry mass of the cake. The cake height has been detected online.

$$\varepsilon = 1 - \frac{m_s}{\rho_s A h_k}$$

with

m_s: mass of dry cake [g]
ρ_s: solids density [g/cm^3]
h_k: cake height [mm]

The cake was dried at 30°C to determine its mass. Prior to drying two samples were taken from the cake rim and the center. The samples were carefully resuspended in the mother liquor and the particle size distribution was determined according to the procedure described before. By this means information on the change in the crystal size distribution in the cake itself is gained.

3. Results

Generally compressibility is visible for all of the three conducted experiments. The porosity of the cake decreases and the cake resistance increases at a pressure increase, as shown in Figure 5. Noticeable is the difference between the calculated cake resistances after the second pressure stage of 5 bars. The reason of the differences is the fact that the cake isn´t totally consolidated after the second pressure step at 5 bar (Stage 6). A linear decrease of the original cake height of around 2% in this pressure stage is observed. At 2.5 bar the cake height stays constant during the second pressure step, hence the consolidation is finished.
For experiments A and C the cake resistance and porosity is similar in spite of the different particle size distributions. Experiment B shows slightly higher resistances for 2.5 bar and larger deviations for 5 bar.

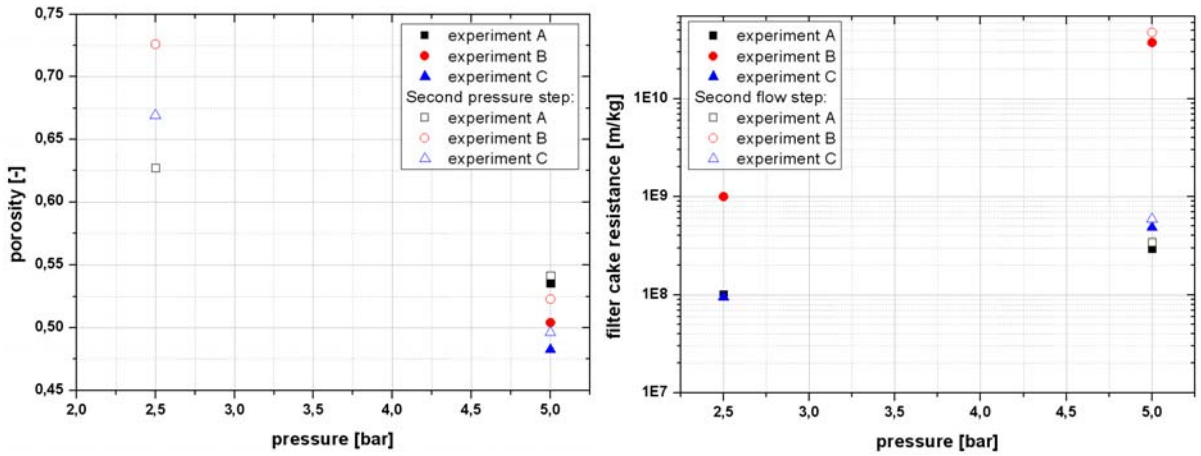

Figure 5: porosity over pressure plot (left) and cake resistance over pressure plot (right)

The porosity at 5 bar corresponds to the porosity of the other two experiments. This do not fit because porosity and cake resistance are related to each other and for a higher resistance a lower porosity was expected.

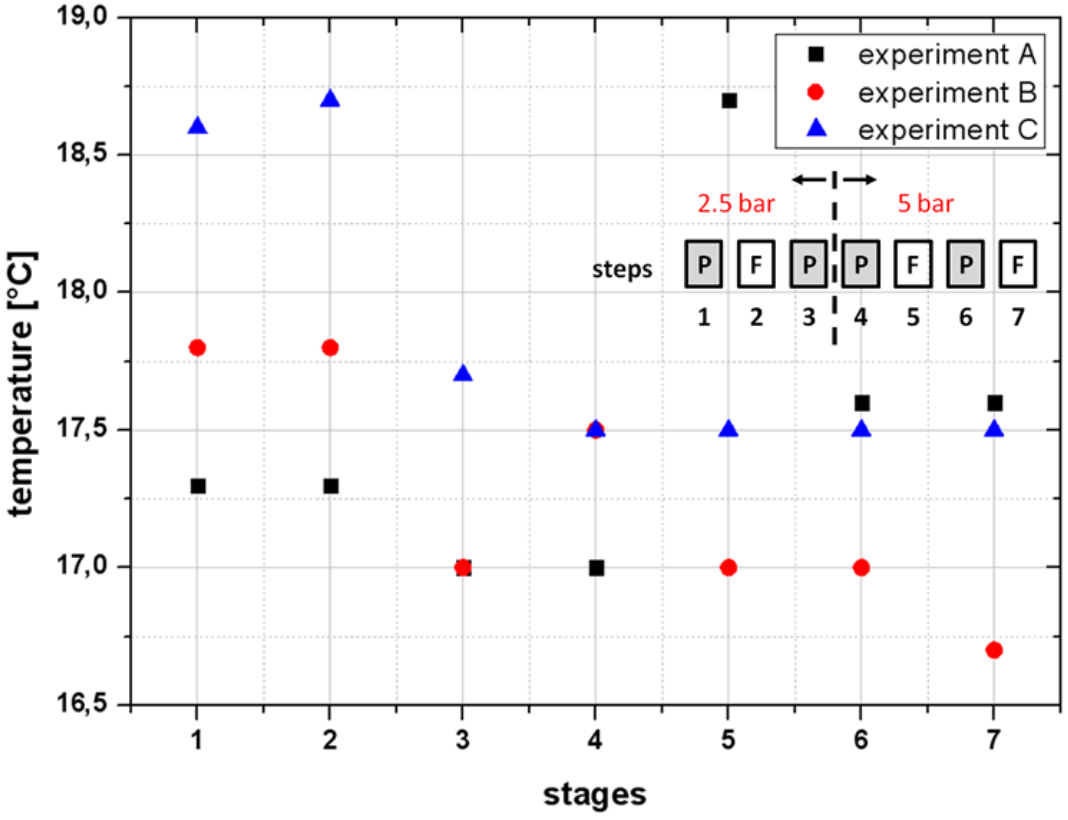

Figure 6: Minimum temperature during the experiments and different stages

A possible source for the increased resistance is crystallizing of lysozyme or salt from the mother liquor in the pores of the filter media. This effect only occurs for experiment B. Figure 6 shows the minimum temperatures measured during the experiments. During experiment B the temperature falls below 17°C, compared to

experiment A and C the temperature is generally lower. This supports crystallizing out of lysozyme or salt of the mother liquor.
In the crystal size distribution a diminution of the distribution is visible after compressing the cake at 5 bar. However the size distribution of the final state is similar despite the initial difference of the suspension, resulting in a similar cake resistance.
In literature forces close to the wall are reported to be weak compared to the center of the cake [Lu 1998], hence we expected a difference between the rim and the center of the cake. In experiment A no difference was measured, while experiment B and C showed a slightly higher particle size at the rim compared to the center, as we expected according to the stress distribution within the cake.
A reference of the original suspension was measured to test the influence of the non-constant temperature on the particle size distribution. Experiment B showed no difference, but during experiment C a diminution of the particle size is observed. Reason for that are stronger temperature fluctuations during experiment C.

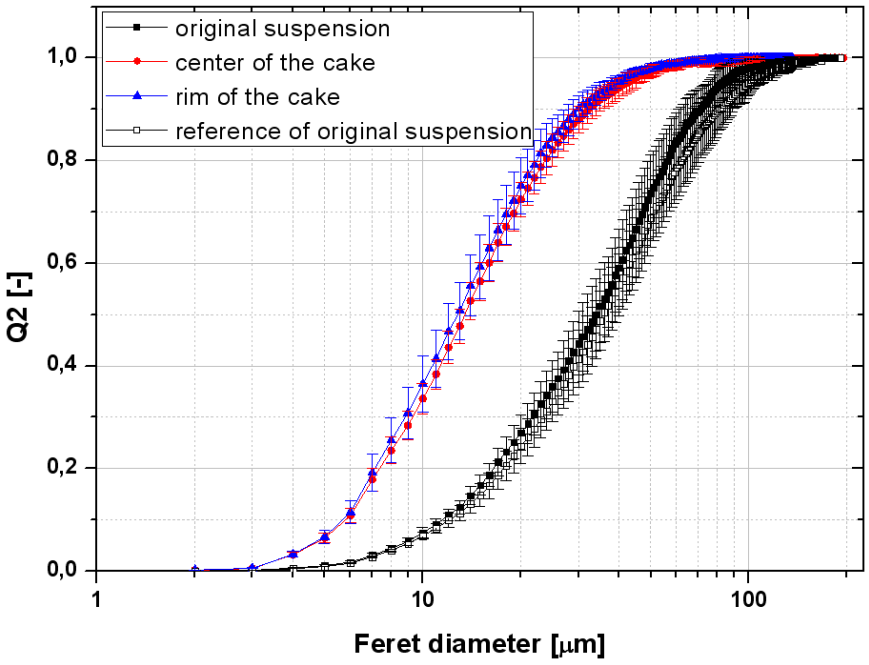

Figure 7: Size distributions of experiment A

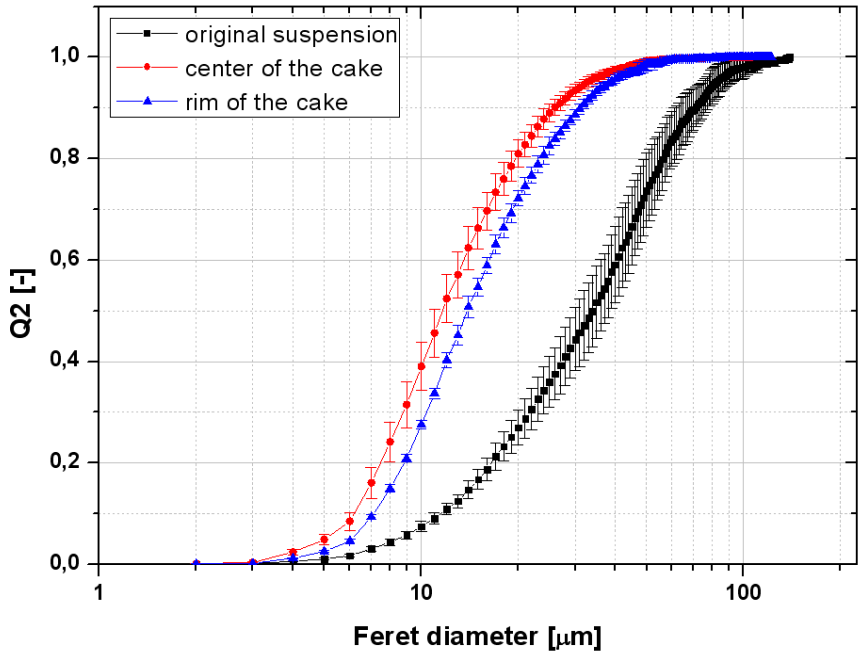

Figure 8: Size distribution of experiment B

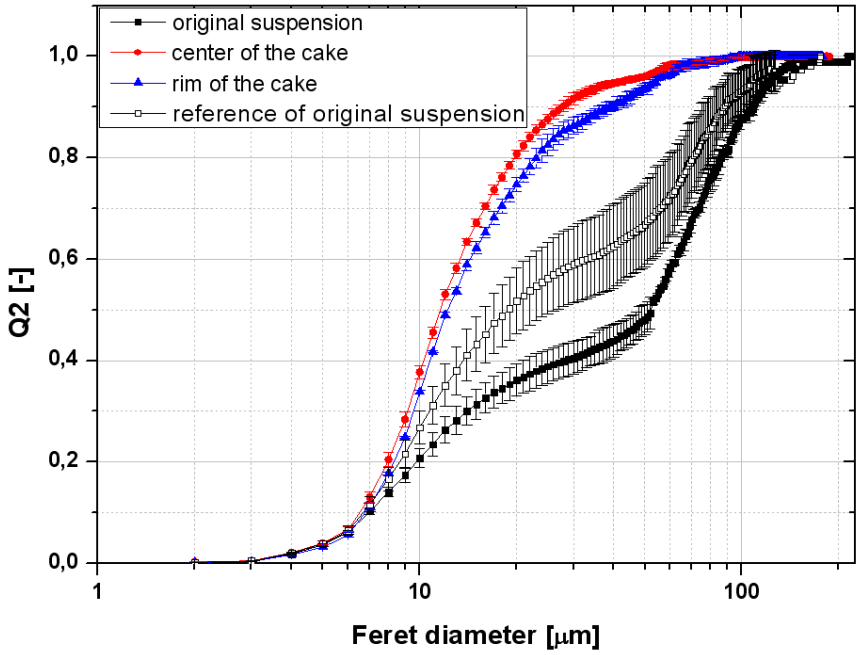

Figure 9: Size distributions of experiment C

4. Conclusions

Tests on crystal breakage have been successfully performed. In the experiments, the diminution of the crystals as a consequence of the applied pressure was strong enough to create the same final size distribution independent of the initial protein crystal size. As a consequence for the separation of proteins by crystallization and

subsequent filtration, care has to be taken to avoid breaking, which might harm the crystals and reduces filtration efficiency.
As an outlook, the size distribution of the filter cake after compression at high and low pressure is interesting. The influence of the median particle size is another important parameter. Furthermore protein crystals which differ in molecular weight might show other effects during compression.

5. Acknowledgements

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