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DOWNSCALING CAKE-FILTRATION - AN INVESTIGATION OF A SEPARATION PROCESS FOR CRYSTALLIZED PROTEINS

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ABSTRACT

The production of pharmaceutical active proteins is in general very expensive. Hence the required amount of product for the determination of crystallization and separation conditions must be reduced. One option for the gentle separation of protein crystals from the mother liquor is cake filtration at low pressure differences. In cake filtration it is possible to reduce the required feed material by a reduction of the filter area.
This work presents a comparison between the filtration behavior of two lysozyme crystal slurries of different mean particle sizes and filtration areas of 20 cm^2 and 3,14 cm^2 respectively. The results show a good agreement between the two filter areas even though the slurries are a rather complex product.

KEYWORDS

Cake Filtration, Microfiltration, Downscaling, Lysozyme crystals

1. Introduction

Crystallization of proteins provides an alternative process to chromatographic techniques for purification purposes. Following crystallization the solids have to be separated mechanically from the mother liquor. The main challenge of the separation process development is the low mechanical stability of protein crystals in comparison to anorganic and other organic crystals. The reduced mechanical stability is due to a higher water content of the protein crystals [1].
One possibility to separate the crystals gently from the mother liquor is cake filtration at low pressure. Standard filtration tests are often performed in a filter nutsche of 20 cm^2 filtration area. The application of a standard nutsche filter implies the need of a large quantity of product to determine optimal filtration conditions. However the production of proteins is in general very expensive; hence the aim is to find optimal separation conditions for small amounts of feed material. This is realized by a reduction of the filtration area. It has been shown for compression-permeability cells that a reduction of the filter area affects the porosity of the filter cake, because boundary effects gain on influence [2]. However investigations on the scaling behavior of pressure nutsches have been carried out at large cake heights (50 mm or more) and non comparable filter apparatuses [3]. Tarleton et al [4] also investigated the scaling behavior of filtration for suspensions of different volume concentrations, varying pressure differences and filter areas. Only filtration behavior of suspensions with mean particle sizes below 15 µm was investigated though. Tarleton concluded from his results that the scaling behavior depends on the suspension characteristics.

The challenge of downsizing cake filtration is to identify the filtration conditions and suspension composition for which wall effects respectively cake structuring effects play an important role. In order to ensure the comparability of experiments with two different filter areas the reduction is realized by inserting a small cake formation ring into the standard nutsche filter. The maximum cake height is limited by the height of the cake formation ring to 17 mm. For compression-permeability cells Grace [5] suggested a cake height to filter diameter ratio of less than 0.6 to minimize the relative influence of the wall. To investigate the influence of the ratio cake height to filter diameter, experiments with two different cake heights were carried out. One cake height above and another below 0.6 was realized (for the small filter area). A membrane instead of a filter cloth was chosen as the filter medium. Membranes avoid solid and gas breakthrough and therefore are advantageous for filtration of crystal slurries. Gas flow through the filter media leads to a crystallization of mother liquor in the pores and hence to pore blocking. This results possibly in a worse dehumidification of the filter cake. The model protein used for the scaling experiments is lysozyme. This protein is relatively cheap, easy to crystallize and has good handling properties.
The work presents a comparison between the two filter areas with regard to the main filtration parameters e.g. filtration time, porosity and permeability of the cake, filter medium resistance and residual moisture content. Furthermore the influence of the sedimentation behavior is discussed.

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. 100 g/l of lysozyme was firstly completely dissolved in a 25 mM 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 are prevented and hence an amorphous precipitation. After 24 hours of stirring the crystallization was stopped. To produce slurries of two different mean crystal sizes the slurries were stirred using different magnetic stir bars and stirring speeds. The small crystal size was realized using a conical magnetic stir bar and a stirring speed of 600 rpm (Suspension 1). A floating magnetic stir bar (Figure 1) and a stir speed of 200 rpm was used to get a crystal slurry containing larger crystals (Suspension 2).

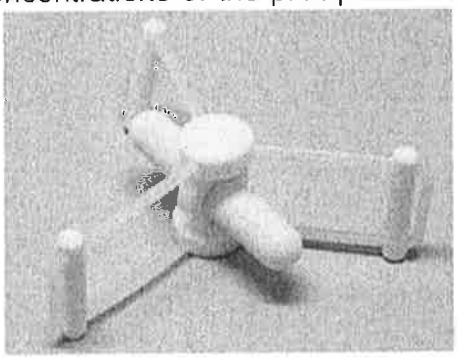

Figure 1: Floating magnetic stir bar

The reached solids volume concentration in the crystal slurries ranged from 3.3 to 3.4 Vol%.
For the determination of the crystal area distributions pictures of the slurries were taken with 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

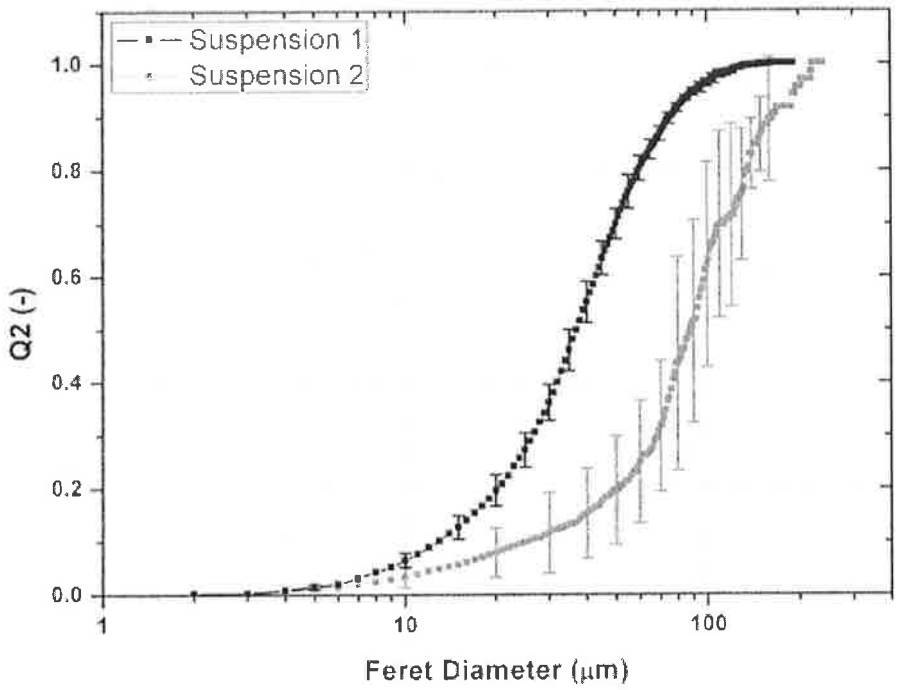

Figure 2: Cumulative area size distribution over the feret diameter of the two produced crystal suspensions

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 the two produced crystal slurries. The error bars display the standard deviation of two taken samples of the crystal slurry. As the graph shows the error between the two samples is larger for suspension 2. This is due to the fast sedimentation of the particles in the slurry. For this reason it is difficult to take uniform samples of the slurry.

Filtration

The filtration experiments were conducted in a standard pressure nutsche from BHS Sonthofen GmbH with an inner diameter of 50 mm. A cake formation ring with a diameter of 20 mm (Figure 3) can be inserted into the nutsche for reduction of the filter area. A polyethersufon membrane (Supor®1.2 µm) of PALL GmbH was used as filter medium. Polyethersulfon has a good chemical resistance and the water flux through this membrane is high.

Figure 3: Cake formation ring

The room temperature was kept constant at 20°C to avoid a change of the suspension during the experiments. The crystallization of lysozyme does not lead to reproducible particle size distributions. To ensure nevertheless a comparable suspension composition the slurry was divided into parts prior to filtration. By prefilling the filtrate outlet with water there is no dead volume. Therefore no correction of the filtration curves is

necessary. Furthermore the clean water flux through the membrane was detected before every filtration experiment. When the flux fell below a certain value the plugged membrane was replaced. All experiments were performed at a pressure difference of 0.5 bar and the dehumidification time was 1000 s. After 1000 s of dehumidification the cake reached its minimum residual moisture at 0.5 bar pressure difference indicated by the fact that the weight on the balance was constant for minimum 200s.
During filtration the mass flow over time was detected. The results were evaluated according to conventional filtration theory. The function time divided by volume versus time is plotted. The permeability of the cake and resistance of the filter media are calculated with the gradient and axis intersection of the following equation:

$$\frac{t}{V} = \frac{\eta \kappa}{2A^2 \Delta p P_c} V + \frac{R_m \eta}{A \Delta p}$$

with

t:time [s]
V: filtrate volume [cm^3]
η: dynamic viscosity of the filtrate [Pas]
κ: concentration parameter [-]
A: filter area [cm^2]
Δp: pressure difference [Pa]
P_c: cake permeability [m^2]
R_m: membrane resistance [m^{-1}]

The concentration parameter κ is defined as:

$$\kappa = \frac{c_v}{1 - \varepsilon - c_v}$$

with

c_v: solids volume concentration [-]
ε: cake porosity [-]

The porosity ε of the cake is calculated as follows:

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

with

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

3. Results

Figure 4 and Figure 5 show the standardized mass versus time curves of the two filtered suspensions. The error bars illustrate the deviations of the curves of the two conducted experiments. The jump at the end of the curves results from the slightly different filtration times of the two experiments. The curves of the two filtered suspensions and filter areas agree well. The deviations between the conducted experiments are larger for suspension 2. The reason for that deviation is the fast sedimentation of the larger crystals. This complicates the exact partitioning of the crystal slurry for the filtration trials. Therefore the crystal size distributions of the filtered crystal slurries differ.

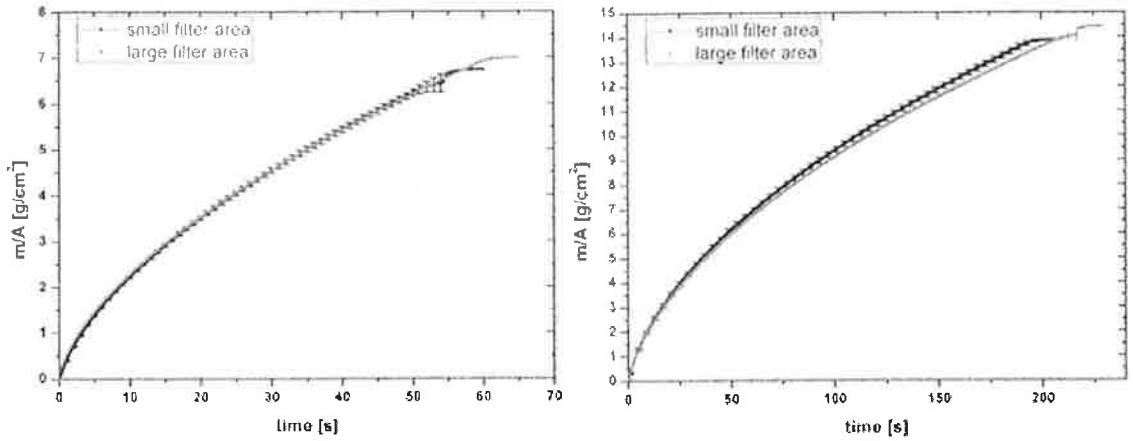

Figure 4: Standardized mass against time curves for small cake height (left) and high cake height (right) of suspension 1

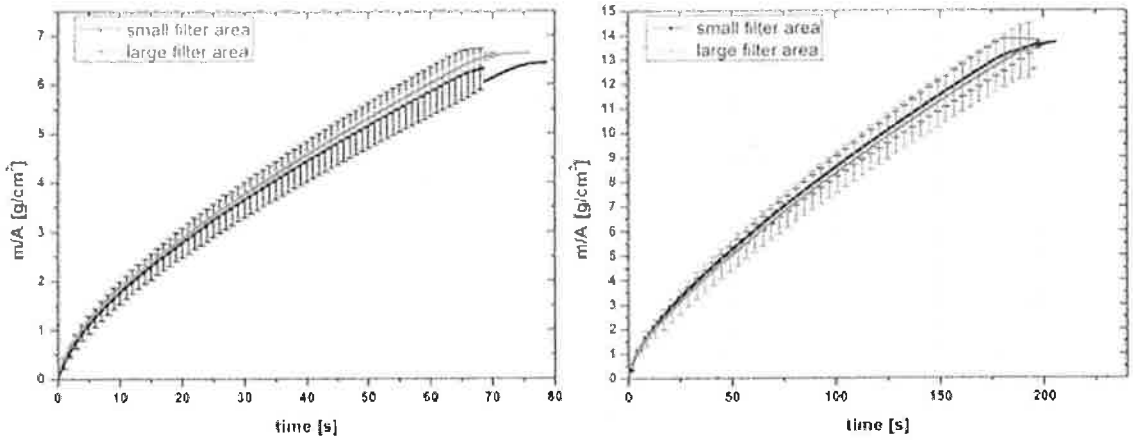

Figure 5: Standardized mass against time curves of small cake height (left) and high cake height (right) of suspension 2

The same is shown in Figure 6. The time over volume against time curves of the performed filtration experiment are displayed. Suspension 1 shows almost linear filtration behaviour and a very small difference of the curve progression (left picture). In the right diagram no linear filtration behaviour is observed. The filtration is overlaid by a sedimentation process and the two conducted experiments show a bigger difference for suspension 1. Furthermore the curve progression is different between the two cake heights. At small cake height there is no linear part of the filtration curve, i.e. during the whole filtration the particles settled down. A flow of clean filtrate through the cake at large cake height is evident by the linear region at the end of the curve.

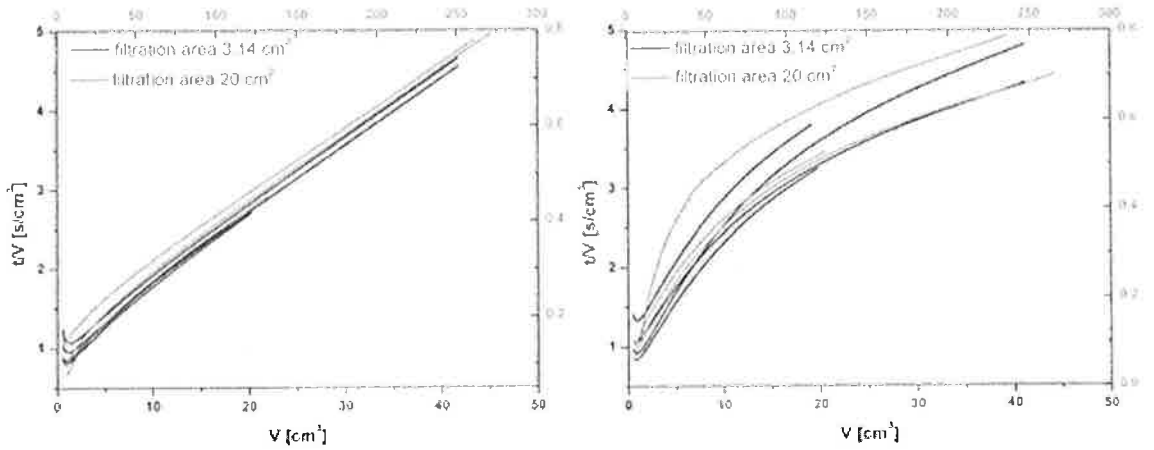

Figure 6: Filtration curves of suspension 1 (left) and suspension 2 (right)

This has certainly an influence on the calculated permeability and membrane resistance, because these two parameters are calculated here with the slope and axis intersection at the end of the curve. Consequently the calculated values for the permeability and membrane resistance of suspension 2 (see Table 2) are not useful for up-scaling. But as expected in accordance to the filtration curves the values for permeability and membrane resistance of the two suspensions and filter areas are close (See Tabel 1 and 2). The same applies for other calculated filtration values. The fact that the filtration time and porosity of the two filtered suspensions doesn't vary very much is unexpected. As the mean particle diameter almost doubles the filtration time of suspension 2 is expected to be shorter than the time of suspension 1. An explanation for this phenomenon is the small ζ-potential of the lysozyme crystals. With decreasing particle size the ζ-potential gains on influence and a more porous cake is formed. Furthermore in suspension 2 there are mainly large single crystals while suspension 1 consists of aggregates. This might be an explanation for the discrepancy of the filtration behavior of the slurries.in comparison to theory.

Table 1: Filtration data of suspension 1

	Large filtration area		Small filtration area	
	Small cake height*	Large cake height	Small cake height	Large cake height
Permeability (m^2)	1.06E-12 ± 0.00E-12	1.07E-12 ± 0.03E-12	1.12E-12 ± 0.08E-12	1.18E-12 ± 0.036E-12
Membrane resistance (m^{-1})	1.21E+09 ± 0.00E+09	1.68E+09 ± 1.27E+09	1.33E+09 ± 0.06E+09	1.65E+09 ± 0.03E+09
Residual moisture (%)	48.81 ± 0.00	49.64 ± 1.63	51.78 ± 0.05	50.53 ± 0.81
Cake height (mm)	7.4 ± 0.00	14.02 ± 0.03	7.39 ± 0.12	14.06 ± 0.13
Porosity (%)	64.98 ± 0.00	62.02 ± 0.02	66.02 ± 0.86	62.87 ± 0.12
Filtration time (s)	59.5 ± 0.00	209.1 ± 8.49	52.45 ± 3.32	192 ± 2.97

* Experiment not reproduced

Table 2: Filtration data of suspension 2

	Large filtration area		Small filtration area	
	Small cake height	Large cake height	Small cake height	Large cake height
Permeability (m^2)	1.33E-12 ± 0.03E-12	2.41E-12 ± 0.08E-12	1.18E-12 ± 0.08E-12	2.34E-12 ± 0.32E-12
Membrane resistance (m^{-1})	2.62E+09 ± 0.04E+09	4.43E+09 ± 0.62E+09	2.63E+09 ± 0.43E+09	4.05E+09 ± 0.17E+09
Residual moisture (%)	43.68 ± 1.11	42.76 ± 0.19	51.31 ± 0.50	46.29 ± 5.98
Cake height (mm)	7.96 ± 0.29	15.27 ± 0.39	7.83 ± 0.15	15.56 ± 0.20
Porosity (%)	67.26 ± 0.18	64.23 ± 1.31	66.58 ± 1.60	64.48 ± 1.76
Filtration time (s)	67.5 ± 2.83	186.05 ± 0.50	68.15 ± 6.01	187.1 ± 13.44

4. Conclusions

The results show good agreement between the two filter areas even though the slurries are a rather complex product. No significant difference is seen between the filtration behaviour at different scales of the two crystal slurries and cake heights. A downscaling of batch filtration seems to be possible for the examined system.
Based on the discussed results it could be interesting to investigate the behaviour of crystal slurries at larger pressure differences and when using a conventional filter media. Furthermore it would be interesting to examine the behaviour of other model products at different solid concentrations and smaller particle sizes.

5. Acknowledgements

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