1. Development and Optimization of a Mammalian Cell Perfusion Culture

Project objective

Continuous manufacturing of complex therapeutic proteins is currently evaluated as alternative production mode to the established batch-wise processing in the biopharmaceutical industry. Given the ability of Chinese hamster ovary (CHO) cells to carry out human-like protein modifications, in particular mammalian cell perfusion cultures [1], [2] regained interest. Long-term term stable, high cell density cultures offer higher volumetric productivities and a more homogeneous product quality.

Project description

The aim of this project is the development and optimization of a mammalian cell perfusion culture for the constant/stable production of an unstable protein. The short residence time of the product in the reactor combined with high viable cell densities is intended to yield in a high fraction of the pure protein. In a first step, the student will determine suitable operating conditions (flow rates, media composition, pH, DOT) by the application of small-scale experiments. In a next step, the identified process parameters are tested in a previously developed and well-characterized bench-scale bioreactor setup. Finally, the reactor operation is further optimized with respect to cell specific productivity and product quality. This project is part of our overall effort to build up an end-to-end integrated continuous production stream for therapeutic proteins.

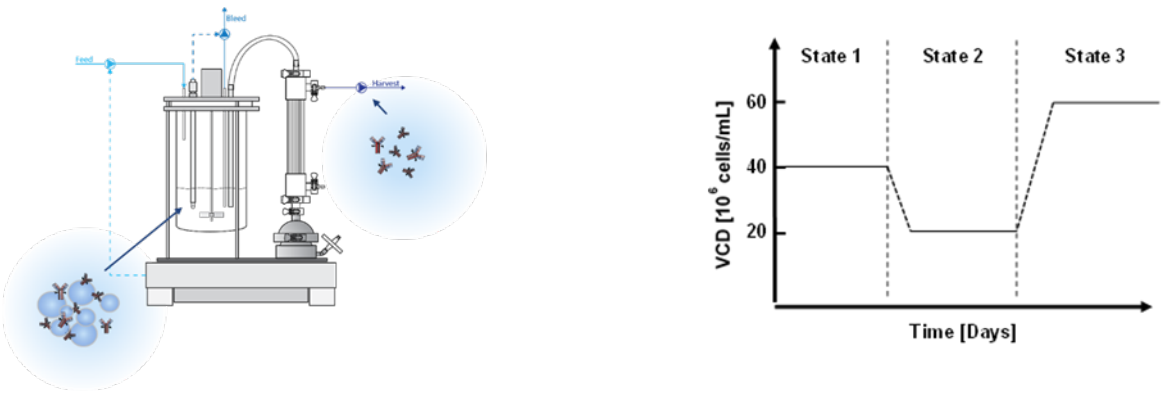


Fig 1: Schematic description of the project

[1] V. Warikoo, R. Godawat, K. Brower, S. Jain, D. Cummings, E. Simons, T. Johnson, J. Walther, M. Yu, B. Wright, J. McLarty, K.P. Karey, C. Hwang, W. Zhou, F. Riske, K. Konstantinov, Integrated continuous production of recombinant therapeutic proteins, Biotechnol. Bioeng. 109 (2012) 3018–3029.

[2] M.-F. Clincke, C. Mölleryd, Y. Zhang, E. Lindskog, K. Walsh, V. Chotteau, Very high density of CHO cells in perfusion by ATF or TFF in WAVE bioreactorTM. Part I. Effect of the cell density on the process., Biotechnol. Prog. 29 (2013) 754–767.

[3] D. Karst, E. Serra, T. Villiger, M. Soos, M. Morbidelli, Characterization and comparison of ATF and TFF in stirred bioreactors for continuous mammalian cell culture, Biochem. Eng. J. 110 (2016) 17-26..

Work description

100% experimental, however chemical engineering concepts will be applied.

Contact person

Moritz Wolf, moritz.wolf@chem.ethz.ch, +41 44 632 30 69, HCI F123

2. Affinity capture chromatography with release of product by a reaction

Project objective

Most proteins are produced in cell cultures. The purification of target protein from other cell culture components is very challenging. In order to simplify the first step in the purification process, a so-called affinity chromatography step is widely used.

The concept of affinity chromatography is to cover the surface of the stationary phase with a molecule that binds specifically to the target. Therefore, the target protein can be bound to the surface and all other products stay in solution. After changing the conditions in the liquid phase, the purified product is released from the stationary phase.

Project description

Such a stationary affinity phase will be tested. Compared to the usual affinity phases, in this case the protein is designed in a way that it has a tag that is recognized by the resin. Instead of eluting the product with the tag, the elution is done by a reaction in which the protein is split from the tag and the untagged protein is collected at a high purity. In a second step, the tag is removed from the stationary phase. Now the stationary phase can be reused to capture new tagged protein.

The thesis will focus on the characterization of the stationary phase. Loading capacities will be determined as well as recovery of purified protein, reusability of the stationary phase and capacity loss over time.

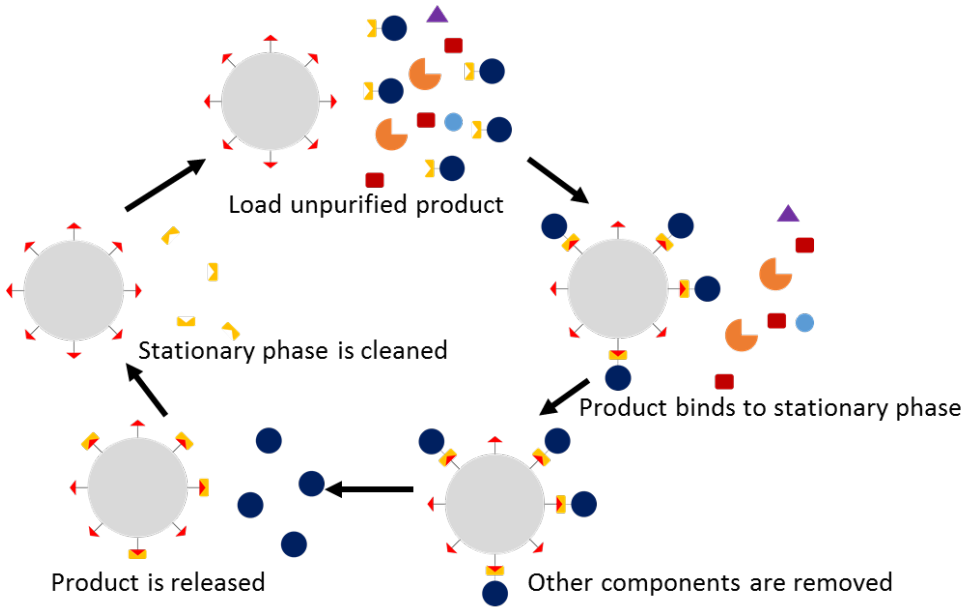


Fig 2: Schematic representation of the resin cycle. Target protein: dark blue, tag on protein: yellow, stationary phase: grey, stationary phase functionalization: red, other colors for impurities.

Work description

100% experimental

Contact person

Nicole Ulmer, Nicole.ulmer@chem.ethz.ch, +41 44 633 45 38, HCI F123

3. Determination of the residence time distribution (RTD) of a therapeutic protein in integrated continuous downstream processing

Project objective

In this project, the residence time distribution of a therapeutic protein in the downstream processing cascade is determined experimentally and compared to simulation results. Due to the internal recycling applied in multi-column processes (Fig. 1), the processing time of a protein is not immediately apparent. When integrating such a process to a continuous cell culture, or integrating two such processes, this effect is even more pronounced. Especially in case of unstable products, knowing the RTD is of great interest.

Project description

In the field of therapeutic protein production, continuous integrated processes have recently gained interest. Regarding the purification, here often multi-column processes are used. In order to measure the RTD, the protein needs to be labeled while retaining its original adsorption behavior. Thus, after labeling the protein, its adsorption behavior on the used chromatographic systems is characterized, comparing it to the untagged form to confirm similar behavior. The experimental determination of the residence time is then performed with step or pulse injections of the labeled protein. The labeled protein is quantified in the eluate, yielding the RTD. These experimental results are then compared to the simulated RTD. The final goal is to quantitatively characterize the RTD in integrated downstream processing. The downstream processing cascade consists of a Protein A capture, virus inactivation and two polishing steps, which are operated in continuous-integrated mode (Fig. 2).

Thereby information about the time-to-recovery after failure in production is obtained. Furthermore the impact on process parameters and production mode on the amount of recycled product, and hence on the residence time, is obtained.

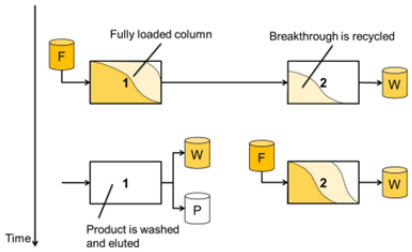


Fig 3.1: Process scheme of CaptureSMB with internal recycling in the first switch.

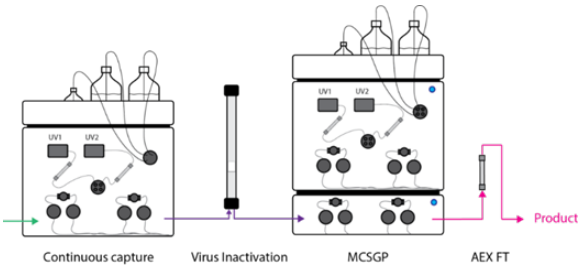


Fig 3.2: Integrated downstream processing cascade consisting of a CaptureSMB step, virus inactivation and two polishing steps (MCSGP and FT).)

Work description

80% experimental, 20% modeling

Contact person

Sebastian Vogg, sebastian.vogg@chem.ethz.ch,+41 44 633 76 62, HCI F128

4. Protein Adsorption into Polyelectrolyte Type Ion Exchangers

Project objective

Chromatography columns packed with ion exchangers have been used for the purification of therapeutic proteins and peptides since the beginnings of the bio-pharmaceutical production industry. Performant ion-exchangers are characterised by high capacity, low mass transfer limitations, and appropriate selectivity between the product and impurities. In order to achieve this in a chromatographic phase, new types of materials containing polyelectrolyte brushes as the ion exchangers were developed. The polyelectrolyte type ion exchangers have since become very popular due to their increased performance.

While these materials are intrinsically different from traditional ion exchangers, very little work detailing the chromatographic behavior of proteins on these resins has been done. In our previous work, we have shown that unusually skewed peaks in chromatographic settings on these polyelectrolyte phases were due to multi-layer adsorption into the charged brush [1]. Further, we have derived a mathematical expression to describe this behavior:

$$q_{eq} = \frac{[S_0]\left(K_1[P] + 2K_1K_2[P]^2 + 3K_1K_2K_3[P]^3\right)}{1 + K_1[P] + K_1K_2[P]^2 + K_1K_2K_3[P]^3}$$

Where q_{eq} and $[P]$ are the adsorbed and bulk protein concentrations, $K_{1\text{-}3}$ are the equilibrium concentrations between the different adsorption layers, and $[S_0]$ is the number of adsorption sites in the first adsorption layer. This expression is derived for a three-layer system.

Project description

The goal of this master thesis is to expand our understanding of the adsorption of proteins and peptides onto polyelectrolyte type ion exchangers. This will be done through the following points:

- Expand experimental knowledge considering different proteins (lysozyme, mAbs, mAb fragments, fusion proteins) and different resins (Eshmuno CPX, Fractogel SO3-, Sepharose XL, Toyopearl, Gigacap)

- Investigate whether cases where more or less than three adsorption layers are present are common. Generalize the isotherm. (note that the limit of infinite adsorption layers is the BET model)

- Develop the techniques and tools to parameterize the unknowns in the isotherm equation and find their dependency to pH and ionic strength
    - The limit of the isotherm equation in infinitely dilute systems is the linear isotherm: $q_{eq}=[S_0]K_1[P]$.
        - Does the parameter K_1 follow traditional models?
    - The parameter $[S_0]$ can be seen as defining the capacity.
        - Can it be related to pH and ionic strength?
    - Develop ways to get the parameters $K_{2\text{-}3}$ independently
        - What assumptions should be made when estimating these?
- Derive a competitive isotherm describing the adsorption of two or more proteins

- Investigate the effect of protein loading, ionic strength and pH on the size exclusion ability of these materials

[1] R. Khalaf, B. Coquebert de Neuville, M. Morbidelli, Protein adsorption in polyelectrolyte brush type cation-exchangers, Journal of Chromatography A. (2016). doi:10.1016/j.chroma.2016.10.024.

Work description

70% experimental, 30% modeling

Contact person

Prof. Dr. Massimo Morbidelli, massimo.morbidelli@chem.ethz.ch,+41 44 632 30 34, HCI F129

5. Palladium incorporation on microporous nitrogen-doped carbon material for catalysis

Project objective

Optimization of the homogeneous incorporation of single-site Palladium atoms during the synthesis of polyacrylonitrile or other polymer nanoparticles. The next step is then the investigation of the effect of thermal treatment on this material towards the single-site metal and the resulting porosity. The catalyst is tested on hydrogenation reactions elsewhere.

Project description

The scope of the project is the production of polymeric support for catalysis. This requires a well-defined pore size distribution: on one side macro- and mesopores to ensure low pressure drop and good diffusion in the material and on the other side micropores to permit the access to the active sites.

Polyacrylonitrile is a semi-crystalline polymer synthetized by free radical polymerization of acrylonitrile, which is our nitrogen-precursor and the bonding site for noble metals. When polymerizing the acrylonitrile with the metallic salt, the metal will be trapped inside the polymeric matrix of the nanoparticle and are therefore difficult to reduce in liquid phase (see Fig. 1). The polyacrylonitrile is then pyrolyzed (N2-atmosphere) on one hand to reduce the metal and on the other hand to introduce porosity, which will permit the access of the adsorbate towards the active sites. The following catalyzed reaction will take advantage of the high porosity of the pyrolyzed polymer, its high mechanical and thermal resistance, high loading as single-site metal and the embedding of the Pd in the carbon-matrix (less risk of sintering).

The same procedure could be also applied for highly crosslinked polymer nanoparticles (without the thermal treatment).

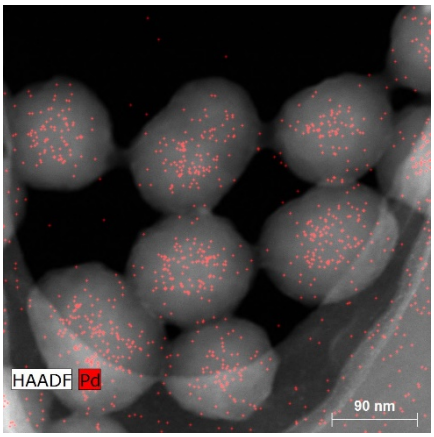


Fig 5: STEM EDX micrograph PAN with Pd-atoms embedded in the structure

Work description

This master thesis project will require mostly experimental work. Please be aware that the lecture “Polymer Reaction & Colloid Engineering” given by Prof. Morbidelli and Prof. Arosio is highly recommended in order to be familiar with colloidal science.

Contact Person

Anna Beltzung, anna.beltzung@chem.ethz.ch, +41 44 632 56 88, HCI F130

6. Synthesis of copolymer having low glass transition temperature via semi-batch emulsion polymerization

Project objective

The production of polymers with low glass transition temperature is of great industrial interest as they would allow the onset of new potential markets. One of the possible application of such innovative polymer is for high performing dry mortars or more aptly for flexible waterproofing membranes. The current state of the art materials guarantee optimal performance at temperatures greater than zero but fail at lowers, due to insufficient crack bridging properties. The development of polymers with lower T_g and therefore higher flexibility at low temperature would eventually overcome this issue.

Project description

The goal of this project is to synthetize a copolymer through emulsion polymerization having defined composition, low glass transition temperature and film forming properties in an appropriate temperature range. One of the standard copolymer used in commercial application is made of ethylene-vinyl acetate, obtained in a high pressure reactor. The percentage of ethylene (T_g ~ -125°C for HDPE) in the copolymer has to be limited to 25% by the existing plant technology. This limits the obtained composition of the copolymer and therefore its glass transition temperature. In order to overcome this, the synthesis of a new copolymer made of different monomers has to be investigated. The operating conditions will be chosen to have free-radical emulsion polymerization and the reaction will be carried out in well mixed glass flasks, under nitrogen atmosphere and at a controlled temperature. To guarantee homogenous composition of the formed polymer particles, semi-batch operations will be required. The reaction will be tested at laboratory scale of 250 mL and subsequently scaled up in a 4L automated lab reactor. Different analytical techniques will be used to monitor the reaction and characterise the product. In particular, conversion, solid content, particle size and size distribution as well as composition will be critical parameters to identify optimal reaction conditions. Differential Scanning Calorimetry (DSC) will be used to evaluate the melting and glass transition temperature of the dried powders. Once a promising polymer has been identified, spray drying of the latex mixture will be performed and redispersibility will be assessed by light scattering measurements. Eventually, the reaction conditions will be optimised to achieve high solid content (>40%) by tuning the operating parameters.

Work description

90% experimental, 10% computational.

Contact person

Stefano Caimi, stefano.caimi@chem.ethz.ch, +41 44 633 32 59, HCI F138

7. Synthesis and Material Development of Polyethylene Furanoate (PEF) as a "green" Substitute for PET

Project objective

Towards a more sustainable future, polyethylene furanoate (PEF) represents a 100% renewable resource-based alternative to polyethylene terephthalate (PET), which is at an annual production of 50M T/a one of the most dominant plastics on the planet. Polyethylene furanoate (PEF) has already shown to possess superior material properties to PET, which allows not only for a reduction in carbon footprint, but also for improved products in typical PET applications such as food packaging, textiles, car tyres, medical devices, solar cells etc. While recent efforts in industry and academia were focused on synthesis of PEF via polycondensation, which is a step-growth polymerization burdened with by-product removal and long reaction times (days), we are exploring a novel route: Ring-opening polymerization (ROP) of cyclic PEF monomers which do not feature endgroups that have to be removed. This approach offers fast reaction times (minutes) and a better control of (co)polymer architecture through a "living" process.

Project description

After this project has progressed over the past 3 years, now comes the final exciting phase where the established knowledge about ROP synthesis has to be applied, optimized and taken further to establish an industrially relevant process that is cost-competitive with polycondensation, specifically in 3 areas:

1) We proved the feasibility high molecular weight PEF synthesis, however, reaction temperature (trade-off between reaction speed, plasticization, degradation and coloring of product) and catalyst type (from tin-based to "green" catalyst) have yet to be optimized.Optional: Application of a computer model to describe the polymerization process and understand the chemistry further

2) Scale-up of the reaction from milligram- to gram-scale in the lab, as well as synthesis at our industrial collaborator SULZER ChemTech in Winterthur

3) Material production from the synthesized high-quality PEF batches: make PEF films, cups, bottles and test the material properties (strength, gas barrier, thermal), as well as apply PEF to new potential areas such as OLED or Solar Cell coatings. Functionalization and copolymerization to tune properties are options.

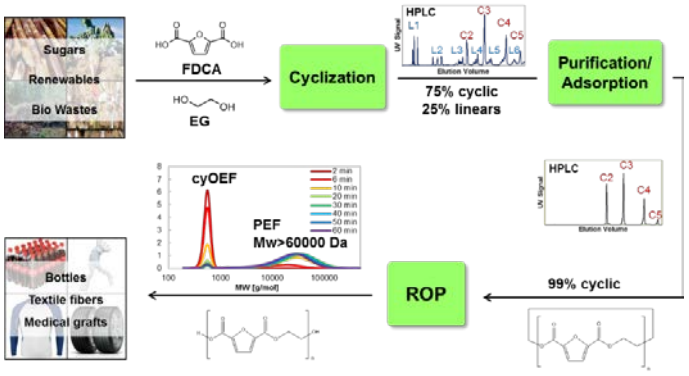


Fig 7: Schematic representation of the process.

Work description

100% experimental

Contact person

Jan-Georg Rosenboom, janr@ethz.ch, +41 44 633 61 23, HCI F136

8. Fragrance encapsulation via miniemulsion polymerization

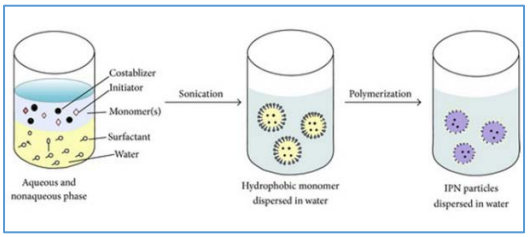
Project objective

The aim of the project is to encapsulate fragrance in polymeric nanocapsules in order to avoid its evaporation and oxidation.

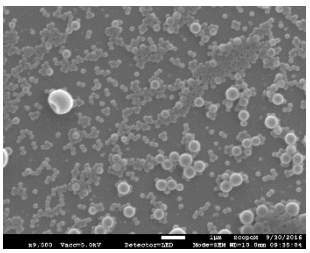
Project description

Encapsulating fragrances in small polymer particles is a popular method to prevent evaporation of volatile components at an early stage of usage of the corresponding product and to increase its shelf life, thus achieving long-term usage for household and cosmetic applications. Miniemulsion polymerization offers several advantages to obtain polymeric nanoparticles which cannot be achieved by other current procedures. The process is initiated from a stable dispersion of very small and narrowly distributed monomer droplets whose size remains practically unchanged throughout the polymerization process. Besides that, the reduced use of surfactants, which is sometimes an irritability agent, is another advantage of this type of polymerization process.

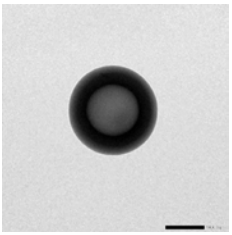
This project will cover the nanocapsules synthesis by miniemulsion polymerization and the characterization using different technics: Dynamic Light Scattering (DLS), Ultracentrifugation (UC), Gel Permeation Chromatography (GPC), Proton nuclear magnetic resonance (NMR) and Differential scanning calorimetry (DSC).



Scheme for miniemulsion polymerization



SEM micrograph



TEM micrograph

Fig 8: Schematic representation of the project.

Work description

100% experimental

Contact person

Dr. Paula M. Nogueira Ambrogi, paula.ambrogi@chem.ethz.ch, +41 44 633 45 26, HCI F127