Technische Universität Braunschweig

ibvt Institut für Bioverfahrenstechnik

pvz Zentrum für Pharmaverfahrenstechnik

Low-Gradient Magnetic Separation in Biotechnology: Parameter Identification and Model-Guided Scale-Up

Jan-Angelus Meyer[1,2], Jennifer Pierick[2,3], Leopold Mareis[4], Dorian Röhring[1,2], Nathalie Opilo[1,2], Georg Garnweitner[2,3] and Rainer Krull[1,2]

[1]Institute of Biochemical Engineering (ibvt), Technische Universität Braunschweig, Germany; [2]Center of Pharmaceutical Engineering (PVZ), Technische Universität Braunschweig, Germany

[3]Institute for Particle Technology, Technische Universität Braunschweig, Germany; [4]TUM School of Computation, Information and Technology, Technische Universität München, Germany

Correspondence: jan-angelus.meyer@tu-braunschweig.de

Introduction

- Efficient downstream processing remains a major challenge in biotechnology, with purification steps representing key bottlenecks.
- Magnetic separation has emerged as a promising alternative to traditional chromatography [1,2].
- Spray-dried superparamagnetic iron oxide nanoparticles (SD-SPIONs) (ø 5.5 µm) were synthesized and are intended to be functionalized for affinity-based protein capture [3].
- This study focuses on identifying critical scale-up parameters for low-gradient magnetic separation using a predictive model derived from experimental data, aiming at biotechnological purification processes.

Magnetsedigraph

- To determine the separation velocity (X_w) of SD-SPIONs, a custom-built unit (**Fig. 1**) was developed, using two vertically aligned pairs of optical sensors. The Magnetsedigraph enables precise quantification of X_w for SD-SPIONs under defined magnetic field gradients ($X_{\nabla B}$).
- The goal was to generate reproducible data for SD-SPION motion in magnetic fields, as X_w is challenging to predict due to agglomeration and fluid dynamic phenomena.
- **Fig. 2** shows how SD-SPIONs agglomerate and align with increasing field strength, with chain formation observed at $X_{\nabla B}$ ≥7 T/m.

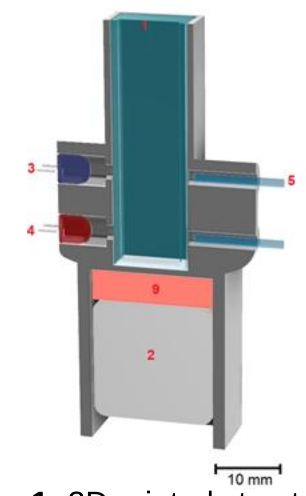

Figure 1: 3D printed structure of the *Magnetsedigraph*: (1) cuvette, (2) magnet, (3, 4) LEDs, (5) glass fibers leading to spectrometers, (9) spacer.

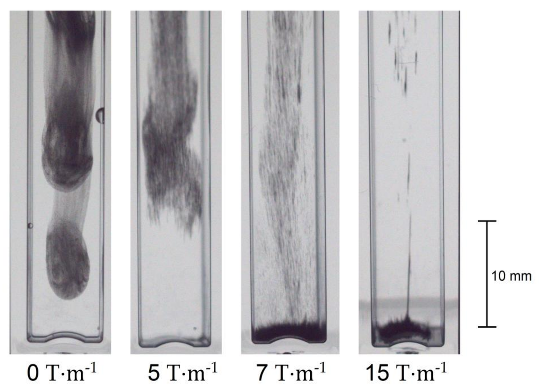

Figure 2: **SD**-SPION agglomeration in a magnetic field ($X_{\nabla B}$ = 0 - 15 T/m)

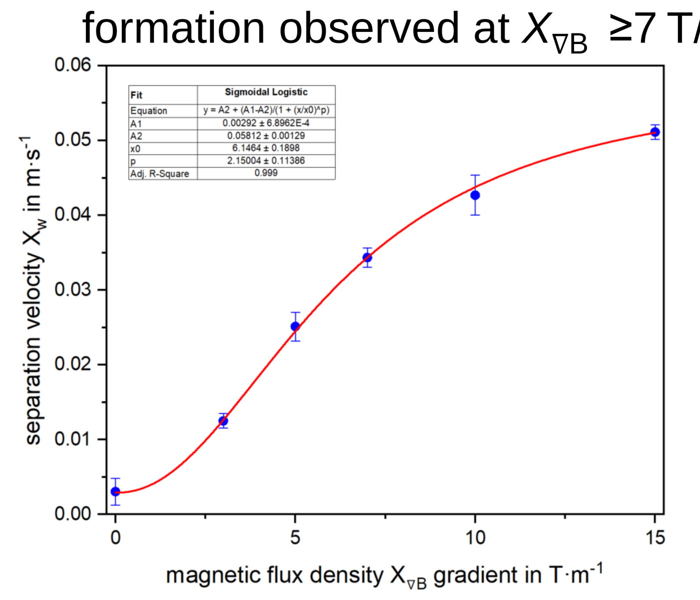

Figure 3: Separation velocity X_w [m/s] of SD-SPIONs as a function of the magnetic flux density gradient $X_{\nabla B}$. Error bars indicate the standard deviation from five measurements determined in the *Magnetsedigraph*.

Results – Separation velocity (Fig. 3):

- This setup allows precise quantification of X_w with minimal deviation.
- X_w increases sharply between 2.5 and 7.5 T/m and reaches a plateau above 10 T/m, probably due to turbulence-induced drag effects.

Parameter identification of a continuous low-gradient magnetic separator

- The magnetic separator (**Fig. 4**) was developed to enable systematic characterization of key separation parameters.
- The modular design allows flexible adjustment of $X_{\nabla B}$, separation area (X_A), and the flow velocity (X_V). A Design of Experiments (DoE) approach was applied to investigate the impact of these parameters on separation efficiency under controlled and reproducible process conditions.

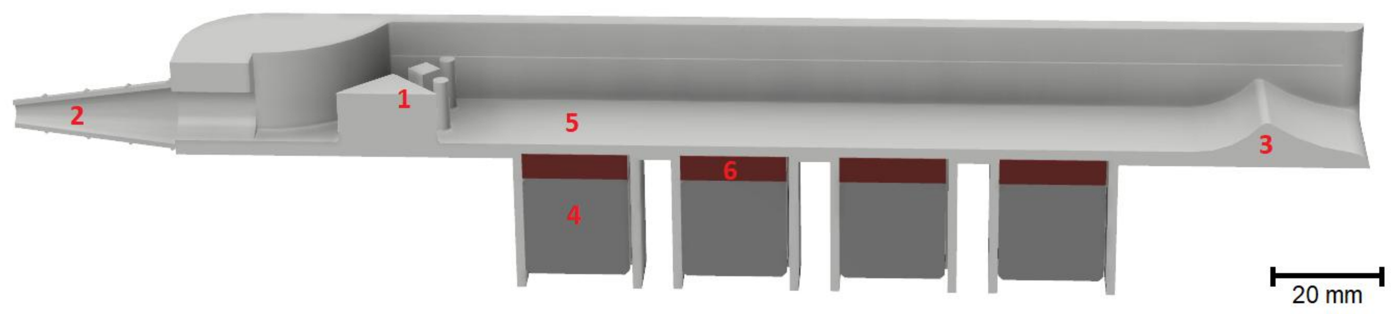

Figure 4: Cross-sectional view of the magnetic separation unit for analyzing the variables influencing magnetic separation: (1) flow distributor, (2) inlet, (3) restrictor, (4) magnets, (5) separation surface, (6) spacer.

- **Fig. 5** illustrates how $X_{\nabla B}$, X_A, and X_V influence the separation efficiency (X_S).
- X_V was identified as the only parameter negatively affecting X_S, due to reduced residence time of SD-SPIONs.**Fig. 5A** demonstrates that increasing X_A consistently leads to higher X_S, especially at low to moderate $X_{\nabla B}$. At $X_{\nabla B}$ ≥10 T/m, X_S exceeds 90 %, with almost complete separation (**Fig. 5B).**
- Beyond a certain threshold, further increasing $X_{\nabla B}$ yields only marginal benefits. Therefore, a higher X_A is the more effective and technically simpler strategy for scaling the process. This observation was further validated through statistical modeling to derive optimal conditions for a scale-up.

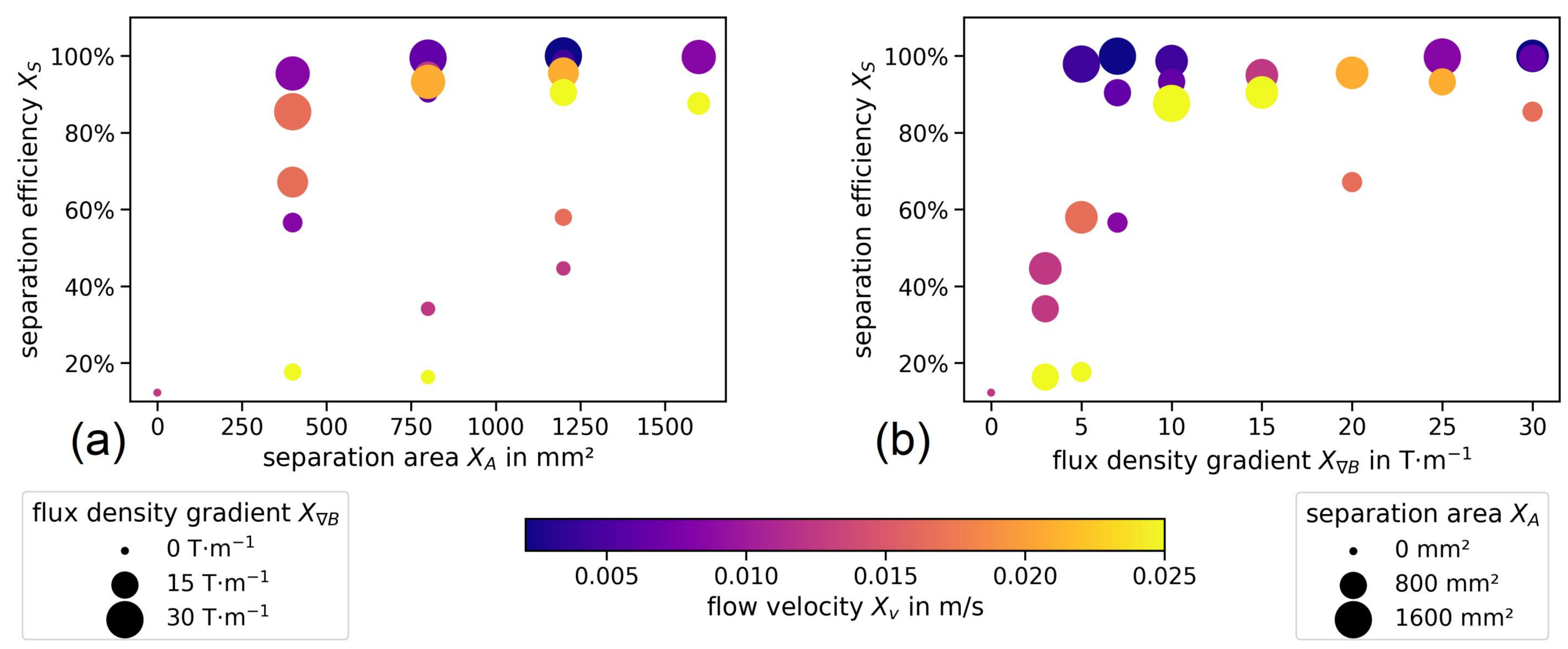

Figure 5: Separation efficiency X_S as a function of (a) separation area X_A [mm²] and (b) magnetic flux density gradient $X_{\nabla B}$ [T/m]. Data points are scaled by (a) $X_{\nabla B}$ or (b) X_A, while color represents separation velocity X_V [m/s].

Statistical modeling and potential for scale up

- To predict X_S under varying process conditions, a statistical model was developed based on experimentally determined X_V.
- It captures the impact of three previous investigated process parameters and translates these relationships into a predictive tool for separator design. The model follows a logistic formulation to account for nonlinear effects and efficiency saturation at high field strengths, thus achieving high predictive quality (R^2 = 94.2 %).
- **Fig. 6** illustrates predicted X_S versus a scale up factor (X_F), assuming increased volumetric throughput, which primarily affects the flow velocity X_V. The 90 % confidence intervals mark threshold values for maintaining X_s > 95 %.

– For the SD-SPION system, a saturation effect is observed at $X_{\nabla B}$ > 10 T/m, where further increases yield only marginal improvements, whereas increasing X_A leads to significant gains in separation efficiency X_S.

– The model enables precise definition of robust operating windows for up scaling.

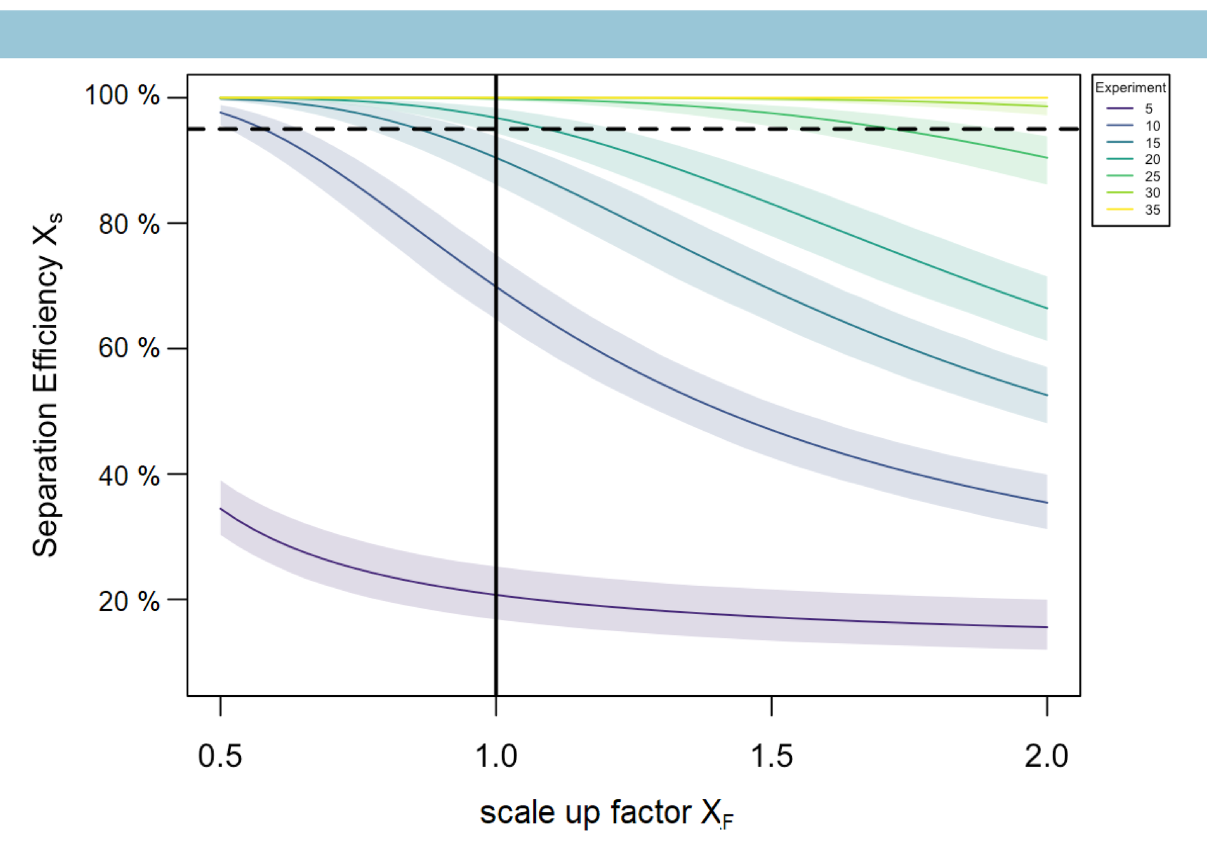

Figure 6: Influence of up-scaling on separation efficiency X_S. The plot illustrates how increasing the volume flow by X_F impacts X_S. Model sensitivity with 90 % pointwise confidence intervals (shaded areas) based on 1000 Monte Carlo simulations.

References and acknowledgement

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Kofinanziert von der Europäischen Union

EUROPA FÜR NIEDERSACHSEN

This research was funded by the European Regional Development Fund (ERDF) under the project title *Development of an innovative biotechnological process for the production and integrated separation of nanobodies using SPIONs* (BioNanoSPION, Project ID: Nds_02x7003115).

Conclusion and outlook

- A predictive model (R^2 = 94.2 %) enables robust separation design by quantifying the impact of $X_{\nabla B}$, X_V, and X_A.
- Identifying X_w as a transferable particle property allows reliable classification of process conditions and scalability.
- It offers a robust engineering tool for designing scalable, cost-efficient magnetic separation systems.