Design and Trial Production of Magnetic Filter for Medical Protein Screening System using High Gradient Magnetic Separation

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Abstract. Biomedicine is indispensable for the treatment of various diseases such as cancer and diabetes mellitus. In particular, antibodies utilized by the immune system are believed to represent the future of medical supplies as they offer an effective treatment for diseases with minor side effects. The development of antibody drugs requires a large-scale, sequential, fast separation, and refinement process for the medical proteins. Thus, in the previous study, we proposed a medical protein screening system that is based on high-gradient magnetic separation using a superconducting magnet. In the proposed system, the protein is specifically attached to the magnetic beads and captured by the magnetic force around a magnetic filter generated by a high magnetic field. In this paper, the behaviour of the magnetic beads was investigated using particle trace simulation coupled with electromagnetic and fluid analyses for three types of commercially available magnetic filters. Simulations were performed using the filter stack intervals and number of filters as design parameters. Finally, we designed the suitable filter for capturing the magnetic beads.

1. Introduction

In recent years, the availability of cutting-edge medical technology has led to the rapid development of antibody drugs to meet its ever-increasing demand. It is even anticipated that these drugs will be the most common type of medical care in the future. The antibody drug market is expected to grow by 10% each year and is predicted to expand to $ 8.6 billion in 2025 [1]. An antibody is a type of protein that protects the body by destroying harmful foreign substances. A medicine equipped with this characteristic is called an antibody drug. Maximum benefits and minimal side effects can be realized by utilizing the specificity and immunochemical properties of the antibody and its ability to bind to a specific antigen, thus establishing the future face of medical care. However, this method has disadvantages such as limited and expensive doses, indicating that there will be an increase in the demand with future improvements. The processes of separation and purification are indispensable for the production of antibody drugs. However, it is difficult to manufacture large quantities of inexpensive antibody drugs using the antibody separation and purification techniques that are currently available. Thus, in order to meet the increasing demand, there is an urgent need to develop a device that is capable of separating and purifying the antibodies at a constant, high-speed rate. In the previous studies, we have been working on research and development of an antibody separation and purification device that uses the magnetic force from a superconducting magnet to achieve a high separation efficiency of 1 g/h as compared to an
efficiency of a few mg/h seen in conventional chromatography [2][3]. We had developed a desktop-type high-gradient magnetic separation system that achieved the trapping and separation of magnetic nano-beads with a high-gradient magnetic force [4-6].

2. High-gradient magnetic separation

The separation of antibodies is a crucial step in the production of antibody drugs. Antibodies attached to a carrier, referred to as affinity beads, are recovered using affinity chromatography and magnetic separation for the non-magnetic and magnetic beads, respectively. The probability of antibody binding dramatically increases when the bead size is reduced by a tenth as this increases the number of beads by $10^3$, while maintaining the same mass and increasing the surface area. In addition, a high-concentration product can be obtained as the magnetic separation method is also capable of separating nano-beads using magnetic force. The purification process using high reaction efficiency is simple and quick. Based on the above, we designed this study investigating magnetic separation using affinity magnetic beads. The magnetic force, $F_{mag}$, on the magnetic beads can be calculated as:

$$F_{mag} = V_p (M_s \cdot \nabla) H$$

where $V_p$ and $M_s$ are the volume and magnetization of the beads, respectively, and $\nabla H$ is the magnetic gradient. The magnetic force can be strengthened by increasing the magnetic gradient. Commercially available magnetic separation systems containing permanent magnets can only generate magnetic fields of up to $\sim 1$ T. Such low magnetic gradients are unable to effectively capture the magnetic nano-beads. On the other hand, a superconducting magnet can be used to generate a high magnetic field. A superconducting magnet is convenient to operate as it can be easily demagnetized and excited. Figure 1 shows a schematic of the superconducting high-gradient magnetic separation system, while Figure 2 illustrates the magnetic gradient. A tube containing a filter with stacked thin-wire meshes is passed through the bore of the magnet. First, a magnetic field of 3 T is applied to the magnetic filter using a superconducting magnet, which generates a large magnetic gradient around the filter. Second, the suspension containing the magnetic beads that bind to the antibodies is poured into the magnetic filter. This causes the magnetic force to capture the magnetic beads following demagnetization of the magnetic filter. Finally, the magnetic beads are recovered by flowing the flushing solution.

Figure 1. A schematic of the high-gradient magnetic separation system.
The magnetic force of the beads increases with a decrease in the wire diameter as shown in the following formula:

\[
F_{\text{max}} = V_p M_p \left[ -4 \frac{\mu_s (\mu_s - 1)}{(\mu_s + 1)^2} \frac{B_0}{a \mu_0} \right]
\]  

(2)

where \(F_{\text{max}}\) is the maximum magnetic force, \(V_p\) is the volume of the bead, \(M_p\) is the magnetization of beads, \(\mu_s\) is the relative magnetic permeability of the magnetic wire, \(a\) is the radius of the magnetic wire, and \(B_0\) is the external magnetic field. It has also been established in a previous study [7] that the capture ratio of the magnetic beads increases with a decrease in the wire diameter. Based on previous studies, we used the magnetic wire mesh marketed by Kansai Wire Mesh Co. Ltd. to prepare filters that are suitable for the separation of magnetic nano-beads. In our system, the filter consists of a stacked magnetic wire mesh. The parameters of the filter must be optimized to achieve a high capture ratio of the magnetic beads as the capture ratio depends on the arrangement and dimensions of the magnetic wire mesh. In this study, three types of the magnetic wire mesh having a small diameter were selected. The filters in the experiment were designed using the electromagnetic, liquid, and particle tracing analyses methods. Finally, the optimal stack intervals and minimum number of required filter layers were estimated.

3. Analysis of magnetic bead movement

3.1. Analysis model and conditions

The electromagnetic, fluid, and particle tracing analyses methods using COMSOL Multiphysics were applied to a filter with stacked wire mesh, which were selected from the commercially available products of Kansai Wire Mesh Co. Ltd. We have previously confirmed that the capture ratio increases with a decrease in the wire diameter [7]. Thus, three types of wire mesh with a small diameter were selected. Figure 3 shows the analysis model. The S43000 filter was arranged along two dimensions. The periodic boundary condition was applied to the left and right ends of the analytical model. Table 1 shows the specifications of the three types of wire meshes. The wire diameter \((d)\) and aperture \((A)\) have been defined in Figure 3. The aperture ratio \((\varepsilon)\) is determined by the wire diameter and aperture as shown below:

\[
\varepsilon = \left(\frac{A}{A + d}\right)^2 \times 100
\]

(3)

Figure 4 shows the top views of Filter A, B, and C. Table 2 shows the specifications of the particles and suspension. The relative permeability is given by the ratio of the permeability of pure water or magnetic particles to that of vacuum. The particles have specifications corresponding to those of the FG beads (TAS8848N1010) manufactured by Tamagawa Seiki using in the magnetic separation test. In the simulations, the magnetic force distribution was calculated assuming that there is a uniform magnetic field.
field of 3 T along the positive $z$ axis. In addition, for the fluid and particle tracing analyses, it was assumed that the suspension containing the magnetic beads in pure water flows uniformly along the negative $z$ axis. A flow rate of 5 mm/s was assumed, similar to that used in a previous experiment [4].

### Table 1. Wire mesh specifications used for the analysis.

| Wire mesh | Wire diameter, $d$ (µm) | Aperture, $A$ (µm) | Aperture ratio (%) |
|-----------|-------------------------|-------------------|-------------------|
| Filter A  | 30                      | 43                | 34.41             |
| Filter B  | 35                      | 50                | 34.41             |
| Filter C  | 40                      | 62                | 36.76             |

**Figure 3.** The analysis model, wherein the region of analysis is shown in colour. The periodic boundary condition was applied to the left and right ends of the analysis region.

**Figure 4.** Top views of the three wire meshes used for the analysis.
Table 2. Specifications of the particles and suspension.

| Specifications             | Value     |
|----------------------------|-----------|
| Mass of the particles (pg) | 0.01      |
| Diameter of the particles (nm) | 200      |
| Relative permeability of the particles | 1.01    |
| Relative permeability of the solvent | 1       |
| Suspension speed (mm/s)    | 5         |
| Suspension concentration (mg/ml) | 1        |

3.2. Analysis of the monolayer filter

We first analysed the monolayer filter and verified the capture ratio and range. The capture ratio is defined as:

$$\text{Capture ratio} \% = \frac{\text{Captured particles}}{\text{All particles}}$$

The particle traces in the monolayer filter are shown in Figure 5. The capture range (denoted by the red double-headed arrow) is defined as the initial position of the particles finally captured by the magnetic filter. Figure 6 shows the numerical results of the capture ratio and range. The maximum capture ratios of Filter A, B, and C were 20%, 17.6%, and 15%, respectively. Filter A had the highest capture ratio and range as the magnetic force increases inversely with respect to the wire diameter (Formula 2). Additionally, the extent of the magnetic force decreases with an increase in the aperture.

3.3. Analysis of the double-layer filter

The capture ratio was investigated for various stack intervals of the double-layer filter. Figure 7 shows the capture ratio for various stacking intervals. It can be seen from Figure 7 that the capture ratio increases with an increase in the stacking interval, but again decreases beyond a certain interval value. In a small stack interval, the fluid flows through the thin magnetic wires of the first layer with high

![Figure 5. Particle traces for the monolayer filter. The colour scheme of the trace shows the various particle speeds.](image)

![Figure 6. Capture ratios and ranges of filters A, B, and C. The line graph corresponds to the capture ratios while the bar graph shows the capture ranges.](image)
velocity. This enables the particles to reach the second layer with high speed, thus making the capture of these accelerated particles difficult. The capture ratio subsequently increases with an increase in the filter stack interval. However, there is a decrease in the capture ratio if the filter interval increases by a large margin and exceeds the peak location. Figure 8 shows the traces of the particles released from the same position for stack intervals of 330 μm and 440 μm in Filter A. Figure 9 shows the particle traces that were not affected by the electromagnetic force. It can be seen in Figure 9 that the particle moves gradually along the positive x axis and flows downwards after passing the first-layer filter. This indicates that the particle was not captured when using an interval of 440 μm as it did not pass within the capture range of the next filter.

3.4. Capture ratio in 10-stacked filter and estimated optimum filter

It is necessary to determine the optimal number of layers of the stacked filter to achieve high efficiency in the separation process. The optimal stack intervals with the highest capture ratio were determined in three types of filters in Section 3.3. In this section, the total number of filters required for the capture of all the particles was determined by examining the capture ratio of each layer in a 10-stacked filter with optimal intervals. The stack interval is built by inserting 110-μm-thickness spacers of S43000 between the filters during its production. Thus, the stacking interval was set equal to a multiple of 110 μm. A smaller interval is selected when multiple maximum capture ratios are available for a certain type of filter. This facilitates a faster processing speed leading to high efficiency in the separation process. Figure 10 shows the capture ratio of the particles corresponding to each layer in the 10-layer filter. It can be seen from Figure 10 that the capture ratio of the first and second layers is very high. This indicates that there is already a decrease in the number of particles at the third and subsequent layers, leading to a corresponding decrease in the suspension concentration. The capture ratio of the third and subsequent layers was less than 7%.

![Figure 7](image1.png)

**Figure 7.** Dependence of the stacking interval on the capture ratio.

![Figure 8](image2.png)

**Figure 8.** The trajectory of the particles released from the same position for stack intervals of 330 μm and 440 μm in Filter A.
Based on the numerical results, the minimum number of filter layers required for capturing all the particles was calculated using the following expression:

$$\text{Required layers (layer)} = \frac{\text{Non-capture ratio of the first and second layers}}{\text{Average capture ratio of the third and subsequent layers}} + 2$$  \hspace{1cm} (4)

The last term ‘2’ in expression (4) refers to the first and second layers that capture many particles. The first term corresponds to the particles that were not captured in the first and second layers but were captured in the third and subsequent layers. Table 3 shows the optimal stack intervals and the minimum number of filter layers necessary for capturing all the particles. In the cases where two or more optimal stack intervals were estimated, the smaller interval was selected to achieve a higher processing speed. Filter A has the shortest stack interval and the least number of layers among the three type of filters.

**Table 3.** Estimated number of filter layers and the optimal stacking interval.

|                | Filter A | Filter B | Filter C |
|----------------|----------|----------|----------|
| Optimal stack intervals (μm) | 220      | 330      | 330      |
| Required layers | 23       | 25       | 25       |

**4. Conclusion**

In this study, electromagnetic, fluid, and particle tracing analyses were conducted for the magnetic filters employed in superconducting high gradient magnetic separation experiments. The behaviour of magnetic particles was clarified for a variable number of filters and stack intervals. The capture ratio increased as the filter stack interval was extended and subsequently decreased after crossing a certain peak threshold. For too short stack intervals, the capture ratio is low due to the fluid pressure and increase in velocity around the wire. For large stack intervals that reached or even exceeded the peak value, the fluid drag caused the particles to flow away from the capture range. This shows that it is critical to select an optimal stack interval. Several particles could be captured in the first and second layers of the multi-stacked filters. There is a decrease in the suspension concentration in the third and subsequent layers, with the capture ratio being less than 7% after the third layer. These results were used to calculate the minimum number of filter layers required to capture all the particles. Filter A has the shortest stack interval (220 μm) and the least number of layers (23 layers) among the three type of filters. In the future,
the results from this study will be used to prepare the magnetic filter and conduct magnetic separation tests.

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