Development of a method based on hydrophobic interaction chromatography for characterizing Eu-chelate labeled antibodies

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BIOTECHNOLOGICAL SYSTEMS (TECH.)

INTRODUCTION

Hydrophobic interaction chromatography (HIC) is a method in which bound proteins are eluted from a stationary phase using a decreasing salt gradient [1]. At high salt concentrations, water molecules move to dissolve salt ions, exposing the hydrophobic regions of proteins and allowing them to interact with the hydrophobic ligands of the solid phase (Figure 1). Separation in HIC depends on the hydrophobicity of protein surface, so that the least hydrophobic proteins are eluted first. Lanthanide chelates have hydrophobic chromophore groups [2], which could increase the hydrophobicity of the labeled antibody.

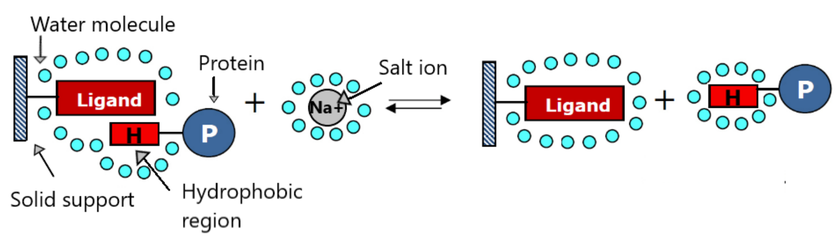

Figure 1. The basic principle of HIC. At high salt concentrations water molecules dissolve salt ions, which exposes hydrophobic regions of proteins and allows hydrophobic interactions. At low salt concentrations proteins are surrounded by water molecules.

The aim of this study was to investigate the applicability of HIC for the characterization of antibodies labeled with lanthanide chelates. The method was intended to separate antibodies according to how many chelates are attached to them. This is also known as the labeling degree. The current method used to determine the labeling degree only gives an average value for it. The purpose of the study was to investigate if the method could be used to separate antibodies based on the number of labels coupled on the antibody.

MATERIALS AND METHODS

A high-performance liquid chromatography (HPLC) with HIC-column was set to allow the separation of the labeled antibodies (Figure 2). A gradient with initial binding time of 10 minutes was set to last 80 minutes (Figure 3).

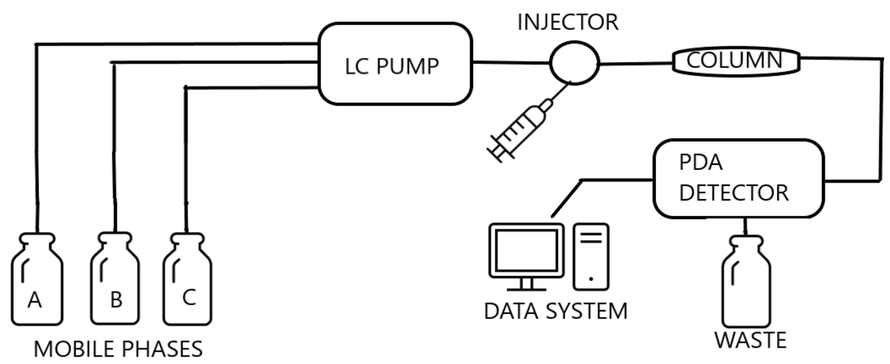

Figure 2. Schematic diagram of the HPLC system. PDA = Photodiode array, allows detection of absorbance at multiple wavelengths simultaneously.

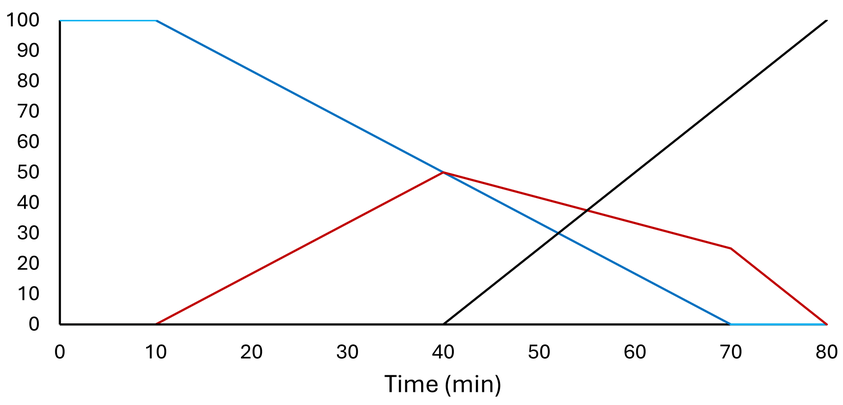

Figure 3. The gradient used. The mobile phases used were A: 0.025 M NaH_2PO_4, pH 6.5 0.75 M $(NH_4)_2SO_4$, B: 0.025 M NaH_2PO_4, pH 6.5 and C: 0.025 M NaH_2PO_4, pH 6.5, 30 vol-% isopropanol. **Blue** = %A, **red** = %B, **black** = %C

RESULTS

The method was able to separate unlabeled antibody from labeled one (Figure 4). The labeling degree during retention can be calculated from the absorbances of the label (330 nm) and the antibody (280 nm). When looking at its development, it can be noticed that the labeling degree increases during the elution of the labeled antibody (Figure 5). High isopropanol proportion was needed to elute antibodies with higher labeling degrees. With smaller labeling degrees the elution occurred earlier in the gradient and better separation was achieved.

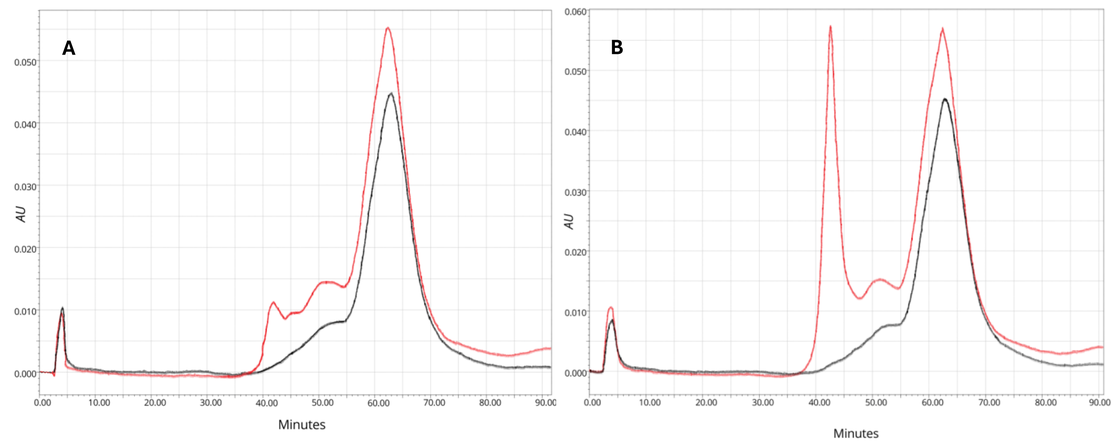

Figure 4. Separation of unlabeled antibodies from labeled ones. A) Labeled antibody sample with labeling degree of 6. B) Labeled antibody sample with labeling degree of 6 spiked with unlabeled antibody. **Red** = absorbance in 280 nm (antibody), **black** = absorbance in 330 nm (label).

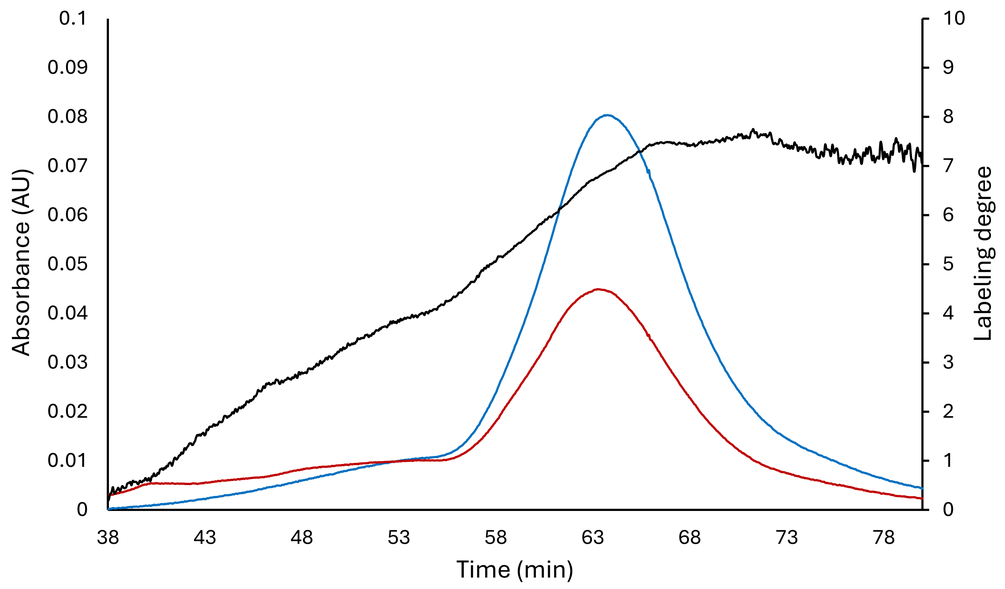

Figure 5. Development of the calculated labeling degree during elution. Labeled antibody sample with labeling degree of 8. **Red** = corrected antibody absorbance (280 nm), **blue** = label absorbance (330 nm), **black** = calculated labeling degree

CONCLUSIONS

HIC can be used to characterize labeled antibodies. The method is especially useful to **separating unlabeled antibodies from labeled ones** and identifying antibodies that have too **small labeling degrees**. At higher labeling degrees, too strong binding can occur and the need for high organic solvent proportions can weaken the method's ability to separate the antibodies.

REFERENCES

[1] Fekete, S., Veuthey, J.-L., Beck, A. & Guillarme, D. (2016) Hydrophobic interaction chromatography for the characterization of monoclonal antibodies and related products. J Pharm Biomed Anal 130:3–18.
[2] Sund, H., Blomberg, K., Meltola, N. & Takalo, H. (2017) Design of novel, water soluble and highly luminescent europium labels with potential to enhance immunoassay sensitivities. *Mol* **22**:1807.

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