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Solvent modulation strategy for superior antibody monomer/aggregate separation in cation exchange chromatography

Simon Kluters[a], Christian Frech[a,*], Thomas von Hirschheydt[b], Andreas Schaubmar[b], Sebastian Neumann[c]

[a] Institute of Biochemistry, University of Applied Sciences Mannheim, Germany
[b] Roche Pharmaceutical Research and Early Development, Large Molecule Research, Roche Innovation Center Penzberg, Germany
[c] F. Hoffmann-La Roche AG, Basel, Switzerland

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ABSTRACT

Cation exchange chromatography (CEX) is an integral part of many downstream processes for monoclonal antibodies (mAbs). However, in some cases CEX methods with standard mobile phase conditions do not lead to a sufficient removal of soluble antibody aggregates. The addition of neutral polymers such as polyethylene glycol (PEG) to the mobile phase can improve the separation of proteins in IEC remarkably. The applicability of this solvent modulation technique is limited by protein precipitation at higher PEG concentrations. To overcome this limitation solubility enhancers like polyols and amino acids can be added to the mobile phase. These additives are known to inhibit PEG-induced protein precipitation in solution.

This new solvent modulation strategy was tested with three different mAbs on two different CEX resins in the presence of PEG in combination with various solubility enhancers. In order to assess the general applicability of this method, mAbs were selected that show major differences with respect to their sensitivity to PEG-induced precipitation and monomer/aggregate resolution performance that is achieved by CEX under standard conditions. For all three mAbs precipitation could be prevented without elimination of the positive PEG-effect. The addition of solubility enhancers gives access to improved separation at elevated PEG concentrations and high protein loadings without running into precipitation issues. Our data indicate that this method is generically applicable and leads to a superior antibody monomer/aggregate separation.

1. Introduction

Currently, mAbs are the most prevalent class of recombinant protein therapeutics in the biopharmaceutical industry [1]. Accordingly, downstream processing strategies for mAbs have advanced greatly within the last years. Today CEX is an integral part of many downstream processes for mAbs. Typically it is employed within a three column purification strategy as an intermediate purification step after Protein A capture and prior to an anion exchange chromatography (AEX) polishing step. Protein A is often used as capturing step due to its high selectivity. AEX step in flow-through mode serves as a polishing step and contributes to viral clearance as well as reduction of host cell proteins (HCP) and host cell DNA (HCDNA) levels [1–3].

As an intermediate purification step CEX is usually operated in bind–elute mode. It typically displays high specificity, high dynamic binding capacity, it can be operated at high flow rates and it shows a high degree of robustness. In addition, cation exchange resins can easily be sanitized. Significant reduction of process-related impurities levels like HCP and HCDNA as well as the removal of product-related impurities like antibody fragments or soluble aggregates can be sufficiently accomplished in most cases by using CEX. Typically the antibody aggregates display a stronger binding compared to the antibody monomer. If the resolution between the respective impurities and the monoclonal antibody is sufficiently high, gradient elution can be replaced by step elution, which has the potential to significantly reduce processing time and elution volumes. In spite of the high potential of CEX methods, in some cases the resolution of antibody monomer and aggregates under typical process conditions is low and leads to a significant loss of

* Corresponding author at: Institute of Biochemistry, University of Applied Sciences Mannheim, Paul-Wittsack-Strasse 10, 68163 Mannheim, Germany. Fax: +49 621 292 6452.
E-mail address: c.frech@hs-mannheim.de (C. Frech).

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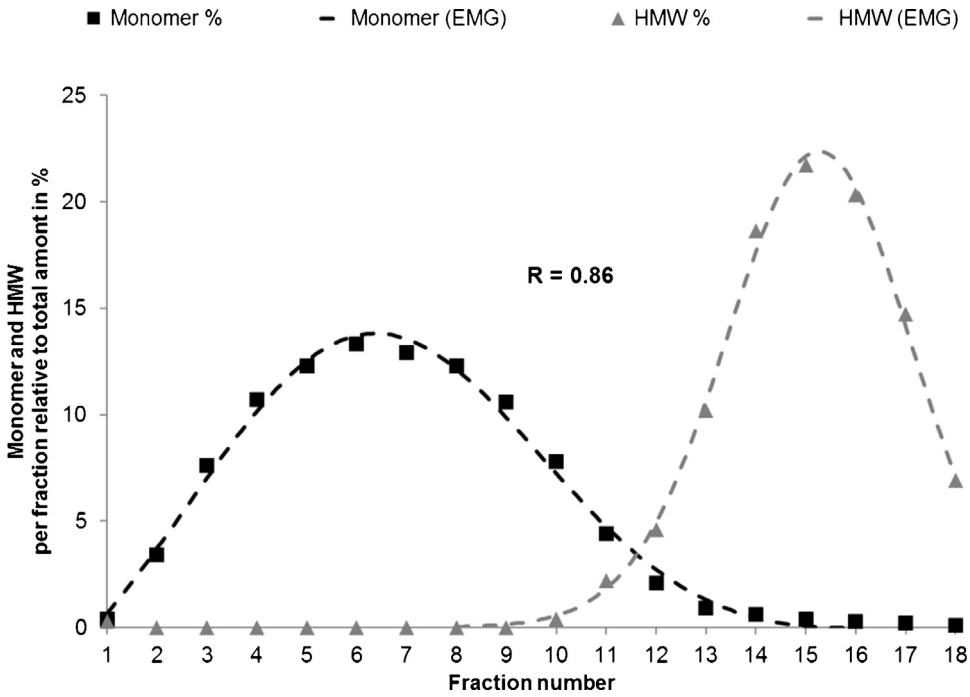

Fig. 1. Distribution graph for monomer (black squares) and HMW species (grey triangles) for Poros 50HS chromatography run with mAb 2 in the presence of 10% (w/v) PEG 4000 and 0.82 M D-sorbitol. Dashed lines are the results of EMG fit using PeakFit software. The corresponding chromatogram is shown in Fig. 7.

the monomeric antibody in the aggregate fraction. This, off course, has a negative impact on the overall efficiency of the production process and directly leads to an increase in the cost of goods.

If a suitable CEX method cannot be established with classical mobile phase buffer systems improved chromatographic performance is possible by employing solvent modulation strategies. Several solvent modulation strategies for cation exchange applications have been published. Besides PEG, ethylene glycol, L-arginine, glycine, urea as well as ammonium sulfate have been successfully used as additive to the mobile phase in order to change the selectivity of CEX. Gagnon [4] and Arakawa et al. [5] give a comprehensive overview on these solvent modulation strategies. The addition of PEG to the mobile phase during CEX is a method that has been known for many years [6,7]. PEGs are water-soluble synthetic polymers (polyether) with the general formula HO-$(CH_2CH_2O)_n$-H. They are widely used, for example as precipitants or crystallization agents [8]. The concept of adding PEG to the mobile phase in chromatographic applications has also been evaluated for hydroxyapatite chromatography [9]. More recently, Gagnon et al. have published innovative purification methods for large proteins utilizing the steric exclusion of PEG [10,11].

The underlying mechanism of the effect that is exerted by PEG on the chromatographic performance has been thoroughly investigated [10–14]. If PEG is added to a protein solution, the large PEG polymers will be sterically excluded from the hydrophilic protein surface due to their sheer size. As a result, PEG will be deficient from a narrow zone in the vicinity of the protein surface i.e., the protein is preferentially hydrated [13,15]. When PEG is used during chromatographic applications as a mobile phase modifier, a PEG deficient zone will also be present on the surface of the stationary phase [12,13]. Because this shell is impenetrable to PEG, the result is an excess of water in adjacent to the protein and the stationary phase i.e., preferential hydration of the protein and stationary phase [12,13,15]. As shown by Kluters et al. [14], the increased retention of proteins in IEC in the presence of PEG can be explained by the increased chemical potential of the protein in solution in the presence of PEG. This leads to an increase of the equilibrium constant for the ion exchange reaction which favors the adsorbed state of the protein. Higher salt concentration for elution are needed although the characteristic charge of the protein remains unchanged [14]. The same mechanism is responsible for the PEG induced precipitation of proteins. The PEG excluded volume will increase with increasing protein size, consequently the increase in retention will be more pronounced for lager solutes (e.g., mAb aggregates). This concept is generally applicable for the improvement of separation of protein species that differ in size [6].

Major practical limitations to the addition of PEG to the mobile phase in chromatographic applications are an increased viscosity that leads to an increased backpressure over the column and protein precipitation at high PEG concentrations. Increased backpressure can be addressed by reducing the operating flow rate accordingly. While precipitation can be modulated by the addition of solubility enhancers.

The beneficial effect of compatible solutes on the stability of biopolymers is well known and a variety of compounds is described in the literature. An overview is given in da Costa et al. [16]. Paleg et al. [17] and Winzor et al. [18] identified a number of polyols and amino acids that reduce the extent of PEG-induced protein precipitation in solution. Their results indicate that compatible solutes can also be used to increase protein solubility in the presence of PEG. Kumar et al. [8] investigated the impact of polyols and PEGs on the structural and thermal stability of proteins in detail.

In this publication, we describe the systematic investigation of the separation of antibody monomers and aggregates during CEX in the presence of different combinations of additives. Different mAbs as well as different cation exchange resins are evaluated. CEX experiments with a mobile phase containing high PEG concentration were conducted. Polyols and amino acids were added to the mobile phase in order to inhibit the PEG-induced protein precipitation. The results show, that the observed resolution is superior to standard CEX as well as to CEX in the presence of moderate PEG concentrations (without further additives). The addition of solubility enhancers to a mobile phase containing high PEG concentrations selectively inhibits the PEG-induced protein pre-