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Non-specific binding and saturation of Polysorbate-20 with aseptic filter membranes for drug substance and drug product during mAb production

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ABSTRACT

An issue identified during filtration of mAb drug substance is Polysorbate-20 absorption by polyethersulfone filter membranes. Experiments determined that both polyvinylidene fluoride filter membranes and polyethersulfone filter membranes bind Polysorbate-20. Saturation point, bound surfactant amount per square cm of membrane, and non-specific binding mechanisms are described in this report. An appropriate approach for preventing Polysorbate-20 loss in drug substance and drug product is presented.

1. Introduction

After expression in mammalian cells, mAb molecules are harvested and then captured and purified with Protein A Affinity chromatography. Partially purified mAb is further polished with anion exchange chromatography (AEX)/cation exchange chromatography (CEX) or other chromatographic steps to remove trace amount of leached Protein A, host cell proteins (HCP), recombinant DNA (rDNA), aggregates (AGG) and charge variants, resulting in a final purified drug substance [1].

During aseptic filtration operation, filter permeability is crucial for mAb production because a high-dose normally is needed for s.c. administration and high concentration of mAb preparation is preferred. When mAb concentration is above 100 mg/mL, extremely high permeability with minimal leach-able becomes the major section choice of aseptic filter. Based on our previous study, PES chemistry-based filters are selected as the aseptic filters (200 nm) of choice for drug substance filtration [1]. A recent report indicated that microfiltration permeability of an IgG solution through virus removal membranes (35 nm) may be affected due to trace DNA contamination [2]. They found that DNase treatment of the IgG solution with micrococcal nuclease enhanced the flux and transmission of IgG through the membranes with or without NaCl. DNase treatment in the protein solution dissociate DNA–IgG complex that plugs the pores in the microfiltration membranes, and the treatment subsequently enhance the flux and transmission of the IgG solution through the membranes [2]. However, this is not the case with the current therapeutic mAb development, since either Q column or membrane is effectively employed [3] to remove DNA and potential virus contamination. In the final drug substance, DNA content in the mAb preparation is almost non-detectable [4] with minimal levels of HCP and AGG to ensure the drug safety for patents.

In protein therapeutics production, after purification and aseptic filtration, other key selections are the components in the formulation solution, which keeps mAb therapeutics soluble with a minimal AGG level at an appropriate pH and osmolarity. Therefore, the formulation aims to provide (1) a minimal pH change during the storage, (2) structural stability by frozen and thaw, and (3) minimal AGG formation. Excipients used in the formulation solution can be classified as buffers, tonicifers and stabilizers. Detergent or surfactant is commonly used as stabilizers. To avoid an over-concentration effect, the buffer system does not contain the surfactant but only the other excipients at a desired pH. Thus, the solution without surfactant is also called pre-formulation buffer. The solution with surfactant is named formulation buffer. The final purified bulk is concentrated and diafiltrated to achieve the desired

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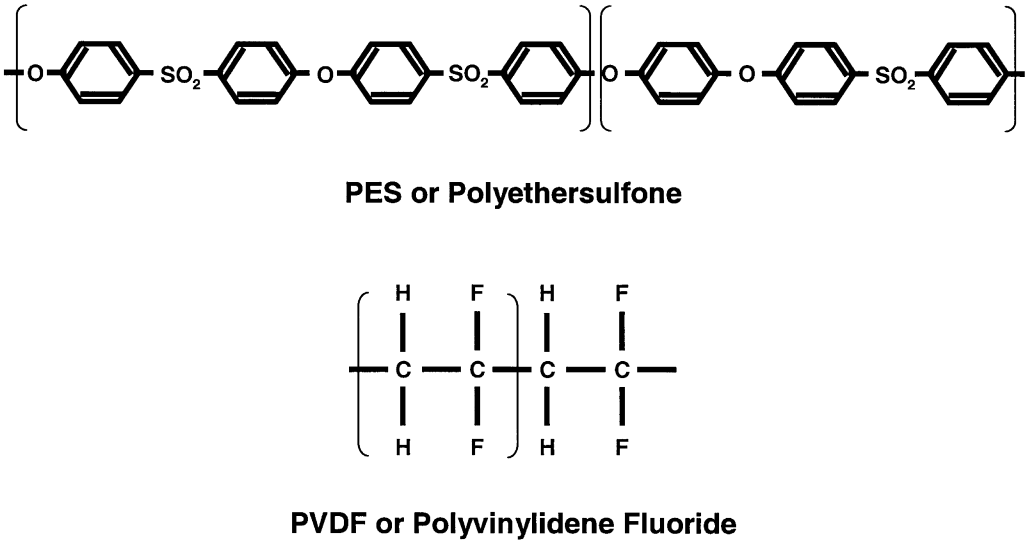

Fig. 1. Structural comparison of polyethersulfone (PES) and polyvinylidene fluoride (PVDF).

pH and target protein concentration using pre-formulation buffer. Surfactant is added and the material is filtered via a high permeability 0.2 μm aseptic filter.

In the mAb industry, commonly used surfactant is Polysorbate series compounds. Polysorbate-80 (PS-80) was used for the first generation mAb drugs such as Remicade [5]. PS-80 was also used as a stabilizer in aerosol formulation [6]. Experimental evidence suggests that PS-80 was found to prevent the formation of protein particles [6,7]. However, in comparison to Polysorbate-20 (PS-20), PS-80 seems readily oxidized due to unsaturated double bonds within the molecule. Later mAb therapeutics, including Herceptin and Xolair, incorporated PS-20 in their formulations [5].

The commonly used surfactant PS-20 or Tween-20 has a critical micelle concentration in the range of 5.5×10^{-5} M or 0.007% to 5.9×10^{-5} M or 0.0077% (FW: 1227.54) [8–11]. Perhaps due to this reason, many mAb drug candidates and therapeutic drugs are currently formulated below 0.007%.

Generation of drug product (DP) normally needs another process, so-called fill and finish. In this process, drug substance is thawed, mixed and then filtered through Durapore polyvinylidene fluoride [PVDF (hydrophilic, surface-modified)] (Fig. 1) filters into drug vials or syringes to become drug product. The final drug product in solution form should have a final PS-20 concentration that meets the release specification. The level of PS-20 is critical to retain product stability especially under stress conditions such as bulk freeze–thaw and agitation during transportation. Adequate concentration of PS-20 is often required for long-term product stability over proposed shelf life. At Amgen, a precise assay was developed to quantify the PS-20 concentration in protein products. However, because of the low concentration (below 0.007%) of PS-20 in the final bulks, a high precision deviation derived from Hurwitz equation (8.9–11.3% R.S.D.) is expected [12].

In this study, partial removal of PS-20 by 0.2 μm filters (polyethersulfone (PES) and PVDF membranes) (Fig. 1) during the GMP drug substance filtration and aseptic filtration in drug product fill/finish process was identified. Based on the determination of bound amount of PS-20 cm^{-2} of membranes, a hydrophobic-based non-specific membrane binding mechanism was suggested. After evaluation of current possible operational proposals together with considerations for future manufacturing feasibility, recommendations were initially made and implemented for the clinical drug substance manufacturing facility. The results of PS-20 concentration in several products after the implementation are summarized, and advantages and disadvantages of this operation procedure are further discussed.

2. Materials and methods

2.1. Materials

Based on our previous evaluation of process capacity, EXPRESS SHC filter was selected as an ideal filter for mAb drug substance filtration [1]. Because of similar membrane (PES) used, Sartopore 2 showed a comparable process capacity. Millipore Durapore hydrophilic PVDF (surface-modified) filters are currently used for mAb drug product filtration. Therefore these filters were used in this study and properties of the filters are summarized in Table 1. As a reference membrane, Posidyne filter cartridges was purchased from Pall and tested. The filter is made of covalent charge-modified Nylon 6,6 membrane which exhibits a net positively charged zeta potential in aqueous solutions.

A 10 mM acetate formulation buffer containing 0.004% PS-20 was made by the buffer group of Pilot Plant. The same buffers containing various concentrations of PS-20 including 0.005, 0.006 and 0.008% provided by the group were also tested. Buffer's pH and conductivity was verified prior to use.

Table 1
Filters used in the study

Filters	Vendors	Membrane types	Layers	Surface area (cm^2)
EXPEESS SHC OptiScale	Millipore	PES[a]	2	17.7
EXPRESS SHC OptiCap 3 in.	Millipore	PES[a]	2	1300
Durapore OptiScale	Millipore	PVDF[b]	1	17.7
Durapore OptiCap 2 in.	Millipore	PVDF[b]	1	900
Durapore OptiCap 4 in.	Millipore	PVDF[b]	1	1394
Durapore OptiCap 10 in.	Millipore	PVDF[b]	1	6875
Millipak 200	Millipore	PVDF[b]	1	1003
Sartopore 2 150	Sartorius	PES[a]	2	150
Posidyne	Pall Life	Nylon 6,6[c]	2	1000

[a] Polyethersulfone.
[b] Hydrophilic polyvinylidene fluoride (PVDF) (surface-modified).
[c] Covalent charge-modified Nylon 6,6 membrane.