Impact of Extractables/Leachables from Filters on Stability of Protein Formulations

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ABSTRACT: Aqueous extractables/leachables from three sterilizing-grade filter membranes [polyvinylidene fluoride (PVDF), polyethersulfone (PES), and mixed cellulose ester (MCE)] were found to significantly reduce the surface tension of aqueous solutions. To evaluate the effect of these extractables/leachables from filter membranes on stability of protein formulations, model IgG2 formulations (with or without added surfactant) were spiked with different levels of filter extractables from stock solutions as a stress study. The stock solutions of extractables were created by processing the filter membranes through autoclaving and soaking steps. The IgG2 formulations were subsequently subject to agitation and temperature stress. Extractables/leachables from the filters were found to have a significant protective (PVDF, PES) and destabilizing (MCE) impact on both visible and subvisible particulates formation under agitation stress for formulations that did not contain any additional surfactant such as polysorbate 80. The impact of filter extractables/leachables on chemical stability of the antibody formulation displayed a more complicated pattern, but was generally destabilizing, causing increases in aggregation, oxidation, and acidic species. In conclusion, extractables/leachables from filter membranes may have impact on protein formulation stability and caution should be exercised during protein filtration, especially when filtering small volumes and in preformulation or high-throughput screening studies. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:4617–4630, 2011

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INTRODUCTION

Filtration is a common unit operation during purification, formulation, and fill–finish processing of therapeutic protein drug products. An ideal filtration device should be noninteractive such that it does not remove any ingredients from the product solution nor should it release anything into the processed solution.[1,2] However, no filtration device is completely inert. A limited number of filter membrane materials are commonly encountered during processing. Filter membranes may also be variously treated to obtain functionality, flexibility, as well as processability.[3,4] The commonly used Durapore™ filtration membranes are based on hydrophobic polyvinylidene fluoride (PVDF) membranes but are grafted on the surface with polyacrylate to make them hydrophilic. Polyethersulfone (PES) membranes are inherently hydrophilic and do not have a grafted wetting agent. Mixed cellulose ester (MCE) membranes are made from biologically inert mixtures of cellulose acetate and cellulose nitrate and are also hydrophilic. Nylon membranes are strong and inherently hydrophilic in nature and are compatible with a broad range of solvents and mainly used for filtration of aqueous and organic mobile phase, filtration of tissue culture media, buffers, solution, or for vacuum degassing, and also as sterilizing filters in bioprocessing.

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Polycarbonate (PC) filter membranes are composed of PC film and are also claimed to be hydrophilic but are not commonly used for protein filtration during processing.[5] The filter membranes may also include oligomeric species of the grafted and/or base polymer, plasticizers, antioxidants, and slip agents, which often become a part of the extractable species from such membranes.[2–4] For instance, filter extractables from hydrophilic-modified PVDF membrane such as Durapore® contain soluble oligomers of hydroxypropyl acrylate, propylene glycol (PG), and crosslinker, for example, tetraethylene glycol diacrylate. These components are all associated with the hydrophilic modification of the PVDF surface.[2,6] Tsui et al.[7] monitored isopropyl alcohol and acetone as extractables from such membranes. In the case of MCE membrane, glycerol was identified as a major component in water extracts, with cellulose acetate and cellulose nitrate also seen in ethanol extracts.[2] No report on PES membranes could be found, although some of the data reported by Reif et al.[8] suggest PES-based cartridges in three of the cartridges analyzed. Unfortunately, the identity of the cartridges/membranes was not provided. Clearly, the list of reported extractables is a function of the extraction conditions and solvents used, as well as the analytical techniques brought to bear. For example, the list of extractables [based on gas chromatography–mass spectrometry (GC–MS), reverse phase high-performance liquid chromatography (RP-HPLC), GPC, and Fourier transform infrared] by Reif et al.[8] for a number of common cartridges contains a very wide range of complex organics that could be potential exractables. The extraction was performed at 50°C (with ethanol) or at 80°C (with water) using a reflux condenser. On the contrary, the extractables reported by Kao et al.,[4] Stone et al.,[9] and Jiang et al.[2,6] are after soaking in the solvent.

The type and amount of extractables that actually become leachables depend on the type of processing (e.g., autoclave cycles) and the solvent/solubilizer in the medium being filtered. Although there is information available on evaluating the extractable and leachable profiles of sterilizing filters during process and manufacturing,[6,9] little is known about the potential impact of inadvertently introduced extractables/leachables from filter membranes on protein stability.

For completeness, the terms "extractables" and "leachables" as employed by US Food and Drug Administration (FDA) are presented here. "Extractables" are compounds that can be extracted by selected solvents from any relevant surface or material that comes in contact with the product during manufacture and storage. "Leachables" on the other hand are compounds that are leached into the product (stream) from these surfaces or materials under actual use or process conditions. Leachables are therefore typically a subset of extractables. Although the FDA usage referred to in the guidance[10] pertains to container/closures, regulatory authorities have the same concern with all materials, which come into product contact, including filters.

Preliminary work in our laboratory showed that filter leachables are readily introduced into protein formulations during filtration of protein solutions and these leachables are surface active. [We observed that filtration of about 4 mL of protein solution through a typical 25 mm syringe filter that had not been flushed led to a decrease of surface tension from about 65 to 53 dyne/cm]. Surfactants are purposely added to protein formulations to prevent adsorption of the protein molecules at air–liquid or solid–liquid interfaces.[11–13] Such interfaces are commonly encountered during manufacturing unit operations, fill and finish, transport, and so on, and can lead to unfolding and subsequent aggregation.[14,15] Surfactants commonly used as excipients are the nonionic polyoxyethylene sorbitan esters, referred to as polysorbates. The primary mechanism for protein stabilization by these surfactants is their higher affinity for the interface, thus blocking the protein from adsorption and subsequent denaturation.[1,16,17] Thus, even though surface-active agents are an essential part of protein formulations, inadvertent inclusion of surface-active leachables would be considered an adulteration. 21 CFR parts 210 and 211, Current Good Manufacturing Practice in Manufacturing, Processing, Packing or Holding of Drugs: General and Current Good Manufacturing Practice for Finished Pharmaceuticals, Subpart D-211.65 states: "Equipment shall be constructed so that surfaces that contact components, in-process materials, drug products shall not be reactive, additive, or adsorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."

The work presented below evaluates the stability of a model IgG2 formulation that has been spiked with filter membrane extractable/leachable stock solution. We created a set of extractables from the filters and spiked them into the protein solution to study their effect. This strategy of "accelerating" or "exaggerating" a factor to understand its impact is similar to that used by Hoehne et al.[18] for assessing the impact of glass particles, and by Thirumangalathu et al.[19] for silicone oil. Although in practice, the filter membranes are on supports and in housings made of plastics that can also contribute to the extractables and leachables, the work here focused on the contribution from filter membranes only. Housing materials vary significantly with manufacturer and would have to be included as part of a product development program,