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Filters and Filtration: A Review of Mechanisms That Impact Cost, Product Quality and Patient Safety

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Abstract

Filters are widely used in the pharmaceutical industry and hospital care for multiple applications such as API processing and purification, pharmaceutical and bio-pharmaceutical operations such as sterile filtration and protein purification, analysis of drug products, while administration to the patients and so on. A multitude of filters with different pore ratings and material of construction are available for use in various applications. The wide variety and type of filters available today also mean that we do not completely understand the nature of such filters and random or un-informed usage could introduce unwanted alterations in the quality or quantity of the product. Thus, one needs to be aware that filters could alter the stability, quality and safety of the product if the right choices are not made. Various mechanisms and examples are described wherein the final quality of the product was affected. Adsorption is the major mechanism of interaction between filters and drug product. Filters can adsorb formulation components such as active pharmaceutical ingredient, preservatives and other excipients which could lead to therapeutic failure or toxicity. Other mechanisms include leaching of filter materials into product during filtration which could adversely affect the product by changing the safety aspect of the product. Despite certain challenges involved while working with filter, usage of filters is the pharmaceutical and biopharmaceutical industry is increasing day by day and many crucial operations such as meeting sterility in aseptic operations, purification of peptides/proteins/Active pharmaceutical ingredients, dissolution analysis etc rely heavily on the quality and right usage of the appropriate filters. It is necessary to minimize the adverse interaction between the filter and the drug product and thus deliver the best to the patient and health care for which the end user needs to thoroughly understand their application, nature of the material being processed and details of the filter to better understand the outcome of the filtration process.

Keywords: Filtration, Pharmaceutical filters, Adsorption on filter membranes, Extractables/ Leachables from filter membranes.

INTRODUCTION

Filtration is a widely used unit operation in the pharmaceutical industry. Filtration is used for clarification purpose (clarification filtration) and/or to sterilize solution using sterilizing grade filter membranes (0.2 µ or smaller pore size filters). Filtration is of great importance in injectable/parenteral formulations where control of visible and sub-visible particulate matter and sterility are of prime importance and a mandate. Mainly filters can be categorized into depth and membrane filters, the former is prone to particle generation and can contaminate the product thus these filters cannot be used as means of sterilization whereas the later one is best suitable for sterile filtration of parenteral dosage forms [1].

Filtration of parenteral products ensures removal of particulate matter and can be used either for clarification or for sterilization purposes. The choice of the filter for the intended purpose needs to be done judiciously since the choice of filter may influence the quality of the product being filtered i.e., constituents of the product may get adsorbed, filters may shed particles or may cause leachables to get into the product under certain conditions which is highly undesirable [2]. Other sources of particulate matter includes silicone oil, rubber, plastic, and cotton etc. Great care needs to be taken to reduce or remove particle shedding/formation during clarification or sterile filtration since these particles will remain in the final product thus impacting product quality, administration of such a product containing particulate matter to patients can have adverse effects such as infusion phlebitis, pulmonary artery granulomata and coronary vasoconstriction and immunogenic reactions causing toxic effects [2, 3]. Similarly, leachables from the filter components could cause undesirable toxicity when administered to patients.

Filtration as a Source of Particulate Contamination

A product that needs to be administered parenterally should be free from any microbial and or particulate contamination that may cause infections and related complications. Thus, the unit operation of filtration is becoming a necessity and different types of filters and filter types can be used depending on the application. However, such filter choices if not carefully made could sometimes become a source of contamination rather removal and may cause several complications associated due to such particulate matter triggering untoward reactions in the body by virtue of its size or nature. In the case of protein solutions, aggregation is a major concern and needs to be handled appropriately during filtration since filter components including filter membranes may shed the particles into the protein solutions leading to protein aggregation.

Lu Liu, *et al.*, studied the hypothesis that foreign particles shedding from filters will accelerate the rate of protein aggregation and particle formation, and that this effect can be observed quickly under stresses such as agitation. The authors investigated the particle shedding

from various commercially available syringe filters (e.g, PES, CA, combination of Glass microfiber as prefilter and the polymer membrane) during filtration of buffer alone and a solution of KGF-2. Besides, the study also evaluated whether preflushing the syringe filter with buffer affected particle shedding into buffer or protein solution during filtration and the effects of particles shedding from syringe filters on protein aggregation under quiescent and agitated conditions. The outcome of the study indicated that the number of particles in filtered buffer varied greatly depending on the filter type used. Preflushing the filter units with buffer prior to filtering buffer did not substantially reduce particle counts for more than 80% of tested filters. There were large differences in the particle counts for KGF-2 samples filtered through the different types of filters, and high variability in the counts for samples filters, and high variability in the counts for samples processed with individual units of a given filter type. The presence of glass microfibers substantially reduced protein concentration in the filtrate for all tested filter types which was attributed to the adsorption of the positively charge KGF-2 molecules (pI = 9.9) to the negatively charged glass surface. Filtration with units that did not contain glass microfibers showed less loss of soluble protein, with no detectable loss with some of the filter types. Agitation of the control KGF-2 solutions resulted in substantially more of particles than quiescent incubation and a detectable loss of soluble protein. It was also concluded that for situations wherein nucleation is rate limiting, addition of heterogeneous nuclei such as particles shed from filters could greatly accelerate the loss of native protein. Thus, the presence of the membrane-derived foreign particles may contribute to additional protein aggregation and particle formation during the shelf life of the product [3].

Makino, *et al.,* studied the suitability of Mixed cellulose esters (MCE; 0.22 µm), polyvinylidene difluoride (PVDF) and Polyethersulfone (PES; 0.2 µm) as in-line filters during the administration of Amphotericin B micellar sodium deoxycholate (DOC) complex. The concentration of amphotericin B and number of particles ranging from 2 to 100 pm in amphotericin B solutions after filtration through various pore sizes and membrane filter materials was the criteria used to determine suitability. The study results indicated that Polyethersulfone (PES; 0.2 µm) was the best of the filters studied in terms of adsorption as well as retention of particulate matter. The mixed cellulose esters (0.22 µm) and polyvinylidene difluoride (0.22 & 0.45µm) filters significantly decreased the concentration of amphotericin B though PVDF with pore size of 0.45 µm was significantly better amongst them. The 0.45 µm PVDF filter however had lower efficiency than the 0.22 µm PES filter in reducing the particulate matter to meet the USP limits and thus the authors recommended the use of 0.2 µ PES filter for filtration of Amphotericin B micellar sodium deoxycholate [4].

Hirakawa, *et al.,* conducted a study where they evaluated the effect of four types of in-line filters on filtration rate, generation of particulate matter and active moiety (Amphotericin B) concentration. Polyethersulphone (0.22 µm & 1.2 µm), Nylon 66 (0.2 µm) and Posidyne positively charged nylon 66 (0.2 µm) were studied in this investigation. The study conclusions indicated that the 1.2 µm and 0.2 µm PES filters were more suitable for use than the 0.2 µm positively charged nylon 66 and the 0.22 µm nylon 66 filters in terms of flow rate, the flow rates were measured under maximum gravity flow. No loss of amphotericin B was observed upon filtration through 1.2µm and 0.2µm PES filters whereas 0.2µm positively charged and uncharged nylon 66 filters rapidly decreased the amphotericin concentration to undetectable levels of low levels respectively. The positively charged nylon 66 filters have amide linkages in the linear polymer which adsorb negatively charged substances such as pyrogens, bacterial, virus and colloidal material from solutions, thus this type of filter was considered inappropriate for filtration of the amphoteric B/DOC complex since is adsorbed micellar amphoteric B/DOC complex and slowly got clogged. The same PES filter showed the reduction in particulate content to below USP limits upon filtration when compared to a unfiltered solution. In conclusion, authors have recommended the use of 0.2µm PES filter for the administration of intravenous amphotericin B infusion [5].

Alteration in Flow Rate

Moce-Llivina, *et al.,* studied the potential of Polyether Sulfone (PES) to allow greater volume of samples to be filtered for filtration of aqueous solutions containing viruses, the study compared the filtration efficacy of PES against polyvinylidene fluoride (PVDF). The outcome of the study indicated that both types of membranes could be used for decontamination of sewage for virus analysis, since the virus reduction after filtration of PES membranes seemed to be quite similar to those presented by PVDF membranes. Untreated PVDF membranes were previously reported to be useful for filtration of viruses, because they showed a low adsorption of the viral particles, allowing good recovery of virus in environmental samples, the use of PES filter membranes appeared as effective as PVDF membranes in sewage samples. Additionally, it was observed that a larger volume of sample could be filtered with the same PES filter unit, with a consequent reduction in the number of filter units used when testing large volumes of sample or when purifying viral suspensions from large volumes of cell culture supernatants. Furthermore, PES membranes were cheaper than PVDF membranes. Thus, the authors recommend the use of PES membrane filters as a suitable method for high recovery of viruses after decontamination by filtration of viral suspensions [6].

Adsorption of Formulation Components to Filter Membranes

Although filtration is widely accepted process for aqueous as well as non-aqueous formulations, a major disadvantage is adsorption of solutes from formulation. Adsorption of solutes broadly depends upon type of filtration used and composition of formulation. Figure 1 shows the factors that affect the adsorption of solutes onto filter membranes [7].

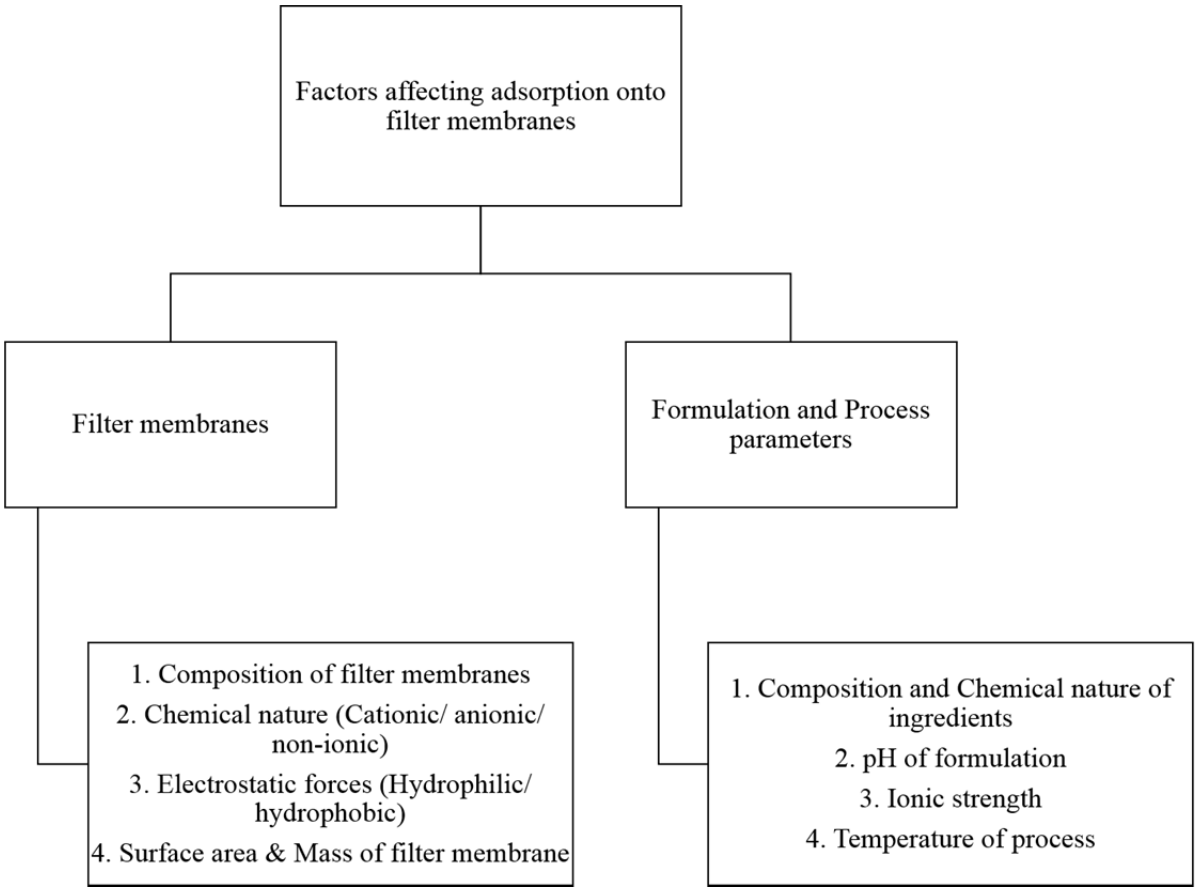

Figure 1: Factors affecting adsorption onto filter membranes

Formulation components sometimes tend to adsorb to the filter membranes and mainly include active moiety and preservatives. This phenomenon results in loss of concentration of the adsorbed component that adversely affects the drug product quality which is highly undesirable. Adsorption phenomenon is dependent on several factors including charge of filter membranes, charge of formulation components, filter surface area, flow rate, temperature while filtration, duration of filtration and many other. In general, the rate and extent of adsorption was inversely related to flow rate and temperature respectively [7].

Adsorption of Preservatives

Naido, *et al.*, studied the loss of preservatives like chlorhexidine acetate 0.01 % w/v, phenylmercuric nitrate 0.002% w/v, benzalkonium chloride 0.02 % w/v and phenylethyl alcohol 0.5% v/v from ophthalmic solutions during filtration sterilization. The loss found was considerable with fibrous asbestos pads, significant with porcelain candles and sintered glass, and slight with membrane filters. The preparation of ophthalmic solutions on a bulk scale is manageable as far as loss of certain preservatives is considered since the initial part of the filtrate is discarded by which time the filter surface gets saturated however the losses could be considerable at small scale. Presaturation with the selected preservative is an option to reduce such losses at small scale. Phenylethyl alcohol was the preferred preservative as per the stud as it showed no adsorption to any of the filters evaluated in this study however the limited spectrum of activity necessitates its use in combination with a broad spectrum agent [8].

Bin, *et al.*, studied the adsorption of Benzalkonium Chloride (BAK) to different filters and elucidated the mechanism(s) of adsorption of BAK by filter membranes. The study was designed to determine the effect of formulation (pH, ionic strength, and ethylene glycol) and processing parameters (flow-rate, temperature, autoclaving, interruption of flow, pre-saturation) on the adsorption of BAK. Six different sterilizing grade filter membranes were used in the study and adsorption was monitored by passing an aqueous solution of BAK at pH 6 through a 47-mm (14.2 cm^2 effective filter area) disk filter membrane and measuring the UV absorption of the filtrate with a UVmicro flow cell.

When the adsorption data were plotted according to the Langmuir equation, graphs exhibiting both monolayer and multilayer adsorption were obtained. The multilayer adsorption of BAK is probably adsorption of BAK on previously adsorbed BAK because of hydrophobic interactions.

Adsorption depends upon the composition of filter membranes. Hydrophilic and nonionic or hydrophilic and cationic filter membranes adsorbed little BAK. However, membranes that were hydrophobic or anionic showed significant BAK adsorption. The probable reason for this could be cationic nature of BAK. Ranking was given to filters w.r.t. amount of BAK adsorption. Electrostatic

forces between the BAK and surface of filter membranes determined the strength of binding. Strength of adsorptive forces are measured by adsorptive coefficient. Lowest adsorptive coefficients were observed with the filter membranes which possessed similar charges as of BAK i.e. cationic charges. It was determined that pH played a major role on adsorption of BAK by modifying the electrostatic forces of filter membranes. Absences of electrostatic repulsive forces between BAK and filter membranes lead to increase in amount of BAK adsorption. Membranes with net positive charge were more sensitive to ionic strength while non-ionic membranes were least affected, this was attributed to decrease in electrostatic repulsive forces with increase in ionic strength leading to higher binding of BAK on membranes. Membranes which adsorb BAK via hydrophobic interactions showed largest decrease in adsorption whereas Hydrophilic membranes were least affected by ethylene glycol, ethylene glycol generally hampered the hydrophobic interactions by stabilization of hydration layer on solute [7].

Bin, *et al.,* did a similar study using a flow through technique to understand the adsorption of esters of p-hydroxybenzoic acid (parabens) by six different filter membranes having different surface composition and charge. Similar formulation and processing factors were studied as in the BAK study. Propyl paraben was more hydrophobic than methyl paraben and the difference in hydrophobicity was directly reflected in adsorption profiles with each filter membrane. Adsorption of propyl paraben was much higher by all filter membranes as compared to methyl paraben. Hydrophobic membranes exhibited higher adsorption of parabens than hydrophilic membranes. Ionic strength affects the adsorption when electrostatic forces are involved but parabens are uncharged molecules and hence adsorption was not affected significantly. Addition of ethylene glycol reduced the adsorption of parabens due the fact that it hindered the hydrophobic interactions. All the results suggested that hydrophobic effect was the major mechanism of adsorption of parabens from filter membranes. Adsorption was directly correlated to the concentration of parabens. Further, several processing parameters were evaluated to understand the effect on adsorption. Adsorption was inversely proportional to flow rate. Temperature and autoclaving had little effect on paraben adsorption. Flow interruption study suggested that additional parabens were adsorbed by almost all six filter membranes after interruption of flow. Presaturation of filter membranes with paraben reduced the adsorption but adsorption process was not completely eradicated in all cases [9].

Adsorption of Active Moieties

Sterile filtration is widely used in case of protein formulations due to the heat sensitive nature of proteins. Proteins and peptides by nature prone to get adsorbed over the filtration membranes during clarification or sterile filtration and this follows time-dependent saturation kinetics phenomenon and thus it is recommended not to use first few mL of filtrate especially while filtering less volumes to reduce the loss of active compound. There is vast amount of literature available regarding the adsorption and its kinetics and conditions under which it gets hastened. The adsorption phenomenon may lead to (i) loss of drug concentration from the product (ii) destabilization or loss of potency of proteins and/or peptides due to conformational changes (iii) formation of aggregates upon incubation post filtration. Magnitude of adsorption also differs with the (a) type of filter used, (b) concentration, ionic strength and pH of product to be filtered and (c) the duration of filtration. So it is critical to decide which filter to use for a particular product type to ensure minimal loss of active moiety. A similar concern exists during filtration of other non-protein parenteral and ophthalmic products as well.

Piet, *et al.,* determined the severity of protein loss due to adsorption using solutions containing bovine serum albumin (BSA), luteinizing hormone releasing hormone (LHRH) and mouse immunoglobulin G (IgG) by five different filters under static and dynamic conditions. Measurement of static adsorption indicated significant differences in protein binding capacities of the filters studied. Nylon and PVDF filters showed much less adsorption than the other filters and among these PVDF proved to be the best one. The relative amount of protein lost decreased with increasing the volume of the protein. It was concluded that the kinetics of the adsorption process are of a time-dependent saturation type. Based on the experiments conducted, the authors stated that number of filtrations, surface area of the filter and duration of filtration should be as low as possible for a better filtration [10].

Different surfactants like polysorbate 80 are used in protein formulations to prevent adsorption of proteins at interfaces during manufacturing and storage. Surfactants have affinity for interface and thus, avoid adsorption of proteins. Mahler, *et al.,* conducted lab scale tests to study the adsorption behavior of polysorbate 80 and a monoclonal antibody (IgG1) to seven different filter materials, the determination of antibody and polysorbate 80 concentrations was performed on aliquots of the unfiltered sample and on aliquots removed at different intervals post filtration. The most prominent adsorption of IgG1 was found on Nylon filter whereas the adsorption found on PES (2 suppliers), CA, PVDF was 100 fold less. PES and PVDF filters of a different supplier showed no detectable protein adsorption, thus the surface properties of the membrane may differ due to differences in the manufacturing or preparation procedures thus imparting different nature inspite of the same material of construction. Significant adsorption of Polysorbate 80 was observed to a 10 in. PES filter when around 5L of a IgG1 (5 mg/mL) formulation with 0.01% w/v polysorbate 80 was filtered to simulate a manufacturing scale operation, calculations indicated a volume of filtrate before the complete surface area is saturated with polysorbate. The authors recommend concentration studies of both the protein and critical stabilizers such as Polysorbate during process development of membrane separation technique and filtration processes, in order to ensure the quality of the product during manufacturing [11].

Zhou, *et al.,* studied the binding of Polysorbate-20 (PS-20) to filter membranes and described the bound surfactant amount per square cm of membrane as well as the non-specific binding mechanism. The outcome of the study indicated that Polyethersulfone (PES) and Polyvinylidene (PVDF) membranes non-specifically bind to PS-20 which could lead to uneven distribution in the drug product. The binding of the PS-20 to the membranes seems to be hydrophobic in nature and measurable. Saturation of the non-specific binding sites using PS-20 present in the formulation buffer or in the product is possible and most feasible approach determined was preconditioning the filters prior to the protein preparation, the pre-condition was found not to be affected by temperature or the flow rate. The authors warn again usage of a over-sized filter in such applications [12].

End-line filtration of intravenously administered fluids has been shown to minimize inadvertent infusion of particulate matter and microbial contaminants thereby the incidence of phlebitis and sepsis associated with intravenous infusion can be reduced. End line filters consisting of a 0.2 µm hydrophilic membrane to prevent the passage of bacteria and particulate matter and a 0.2 µm hydrophobic membrane to remove air, are available. With the widespread use of these filtration devices, the problem of drug binding, and consequently the reduction in the potency of the administered drug substance arises [13].

Gasch, *et al.* investigated the interaction of five different drugs (negative charged, lipophilic uncharged, hydrophilic uncharged) with a positively charged PES filter as compared to a un-charged PES filter. Solutions of electrolytes with different hydrodynamic volume were used as eluents to understand the ion-dependency characteristics. For a positively charged PES filter, chloride ions are the counter ions of the PES and thus anionic drugs will compete with these chloride ions to replace them during the beginning of the filtration process. The smaller the hydrodynamic radius of the anion, the faster is the diffusion into the membrane inner structure and more efficient is the replacement of the chloride ions. The larger the amount of these anions, the higher the number of drug molecules that are replaced. Thus, higher the electrolyte concentration in the eluent, the smaller the amount of drug molecules that can interact with the positive charges inside the membrane and are retained by the membrane. The results of the study indicate that for each infusion formulation, a careful selection of the filter material is essential [14].

Gasch, *et al.,* studied the influence of Furosemide on the Zeta Potential of positively charged polyethersulfone membranes while using uncharged filters also in the study. The study left a question on the extent of advanantage that positively charged filter membranes have over “uncharged” membranes in retaining negatively charged ions or endotoxins [15].

Muynck, *et al.,* evaluated drug adsorption by end-line filters in intensive care units. The authors have observed significant loss in digoxin and diazepam delivery to patients in the first 20 min to 60 minutes of infusion. This kind of loss was less predominant with dopamine infusion. The loss observed during the initial period of infusion is due to adsorption of the drugs to the hydrophilic membrane of the filters. Once binding sites are saturated, no more drugs seems to be adsorbed [13].

Ennis, *et al.,* investigated the in-vitro study of inline filtration of medications commonly administered to pediatric cancer patients. The drugs selected for the study were amongst the most commonly administered to acut nonlymphocytic leukemia such as Adriamycin, cytarabine, vincristine, dactinomycin, cephalothin, carbenicilin and gentamicin. The filtration of the drugs was simulated by intravenous bolus injection where each drug was individually pushed through a new Pall Ultipor 0.2 µm air-eliminating filter. Priming of the filters with Normal slaine or dextrose solution was done to wet the filters, similarly the filters were flushed with normal saline post passage of the drug solution. The study results indicated that there was loss of each drug to varying degrees upon passage through the filter, gentamicin showed the highest loss and thus was recommended not to be filtered through the studied membrane. The mechanism of drug loss in the filtration process was concluded to be largely due to a trapping phenomenon where the number of moles lost was a function of the number of moles administered. The authors refer to a study done by Rajchgot et al which determined that gentamycin maybe retained in certain filters deu to the low specific gravity of gentamicin-containing solutions under upright and inverted conditions [16].

Filtration of anti-sera through microfilters often causes loss of immunoglobulin’s (IgA, IgG, IgM), albumin and transferrin. Generally, cellulose nitrate filters adsorb immunoglobulins (majorly IgG) from diluted anti-sera. However, no significant adsorption of albumin and transferrin was observed. Filtration is a general practice for diluted anti-sera in nephelometric and turbidometric assays to get rid of particulate matter. But during filtration through microfilters (pore size 0.2-0.4 µ), removal of IgG was observed. Walsh, *et al.,* evaluated the loss of IgG on filtration with 10 different microfilters. Also, they investigated the effect of filtration on diluted anti-sera. Ten types of microfilters with different composition and vendors were evaluated including cellulosic and non-cellulosic filters. They observed that adsorption of albumin and transferrin was insignificant for all the microfilters. IgG was majorly adsorbed by the filters with either cellulose nitrate or combination of cellulose nitrate and cellulose acetate. Only cellulose acetate filters did not show adsorption of IgG. Further, they found out that adsorption of IgG from cellulose nitrate filters is directly proportional to concentration of IgG. Authors concluded that adsorption of IgG to depend upon filter composition in following manner: cellulose nitrate > mixed cellulose esters > cellulose diacetate > cellulose triacetate [17].

Kanke, *et al.,* evaluated the binding of several drugs to a inline i.v filter that had been “treated” to inhibit drug binding. The authors based on prior studies suggested that by treating the membrane with an agent capable of both hydrophilic and hydrophobic hydration, the polar groups as well as the linear cellulose moiety would be blocked, and binding of drugs would be minimized. Solutions of mithramycin, vincristine sulfate, digitoxin, insulin,

dactinomycin and nitroglycerin in 5% dextrose injection and 0.9% sodium chloride injection were allowed to flow through an i.v.administration set containing a 0.22 µm cellulose ester filter that had been treated with a proprietary agent [18].

Weltje, *et al.,* evaluated the suitability of eight hydrophilic filter types for the sterilization of nutrient solutions with respect to the adsorption of mono-, di-, and trivalent metal cations or complexes. Chemical speciation of the metals was calculated to assess its importance for adsorption, while the use of radioisotopes permitted sensitive measurements of metal concentrations in both filters and filtrates. It was anticipated that the filters had some cation exchange capacity and thus would electrostatically bind free metals and positively charged complexes. Polycarbonate and Nylon showed the lowest affinity for metals and are as such recommended for the filtering of culture media and other metal-containing solutions. Further, it is recommended to preequilibrate filters in the solutions to be filtered and thus saturate possible binding sites. Filter membranes have certain saturable capacity to being cations and thus the amount of metals that may adsorb is best conceived as an absolute value. The study concludes that high pH, small sample volume and low metal concentrations make the choice of the filter crucial to get the correct analytical results [19].

Filters as a Source of Extractables/ Leachables

Several processes are involved in manufacturing of filters with the use of different ingredients depending on the type of application and these can be leached out into the product upon filtration. Leachables from sterilizing grade filters could affect the stability of the formulation; some of them are even toxic. Especially, protein formulations are more sensitive. Leachables generally destabilize, increase aggregation or oxidize the protein structure. Different extractables and leachables are observed depending upon the type and material of construction of the filters. For example, oligomers of hydroxypropyl acrylate, propylene glycol and cross linker like tetraethylene glycol diacrylate could be the filter extractables from hydrophilic-modified PVDF membrane. Isopropyl alcohol and acetone have also been found as extractables from similar membranes. Glycerol was identified as the major extractable from mixed cellulose ester filers. Leachables which are readily introduced into formulation generally decrease surface tension. Different organic compounds could be observed as leachables from various filter membranes. Nature of leachables depends upon material of construction of filters and chemicals used to modify filters hydrophilicity/ hydrophobicity.

Otsuki, *et al.* identified the leaching of a non-ionic surfactant polyoxyethylene nonylphenyl ether from mixed cellulose ester filters (Millipore HA). Its presence in sufficient amount for its absorption maxima at 224 nm to be directly observable in the spectrum of the filtrate (double distilled water) during analysis lead to its identification. Rinsing the filter with sufficient amounts of water is necessary to remove such leachables especially while measuring low levels of dissolved organic carbon in a sample and when micro-organisms in the filtrate are to be cultured [20].

Huang, *et al.,* studied the impact of extractables and thus possible leachables on stability of protein formulations since little is known about the potential impact of inadvertently introduced leachables from filter membranes on protein stability. IgG2 monoclonal antibody formulation was used as a model formulation. Surface tension analysis, observation of visual and sub-visible particles, dynamic light scattering, nephelometry, size-exclusion chromatograph, imaging capillary gel electrophoresis, reverse phase HPLC, differential scanning calorimetry and thermal desorption GC-MS analysis were used to evaluate the effects of the extractables. The study design was such that stock solutions of extractables were intentionally spiked into the formulation, the formulations were subsequently subjected to agitation and thermal stress. In some cases, the extractables tended to decrease the surface tension and provided protective effect similar to a surface-active agent though this was not demonstrated by the extractables from mixed cellulose ester filter thus clearly demonstrating that all surface active agents are not equally effective in protecting proteins against interface-induced stresses. The impact of the extractables was higher where the product did not contain a surfactant; the effect was more prominent under shear stress. The amount of protein particulate was inversely proportional to the amount of filter extractables. The authors recommend a thorough flush of the filter before use to reduce leachables from the filter which also has the added benefit of removing particulates shed from the filter and housing [21].

Gasch, *et al.,* made an attempt to find out the compound that caused unexpected maximum at 234.5 nm in the ultraviolet spectrum of a filtered digoxin solution using a filter (Intrapur Neonat by Braun). With the aid of mass spectrometry and ultraviolet spectroscopy the unknown compound was found to be N,N-dimethylacrylamide. There was high variability in the released amount of the leachable between the batches as well as between the filters of the same batch. N,N-dimethylacrylamide is a acrylamide derivative and is used commonly as an adhesive, its use in the filter was associated to the housing rather than the membrane itself. Though N,N-dimethylacrylamide could not be concluded to be toxic the neurotoxicity of the metabolites was not ruled out. The study concluded that N,N-dimethylacrylamide should be completely removed from the final filter product [1].

Effect of membrane filtration on sample preparation for chemical analysis

Analysis of drug products by chromatographic techniques involves the dissolution of product in suitable media followed by filtration or centrifugation as to remove undissolved particulates because particulates can interfere with analysis and also damage the columns. Filtration is preferred over centrifugation due to its advantages of less time, low cost and labor with equal particulate removing efficiency. The major concern with filtration is adsorption of APIs which leads to false results [22].

Adsorption of APIs from filter membrane during sample preparation mainly depends upon interaction between API and membrane, which majorly categorized into three:

- **Physico-chemical properties of APIs and formulation**
  Various physico-chemical parameters of API affects the type and intensity of interaction with filter membranes viz. molecular weight, molecular structure, ionization state etc.
- **Type and nature of filter membranes**
  Composition of filter membrane, nature of polymer used (ionic/ non-ionic, hydrophobic/ hydrophilic) etc.
- **Sample medium**
  Sample medium is also important factor that affect adsorption, as it can directly affects the solubility of API and wettability of filter membrane. Table 1 shows the examples of filter that could be used for specific type of samples.

Table 1: Recommended filters for different type of samples.

S.No.	Type of sample	Recommended filters
1.	Aqueous samples	Hydrophilic filters viz. cellulose acetate (CA), polyethersulfone (PES)
2.	Organic samples	Hydrophobic filters viz. polypropylene (PP), polytetrafluoroethylene (PTFE)
3.	Mixture of aqueous and organic samples	PP, PTFE, nylon, PES and regenerated cellulose (RC) filters can be used depending upon the percent of aqueous/ organic solvent

The phenomenon of membrane filter adsorption in high performance liquid chromatography (HPLC) was investigated utilizing 16 brands of filters representing 3 polymeric materials: cellulose acetate (CA), nylon, and polyvinylidene difluoride in a variety of diameters (3, 4, 7, 13, and 25 mm). Sixteen compounds commonly encountered in drug preparations were selected as sample analytes and classified as acidic, basic, and neutral in chemical behavior. Six mobile phase/sample solvent mixtures were included: 3 with methanol–water and 3 with acetonitrile–water as major constituents. When using methanol as the mobile phase organic component, CA, nylon, and polyvinylidene difluoride (PVDF) filters exhibited negligible to moderate adsorption levels with regard to the neutral and basic drug compounds. The acidic drug test compounds were adsorbed by 50% of all 3 filter materials tested in methanol–water. In acetonitrile, neutral compounds were affected by 31.4%, basic compounds were affected by 47.0%, and acidic compounds are affected by 53.6% of the nylon and PVDF filters. Membrane filter adsorption effects can be reduced by saturating the filter with a few milliliters of sample solution during the injection step. Filter extractables are removed during this process, and the adsorptive sites are gradually occupied by the sample matrix ingredients. When the available active sites are occupied, additional filtration will no longer reduce adsorption effects. The compound of interest may be tested by injecting a standard solution or the sample solution into the liquid chromatograph with and without filtration in order to observe any response differences. If significant losses are observed, consideration should be given to the use of the same type of membrane filter material from another manufacturer or the choice of a different membrane filter material [23].

There are sufficient reports that indicate that not all filter materials are suitable for dissolution testing. Therefore, the recovery of a drug from the analyte should always be validated, since this is the only way to rule out adsorption to the filter. Dressman, *et al.*, studied the adsorption on a larger range of filters. They also evaluated the influence of soluble residues from filter materials on the drug determination by UV spectroscopy. Ten filter materials from three different filter suppliers were chosen for the study where acetylsalicylic acid (hydrophilic drug, high dose) and prednisolone (lipophilic, low dose) were chosen as the model drugs for evaluation in three different buffers which where the recommended media for biowaiver requests (Simulated Gastric Fluid USP without enzymes, a pH 4.5 buffer, and Simulated Intestinal Fluid USP without enzymes). The study determined that there was minimal adsorption of acetylsalicylic acid and prednisolone on most of the investigated filter materials. When the first 2 mL of filtrate was discarded before analysis, most of the tested filter materials showed a recovery >95% and should therefore be suitable for dissolution testing in the biowaiver buffers. After the available active adsorption sites are saturated, the subsequent filtration process does not further decrease the drug concentration in the filtrate. An additional benefit is that soluble residuals that could be present from the manufacturing process are eliminated by discarding the first 2 mL. Without discard of 2 mL before analysis, the UV absorbance of the soluble residues could result in a higher cumulative absorbance and thus an overestimate of drug recovery. In summary, the studies indicate that drug recovery should always be validated for a specific filter material, since the absence of filter adsorption and soluble residuals cannot otherwise be assured. Under these conditions, unacceptable drug loss due to adsorption was only observed for the nylon/polyamide material. With respect to both chosen model drugs prednisolone and acetylsalicylic acid, the results indicate that the nylon/polyamide material is unacceptable for dissolution testing [24].

CONCLUSION

The variety of filter materials available to process development scientists is large—from depth media containing nominally-rated micron-sized filtration-matrices to validated sterile filtration membranes containing submicron-sized pores. The criteria by which one chooses the optimal filter is commonly application-specific, and it is therefore important to understand these criteria when designing experiments, analyzing data, and comparing product attributes. Due to the difference in membrane characteristics, different products and operating methods have developed to take best advantage of the strengths of various membranes.

REFERENCES

1. Gasch J, Oertel R, Leopold C.S, Knoth H., *J Crit Care.* 2010, 25, 172. e9-72. e14.
2. Werner B.P, Winter G., *Int J Pharm*. 2015, 496, 250-67.
3. Liu L, Randolph T.W, Carpenter JF., *J Pharm Sci.* 2012, 101, 2952-59.
4. Makino K, Hirakawa M, Goto Y, Nakashima K, Kataoka YOishi R., *Electrophoresis.* 1998, 19, 2930-34.
5. Hirakawa M, Makino K, Nakashima K, Kataoka YOishi R., *J Clin Pharm Ther*. 1999, 24, 387-92.
6. Moce-Llivina L, Jofre J, Muniesa M., *J Virol Methods*. 2003, 109, 99-101.
7. Bin T, Kulshreshtha A.K, Al-Shakhshir R, Hem S.L., *Pharm Dev Technol*. 1999, 4, 151-65.
8. Naido N.T, Price C.H, McCarthy T.J., *Australian Journal of Pharmaceutical Sciences.* 1972, 16-18.
9. Bin T, McCrosky L, Kulshreshtha A.K, Hem S.L., *Pharm Dev Technol*. 2000, 5, 95-104.
10. Van den Oetelaar P.J, Mentink I,M, Brinks G.J., *Drug Dev Ind Pharm*. 1989, 15, 97-106.
11. Mahler H.C, Huber F, Kishore R.S, Reindl J, Rückert P, Müller R., *J Pharm Sci*. 2010, 99, 2620-27.
12. Zhou J.X, Qiu J, Jiang G, Zhou C, Bingham N, Yeung H, *et al.*. *J Membrane Sci.* 2008, 325, 735-41.
13. Muynck C.D, Vroe C.D, Remon J, Colardyn F., *J Clin Pharm Ther*. 1988, 13, 335-40.
14. Gasch J, Leopold C.S, Knoth H., *Eur J Pharm Sci.* 2011, 44, 49-56.
15. Gasch J, Leopold C.S, Knoth H.,. *J Membr Sci Technol.* 2013, 3, 121-25.
16. Ennis C.E, Merritt R.J, Neff D.N., *JPEN-Parenter Enter*. 1983, 7, 156-58.
17. Walsh R.L, Coles M.E., *Clin Chem.* 1980, 26, 496-98.
18. Kanke M, Eubanks J.L, DeLuca P.P., AM J Health-Syst Ph. 1983, 40, 1323-28.
19. Weltje L, den Hollander W, Wolterbeek H.T., *Environ Toxicol Chem*. 2003, 22, 265-71.
20. Otsuki A, Fuwa K., *Talanta.* 1977, 24, 584-86.
21. Huang M, Horwitz T.S, Zweiben C, Singh S.K., *J Pharm Sci*. 2011, 100, 4617-30.
22. Zhao L, Long W. Syringe filter suitability for sample preparation in drug assay. *Application note- Agilent technologies*, 1-11.
23. Carlason M, Thompson R.D., *J Chromatogr Sci*. 2000, 38, 77-83.
24. Kiehm K, Dressman J.B., *Dissolution technologies.* 2008, 13-17.