Received: 16 May 2019 | Revised: 13 September 2019 | Accepted: 17 September 2019

DOI: 10.1002/btpr.2923

BIOTECHNOLOGY PROGRESS

RESEARCH ARTICLE

Clearance of solvents and small molecule impurities in antibody drug conjugates via ultrafiltration and diafiltration operation

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Peer Review
The peer review history for this article is available at https://publons.com/publon/10.1002/btpr.2923.

Abstract

Ultrafiltration and diafiltration (UF/DF) processes by tangential flow filtration (TFF) are frequently used for removal of solvents and small molecule impurities and for buffer exchange for biopharmaceutical products. Antibody-drug conjugates (ADCs) as an important class of biological therapeutics, carry unique solvents and small molecule impurities into the final UF/DF step as compared to standard antibody preparation. The production process of ADCs involves multiple chemical steps, for example, reduction and conjugation. The clearance of these solvents and small molecules by UF/DF, specifically the DF step, has been assessed and described herein. The rates of clearance for all the impurities in this study are close to the ideal clearance with no apparent interaction with either the protein or the TFF membrane and system. The effect of process variables during DF, such as pH, temperature, membrane loading, transmembrane pressure, and cross flow rate, has also been evaluated and found to have minimal impact on the clearance rate. These results demonstrate efficient clearance of solvents and small molecule impurities related to the ADC process by the DF process and provide a general data package to facilitate risk assessments based on the sieving factors and program specific needs.

KEYWORDS
ADC, impurity clearance, UF/DF

1 | INTRODUCTION

Ultrafiltration and diafiltration (UF/DF) by tangential flow filtration (TFF) is a frequently used final purification and buffer exchange step for biopharmaceutical products. TFF allows liquid to flow in the direction parallel to the membrane, generating crossflow current that reduces accumulation of protein on the membrane surface and improves permeation of solvents through the ultrafiltration membrane.[1] This unit operation is a mainstay in concentration of proteins and buffer exchanges and has shown to be effective at removing solutes when the molar masses of the solutes are significantly lower than the protein.[2,3] In one study, seven extractables and leachables from single-use technologies, of different chemical families and molecular weights, were spiked in and evaluated for clearance from proteins by UF/DF process. The results demonstrated effective removal of all seven extractables and leachables.[4]

Antibody drug conjugates (ADCs) have emerged as a new class of biotherapeutics in areas for oncology, infectious diseases, and immunological indications.[5,6] ADCs are comprised of antigen-targeting monoclonal antibodies (mAbs) and therapeutic drugs that are attached covalently via chemical linkages (i.e., linkers). Most ADCs rely on chemical methods to link therapeutic drugs to lysines or reduced interchain cysteines on the native antibody, for example, the commercial ADCs, Kadcyla, Mylotarg, Besponsa, and Adcetris.[6-8] The chemical

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processes involved in the ADC production generate a unique collection of solvents and small molecule impurities that is not typically associated with traditional mAb production. However, there is a general lack of reported data on the UF/DF removal of small molecule impurities from ADC processes, prompting the herein described study.

For ADCs using reduced interchain cysteines for conjugation, the chemical steps include reduction of interchain disulfide bonds, conjugation, and quench of thiol-reactive drug-linkers before the final UF/DF operation.[9-11] In this article we focus on two commonly utilized cosolvents and three small molecules (see Table 1) that are frequently present in the UF/DF unit operation. The clearance of drug-linker related species by UF/DF is specific to each ADC program and needs to be studied separately to meet the specification for each program, therefore is beyond the scope of this article. Tris (2-carboxyethyl)phosphine (TCEP) is typically used for reduction and is converted to the oxidized form (TCEP=O) upon completion of the reduction. Ethylenediaminetetraacetic acid (EDTA) is typically added for metal chelation to minimize re-oxidation of the newly freed cysteines. Dimethyl sulfoxide (DMSO) and *N*,*N*-dimethylacetamide (DMA) are commonly used to dissolve the typically hydrophobic drug-linker and serve as cosolvents during the conjugation. Once the conjugation is complete, *N*-acetyl-L-cysteine (NAC) can be used to quench any excess amount of thiol-reactive drug-linkers, often maleimide-containing drug-linkers.[12] In this article, the clearance of these five molecules, with molecular weights ranging from 78.1 to 292.2 Da, is studied in the presence of one mAb to determine the clearance rate. The operational variables of UF/DF and incorporation of two distinct mAbs are also studied to probe the robustness of the clearance. Here mAbs are used as surrogate to the ADCs assuming minimal impact of drug-linker conjugation on the clearance rate of the listed solvents and small molecules, as well as nearly complete retention of mAb or ADC by the membrane.

2 | MATERIALS AND METHODS

2.1 | Materials

Antibodies employed in this study were manufactured at AbbVie. Two mAbs were used, 34C3 monoclonal antibody formulated at 50 mg/ml in 15 mM Histidine, pH 6.0 buffer, and anti-TeTx (AB095) monoclonal antibody formulated at 11.4 mg/ml in 30 mM Histidine, 8% sucrose, pH 6.0 buffer. TCEP=O was synthesized at AbbVie. EDTA disodium salt dihydrate, DMSO, DMA, and NAC were purchased from Sigma Aldrich. PBSE buffer (potassium phosphate, NaCl, EDTA, pH 7.2) and 15 mM Histidine buffer pH 6.0 were purchased from SAFC.

2.2 | Load sample preparation

The load samples were prepared by a gravimetric method based on the density of each component. For a 50 ml load solution containing the 34C3 mAb, 20 ml of mAb solution was diluted with 25 ml of PBSE, followed by ~5 ml of the solution containing specific amounts of the solvents or small molecules shown in Table 1. As shown in Table 2, the spiked solvents and small molecule solutions were: neat DMA, neat DMSO, 4.4 mg/ml of TCEP=O in water, and 11 mg/ml of NAC in water. EDTA was present at 1.84 mg/ml as a component in PBSE solution. Each load sample contained 20 mg/ml of mAb with different final concentrations of the spiked impurities listed in Table 2.

For the AB095 mAb, the mAb solution was mixed in a 9:1 ratio with either neat DMA or 11 mg/ml of NAC in water to give 10.3 mg/ml of mAb with the corresponding final concentration of the spiked impurities listed in Table 2.

2.3 | TFF operation: Setup and sampling

A bench-top UF/DF system shown in Figure 1 was used for the study. A 30 kDa Millipore Pellicon-3 Ultracel (regenerated cellulose) membrane cassette with 88 cm^2 surface area and Type C screen (catalog number P3C030C00) was implemented. Prior to each run, the system was flushed and sanitized overnight with sodium hydroxide (0.1 N), then flushed with purified water and equilibrated with the DF buffer (15 mM Histidine pH 6.0 buffer).

After the system was emptied, ~50 ml of load sample containing the solvent or small molecule impurity was added to the UF/DF system to yield a loading of 113 g protein per m^2 membrane. The system was then operated in constant volume and constant transmembrane pressure (TMP) DF mode, where solution in the reactor was pumped across the membrane and filtered, while fresh buffer was fed continuously to keep the constant volume in the reactor. The resulting constant volume in the system is referred to as diavolume (DV). The number of DVs (*N*) is defined as the volume of the permeate removed divided by DV. In this study, DF operation typically ranged from 8 to 10 DVs. The standard UF/DF conditions were as follows: room temperature at around 21°C, cross flow rate, or feed flow rate, at 300 L m^{-2} hr^{-1}, or 44 ml/min, and TMP ([P1 + P2]/2 – P3) at 18–20 psi. Under this operational condition, the average permeate flux was about 66 L m^{-2} hr^{-1} at the first DV, and increased to about 108 L m^{-2} hr^{-1} at 8 DV of DF due to the change in solution composition by buffer exchange.

TABLE 1 Solvents and small molecule impurities studied for UF/DF clearance

Compound	Purpose in ADC process	MW (Da)
Tris(2-carboxyethyl) phosphine oxide	Oxidized form of reducing reagent	266.2
Ethylenediaminetetraacetic acid	Chelation reagent for reduction	292.2
Dimethyl sulfoxide	Solvent for drug-linker	78.1
N,*N*-Dimethylacetamide	Solvent for drug-linker	87.1
N-acetyl-L-cysteine	Quench reagent for drug-linker	163.2

Abbreviations: ADC, antibody-drug conjugate; MW, molecular weight; UF/DF, ultrafiltration and diafiltration.

TABLE 2 Preparation of 50 ml of load sample from spiked compounds at different concentrations

mAb solution	5 ml stock solution of spiked compounds	Stock concentration of spiked compounds (mg/ml)	Final concentration of spiked compounds (mg/ml)	Final concentration of mAb (mg/ml)
20 ml of 34C3 at 50 mg/ml, diluted with 25 ml of PBSE	DMA	Neat	94	
	DMSO	Neat	110	
	TCEP=O	4.4	0.43	20
	NAC	11	1.1	
	Water (EDTA in PBSE)	1.84	0.92	
45 ml of AB095 at 11.4 mg/ml	DMA	Neat	94	10.3
	NAC	11	1.1	

Abbreviations: DMA, *N*,*N*-dimethylacetamide; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; NAC, *N*-acetyl-L-cysteine; TCEP, tris (2-carboxyethyl)phosphine.

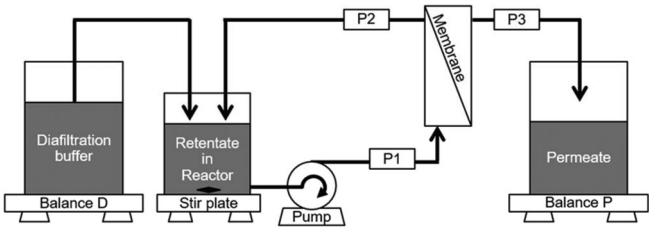

FIGURE 1 Set-up of the bench-top UF/DF system. UF/DF, ultrafiltration and diafiltration

Samples (1 ml) were taken at different number of DVs of permeate, at every one or two DVs from both the permeate port and from the reactor (retentate). Before taking retentate samples from the reactor, the valves of DF buffer and permeate were closed, and the retentate solution was recirculated through the membrane for ~5 min to ensure homogeneity of the solution in the system. Sampling also contributed to a volume decrease of the retentate in the reactor and was corrected for the following DF operation.

2.4 | HPLC analytical method for DMSO

An amount of 200 μl of each sample solution was accurately transferred into a 5 ml volumetric flask and diluted to volume with acetonitrile. The mixture was agitated and the resulting protein precipitation was allowed to settle for ~5 min, followed by filtration through a 0.2 μm PTFE filter to remove the precipitate. Each filtrate was either diluted with 15 mM Histidine buffer or injected neat onto an Ascentis Express HILIC column (3.0 × 150 mm, 2.7 μm, Supelco, Catalog No: 53972-U). The concentration of DMSO in each sample was calculated by comparing the peak area to that of a 0.1 mg/ml DMSO working standard and accounting for the dilution factor of the sample preparation. Recovery of DMSO was performed for the analytical method and was above 95% at both working standard level and quantitation limit level.

2.5 | HPLC analytical method for DMA

Each sample was either directly diluted with 15 mM Histidine buffer or injected neat onto an ODS-2 HYPERSIL column (4.6 × 150 mm, 5 μm, Thermo Scientific, Catalog No: 31605-154630). An isocratic method with mobile phase of 5% ACN in 20 mM Potassium Phosphate, pH 6.5 was used. The concentration of DMA in each sample was calculated by dilution factor and by comparing the peak area to that of a 0.1 mg/ml DMA working standard.

2.6 | LCMS analytical method for NAC and TCEP=O

Each sample was mixed in a 1:3 ratio with 600 mM HCl in 90/10 methanol/water to precipitate the protein, followed by centrifugation for 10 min at 16.1 RCF. The supernatant was collected, diluted with water, and injected onto an Ascentis Express RP-Amide column (4.6 × 150 mm, 2.7 μm, Supelco). NAC and TCEP=O were quantified by single quad LC–MS under selective ion monitor (SIM) mode. The concentration of NAC and TCEP=O in each sample were calculated by dilution factor and by comparing the peak area to that of a 0.1 mg/ml NAC and TCEP=O working standard. Recovery of NAC and TCEP=O was between 90 and 105% depending on the type of protein and concentration of the analytes.

2.7 | HPLC analytical method for EDTA

Each sample was 1:1 mixed with acetonitrile to precipitate the protein, and was placed on the centrifuge for 10 min at 16.1 RCF. The supernatant was collected, and was either diluted with water or injected neat onto a PRIMESEP D column (4.6 × 150 mm, 5 μm, SIELC). Mobile phases contained 1.26 mM $CuSO_4$, and EDTA formed a UV active complex with copper ion when mixed with mobile phase through the HPLC system. The concentration of EDTA in each sample was calculated by dilution factor and by comparing the peak area to that of a 0.012 mg/ml EDTA working standard.

2.8 | TFF parameter exploration

Several TFF parameters were explored for DMA to determine their impact on the clearance rate. Two parameters were studied separately while keeping the other parameters the same, such as the pH of the DF buffer (15 mM Histidine), which varied from pH 6.0 to 5.0 and pH 7.0, and the membrane loading, which was increased to 454 g protein per m^2 membrane. Three other parameters were studied in four

experiments with different combinations of the following: the temperature was varied from 21 to 28°C and 12°C; cross flow rate was varied from 300 to 186 L m^{-2} hr^{-1} and 420 L m^{-2} hr^{-1}; TMP deviated from 18 to 30 psi and 12 psi. Some of the conditions might be in the pressure dependent regime for this TMP range explored.

3 | RESULTS AND DISCUSSION

3.1 | Determination of clearance rate of solvents and small molecule impurities by UF/DF

During UF/DF process, sieving factor (*S*), defined as the ratio of concentration of the species in the permeate to the concentration of the species in the retentate,[13] is often used to characterize the clearance rate. The sieving factor can be species dependent. A species that is completely retained will have a sieving factor of 0, while a species that is ideally cleared or freely permeable will have a sieving factor of 1. The clearance of each solvent and small molecule species for a constant volume DF can be described by Equation (1),[2] where C_f is the concentration of the species of interest at certain number of DVs into the DF, and C_i is the initial concentration of the species of interest at the start of DF, *S* is the sieving factor, and *N* is the number of DVs.

$$C_f = C_i e^{-(S \times N)} \quad (1)$$

To evaluate the clearance rate of the solvents and small molecule impurities listed in Table 1, each was spiked at different levels in 34C3 mAb solution and was subjected to a constant volume DF as described above. The samples collected at different DVs were analyzed for the concentration of each compound in both retentate solution and permeate solution. To determine the sieving factor for each species, the natural logarithm of the concentration (mg/ml) of each species was plotted against the number of DVs, and the negative slope from the linear regression represents the sieving factor. Here, the calculated sieving factor represents the apparent sieving factor that is based on the permeate concentration and mixing-cup concentration of the retentate.[14] Figure 2 shows the plot, the linear regression, and the corresponding sieving factor from both the retentate solution and the permeate solution for the solvents and small molecule impurities studied here. The clearance trends and the sieving

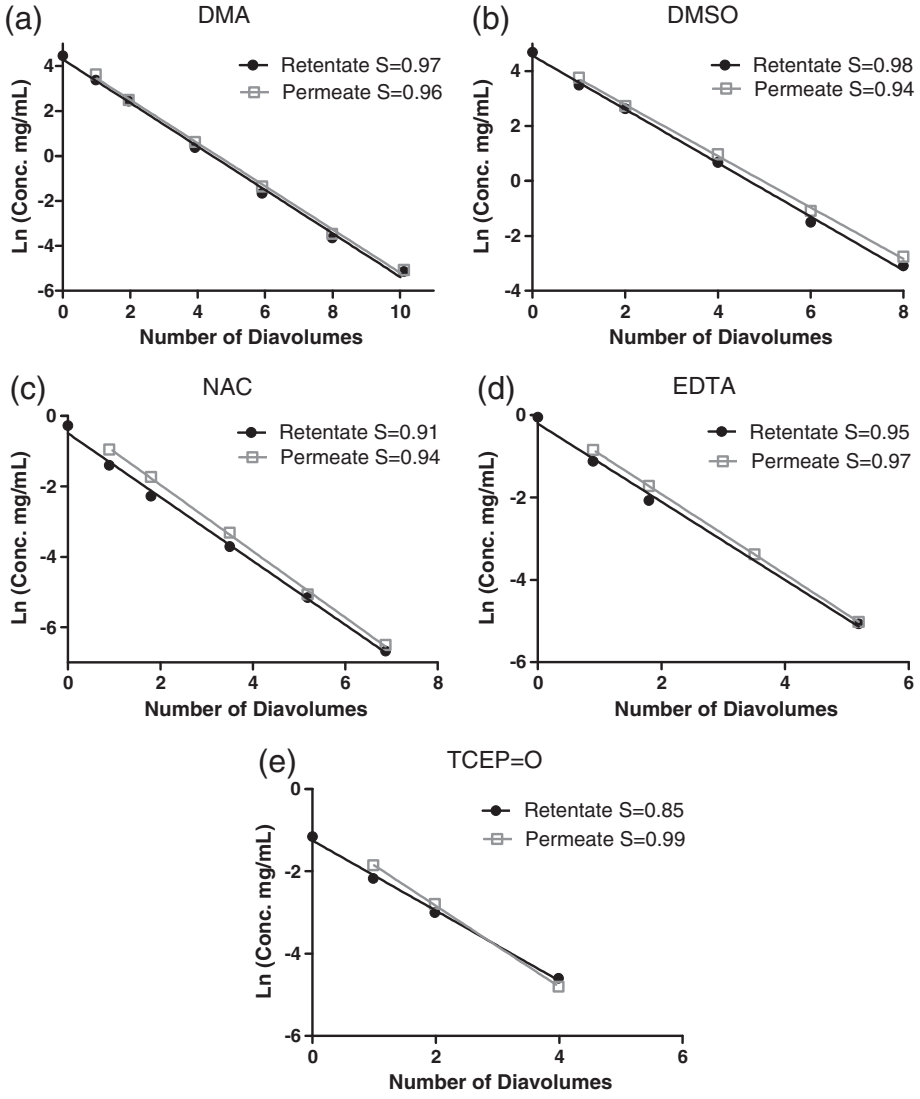

FIGURE 2 The UF/DF clearance trend and sieving factor for solvents and small molecule impurities in 34C3 mAb solution; each line represents the linear regression result and the R^2 values are all equal to or greater than .99. UF/DF, ultrafiltration and diafiltration

factors matched well for the retentate solution and the permeate solution of each species, and all the sieving factors approximated ideal clearance. Except for the study of DMA where no protein precipitation method was used, there were slight differences observed between the concentrations and sieving factors obtained from the retentate solution and permeate solution for the other four molecules, possibly due to the protein precipitation during sample treatment that impacted differently for the retentate solution that contained mAb and permeate solution that contained no mAb.

Both solvents, DMA and DMSO, were spiked at 10% vol/vol into the mAb solution, representing a typical level in a conjugation reaction mixture. They were both cleared efficiently with sieving factors close to 1. DMA had closely matching clearance trends of the retentate solution and permeate solution, while DMSO showed slight differences between these two, possibly due to the sample treatment during the analysis (Figure 2a,b). Starting at around 10^5 ppm in the solution or 5×10^6 ppm relative to the mAb, both solvents were below 2,500 ppm and 50 ppm in the solution after 4 DV and 8 DV of DF, respectively, or below 2,500 ppm relative to the mAb after 8 DV of DF.

NAC and EDTA were spiked at ~1 mg/ml in the mAb solution. Both impurities were cleared efficiently, and the sieving factors were determined to be 0.91 and 0.95 in the retentate solution, respectively (Figure 2c,d). The concentrations measured in the retentate solution were slightly lower than that in the permeate solution, which could be due to the protein precipitation in the retentate solution and slightly lower recovery of the analytes when preparing the analytical sample. In the retentate solution, both impurities reached <0.032% wt/wt relative to the 20 mg/ml mAb after 5.2 DV of DF.

TCEP=O was spiked at 0.43 mg/ml in the mAb solution, which corresponded to 1.6 mM, or 11.8 equivalents relative to the 34C3 mAb at 20 mg/ml. Samples were collected from both retentate solution and permeate solution up to 8 DVs. The 6 DV and 8 DV samples showed concentrations of ≤4 μg/ml, which were lower than the detection limitation of the analytical method for TCEP=O and were therefore excluded for the determination of fit (i.e., linear regression). As shown in Figure 2e, the linear regression based on the first 4 DVs yielded sieving factors of 0.85 and 0.99 for retentate solution and permeate solution, respectively. In the retentate solution, TCEP=O reached 0.05% wt/wt relative to the 20 mg/ml mAb after 4 DVs of DF.

Based on these results, both solvents and all three small molecule impurities tested here exhibited clearance rates close to ideal clearance, and their levels were well controlled for a typical process using 7–10 DVs of DF.

3.2 | Clearance of solvents and small molecule impurities with different antibodies

One solvent (DMA) and one small molecule impurity (NAC) were also tested with a second mAb, AB095. AB095 mAb has higher isoelectric point (pI 9.24) than that of 34C3 mAb (pI 8.7). Also, AB095 was formulated at 11.4 mg/ml in 30 mM Histidine, 8% sucrose, pH 6.0 buffer, therefore the load samples were prepared directly in a 9:1 ratio of the mAb solution to the solution of spiked impurity. While maintaining similar starting concentration of the spiked impurities, the compositions of the load samples were very different for these two mAbs, with AB095 samples containing 10.3 mg/ml mAb in 27 mM Histidine buffer, 7.2% sucrose and pH around 6, while the 34C3 mAb containing 20 mg/ml mAb in 6 mM Histidine, 50% PBSE and pH around 7.

Despite the differences in mAb properties and load sample compositions, DMA and NAC showed similar clearance trends for these two mAb solutions, using 15 mM Histidine pH 6.0 as the DF buffer (Figure 3). In the case of DMA, the clearance trend between these two mAbs solutions aligned, and the calculated sieving factors were similar and close to ideal clearance ($S = 1$). The sieving factors of NAC in both mAb solutions were calculated as >0.9 and NAC reached <0.03% wt/wt after 6 DV of DF. Despite the difference of sieving factors of 0.97 versus 0.91 for the two mAbs, both resulted in NAC concentrations below the acceptable levels during a typical process involving more than 6 DVs of DF. Therefore, both clearance trends of DMA and NAC were close to ideal clearance with different mAbs and sample compositions. We propose that these results would hold true as well for other impurities, other mAbs, or other buffer components, provided there is no interaction between the impurities and the mAb or buffer components.

3.3 | Clearance of DMA under various UF/DF conditions

Multiple UF/DF parameters were studied for their impact on DMA clearance, including the pH of DF buffer, the membrane loading of the mAb, temperature, TMP, and cross flow rate.

The DF buffer was added to the retentate to maintain constant volume during DF, and 15 mM Histidine, pH 6.0 buffer was used. The buffer pH was adjusted to 5.0 by addition of HCl, or to 7.0 by addition of NaOH. As shown in Figure 4a, when the DMA clearance in 34C3 mAb solution was tested with the DF buffer at pH of 5.0 or 7.0, no impact was observed on the clearance trend and sieving factor from the retentate solution. Both pH conditions showed the clearance of DMA close to ideal clearance, similar to that encountered with the nominal pH 6.0 experiment.

Membrane loading is an important UF/DF parameter. In this study, the membrane loading was increased four folds for the 34C3 mAb, from 113 to 454 g/m^2, by increasing the volume of the load sample from 50 to 200 ml, while keeping the concentration of all the solution components the same. As shown in Figure 4b, at the range tested, the DMA clearance rate was not impacted by increased membrane loading, and the sieving factors were close to 1 for both low and high loadings. This result indicated a wide operational range for the membrane loading, and possible higher loadings could be tolerated without impacting the clearance of small molecule impurities.

Finally, temperature, TMP, and cross flow rate were studied in combination in four sets of experiments. During UF/DF, retained solutes, mostly protein, build up on the membrane surface and form a gel

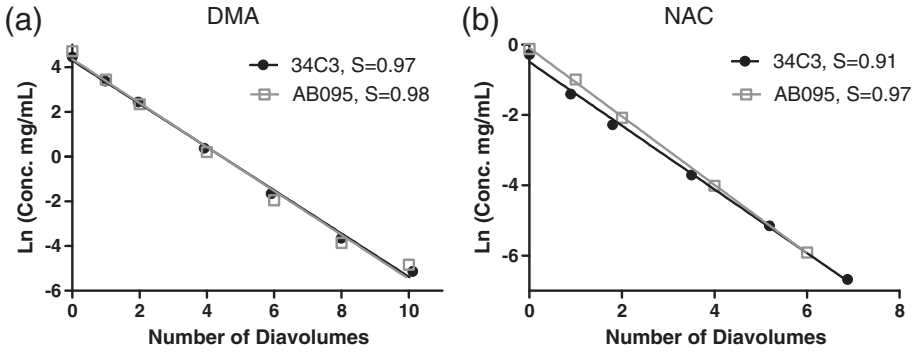

FIGURE 3 The UF/DF clearance trend and sieving factor of the retentate solution for DMA and NAC in 34C3 and AB095 mAb solutions; each line represents the linear regression result and the R^2 values are all equal to or greater than .99. DMA, dimethylacetamide; NAC, *N*-acetyl-L-cysteine; UF/DF, ultrafiltration and diafiltration

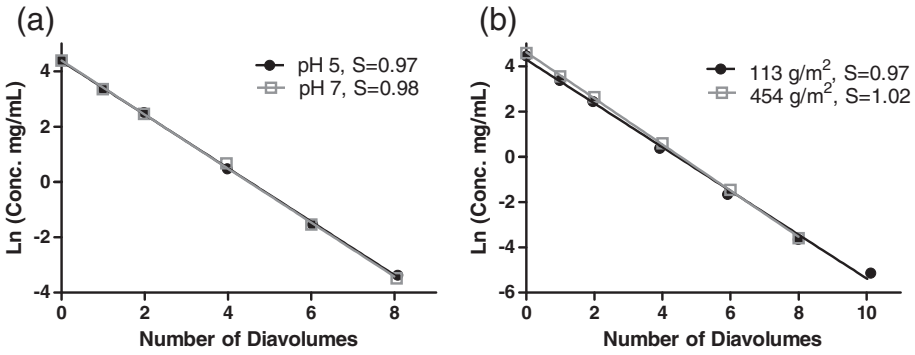

FIGURE 4 The UF/DF clearance trend and sieving factor of retentate solution for DMA in 34C3 mAb solution with different pHs (a) or mAb loadings (b); each line represents the linear regression result and the R^2 values are all greater than .99. DMA, dimethylacetamide; UF/DF, ultrafiltration and diafiltration

TABLE 3 Effect of different UF/DF operational conditions on the clearance rate of DMA

	Temperature (°C)	TMP (psi)	Feed flux (L m^{-2} hr^{-1})	Permeate flux at first DV (L m^{-2} hr^{-1})	Conversion ratio at first DV (%)	Sieving factor (retentate)	Sieving factor (permeate)
Nominal	21	18	300	68	23	0.97	0.96
Cond. A	12	30	186	46	25	0.95	0.93
Cond. B	12	12	420	59	14	0.95	0.94
Cond. C	28	12	186	56	30	0.98	0.96
Cond. D	28	28	420	97	23	0.99	0.97

Abbreviations: DV, diavolume; TMP, transmembrane pressure; UF/DF, ultrafiltration and diafiltration.

layer. The thickness of this layer could depend on diffusivity of the solute, permeate flux, and the hydrodynamic shear tangential to the membrane.[15] Temperature has an influence on the viscosity of the solution and diffusivity of the solute. The cross flow rate directly impacts the shear force on the membrane surface. The protein concentration at the membrane surface increases with TMP until it reaches the gelation concentration. As a result, all of these factors, temperature, TMP, and cross flow, could have an effect on gel layer formation and membrane performance. Four conditions (A–D) listed in Table 3 with either higher or lower temperature, TMP, and/or cross flow rate than the nominal conditions were studied for DMA clearance in 34C3 mAb solution. The parameter ranges were chosen as high or low end toward the nominal condition, in order to show the tolerance of operational variables during UF/DF process. The permeate flux at the first DV for each condition is listed in Table 3 to indicate the differences. The permeate flux typically increases in subsequent DVs due to the change in buffer composition by buffer exchange. Condition D showed higher permeate flux rate than the nominal condition, while all the other three conditions showed lower permeate flux rate than the nominal condition. In terms of the conversion ratio in Table 3, defined as the ratio of permeate flux to the feed flux, condition A and D showed similar conversion ratio as the nominal condition, while condition B and C showed lower and higher conversion ratio than the nominal condition, respectively. The conversion ratio here reflected the impact of both TMP and temperature. The processing time and the impact on mAb and ADC properties are other factors to consider for the UF/DF unit operation, and are beyond the scope of this study. As shown in Figure 5, despite very different processing conditions used for these four experiments, the DMA clearance trends aligned well. The sieving factors calculated from both the retentate solution and permeate solution were all very close to those obtained from the nominal condition, which were close to ideal clearance (Table 3). Therefore, the operational range

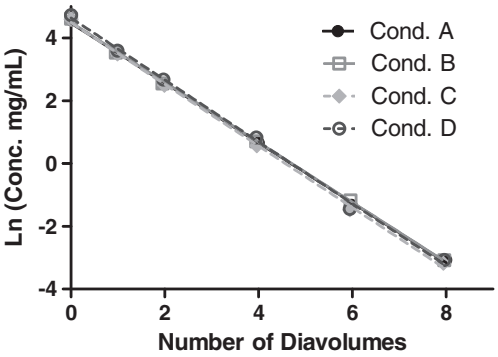

FIGURE 5 The UF/DF clearance trend of the retentate solution for DMA in 34C3 mAb solution under different operational conditions; each line represents the linear regression result and the R^2 values are all greater than .99. DMA, dimethylacetamide; UF/DF, ultrafiltration and diafiltration

for temperature at 12–28°C, TMP at 12–30 psi, and cross flow rate at 186–420 L m^{-2} hr^{-1} had no impact on the clearance rate of DMA.

These results demonstrate that the operational variables during UF/DF, including the pH of DF buffer, the membrane loading, temperature, TMP, and cross flow rate, were all well tolerated and had minimal impact on DMA clearance. Since all the other solutes of interest evaluated in the study, that is, DMSO, EDTA, TCEP=O, and NAC, had similar behavior to DMA at the nominal condition, it is plausible that the operational variables have minimal impact on clearance of these molecules as well.

4 | CONCLUSION

This study showed conditions when solvents and small molecule impurities from a typical ADC process (Table 1) were spiked at relevant concentrations into mAb solutions to provide a simulated post-conjugation reaction mixture. From this study, all small molecule impurities were cleared efficiently by UF/DF, specifically the DF step. The rates of clearance for the species were characterized by sieving factor, which were all close to the ideal clearance (S = 0.9–1). Based on the ICH guideline, the permissible daily exposure of DMA and DMSO are 10.9 and 50 mg/day, or 1,090 ppm and 5,000 ppm relative to the pharmaceutical products based on 10 g/day dosage, respectively. In our study, without taking into account of further ultrafiltration step, DMA and DMSO were cleared more than 2,000 times at 8 DV of DF and reached below the conservative 1,090 ppm after 9 DV, and 5,000 ppm after 8 DV, respectively. As for the other three small molecules, the ICH guidance is to limit to 0.15% wt/wt relative to the ADCs if dosed ≤2 g/day, or 0.05% wt/wt relative to the ADCs if dosed >2 g/day. In our study, the impurities were cleared below 0.05% wt/wt after 4 DV of DF for TCEP=O and after 5 DV of DF for NAC and EDTA. Their levels should all be well controlled below the acceptable level in a UF/DF process that typically employs 7–10 DVs of DF. For a specific program, the acceptable levels of these impurities would further depend on the actual dose, duration, and indication of the ADC. The findings from this study can serve as a foundation to assess the risk that an impurity poses to the quality of the ADC on a case-by-case basis. The levels would likely be further reduced if a chromatography purification step is involved in the ADC process, which would reduce the amount of solvents and small molecule impurities prior to the UF/DF operation. Additionally, this study evaluated the impact of process variables during DF, such as pH, temperature, membrane loading, TMP and cross flow rate, and demonstrated minimal impact of these variables on the clearance rate. These results demonstrate efficient and robust clearance of solvents and small molecule impurities that are unique to ADC process by the DF operation, and provide a general data package to facilitate risk assessments of controlling their levels.

ACKNOWLEDGMENTS

We thank Anthony Haight, Dennie Welch, Calvin Becker, Steve Richter, and Robert Leanna from AbbVie for helpful discussions and manuscript reviewing.

DISCLOSURE OF INTERESTS

All authors are AbbVie employees or summer worker, and may own AbbVie stock. AbbVie sponsored and funded the study; contributed to the design; participated in the collection, analysis, and interpretation of data, and in writing, reviewing, and approval of the final publication.

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How to cite this article: Gates TJ, Lyu YF, Fang X, Liao X. Clearance of solvents and small molecule impurities in antibody drug conjugates via ultrafiltration and diafiltration operation. *Biotechnol Progress*. 2019;e2923. https://doi.org/10.1002/btpr.2923