Comparative Study on Elution of Polyvinylpyrrolidone on Dialyzers Using Ultraviolet Analysis and Iodine Method

Claudia Woiterski, Sandra Jäger, and Stefan Dröschel

Abstract: It is known that poly(arylethersulfone)-based dialyzers can elute poly(*N*-vinyl-2-pyrrolidone) (PVP). With regard to chronic renal replacement therapy, this is a burden for the patient, because PVP is deposited in different organs and cannot be degraded or released from there; so elutable PVP has to be minimized. Usually, the iodine method is used for quantification of extractable PVP. To overcome the chain length dependency of this method, we used an ultraviolet method that is independent from the PVP chain lengths; so the absolute amount of eluted PVP can be quantified. The current study shows the amount of eluted PVP on differently sterilized low flux dialyzers (1.6 m², similar storage time, n = 12)—PS160 (Allmed, Egypt), F7HPS (Fresenius Medical Care, Germany), F16 (Wego, China), and B-16P (Bain, China). Using the ultraviolet method, the irradiated filters show a sum total of approximately 9 mg more eluted PVP compared with the steam-sterilized ones, whereas the iodine method shows a value about three times lower between different types of sterilization. The boundary conditions during the radiation sterilization could lead to PVP degradation instead of cross-linking. The resulting shorter PVP chains can be more easily rinsed out and can falsely decrease the calculated eluted PVP amount by using the iodine complexation method. *ASAIO Journal* XXX; XX;00–00

Key Words: hemodialysis, polyarylethersulfone membrane dialyzer, polyvinylpyrrolidone, UV spectrum, iodine method

Various poly(arylethersulfone)-based dialyzer membranes elute poly(*N*-vinyl-2-pyrrolidone) (PVP), which is responsible for pore size building during membrane manufacturing as well as improvement of the hydrophilicity of these kind of membranes.[1,2] Because PVP accumulation in different organs of the patients leads to organ injury or failures,[1,3] the elution of PVP out of a dialyzer has to be minimized. This led to numerous publications dealing with this topic.[1,3–9]

Different studies described dependence of PVP elution on methods of sterilization,[1,10,11] aging[3] as well as other conditions.[11] Others focused on investigating the biocompatibility of dialyzers with respect to sterilization[11,12] and further parameters.[2,13] The potential influence of PVP on synthetic dialysis membranes in relation to observed patient reactions during dialysis treatment is also discussed in the literature.[3,14,15]

It is known that environmental conditions for the dialyzer such as oxidative degradation as well as sterilization conditions can change PVP chain lengths.[3,10] Cross-linking can decrease the mobility of PVP,[5,16,17] whereas degradation can increase PVP elution.[10]

In most cases, a potassium triiodide reagent[18,19]—sometimes called the Müller method—is used to quantify the amount of eluted PVP by PVP-iodine-complex formation in dialyzer elution studies.[1,3,4] In these investigations, different commercially available dialyzers were investigated. Comparatively small amounts of leached PVP were found. High variations were obtained between PVP K30 and K90 detection using the Müller method (0.006 N iodine solution)[3] as well as differences between NMR determinations and the iodine method were discussed.[10]

Another method that does not require formation of a PVP-iodine complex to detect PVP eluted from polysulfone-based dialyzers has been previously published. This ultraviolet (UV) method can be used for online (while priming and recirculating) PVP elution experiments easily.[20]

In poly(arylethersulfone)-dialyzer membrane manufacturing PVP K85 or K90 are the most common components. The sterilization carried out during dialyzer production as well as other conditions can change the PVP chains.[10] For the aforementioned reasons, PVP with different chain lengths were investigated first in a concentration-dependent manner by UV method and with iodine method. We used PVP K17—K90, which are available on the market for different applications.[17,21,22]

Subsequently, four different types of commercially available low flux dialyzer filters of the same surface area and similar storage time were rinsed by reverse osmosis (RO) water using a similar experimental setup and conditions as others.[1,4] The obtained elutes were examined by UV and iodine method. Based on the Beer-Lambert's law and the extinction coefficients found previously, absolute amounts of eluted PVP can be calculated.

The aim of this study is the comparative quantification of PVP from dialyzer eluates by the UV method and the iodine method. Limitations in the interpretation of the results are discussed.

Materials and Methods

UV Method—Comparison of Different PVP Chain Lengths

We started with the investigation of different concentrations (15–150 ppm) of aqueous solutions of different types of

From the Allmed Dialysis Technologies GmbH, Pulsnitz, Germany.

Submitted for consideration September 2021; accepted for publication in revised form March 2022.

Disclosure: All authors are from the Allmed Dialysis Technologies GmbH, Pulsnitz, Germany. Allmed Dialysis Technologies GmbH is a company of the Allmed Group, United Kingdom, as are the dialyzer manufacturers Allmed Medical GmbH, Germany, and Allmed Middle East, Egypt.

Correspondence: Claudia Woiterski, Allmed Dialysis Technologies GmbH, Mittelbacher Str. 18, 01896 Pulsnitz, Germany. Email: Claudia.Woiterski@allmedgroup.com.

DOI: 10.1097/MAT.0000000000001751

PVP (CAS No. 9003-39-8, BASF Luvitec powder [K17 (M_w/M_n = 9/2 kDa), K30 (50/14 kDa), K85 (1,100/250 kDa), K90 (1,400/325 kDa)],[23] and older Luvitec PVP K85 degraded to K70). All K values,[17,23] corresponding with molecular weight or chain length,[23,24] were checked and confirmed by viscosity determinations before further investigations. All samples were measured by UV/Vis spectroscopy using a Biochrom UV/Vis Spectrophotometer LIBRA S22 (United Kingdom).

The examination of the solutions was carried out in the wavelength range between 190 and 250 nm. Absorbance depending on wavelength for PVP of different molecular weight showed the same values for the same concentrations (see Figure 1). Test for linear regression is determined to be $R^2 \geq 0.999$ using a wavelength approximately 220 nm. PVP can be quantified in a concentration range between 0.0015 and 0.015 m% independent from PVP chain length. An average extinction coefficient of 60.25 ± 1.02 $m\%^{-1}cm^{-1}$ can be used for PVP quantification at 220 nm (0.0015–0.015 m%, see Figure 2).

Smaller amounts of PVP in aqueous solutions, as expected in dialyzer elution tests, can also be detected by UV. They can be measured better at 210 nm because of the higher sensitivity (higher extinction coefficient) and peak shift. Linear regression was checked as well for K30 and K85 (see Figure 3).

Iodine Method—Comparison of Different PVP Chain Lengths

The same concentrations of aqueous solutions of different types of PVP as mentioned above (0.0015–0.015 m%) were analyzed by iodine method. Thus, 2.5 ml of 0.2 M citric acid and 1 ml of 0.006 M iodine solution (0.8 g/L iodine + 1.44 g/L potassium iodide) were added to 5 ml of the PVP solution sample, well mixed and measured after 10 minutes.[18] The results between 300 and 700 nm for the lowest (0.0015 m%) and highest concentration (0.015 m%) can be seen in Figure 4. The rather complex iodine UV/Vis spectra are described in detail elsewhere.[25]

Results showed smaller linear ranges (0.0015–0.009 m%, $R^2 \geq 0.99$) compared with the UV method. The adjustment of the concentration range is necessary because of the fact that the extinction coefficient can only be determined if the test is positive. For K17, the usable concentration range is even smaller compared with the other K30-K90.

Calculation of extinction coefficients lead to distinct extinction coefficients for certain PVP chain lengths as expected.[18,19] For shorter PVP chains as K17 or even K30, a smaller extinction coefficient was obtained compared with larger PVP as K90 between approximately 400 and 550 nm for concentrations between 0.0015 and 0.009 m%. That means, that the same concentration of PVP leads to lower absorbance values by the iodine method for K17 or K30 compared with larger PVP as K85 or K90.

The influence of this result on discussion on elution studies of dialyzers is discussed below.

Figure 5 shows standard curves at 422 nm of different types of PVP (0.0015–0.009 m%) for the used iodine method. Quantification of PVP is rather inaccurate for very low concentrations of <0.0002 m% (see Figure 6).

So the following investigation of dialyzer eluates were analyzed using aforementioned UV method compared with iodine method taking into account the limit of quantification.

Elution of Filters—Dialyzers and Experimental Setup

Because aging in dialyzers increases the amount of elutable PVP,[3,6] filters (see Table 1) of a similar storage time (average 6 months) were used for the study shown here—PS160 (Allmed Middle East S.A.E. [Egypt]), F7HPS (Fresenius Medical Care AG & Co. KGaA [Germany]), F16 (Wego Blood Purification products Co., Ltd. [China]), and B-16P (Bain Medical Equipment Co., Ltd. [China]). All low flux filters had the same surface area (1.6 m^2), allowing comparability.

Twelve filters of each type were primed on the blood compartment with RO water first (37°C, 200 ml/min, 5 min, single pass—priming) and then rinsed in a closed loop with RO water for 4 hours (37°C, 675 ml, 200 ml/min, recirculation). The dialysate compartment was closed.

Results

The absorbance values of the obtained eluates from blood compartment are measured by UV and iodine method. The blank value for the tubing system has already been subtracted from the absorbance values. The corresponding calculated amounts of eluted PVP using the UV (at 210 nm) and the iodine method (at 422 nm) are shown in Figure 7.

Both the UV method and the evaluation according to the iodine method show that only very small amounts of PVP were detected in eluates from the steam-sterilized products PS160 and F7HPS (below limit of quantification), whereas the two irradiated filters F16 and B-16P eluted significantly more PVP per filter of the same surface area (Mann-Whitney *U* test, $\alpha = 0.05$). According to the UV method, the irradiated filters showed approximately two times as high values for the recirculation compared with the single pass.

A different picture emerged for single pass and after 4 hours recirculation, when the eluted PVP amount is interpreted using the iodine method for evaluation. The measured values for PS160 and F7HPS are below the quantification limit, whereas the dialyzers F16 and B-16P elute considerably higher amounts of PVP in 4 hours recirculation. Absolute eluted PVP contents of irradiated filters appear lower for the iodine method investigations compared with the UV analyzed ones.

Discussion

The previously described results of the analysis of certain PVP compounds K17–K90 show that the UV method is more suitable for PVP quantification because it is independent of the PVP chain length. The iodine method, on the other hand, can only lead to the same results if the length of the PVP chains is known or has been determined beforehand (*e.g.,* by gel permeation chromatography). In the case of a wide molecular weight distribution or changing of the PVP chain length, which can occur by aging or processing of PVP-containing products (*e.g.,* by sterilization), the iodine method is very limited because of the lack of a specific extinction coefficient.

The design of experiment included dialyzers of different kinds of sterilization but the same size (1.6 m^2), flux and similar storage time because an age-dependence of PVP elution because of oxidative degradation of PVP. The choice of sterilization method and sterilization conditions can significantly

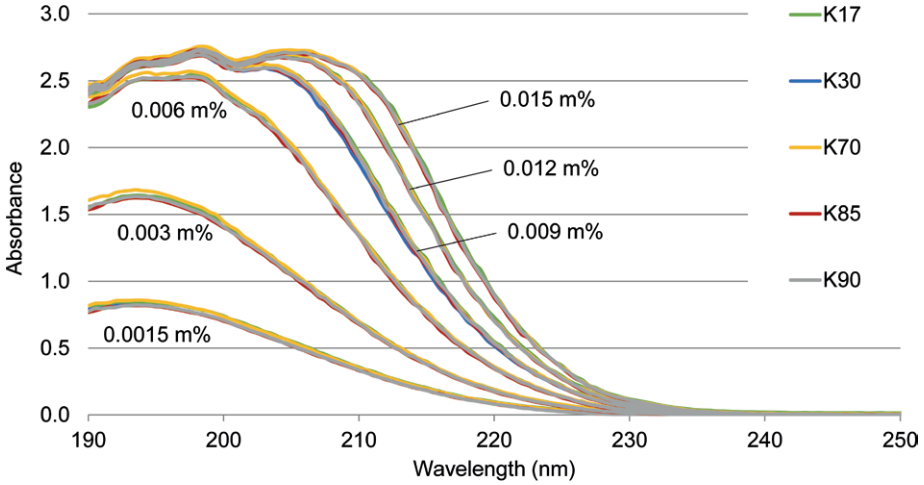

Figure 1. Absorbance of aqueous PVP solutions of different types of PVP (K17, K30, K70, K85, K90) depending on concentration (0.0015–0.015 m%) and wavelength—UV method (PVP chain length independent). PVP, poly(*N*-vinyl-2-pyrrolidone).

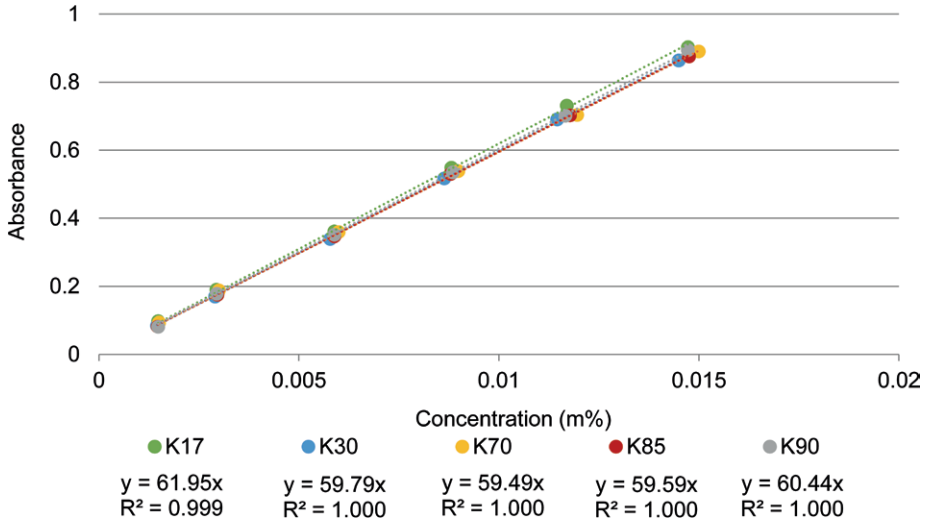

Figure 2. Absorbance of aqueous PVP solutions of different types of PVP (K17, K30, K70, K85, K90) depending on concentration (0.0015–0.015 m%) at 220 nm—UV method. PVP, poly(*N*-vinyl-2-pyrrolidone).

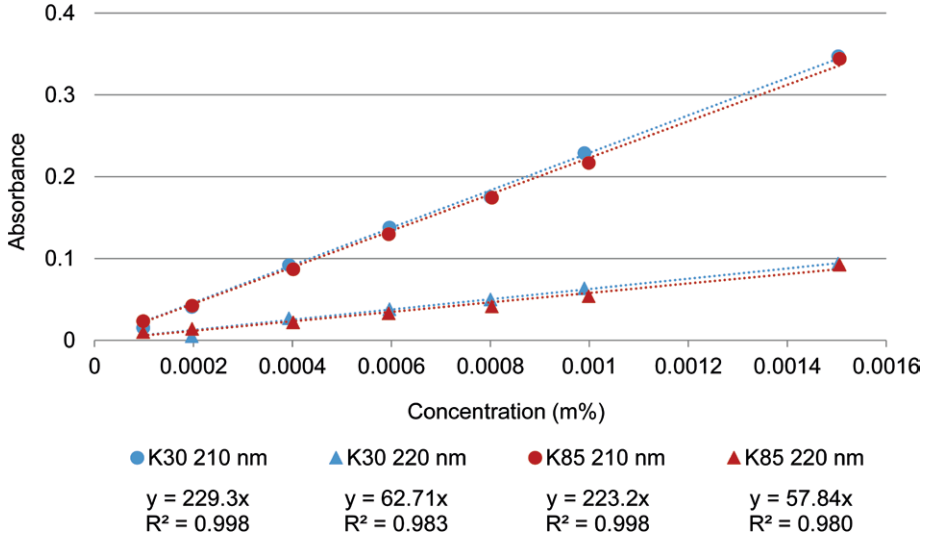

Figure 3. Absorbance of K30 and K85 solutions depending on concentrations (0.0001–0.0015 m%) at 210 and 220 nm—UV method.

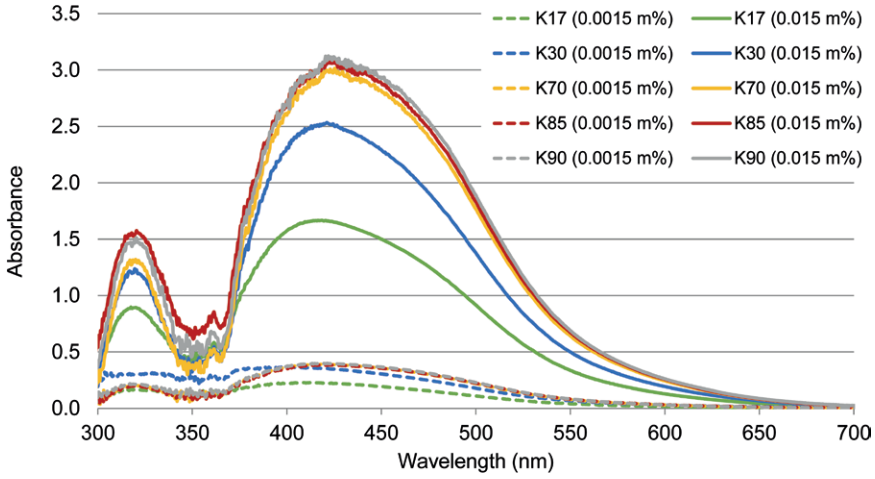

Figure 4. Absorbance of aqueous PVP solutions of different types of PVP (K17, K30, K70, K85, K90) with iodine depending on concentration (0.0015 and 0.015 m%) and wavelength—iodine method (PVP chain length dependent). PVP, poly(*N*-vinyl-2-pyrrolidone).

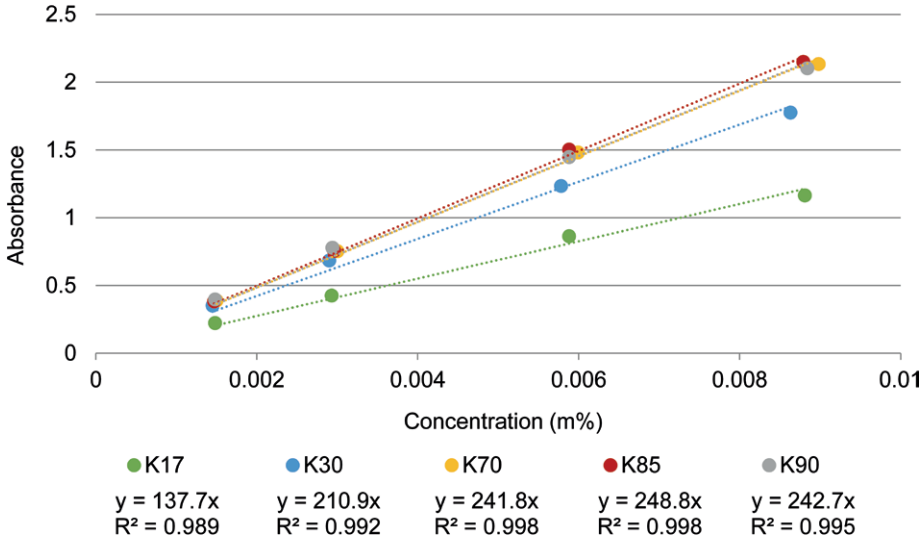

Figure 5. Absorbance of aqueous PVP solutions of different types of PVP (K17, K30, K70, K85, K90) depending on concentration (0.0015–0.09 m%) at 422 nm—iodine method. PVP, poly(*N*-vinyl-2-pyrrolidone).

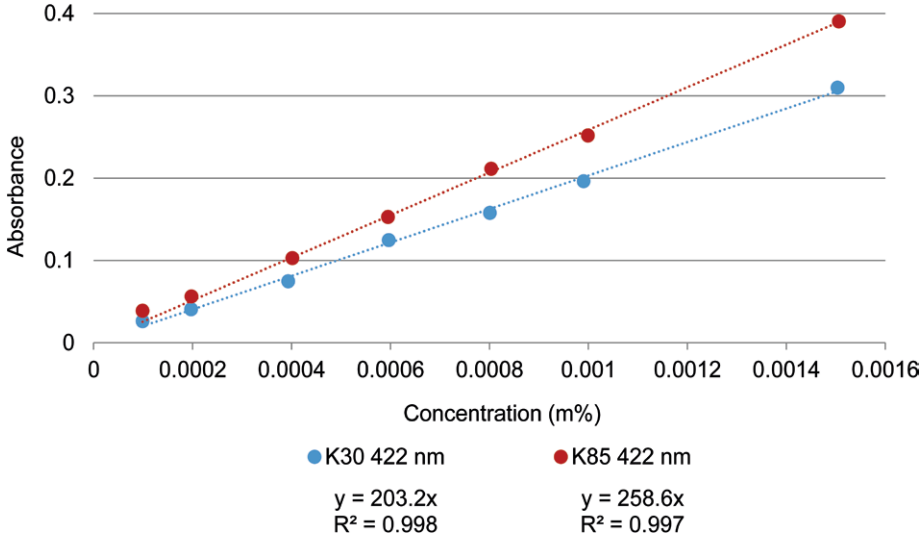

Figure 6. Absorbance of K30 and K85 solutions depending on concentrations (0.0001–0.0015 m%) at 422 nm—iodine method.

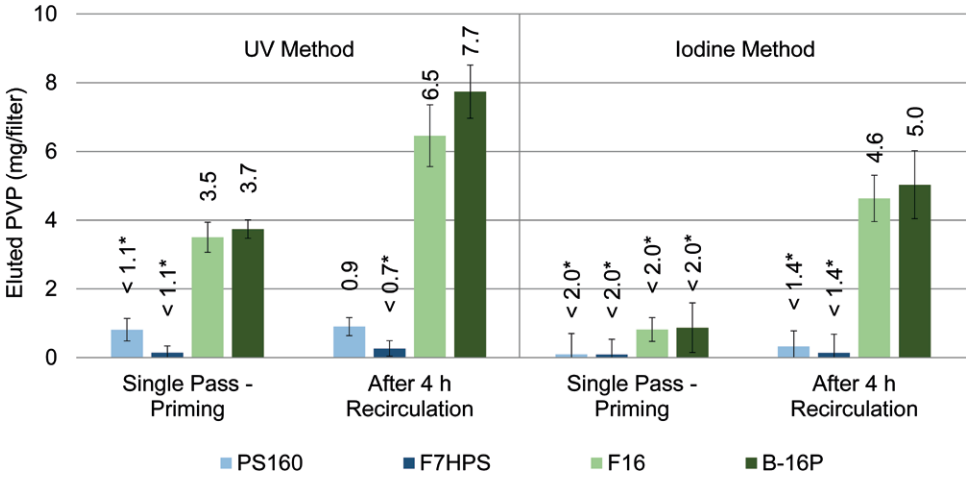

Figure 7. Eluted PVP (averages and standard deviations) from blood compartment of PS160, F7HPS, F16, and B-16P (n = 12)—calculated at absorbances at 210 nm (UV method) and 422 nm (iodine method) for single pass and after 4 hours recirculation. *Quantification limits of 1.1/2.0 mg/filter for the single pass and 0.7/1.4 mg/filter for the recirculation (UV/iodine method) result because of the limits of quantification of the analytical methods (calibration curve 1–15 ppm, K30/K85, UV method 1.1/1.1 ppm [α, β = 0.05, k = 3], iodine method 2.1/2.0 ppm [α, β = 0.05, k = 3]) and the eluate volumes. PVP, poly(*N*-vinyl-2-pyrrolidone).

Table 1. Technical Data of Investigated Dialyzers (Per Dialyzers Group, n = 12)

Dialyzer	Flux	Surface Area (m^2)	Membrane Material	Sterilization
PS160	Low flux	1.6	PSu	Steam
F7HPS			PSu	Steam
F16			PSu	Irradiation
B-16P			PES	Irradiation

PES, polyethersulfone; PSu, polysulfone.

affect PVP chain length.[1,10] In our study, by totaling up the single pass—priming and after 4 hours recirculation results, the radiation-sterilized dialyzers show a multiple compared with the steam-sterilized ones by the UV method—10.0 and 11.4 mg/filter eluted PVP (F16, B-16P) compared with <2 mg/filter (PS160, F7HPS). The iodine method shows significantly lower amounts of eluted PVP especially for the investigated irradiated filters (Wilcoxon signed-rank test, α = 0.05), possibly because the shorter PVP chains degraded by the irradiation show a lower absorbance (see above). These shorter chains are easier to mobilize and thus also to wash out first during priming. Thus, this leads to significantly lower absorbance values using the iodine method and the eluted amount of PVP during priming appears disproportionately lower compared with the recirculation compared with the UV method.

Steam sterilization or autoclaving can result in a hydrophilic, well-biocompatible dialyzer membrane.[2,13] Gamma irradiation can lead to cross-linking of PVP[16,23] and main polymer as polysulfone,[2,5] which effectively reduce PVP elution from dialyzer membranes[1] or can destruct PVP chains.[10] The influence of the sterilization on PVP amounts eluted from dialyzers investigated here cannot be discussed further, because nonsterile references as well as details of membrane composition (PSu/PVP, PES/PVP) and sterilization conditions are not accessible.

In general quantification of PVP by UV spectroscopy is limited,[26] not only because of the detection limit and quantification limit but also because other extractable substances may increase the absorbance values in this wavelength range and thus can falsely increase the calculated eluted PVP amount. However, small water-soluble molecules such as residual solvent from the fiber-spinning process, which can be such a disturbance variable, should no longer be present after the priming procedure.

Furthermore, it has been published that similar amounts of PVP are eluted with RO water as with saline during priming and recirculation on the blood side.[1] Thus, the recirculation (4 hours, 37°C) modeled on a patient treatment in terms of duration and temperature can be used well for absolute comparisons regarding PVP exposure between individual filter types.

Conclusion

Both the UV method and the iodine method are established methods for quantifying the amount of PVP eluted from dialyzers. Our results show the interpretation of the eluted amounts in other publications must also take into account the method used—UV method as a PVP chain length independent method and iodine method as a PVP chain length dependent method. The iodine method may result in detection of a falsely low amount of eluted PVP if the PVP is degraded compared with the commonly used reference K90.

The dialysis filters studied here show that a steam-sterilized filter such as PS160 or F7HPS can release significantly less PVP than the radiation-sterilized products F16 and B-16P. Although steam sterilization results in a well-biocompatible membrane,[2,13] irradiation under certain sterilization conditions can destroy the PVP chains,[10] which are thus more easily washed out.

References

1. Namekawa K, Matsuda M, Fukuda M, Kaneko A, Sakai K: Poly(*N*-vinyl-2-pyrrolidone) elution from polysulfone dialysis membranes by varying solvent and wall shear stress. *J Artif Organs* 15: 185–192, 2012.
2. Hayama M, Yamamoto K, Kohori F, Sakai K: How polysulfone dialysis membranes containing polyvinylpyrrolidone achieve excellent biocompatibility? *J Membrane Sci* 234: 41–49, 2004.
3. Namekawa K, Kaneko A, Sakai K, Kunikata S, Matsuda M: Longer storage of dialyzers increases elution of

poly(*N*-vinyl-2-pyrrolidone) from polysulfone-group dialysis membranes. *J Artif Organs* 14: 52–57, 2011.
4. Matsuda M, Sato M, Sakata H, *et al*: Effects of fluid flow on elution of hydrophilic modifier from dialysis membrane surfaces. *J Artif Organs* 11: 148–155, 2008.
5. Matsuda M, Yakushiji T, Sakai K: Effect of γ irradiation on surface properties of wet polysulfone films containing poly(vinylpyrrolidone). *Surf Interface Anal* 43: 976–983, 2011.
6. Miyata M, Konishi S, Shimamoto Y, Kamada A, Umimoto K: Influence of sterilization and storage period on elution of polyvinylpyrrolidone from wet-type polysulfone membrane dialyzers. *ASAIO J* 61: 468–473, 2015.
7. Konishi S, Fukunaga A, Yamashita H, Miyata M, Usami M: Eluted substances from hemodialysis membranes elicit positive skin prick tests in bioincompatible patients. *Artif Organs* 39: 343–351, 2015.
8. Wenten IG, Aryanti PTP, Hakim AN, Himma NF: Advances in polysulfone-based membranes for hemodialysis. *J Membr Sci Res* 2: 78–89, 2016.
9. ter Beek OEM, Pavlenko D, Stamatialis D: Hollow fibre membranes for long-term hemodialysis based on polyethersulfone SlipSkin polymer blends. *J Membrane Sci* 604: 1–11, 2020.
10. Murakami J, Kaneko I, Kimata N, Mineshima M, Akiba T: Problems in the evaluation of polyvinylpyrrolidone (PVP) elution from polysulfone membrane dialyzers sterilized by gamma-ray irradiation. *Ren Replace Ther* 2: 36, 2016.
11. Madsen B, Britt DW, Griffiths F, McKenna E, Ho C: Effect of sterilization techniques on the physicochemical properties of polysulfone hollow fibres. *J Appl Polym Sci* 119: 3429–3436, 2011.
12. Togo K, Yamamoto M, Ono T, *et al*: Comparison of biocompatibility in polysulfone dialysis membranes with different sterilization. *Hemodial Int* 22(S2): S10–S14, 2018.
13. Erlenkötter A, Endres P, Nederlof B, Hornig C, Vienken J: Score model for the evaluation of dialysis membrane hemocompatibility. *Artif Organs* 32: 962–969, 2008.
14. Álvarez-de Lara MA, Martín-Malo A: Hypersensitivity reactions to synthetic haemodialysis membranes - an emerging issue? *Nefrologia* 34: 698–702, 2014.
15. Martin-Navarro J, Esteras R, Castillo E, *et al*: Reactions to synthetic membranes dialyzers: Is there an increase in incidence? *Kidney Blood Press Res* 44: 907–914, 2019.
16. Jeong J, Park J, Kim Y, *et al*: Gamma ray-induced polymerization and cross-linking for optimization of PPy/PVP hydrogel as biomaterial. *Polymers (Basel)* 12: 111, 2020.
17. Reintjes T (ed): *Solubility Enhancement with BASF Pharma Polymers—Solubilizer Compendium*. Lampertheim, Germany, BASF SE Pharma Ingredients & Services, 2011.
18. Levy GB, Fergus D: Microdetermination of polyvinylpyrrolidone in aqueous solution and in body fluids. *Anal Chem* 25: 1408–1410, 1953.
19. Müller K: Nachweis und Bestimmung von Polyvinylpyrrolidon (PVP) sowie Bestimmung von Wirkstoffen in PVP-haltigen Arzneimittelzubereitungen. *Pharm Acta Helv* 43: 107–122, 1968.
20. Shimamoto Y, Matsushita S, Yamamoto T, *et al*: Real-time measurement of polyvinylpyrrolidone eluted from polysulfone membrane dialyzers based on the ultraviolet spectrum. *ASAIO J* 65: 522–526, 2019.
21. Teodorescu M, Bercea M: Poly(vinylpyrrolidone)—A versatile polymer for biomedical and beyond medical applications. *Polymer Plast Technol Eng* 54: 923–943, 2015.
22. Kurakala M, Koteswara Rao GSN: Pharmaceutical assessment of polyvinylpyrrolidone (PVP): As excipient from conventional to controlled delivery systems with a spotlight on COVID-19 inhibition. *J Drug Deliv Sci Tec* 60: 1–24, 2020.
23. *PVP and more ...LUVITEC®, LUVICROSS® and COLLACRAL®VAL Versatile specialty polymers for technical applications*. Ludwigshafen, Germany, BASF SE Operating Division Dispersions and Pigments, 2009.
24. Haaf F, Sanner A, Straub F: Polymers of *N*-vinylpyrrolidone: Synthesis, characterization and uses. *Polym J* 17: 143–152, 1985.
25. Makhayeva DN, Irmukhametova GS, Khutoryanskiy VV: Polymeric iodophors: Preparation, properties, and biomedical applications. *Rev J Chem* 10: 40–57, 2020.
26. Pedersen G, Kristensen HG: Quantitative analysis of povidone (PVP) in drug-PVP matrix using multicomponent analysis. *Drug Dev Ind Pharm* 25: 69–74, 1999.