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Short communication

Determination of total leachable bisphenol A from polysulfone membranes based on multiple consecutive extractions

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ABSTRACT

The paper presents the development of a multiple consecutive extraction method enabling the determination of the total amount of bisphenol A (BPA) released from porous polysulfone (PS) membranes in a hemodialyzer or hemoconcentrator under simulated-use conditions. The levels of BPA were determined using solid phase extraction (SPE) coupled with high performance liquid chromatography–mass spectrometry (HPLC–MS). We demonstrated that it was difficult to determine the total amount of BPA released from the PS membranes using a single extraction method with finite solvent volume because of the chemical equilibrium between the extraction solution and the polymer phase. Repeated extractions gave more accurate results than a single extraction for the determination of the total amount of leachables in porous membranes. A general equation was derived to fit the elution profile of BPA released during multiple consecutive extractions.

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1. Introduction

Leachables can be described as trace amounts of chemicals that are released into contacting media from a component/product such as a medical device, container, or process equipment under normal conditions [1,2]. Certain leachables have been of concern to the scientific, industrial, and/or public communities. They may interfere with an experimental system [3,4]. Others may be unintentionally introduced to consumer products through foods, drinks, and drug containers [1,2].

Bisphenol A (BPA) is a high-production-volume chemical widely used to manufacture polysulfone (PS), polycarbonate, epoxy resin, etc [5–8]. Over the past ten years, BPA has been the subject of numerous risk assessment reviews and research worldwide because of its potential to produce adverse health effects through endocrine disruption [5–7]. Although there is a significant body of literature focused on the adverse effects of BPA at low doses, there are discrepancies in the relevance and reliability of the published results [5–7]. These make it difficult to properly evaluate the hazards of BPA. To reduce discrepancies and variation in research results, it is essential to establish reproducible/accurate analytical methods.

Porous PS membranes are widely used to purify or concentrate fluids in medical devices such as hemofiltration and hemodialysis or scientific apparatus for ultrafiltration [9,10]. Because of their high surface area and low density, they have the tendency to readily release leachable contaminants. For example, we recently showed that a PS ultrafilter used for type I water purification system releases BPA that contaminates product ultrapure water with up to 0.70 ± 0.06 ng/mL during normal use [11]. Haishima et al. also reported that PS hemodialyzers released BPA during single circulation of 17.2% ethanol solution or bovine serum at wide range from 0.14 to 2.1 μg per device [12]. Thus, the determination of the total amount of leachable BPA from PS membranes is important for risk assessors to evaluate BPA exposure and determine its interference during clinical use of medical devices. Currently, a reliable extraction method that accurately analyzes total BPA leachables released from porous PS used in medical devices has not been reported.

This paper presents the development of a new extraction method that accurately determines the total amount of BPA leachables in a bundle of PS hollow fiber membranes used in hemodialyzers and hemoconcentrators. We used solid phase extraction (SPE) coupled with high performance liquid chromatography–mass spectrometry (HPLC–MS) to concentrate and analyze BPA released from PS hollow fiber membranes. We investigated the effect of the extraction solution volume, number of extractions, and extraction time to understand the leaching phenomenon of hollow fiber membranes. The extraction profile

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was analyzed using a general equation derived for multiple consecutive partitions of leachables between extraction solution and the polymer phase.

2. Experimental section

2.1. Chemicals and materials

PS hemodialyzers and hemoconcentrators were obtained from two different companies (not disclosed). According to the manufacturer's specifications, the surface area of the hemodialyzers was 0.4 or 0.7 m^2 while that of the hemoconcentrators was 0.5 m^2. HPLC-grade water, ethanol, and acetonitrile were purchased from Fisher Scientific (Raleigh, NC). BPA and $^{13}C_{12}$-BPA standards were obtained from Sigma-Aldrich (St. Louis, MO) and Cambridge Isotope Laboratories (Andover, MA), respectively.

2.2. Sample extraction and preparation

To extract leachables from hollow fiber membranes, the extraction solution was circulated through a hemodialyzer or hemoconcentrator at 37 °C at flow rate of 200 mL/min for 1 h, unless specified otherwise. We used 17.2% (v/v) solution of ethanol in water as an extraction solution. The volume of the extraction solution was set at 0.25, 1, 2, or 4 L depending on experimental needs. After extraction, 10 mL of the extraction solution was taken for further analysis.

2.3. SPE-based enrichment and LC–MS analysis

Extracted samples were directly analyzed without chemical derivatization using an online SPE coupled with HPLC–MS (Alliance 2695-ZQ4000; Waters, Milford, MA). To compensate for run-to-run variations in instrumental performance, $^{13}C_{12}$-BPA was introduced as an internal standard at fixed concentration of 2.67 ng/mL prior to the LC–MS experiments. A large volume of sample (1500 μL) was injected and enriched by SPE using a Waters symmetry shield C_{18} column (3.9-mm width × 20-mm length, 3.5-μm particle size). BPA and $^{13}C_{12}$-BPA retained in the C_{18} SPE column were eluted with acetonitrile and directly introduced into a HPLC column for further analysis.

The separation of BPA was achieved using a Waters Xterra MS C_{18} column (3.0 × 150 mm, 3.5 μm) at a flow rate of 250 μL/min with a linear gradient of 80 to 95% of acetonitrile (solvent A) relative to HPLC-grade water (solvent B). The column was re-equilibrated at least 10 min before each analysis. BPA and $^{13}C_{12}$-BPA were ionized by negative ion electrospray ionization. The deprotonated BPA and $^{13}C_{12}$-BPA were measured in a selected ion monitoring mode at m/z 227 and 241, respectively.

3. Results and discussion

3.1. Effect of number of extractions and solution volume

Haishima et al. showed that BPA release from a PS hemodialyzer reached a maximum amount at 2 to 4 h after circulation started with 250 mL extraction solution [12]. They also showed that 17.2% ethanol/water has similar extraction strength to bovine serum in extracting BPA from hemodialyzers [12]. Thus, the authors recommended 17.2% ethanol/water mixture as an extraction solution to mimic physiological extraction conditions and avoid further sampling processes like the purification of BPA from serum. To determine the total amount of BPA leachables from PS hollow fibers in hemodialyzers or hemoconcentrators, we adopted Haishima et al.'s extraction conditions: 250 mL of 17.2% ethanol solution for 6 h circulation.

To ensure that BPA was completely released from hemodialyzer membranes, we performed a second extraction on the membranes which had been extracted only once. Unexpectedly, however, a similar amount of BPA was released in the second extraction (Fig. 1a). The amount of BPA release was similar with increasing number of extractions. This may have been caused by the hydrolysis of polysulfone membranes at the given condition. To investigate this possibility, we changed solution volume from 250 to 4 L. If the continuous release of BPA by multiple extractions is related with polymer hydrolysis, the amount of BPA released is expected to be the same or decrease with the increase of the solution volume because the hydrolysis is mainly governed by temperature, pH, catalyst, etc. As shown in Fig. 1b, however, the amount of BPA released continuously increased with the volume of extraction solution. These findings indicate that BPA leachables in PS membranes might not be completely released at the given condition. The plateau, observed by Haishima et al. in the kinetic elution profile of BPA release for the extraction time up to 24 h [12], might not represent the maximum amount of BPA eluted from membranes. Eluted BPA is likely to be in equilibrium with both the membrane and the solvent used. The problem that arises in extraction is the following: given limited quantity of the solvent, the partition law would give best result when repeated extractions were used compared to single extraction. Therefore, we performed multiple consecutive extractions using a finite volume of solution.

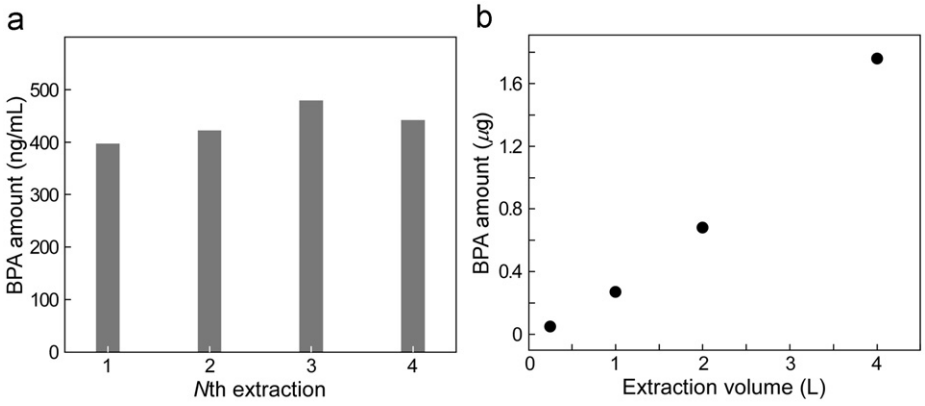

Fig. 1. Effect of (a) number of extractions and (b) volume of extraction solution on the amount of BPA released from PS membranes. (a) Four repeated extractions were performed by circulating 250 mL of 17.2% ethanol solution at 37 °C at the flow rate of 200 mL/min for each 6 h. (b) Except the volume of extraction solution, other experimental conditions are the same in Fig. 1a.

3.2. Kinetic elution profile of BPA release at single prolonged extraction

To establish a proper extraction method, we first investigated the elution profiles of BPA released from hemodialyzer membranes with two different capacities (0.4 and 0.7 m^2 in surface area of membranes). One liter of 17.2% (v/v) solution of ethanol in water was circulated at a flow rate of 200 mL/min at 37 °C for 6 h. A small amount of solution (10 mL) was taken to measure BPA concentration at the time points of 0.33, 0.66, 1, 1.5, 2, 3, 4, and 6 h, and the loss of solution volume was corrected. As observed by Haishima et al. [12], BPA release from both hemodialyzer membranes reached a plateau (Fig. 2) approximately 2 h after circulation started. The difference in these two profiles is the final equilibrium concentration. This can be explained by the fact that one (closed circle) contains 1.8 times larger amount of membranes than the other (open circle). As the flow rate of the extraction solution was reduced to half, equilibrium was reached approximately at 4 h (data not shown). However, the final concentration was independent of flow rate. This supports that the leaching process should be understood as a chemical equilibrium as opposed to a total extraction. In further analysis, we circulated extraction solution at the flow rate of 200 mL/min for 1 h to save time.

3.3. Extraction profile of BPA release by multiple consecutive extractions

On the basis of these experimental results, we performed the following multiple consecutive extractions to fully extract BPA leachables from PS membranes. First, one liter of 17.2% (v/v) solution of ethanol in water was circulated at a flow rate of 200 mL/min at 37 °C for 1 h. Then, the solution was refreshed with a new solution (1 L) every hour. Extraction continued until the concentration of released BPA was lower than the limit of quantification (=0.02 ng/mL). BPA in each extraction solution was concentrated and analyzed using the online SPE–HPLC–MS technique. Fig. 3a shows a representative extraction profile for BPA released from PS membranes. One liter volume of extraction solution rendered the amount of BPA released to decrease continuously with increasing number of extractions (*cf.* extraction at 0.25 L of 17.2% ethanol in Fig. 1a). This supports that the contribution of hydrolysis of PS is negligible under this extraction condition. Furthermore, this result provides basis to reduce the total amount of BPA leachables in hemodialyzers or hemoconcentrators during the manufacturing processes, which may be achieved through multiple consecutive washings of PS hollow fiber membranes.

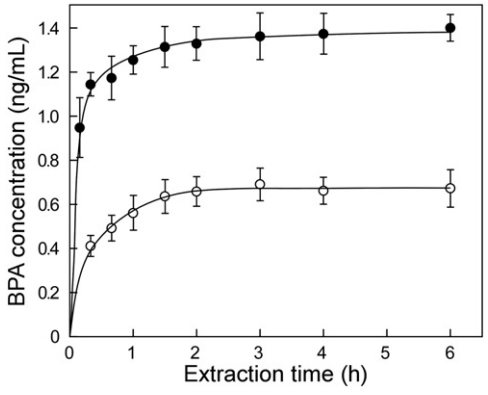

Fig. 2. Kinetic elution profile of BPA released from PS membranes with two different capacities. The surface area of the PS membranes was 0.4 m^2 (open circle) or 0.7 m^2 (closed circle). The volume of extraction solution was 1 L, and other experimental conditions were as in Fig. 1a.

To predict total weight of leachables (w_t; μg in unit), a general equation was derived for multiple consecutive extractions with the following assumptions. First, physical/chemical properties of polymer membranes do not change during repeated extractions. Second, the variation in degree of equilibrium (α; one at complete equilibrium) is negligible at a given time point. The partition coefficient (P) is defined as the ratio of concentration of the leachables in polymer to that in solution [13]. Then, the weight of leachables in extraction solution at n-th extraction (w_n; μg in unit) can be expressed by

$$w_n = w_t \frac{K^{n-1}}{(K+1)^n} \quad (1)$$

here, K is constant ($=PV_{polymer}/\alpha V_{solution}$, where $V_{polymer}$ and $V_{solution}$ represent the volumes of polymer and solution, respectively). The multiple consecutive extraction profile was fitted using Eq. (1) (dashed line in Fig. 3a). A linear relationship can be also established by taking the log of both sides of Eq. (1) and rearranging the terms, to give

$$\log w_n = \log \frac{w_t}{K} - n\log\left(1+\frac{1}{K}\right) \quad (2)$$

A plot of the log w_n against n should be a straight line whose slope and intercept allow K and w_t to be determined. Thus obtained linear regression line (solid line in Fig. 3b) has the slope of −0.125 and the intercept of 3.84, which can be solved to give K=3.01 and w_t=20.7 μg. For comparison, the sum of BPA amounts for ten extractions of the PS hollow fibers, which have total surface area of 0.5 m^2 and total polymer weight of 7.9 g, was

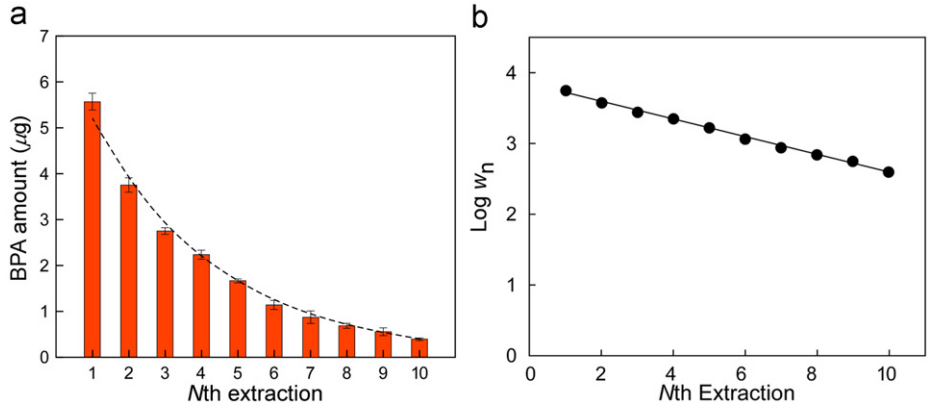

Fig. 3. (a) Typical multiple consecutive extraction profile of BPA released from PS membranes and (b) plot of logarithmic extraction profile against n. Each extraction was performed by circulating 1 L of 17.2% ethanol solution at 37 °C at the flow rate of 200 mL/min for 1 h. The best fit using Eq. (1) or Eq. (2) for each plot was expressed as dashed (a) and solid (b) line, respectively. See text for detail.

determined to be 19.7 μg, indicating that 95% of total leachables was released. The successful fitting with correlation coefficient of 0.9965 implies that the leaching process is governed by chemical equilibrium, and single prolonged extraction may underestimate total content of leachables.

While our extraction method provides a precise way to experimentally predict the total weight of leachables, it may not be practical to perform the full set of extractions until the level of leachables is less than limit of quantification. For practical applications, we recommend at least four extractions, which may produce a good linear regression line. In addition, the extraction can also be completed when the leachable level is one-tenth of the level of the initial extraction as an exhaustive extraction condition [14]. This condition is satisfied at 9th extraction in our experiment to give 19.3 μg in total amount of BPA leachables.

4. Conclusion

We developed a new extraction method based on multiple consecutive extractions to accurately determine the total amount of leachable BPA from PS membranes of hemodialyzers/hemoconcentrators under simulated-use condition. The multiple consecutive extraction profiles were well fitted with a general equation to show that there is a chemical equilibrium between the extraction solution and the polysulfone solid phase. This method is not limited to BPA-leachable study and would very likely be useful in determining the total amount of leachables in any products, in particular, those with low density and high surface area. This method will be helpful in evaluating the risk of material use in medical devices because this can provide the upper limit for the real amount of leachables released from the medical devices during their intended use.

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