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RELEASE PROFILE OF BISPHENOL-A FROM DENTAL RESINS IN WATER ASSESSED BY LC-MS/MS

SILVA, Bruno Pereira da^{1*}; RÜBENSAM, Gabriel²; SAAFELD, Claudiela Wachholz³; WEBER, João Batista Blessmann¹

¹ Pontifícia Universidade Católica do Rio Grande do Sul, Escola de Ciências da Saúde e da Vida, Programa de Pós-Graduação em Odontologia

² Pontifícia Universidade Católica do Rio Grande do Sul, Escola de Ciências da Saúde e da Vida, Centro de pesquisa em Farmacologia e Toxicologia - INTOX

³ Universidade Federal de Ciências da Saúde de Porto Alegre, Faculdade de Biomedicina

* Correspondence author

e-mail: gabriel.rubensam@pucrs.br

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ABSTRACT

Dental resins have been designed to replace amalgam restorations due to their more favorable physical, chemical, and biological properties. However, depending on its composition, the efficiency of its polymerization, and the degradation of the polymer matrix, Bisphenol-A can be present and therefore released from this material to the human body. It has been reported that residues of additives and minority by-products of polymer reactions, such as Bisphenol-A, can be released from plastics into aqueous media through polymer hydration, water-polymer diffusion, residue dissolution, and equilibrium between dissolved residues in water and polymer. Over time, this could lead to a polymer material with an external layer practically free of Bisphenol-A. However, the newly formed layer could be removed by brushing during oral cleaning, similar to toothbrushing, exposing the new layer containing Bisphenol-A to the aqueous media. Due to the toxic effects of this compound, an increasing number of plastics labeled as BPA-free have been introduced to the dental market, including tooth coating, dental sealants, and resins. Nevertheless, more specific studies on analytical chemistry have revealed a trace of Bisphenol-A in dental resins labeled as BPA-free and pointed out the need for even more sensitive and accurate detection methods to help manufacturers evaluate the presence of background contaminations in their products and to avoid false-negative results. In this way, liquid chromatography-tandem mass spectrometry has been considered one of the most suitable methods for confirming Bisphenol-A even at low concentrations, in high complex matrices, due to its high select sensitivity. In the present work, we developed a sensitive, reliable, and efficient approach to trace the release profile of Bisphenol-A by LC-MS/MS in dental resin samples purchased in the Brazilian dental market. With the analysis of five different brands of resin composites performed in eight days of exposition to water, four of them released Bisphenol-A from 3.4 pg/mm² to 10.1 ng/mm². The brand labeled as BPA-free released BPA at concentrations of 1.1 ng/mm². However, one sample reached the maximum of released BPA in only 3 days, one in 4 days, and two virtually did not reach a maximum of BPA released into the water in the window time assessed. Limits of detection and quantification of the LC-MS/MS method were 40 pg/mm² and 100 pg/mm², respectively, and allowed the quantification of BPA released from a composite labeled as BPA-free. For future analysis, we will conduct a more comprehensive study on the release profile of BPA from resin composites into the water using a tooth brushing simulator to determine if the obtained profiles might have clinical implications.

Keywords: Dental resins, Bisphenol-A, LC-MS/MS, release profile.

1. INTRODUCTION

Today, dental restoration is one of the basic specialties of dentistry in which the use of resinous materials has exponentially increased because of

their better physical, chemical, and biological characteristics than dental amalgam (Cho et al., 2022) and, in part, due to the race against the time generated by Minamata Convention on Mercury, which considered a proposal of phase-out amalgam by 2024 (Reher et al., 2021). But, on the

other hand, a number of leachable and extractable chemicals from these materials have been reported, including Bisphenol-A (BPA), a well known potent endocrine disruptor (Cho *et al.*, 2022; Nys *et al.*, 2022).

The presence of BPA in dental resins can occur depending on the resin composition, on the efficiency of its polymerization, and on the degradation of the polymer matrix (Nys *et al.*, 2022) and be released into an aqueous media through polymer hydration, water-polymer diffusion, residue dissolution, and equilibrium between dissolved residues in water and polymer (Asaoka *et al.*, 2008). This situation could lead to a polymer material with an external layer practically free of Bisphenol-A. However, the newly formed layer could be removed by brushing during oral cleaning, similar to toothbrushing, exposing the new layer containing Bisphenol-A.

Alternative materials have been used to develop new dental resins called BPA-free. However, more specific studies on analytical chemistry have been revealed a trace of Bisphenol-A in dental resins labeled as BPA-free and pointed out the need for even more sensitive and accurate detection methods to help manufactures to evaluate the presence of background contaminations in their products and to avoid false-negative results (Tichy *et al.*, 2022). In this way, liquid chromatography-tandem mass spectrometry has been considered one of the most suitable methods for confirming Bisphenol-A even at low concentrations, in high complex matrices, due to its high selectivity and sensitivity (Goery *et al.*, 2022).

In the present work, we developed a sensitive, reliable, and efficient approach to trace the release profile of Bisphenol-A by LC-MS/MS from dental resin samples of different brands purchased in the Brazilian dental market, including one declared as BPA-free material, to water.

2. MATERIALS AND METHODS

2.1. Chemicals

Methanol LC-MS grade (Riedel-de Haën, Germany), ammonium hydroxide 25% LiChropur (Merck, Germany), formic acid LC-MS LiChropur (Supelco, Germany), Bisphenol A 99% (Supelco, Germany), were purchased. Type 1 water was obtained by using a purifier system Milli-Q UV (Millipore, USA).

2.2. Sample Preparation

Cylinders of resin composite (8 x 6 mm) were prepared and stored according to the manufacturer. A total of five different brands of resin composites were assessed (sample 1: intermediate viscosity; sample 2: low viscosity; sample 4: high viscosity; sample 5: with nanoclusters), including one labeled as BPA-free (sample 3). Two groups for each sample composed of five pellets of finished resin composite, one without and other with burnish performed at the lower and upper extremities of the pellets (Abrasive 4000 grit, 4 microns, Agilent, USA), were gently washed with type 1 water, and transferred into 15 mL glass falcon tubes. Type 1 water (2 mL) was added into tubes; the mixture was gently agitated for 10 s, and the samples were left to stand for 8 days at room temperature after the first collection (20 μ L). All collections were carried out every 24h, and the generated aliquots were stored at -20 °C before LC-MS/MS analysis. The grinding experiment was performed using sandpaper (Abrasive 4000 grit, 4 microns, Agilent, USA). All experiments were performed in triplicate. An example of a finished sample was presented in Figure 1.

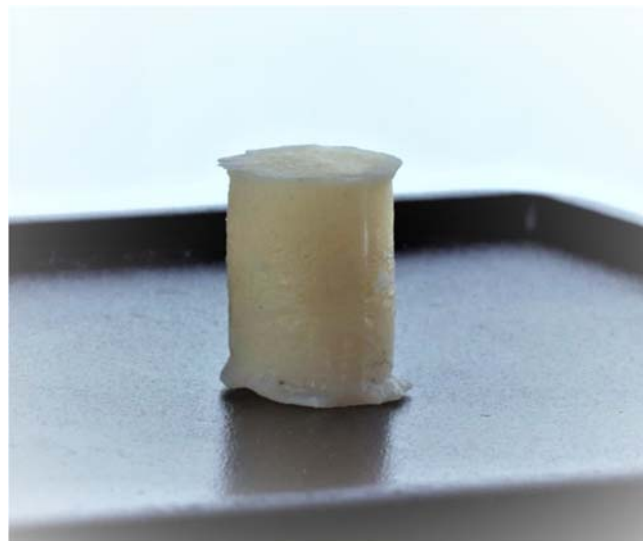


Figure 1. Example of a finished sample used to evaluate BPA release profile to water.

2.3. LC-MS/MS analysis

LC-MS/MS was used in the quantification of BPA released from resin composites. Sample aliquots were thawed at room temperature and injected (5 μ L) into the liquid chromatograph

Agilent 1290 Infinity coupled to a mass spectrometer Agilent 6460 Triple Quadrupole (Agilent Technologies, USA). Chromatographic separations were performed by using a Zorbax® Extended C18 RRHD 2.1 x 50 mm 1.8- μ m particle size (Agilent Technologies, USA), and mobile phase consisted of (A) ammonium formate 5 mM pH 10, and (B) methanol, in gradient mode. The gradient initiated with 5% of B, subsequently increasing to 90% in 2.5 min, remaining at this condition for one min. The initial condition was recovered and kept for 0.5 min. The flow rate was 0.4 mL/min, with a column temperature of 40 °C BPA was ionized with an electrospray (ESI) source operated in negative mode, and the mass spectrometer was optimized to monitor the transitions m/z 227>212 and 227>133, in MRM mode, for quantification and confirmatory purpose, respectively. The calibration curve was constructed in the range of 5 to 100 ng/mL, and external standardization was considered in the quantification. Results were obtained in terms of cumulative data.

3. RESULTS AND DISCUSSION:

To obtain a sensitive method for determining BPA released from dental resin composites, even in products declared as BPA-free, it was necessary to perform a more comprehensive analytical optimization. The optimal condition was reached by adding ammonia to the chromatographic mobile phase since the deprotonation of BPA is facilitated in alkaline conditions, leading to an enhancement of its ionization and analytical sensitivity (Tan *et al.*, 2018). In addition, formic acid was added to pair the ionized BPA, enhancing its retention on a C18 column. An example of a chromatogram obtained with the optimized method was presented in Figure 2.

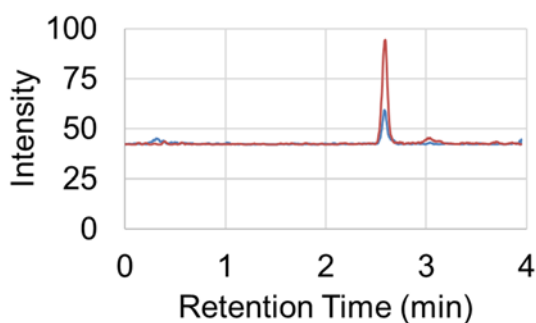


Figure 1. Representative chromatogram of a sample containing 10 ng/mL of BPA, analyzed

by LC-MS/MS. Redline for the quantification transition (m/z 227>212) and blue line for the confirmatory transition (m/z 227>133).

The established conditions allowed limits of detection and quantification of 40 and 100 $\mu\text{g}/\text{mm}^2$ and the quantification of BPA released from a sample labeled as BPA-free. All samples that did not received burnish treatment released BPA at concentrations below 10 ng/mL after 8 days of exposition on water, except for the one labeled as BPA-free, which presented BPA at concentrations below the limit of quantification (data not shown). Burnished samples released BPA between the concentrations of 3.4 $\mu\text{g}/\text{mm}^2$ to 10.1 ng/ mm^2 , except the one declared as BPA-free, which released BPA at concentrations below 1.1 ng/ mm^2 . Most of them released BPA from the fourth day, while only one released BPA from the onset of exposure to water. The sample labeled as BPA-free started releasing BPA after 7 days. The release profile of the tested samples is presented in Figure 2.

It was observed two distinct behavior patterns for releasing BPA from the composite to water with the results herein presented. Both are related to the product viscosity and polymer conversion degree. Products with higher viscosity and lower conversion presented a faster water diffusion and provided more release of BPA to water. On the other hand, composites with lower viscosities and higher polymer conversions had lower release rates. A special situation was observed for a sample with low viscosity but composed of nanoclusters, which may have contributed to an increased water diffusion through the composite after sanding.

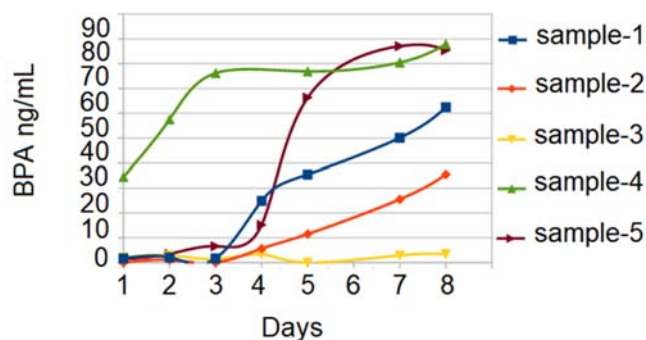


Figure 3. Release profile of BPA from the assessed composite samples to water.

The obtained results were in accordance with findings reported in the literature in terms of BPA released (Durner *et al.*, 2012) and in terms of the physical properties of the composites (Schneider *et al.*, 2012). However, more comprehensive studies involving brushing simulation must be performed to evaluate and understand the release profile of BPA in a more realistic approach and to allow more precise information for the preparation and use of the assessed resin composites.

4. CONCLUSIONS:

An LC-MS/MS method was presented as capable of determining low BPA levels, even for resin composites labeled as BPA-free. The BPA profile released from different resin composites brands into the water after the sanding process was presented and related to their viscosity and polymer conversion degree and structure. More comprehensive testing using a tooth brushing simulator should be conducted to determine if the obtained profiles might have clinical implications.

5. REFERENCES:

1. Cho K., Rajan G., Farrar P., Prentice L., Prusty B.G. *Composites Part B*, **2022**, 230, 109495.
2. Reher V., Reher, P., Peres K.G., Peres M.A. *Aust Dent J*, **2021**, 66, 61-66.
3. Nys S., Duca, C.R., Vervlietd P. *et. al.*, *J Dent*, **2022**, 116, 103894.
4. Asaoka, K., Hirano, S., *Biomat.*, **2008**, 24, 975-979.
5. Tichy, A.; Simkova, M.; Vrbova, R.; Roubickova, A.; Duskova, M.; Bradna, P. *Polymers*, **2022**, 14, 46.
6. Goeury K, Duy S.V., Munoz G., Prévost M., Sauvé, S. *Talanta*, **2022**, 241, 123216.
7. Tan D., JinJ., Wang L., Zhao X., Guo C., Sun X., Dhanjai, Lu X., Chen J., *Talanta*, **2018**, 182, 590-594.
8. Durner J., Obermaier J., Draenert M., Ilie N., *Dent mat*, **2012**, 28, 11, 1146-1153.
9. Schneider, A.C., Mendonça, M.J., Rodrigues, R.B., Busato P.M.R., Camilotti, V. *Polímeros*, **2016**, 26, 37-42.