http://dx.doi.org/10.1590/1981-86372020000372019011 3

| ORIGINAL

Qualitative and quantitative analysis of Mercury ions on the surface of amalgam restorations after home bleaching

Análise qualitativa e quantitativa de íons Mercúrio na superfície de restaurações de amálgama após o clareamento caseiro

Natália Almeida **BASTOS**[1] https://orcid.org/0000-0002-8365-0978

Sandro Basso **BITENCOURT**[2] https://orcid.org/0000-0001-9140-7516

Juliana Fraga Soares **BOMBONATTI**[1] https://orcid.org/0000-0002-4046-8375

Ana Paula Camatta **DO NASCIMENTO**[3] https://orcid.org/0000-0002-1565-6964

Claudia Machado de Almeida **MATTOS**[3] https://orcid.org/0000-0001-9942-6586

Valéria da Penha **FREITAS**[4] https://orcid.org/0000-0002-2037-5841

Jackeline Coutinho **GUIMARÃES**[3] https://orcid.org/0000-0002-0054-6033

ABSTRACT

Objective: The bleaching agents may interact with restorative materials, such as dental amalgam, providing an increase mercury ions (Hg) release, whose toxic effect is known. Whereas many patients have amalgam restorations (AR) and seek bleaching treatments, the present study aimed to evaluate in vitro the amount of Hg released from AR made with spherical alloy, after being subjected to different concentrations of carbamide peroxide (CP) for home bleaching. **Methods**: 40 class I AR were prepared in bovine teeth. After the restoration, the samples were randomly allocated into 4 groups (n = 10): C (control group), CP10 (CP 10%) CP15 (CP 15%) CP20 (CP 20%) and its treatments were simulated for 14 days for 6 hours daily. The Hg ions released was measured by energy dispersive X-ray spectroscopy (EDS) and surface changes were assessed in the Scanning Electron Microscope (SEM). Hg levels (percent mass) were recorded and the differences were statistically analyzed using the Kruskal-Wallis test and Dunn's "Post hoc" test. **Results**: Statistical analysis showed the bleaching treatment resulted in a higher Hg release ($p < 0.05$), although no visible changes were identified in micrographs when comparing the control group with the other groups (CP10, CP15, CP20). **Conclusion**: Increased Hg release may be observed during simulated home bleaching.

Indexing terms: Dental amalgam. Mercury. Tooth bleaching.

[1] Universidade de São Paulo, Faculdade de Odontologia de Bauru, Departamento de Dentística, Endodontia e Materiais Odontológicos. Al. Dr. Otávio Pinheiro Brisolla, 9-75, Vila Universitária, 17011-220, Bauru, SP, Brasil. Correspondence to: NA BASTOS. E-mail: <nembastos@hotmail.com>.
[2] Universidade Estadual Paulista, Faculdade de Odontologia de Araçatuba, Departamento Materiais Odontológicos e Prótese. Araçatuba, São Paulo, Brasil.
[3] Universidade Federal do Espírito Santo, Departamento de Prótese. Espírito Santo, Vitória, Brasil.
[4] Faculdade Brasileira Multivix, Departamento de Prótese. Espírito Santo, Vitória, Brasil.

How to cite this article

Bastos NA, Bitencourt SB, Bombonatti JFS, Nascimento APC, Mattos CMA, Freitas VP, et al. Qualitative and quantitative analysis of Mercury ions on the surface of amalgam restorations after home bleaching. RGO, Rev Gaúch Odontol. 2020;68:e20200037. http://dx.doi.org/10.1590/1981-86372020000372019011 3

RESUMO

***Objetivo**: Os agentes clareadores podem interagir com materiais restauradores, como o amálgama, proporcionando um aumento na liberação de íons de mercúrio (Hg), cujo efeito tóxico é conhecido. Considerando que muitos pacientes possuem restaurações de amálgama (AR) e buscam tratamentos clareadores, o presente estudo teve como objetivo avaliar in vitro a quantidade de Hg liberado a partir da AR confeccionada com liga tipo esférica, após serem submetidas a diferentes concentrações de peróxido de carbamida (PC) para clareamento caseiro. **Métodos**: 40 AR foram confeccionadas em dentes bovinos classe I. Após a restauração, as amostras foram distribuídas aleatoriamente em 4 grupos (n = 10): C (grupo controle), CP10 (PC 10%) CP15 (PC 15%) CP20 (PC 20%) e seus tratamentos foram simulados por 14 dias, por 6 horas diárias. Os íons de mercúrio liberados foram medidos por espectroscopia de energia dispersiva de raios-X (EDS) e as mudanças de superfície foram avaliadas pelo Microscópio Eletrônico de Varredura (MEV). **Resultados**: A análise estatística demonstrou que o tratamento clareador resultou em uma liberação de íons mercúrio (p <0,05), embora nenhuma alteração visível tenha sido identificada nas micrografias quando compara-se o grupo C com os outros grupos (CP10, CP15, CP20). **Conclusão**: Pode-se observar aumento da liberação de mercúrio durante o clareamento caseiro simulado.*

***Termos de indexação**: Amálgama dentário. Mercúrio. Clareamento dental.*

INTRODUCTION

Dental bleaching has become very popular in dentistry [1] and is considered as an effective and non-invasive treatment [2,3]. Besides, this technique is relatively safe in terms of potential risk for an alteration of hard dental tissues [4]. When done under professional supervision, it is considered an efficient and reasonable method that requires reduced office time and still preserves the dental structure [5].

Among the techniques for vital teeth bleaching, the home bleaching stands out [6]. In general, the bleaching agent is applied on the tooth, using a flexible tray, for a few hours [3,7,8]. During this procedure, hydrogen peroxide (HP) or peroxide-releasing agents, such as carbamide peroxide (CP), are used, being commercially available in different concentrations [9,10].

Home bleaching has been extensively researched since 1989. However, not all questions about its use have been clarified. Some of them are related to their effects on restorative materials. Therefore, the influence of several bleaching agents on the physical properties and surface morphology of different restorative materials has been investigated in some studies [10,11].

Considering dental amalgam restorations, some studies have shown that the exposure of amalgam to bleaching agents causes an increase in Mercury (Hg) levels on the amalgam surface [12,13], as well as an additional Hg release [14-17]. Besides, a variation in the Hg release was found among the different types of amalgam exposed to the bleaching agent [15,16]. The factors that may increase the Hg release are related to the bleaching treatment duration, the bleaching gel concentration, the amalgam age, the unpolished surfaces of the restorations and the acidic pH of the bleaching material, promoted by the oxidation from the bleaching agents [14,18]. In a study that used different dental alloys, the authors observed that all groups with spherical alloy exposed to bleaching agents showed an increase in the Hg release over time [18].

Dental amalgam is still the most common material for premolars and molars restoration [19]. Many patients undergoing home bleaching have dental amalgam restorations, becoming the possible increase of Hg release a concern leading to the deleterious effects of this metal, including the development of chronic diseases [20]. As far as this problem is concerned, the studies mostly use amalgam samples, not simulated restorations, which can give erroneous results when it is considered clinically. The simulation of restoration may reduce the number of surfaces in contact with the bleaching agent, reducing the Hg release. Therefore, the present study aimed to evaluate the amount of Hg released from amalgam restorations made with spherical alloy, after being subjected to different concentrations of CP for home bleaching.

METHODS

For this study, 40 freshly extracted bovine incisors were selected, obtained by donating a local slaughterhouse, and were stored at -4°C until the beginning of the research procedures. The teeth were sectioned perpendicularly to the long axis, at the height of the cement-enamel junction boundary, and was cut using an Isomet 1000 cutter (Buehler, Lake Bluff, IL, USA) and diamond disc (series 15LC Diamond no. 11-4254, Buehler, Lake Bluff, IL, USA) at a 275 rpm low speed under water cooling. The root portions

were appropriately discarded as biological waste and the root canal was restored with composite resin (Filtek™ Z350 XT, 3M ESPE, Brazil).

Class I preparations were performed on the vestibular face using the diamond tip (#3131, KG Sorensen, Cotia, Sao Paulo, Brazil), with the depth corresponding to the height of the diamond point active part (4 mm) with an extension of 5 mm by 2.5 mm. The preparations were cleaned with cavity detergent (Tergentol, Formula & Action Pharmacy, Sao Paulo, Brazil) and restored with spherical amalgam alloy (DFL Alloy, DFL, Rio de Janeiro, Brazil).

The pre-dosed amalgam capsules were subjected to the amalgamation process (Duomat 2, Degussa, Germany) according to the manufacturer's instructions, and the material was condensed into the cavity following the condensers sequence. After the condensation, the pre-sculpture honing, the sculpture and the post-sculpture honing were performed, always following the tooth vestibular face contour. After the initial settling time/ crystallization indicated by the manufacturer (8 to 12 minutes), the restored samples were kept in distilled water for 48 hours until the complete material cure. Afterward, all restorations were polished, since the release of Hg is higher when this procedure is not performed [21]. Abrasive rubbers (Amalgam Kit, Microdont, São Paulo, Brazil) and diamond paste (Diamond Excel, FGM, Joinville, Brazil) were used for polishing. All preparation and restoration steps were performed by the same researcher.

Bleaching stage

The 40 samples were randomly distributed into four groups (n = 10) (table 1) [10], according to the different concentrations of CP (Opalescence™ PF, Ultradent, South Jordan, UT, USA). The bleaching gel was kept on the surface of the restoration for 6 hours daily. After this time, the samples were washed and stored in distilled water. The total bleaching time was 14 days, which corresponds to the mean time required to obtain a satisfactory treatment [6,22].

Table 1. Groups according to bleaching gel concentration.

Groups	Surface treatment
C	No bleaching treatment
CP10	Bleaching with 10% carbamide peroxide
CP15	Bleaching with 15% carbamide peroxide
CP20	Bleaching with 20% carbamide peroxide

Scanning Electron Microscopy

The surface morphology evaluation was performed by a qualitative analysis with Scanning Electron microscopy (SEM) (Shimadzu Corporation, model SSX-550, Japan). In the SEM analysis, the humidity of the samples was removed and positioned on a rotating base with the carbon tape, in order to the sample face was perpendicular to the incident electron beam, during the analysis. Micrographs were performed at 1000X with 1kV of acceleration voltage.

Energy dispersive X-ray spectrometer (EDS)

For quantitative analysis of the chemical elements on the sample surface, an analysis was performed by Energy Dispersive X-ray Spectrometer (EDS) (Shimadzu Corporation, model SSX-550, Japan). Three measurements of each sample with 100X amplification, 25kV voltage were performed. All specimens were evaluated using the average of 3 measurements for each sample to quantify the Hg present in each sample.

Statistical analysis

Hg levels (percent mass) were recorded and the differences were statistically analyzed using the Kruskal-Wallis test and Dunn's "Post hoc" test. Statistical software (SPSS version 20.0 – Statistical Package for the Social Sciences, IBM Corporation, Armonk, NY, USA) was used, and all tests were conducted at a significance level of 5%.

RESULTS

In the EDS analysis, several chemical elements were observed (figure 1). However, only the quantitative analysis of Hg was performed.

The bleaching treatment resulted in an increase in Hg release when compared to the control group (table 2). However, there were no differences between the experimental groups (CP10, CP15 and CP20).

It was possible to observe that the SEM micrographs presented similar surface topography for all groups. Figure 2 shows the surface texture in the different samples is similar, there being no cracked regions or characterizing the structural loss in the restorative material.

Table 2. The Comparison of Hg levels between groups.

Chemical element	Groups	Median	Mean	Standard deviation	p-valor
Hg**	Control	$44,52_{A}$	44,72	2,40	0,044
	CP10	$46,46_{B}$	47,00	3,16	
	CP15	$45,97_{B}$	47,01	3,68	
	CP20	$47,10_{B}$	47,35	1,67	

Note: **Kruskal-Wallis test. ***Different letters denote statistically differences through the Dunn's Test.

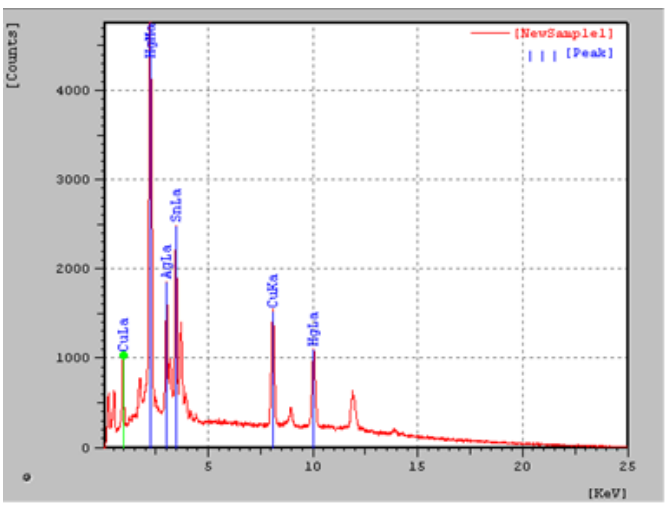

Figure 1. EDS Analysis showing peaks of various chemical elements.

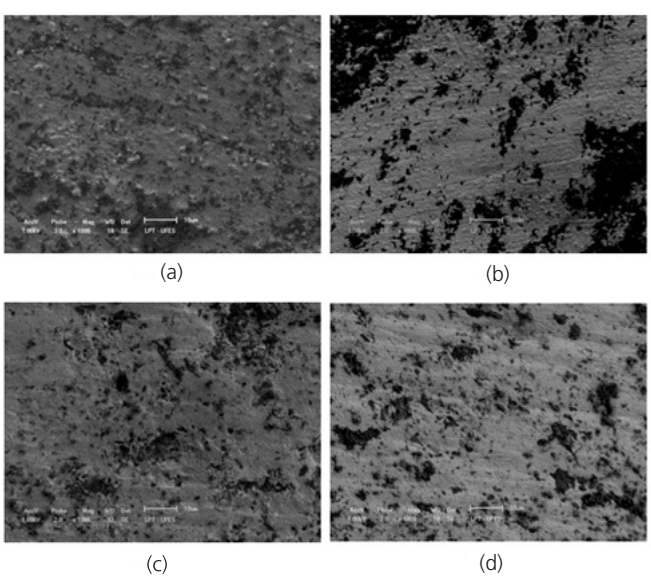

Figure 2. Absence of differences in surface topography. Photomicrograph of (a) control group, (b) CP10 group, (c) CP15 group and (d) CP20. The presence of Hg is represented by the darker regions.

DISCUSSION

The evaluation of the SEM micrographs did not present significant differences between all the groups studied. In the study of Gurgan et al. [23], subtle differences in micrographs were observed only when 30% CP was used, and the concentration of 16% showed no significant changes. As the 30% CP is not indicated for the home bleaching technique, this concentration was not tested in the present study.

All CP concentrations studied (10%, 15% and 20%) showed an increase in the Hg ions release. These results are similar to Rotstein et al. [12], who reported a significant increase in Hg levels after 14 days of 10% CP bleaching. These authors state that prolonged treatment (more than 14 days) with bleaching agents can promote microstructural changes in the amalgam surfaces, possibly resulting in increased patient exposure to toxic byproducts. Similar results were observed by Oskoee et al. [13] who evaluated the effect of CP at 15% applied for 6h/day and observed that Hg levels were significantly higher than control group. At 16% and 30% CP concentrations, Gurgan et al. [23] also founded an increase in the Hg release in the samples tested. On the other hand, the study by Al-Salehi et al. [9] did not find an increase Hg release in amalgam samples after 10% CP treatment. A systematic review reported that increasing CP concentrations led to an increase in Hg release [10].

Increased Hg levels have also been studied in sample stock solutions and some studies have shown an increase in solutions Hg levels [14-17,24]. In our study the stock solution analysis of the samples was not carried out, which could bring additional information of Hg in the saliva, considering that Hg present in saliva can be absorbed by oral tissues and cause adverse effects [20]. These effects are related to the active oxidation, that was assumed to be responsible for the increased release of amalgam components during extended 10% CP bleaching [10].

As for the amalgam restorations polishing, several studies [10,14,25] reported higher Hg concentrations in the solution from non-polished amalgam samples compared to the polished one and that the bleaching agent caused minor corrosion potential for the polished amalgam when compared to non-polished. The non-polished surfaces lead to active oxidation, and it was reported that the use of 10% CP solution on non-polished amalgam samples and nickel-chromium specimens may cause corrosion of these materials, but not of noble alloys [10]. In the present study all samples were polished, which possibly provided a quantitative reduction in the Hg release.

Hydrogen peroxide at 3%, 5% and 6% concentrations are also indicated for home bleaching, however, additional experimental groups of these bleaching agents were not included as there is an equivalence between the agent's concentrations with the CP. It is important to emphasize that the CP can be subdivided into 6.4% urea, ammonia, carbon dioxide, and approximately 3.6% HP [24]. Subsequently, the HP decomposes and yields free radicals of OH– and O–, that are strong oxidizing agents, promoting changes in the surface levels of the amalgam restorations, being a potential source for mercury release [13]. The typical bleaching method uses 10 to 15% CP, that decomposes into 3 to 5% HP and 7 to 10% of urea once the bleaching interacts with the oral environment [24]. According to Ahn et al. (2006) [24], 10% CP corresponds to about 3.5% HP, leading to observe a close proximity in the concentrations between the different bleaching gels (CP 10% = HP 3%, CP 15% = HP 5.25%, CP 20% = HP 7%), therefore, so we chose not to include the groups with HP in this study.

In this study, all teeth were restored with spherical type alloy, however, differences in the Hg release have been reported depending on the type of alloy used26. When comparing a spherical alloy to mixed alloy, Certosimo et al. (2003)26 showed the Hg release from spherical alloy exceeded than mixed alloys. In addition, it was observed that all the groups with spherical alloy amalgam exposed to bleaching agents showed an increase Hg release over time18. These results are similar to observed in the present study.

CONCLUSION

All CP concentrations indicated for home bleaching were able to increase the Hg release in amalgam restorations when compared to the control. Although it is not possible to identify visible changes in micrographs when comparing all groups. In addition, prudence is required to indicate home bleaching for patients with amalgam restorations.

Collaborators

All authors contributed equally to the study. NA BASTOS participated in the acquisition, analysis, and interpretation of data and drafting the manuscript. SB BITENCOURT contributed on interpretation of data, critical review of relevant intellectual content, and final approval of the version to be published. JFS BOMBONATTI was responsible for critical review and final approval of the version to be published. APC DO NASCIMENTO participated in the analysis and interpretation of the data and final approval of the version to be published. CMA MATTOS participated in critical review and final approval of the version to be published. VP FREITAS was responsible for critical review and final approval of the version to be published. JC GUIMARÃES participated in conception and design of the study, interpretation of data, and final review of the version to be published.

REFERENCES

1. Attin T, Betke H, Schippan F, Wiegand A. Potential of fluoridated carbamide peroxide gels to support post-bleaching enamel re-hardening. J Dent. 2007;35(9):755-9. http://dx.doi.org/10.1016/j.jdent.2007.06.005
2. Haywood VB. History, safety, and effectiveness of current bleaching techniques and applications of the nightguard vital bleaching technique. Quintessence Int. 1992;23(7):471-88.
3. Haywood VB, Leonard RH, Nelson CF, Brunson WD. Effectiveness, side effects and long-term status of nightguard vital bleaching. J Am Dent Assoc. 1994;125(9):1219-26. https://doi.org/10.14219/jada.archive.1994.0154
4. Dadoun MP, Bartlett DW. Safety issues when using carbamide peroxide to bleach vital teeth--a review of the literature. Eur J Prosthodont Restor Dent. 2003;11(1):9-13.
5. Campos I, Briso AL, Pimenta LA, Ambrosano G. Effects of bleaching with carbamide peroxide gels on microhardness of restoration materials. J Esthet Restor Dent. 2003;15(3):175-82. https://doi.org/10.1111/j.1708-8240.2003.tb00187.x
6. Bernardon JK, Sartori N, Ballarin A, Perdigao J, Lopes GC, Baratieri LN. Clinical performance of vital bleaching techniques. Oper Dent. 2010;35(1):3-10. https://doi.org/10.2341/09-008CR.
7. Heymann HO. Nonrestorative treatment of discolored teeth: reports from an International Symposium. J Am Dent Assoc. 1997;128(6):710-1. https://doi.org/10.14219/jada.archive.1997.0292
8. Kihn PW, Barnes DM, Romberg E, Peterson K. A clinical evaluation of 10 percent vs. 15 percent carbamide peroxide

tooth-whitening agents. J Am Dent Assoc. 2000;131(10):1478-84. https://doi.org/10.14219/jada.archive.2000.0061

9. Al-Salehi SK. Effects of bleaching on mercury ion release from dental amalgam. J Dent Res. 2009;88(3):239-43. https://doi.org/10.1177/0022034508328676.
10. Attin T, Hannig C, Wiegand A, Attin R. Effect of bleaching on restorative materials and restorations--a systematic review. Dent Mater. 2004;20(9):852-61. https://doi.org/10.1016/j.dental.2004.04.002
11. Swift EJ, Jr., Perdigao J. Effects of bleaching on teeth and restorations. Compend Contin Educ Dent. 1998;19(8):815-20.
12. Rotstein I, Mor C, Arwaz JR. Changes in surface levels of mercury, silver, tin, and copper of dental amalgam treated with carbamide peroxide and hydrogen peroxide in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1997;83(4):506-9. https://doi.org/10.1016/s1079-2104(97)90154-2
13. Oskoee PA, Kahnamoui MA, Oskoee SS, Zadfattah F, Pournaghi-Azar F. Effects of in-office and home bleaching gels on the surface mercury levels of dental amalgam. Eur J Dent. 2010;4(1):23-7.
14. Rotstein I, Avron Y, Shemesh H, Dogan H, Mor C, Steinberg D. Factors affecting mercury release from dental amalgam exposed to carbamide peroxide bleaching agent. Am J Dent. 2004;17(5):347-50.
15. Rotstein I, Dogan H, Avron Y, Shemesh H, Steinberg D. Mercury release from dental amalgam after treatment with 10% carbamide peroxide in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;89(2):216-9. https://doi.org/10.1067/moe.2000.102160.
16. Rotstein I, Dogan H, Avron Y, Shemesh H, Mor C, Steinberg D. Protective effect of Copalite surface coating on mercury release from dental amalgam following treatment with carbamide peroxide. Endod Dent Traumatol. 2000;16(3):107-10. https://doi.org/10.1034/j.1600-9657.2000.016003107.x
17. Salomone P, Bueno RP, Trinidade RF, Nascimento PC, Pozzobon RT. Assessment of the release of mercury from silver amalgam alloys exposed to different 10% carbamide peroxide bleaching agents. Gen Dent. 2013;61(1):33-5.
18. Canay S, Cehreli MC, Bilgic S. In vitro evaluation of the effect of a current bleaching agent on the electrochemical corrosion of dental alloys. J Oral Rehabil. 2002;29(10):1014-9. https://doi.org/10.1046/j.1365-2842.2002.00934.x
19. Brunton PA, Burke FJ, Sharif MO, Creanor S, Hosey MT, Mannocci F, et al. Contemporary dental practice in the UK in 2008: aspects of direct restorations, endodontics and bleaching. Br Dent J. 2012;212(2):63-7. https://doi.org/10.1038/sj.bdj.2012.46.
20. Weidenhammer W, Bornschein S, Zilker T, Eyer F, Melchart D, Hausteiner C. Predictors of treatment outcomes after removal of amalgam fillings: associations between subjective symptoms, psychometric variables and mercury levels. Community Dent Oral Epidemiol. 2010;38(2):180-9. https://doi.org/10.1111/j.1600-0528.2009.00523.
21. Azarsina M, Kasraei S, Masoum T, Khamverdi Z. Effect of surface polishing on mercury release from dental amalgam after treatment 16% carbamide peroxide gel. J Dent (Tehran). 2011;8(1):33-8.
22. Cardoso PC, Reis A, Loguercio A, Vieira LC, Baratieri LN. Clinical effectiveness and tooth sensitivity associated with different bleaching times for a 10 percent carbamide peroxide gel. J Am Dent Assoc. 2010;141(10):1213-20. https://doi.org/10.14219/jada.archive.2010.0048
23. Gurgan S, Kiremitci A, Yalcin F, Alpaslan T, Yazici E. Effect of carbamide peroxide treatments on the metal-ion release and microstructure of different dental amalgams. Oper Dent. 2007;32(5):476-81. https://doi.org/10.2341/06-136.
24. Ahn HJ, Song KB, Lee YE, Lee JT, Cho SA, Kim KH. Surface change of dental amalgam after treatment with 10% carbamide peroxide. Dent Mater J. 2006;25(2):303-8. https://doi.org/10.4012/dmj.25.303
25. Ferracane JL, Mafiana P, Cooper C, Okabe T. Time-dependent dissolution of amalgams into saline solution. J Dent Res. 1987;66(8):1331-5. https://doi.org/ 10.1177/00220345870660080801.
26. Certosimo A, Robertello F, Dishman M, Bogacki R, Wexel M. The effect of bleaching agents on mercury release from spherical dental amalgam. Gen Dent. 2003;51(4):356-9.

Received on: 11/7/2019
Final version resubmitted on: 18/10/2019
Approved on: 22/11/2019