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Dependence of kinetic variables in the short-term release of Hg^{2+}, Cu^{2+} and Zn^{2+} ions into synthetic saliva from an high-copper dental amalgam

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Abstract The short term (up to 14 days after restoration) release of selected ions (i.e., Hg^{2+}, Cu^{2+} and Zn^{2+}) from Dispersalloy® into artificial saliva has been evaluated in regards to the nature of the saliva (Fusayama and McCarty and Shklar's solutions), the amount of amalgam, the time of contact and the periodical renewal (every 48 h interval) of artificial saliva. The evaluation of the ionic fraction of such metals has been accomplished by using anodic stripping methods (i.e., Differential Pulse Anodic Stripping Voltammetry, DPASV) with a 7 μm graphite disk microelectrode as a working electrode. Data obtained in this work are almost unprecedented in the literature due the fact that such analytical method exclude metals in non-ionic forms (e.g., metals or organometallic compounds). The high concentrations measured in every experimental condition confirm the concern for the short-term release of metals from amalgam into saliva.

Introduction

Dental amalgam was introduced more than 150 years ago as a tooth filling material. Today it is still the most popular restorative material, despite the introduction of new types of fillings and its potentially 'frightening' composition. Mercury is the main constituent of the amalgam, usually accounting for about 50% by weight. Other metals include silver, tin and copper and, to a lower extent, zinc. Amalgam contributes significantly to elemental Hg uptake. Several studies [1–3] demonstrated that amalgam filling is able to release mercury vapor into oral cavity. Mouth breathing carries the vapor to the lung, where it is absorbed and distributed to tissues. Mercury levels in autopsy tissue samples, including brain, showed a good correlation with the total number of surfaces of amalgam restorations [4, 5]. An estimate for the rate of release in people with amalgam restoration is 2–17 μg Hg day^{-1} [6]. The urinary excretion of mercury was correlated with amalgam surfaces: it was estimated that 10 amalgam surfaces would raise urinary levels by 1 μg Hg L^{-1} [7]. These amounts are far below toxic levels. It is well documented that a certain amount of inorganic mercury is released from dental amalgam fillings [5, 8–11]. In particular, the uptake of mercury in natural

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or artificial saliva was extensively studied in past decades. In vivo and in vitro methodological approaches often generated conflicting results, as well summarized by Ferracane et al. [12]. Different experimental and analytical conditions and heavy procedural errors (i.e., the massive adsorption of Hg^{2+} onto the walls of plastic vials) played an important role in the spreading of the analytical results over a range of concentration encompassing four orders of magnitude. The major mechanisms and the kinetics of release of mercury from amalgam were also elucidated [13].

Moreover, the toxic effects of mercury species depend on the chemical nature of the pollutant. In particular, metal mercury, mercury(I) and mercury(II) inorganic species and organomercuric compounds (e.g., methylmercury) exhibit very different toxicity levels [14, 15].

Despite this, only a slight attention has been devoted to the identification and quantification of the chemical species released into saliva by Hg and other heavy metals. Actually, speciation is not possible if spectroscopic techniques (i.e., atomic absorption spectroscopy) are used and in some cases needs electroanalytical methods. Our research group [16–19] became skilled in the assessment of stripping methods involving microelectrodes for the determination of heavy metal traces directly in biological matrices, with or without any pre-treatment of the sample. Recently [17] we showed that graphite microdisk electrodes are most suitable for determining heavy metal traces (e.g., Hg) in aqueous solutions, even at pH close to neutrality. Using this electrode it was possible to detect a number of metal ions in a single stripping run without suffering interferences. In a previous study we used this method to preliminarily evaluate the ionic fraction of heavy metals released from a high-copper dental amalgam (Dispersalloy®) into synthetic saliva [18]. It was shown that Hg concentration was at the highest level of risk (HBM III), as identified by the three human biomonitoring categories suggested by the German *Institut für Wasser-, Boden- und Lufthygiene des Umweltbundesamtes*, for the estimation of potential harmful effects on health due to exposure to heavy metals [20]. Therefore, the main aim of the present study was the evaluation of the ionic species of Hg, Cu and Zn released from Dispersalloy® in artificial saliva as a function of several parameters, namely: (i) the nature of the solution; (ii) the amount of amalgam; (iii) the amalgam/saliva time of contact; (iv) the periodical renewal, every 48 h, of artificial saliva.

Materials and methods

Instrumentation, electrodes, and techniques used

Electroanalytical experiments were carried out using a computerised electrochemical system mod. CHI-650 (CH Instruments, Austin, TX, USA), associated with a current booster mod. CHI-200. A specific software was used. The electrochemical cell was inside a Faraday cage, where also the current amplifier was positioned. The working disk electrode was made by graphite fiber (7 μm diameter), assembled by 'sinking' the fiber into a double-component insulating epoxy glue. The resulting electrode was tested according to published methods [21, 22]. These methods allowed also evaluating the electrochemical area. A three-electrode system was used; the auxiliary electrode was a glassy carbon rod and the reference electrode was a Saturated Calomel Electrode (SCE). All potential values given are referred to this electrode.

Differential pulse anodic stripping voltammetry (DPASV) was used in this work [23, 24] under the following experimental conditions: $E_{dep} = -1.5$ V; $(\partial E/\partial t)_{stripping\ sweep} = 0.08$ V s^{-1}, $E_{step\ height} = 0.05$ V, $t_{step} = 0.1$ s, $t_{delay} = 0.1$ s. The deposition time (t_{dep}) was between 15 and 60 s. The solution was de-aerated with 99.999% pure argon before the measurements and was kept under argon continuous flow during the tests. In order to avoid CO_2 stripping from the FS solution, argon was pre-bubbled through a 2×10^{-2} mol dm^{-3} $NaHCO_3$ solution. For the Zn^{2+} ionic concentration, the quantification was done against a linear calibration function in the range 100 ÷ 1,000 μg dm^{-3}. When the Zn^{2+} concentration was below the range of the linear calibration plot, the quantification was performed using the standard multiple additions method. This analytical approach was always used for measuring Cu^{2+} and Hg^{2+} releases in saliva.

Standard solutions were prepared either by dissolving salts of suitable purity ($ZnSO_4$, $Cu(NO_3)_2$, $Hg(NO_3)_2$ from Merck, *pro analisi*) or by dilution of standard solutions for Atomic Absorption Spectroscopy (Zn, Cu and Hg, 1,000 mg dm^{-3} concentration, from Fluka).

Artificial saliva

Artificial saliva, namely Fusayama and McCarthy and Shklar [25, 26] solutions, is well known in dental research. In particular, McCarthy and Shklar saliva [26] (MCSS) has physical characteristics most similar to human saliva, while the closest biochemical similarities are found in Fusayama saliva [25] (FS).

FS consists of an aqueous solution with the following composition: 5.3×10^{-3} mol dm^{-3} KSCN; 1.5×10^{-2} mol dm^{-3} $NaHCO_3$; 2×10^{-2} mol dm^{-3} KCl; 1.4×10^{-3} mol dm^{-3} NaH_2PO_4; 1×10^{-2} mol dm^{-3} lactic acid; pH = 6.7. Instead, MCSS solution contains 1.5×10^{-2} mol dm^{-3} NaCl; 8.4×10^{-3} mol dm^{-3} KCl; 5.9×10^{-3} mol dm^{-3} Na_2HPO_4; 2.4×10^{-3} mol dm^{-3} NaH_2PO_4; 9.5×10^{-5} mol dm^{-3} NaF; 1.3×10^{-2} mol dm^{-3} methyl *p*-hydroxybenzoate; 10 g dm^{-3} sodium carboxymethylcellulose; pH = 7.2.

These solutions were prepared using NaCl and NaH_2PO_4, (Merck, *pro analisi*), Na_2HPO_4, $NaHCO_3$, and KSCN (RPE-ACS, Carlo Erba, Milan, Italy), KCl and NaF (Aldrich, *pro analisi*), *p*-hydroxymethyl benzoate, sodium carboxymethylcellulose (Merck, low viscosity), and lactic acid 85% solution (Aldrich, ACS Reagent).

Release of metals from amalgam into artificial saliva

For the preparation of the dental amalgam, the commercial product Dispersalloy® (Johnson & Johnson) was employed. The alloy was mixed with an equal amount of Hg inside a capsule. An amalgam mixer (Eurodigital2, Ghimas, Italy) ensured proper mixing; the time of vibration selected was 5 s. The final composition was: Ag, 34.65%, Sn, 8.95%, Cu, 5.90%, Zn, 0.50%, Hg, 50.00%. The spherical amalgam particle so obtained was modeled first by hand to ensure suitable mechanical characteristics and to better simulate the actual manipulation inside the dental cavity and then by an amalgam knife (Hollenback) in order to remove the excess of elemental Hg. 3 min after the preparation, one or more spherical particles (each ca. 800 mg weight and ca. 30 mm^2 surface) were placed in contact with 150 cm^3 of artificial saliva, from 6 h to 336 h, inside small HDPE test tubes [27]. The test tubes were previously treated for 24 h with 0.5 mol dm^{-3} Suprapur HNO_3 in water (Ultrapure, Merck, Pro Analysis) and then washed carefully with the same pure water. The suspension was gently stirred in a water bath at 37 ± 0.5 °C. At the end of the procedure, artificial saliva was separated from the amalgam by means of a syringe bearing a cellulose nitrate filter (Whatman, 0.2 μm pore diameter) at the end tip, and analyzed by DPASV. Every release test was repeated five times.

Table 1 Amounts of Hg^{2+}, Cu^{2+} and Zn^{2+} released into Fusayama's (FS) and McCarthy and Skhlar's (MCSS) saliva, after contacting one unit (area = 30 mm^2) of dental amalgam for 6–336 h

Contact time between FS and amalgam (h)	C_{Hg} (ng mm^{-2})	s[a]	C_{Cu} (ng mm^{-2})	s[a]	C_{Zn} (ng mm^{-2})	s[a]
6	75[b]	15	190[b]	30	1,440[b]	60
12	95[b]	20	245[b]	30	1,485[b]	60
24	205[b]	15	385[b]	20	1,590[b]	60
48	320[b]	20	445[b]	25	1,625[b]	60
72	385[b]	15	480[b]	20	1,690[b]	60
90	505[b]	10	525	30	1,730[b]	60
96	530	25	555	30	1,745	65
120	655	25	605	35	1,795	60
144	755	30	650	35	1,850	65
168	835	30	695	40	1,890	65
336	1,005	40	925	50	2,125	60
Contact time between MCSS and amalgam (h)	C_{Hg} (ng mm^{-2})	s[a]	C_{Cu} (ng mm^{-2})	s[a]	C_{Zn} (ng mm^{-2})	s[a]
6	40	30	250	45	1,375	70
12	65	30	340	40	1,405	65
24	145	30	395	45	1,450	70
48	275	30	465	45	1,510	60
72	340	30	550	50	1,550	65
90	405	35	615	50	1,590	60
96	430	35	645	45	1,610	65
120	480	40	730	55	1,655	70
144	525	40	810	55	1,700	60
168	570	40	885	65	1,755	70
336	825	50	1,105	75	2,010	70

[a] s is the standard deviation on five independent measurements

[b] ref [18]

Results

Data relative to the amount (ng) of Hg^{2+}, Cu^{2+} and Zn^{2+} ions released for surface unit (1 mm^2) of amalgam are summarized in Tables 1 and 2 and in Fig. 1. Table 1 reports the dependence of metal ion losses from amalgam as a function of the time of contact with two different artificial saliva samples. The behavior shows that, for both saliva samples, the concentration of all ionic releases tends to increase with increasing contact time between amalgam and saliva.

The comparison between ionic concentrations measured at the same contact time reveals that the amount of Hg^{2+} and Zn^{2+} ions is higher in FS than in MCSS, whereas the contrary is observed for Cu^{2+}.

Figure 1 reports the behavior of all metal ions released in FS over a 144 h period at varying the amount of amalgam.

The higher the amount of amalgam is, the higher is the concentration of metal ions in FS (from 75 ng mm^{-2} with 1 unit to 100 ng mm^{-2} with 4 units after 6 h). A comparison of Fig. 1a, b, c shows that, for all metal ions considered, the density of release decreases with increasing amount of amalgam units.

For Hg^{2+} and Zn^{2+} ions the density of release is the highest if only one amalgam unit is taken into account. On the other hand, the situation is quite different for Cu^{2+}. In this case, it is possible to observe that, below 72 h, the density of release is the highest for one amalgam unit, whereas for higher contact times the release is more pronounced using two units.

By taking into account that saliva is fully renewed in the oral cavity over 48 h, we tried to evaluate Hg^{2+}, Cu^{2+} and Zn^{2+} released from one amalgam unit in Fusayama's saliva after its renewing for seven times. Table 2 shows the results of these experiments.

From the first to the last (seventh) cycle, a heavy reduction of the density of the release is observed for all metals (higher than 62%, 85% and 95% for Hg^{2+}, Cu^{2+} and Zn^{2+} ions, respectively).

Discussion

The aim of our study was to investigate the short-term release of heavy metal ions from dental alloys into artificial saliva. Only a few studies have been published focusing on the kinetic evaluation of the short-term ionic losses of heavy metals from hardening amalgams.

Our results substantiate that dental amalgam fillings are a continuous source of mercury and other heavy metals mainly during the first days after restoration.

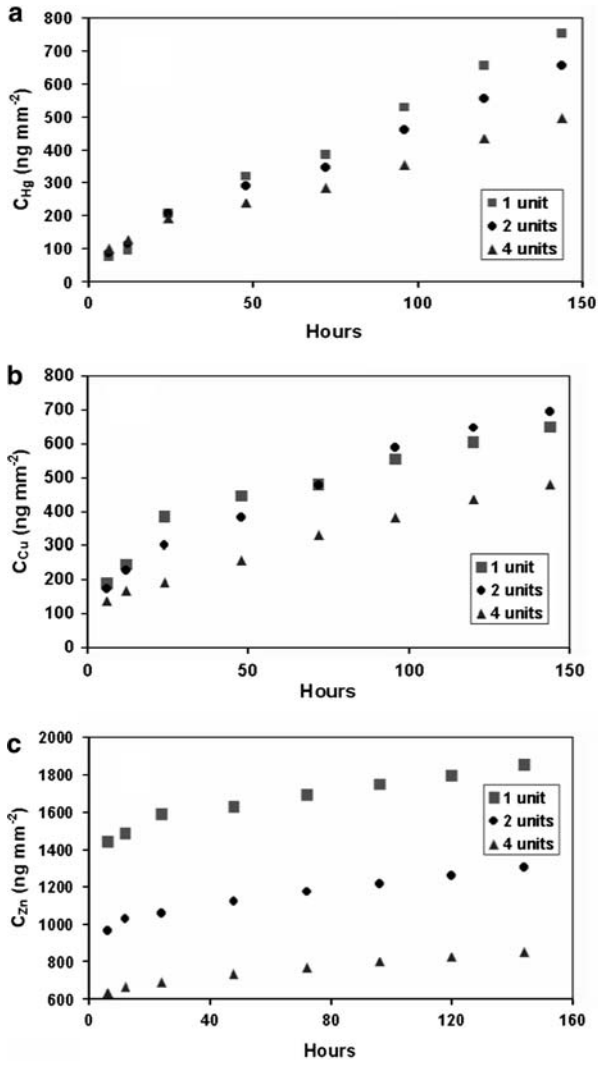

Fig. 1 Density of release of metal ions versus time from units of Dispersalloy® into Fusayama's (FS) artificial saliva. (**a**), Hg^{2+}; (**b**), Cu^{2+}; (**c**), Zn^{2+}

Moreover, the amounts measured are only a fraction of the metal losses in artificial saliva. The stripping methods used determine only the ionic fraction of such metals, inasmuch as different chemical forms, i.e., elemental mercury [1–3] or organometallic species [10, 28] are not taken into account. For this reason our data cannot be compared with those reported in the literature, in which the metal uptake was evaluated irrespectively of the chemical form (e.g., by atomic spectroscopy).

The evaluation was discontinued within 14 days after restoration. Phenomena taking place over a longer time scale, e.g., corrosion or passivation-dissolution were not considered. Also additional complications, like abrasion due to the saliva itself, chewing and routine oral hygiene, which could play an important

Table 2 Evaluation of Hg^{2+}, Cu^{2+} and Zn^{2+} releases from one amalgam unit in Fusayama's saliva after its renewing for seven times. Each cycle was of 48 h

Renewal cycle of FS	C_{Hg} (ng mm^{-2})	s[a]	C_{Cu} (ng mm^{-2})	s[a]	C_{Zn} (ng mm^{-2})	s[a]
1	320	20	445	25	1,625	60
2	300	25	455	35	1,315	60
3	280	25	310	35	625	40
4	225	30	205	30	300[b]	40
5	160	30	150	30	210[b]	35
6	125	25	105	35	100[b]	35
7	100	30	65	35	75[b]	40

[a] s is the standard deviation on five independent measurements

[b] measured with the multiple standard addition method

role in the mechanical release of heavy metals, were not taken into account in this study.

In the first phase of the research, the release of Hg^{2+}, Cu^{2+} and Zn^{2+} into two different saliva samples, FS and MCSS, was compared. The ion losses were found to be time dependent and statistically different in the two solvent media used. At comparable times of contact between amalgam and saliva, FS exhibits always a higher concentration of Hg^{2+} and Zn^{2+}, whereas the concentration of Cu^{2+} is higher in MCSS than in FS. Most probably, for Hg^{2+} this behavior is due to the significant amounts of SCN^{-}, a strong binder for such metal ion, present in FS.

The concentration of Hg^{2+} in both saliva samples after 24 h of amalgam unit immersion are 205 and 145 ng mm^{-2} for FS and MCSS, respectively. Only for comparison purposes, we remark that such values are in the middle of the very wide range previously reported [12] and quite close to those observed by Tomoda and Ohta [29, 30]. On the other hand, the amount of Hg^{2+} monitored in FS after 7 and 14 days was ten times higher than that observed by Brune [31], whereas the Cu^{2+} release was 10 times lower. The use of a different copper-rich alloy (Neo Silbrin®, Cu = 31.3%) could explain the differences.

According to Marek [13], the metal dissolution from amalgam is very fast during the first 24 h of contact with saliva. Between 24 h and 96 h it is possible to observe a non-linear dissolution rate (mainly for Hg^{2+} and Cu^{2+}), probably due to the rapid transformations occurring in the amalgam during the hardening process. Finally, after the first 100 h, a monotonic reduction of the dissolution rate of the metal ions is observed. The complexity of the dissolution mechanisms during the hardening phase prevents us from making additional comments.

After establishing that the most significant release of all metal ions occurs in the first week, we monitored the effect of the amount of amalgam (1, 2 or 4 units) as a function of the time of contact with FS (i.e., the saliva most efficient in the Hg^{2+} dissolution). As expected, the higher the amount of amalgam, the higher the concentration of metal ions in FS. However, also in this case a more convenient expression of analytical data (i.e., the amount of release per surface unit of amalgam—density of release—in the place of concentration) is more informative. For Hg^{2+}, within the first 24 h the density of release is roughly irrespective of the overall surface of amalgam. On the other hand, with increasing saliva/amalgam time of contact, the density of release diminishes if the surface of amalgam is increased. The effect is more pronounced for prolonged times of contact between amalgam and saliva. A different situation is evidenced for Cu^{2+}. Whereas the highest surface of amalgam always gives rise to the least density of release (this is evident even in the first 24 h), the density of release for 1 and 2 amalgam units is the same at two different times, i.e., after 6 h and 72 h, respectively. Within this range, the density of Cu^{2+} release from the solution bearing one amalgam unit is higher, whereas, the trend is reversed for higher contact times. Until now, we are unable to propose a *rationale* for this behavior.

Moreover, the density of release for Zn^{2+} ion shows a strong relationship with the number of amalgam units immersed into saliva. The higher is the overall surface exposed to FS, the lower the density of Zn^{2+} ion release is. The behavior is well noticeable even at low contact times between amalgam and saliva. Our last purpose was to evaluate the release of target ions from one amalgam unit in FS media after renewing it every 48 h for seven times. This experiment was aimed at reproducing saliva in the oral cavity (which is fully renewed over 48 h). A similar approach was recently reported [2]; however, the solvent was acidified or pure water and a flow system were used. Although the different experimental conditions hinder a reliable comparison between the results, it is interesting to evaluate the relevant trends. In both situations, a monotonic decrease for Hg^{2+} is observed but, in contrast to the Okabe's study, in our study it corresponds to an exponential trend; a sigmoid-like trend was observed for all ions. Also the density of releases of Cu^{2+} and Zn^{2+} exhibits a similar behavior. Factors like the ionic strength and the presence of strong complexing anions, e.g., SCN^- and Cl^-, in FS could account for these differences. Moreover, it is interesting to note that the dynamic system used by Okabe [2] is more efficient than our ''static'' system in decreasing the mercury release: after six days, Okabe detected only 33% of the initial concentration of Hg, whereas in our system a similar Hg^{2+} depletion was measured only after 2 weeks. Again, it is important to notice that the data reported by Okabe [2] refer to the overall mercury concentration, not only to the ionic fraction. Moreover, the concentration of Cu^{2+} and Zn^{2+} ions in FS is heavily depleted after 2 weeks: these values are only 15% and 5% of the initial releases, respectively.

Conclusions

In this paper the short-term (up to 2 weeks) release of Hg^{2+}, Cu^{2+} and Zn^{2+} from Dispersalloy® was evaluated in two different artificial saliva samples using a previously established electroanalytical procedure. The data obtained are difficult to compare with those reported in the literature due to the specificity of the analytical procedure used. Concentrations of Hg^{2+} ranging from 40 ng to 1,005 ng mm^{-2} were found between 6 h and 336 h, respectively, in both solvent media. These values are within the wide interval of experimental releases reported in literature for ''total'' mercury. Also the losses of Cu^{2+} (190–1,105 ng mm^{-2}) and Zn^{2+} (1,375–2,125 ng mm^{-2}) are meaningful. FS is more active in extracting Hg^{2+} and Zn^{2+} ions, whereas a higher release of Cu^{2+} ion was observed in MCSS. Also the dependence from the exposed surface of amalgam and its time of contact with FS was studied. As a general rule, the higher the exposed surface is, the lower the density of release is. This is true mostly for Zn^{2+}, whereas Hg^{2+} releases were irrespective of the amalgam surface in the first 24 h of contact with artificial saliva. On the other hand, a more complex dependence from amalgam area was found for Cu^{2+}. Furthermore, the decrease of ionic releases was monitored during seven FS renewals every 48 h of contact. After 2 weeks, only 33%, 15% and 5% of the initial concentrations of Hg^{2+}, Cu^{2+} and Zn^{2+} ions, respectively, were detected.

Finally, our study confirms that DPASV with graphite microelectrodes is a reliable and accurate analytical tool for evaluating metal ions released from amalgam into artificial saliva.

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