



EFFECT OF PH OF IMMERSION MEDIA AND LENGTH OF IMMERSION PERIOD ON ELEMENTAL ION RELEASE FROM COMMERCIALY AVAILABLE NI-CR ALLOYS: AN IN VITRO STUDY

Dental Science

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ABSTRACT

Purpose: Biocompatibility of dental casting alloys is of paramount importance in rendering successful treatment to the patients. Controversies have been cited in literature related to elemental ion release based on pH and length of immersion period. Hence the study was conducted with the purpose of evaluating the effect of the pH of immersion media and length of immersion period on the release of elemental ions from the commercially available Ni-Cr alloys.

Materials and Methods: A total of 270 disc shaped specimens (5 mm x 3 mm) were prepared from 2 commercially available Ni-Cr alloys. One hundred and thirty five samples were fabricated per alloy which were divided into three groups of 15 each. The samples were immersed in immersion media of pH 4.0, 6.8 and 8.0 for time intervals of 1 hour, 1 day and 1 month. The elemental ion release was estimated using inductively coupled plasma atomic spectrophotometer.

Results: The elemental ion release of the major elements increased significantly ($p < 0.05$) from both alloys with decrease in the pH of the immersion media. Maximum ion release was seen at pH 4.0 followed by pH 8.0 and 6.7. Significant increase ($p < 0.05$) in the elemental ion release was seen only at an acidic pH of 4.0 when the release at 1 month was compared with 1 hour and 1 day.

Conclusion: Decrease in pH of immersion media and increase in the length of immersion period led to increase in the elemental ion release from commercially available Ni-Cr alloys.

KEYWORDS

Introduction

During the past few years, the biocompatibility of dental materials has evolved into a comprehensive, complex and independent discipline of dental material science.¹ Biocompatibility refers to compatibility with living tissues or a living system by not being toxic, injurious or physiologically reactive and not causing immunological rejection. Many a times, it is often assumed that, if the alloy is in the market, its biocompatibility need not be questioned.¹⁻³

Various high noble, noble and predominately base metal alloys are available for the fabrication of dental prosthesis. The search for less expensive alternative alloys has led to the use of base metal alloys both for fixed and removable dental prosthesis. Unlike removable dental prosthesis, a fixed dental prosthesis cannot be removed by the patient and remains in the oral cavity in direct contact with enamel, dentin, bone and saliva for extended duration. Their close proximity to the gingiva and their extension into the subgingival area may cause various adverse effects (toxic or allergic) like gingival swelling, erythema, mucosal pain, lichenoid reaction and crevice corrosion.⁴ These effects may be linked to type of ions released, the quantity released, synergistic or antagonistic effects of released ions, the time the tissues are exposed and the corrosion rates of the casting alloys. Various reports have detected these elements in tongue scrapings and in saliva.⁵ Ions released during corrosion may be detectable by the patient as a shock that can be disconcerting and even debilitating. Released metallic components may cause adverse health problems and undesirable metallic taste.⁶

Clinical performance of a dental alloy is affected by dynamic conditions prevailing within the oral cavity. Consumption of acidogenic food and beverages like coca-cola, pepsi, sport drinks, milk with bournvita, fruit juice like orange juice, mango juice and apple juice (carbonated and non-carbonated), various adverse habits like smoking, consumption of alcohol and other tobacco products, dental plaque and calculus accumulation, state of health and disease such as gastro-esophageal reflux disorder leads to the reduction of the oral pH.⁷

Likewise, metabolic acidosis, respiratory alkalosis and various states involving the accumulation of plaque in the oral cavity can lead to alkaline pH of saliva. Further, fluctuations in temperature, a continuous flow of saliva, microbiological activity, occlusal load, variations in oxygen pressure, decomposition of food and unconscious regular contact with metal ions (contact with jewellery and cooking utensils) contribute to changes in the electrochemical balance leading to increase in elemental ion release from dental casting alloys.⁸ In addition, presence of multiple alloys in the oral cavity lead to the production of galvanic currents that might lead to elemental ion release, corrosion, increase in surface roughness and deterioration of the alloys. As reduced pH probably acts by altering the alloy surface, it was hypothesized that release of elements subsequent to low pH exposure would also be higher as the surface re-equilibrated in the neutral environment. If alloys release more mass from brief exposures to low pH environments, then the long term burden of elements released into the body will be substantially increased. This increased burden may alter the biologic response of adjacent tissues to the alloy. Hence, evaluation of elemental ion release is important both qualitatively and quantitatively to assess the adverse effects and sensitisation of the body at different doses of exposure.^{9,10}

Studies have reported that many heavy metals are carcinogenic, hazardous and toxic pollutants like beryllium, cadmium, cobalt, copper, iron, palladium, silicon and vanadium are harmful and should be used in lower ratios in the alloys.⁷ Allergic reactions caused by ion release from dental alloys are important for two reasons. Firstly, a patient who is already sensitized may exhibit reactions resulting from the dissolution of metal in saliva which is not dose dependent. Secondly, a non-sensitized patient may become sensitized by the alloy. Since both possibilities are undesirable, the alloys used for dental restorations must exhibit the lowest possible corrosion rates.

The release of metallic biomaterials from Ni-Cr alloys into the alloys has been controversial for many years. Previous studies conducted by various authors^{1-3,5,6,8-10} have reported that there is a significant increase

in elemental ion release with decrease in pH. The effect of a constant reduced pH on elemental ion release from Ni-based alloys has been reported to increase the release of nickel.¹¹ According to these studies the protective passive layer usually disintegrates at reduced pH leading to an accelerated corrosion process. However, conflicting reports have been documented by Denizoglu et al.¹² who reported that there is no effect of pH on the elemental ion release from Ni-Cr alloys. Several authors¹³⁻¹⁷ demonstrated that addition of minimum 2% molybdenum to Ni-Cr alloys stabilizes the oxide film in saline environment and reduces grain boundary corrosion to the extent that no increased corrosion is recognizable even at a pH value of 1. Previous study conducted by Holm et al.¹³ stated that Ni-Cr alloys expressed low elemental release of ions (<200 µg/cm² per 7 days) at a reduced pH of 2.3. Hence, Ni-Cr alloys are sufficiently corrosion resistant and can be safely used for fixed prosthodontics.

Tai et al.¹⁴ reported that that dissolution and occlusal wear are both factors in the release of nickel and beryllium metal. Occlusal wear increases the concentrations of metals in the leachate two to three times compared with dissolution alone at reduced pH within the oral cavity. Previous studies conducted by some authors¹⁵⁻¹⁷ documented that increased elemental ion release is seen in Ni-Cr alloys with Cr content less than 25% at reduced pH. Beck et al.¹⁶ reported that increased corrosion resistance was seen in palladium containing Ni-Cr alloys compared to traditional Ni-Cr alloys at a reduced pH of 2.3.

There is a significant increase in the elemental ion release with increase of length of immersion period as reported by several authors.^{15-17,14} In contrast, Geis-Gerstorfer et al.¹⁷ and Estill et al.¹⁸ determined that the element release from Ni-Cr based alloys decreases with time. Wataha and Lockwood (1998)¹⁹ measured the release of elemental from dental alloys at monthly intervals for 10 months. They hypothesized that element release should decrease as a function of time of exposure to the medium and that the cytotoxic effects of the alloys should also decrease. They also stated that the initial release rates were the highest. Although majority of the previous studies have reported that the elemental ion release does not cross the threshold values, however according to Wataha et al.,¹⁹ on comparing the in vitro elemental ion release with the dietary intake of many dental metals, it was reported that for some elements such as nickel, the release can approach dietary levels. The tolerable upper intake level is the highest daily nutrient intake, from food plus other sources of supply, which can be safely ingested by the vast majority of individuals without posing any adverse health effects. Given that prosthodontic restorations are a supply source for metal ions, the amount of ion released must be below the tolerable upper intake level.²

Owing to controversial findings of previous studies related to the effect of pH and length of immersion period on elemental ion release and lack of data on the corrosion behaviour of Ni-Cr alloys in alkaline pH, the present study was taken up to evaluate the effect of different pH and length of immersion media on elemental ion release from commercially available Ni-Cr alloys. The null hypothesis of the present study was that there was no effect of pH of immersion media and length of immersion period on the elemental ion release from commercially available Ni-Cr alloys.

Materials and Methods

The present in-vitro study was conducted in the Department of Prosthodontics and Crown & Bridge in Kothiwal Dental College and Research Centre, Moradabad, Uttar Pradesh. The test for elemental ion release was carried out in the Department of Metallurgy, Research Testing and Calibration Laboratory, Moradabad, Uttar Pradesh.

Two commercially available Ni-Cr alloys namely Alloy A of composition Ni 62 %, Cr 25 %, Mo 9.5%, Si3.5 % and Alloy B of composition Ni 65.2%, Cr 22.5% and Mo 9.5% and 2.8% of other trace elements (Fe, Si, Nb and Mn) were employed for the study. A total sample size of two hundred and seventy (135 samples per Ni-Cr alloy) was used in order to have significant parametric evaluation of the test results. The sample for each alloy was divided into three groups of 45 samples each. Each group was further sub-divided into 3 subgroups of 15 each. Inlay wax pattern of diameter 5 mm and thickness 3 mm was made in accordance with the studies conducted by Nelson et al.,¹ Al-hiyasat et al.,⁴ Oyar et al.,⁵ Denizoglu et al.¹² and Can et al.²⁰ Sprue wax of 10 gauge (2.6 mm) was attached to the wax pattern. The sprued wax pattern was invested, casted, retrieved, finished and polished according to manufacturer's instructions. This served as the metal

master model which was used for taking multiple impressions of the same using putty consistency addition silicone.

Immersion media of 3 different pH (4.0, 6.8 and 8.0) were selected to simulate the pH changes occurring within the oral cavity. The immersion media of pH 4.0 simulated acidic conditions within the oral cavity. Immersion media of pH 6.7 mimicked the resting pH condition of saliva and of pH 8.0 corresponded to alkaline state of saliva.

For formulation of immersion medium of pH 4.0, tris buffer of pH 4.0 in the form of capsules were first dissolved in 100 ml distilled water. The solution was titrated using 15.6 ml of 0.1 M hydrochloric acid to form the immersion media of pH 4.0. For preparation of pH 8.0, 1.21 gm of tris buffer was added to 100 ml of distilled water. 0.1 M HCl of volume 58.4 ml was added to it with continuous stirring and the pH was measured using a pH meter until the pH was 8.0. The amount of tris buffer to be added to distilled water was calculated using the formula:

moles of tris = molarity × volume

Next the moles of tris was used to calculate the grams of tris needed to prepare 200 ml of tris-HCl solution of pH 8.0 using the formula:

grams of tris = moles of tris × mol wt of tris

Artificial saliva (Saleva) by Dent Aids of pH 6.8 was used as the third immersion media. It was composed of 18% glycerine, 0.15% sodium methyl paraben, 0.05% sodium propyl paraben, lubrajel, carboxymethyl cellulose sodium and carrageenan

All the samples were immersed in each of the immersion media of pH 4.0, 6.8 and 8.0 for 1 hour, 1 day and 1 month respectively. The ions released within these solutions were measured using an inductively-coupled plasma atomic spectrophotometer in accordance with the previous studies conducted by Nelson et al.,¹ Oyar et al.,⁵ Mutlu-Sagesen et al.,⁶ Wataha et al.,⁹ Denizoglu et al.,¹² Geis-Gerstorfer et al.,¹⁷ Can et al.,²⁰ Geis-Gerstorfer et al.,²¹ Galo et al.,²² Geis-Gerstorfer et al.²³ and Stipetic et al.¹¹

The data was statistically analysed using SPSS (21.0 version). Analysis was performed using the parametric tests i.e. One way ANOVA and Unpaired Student's T-test. Level of statistical significance was set at p-value less than 0.05.

Results

The results of the present study showed that when elemental ion release from alloy A at pH 4.0, 6.7 and 8.0 in various time intervals of immersion period was evaluated (table 1), nickel was released the most at all pH of immersion media followed by chromium although maximum release was observed at pH 4.0 in 1 month.

Likewise, the elemental ion release from Alloy B at pH 4.0, 6.7 and 8.0 in various time intervals of immersion period (table 2) demonstrated that iron was released the most followed by nickel at all pH of immersion media. However maximum release was seen at pH 4.0 in 1 month.

The intra-group comparison of elemental ion release from alloy A at pH 4.0 at 1 hour and 1 month and 1 day and 1 month using unpaired Student's t-test showed statistically significant (p<0.05) results. Significant increase in elemental ion release was observed at 1 month compared to 1 day and 1 hour. However, no significant difference in elemental ion release was observed at 1 day compared to 1 hour.

The intra-group comparison of elemental ion release from alloy A at pH 6.7 and 8.0 at 1 hour and 1 day, 1 hour and 1 month and 1 day and 1 month using unpaired Student's t-test showed statistically insignificant (p>0.05) results. Similar trend was reported for alloy B for 3 different pH of immersion media for different intervals of time.

When subjected to one-way ANOVA, the intergroup comparison of the elemental ion release using from alloy A at pH 4.0 (Group I), 6.7 (Group II) and 8.0 (Group III) in 1 hour (table 3) showed statistically significant results (p<0.05). The maximum elemental ion release was observed in group I followed by group III and least in group II. Similar trends were observed at 1 day (table 4) and 1 month (table 5) for different pH of immersion media.

Likewise, for alloy B, when subjected to one-way ANOVA, the

intergroup comparison of the ionic release of major elements at pH 4.0 (Group I), 6.7 (Group II) and 8.0 (Group III) in 1 hour (table 6) showed statistically significant results ($p < 0.05$). The maximum elemental ion release was observed in group I followed by group III and least in group II. Similar trends were observed at 1 day (table 7) and 1 month (table 8) for different pH of immersion media. However, the release of the minor elements from alloy B for all three groups at various intervals of time showed statistically insignificant results ($p > 0.05$).

Discussion

The null hypothesis of the present study that there was no effect of pH of immersion media and length of immersion period on the elemental ion release from commercially available Ni-Cr alloys was partially rejected as statistically significant increase in elemental ion release was seen at acidic pH (pH 4.0) as compared to pH 6.7 and 8.0 at different time intervals. However no statistically significant difference in elemental ion release was found at pH 6.7 and 8.0 at different time intervals. Significant release of elements was seen at one month when compared to one hour and one day at pH 4.0. However there was no statistically significant difference in elemental ion release for different lengths of immersion period at pH 6.7 and 8.0.

The evaluation of elemental ion release between Group I (pH 4.0), II (pH 6.7) and III (pH 8.0) of alloy A showed statistically significant difference in the elemental ion release at varying time intervals. At all three time intervals, it was seen that the maximum elemental ion release was seen at pH 4.0 followed by pH 8.0 and the least was seen at pH 6.7. Similarly while comparing Group I, II and III of alloy B, it was seen that there was a statistically significant increase in the release of the major elements at pH 4.0 followed by pH 8.0. The least elemental ion release was seen at pH 6.7 at all three time intervals. As minor elements contributed to less than 1 % of the composition of alloy, there was no significant difference in the elemental ion release of the minor elements present in Alloy B. The inference that increase in elemental ion release with decrease in the pH of the immersion media can be attributed to the fact that acidic environments leads to shift of equilibrium towards unfavourable conditions that generally lower the polarization resistance and enhances the release of ions from metal alloys. Studies have documented that electrochemical corrosion is more pronounced in acidic environments and is accelerated by the presence of chloride ions which can be present in the oral cavity.²⁴ Galvanic corrosion may occur due to heterogenous composition of the surface of dental alloys. The corrosion resistance of a multiphase alloy is less than that of a single phase solid solution owing to the differences in electrode potential caused by microsegregation and variations in composition between individual dendritic microstructures. In acidic pH it is possible that the alloy surface also plays an important role in enhanced elemental ion release from alloys leading to accelerated corrosion; for example, lack of polishing may increase micro-porosity.^{10,11} Moreover, concentration cell corrosion may also be evident due to the difference in the oxygen concentration in different parts of the restoration. Intra-orally, the parts of the restoration over which there is evidence of food and plaque accumulation has lower oxygen concentration which acts as the anode compared to the rest of the restoration that acts as the cathode. The acidic pH of the saliva acts as the electrolyte leading to accelerated elemental ion release from the anodes. The results of the present study are in accordance with the previous studies conducted by Oyar et al.,⁵ Stipetic et al.,¹¹ Wataha et al.,¹⁹ Geis-Gerstorfer et al.,²¹ Mutlu-Sagesen et al.,⁶ Wylie et al.,¹⁵ Nelson et al.,¹ Lopez alias et al.,² Elshahawy et al.,³ Can et al.,²⁰ Beck et al.,¹⁶ Al-hity et al.,¹⁰ Tamam et al.,²⁵ Wataha et al.,⁹ Convington et al.,²⁴ He et al.⁸ However, the results are in contradiction to the study conducted by Denizoglu et al.¹² who reported that there is no effect of pH on the elemental ion release from Ni-Cr alloys.

The normal physiologic pH of resting saliva is pH of 6.7 and is least reactive in the oral cavity. Therefore, the least elemental ion release from the Ni-Cr alloys was observed at this pH. Alkaline pH led to elemental ion release that was less than that seen in acidic pH but was more compared to the resting pH of saliva. Although there is lack of scientific evidence in this regard, it can be attributed to the change in the alloy microstructure occurring due to leaching of ions as pH shifts away from the normal salivary resting pH.

In the present study, a statistically significant increase ($p < 0.05$) in the elemental ion release was seen in 1 month (Group Ic) compared to 1 day (Group Ib) and 1 hour (Group Ia) at pH 4.0. Although group Ib (1 day) showed more elemental ion release compared to Group Ia (1

hour), there was no statistically significant difference in the elemental ion release between Group Ia and Ib. This trend is in accordance with previous studies conducted by Oyar et al.,⁵ Convington et al.,²⁴ Denizoglu et al.,¹² Stipetic et al.,¹¹ Wataha et al.,⁹ Pangi et al.,²⁶ Mutlu-Sagesen et al.⁶ and Tamam et al.²⁵ Increased elemental ion release with increase in the length of immersion media was due to long term effect of the reduced pH on the alloys that may cause a change in the alloy microstructure leading to dissolution of ions from the alloy surface. Hence, there is a synergistic effect of reduced pH on the elemental ion release in 1 month compared to 1 day. However conflicting findings were observed by Lockwood et al.,¹⁹ Geis-Gerstorfer et al.,²² and Estill et al.¹⁸ who reported that initial elemental ion release was maximum followed by a gradual decrease with increase in length of immersion period. They hypothesized that elemental release should decrease as a function of time of exposure to the medium owing to the formation of passive oxide layer formation on the surface of the alloy. Moreover, as the ionic concentration of an element increases in the environment, the tendency for that element to dissolve decreases. Hence, cytotoxic effects of the alloys should decrease as a function of time.

When comparing the elemental ion release from Alloy A as well as Alloy B for different time intervals at pH 6.7, maximum elemental ion release was seen with group IIc (1 month) followed by IIb (1 day) and IIa (1 hour), though there was no statistically significant difference between them. As mentioned earlier that pH 6.7 is the least reactive in the oral cavity, therefore even though the ion release was maximum for one month compared to 1 hour and 1 day, the values did not vary so much so as to be statistically significant. This was in accordance with studies conducted by Oyar et al.,⁵ Mutlu-Sagesen et al.,⁶ Wataha et al.⁹ and Wylie et al.¹⁵

On evaluation of the elemental ion release from Alloy A as well as Alloy B for different time intervals at pH 8.0, maximum elemental ion release was seen with group IIIc (1 month) followed by IIIb (1 day) and IIIa (1 hour), though there was no statistically significant difference between them. This can be attributed to increased elemental ion release as pH shifts away from the normal resting pH of saliva. Also, alkaline pH is less reactive as compared to acidic pH, hence the total elemental ion release in different time intervals is statistically insignificant in comparison to acidic pH.

In the present study, nickel released in 1 day at pH 4.0 was 1.709 $\mu\text{g/L}$ for Alloy A and 2.856 $\mu\text{g/L}$ for Alloy B. The maximum release of Ni seen in one month was 14.125 $\mu\text{g/L}$ for Alloy A and 15.255 $\mu\text{g/L}$ for Alloy B. The tolerable upper intake limit of nickel is 1 mg/day. Hence, the quantity of release was far below the daily intake threshold.⁴ However, Wataha et al.¹⁹ reported that the release of some elements like nickel can approach dietary levels. The readiness with which nickel may induce carcinogenicity depends on its solubility and the form in which it involves the tissue. Nickel carbonyl, nickel subsulfide, and nickel sulfide have been shown to produce carcinogenesis.¹⁶ Also, nickel is one of the most potent allergens and can lead to hypersensitivity reactions at any dose since allergenicity is dose independent. Information on the acute oral toxicity of nickel in humans comes from reports of accidental exposures and studies of nickel-sensitized individuals. Gastrointestinal upset (vomiting, cramps, diarrhoea) and neurological symptoms (giddiness, headache, weariness) were observed in workers accidentally ingesting water containing approximately 7.1–35.7 mg Ni/kg in the form of nickel sulphate and nickel chloride. A number of animal studies have assessed the toxicity of nickel following intermediate-duration oral exposure. Significant decrease in body weight and organ weight (liver, kidney, pituitary) were consistently observed in rats exposed to 8.6 mg Ni/kg/day or higher doses in the form of nickel chloride, nickel acetate, or nickel sulphate. Other systemic effects included kidney damage (minimal convoluted tubular damage) at 108 mg Ni/kg/day in the form of nickel sulphate and adverse lung effects at 8.6 and 20 mg Ni/kg/day in the form of nickel chloride. Inconsistent results have been reported for the reproductive toxicity of nickel. Decreased sperm motility and count and sperm abnormalities were observed at 1.9 mg Ni/kg/day and higher doses in the form of nickel sulphate and decreased fertility was observed in studies where subjects were exposed to 3.6 mg Ni/kg/day in the form of nickel chloride.²⁷

The release of chromium was found to be 1.515 $\mu\text{g/L}$ for Alloy A and 1.395 $\mu\text{g/L}$ for Alloy B in 1 day at pH 4.0. The quantity of chromium released in 1 month at pH 4.0 from Alloy A and Alloy B was estimated at 4.832 $\mu\text{g/L}$ and 5.688 $\mu\text{g/L}$ respectively which was very less in

comparison to the lethal dose of chromium. Acute oral toxicity of chromium ranges between 1900 µg/kg and 3300 µg/kg. Tai et al.¹⁷ has reported that chromium in the form of chromate salts in concentrations of 885 ppm were sufficient to cause positive patch tests in patients. The oral exposure of humans to lethal (1-3 g) or near-lethal doses of chromium (VI) has produced adverse gastrointestinal effects, including abdominal pain, vomiting, gastrointestinal ulceration, haemorrhage and necrosis, and bloody diarrhoea.²⁸ Several in vitro studies indicated that high concentrations of chromium (III) in the cell can lead to DNA damage.²⁹

The quantity of molybdenum released from Alloy A and Alloy B in 1 day at pH 4.0 was 0.91 µg/L and 1.385 µg/L respectively. The molybdenum release from Alloy A in 1 month at pH 4.0 was estimated to be 4.97 µg/L and that of Alloy B to be 4.078 µg/L. The recommended dietary allowance of molybdenum in human is 45 µg/day. The tolerable upper intake level is 2 mg/day for molybdenum. Therefore, the amount of molybdenum released from the alloys used in the present study even over a period of one month is not toxic to the human body.²⁷

The minor elements released from Alloy B were iron and manganese. The elemental ion release of iron was found to be 12.172 µg/L in one day and 35.011 µg/L in one month at a pH of 4.0. The recommended dietary allowance of iron for all age groups of men and postmenopausal women is 8 mg/day; the recommended dietary allowance for premenopausal women is 18 mg/day. The tolerable upper intake level for adults is 45 mg/day of iron, a level based on gastrointestinal distress as an adverse effect. Hence the total elemental ion release of iron in one month was below the tolerable upper intake level of adults. Release of manganese was estimated at 0.341 µg/L in one day and 1.524 µg/L in one month at pH 4.0. Tolerable upper intake level of 11 mg/day was set for adults based on a no-observed-adverse-effect level for Western diets for manganese. Thus the elemental ion release of manganese was far below the upper intake limit.²⁷

The clinical implications of the results of the present study is that though the levels of elemental ion release from the Ni-Cr alloys used in the study are far below the tolerable upper intake level, a cumulative effect of released ions over a long period of time may render these alloys toxic. Moreover, clinical situations such as full mouth rehabilitation cases may require multiple crowns. In such cases, the total ions released over a period of time will be more as compared to a single crown. Furthermore, allergenicity of Ni is dose independent. Hence even minor amount of elemental ion release of Ni from the alloys may trigger an allergic reaction. Therefore, it is questionable whether the Ni-Cr alloys that are regularly used for fabrication of fixed dental prosthesis are biocompatible in the true sense.

However, there are certain limitations in the present study. Natural saliva is a complex system with numerous constituents and variables according to the time of day. The dynamic nature of intra oral conditions extends beyond a simple reduction in pH. Ni-Cr alloys may undergo accelerated corrosion process in the oral environment with fluctuations of quantity and quality of saliva, temperature and pH changes owing to variations in the diet, healthy or diseased states oral hygiene and polishing of the alloy. Moreover, alloys also experience mechanical disruption from occlusal forces and tooth brushing. The role of these dynamic conditions on the elemental ion release were not considered in the present study. It should also be kept in mind that no fewer than 60 formulae for artificial saliva solutions have been reported in literature which reveals the relative difficulty to optimize the simulation of the chemical conditions pertaining in the oral cavity. Thus, exact duplication of saliva was not possible in the present study.

Further in vivo studies can be conducted mimicking the dynamic nature of the oral cavity. The results of the in vitro studies should be correlated with clinical data. Long term studies are needed to assess the elemental ion release from Ni-Cr alloys and other base metal alloys that are used regularly for fabrication of fixed dental prosthesis.

Conclusion

Within the limitations of the study, following conclusions were drawn:

- Highest elemental ion release was seen at pH 4.0 followed by pH 8.0 and least at pH 6.7 at different time intervals for both types of commercially available Ni-Cr alloys. Hence, decrease in pH of immersion media had a significant effect on the elemental ion release from these alloys.
- Highest elemental ion release was seen at 1 month followed by 1

day and least at 1 hour for both the alloys. The difference in elemental ion release was significant in 1 month when compared to 1 day and 1 hour at pH 4.0 only. Hence, increase in length of immersion period had a significant effect on the elemental ion release from the two commercially available Ni-Cr alloys used.

TABLES:

Table 1: Elemental ion release from alloy A at pH 4.0, 6.7 and 8.0 at various time intervals

Elements released	pH	1 hour	1 day	1 month
Ni	4	1.515	1.709	14.125
Cr		1.463	1.515	4.832
Mo		0.847	0.91	4.97
Si		0.035	0.205	3.87
Ni	6.7	1.369	0.498	3.024
Cr		0.093	0.03	0.512
Mo		0.043	0.151	0.066
Si		0.074	0.012	0.161
Ni	8.0	0.429	0.796	4.312
Cr		0.477	0.477	0.202
Mo		0.084	0.084	0.012
Si		0.012	0.012	0.113

Table 2: Elemental ion release from alloy B at pH 4.0, 6.7 and 8.0 at various time intervals

Elements released	pH	1 hour	1 day	1 month	
Ni	4.0	1.908	2.856	15.255	
Cr		0.242	1.395	5.688	
Mo		0.058	1.385	4.078	
Si		0.027	0.032	3.28	
Mn		0.231	0.341	1.524	
Nb		0.447	0.129	0.482	
Fe		13.611	12.172	35.011	
Ni		6.7	0.422	0.548	2.251
Cr			0.08	0.019	0.511
Mo			0.68	0.108	0.024
Si			0.009	0.032	0.19
Mn			0.057	0.029	1.265
Nb			0.095	0.073	0.321
Fe	1.073		0.619	25.522	
Si	8.0			1.234	3.51
Cr			0.3	0.267	0.621
Mo			0.104	0.162	0.23
Si			0.035	0.108	0.12
Mn			0.076	0.104	1.22
Nb			0.034	0.056	0.352
Fe		2.398	2.78	19.621	

Table 3: Inter-group comparison of elemental ion release from alloy A in Group I, Group II and Group III in 1 hour using One way ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.592671	2	0.796336	4.303427	0.04881	4.25649
Within Groups	1.665422	9	0.185047		2	5
Total	3.258093	11				

SS=Sum square; df=Degrees of freedom; MS=Mean of squares; F=F test statistic; 'p'-value=level of significance

Table 4: Inter-group comparison of elemental ion release from alloy A in Group I, Group II and Group III in 1 day using One way ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.008385	2	1.004192	4.776384	0.03857	4.256495
Within Groups	1.89217	9	0.210241			
Total	3.900555	11				

SS=Sum square; df=Degrees of freedom; MS=Mean of squares; F=F test statistic; 'p'-value=level of significance

Table 5: Inter-group comparison of elemental ion release from alloy A in Group I, Group II and Group III in 1 month using One way ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	93.1168	2	46.5584	4.744854	0.039166	4.256495
Within Groups	88.3116	9	9.8124			
Total	181.4284	11				

SS=Sum square; df=Degrees of freedom; MS=Mean of squares; F=F test statistic; 'p'-value=level of significance

Table 6: Inter-group comparison of elemental ion release of major elements from Alloy B in Group I, Group II and Group III in 1 hour using One way ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.024822	2	1.012411	5.754472	0.040241	5.143253
Within Groups	1.055608	6	0.175935			
Total	3.08043	8				

SS=Sum square; df=Degrees of freedom; MS=Mean of squares; F=F test statistic; 'p'-value=level of significance

Table 7: Inter-group comparison of elemental ion release of major elements from Alloy B Group I, Group II and Group III in 1 day using One way ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4.596933	2	2.298466	6.017696	0.036819	5.143253
Within Groups	2.291707	6	0.381951			
Total	6.88864	8				

SS=Sum square; df=Degrees of freedom; MS=Mean of squares; F=F test statistic; 'p'-value=level of significance

Table 8: Inter-group comparison of elemental ion release of major elements from Alloy B Group I, Group II and Group III in 1 month using One way ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	100.9178	2	50.45888	4.403724	0.046386	4.256495
Within Groups	103.1241	9	11.45823			
Total	204.0418	11				

SS=Sum square; df=Degrees of freedom; MS=Mean of squares; F=F test statistic; 'p'-value=level of significance

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