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Tribological study of metal alloys subject to dental wear

Tese no âmbito do Programa Doutoral em Ciências da Saúde, ramo de Ciências Biomédicas orientada pelo Professor Doutor Pedro Miguel Gomes Nicolau e pelo Professor Doutor Fernando Alberto Deométrio Rodrigues Alves Guerra e apresentada à Faculdade de Medicina da Universidade de Coimbra

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Tribological study of metal alloys subject to dental wear

Doctoral Thesis in the Doctoral Program in Health Sciences branch of Biomedical Sciences under the supervision of Professor Pedro Miguel Gomes Nicolau and Professor Fernando Alberto Deométrio Rodrigues Alves Guerra and presented to the Faculty of Medicine of the University of Coimbra.

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À minha Mãe,
minha estrela-guia.

“Recomeça...
Se puderes
Sem angústia
E sem pressa.
E os passos que deres,
Nesse caminho duro
Do futuro
Dá-os em liberdade.
Enquanto não alcances
Não descanses.
De nenhum fruto queiras só metade.

E, nunca saciado,
Vai colhendo ilusões sucessivas no pomar.
Sempre a sonhar e vendo
O logro da aventura.
És homem, não te esqueças!
Só é tua a loucura
Onde, com lucidez, te reconheças...”

Miguel Torga, Sísifo, in “Diário XIII”

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RESUMO

As ligas metálicas utilizadas nos componentes das próteses que são utilizadas na reabilitação oral de pacientes desdentados, estão sujeitas a fenómenos corrosivos, tais como a tribocorrosão e a corrosão galvânica, devido às forças a que estão sujeitas e à coexistência de diferentes tipos de ligas metálicas sob a ação da saliva. Esta situação pode originar falência das reabilitações protéticas e libertação para o organismo de diferentes tipos de iões metálicos.

Neste trabalho efetuaram-se três estudos in vitro: 1) a avaliação das propriedades tribológicas da liga Ti-6Al-4V; 2) caracterização da difusão dos iões alumínio e vanádio e 3) caracterização da difusão dos iões cobalto e cromo.

No estudo da avaliação das propriedades tribológicas, amostras de Ti-6Al-4V foram sujeitas a desgaste num equipamento de deslizamento alternativo, por um contra-corpo de alumina e uma carga de 15N em saliva artificial e saliva artificial fluoretada. Avaliou-se se o desgaste era influenciado pela coexistência de amostras de amálgama dentária testadas simultaneamente e pela presença de flúor. Os parâmetros testados foram a dureza das ligas, a rugosidade superficial, a perda de massa e o volume das pistas de desgaste. Verificámos que o desgaste das amostras Ti-6Al-4V aumenta significativamente quando testadas simultaneamente com amostras de amálgama dentária em presença de flúor. Dos resultados obtidos podemos concluir que a liga de Ti-6Al-4V não deve ser utilizada em zonas sujeitas a desgaste e em que possa estar em contacto com substâncias contendo flúor, especialmente se restaurações em amálgama também estiverem presentes.

Devido à comprovada libertação de iões para o meio, resultantes do desgaste e corrosão dessas ligas, com inerentes consequências a nível local e sistémico, através de um modelo experimental baseado na técnica de dispersão de Taylor, realizámos dois estudos envolvendo diferentes iões metálicos: alumínio e vanádio provenientes das ligas de titânio-alumínio-vanádio, e cobalto e cromo, provenientes das ligas de cobalto e cromo. Nestes dois estudos experimentais foram avaliados os coeficientes de difusão mútua vestigiais dessas espécies iónicas em diferentes meios, tais como saliva artificial com e sem flúor, a diferentes pH, e em digluconato de clorexidina, a 298.15 K. Foram também medidos os coeficientes de difusão mútua de digluconato de clorexidina em soluções aquosas a diferentes concentrações.

Verificou-se que os coeficientes de difusão vestigiais para os iões estudados sofrem variações em função do pH do meio.

Constatou-se também uma significativa interação entre os dois fluxos envolvidos (componente iónico + digluconato de clorexidina), explicando-se assim a obtenção de elevados valores negativos para os coeficientes de difusão D_{21}. Os estudos de difusão mútua foram complementados com outros estudos teóricos e experimentais, nomeadamente simulações de dinâmica molecular, no caso do alumínio e espectroscopia UV-Vis para o cromo e o cobalto. As interações observadas entre os iões metálicos de alumínio, cromo e cobalto, e o digluconato de clorexidina sugeriram que este composto farmacológico pode ser considerado um agente terapêutico vantajoso, pois pode contribuir para a redução da concentração desses iões libertados na cavidade oral.

Palavras-chave

Ligas dentárias

Difusão mútua

Ligas de titânio

Tribocorrosão

Desgaste

ABSTRACT

The metal alloys used in the components of prostheses that are used in the oral rehabilitation of edentulous patients are subject to corrosive phenomena, such as tribocorrosion and galvanic corrosion, due to the forces to which they are subjected and the coexistence of different types of dental alloys, under the action of saliva. This situation can lead to failure of prosthetic rehabilitations and release in the body of different types of metal ions.

In this work, three in vitro studies were carried out: 1) the evaluation of the tribological properties of the Ti-6Al-4V alloy; 2) characterization of the diffusion of aluminium and vanadium ions and 3) characterization of the diffusion of cobalt and chromium ions.

In the study that evaluated the tribological properties, samples of Ti-6Al-4V were subjected to wear in an alternative sliding device, by an alumina counter-body and under a load of 15N in artificial saliva and fluoridated artificial saliva. We evaluated whether wear was influenced by the coexistence of dental amalgam samples tested simultaneously and by the presence of fluoride. The parameters tested were the hardness of the alloys, the surface roughness, the mass loss and the volume of the wear tracks. We found that the wear of Ti-6Al-4V samples increases significantly when tested simultaneously with dental amalgam samples in the presence of fluoride. From the results obtained, we can conclude that the Ti-6Al-4V alloy should not be used in areas subjected to wear, where it may be in contact with substances containing fluoride, especially if amalgam restorations are also present.

Due to the proven release of ions to the environment, resulting from the wear and corrosion of these alloys, with inherent consequences at a local and systemic level, through an experimental model based on the Taylor dispersion technique, we carried out two studies involving different metallic ions: aluminium and vanadium from titanium-aluminium-vanadium alloys, and cobalt and chromium from cobalt-chromium alloys. In these two experimental studies, the tracer mutual diffusion coefficients of these ionic species in different media, such as artificial saliva with and without fluoride, were evaluated, at different pH, and in chlorhexidine digluconate, at 298.15 K. The mutual diffusion coefficients of chlorhexidine digluconate in aqueous solutions at different concentrations were also measured.

It was found that the tracer diffusion coefficients for the studied ions vary as a function of the pH of the medium.

There was also a significant interaction between the two fluxes involved (ionic component + chlorhexidine digluconate), which explains the high negative values obtained for the D_{21} diffusion coefficients.

The mutual diffusion studies were complemented with other theoretical and experimental studies, namely, molecular dynamics simulations for aluminium and UV-Vis spectroscopy for chromium and cobalt. The interactions observed between the aluminium, chromium and cobalt metal ions, and chlorhexidine digluconate suggested that this pharmacological compound can be considered an advantageous therapeutic agent, because can contribute to the reduction of the concentration of these ions released in the oral cavity.

Keywords

Dental alloys
Mutual diffusion
Titanium alloys
Tribocorrosion
Wear

List of Publications, Oral and Posters Communications

Within the scope of this thesis, the following full **papers** were published in international peer-review journals:

1. Fangaia, S. I. G., Nicolau, P. M. G., Guerra, F. A. D. R. A., Rodrigo, M. M., Ribeiro, A. C. F., Valente, A. J. M., Santos, J. R. C., Marques, J. M. C., & Esteso, M.A. (2022). **The behaviour of aluminium ions in artificial saliva and the impact of the chlorhexidine digluconate on its removal – A diffusion model.** *Journal of Molecular Liquids, 353,* 118749. https://doi.org/10.1016/j.molliq.2022.118749

2. Fangaia, S. I. G., Nicolau, P. M. G., Guerra, F. A. D. R. A., Rodrigo, M. M., Utzeri, G., Cabral, A. M. T. D. P. v., Valente, A. J. M., Esteso, M. A., & Ribeiro, A. C. F. (2021). **Effect of Cobalt and Chromium Ions on the Chlorhexidine Digluconate as Seen by Intermolecular Diffusion.** *International Journal of Molecular Sciences, 22(24),* 13266. https://doi.org/10.3390/ijms222413266

3. Ribeiro, A. C. F., Barros, M. C. F., Lobo, V. M. M., Sobral, A. J. F. N., Fangaia, S. I. G., Nicolau, P. M. G., Guerra, F. A. D. R. A., & Esteso, M. A. (2011). **Interaction between calcium chloride and some carbohydrates as seen by mutual diffusion at 25°C and 37°C.** *Food Chemistry, 124(3),* 842–849. https://doi.org/10.1016/j.foodchem.2010.07.005

Within the scope of this thesis, the following **chapters** were published in books:

1. Fangaia, S. I. G., Nicolau, P. M. G., Guerra, F. A. D. R. A., Lobo, V. M. M., & Ribeiro, A. C. F. (2021). **Effect of Light on Transport of Potassium Thiocyanate in Aqueous Solutions.** In *Applied Pharmaceutical Practice and Nutraceuticals*. Apple Academic Press. https://doi.org/10.1201/9781003054894-12

2. Ribeiro, A., Victor, L., Valente, A., Cabral, A., Veiga, F., Fangaia, S., Nicolau, P., Guerra, F., & Esteso, M. (2012). **"Transport properties and their impact on biological systems."** In J.C Taylor (Ed.), *Advances in Chemistry Research* (Vol. 10, pp. 379–391). Nova Publ.

From the data of this thesis, the following **abstract** was published in a scientific journal:

Fangaia, S. I. G., Ribeiro, A. C. F., Valente, A. J. M., Esteso, M. A., Guerra, F. A. D. R. A., & Nicolau, P. M. G. (2022). **Comportamento de iões metálicos cromo e cobalto – Coeficientes de difusão mútua.** *Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial, 62(s1).*
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From the scope of this thesis, the following **oral communications** were presented in national or international scientific meetings:

1. **Avaliação qualitativa com microscopia SEM de duas ligas metálicas sujeitas a desgaste.** Sónia Fangaia, Ana Ribeiro, Fernando Guerra, Pedro Nicolau, XX Reunião Anual de Medicina Dentária e Estomatologia de Coimbra e do 1º Encontro Internacional de Implantologia de Coimbra. Centro de Congressos dos Hospitais da Universidade e Coimbra, 2011.

2. **Mutual Diffusion and their Impact on Biological Systems.** Ana Ribeiro, Vítor Lobo, Ana Cabral, Francisco Veiga, Sónia Fangaia, Pedro Nicolau, Fernando Guerra, Miguel Esteso, XI Encontro Ibérico de Electroquímica, Adeje, Tenerife (Espanha), 2009.

3. **Comportamento de ligas metálicas sujeitas a desgaste- estudo in vitro.** Sónia Fangaia, Fernando Guerra, Pedro Nicolau, XV Reunião Anual e Medicina Dentária e Estomatologia, Coimbra, 2006.

4. **A ação do flúor na reabilitação oral protética.** Sónia Fangaia, Rita Reis, Salomão Rocha, Pedro Nicolau, XII Congresso Anual da Ordem dos Médicos Dentistas, Santa Maria da Feira, 2003.

5. **Comportamento corrosivo do Titânio na cavidade oral em contacto com outras ligas metálicas.** Sónia Fangaia, Rita Reis, Salomão Rocha, Pedro Nicolau, XI Congresso Anual da Ordem dos Médicos Dentistas, Lisboa, 2002.

From the scope of this thesis were presented in scientific meetings the following **poster communications**:

1. **Cobalt-chromium Dental Alloys: Concern about the Toxic Impact of Ions released in the Oral Cavity analyzed by Diffusion Measurements.** Sónia I.G. Fangaia, Ana C.F. Ribeiro, Artur J.M. Valente, Miguel A. Esteso, Melia Rodrigo, Fernando A.D.R.A. Guerra, Pedro M.G. Nicolau, 7th International Iberian Biophysics Congress, 2021.

2. **Comportamento de iões metálicos cromo e cobalto: coeficientes de difusão mútua.** Sónia I.G. Fangaia, Ana C.F. Ribeiro, Artur J.M. Valente, Miguel A. Esteso, Fernando A.D.R.A. Guerra ,Pedro M.G. Nicolau, XLI Congresso anual da SPEMD, 2021.

3. **Diffusion behaviour of aluminium and vanadium metal ions present in dental prosthesis, in different environments.** Víctor M.M. Lobo, Sónia I.G. Fangaia, Pedro M.G. Nicolau, Fernando A.D.R.A. Guerra, Melia Rodrigo, Ana C.F. Ribeiro, Artur J.M. Valente, Miguel A. Esteso, XXIV Congresso da sociedade Portuguesa de Electroquímica, Tomar, 2021.

4. **Diffusion of aluminium and vanadium metal ions, in different environments.** Sónia I.G. Fangaia, Pedro M.G. Nicolau, Fernando A.D.R.A. Guerra, M. Melia Rodrigo, Ana C.F. Ribeiro, Artur J.M. Valente, Jorge M.C. Marques, Joana R.C. Santos, Miguel A. Esteso, Ana M.T.D.P.V. Cabral, Víctor M.M. Lobo, Coimbra Chemistry Centre Day, Coimbra, 2021.

5. **Transport Properties of Calcium Chloride with some Carbohydrates at 298.15 K and 310.15 K.** Ana Ribeiro, Cecília Santos, Miguel Esteso, Vítor Lobo, Abílio Sobral, Sónia Fangaia, Pedro Nicolau, Fernando Guerra, Marisa Barros, XI Encontro Ibérico de Electroquímica, Adeje, Tenerife (Espanha), 2009.

6. **Avaliação da microdureza de Vickers em duas ligas metálicas.** Sónia Fangaia, Pedro Nicolau, XVI Reunião Anual de Medicina Dentária e Estomatologia, Coimbra, 2007.

Table of contents

List of abbreviations and list of symbols

List of abbreviations

ADA	American Dental Association
AAS	Atomic Absorption Spectroscopy
AINSI	American National Standards Institute
AS	artificial saliva
BCC	body-centered cubic
cf	commercial formulation
CHDG	chlorhexidine digluconate
cp	commercially pure
ECHA	European Chemicals Agency
EIS	Electrochemical Impedance Spectroscopy
FCC	face-centered cubic
GLU	gluconate
HCP	hexagonal close packed
HPLC	High Performance Liquid Chromatography
ICP	Inductively Coupled Plasma
ISO	International Standardization Organization
IUPAC	International Union of Pure and Applied Chemistry
MD	Molecular Dynamics
NMR	Nuclear Magnetic Resonance
PBS	phosphate buffer saline
PTFE	polytetrafluoroethylene
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RPD	removable partial denture
rpm	revolutions per minute
SVHC	Substances of Very High Concern

List of symbols

Ag	Silver
Al	Aluminium
Au	Gold
Be	Beryllium
C	Carbon
C	molar concentration
Cl	Chlorine
Co	Cobalt
cp	commercially pure
Cr	Chromium
Cu	Copper
D	diffusion coefficient
D_0	tracer diffusion coefficient
Fe	Iron
GPa	gigapascal
HV	hardness Vickers
In	Indium
Ir	Iridium
λ	wavelength
J	molar flux
mA	miliampere
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
MPa	megapascal
Ni	Nickel
K	Kelvin
k	Boltzman constant
kPa	kilopascal
keV	kiloeletronvolt
kV	kilovolts
N	Newton
N	Nitrogen
Nb	Niobium

n	number of experiments
HVN	Vickers hardness number
O	Oxygen
P	pressure
Pd	Palladium
Pt	Platinum
ppm	parts per million
R	detector sensitivities
Ra	arithmetic average height
Rz	ten-point height
S	standard deviation
Sn	Tin
$\boldsymbol{t}$	time
$\boldsymbol{t_R}$	retention time
$\boldsymbol{T}$	absolute temperature
Ti	Titanium
$\boldsymbol{u}$	standard uncertainties
V	Vanadium
V	voltage
$\boldsymbol{V_0}$	base line voltage
$\boldsymbol{V_1}$	base line slope
$\boldsymbol{V_{max}}$	peak height
$\nabla \boldsymbol{c}$	gradient in the concentration of solute
W	Tungsten
Wt%	percentage by weight
$\boldsymbol{X}$	molar fraction
Zr	Zirconium

Preface

The oral rehabilitation of a patient with missing teeth remains a major challenge in the clinical practice of a dentist. Although there is currently a wide range of materials available, the choice is not always easy and obvious.

When we carry out the clinical examination of a previously rehabilitated patient, it is common to find the use of different types of metal alloys; knowing that metallic alloys have different electrochemical and mechanical properties, it seems to us that their coexistence in the oral cavity should not be taken lightly.

It is not unusual to have patients rehabilitated with pure titanium dental implants with Titanium-Aluminium-Vanadium superstructures and that eventually, on the antagonist arch have a removable prosthesis with a Cobalt-Chromium framework supported on a tooth restored with a dental amalgam and another with a porcelain fused-metal crown made of a Nickel-Chromium alloy.

Although dental implants are manufactured from pure titanium or one of his alloys, the choice for the material of the superstructure can be more diverse and does not always take into account all the biological and mechanical factors required in terms of clinical performance or biocompatibility.

The electrochemical corrosion suffered by metallic alloys is widely described in the literature, however there is a gap concerning the behavior of different dental alloys concomitantly in a dynamic environment when subjected to forces similar to those that occur during chewing. Although there are some studies, none of them that we know of can simulate the specific conditions existing in the oral cavity.

Therefore, the coexistence of different metal alloys in the oral cavity associated with tooth wear caused by chewing is a matter of concern to us and the great motivation for this work.

It is predictable that in these situations there is a risk of failure of restorations and release into the body different types of metal ions, with health and economic consequences.

The metallic particles, released from the prosthetic devices, are a matter of great concern; they potentially follow two paths, pass through the oral mucosa, or are ingested and eventually accumulate in different organs at a distance, both of these situations may have serious health implications.

So, all the scientific evidence that we can bring about on this topic is of great importance to supplying the scientific and technological communities with data that facilitate the choice of materials to use in a rehabilitation, as well as a great benefit to patients, in the maintenance of their oral and systemic health, providing rehabilitations with greater longevity without causing any adverse, toxic or carcinogenic reactions to the body.

Outline of the thesis

This thesis comprises two main paths for research, such as an experimental study about the tribological properties of the Ti-6Al-4V and the analysis of the diffusion behavior of metal ions in different mediums and is structured into 6 chapters:

The **first chapter** starts with a review of dental alloys and the events related to their existence in oral cavity such as corrosion, wear and diffusion of metal ions released by them.

The **second chapter** describes an in vitro study that analizes the structure of some metallic alloys in different solutions (artificial saliva and fluoridated artificial saliva) after being subjected to wear in a sliding equipment designed for the effect, and was written in a scientific paper format organized into five parts: Introduction, Materials and Methods, Results, Discussion, and Conclusions,

The **third** and **fourth chapters** are dedicated to the transport properties of metal ions released from prosthetic devices, in particular to mutual diffusion.

These two chapters are both written in the format of a scientific paper.

On third chapter, through an experimental model based on the Taylor dispersion technique, the behaviour of aluminium and vanadium ions, present in Ti-6Al-4V alloys, was assessed in different mediums. The interaction of aluminium ion with chlorhexidine digluconate was also clarified, resorting as well to Dynamics Molecular Simulations.

Considering that Co-Cr alloys are also widely used in oral rehabilitation; and due to the potential toxicity of the cobalt and chromium ions, in fourth chapter we evaluated the diffusion coefficients of these ions using the Taylor dispersion technique. This study also analysed the UV-visible spectroscopy to assess the interaction between cobalt and chromium ions and chlorhexidine digluconate.

Finally, the **fifth chapter** intends to aggregate the information obtained on both paths of research and to present the main conclusions, as well as to suggest future perspectives.

The last chapter presents the bibliographic support for the present investigation.

Chapter 1

Introduction

Metal alloys have been applied in the most diverse areas of Dentistry for decades, we can find them in Orthodontics, Fixed and Removable Prosthodontics, Implantology and as a restorative material. They are also used in other medical specialties such as orthopedics, maxillofacial surgery and in cardiovascular stents, it is therefore of main importance to have a thorough knowledge of its composition, properties and clinical implications.

In recent years, the appearance of numerous dental alloys has increased overwhelmingly, with the constant search for a material with good price-quality ratio and that meets all the requirements demanded; making it very complicated for clinicians to know all the available dental alloys and make the right choice for each clinical case. In full or partial edentulous patients, who were prosthetically rehabilitated, it is possible to detect different metals, for example in Figure 1.1, a maxillary rehabilitation with Titanium (Ti) implants in 1.4, 2.1, 2.3 and 2.4 with respective abutment components in Titanium-Aluminium-Vanadium (Ti-6Al-4V) alloy, porcelain fused metal (PFM) crowns in 2.3 and 2.4 in Cobalt-Chromium (Co-Cr) alloy, 1.3, 1.2, 1.1 and 2.2 in zirconia and total metal crowns on tooth 1.7 and 2.7 made of Co-Cr alloy and a removable prosthetic denture with a metal framework also in a Co-Cr alloy.

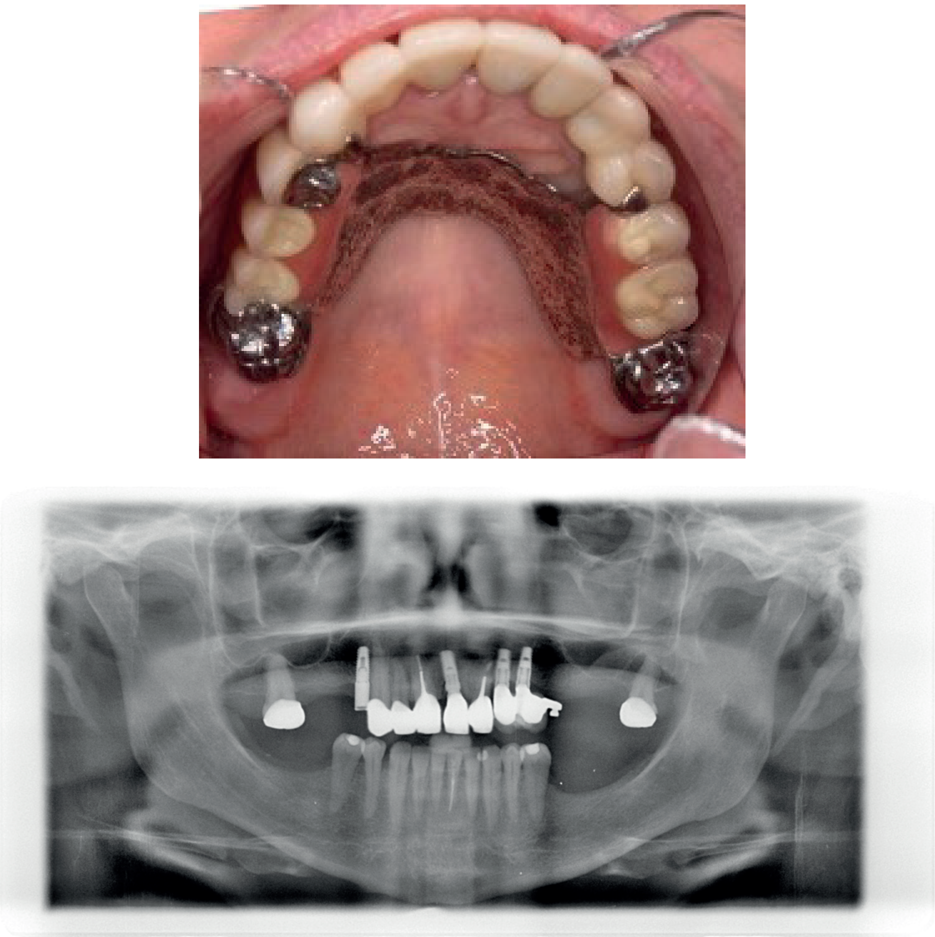


Figure 1.1. Maxillary Rehabilitation of a partial edentulous patient, photo courtesy of Professor Pedro Nicolau (top) and respective orthopantomography (bottom).

The metal alloys used in the oral cavity must meet specific requirements, namely to have mechanical properties for longevity, as well as being biocompatible.

The definition of biocompatibility has been updated over the years and it is now accepted that the biocompatibility of a long term implantable medical device refers to the ability of the device to perform its intended function, with the desired degree of incorporation in the host, without eliciting any undesirable local or systemic effects in that host (Williams, 2008).

Due to the presence of saliva, the oral cavity presents a favorable environment for the occurrence of corrosion of dental alloys. The corrosion parameters, ion release profile and metal ion toxicity, with the characteristics of the bulk material and his surface represent the major material characteristics that may conceivably influence the host response (Williams, 2008).

1.1. Metallic Alloys with application in Prosthodontics

Metal alloys are mixtures consisting of 2 or more chemical elements, at least one of which is a metal, so a binary alloy has 2 constituents, a ternary alloy has 3 constituents and so on.

In metallic alloys we can find several metals, the combination between them and the different proportions that each assumes in the constitution of the alloy will determine its mechanical properties.

While a metal in its pure state has a defined melting point and when it reaches this temperature, it undergoes a transition from solid to liquid; a metallic alloy has a melting zone because its constituents have different melting points (Figure 1.2).

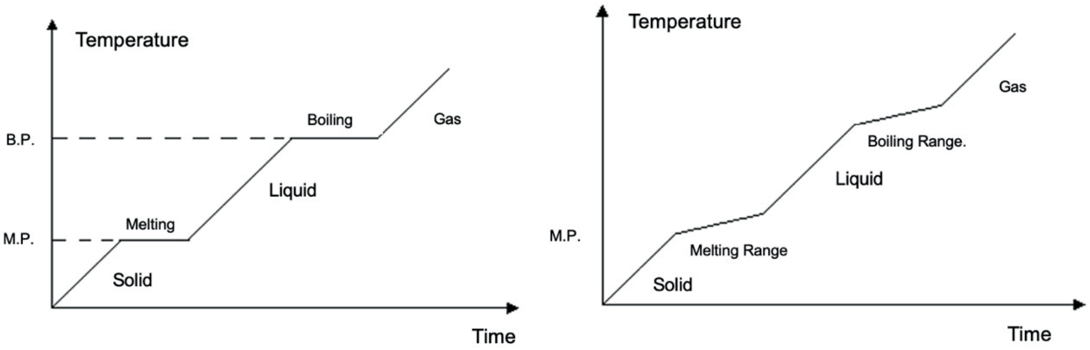


Figure 1.2. Graph of the heating: a) pure substance and b) mixture, adapted from http://webinfobrasil.blogspot.com/2015/04/graficos-de-substancias-puras-e.html (2015)

However, there are special cases of alloys that do not fit these standards, the eutectic ones, that behave like a pure substance during the process of fusion (or solidification). This means that when the mixture is heated and starts to change from a solid to a liquid state, the melting temperature is fixed and remains constant until the entire mixture goes into a liquid state (Donachie, 2000).

Approximately all metals and alloys have a crystalline structure, atoms form a crystal, with an orderly three-dimensional arrangement.

The most common structures in metals are body centered cubic (BCC), face centered cubic (FCC) and hexagonal close packed (HCP) (Figure-1.3).

In the crystalline plane, the metal appears as a set of grains or crystallites, separated from each other by joints of crystals (Burdairon, 1990).

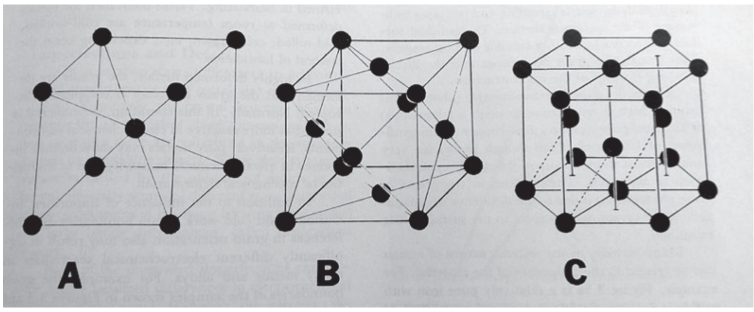
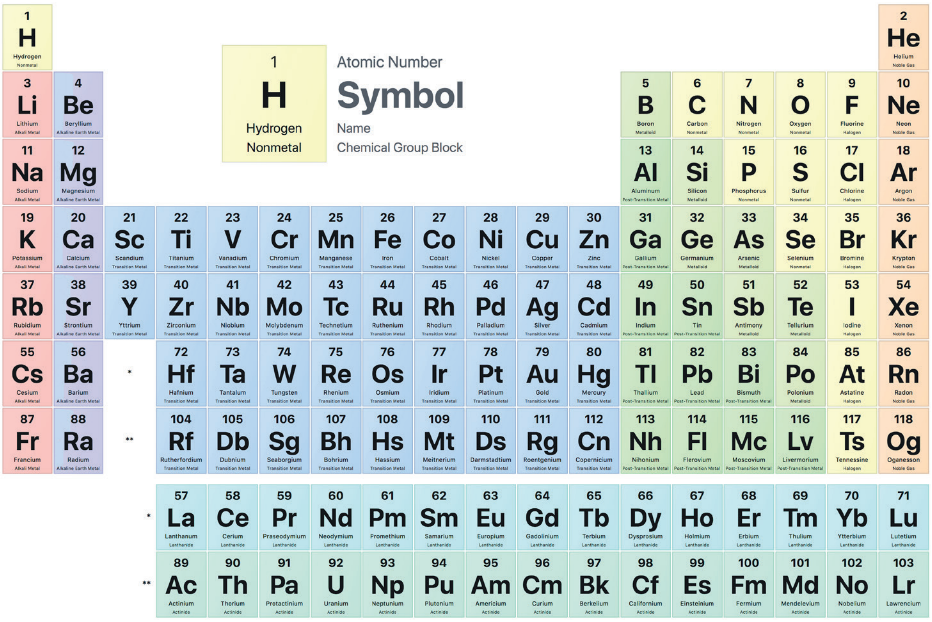


Figure 1.3. Schematic representation of the unit cells of the most common crystal structures found in metals. A- body-centered cubic (BCC), B- face-centered cubic (FCC) and C- hexagonal close packed (HCP). Original image from van Delinder L.S. (1984).

Some metals are allotropic, because they may exist in more than one crystal structure (Donachie, 2000), and can be classified in: ferrous metals, those who contain iron and non-ferrous metals, which include noble and base metals (Givan, 2007).

Considering the main and most important alloying elements present in dental alloys: gold (Au), silver (Ag), palladium (Pd), platinum (Pt), chromium (Cr), cobalt (Co), titanium (Ti), nickel (Ni), molybdenum (Mo), coper (Cu), beryllium (Be), aluminium (Al) and Vanadium (V) as all metals they are metallic shine and are solid, with the exception of mercury, which is a liquid at room temperature; conduct electric current and heat, are malleable and ductile and have a tendency to lose electrons and form cations.

In figure 1.4 it is possible see the localization of those elements on the periodic table, according to their chemical properties.



1
H
Hydrogen
Nonmetal

Atomic Number
Symbol
Name
Chemical Group Block

1 H Hydrogen																	2 He Helium
3 Li Lithium	4 Be Beryllium											5 B Boron	6 C Carbon	7 N Nitrogen	8 O Oxygen	9 F Fluorine	10 Ne Neon
11 Na Sodium	12 Mg Magnesium											13 Al Aluminum	14 Si Silicon	15 P Phosphorus	16 S Sulfur	17 Cl Chlorine	18 Ar Argon
19 K Potassium	20 Ca Calcium	21 Sc Scandium	22 Ti Titanium	23 V Vanadium	24 Cr Chromium	25 Mn Manganese	26 Fe Iron	27 Co Cobalt	28 Ni Nickel	29 Cu Copper	30 Zn Zinc	31 Ga Gallium	32 Ge Germanium	33 As Arsenic	34 Se Selenium	35 Br Bromine	36 Kr Krypton
37 Rb Rubidium	38 Sr Strontium	39 Y Yttrium	40 Zr Zirconium	41 Nb Niobium	42 Mo Molybdenum	43 Tc Technetium	44 Ru Ruthenium	45 Rh Rhodium	46 Pd Palladium	47 Ag Silver	48 Cd Cadmium	49 In Indium	50 Sn Tin	51 Sb Antimony	52 Te Tellurium	53 I Iodine	54 Xe Xenon
55 Cs Cesium	56 Ba Barium	*	72 Hf Hafnium	73 Ta Tantalum	74 W Tungsten	75 Re Rhenium	76 Os Osmium	77 Ir Iridium	78 Pt Platinum	79 Au Gold	80 Hg Mercury	81 Tl Thallium	82 Pb Lead	83 Bi Bismuth	84 Po Polonium	85 At Astatine	86 Rn Radon
87 Fr Francium	88 Ra Radium	**	104 Rf Rutherfordium	105 Db Dubnium	106 Sg Seaborgium	107 Bh Bohrium	108 Hs Hassium	109 Mt Meitnerium	110 Ds Darmstadtium	111 Rg Roentgenium	112 Cn Copernicium	113 Nh Nihonium	114 Fl Flerovium	115 Mc Moscovium	116 Lv Livermorium	117 Ts Tennessine	118 Og Oganesson
		*	57 La Lanthanum	58 Ce Cerium	59 Pr Praseodymium	60 Nd Neodymium	61 Pm Promethium	62 Sm Samarium	63 Eu Europium	64 Gd Gadolinium	65 Tb Terbium	66 Dy Dysprosium	67 Ho Holmium	68 Er Erbium	69 Tm Thulium	70 Yb Ytterbium	71 Lu Lutetium
		**	89 Ac Actinium	90 Th Thorium	91 Pa Protactinium	92 U Uranium	93 Np Neptunium	94 Pu Plutonium	95 Am Americium	96 Cm Curium	97 Bk Berkelium	98 Cf Californium	99 Es Einsteinium	100 Fm Fermium	101 Md Mendelevium	102 No Nobelium	103 Lr Lawrencium

Figure 1.4. Periodic table of elements reproduced from *https://pubchem.ncbi.nlm.nih.gov/periodic-table/* (National Center for Biotechnology Information, 2021).

More than 25 elements of the periodic table can be used in dental alloys in Dentistry, but only Titanium, Platinum and Gold are used in their pure state (Wataha, 2000; Wataha & Messer, 2004). Metal alloys with application in Prosthodontics can be classified according their composition, dominant phase system and application.

As for the phases, the alloys are distinguished between single-phase or multi-phase alloys, single-phase alloys have a homogeneous structure while multi-phase alloys have a heterogeneous structure and are more prone to corrosion (Wataha, 2000). This characteristic of alloys is related to the capacity of the metals of an alloy to dissolve into each other, this phenomenon happens in single phase alloys, but not in multiple phase alloys, being the first easier to manipulate. However, multi-phase ones can be etched for bonding and can be stronger (Wataha & Messer, 2004).

The importance of the alloy phase structure is due to its relationship with the clinical performance; because it affects the corrosion and strength of the alloy. In general, multi-phase alloys are prone to higher corrosion rates than single-phase alloys because of galvanic effects between the microscopic areas of different compositions. In some cases, multiple phases greatly strengthen an alloy but in others they weaken the alloy (El-Bediwi et al., 2009). Base metal dental prostheses with single-phase alloys are less cytotoxic and inhibit human cell growth less than those with multi-phase alloys (Can et al., 2007; Wataha & Lockwood, 1998).

Another important consideration, about dental alloys, is the grain-structure, that is also closely related with corrosion resistance, that is favored by impurities between small and large grains. The tendency to corrode is lower in case of a more homogeneous distribution of metallic atoms in the alloy. That is the reason why most alloys are submitted to a homogenizing heat treatment, to minimize the possibility of electrochemical corrosion (Ardelean et al., 2016).

A consensus of the National Bureau of Standards dental materials group in 1932 formulated the following classification for the hardness of the alloys in use at the time: Type I (soft: Vickers hardness number (VHN) between 50 and 90), Type II (medium: VHN between 90 and 120), Type III (hard: VHN between 120 and 150), Type IV (extra hard: VHN ≥ 150).

However, as the number of dental alloys has vastly increased, as well as their application, this classification has fallen into disuse, and other types of classifications are currently accepted according to composition, application or mechanical properties (Anusavice, 2003).

According to the American Dental Association (ADA) Standard, dental alloys can be classified according to their noble metal content in high noble alloys, noble alloys, titanium and titanium alloys, and predominantly base alloys (Table 1.1), in this revised classification titanium was include in a separate category due to his extensive use and similar properties with noble metals (Givan, 2007):

Table 1.1. ADA dental alloy classification, ADA Council on Scientific Affairs, 2003 (Department of Scientific Information, 2021)

High Noble Alloys	≥ 60% (gold and platinum group) and gold ≥ 40%
Titanium and Titanium Alloys	Titanium ≥ 85%
Noble Alloys	≥ 25% (gold and platinum group)
Predominantly Base Alloys	< 25% (gold and platinum group)

There are, however, other ways in the literature to classify dental alloys for casting according to their composition (Ardelean et al., 2015) :

- Noble metal alloys including gold alloys, with high and low gold content, silver-palladium alloys (Ag-Pd), with and without the addition of copper and Palladium alloys.
- Predominantly Base Metal Alloys, which include Nickel-Chromium alloys, Cobalt-Chromium alloys and Titanium alloys

With regard to the mechanical property requirements for each application in 2016, ANSI/ADA determined the following classification (Table 1.2):

Table 1.2. ADA/ American National Standards Institute (ANSI) Standard N°134/22674:2016 Dental Casting Alloy (Department of Scientific Information, 2021)

Type	Applications	Yield Strength	Elongation
0	Low-stress small single tooth fixed restorations.	–	–
1	Low-stress single-tooth fixed restorations: one-surface inlays, veneered crowns.	80	18
2	Single-tooth fiex restorations: crowns or inlays without restriction on the number of surfaces.	180	10
3	Multiple-unit fixed prostheses, e.g. bridges	270	5
4	Appliances with thin sections subject to very high forces: removable partial dentures, clasps, etc.	360	2
5	High stiffness (greater than 150 GPa) and strength: thin removable partial dentures, parts with thin cross sections, clasps.	500	2

1.1.1. Noble metals used in Dental alloys

Noble metals are characterized by a relatively high potential in relation to the hydrogen electrode, excellent corrosion resistance and a high price. In the area of Dentistry, we have to consider among these metals: gold, silver, and the platinum group metals that include platinum, palladium, rhodium, iridium, osmium and ruthenium.

Gold is found in Nature in its pure state and is the most malleable and the most ductile of metals, is a good conductor of heat, but if pure is too soft for most dental applications (Upadhyay et al., 2006), has an elastic modulus and hardness similar to enamel, which results in a desirable wear coupled with teeth (Givan, 2007). **Silver** is the preferentially corroded element in noble alloys (Canay & Oktemer, 1992), therefore its inclusion in the group of noble metals is very controversial, being included in the group of base metals by several authors (Givan, 2007). Has a low melting temperature and readily uptakes oxygen (O), which makes it difficult to cast without porosity (Givan, 2007).

Palladium has a high melting point, over 1600 °C (Wataha & Messer, 2004), but may absorb hydrogen (H) when heated, resulting in undesirable properties (Givan, 2007). Palladium is a metal resistant to corrosion and associated to silver gives a stable protection and decrease it´s density (Canay & Oktemer, 1992).

Platinum is a mineral of the cubic system, usually found in irregular grains, very rarely in octahedra or cubes, is malleable and ductile, with a very high density, therefore imparts excellent resistance to wear, being a common constituent in precision prosthetic attachments (Givan, 2007) and relatively corrosion resistant. Like palladium it also absorbs hydrogen (Lansdown, 2014).

1.1.1.1. Noble Metal Alloys

As mentioned above, Noble Metals alloys are subdivided according to the percentage of their noble metal content.

We can also consider the sub-division of alloys with high content in Gold: Gold-Platinum (Au-Pt), Gold-Palladium (Au-Pd) or Gold-Copper-Silver (Au-Cu-Ag), taking into account the elements that compose them (Table 1.3) (Wataha, 2002; Wataha & Messer, 2004).

Table 1.3. Selected properties of major types of high-noble alloys (Wataha & Messer, 2004).

Alloy type	Solidus-liquidus (°C)	Color	Phase structure	Elastic modulus (static, GPa)	Vicker's hardness (kg/mm^2)	Yield strenght (tension, 0.2%, MPa)
Au-Pt (Zn)[a]	1060-1140	Yellow	Multiple	65-96	165-210	360-580
Au-Pd (Ag)[a]	1160-1260	White	Single	105	280	385
Au-Cu-Ag	905-960	White	Single	100	210	450

The Au-Pt, and Au-Pd, alloys are suitable for pure metallic and metal-ceramic restorations while the Au-Cu-Ag alloys only for pure metallic restorations due to their high silver and copper content and low melting zone (Givan, 2007; Wataha & Messer, 2004).

With regard to alloys with low gold (or noble metal) content, we can consider the alloys: Au-Cu-Ag-(Pd), Pd-Cu-Ga, Pd-Ag and Ag-Pd. In Table 1.4 are shown some of the chemical and mechanical properties of those alloys.

Table 1.4. Selected properties of major types of noble alloys (Wataha & Messer, 2004).

Alloy type	Solidus-liquidus (°C)	Color	Phase structure	Elastic modulus (static, GPa)	Vicker's hardness (kg/mm^2)	Yield strenght (tension, 0.2%, MPa)
Au-Cu-Ag-(Pd)[a]	880-930	Yellow/white	Single	100	250	690
Pd-Cu-Ga	1145-1270	White	Single	127	280	580
Pd-Ag	1185-1230	White	Single	125	275	620
Ag-Pd	990-1045	White	Multiple	93	230	480

[a] In many formations.

These alloys have decreased gold content and compensate for the reduced gold by increasing copper, silver, or palladium. They have moderate modulus of elasticity but are higher in hardness and yield strength than the high-noble homologs (Wataha & Messer, 2004).

Ag-Pd alloy is white, and has at least 25% of palladium to promotes de resistance to discoloration of the silver. Increasing the content in silver, increase flexibility and corrosion and decreases hardness, such as Pd-Cu-Ga alloys are a low-cost alternative alloy with a hardness similar to tooth structure and excellent casting accuracy (Givan, 2007).

In alloys containing silver, silver-rich zones have minimal passivation and therefore little protection against corrosion(Canay & Oktemer, 1992), so in low gold content alloys, corrosion occurs primarily in that silver zones, and secondarily in copper-rich regions (Laub & Stanford, 1981).

Gold alloys were for many years considered the material of choice for superstructures due to their excellent biocompatibility, corrosion resistance and mechanical properties, however, their use was replaced by others less expensive alloys, which were developed over time, such as Ni-Cr, Co-Cr and titanium alloys (Taher, 2003).

1.1.2. Base metals most used in dental alloys

Among the most commonly base metals used in dental alloys, we highlight chromium, cobalt, nickel, molybdenum, copper and beryllium.

Chromium is a brittle, hard and reactive element, but both pure or in this alloy have a high resistance to various media due to its passivation capacity (Vaicelyte et al., 2020). Chromium improves the corrosion resistance of alloys in a corrosive environment due to the formation of a Cr-rich, passive oxide film Cr_2O_3 formed on the alloy surface. The Cr content determines passive film stability (Golgovici et al., 2021).

Their atoms are arranged in a face-centered cubic system (FCC), Cr(III) and Cr(VI) are the states more widespread. Cr(VI) is a toxic element and is released from alloys, although it is quickly transformed in Cr(III), that is a non-toxic state. An alloy must contain a minimum of 12% Cr, to give it an adequate passivation (Anusavice, 2003) and at a maximum of 30%, otherwise it makes it brittle (Wataha, 2002).

Cobalt exists in two crystalline forms: closed-packed hexagonal (HCP) and face-centered cubic (FCC) at room temperature and 421°C respectively; it is a hard metal after reductive melting (Vaicelyte et al., 2020) and increases the modulus of elasticity, strength and hardness of an alloy, being ductile at room temperature. It can be replaced by Ni and vice versa, and is considered the second most common metal allergen, after Ni (Wataha & Messer, 2004). According to the EU Medical Devices Regulation (MDR) (2017/745), cobalt metal is considered carcinogenic, suspected to be mutagenic, and a substance toxic to reproduction so their application and invasive medical devices might be forbidden if more than 0.1% (m/m) is present in their composition (Golgovici et al., 2021; Vaicelyte et al., 2020).

Nickel It has good corrosion resistance at high temperatures and in neutral or slightly acidic media, but it is not resistant to oxidizing solutions. Has a low melting temperature, and as for its mechanical properties, it is a malleable and ductile element, however is a known allergen (Anusavice, 2003; Setcos et al., 2006).

Molybdenum has good corrosion resistance subject to hydrofluoric acids but oxidizing agents attack it quickly. One of its great advantages is its high modulus of elasticity (345GPa) which makes it more rigid and suffers less deflection under a given load (van Delinder L.S., 1984).

This element added to an alloy can improve its corrosion resistance, it is also a hardener of the alloy, and decreases the thermal coefficient of expansion of the alloy (Piron, 1991).

Copper is the principal hardener; in addition to being resistant to corrosion, has high thermal and electrical conductivity. Does not corrode with acids, except in the presence of oxygen or other and oxidizing agents (van Delinder L.S., 1984). Similarly, to what happens with Ni, Mo and Cr in a percentage less than 12% improves corrosion resistance(Piron, 1991).

Beryllium present in some metal alloys improves its melting capacity and bond strength (Wataha, 2002), however in the casting, finishing and polishing process there is release of particles and vapors containing beryllium depending on the intensity, frequency and duration of exposure. For this reason, these alloys are used less and less frequently, and laboratory technicians who handle them are required to wear suitable protective equipment, according to the American Dental Association (ADA). Although beryllium is toxic, the primary risk is in the form of vapor, so patients and dentists are not affected, but prosthetic technicians are (López-Alías et al., 2006). When using alloys containing Be, this metal should be limited to 0.9% (w/o) because of the potential health risk(Bezzon et al., 1998).

Some elements, metals and non-metals, are considered residual elements in alloys because they are less than 0.1% by weight or because are considered impurities, but some of them are particularly important in their properties. Silicon (Si) improves the fusibility and ductility of the alloy, and carbon (C) determines the hardness of the alloy, however a percentage greater than 0.25 makes it brittle. Indium (In) for example at a concentration of about 0.1% is a grain size refiner (Wataha & Lockwood, 1998).

1.1.2.1. Predominantly Base Metal Alloys

Base metal alloys can be divided into 4 types: Ni-Cr-Be, Ni-Cr, Ni-high-Cr and Co-Cr alloys.

Nickel-Chromium alloys

The composition of Ni-Cr alloys is Ni with a % between 68-80% and Cr (11,9-26,3%), are always multiple-phase. This alloy has good mechanical properties, corrosion resistance and high elastic modulus, however Ni is an element strongly related to hipersensivity reactions. (Bezzon et al., 1998; El-Bediwi et al., 2009) . This kind of alloys are more ductile and they're melting temperature is lower than the Co-Cr alloys (Bezzon et al., 1998). For an adequate protective oxide film, this alloy must have between 16% to 27% chromium content (Rao & Chowdhary, 2011; Reddy et al., 2011), and may have 1 to 2 wt% beryllium on their composition (Wataha, 2002).

Due to their low-price and relative resistance to heat treatment, it is an alloy commonly used in porcelain fusion, it is also very resistant to distortion at high temperature (Ozden et al., 2002).

According to some authors, Nickel-Chromium alloy is susceptible to localized corrosion in oral cavity while Co-Cr appears to be more resistant (al Jabbari, 2014; Borg et al., 2018; Canay & Oktemer, 1992).

Alloying Beryllium to Ni-Cr alloys improves both the alloy castability and adherence of the veneering porcelain, but decreases alloy corrosion resistance, so it should be added with less than 0.6 wt% (Wataha & Messer, 2004; Wylie et al., 2007).

Cobalt-chromium Alloys

The application of Co-Cr alloys in Prosthodontics focuses on the construction of frameworks for removable prostheses, in Fixed Prosthodontics and Implantology substructures, which are also widely used in Orthopedics and in stents (Puskar et al., 2014b; Vaicelyte et al., 2020).

These alloys are divided into 2 large groups that are distinguished by their concentration in cobalt, so the composition of the alloy in one of the groups has the following formulation: 60% Co, 30% Cr, 5% Mo, C, Si, Ni and magnesium in small quantities. In the other group the percentage of Co decreases and is replaced by Ni which can reach 30%, in this situation Be is also present as an impurity, and is always a multi-phase alloy (Wataha, 2002).

Co-Cr alloys have good corrosion resistance, although less than noble metal alloys; as well as good mechanical properties, are rigid, a relatively high elastic modulus, strength (Table 1.5) and high biocompatibility, due to the formation of Cr-based oxides on the surface (Borg et al., 2018; el Sawy & Shaarawy, 2014; Haugli et al., 2020; Vaicelyte et al., 2020). This passive layer in the surface of the Co-Cr alloy, as well as being beneficial in increasing corrosion resistance, it also prevents the oxidation of cobalt, a toxic element as we have seen previously.

Their high elastic modulus, is almost the double of titanium alloys, consideration to be taken into account in length and retention of the clasps of partial removable dental prostheses, providing the requisite strength and rigidity without the need for heavy cross-sections, thus reducing the weight of metal substructures (al Jabbari, 2014).

Table 1.5. Mechanical properties of cobalt-chromium alloy. Adapted from Jang et al., 2001.

Alloy	Ultimate tensile strength (MPa)	Tensile yeld strength (0,2%)	Elongation at fracture (%)	Hardness (VHN)
Co-Cr	1277	500-650	4-10	300-400

Co-Cr alloys are used for the production of RPD frameworks and as alternative to Ni-Cr alloys for the production of PFM crowns and bridges.

1.1.3. Titanium and its alloys

1.1.3.1.Titanium

Titanium is one of the elements most frequently found on earth, and that despite having been discovered in the 18th century , only in the first half of the 20th century did it begin to be used, becoming one of the metals with the greatest industrial use, namely in the aerospace and defense industries due to its excellent mechanical properties, such as fatigue resistance, high strength to weight and good corrosion resistance, that associated with its biocompatibility makes it of special interest in dentistry (Blackman et al., 1991;Tschernitschek et al., 2006).

Its use in dentistry is quite wide, in Orthodontics, in wires and brackets, in endodontic files, endosseous implants, surgical screws and in the laboratory manufacture of inlays, crowns and prostheses frameworks (Tschernitschek et al., 2006).

Titanium has the atomic number 22, appears in group 4 of the periodic table, and is a transition metal, nonmagnetic.

Commercial pure (cp) titanium is available in 4 grades (I to IV), according to the degree of impurity, that classification is based on the incorporation of small amounts of oxygen, nitrogen (N), hydrogen, iron (Fe) and carbon during purification procedures. The percentage of oxygen can range from 0.18 to 0.40 wt%, while that of iron can range from 0.20 to 0.50 wt%. These apparent slight percentage differences play a substantial role in the effects of physical and mechanical properties.(Craig & Powers, 2001; Donachie, 2000) , for example grade 1 despite having greater purity, has less resistance to mechanical stress.

The density of cp Ti is 4,5 g/cm^3, and its elastic modulus is 120 GPa, both are almost half of other base metals, which is very useful in extensive prostheses (Table 1.6).

Table 1.6. Physical and mechanical properties of elemental titanium. Adapted from Donachie (2000).

Property	Description or value
Atomic number	22
Atomic weight	47.90
Atomic volume	10.6 *W/D*
Covalent radius	1.32 Å
Ionization potential	6.8282 V
Thermal neutron absorption cross section	5.6 barns/atom
Crystal structure	
Alpha (≤ 8882.5°C, or 1620°F)	Close-packed hexagonal
Beta (≥ 882.5°C, or 1620°F)	body-centered cubic
Color	Dark gray
Dentiy	4.51 g/cm^3 (0.163 lb/in.^{3})
Melting point	1668 ± 10°C (3035°F)
Solidus/liquidus	1725°C (3135°F)
Boiling point	3260°C (5900°F)
Specific heat (at 25°C)	0.5223 kJ/kg · K
Thermal conductivity	11.4 W/m · K
Heat of fusion	440 kJ/kg (estimated)
Heat of vaporization	9.83 MJ/kg
Specific gravity	4.5
Hardness	70 to 74 HRB
Tensile strenght	240 MPa (35 ksi) min
Young's modulus	120 GPa (17 x 10^6 psi)
Poisson's ratio	0.361
Coefficient of friction	
At 40 m/min (125 ft/min)	0.8
At 300 m/min (1000 ft/min)	0.68
Coefficient of linear thermal expansion	8.41 μm/m · K
Electrical conductivity	3% IACS (where copper = 100% IACS)
Electrical resistivity (at 20°C)	420 nΩ · m
Electronegativity	1.5 Pauling's
Temperature coefficient of electrical resistance	0.0026/°C
Magnetic susceptibility (volume, at room temperature)	180 (±1.7) x 10^{-6} mks

Titanium is an allotropic element, so it may exist in more than one crystallographic form (Donachie, 2000). At low temperature, c.p. Ti is in a state called α-phase, with a hexagonal close-packed crystal structure (HCP), it occurs below the β trans. With heat, an allotropic phase transformation occurs and at 883°C it forms a body-centered cubic (BBC) called the β phase. A component with a predominant β phase is harder but more brittle than a component with a predominant α microstructure phase (Craig & Powers, 2001; Donachie, 2000).

The surface of this metal is almost always different from its body, either in shape or in chemical composition and structure, due to molecular arrangements, surface reactions as well as the contamination that the surface is exposed to (Donachie, 2000)

Titanium forms spontaneously on its surface a stable and dense layer of titanium oxide, mainly TiO_2 which is responsible for its resistance to corrosion, this is the basis for the corrosion–resistance property and biocompatibility (Craig & Powers, 2001).

Titanium is a reactive metal but the thin oxide film that instantly and spontaneously forms in air on its surface transforms the metal from an active to a passive condition (Upadhyay et al., 2006) ; therefore, the nature of its corrosion resistance is different from that of gold and other noble metals(Donachie, 2000). This characteristic called passivation, determines its great resistance to corrosion once these oxides are considerably stable, and practically insoluble.

In cp Ti, this passive film is composed by amorphous TiO_2 and small amounts of sub-oxides such as TiO and Ti_2O_3 (Donachie, 2000) the thickness of oxides varies between 1.8 and 17 nm, being mainly composed of TiO_2, the most stable and commonly formed, has an amorphous or very-low-crystalline structure, this oxide is also responsible, as already mentioned, for the excellent biocompatibility of titanium, due to its low level of electroconductivity, high resistance corrosion and stable thermodynamic state at physiological pH (Rocha et al., 2013; Sul et al., 2002).

High-temperature oxidation tends to promote the formation of the chemically resistant form of TiO_2 known as rutile-type tetragonal structure, whereas lower temperatures often generate the more amorphous form of Ti_2O_3 known as anatase, or promote formation of a mixture of rutile and anatase. The TiO_2 oxide is generally chemically resistant and is attacked by very few substances, that includes hot concentrated hydrochloric acid, sulfuric acid, sodium hydroxide, phosphoric acid, and, most notably, hydrofluoric acid (Donachie, 2000).

The passive layer of titanium possesses inferior mechanical and wear properties and can be disrupted at very low shear stresses, which is particularly worrying in the interface implant/ abutment that suffers relative movements between them and can undergo deterioration of the passive layer (Licausi et al., 2013; Sivakumar et al., 2011).

Among the characteristics of titanium, good resistance to electrochemical degradation, benign biological response, relatively low weight, low modulus of elasticity, low density and high tensile strength are highlighted (Ban et al., 2000; Craig & Powers, 2001; el Sawy & Shaarawy, 2014), and make cp Ti and its alloys the most used in implants and their prosthetic components.

Despite its excellent corrosion resistance, titanium is susceptible to hydrogen embrittlement, and can propagate cracks very quickly during stress corrosion in fluoride solution (Könönen et al., 1995), additionally, mechanical stress, oxygen deficit and low pH also can undergo corrosion on Ti alloys, other disadvantage that can be enumerated is the low thermal conductivity (Tschernitschek et al., 2006).

When it cools after being melted, titanium crystallizes in α phase below 883°C, in this α phase the mechanical properties are similar to gold alloys type III and IV.

Above 883°C crystallization occurs in a β phase, in which the alloy is more brittle and therefore, the temperature after melting should be highly controlled (Blackman et al., 1991).

Due to the high melting point (1700°C to cp Ti) and his reactivity, casting process of titanium is a very sensitive procedure, casting equipment that operates in vacuum is required to prevents contamination, since titanium reacts with gaseous elements like H, O and N at high temperatures. Contamination during the casting impairs the formation of the surface layer, making it thinner and therefore weaker and ductile, and which is more likely to cracking (Craig & Powers, 2001). Additionally, Ti can react also with the surface of the investment; if contamination occurs a hard surface contamination layer, $\alpha-$ case is created, with compounds of Ti with Si, phosphorus and O, with 80-150 mm; due to this increased surface hardness, and roughness, polishing is a complicated procedure, beyond that the elongation and fatigue limit is reduced (Ohkubo et al., 2006; Oliveira et al., 2006). It should also be noted that, due to the specifics of the casting technique, if there is any failure during the melting procedure, casting porosities may occur.

Ti also presents metastable phases, such as the hexagonal martensite α' and orthorhombic α'' phases, which are dependent on processing conditions (D. R. N. Correa et al., 2014) .

1.1.3.2. Titanium alloys

Regarding titanium alloys, adding alloying elements alters their mechanical and physical properties, and the structural change modifies also the corrosion resistance.

With respect to their microstructure, titanium alloys are classified according to their phase proportion and can be near α, α, $\alpha+\beta$, near β, and β. The mechanical properties of titanium alloys are directly related to their microstructure (D. R. N. Correa et al., 2014; Donachie, 2000).

As α stabilizers we can enumerate Al and tin (Sn), β stabilizers are V, Mo, Cr and Cu for example (Donachie, 2000; Trethewey & Chamberlain, 1998). The effect of alloying elements one some properties of titanium alloys is shown in Figure 1.5.

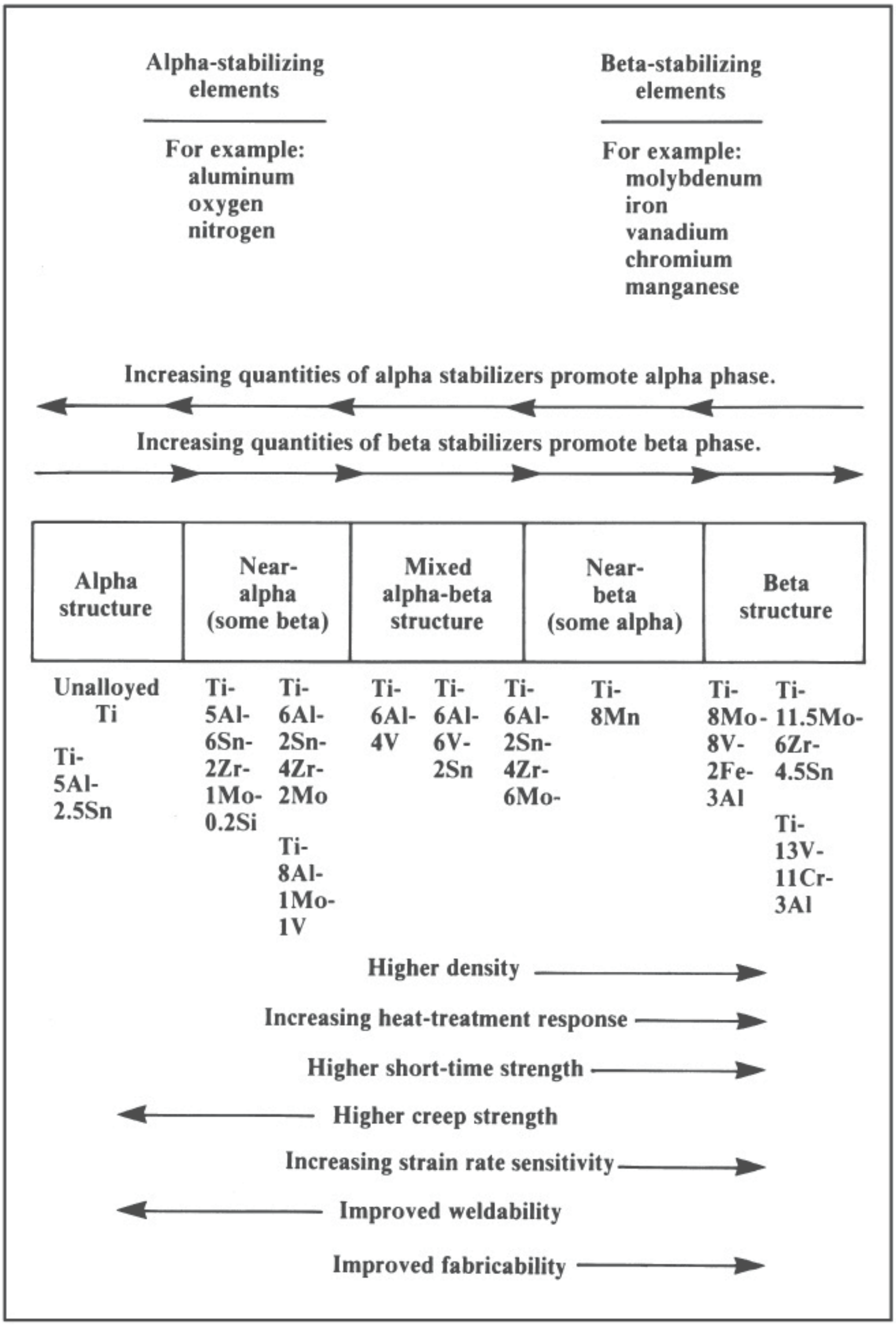


Figure 1.5. Schematic showing effects of alloy elements on structure and some selected properties (representative alloys noted). Image from the original publication (Donachie, 2000).

α-Type titanium alloys have higher corrosion resistance, elasticity modulus, and resilience than β- type (D. R. N. Correa et al., 2014) . Due to this corrosion resistance α-alloys are preferred for implant fabrication, unless they are narrow diameter implants because they have lower strength than β- type.

In Table 1.7 we can see some commercial and semicommercial grades and alloys of titanium, their composition, tensile strength, yield strength and impurity limits.

Table 1.7. Some commercial and semicommercial grades and alloys of titanium.
Image from the original publication (Donachie, 2000).

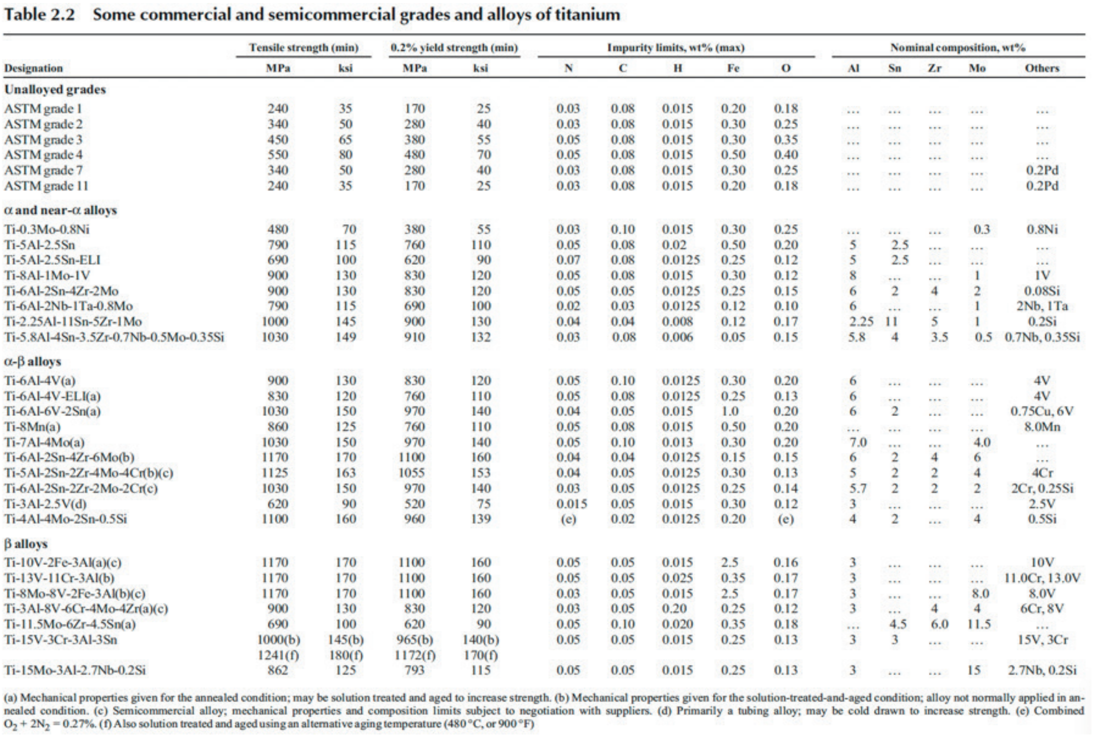


Table 2.2 Some commercial and semicommercial grades and alloys of titanium

	Tensile strength (min)		0.2% yield strength (min)		Impurity limits, wt% (max)					Nominal composition, wt%				
Designation	MPa	ksi	MPa	ksi	N	C	H	Fe	O	Al	Sn	Zr	Mo	Others
Unalloyed grades														
ASTM grade 1	240	35	170	25	0.03	0.08	0.015	0.20	0.18	...	...	...	...	...
ASTM grade 2	340	50	280	40	0.03	0.08	0.015	0.30	0.25	...	...	...	...	...
ASTM grade 3	450	65	380	55	0.05	0.08	0.015	0.30	0.35	...	...	...	...	...
ASTM grade 4	550	80	480	70	0.05	0.08	0.015	0.50	0.40	...	...	...	...	...
ASTM grade 7	340	50	280	40	0.03	0.08	0.015	0.30	0.25	...	...	...	...	0.2Pd
ASTM grade 11	240	35	170	25	0.03	0.08	0.015	0.20	0.18	...	...	...	...	0.2Pd
α and near-α alloys														
Ti-0.3Mo-0.8Ni	480	70	380	55	0.03	0.10	0.015	0.30	0.25	...	...	...	0.3	0.8Ni
Ti-5Al-2.5Sn	790	115	760	110	0.05	0.08	0.02	0.50	0.20	5	2.5	...	...	...
Ti-5Al-2.5Sn-ELI	690	100	620	90	0.07	0.08	0.0125	0.25	0.12	5	2.5	...	...	...
Ti-8Al-1Mo-1V	900	130	830	120	0.05	0.08	0.015	0.30	0.12	8	...	...	1	1V
Ti-6Al-2Sn-4Zr-2Mo	900	130	830	120	0.05	0.05	0.0125	0.25	0.15	6	2	4	2	0.08Si
Ti-6Al-2Nb-1Ta-0.8Mo	790	115	690	100	0.02	0.03	0.0125	0.12	0.10	6	...	...	1	2Nb, 1Ta
Ti-2.25Al-11Sn-5Zr-1Mo	1000	145	900	130	0.04	0.04	0.008	0.12	0.17	2.25	11	5	1	0.2Si
Ti-5.8Al-4Sn-3.5Zr-0.7Nb-0.5Mo-0.35Si	1030	149	910	132	0.03	0.08	0.006	0.05	0.15	5.8	4	3.5	0.5	0.7Nb, 0.35Si
α-β alloys														
Ti-6Al-4V(a)	900	130	830	120	0.05	0.10	0.0125	0.30	0.20	6	...	...	...	4V
Ti-6Al-4V-ELI(a)	830	120	760	110	0.05	0.08	0.0125	0.25	0.13	6	...	...	...	4V
Ti-6Al-6V-2Sn(a)	1030	150	970	140	0.04	0.05	0.015	1.0	0.20	6	2	...	...	0.75Cu, 6V
Ti-8Mn(a)	860	125	760	110	0.05	0.08	0.015	0.50	0.20	...	...	...	...	8.0Mn
Ti-7Al-4Mo(a)	1030	150	970	140	0.05	0.10	0.013	0.30	0.20	7.0	...	...	4.0	...
Ti-6Al-2Sn-4Zr-6Mo(b)	1170	170	1100	160	0.04	0.04	0.0125	0.15	0.15	6	2	4	6	...
Ti-5Al-2Sn-2Zr-4Mo-4Cr(b)(c)	1125	163	1055	153	0.04	0.05	0.0125	0.30	0.13	5	2	2	4	4Cr
Ti-6Al-2Sn-2Zr-2Mo-2Cr(c)	1030	150	970	140	0.03	0.05	0.0125	0.25	0.14	5.7	2	2	2	2Cr, 0.25Si
Ti-3Al-2.5V(d)	620	90	520	75	0.015	0.05	0.015	0.30	0.12	3	...	...	...	2.5V
Ti-4Al-4Mo-2Sn-0.5Si	1100	160	960	139	(e)	0.02	0.0125	0.20	(e)	4	2	...	4	0.5Si
β alloys														
Ti-10V-2Fe-3Al(a)(c)	1170	170	1100	160	0.05	0.05	0.015	2.5	0.16	3	...	...	...	10V
Ti-13V-11Cr-3Al(b)	1170	170	1100	160	0.05	0.05	0.025	0.35	0.17	3	...	...	...	11.0Cr, 13.0V
Ti-8Mo-8V-2Fe-3Al(b)(c)	1170	170	1100	160	0.03	0.05	0.015	2.5	0.17	3	...	...	8.0	8.0V
Ti-3Al-8V-6Cr-4Mo-4Zr(a)(c)	900	130	830	120	0.03	0.05	0.20	0.25	0.12	3	...	4	4	6Cr, 8V
Ti-11.5Mo-6Zr-4.5Sn(a)	690	100	620	90	0.05	0.10	0.020	0.35	0.18	...	4.5	6.0	11.5	...
Ti-15V-3Cr-3Al-3Sn	1000(b) 1241(f)	145(b) 180(f)	965(b) 1172(f)	140(b) 170(f)	0.05	0.05	0.015	0.25	0.13	3	3	...	...	15V, 3Cr
Ti-15Mo-3Al-2.7Nb-0.2Si	862	125	793	115	0.05	0.05	0.015	0.25	0.13	3	...	...	15	2.7Nb, 0.2Si

(a) Mechanical properties given for the annealed condition; may be solution treated and aged to increase strength. (b) Mechanical properties given for the solution-treated-and-aged condition; alloy not normally applied in annealed condition. (c) Semicommercial alloy; mechanical properties and composition limits subject to negotiation with suppliers. (d) Primarily a tubing alloy; may be cold drawn to increase strength. (e) Combined $O_2 + 2N_2 = 0.27\%$. (f) Also solution treated and aged using an alternative aging temperature (480 °C, or 900 °F)

1.1.3.2.1. Ti-6Al-4V alloy

Ti-6Al-4V, also known as titanium grade 5, with the following composition: Titanium 90%, Aluminium 6% and Vanadium 4%, represent 45% of total production of titanium as biomaterial, due to its physical, chemical and biocompatibility properties(Donachie, 2000; el Sawy & Shaarawy, 2014) .

Aluminium is a soft and weak element in its pure state, but in alloys and after heat treatment it improves its mechanical properties. It is a reactive material, but it develops an aluminium oxide film or layer that makes it quite stable in neutral pH solutions and in many acidic pH solutions.(van Delinder L.S., 1984). Due to this thin film that is formed when a surface is cut and exposed to contact with air, aluminium alloys have good corrosion resistance.The oxide layer is transparent, hard and adherent (Trethewey & Chamberlain, 1998) .

Vanadium is a transition metal distributed in soil, crude oil, water and air, that despite having a role in biological systems it is also associated with toxicology (Pessoa et al., 2015), has a high resistance to deformation and corrosion at high temperatures.

Ti-6Al-4V has higher strength compared to cp Ti, and his passive layer is more complex, as well as TiO_2, this alloy contains both aluminium and vanadium in the surface layers, usually as the appropriate metal oxides. The Ti-6Al-4V passive film is formed by a compact inner layer, mainly composed by TiO_2 and responsible for the higher corrosion resistance of the passive film and a porous outer layer, with lower corrosion resistance, the nature of the porous layer is dependent of the alloy and the solution anion species where it is inserted, so it is also composed by TiO_2 but enriched with aluminium (Al_2O_3) and vanadium (V_2O_5) oxides , present in small amounts.(Aziz-Kerrzo et al., 2001; B. C. Costa et al., 2019; Gudić et al., 2021). This alters the metal–cell interactions with the different mechanical properties previously mentioned, these are the reason why dental implants are usually made from cpTi (W. Nicholson, 2020).

At room temperature T-6Al-4V is a two-phase $\alpha\beta$ alloy, and except for the modulus of elasticity, it has similar properties to Ni-Cr and Co-Cr dental alloys, meanwhile cp Ti have properties similar to gold alloys (R. R. Wang & Fenton, 1996).

The melting point of Ti-6Al-4V is lower than of the cp Ti, and has a higher mechanical resistance being the choice material in medical applications that involve high stresses, such as orthopedic prostheses (Elias et al., 2015) and implant suprastructures, however both cp Ti and Ti-6Al-4V have poor abrasive and adhesive wear resistance and according to Budinski (1991), should be avoided even in systems involving low stress abrasion, however, Ti alloys have the ability to repassivate after being damaged by wear (Manhabosco et al., 2011).

Due to the potential toxicity of Al and V, new titanium alloys, with adequate mechanical properties, have been developed replacing these elements by others more biocompatible such as niobium, molybdenum, tantalum, and zirconium (D. R. N. Correa et al., 2014; Khan et al., 1996; Okazaki et al., 1998; Watanabe et al., 2004).

1.2. Corrosion

Due to their granular structure, metallic alloys are predisposed to the occurrence of corrosion, associated with the fact that the oral cavity presents an ideal environment for the existence of electrolytic corrosive phenomena (Ardelean et al., 2016) so the study of its behavior is critical.

According to the European Corrosion Federation, Corrosion can be defined as the physicochemical interaction between a metal and its surroundings, which result in changes in the properties of the metal, often leading to its disuse or the technical system of which it is part or even to changing the environment (European Corrosion Federation, ISO 8044-1986).

All metals are subject to corrosive attack if the medium in which they are inserted is sufficiently aggressive. For example, gold and platinum are practically unassailable in common media, but they are not resistant to aqua regia, constituted by hydrochloric acid (HCL); and nitric acid (HNO_3). Titanium, in turn, although extremely resistant, suffers corrosion in hydrofluoric acid (HF) (Gentil, 2003).

Various studies suggest that the corrosive processes, depends on the metallic material, the corrosive medium and the operating conditions (Arregui et al., 2021a; Burdairon, 1990; Gentil, 2003; Trethewey & Chamberlain, 1998).

For the metallic material, concern should be with the chemical composition, structure, presence of impurities, obtaining process, surface condition, and heat treatments. The corrosive medium is influenced by the chemical composition, concentration, pH, temperature, oxygen content, volume, conductivity, microbiological and enzymatic conditions. For the operating conditions, concerns should be on the values of mechanical stresses, if the relative movement between the metal and the medium are continuous or intermittent, partial or total immersion in the medium, and time of contact (Arregui et al., 2021a; Burdairon, 1990; Gentil, 2003; Toumelin-Chemla et al., 1996).

With regard to the metal structure, it matters to emphasize the influence of the grain size: if there is a precipitation of impurities between grains, the larger the grains, the more intense is corrosion along them; the structural heterogeneity: the more homogeneous the distribution of metallic atoms in an alloy, the less present is the tendency to corrode. Most of the alloys are submitted to a homogenizing heat treatment, in order to minimize the possibility of electrochemical corrosion.

The surface condition of the material depends on the polishing degree, surface properties, absence of pores, voids or cracks. Deformations, and mechanical tensions in the metal contribute to decreasing the potential, as fatigue is more accentuated in corrosive environment (Ardelean et al., 2015).

For a material to be considered corrosion resistant it must behave as either inert or passive. Inert is when the material is unaffected by the environment. Passive when it oxidizes and forms a layer on its surface called a passive layer that is insoluble and resistant to corrosion by itself, that´s the case of the cp as previously described, in which the titanium oxide is much more stable than the titanium metal.

Passivation results from the formation of a strong, adherent and protective oxide film on the metal surface, however that protection does not exist in all the media (Piron, 1991).

This phenomenon can be defined as the loss of chemical reactivity exhibited by certain metals and alloys under specific environmental conditions, these metals become inert (passive) and act as if they were noble metals (van Delinder L.S., 1984).

In our field the passivate layer of oxides that forms on the surface of the titanium and his alloys, that we previously described, is the most known example, however chromium and aluminium also forms that oxide layer.

Under certain circumstances the passive oxide layer may be disrupted, however the material has the capacity to regenerate the passive film on his deformed surface, this phenomenon is known as repassivation, and it has a great impact on the tribocorrosion behavior of dental alloys (Mary et al., 2020; Rocha et al., 2013). According to Rocha et al. (2013) higher repassivation rates lead to a better electrochemical response from the material and better protection against corrosion; however, it can also contribute to generating a higher amount of wear debris due to the poor mechanical properties of the regenerated oxide.

When the material suffers alterations in a dry environment a **chemical corrosion will occur**, if the environment is wet or in presence of an electrolytic solution than an **electrochemical corrosion will take place**, that´s the kind of corrosion that occurs in oral cavity because saliva act´s like an electrolytic solution.

Corrosion is an oxidation reaction, it proceeds at the anode, the place at which oxidation occurs, and where electrons are released. In an acidic environment, corrosion may be expressed by the following equations: (1.1) oxidation reaction, (1.2) reduction reaction, and (1.3) redox equation. (Gentil, 2003; van Delinder L.S., 1984)

$$\text{Metal (Me)} \rightarrow \text{Me}^{n+} + \text{ne} \quad (1.1)$$

$$2\text{H}^{+} + 2\text{e} \rightarrow \text{H}_2 \quad (1.2)$$

$$\text{M} + \text{nH}^{+} \rightarrow \text{M}^{n+} + \text{n}/2\text{H}_2 \quad (1.3)$$

The standard electrode potential of an element is then the potential difference expressed in volt between the element and a 1M solution of its ions in relation to the normal hydrogen electrode (Table 1.8).

Table 1.8. Standard electrode potential referred to standard hydrogen electrode.

Metal	Ion	Standard electrode potential
Gold	Au^{+}	+1,5
Gold	Au^{3+}	+1,36
Platinium	Pt^{3+}	+0,86
Palladium	Pd^{2+}	+0,82
Silver	Ag^{+}	+,80
Copper	Cu^{+}	+0,47
Nickel	Ni^{2+}	-0,23
Chromium	Cr^{2+}	-0,56
Aluminium	Al^{3+}	-1,70

The lower position of an element in the standard electromotive force series, the more active it is, and by opposing the higher the metal is on the series, the more inert (noble) it is (Anusavice, 2003).

1.2.1. Oral cavity

The oral environment is very favorable for the occurrence of corrosive phenomena, temperature fluctuations, being a humid environment, pH changes due to diet and food decomposition contribute to this phenomenon.

Saliva is constituted by 99,4% of water and minerals (calcium, magnesium, sodium, phosphorus, chlorine, etc.) and organic substances (urea, uric acid, proteins, lipids, hormones, enzymes, etc.)(Burdairon, 1990; Rocha et al., 2013).

Usually, saliva has a pH between 6 to 7, but these values can suffer alteration with diet, hygiene and bacteria action, such variances may be classified as short or long-term pH variances. Example of short variances are the intake of acidic beverages and long-term variances occur in plaque films where pH in gingival fluid may be 2,3; and in the narrow crevices of restorations and attachments 1,5-2,5 (Geis-Gerstorfer, 1994).

Special attention should be given to patients that are exposed to an extrinsic factor such as ingestion of some acidic beverages with the pH ranges between 2-3, or patients with gastroesophageal reflux, because pH of gastric fluids is above 1 (Arregui et al., 2021a; M. Edgar et al., 2012; Koike & Fujii, 2001; Kunrath et al., 2021), however saliva has an important role in oral lubrication and buffer capacity (Zheng et al., 2021; Zhou & Jin, 2015).

Oral cavity is also subject to a wide amplitude of temperature. During ingestion of food and drinks, temperature can vary 65 °C if a person eats an ice-cream and immediately drinks a coffee (Upadhyay et al., 2006).

Microorganisms can also interfere with metal corrosion, certain species can absorb and metabolize metal from alloys and specially their metabolic products can change de conditions of the medium, making them more conductive, changing de pH values for example (House et al., 2008). Within the oral cavity there are specific locations and situations that can trigger the surface titanium corrosion, inflammation of the surrounding tissues with consequent local acidification and the acidic environment created by lactic acid released by bacteria. The lipopolysaccharide of gram-negative bacteria can increase the inflammation of peri-implant tissues by its marked effects on macrophages, lymphocytes, fibroblasts, and osteoblasts. The oxide film of the titanium surface is attacked and gaps arise in the oxide film. The Ti surface is exposed and ion exchange between the exposed surface and the saliva initiates the corrosion process (Delgado-Ruiz & Romanos, 2018).

The corrosion that occurs in the mouth is a continuous process, because the corrosive products formed are removed from the surface of the alloy with the abrasion of the ingested food and with brushing (Canay & Oktemer, 1992).

Clinically the signals and symptoms reported that may indicate corrosion are: metallic taste, burning sensation, xerostomia, or on the contrary hypersalivation, pulp pain.

More specifically clinicians can find inflammation of the gingiva and of the tongue, accompanied by slight whitening of the mucous membranes, peri-oral stomatitis, hypertrophy and turgescence of the lingual papillae, erosion and ulceration of the oral mucosa and a late leukoplakia, about 4 or 5 years after the beginning of signs. (Ardelean et al., 2015; Burdairon, 1990). Extra-oral manifestations, such as headache due to the occurrence of galvanic current or even trigeminal neuralgy, fatigue, dyspepsia, hair loss, eczematous rashes, and even episodes of brain-fog are also described in literature (Noumbissi et al., 2019) .

1.2.2. Types of corrosion

As we saw previously the oral cavity is a very specific medium where many corrosive phenomena can exist.

The corrosion issue focuses essentially on two problems, corrosion products can be absorbed into the body and cause local or systemic effects, on other hand, corrosion may affect the tribological properties of the dental alloys with serious implications on the clinical performance.

Corrosion can be measured in different ways: through surface analysis, electrochemical tests and measuring ion release with spectroscopy tests (Arregui et al., 2021a), and it is possible to distinguish several forms of corrosion (Figure 1.6).

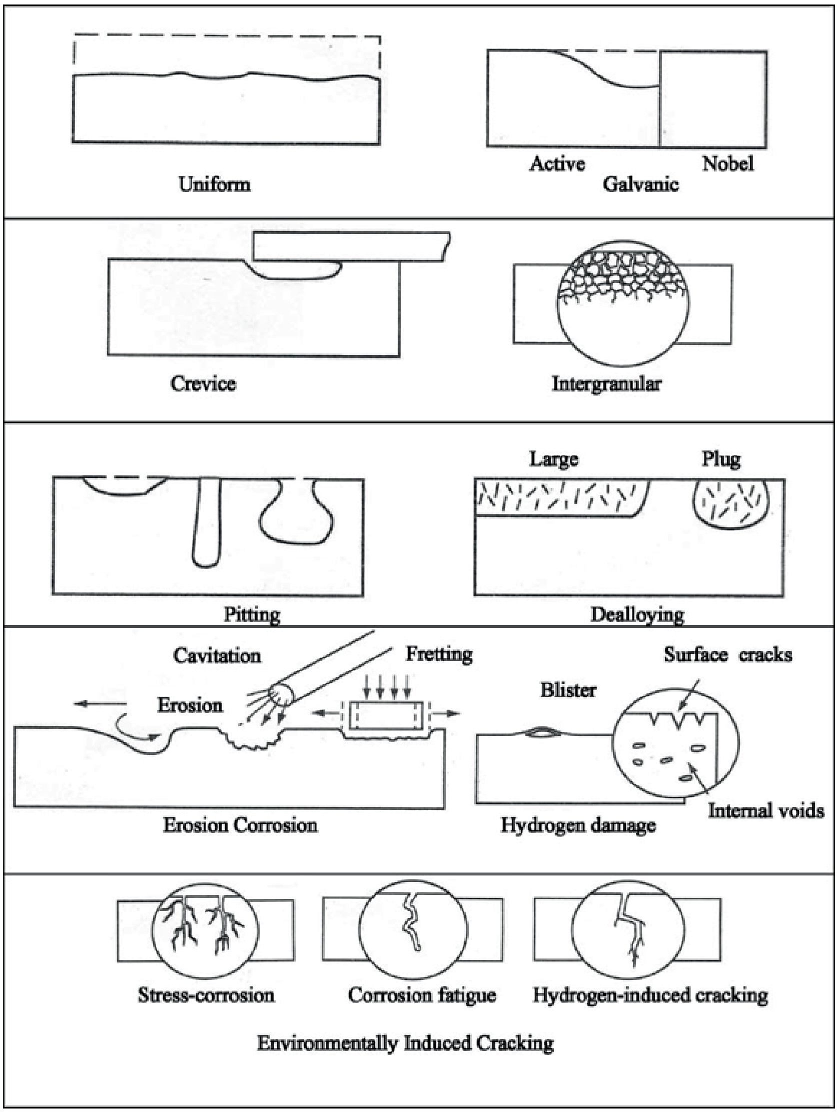


Figure 1.6. Schematic representation of various forms of corrosion. Original image from Upadhyay et al. (2006).

The **uniform** corrosion is characterized by a uniform and generalized reduction of the thickness, meanwhile the **localized corrosion** is presented in a specific area, and is usually a slow process that affects all the metals (House et al., 2008; Upadhyay et al., 2006).

Crevice corrosion is a localized corrosion resulting from the formation of a concentration cell in a crevice formed between a metal and a nonmetal, or between two metal surfaces (van Delinder L.S., 1984) , results from differences in oxygen concentration of electrolytes or changes in pH in a confined space.

Chemically is characterized by high chlorine ion concentration, low oxygen concentration and low pH, and is an extremely aggressive form of corrosion.

In crevice sites, local flux of ions is enhanced and tissue impregnations follows, this is specially cause of concern in implants and screws because they don´t have regular shapes (Upadhyay et al., 2006).

Pitting corrosion is highly localized corrosion resulting in deep penetration at only a few isolated spots, while the surrounding areas are not affected (van Delinder L.S., 1984). According to Noumbissi et al. (2019) this occurs usually along with fluoride-based solutions used during dental procedures and home care.

Selective leaching or Selective corrosion is a form of corrosion that occurs by selective dissolution of certain elements of the alloy. It results from the action of specific environments that remove the most active element from the solid, leaving a porous on the structure (Piron, 1991).

In the **intergranular corrosion** the metal corrodes in a depth way, preferentially at grain boundaries. Due to more reactive nature of grain boundaries, intergranular corrosion occurs adjacent to grain boundaries with relatively little corrosion of grains. This type of corrosion occurs due to the inhomogeneity and mainly technological errors. Heat treatments may improve the resistance to this kind of corrosion (Upadhyay et al., 2006).

Galvanic corrosion

When two metallic metals, with different electrode potentials, are in contact in the presence of an electrolytic solution, a potential difference occurs and consequent transfer of electrons, we are in presence of galvanic corrosion (Figure 1.7) (Gentil, 2003). In the galvanic couple, if galvanic corrosion develops, the less noble metal corrodes (anode) and the more noble (cathode) is protected. The 3 prerequisites of galvanic corrosion: metal alloys with different corrosion potentials connected by the same electrolytic solution.

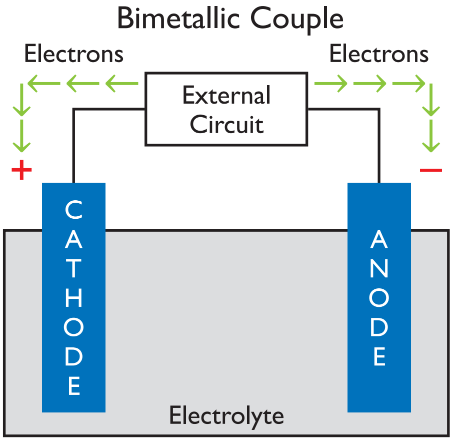


Figure 1.7. Schematic representation of galvanic *corrosion reproduced from* https://galvanizeit.org/corrosion/corrosion-process/galvanic-corrosion (American Galvanizers Association, n.d.)

The corrosion of the metallic material that works as an anode is more accentuated than the corrosion of this material if it exists alone in the same medium. The corrosion of the metal acting as the cathode is very low and markedly less than that which occurs when the material corrodes alone.

The surface area of the different components is one of the factors to be taken into account in galvanic corrosion, with the most unfavorable situation being when a small anode is connected to a large cathode (Grosgogeat et al., 1999).

Besides the factors already enumerated that affect corrosion, galvanic corrosion in particularly can be affected by mass transport events like migration, diffusion and convection.

It is therefore extremely important that in an implant rehabilitation, the suprastructure material has a galvanic potential close to that of the implant material, if this is not taken into account, a galvanic current may occur with regrettable biological effects such as dissolution of the metallic alloy and bone resorption (Geis-Gerstorfer, 1994; Taher, 2003).

Noumbissi et al. (2019) also consider **microbial corrosion** as a result of the inflammatory process triggered by the host response to the materials and the products released by anaerobic bacteria, that create an acidic environment that is corrosive to the implant and its prosthetic parts.

If there is an association between a corrosive medium and mechanical stresses, the metal can undergo an accelerated corrosive process, even without an accentuated loss of mass. Within these situations we can consider: corrosion under fatigue, corrosion with erosion, cavitation and impingement, corrosion under friction, corrosion under tension, etc. (Gentil, 2003).

In the **Erosion corrosion**, the corrosion is increased or accelerated because of the abrasive action of a moving stream, the presence of suspended particles greatly accelerates abrasive action. The presence of a corrosive medium is a condition to occur this type of corrosion (van Delinder L.S., 1984).

Cavitation corrosion is not important in dentistry, because is due to a phenomenon that does not exist on oral cavity, the repeated collapse of vapor bubbles and subsequent electrochemical attack of the damaged area (Piron, 1991; van Delinder L.S., 1984).

Fretting corrosion can be considered a type of tribocorrosion that is caused by small cyclic and repetitive vibrational or slip movements between two materials, combined with corrosive attack from the environment (Upadhyay et al., 2006; van Delinder L.S., 1984). The amplitude of those movements is smaller than 100μm. (Sivakumar et al., 2011). This type of corrosion is one of the mode failures of orthopedic and dental implants (Mathew et al., 2009).

The damage caused by this type of corrosion is characterized by discoloration of the metal surface, formation of corrosion products and in some cases pits, which can serve as cores for the occurrence of fatigue fractures (Gentil, 2003).

Stress corrosion or **Stress corrosion cracking** refers to failure under simultaneous presence of a corrosive medium and a tensile stress, and **Fatigue corrosion** is the joint action between corrosion and fatigue leading to the metal fracture. Stress corrosion and fatigue corrosion are very similar, but fatigue corrosion is extremely non-specific (Trethewey & Chamberlain, 1998).

Stress corrosion is a very dangerous process that can impair the integrity of an implant.

1.2.3. Tribocorrosion

The synergistic action between corrosion and wear results in an irreversible transformation of a material called tribocorrosion (Landolt et al., 2004). If that process occurs in a biological environment can be also called biotribocorrosion. The material degradation occurs due to a mechanical action, a tribological process; and a chemical or electrochemical process due to a corrosive environment (Figure 1.8).

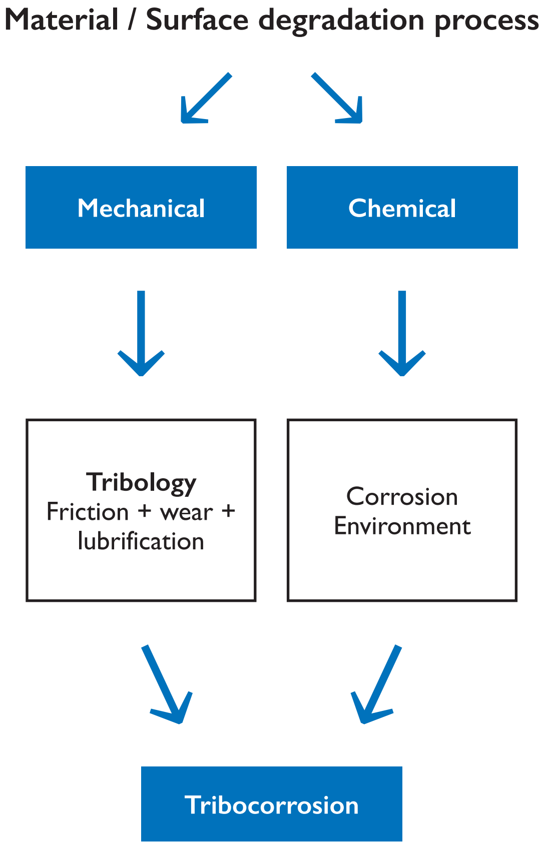
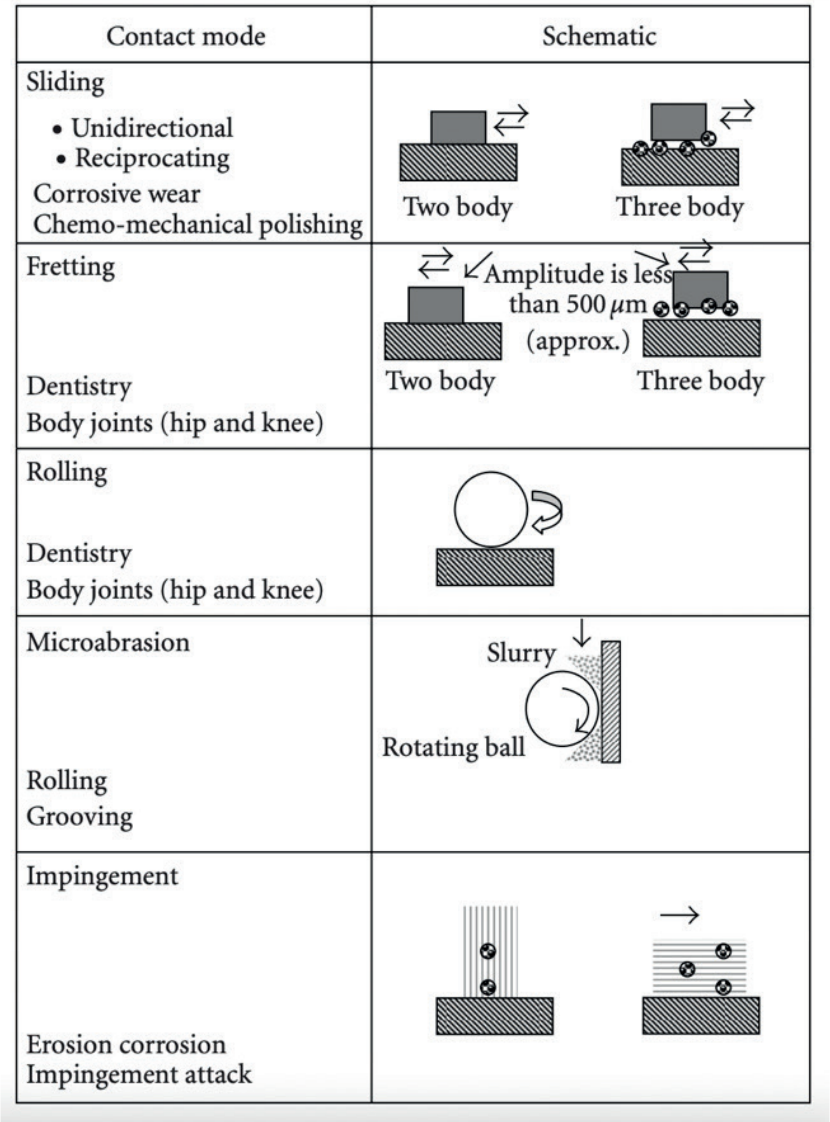


Figure 1.8. Schematic presentation of tribocorrosion. Adapted from Mathew et al. (2009).

The factors that affect tribocorrosion are the same that are related to corrosion and additionally the ones that affect wear such as the force, velocity of the movement, types of motion (unidirectional, sliding, reciprocating, rolling or impact loads), duration of contact, lubrification, shape and size of contacting bodies, alignment and vibration (Mathew et al., 2009). In Figure 1.9 is demonstrated some of the contact modes that can occur in the wear phenomenon.



Contact mode	Schematic
Sliding • Unidirectional • Reciprocating Corrosive wear Chemo-mechanical polishing	Two body Three body
Fretting Dentistry Body joints (hip and knee)	Amplitude is less than 500 μm (approx.) Two body Three body
Rolling Dentistry Body joints (hip and knee)	
Microabrasion Rolling Grooving	Slurry Rotating ball
Impingement Erosion corrosion Impingement attack	

Figure 1.9. Different contact modes that can occur in the wear phenomenon.
Image from de original publication (Mathew et al., 2009).

When we consider the different types of prosthetic devices existing in the oral cavity, in the crown-abutment-implant-bone system, due to movements resulting from mastication, micro-movements propagate to the implant/bone; abutment/implant and crown/abutment interfaces, so the mechanism that affects these areas is the fretting tribocorrosion.

In the occlusal contacts arising from mastication or bruxism, the mechanism existents are the sliding or fretting wear, depending of the amplitude of the movements. In fretting wear the amplitude of the movements, which is much smaller than in sliding wear.

Wear can be defined as a process where interaction between two surfaces within the working environment that results in dimensional loss of one solid, with or without any actual decoupling and loss of material (Affatato & Grillini, 2013).

Wear along with friction and lubrication are the branches of tribology, the science of interacting surfaces in relative motion (Figure 1.8).

The kind of surface contact that exist in wear process is shown in Figure 1.9 and we can distingue several types of wear (Figure 1.10).

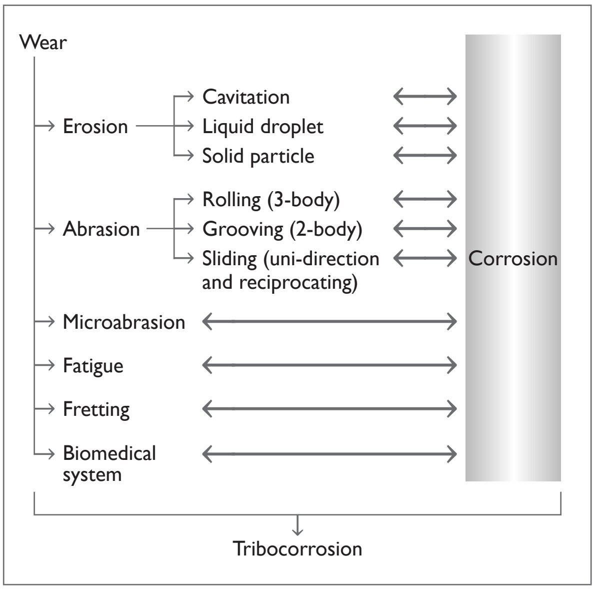


Figure 1.10. Types of wear. Image from the original publication (Mathew et al., 2009).

Regarding wear, it is important to clarify several concepts:

Abrasive wear occurs during the sliding of a hard and rough surface on a smoother surface and is sub-divided in two types: two-body abrasion, when surfaces are kept in direct contact during the rubbed movement and three-body abrasion when there´s a third intervenient such as abrasive particles.

Adhesive wear takes place when two surfaces are pulled one against the other under a certain load, due to a high attraction between them, during sliding fragments will be pulled off one surface and adhere to the other, a phenomenon also called "cold welds". This is the most common and least preventable type of wear, and is normally associated with metals (Devaraju, 2015; Lambrechts et al., 2006; Pourasiabi et al., 2013; *Types of Wear*, n.d.).

Fretting wear is a type of wear that occurs during slight, on the order of a fraction of a mm, reciprocating movement of bodies that are in contact with each other (Chen & Zhou, 2001; Sidun et al., 2012), in a slip and low frequency (Waterhouse, 1984). It is a very complex phenomenon that may occur, often at rest, between contacting surfaces subjected to such as mechanical vibration, cyclic loads, electromagnetic shocks or thermal cycling (Swain et al., 2021; Zhu & Zhou, 2011). The damages of fretting wear are strongly dependent upon the normal load, displacement amplitude, frequency, properties of material (Zhu & Zhou, 2011).

Fatigue wear occurs when a repeated sliding or rolling over a surface causes subsurface cracks that initiate, grow and eventually lead to material breakup. Applying alternative stress on materials surface layers can result in formation of fatigue stress. Stress increase under the surface makes microcracks in this area. These cracks coalesce from maximum shear stress points and then propagated towards the surface, then there can be loss of a fragment of material (Lambrechts et al., 2006; Pourasiabi et al., 2013).

Erosive wear is due to mechanical interaction between solid surface and fluid, or impinging liquid or solid particles. When particles with some velocities are impacted on the surface of metal, the pits and large-scale subsurface deformation occur on the metal surface (Devaraju, 2015).

Oxidative wear happens when dry metals sliding in air or exposure to oxygen. At elevated temperature, oxygen can interact with sliding surface and form oxides called oxidative wear, temperature rise because of metallic pairs sliding makes an oxide layer with few microns thickness on counterfaces. This oxidative layer is destroyed a few minutes after being formed due to contact sever effects. After a while the layer will be made again, because this layer is weak and mechanically incompatible. This repetitive mechanism and depends on the speed of the movement and temperature (Devaraju, 2015; Pourasiabi et al., 2013).

Lambrechts et al. (2006) made an overview of the existing methods to simulate wear in the oral cavity, where wear testing devices, wear simulation techniques and wear terminology was widely clarified, So the two-body abrasion was considered by them, the wear that occurs in non-masticatory tooth movement such as in bruxism, and the three-body abrasion occurring in masticatory movements. The wear located in occlusal contact areas and in free occlusal contact areas is also distinguished.

The mechanical behavior of several types of wear is affected by the microstructure of the materials and the presence of defects, like phase distribution, grain size and orientation, nonmetallic inclusions, segregations, dislocation density affect the mechanical behavior of several types of wear (Mathew et al., 2009).

The progression of wear depends of several factors such as the presence of lubrification, the duration and area of contact, temperature and load applied. (Yap et al., 1999) According to several authors the synergy between wear and corrosion increases the rate of degradation of the biomaterial, being considered one of the most destructive processes on the surface of the material, due to the destruction of the protective passive layer that occurs when two surfaces rub against each other, leading to a reduction in its useful life (Kim et al., 2006; Rocha et al., 2013).

Landolt et al.(2004) proposed a tribocorrosion approach involving metals (first body) and an inert counter body (second body) in sliding contact, immersed in an electrolytic solution. According to them, the tribocorrosion mechanism leads to the detachment of worn particles that may form a third body and depending on their properties, corrosion can accelerate or slow down the rate of mechanical material removal from the first body. Additionally, the material removed during this process, generates different material fluxes: metal particles may eject from the contact area, transfer to the counter body, spread on the first body, suffer dissolution or create a third body. The metallic third body particles created may also undergo electrochemical oxidation, leading to formation of solid oxides or dissolved ions. The metal (first body) for being immersed in the electrolytic solution is also a source of ions by itself, either through its dissolution or through the dissolution of the oxide film formed on its surface (Landolt et al., 2004).

Some metals, such as titanium have high repassivation rates, that provide them a better electrochemical behavior and protection against corrosion; however, it can also contribute to generating a higher amount of wear debris due to the poor mechanical properties of the regenerated oxide (Rocha et al., 2013).

The biological impact of these released particles will be developed in the next section of this work.

Considering the wear that occurs in dental materials, is not an isolated process but the result of various types of wear such as adhesive, abrasive, fatigue and corrosive; that may occur simultaneously or sequentially and may be influenced by each other, which makes intraoral wear a complex phenomenon.

Although there are several studies on tribocorrosion, namely that assess stress corrosion Könönen et al., 1995;Vieira et al., 2006), fatigue (Zavanelli et al., 2004), elastic tensile strain on dental alloys(H.-H. Huang, 2002), as far as we have knowledge on those studies, alloys were tested isolated. In the study we undertook and included in chapter 2, two different dental alloys were tested simultaneously in the same medium, being submitted to a sliding wear test, two-body abrasion in a wear simulator designed to simulate the chewing movement.

During mastication, implants are subject to several forces that create micromovements in the interfaces bone/implant/abutment/crown (Rocha et al., 2013; Zhu et al., 2009).

The tribocorrosion phenomenon, that fits in corrosion fretting, and occurs in that situation, results in a release of debris under a form of metallic particles and/or ions, that leads to a biological response, commonly associated with inflammatory responses and the activation of bone resorptive cells (osteoclasts), which may lead to osteolysis (bone resorption) and, ultimately, implant loosening locally, but also to a harmful effect systemically (B. C. Costa et al., 2019; Rocha et al., 2013).

The problem of tribocorrosion in prosthetic devices is therefore focused on the reduction in their use time due to material failure, such as the release of worn material into the oral environment, the biological implication of this situation, although reported by several authors, is still unclear and subject of many investigations.

1.2.4. Fluoride impact on titanium alloys corrosion

Titanium offers excellent corrosion resistance in most environments, except those media that contain fluoride ions (Donachie, 2000).

Fluoride ions can infiltrate and dissolve the stabilizing oxide layer of titanium and titanium alloys. Fluoride-based formulations, used in prevention of dental caries, often with acidic pH and in gel presentation increase corrosion and surface roughness due to their pronounced adhesion (Tschernitschek et al., 2006).

With low levels of pH, even small amounts of fluoride can cause titanium corrosion, because fluoride ions form hydrofluoric acid, that with a concentration higher than 30 ppm leads to the destruction of the titanium passive film (Nakagawa et al., 1999).

According to Her-Hsiung Huang, notable corrosion occurs on cpTi surface when the concentration of NaF increased up to 1% (H.-H. Huang, 2002).

Because of the discussed mechanism, the solvation of the passive oxide layers and the accelerated solubility of titanium oxide, will occur by decreasing pH-values concentration of hydrogen cations, which will cause the ion release of titanium (Strietzel et al., 1998).

It is consensual that passive layer of the titanium and titanium alloys reacts in fluoride solutions, with the formation of complex titanium–fluoride molecules which are very stable and soluble in the solution. The complexes which can be found on the surface of the alloy can either be of titanium fluoride, titanium oxide fluoride, or sodium titanium fluoride (Kaneko et al., 2003; Toumelin-Chemla et al., 1996). These soluble molecules replace the titanium oxide film, allowing corrosion initiation; when it happens, the alloy becomes more susceptible to the hydrogen embrittlement due to the high affinity from the titanium to the hydrogen leading to an accentuated decreasing of the corrosion resistance.

Fluoride ions have the capability to bond to the titanium oxide layer and create compounds that dissolve easily in acidic media, thereby facilitating titanium or metal dissolution and particle and ion release (Delgado-Ruiz & Romanos, 2018). Some authors recommended to utilize non-fluoride rinses or gels for daily care and use non-acidic substances in patients with titanium dental implants, others advise placing physical barriers such as PTFE tap or rubber dam in areas rehabilitated with implants before making topical applications of fluoride with trays in the office (Delgado-Ruiz & Romanos, 2018; H. H. Huang & Lee, 2005).

1.3. Ion metal release from Prosthodontic Devices

According to Wataha, probably the most relevant measurement of corrosion from the point of view of biocompatibility is the quantification and identification of the released ions (Wataha, 2000).

The amount and nature of released ions varied depending upon the type of alloy and other parameters, e.g., type of corrosion, composition and chemical characteristics of the corrosive solution—such as pH and ionic composition, artificial saliva, cell culture medium, serum, etc. (Geurtsen, 2002).

Studies on metallic ion release under different conditions proved that it was not directly proportional to ion concentration in the dental alloy, some elements are more unstable than others, this phenomenon is called selective dissolving (Can et al., 2007; Wataha & Lockwood, 1998).

As seen previously in Landolt et al. report (2004) a metal tribocorrosion system is a considerable source of metal ions and particles, that spread into the environment. In a review article on release metal ions from dental casting alloys, Geurtsen (2002) concluded that cations released from dental alloys may cause significant biological alterations even at non-cytotoxic concentrations.

When analyzing the issue of ion release, we have to differentiate two types of clinical applications of dental alloys; when they are used in prosthetic devices, which, despite not being inserted into the body, release ions into the oral cavity and which can be accessed by the inside of the body through the epithelium in the gut or through the gingiva and oral mucosa (Wataha, 2000) ; and when ions are released from dental implants into the bone tissues around the implant that are clearly into human body.

Delgado-Ruiz & Romanos (2018) made a systematic review where they clarified the different sources of titanium particles and ions that can be released, in oral cavity, during the surgical, prosthetic and maintenance phases due to different causes during the life span of a dental implant. The rectangles filled in red are the three phases in implant dentistry procedures in which titanium particles and ions can be released. Within the experimental part of this work, on chapters 3 and 4, we consider ions released on the Prosthetic/ Rehabilitation phase (Figure 1.11). In that phase the potential sources of titanium particles and ion release are the implant abutment material interface, the loss of superficial oxide layer, from the microgap at the implant abutment interface resulting from micromovements, fretting at the implant/bone interface by corrosion and tribocorrosion and also the fluoride effect that may act as an initiator of this release.

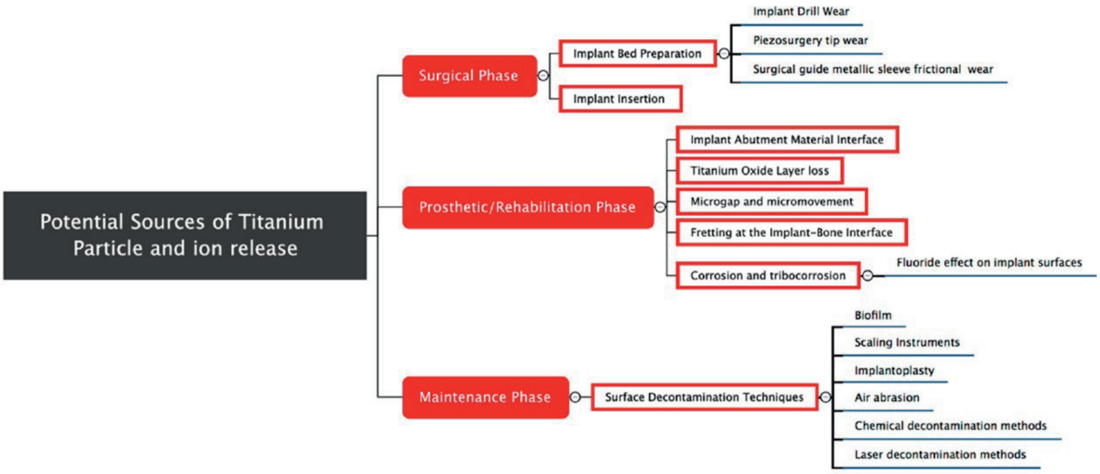


Figure 1.11. Potential sources of titanium particle and ion release.
Reproduction of the original image of the author (Delgado-Ruiz & Romanos, 2018).

Wear particle release at the interface of dental implant components is a current issue, being the object of several recent studies. Noumbissi et al. (2019) made a literature review study on atomic ions dissolution of titanium and its alloys in implant dentistry and by the analysis of 64 papers, published between 1940 and 2018, concluded that the presence of the long-term corrosion reaction along with continuous corrosion leads to the release of ions into the peri-implant tissue but also to a disintegration of the implant that contribute to material fatigue and even fracture of the abutments and implant body or both.

Olander et al. (2022) evaluated the effect of combining implant and abutment materials on wear and particle release in a dynamical loading setup. They observed that signs of wear deformation were found on all couplings and that the wear was concentrated around three areas; abutment base, inside the implant connecting surface, and on top of the implant head.

Implantoplasty is a procedure that also may undergo ion release, according to Barrak et al. (2020) there are release of nano and fine particles from both commercially pure grade 4 titanium and grade 5 Ti-6Al-4 V alloy implants following implantoplasty procedure, therefore, the authors do not recommend this treatment as a safe procedure in peri-implantitis, regardless of the material of the implant, because this procedure may release nanometer-sized particles, whose full systemic effect is not fully understood.

As we have seen previously some particulate metals released from implant surfaces, due to tribocorrosion, these particles can assume several forms that include nanometric and micrometric particles, colloidal and ionic forms, organic forms and inorganic oxides(Wilson, 2021); and their degradation products exhibit local and systemic reactions after implant installation and osseointegration, particularly at local level there´s a release of inflammatory mediators from macrophages, that lead to a bone resorption and encapsulation of implant, contributing to unexpected treatment complications and adverse effects, that may culminate with the implant failure (Figure 1.12) (Apaza-Bedoya et al., 2017; Azzi & Szpunar, 2007; Dini et al., 2020; Kunrath et al., 2021; Rocha et al., 2013; Scortecci et al., 2001)

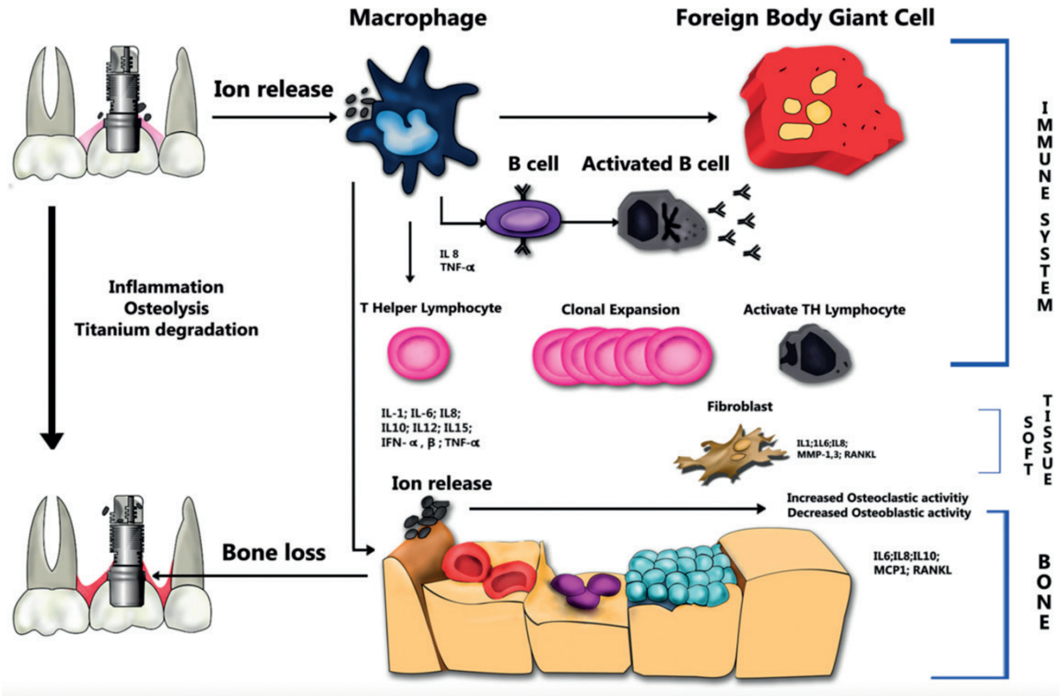


Figure 1.12. Schematic representative diagram of the inflammatory process in the peri-implantar zone. Reproduction of the original image of the author (Dini et al., 2020).

Those findings have been corroborated by more recent studies, Biguetti et al. (2021), were they observed that corrosion of Ti-based biomaterials induced exacerbated inflammatory response in both connective tissue and bone, linked to the upregulation of fibrosis, pro-inflammatory and osteoclastic markers and resulted in unfavorable healing and osseointegration outcomes.

The biological response to the particles that are released due to corrosion depends on the type, size, surface area, materials chemistry and amount of the element and level of corrosion of the alloy on the metal alloy, the duration of exposure to tissues and the amount of the ion already present in the organism (Azzi & Szpunar, 2007; Wataha, 2000).

This problem happens not only in dentistry but also with orthopedic implants, the low frictional wear resistance of Ti implants led to the release of fine metallic and polymer particles to the body. As the body attempts to eliminate these wear particles, it triggers an autoimmune reaction, which causes resorption of living bone tissues, causing joint failure (Durgalakshmi et al., 2013a).

Kumazawa et al. (2002) studied the cytotoxicity of soluble and particulate Titanium, Vanadium, Nickel, and conclude that the cytotoxic effect of Ti particles is size dependent, and that they must be smaller than that of cells, Ti particles below 10 μm in diameter induce cytotoxicity both in vivo and in vitro. When the Ti particle is smaller than the cell (10 µm), cytotoxicity is induced, and are swallowed by inflammatory cells. Ni in solution destroys the cell membrane of neutrophils, whereas Ti and V in solution stimulate neutrophils and increase the quantity of released superoxide anions. The release of superoxide anions and TNF-a due to stimulation and phagocytosis are factors that can enhance the further process of phagocytosis and inflammation. Therefore, the effects of fine particles of Ti that are produced during various clinical processes must be carefully considered.

For having a high impact on the success of implant-supported rehabilitations, this issue has been widely documented in the literature; in another review are related the consequences of Ti particles released in the peri-implant soft tissues and the inflammatory process induced by Ti wear particles. This process causes the activation of immune-system cells (monocytes, macrophages, neutrophils, and T lymphocytes) with the release of pro-inflammatory cytokines, such as TNF-a, IL-1a, 1b, IL-6, IL-10, and prostaglandin (PGE2) which leads to damages in the epithelial barrier, facilitating bacterial colonization, increasing the inflammatory, and infectious reactions (Dini et al., 2020).

An inflamed or infected environment can generate an intense tendency to biocorrosion. Dead cells, as well as contaminated and inflamed regions have lower pH and oxygen vacancy leaving more reactive areas promoting corrosion, this process over time causes surface failures and the release of particles/ions from the implant (Kunrath et al., 2021).

Due to the cytotoxicity associated to V e Al, Park et al. (2013) investigated the biocompatibility of nine ions, possible alloying elements to Ti, and suggested that the ranking of pure metal cytotoxicity from most potent to least potent was as follows:

$$\mathrm{Cu} > \mathrm{Al} > \mathrm{Ag} > \mathrm{V} > \mathrm{Mn} > \mathrm{Cr} > \mathrm{Zr} > \mathrm{Nb} > \mathrm{Mo} > \mathrm{cp\,Ti}.$$

There are also several studies concerning the release of ions in saliva; the concentration of metal ions in saliva, in patients with metal devices, seems to be bigger and directly related to the acidity of the environment (Borg et al., 2018; Garhammer et al., 2004).

In an *in vitro* and *in vivo* study, El Sawy & Shaarawy (2014) investigated the type and amount of ions released from Ti-6Al-4V and Co-Cr-Mo alloys over a period of 28 day, in buffer saline and human saliva, the results showed that three types of metal ions were released from the Co-Cr-Mo alloy during the study period: Co, Cr and Mo and two types of metal ions were released from the Ti-4Al-6V alloy during the study period: Ti and Al. Some studies suggest that the maximum rate of metallic ions released was reached after approximately 2 weeks, and then the rate diminished with time, thus did not happen on this study.

Furthermore, a study with five different mini-implant systems, composed of Ti and

Ti-6Al-4V alloys, revealed a significant release of Ti, Al, and V ions after 30 days of immersion in artificial saliva, showing that degradation of these alloys in induced oral environment does not depend on long periods (Kunrath et al., 2021).

Puskar et al. (2014b) studied the ion release on Co-Cr-Mo dental alloy, at different pH and conclude that the acidity of artificial saliva increased the ion release in the alloys tested and that Co was the ion most released; similar findings were reported by Luchetti et al. (2015), who concluded that Co-Cr casting alloys shows higher ion release under acidic conditions.

The material degradation in implants and cobalt-chromium or titanium frameworks, before and after exposure to artificial saliva was evaluated by Hjalmarsson et al. (2011) with the following conclusions: cobalt release was higher than release of titanium or chromium, what is consistent with Puskal et al. reports ; the contact surfaces of the implants, as well as of the frameworks, became rougher after saliva exposure; which would indicate active material degradation processes for both implants and framework materials.

According to Hanawa et al. (2004), Ti ion for being very active, reacts with hydroxyl radicals and anions and forms oxide and salt in body fluid, so the possibility of combination with biomolecule is lower, but still can occur, from the same type of ion are zirconium, niobium, and tantalum. Ni and Cu ions are inactive, do not combine immediately with water molecules and inorganic anions and remain in an ionic state for relatively long time, having more probability to combine with biomolecules with inherent toxicity (Figure 1.13).

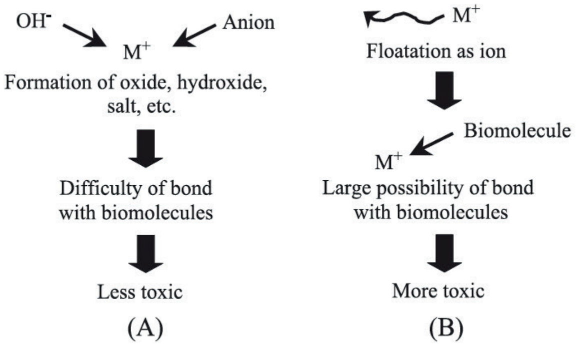


Figure 1.13. Toxicity of metal ions classified with the possibility of bind with water molecules and anions. Reproduction of the original image of the author (Hanawa, 2004).

Beyond the implications at the local level, metal ions may be distributed by diffusion through tissues, the lymphatic system, or the bloodstream and as previous described metallic particles (0.5 to 10.0 µm) may also be ingested by cells such as macrophages, which are themselves transported by the lymphatics or blood vessels, and can be accumulated in different organs or excreted (Kunrath et al., 2021; Wataha, 2000; Zarb et al., 1978).

The long-term local and systemic effects of titanium particles and ions released into the oral environment and their potential effects on cells, tissues and organs remain unknown due to the rapid evolution and variability of new implant surfaces, new implant-abutment connections and new restorative materials (Delgado-Ruiz & Romanos, 2018).

There has been in Europe in recent years, an increasing concern about the toxicology of chemicals in humans, through the European Chemical Agency (ECHA), metal ions released by dental alloys can induce toxic, allergic, inflammatory or mutagenic reactions.

REACH (registration, evaluation, authorization, and restriction of chemicals) is a regulation of the European Union, adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, and regulate the substances of very high concern (SVHC), namely endocrine disruptors, carcinogens, mutagens, toxics for reproduction, sensitizers, and allergens. For instance, Ni derivates, Cr(VI), were classified as carcinogenic to humans, and metallic nickel, titanium dioxide, cobalt and its compounds as possibly carcinogenic to humans(Ardelean et al., 2016; *European Chemicals Agency*, n.d.; Ionescu et al., 2019; Mulware, 2013a; Reclaru et al., 2020a).

By the exposed and considering the new medical device regulation (*Medical Devices- European Medicines Agency*, n.d.) to be implemented in 2022, the study of metal ions used in biomedical devices is increasingly needed.

1.4. Diffusion in aqueous solutions

Several methods to assess corrosion, metal ion release and biomaterials subject to tribocorrosion are found in the literature, as for example, corrosion tests under static and dynamic conditions, varying the environment conditions in different ways (pH, time, presence of bacteria, fluoride, enzymes, etc.), with biomaterials being analyzed from a tribological point of view by mechanical tests in simulated body fluids with subsequent analysis of the surface.

While numerous studies have been carried out on the detection and quantification of ions released using different techniques, (e.g. Polarization Tests, Atomic Absorption Spectroscopy (AAS), Electrochemical Impedance Spectroscopy (EIS), Inductively coupled Plasma (ICP) (Vaicelyte et al., 2020), few have taken into account the transport behavior (diffusion, viscosity, transport number, conductivity) of these complex chemical systems resulting of the presence of these ions in different environment, such as, in the oral cavity.

In our previous works(A. Ribeiro et al., 2012), the metal ions released after *in vitro* tribological studies were evaluated by using atomic absorption spectroscopy, and the transport properties of these systems containing these entities (i.e., diffusion, viscosity and conductance) were characterized.

The measurements of transport properties provide data of outmost importance for different areas, such as corrosion and drug delivery. However, information on many systems, of practical importance, is still lacking in the scientific literature probably because the measurements, require a lot of attention and a learning curve to eliminate possible errors in laboratory procedures.

In recent decades, the main sources and methods of measurements of these properties have been the subject of study by some authors, improving their precision and accuracy(Asfour, 1985; Callendar & Leaist, 2006a; Price, 1988a; A. C. F. Ribeiro et al., 2010).

Among the transport properties, mutual diffusion has been one of the focus of this dissertation, due to its importance in different areas such as fundamental and applied sciences.

1.4.1. Diffusion - the concept

The diffusion is an irreversible phenomenon that involve mass transfer, can be observed in physical, chemical and biological processes, being therefore of great importance (Tyrrell & Harris, 1984).

The mass transport processes in aqueous solutions result from a situation where the systems are far from their thermodynamic equilibrium states caused for heterogeneities of temperature, composition or electrical potential, for example (A. C. F. Ribeiro, 2020).

Many techniques are used to study mutual diffusion such as optical, conductimetric or diaphragm-cell techniques (Robinson & Stokes, 1959; Tyrrell & Harris, 1984); these techniques, although reliable, involve extremely laborious laboratory procedures, so that, the Taylor Dispersion technique has gained popularity since it presents several advantages over the others, namely because it is simpler and it is immune to errors from convection.(Deng & Leaist, 1991b; A. C. F. Ribeiro et al., 2005a).

The diffusion phenomenon on solutions (known to be irreversible process) is based on the gradient of concentration of a given component inside this medium (without migration or convection) that produces a flow of matter in opposite direction, which arises from random fluctuations in the positions of molecules in space. Although the chemical potential gradient in the real solution is considered the true virtual force producing diffusion, however, in ideal solutions, this force can be quantified by the concentration gradient at constant temperature. Thus, we may consider the following approaches to describe the isothermal diffusion: the thermodynamics of irreversible processes and Fick´s laws (Robinson & Stokes, 1959).

Diffusion coefficient, *D,* in a binary system (i.e., with two independent components), may be defined in terms of the concentration gradient by a phenomenological relationship, known as Fick´s first law (Eqs. 1.4 and 1.5),

$$J = -D\frac{\partial C}{\partial x} \tag{1.4}$$

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial C}{\partial x}\right) \tag{1.5}$$

where J represents the flow of matter across a suitable chosen reference plane per area unit and per time unit (t), in a one-dimensional system, and C is the concentration of solute in moles per volume unit at the point considered.

The resolution of equation (1.5) for a unidimensional process is much easier if we consider D as a constant. This approximation is applicable only when there are small differences of concentration, which is the case in the open-ended condutimetric technique, and in the Taylor technique (Robinson & Stokes, 1959; Tyrrell & Harris, 1984). In these conditions, it is legitimate to consider that our measurements of differential diffusion coefficients obtained by the above techniques are parameters with a well-defined thermodynamic meaning (Robinson & Stokes, 1959; Tyrrell & Harris, 1984).

However, in the case of ternary systems (i.e., with three independent components), Fick's laws (Equations (1.4) and (1.5)) are, in general, inadequate to describe the diffusion process.

The flux of each component can be affected by the concentration gradient of the other component, and this interaction can occur whether the components are ionic or molecular (Asfour, 1985; Hertz, 1975; Tyrrell & Harris, 1984).

Restricting diffusion to a one-dimensional process, the equations that describe the flows of the components in a ternary system are given by the equations (1.6) and (1.7).

That is,

$$J_1 = -D_{11}\nabla C_1 - D_{12}\nabla C2 \tag{1.6}$$

$$J_2 = -D_{21}\nabla C_1 - D_{22}\nabla C2 \tag{1.7}$$

where J_1 and J_2 the molar fluxes of component 1 and component 2 driven by the concentration gradients ∇C_1 and ∇C_2 of each solute 1 and 2, respectively. Main diffusion coefficients D_{11} and D_{22} give the flux of each solute driven by its own concentration gradient. Cross-diffusion coefficients D_{12} and D_{21} give the coupled flux of each solute driven by a concentration gradient in the other solute. A positive D_{ik} cross-coefficient ($i \neq k$) indicates co-current coupled transport of solute i from regions of higher to lower concentrations of solute k. On the other hand, a negative D_{ik} coefficient indicates counter-current coupled transport of solute i from regions of lower to higher concentration of solute k.

1.4.2. Self-diffusion and mutual diffusion

Self-diffusion D^* and mutual diffusion are two different phenomena but often confused with each other. However, IUPAC's compendium of chemical terminology distinguishes clearly these terms. That is, self-diffusion D^* (or intradiffusion, tracer diffusion, single ion diffusion or ionic diffusion), and mutual diffusion *D* (also known as salt diffusion, interdiffusion or concentration diffusion) (A. Ribeiro et al., 2012) .

Polarography, NMR, and capillary-tube techniques with radioactive isotopes are used in the measurements of self-diffusion coefficients, but not in mutual diffusion.

As a matter of fact, mutual diffusion coefficients *D*. is the parameter indicated for bulk substance transport, giving useful information about the transport in solutions of molecules and ions (A. C. F. Ribeiro et al., 2005a).

Theoretical relationships derived between self-diffusion and mutual diffusion coefficients, D^* and *D*, respectively, have had limited success for estimations of *D* (as well as theoretical formulae for the calculation of *D*) and consequently experimental mutual diffusion coefficients are absolutely necessary (A. Ribeiro et al., 2012).

In binary solutions (i.e., containing water and a solute) for example, mutual diffusion of the solute is accompanied by a flux of solvent in the opposite direction. If there is a concentration gradient in a binary electrolyte solution, then the constraint of maintaining electrical neutrality ensures that positive and negative ions move from the region of higher to lower concentration at the same speed. On a volume-fixed and most other reference frames, there is only one mutual or inter diffusion coefficient in this system.

In multicomponent systems, it is not uncommon for a diffusing solute to drive substantial co-current or counter-current coupled fluxes of other solutes, especially in solutions of mixed electrolytes, polymers or associating components. For these reasons, mutual diffusion data are used to understand the rates of many processes involving mass transport driven by chemical concentration gradients for instance corrosion and diffusion-limited chemical reactions (Callendar & Leaist, 2006a).

Table 1.9 shows some examples of binary systems and multicomponent systems already studied (A. Ribeiro et al., 2012).

Table 1.9. Some examples of binary systems and multicomponent systems (A. Ribeiro et al., 2012).

Binary systems	Ternary and quaternary systems
I. System with one weak electrolyte in aqueous solutions at different concentrations and temperatures (e.g., lactic acid).	III. System with one non -electrolyte in aqueous solutions of different components, and at different concentrations and temperatures. (e.g. drug /water)
II. System with non-electrolyte in aqueous solutions of different components, and at different pH and temperature (carbohydrates, cyclodextrins, such as βCD, DM-β-CD, HP-β-CD, SBE-β-CD, and drugs (analgesic and anti-inflamatory)).	IV. System with two electrolytes (or one electrolyte and one non –electrolyte) in aqueous solutions of different components, and at different concentrations and temperatures (e.g., KCl/drug /water).

In Chapters III e IV of the present work, from an experimental model based on the Taylor dispersion technique, we have studied the mutual diffusion coefficients of some salts in aqueous solutions (that is, $CoCl_2$, $CrCl_3$, $AlCl_3$ and NH_4VO_3), because these metal ions can exist in the oral cavity, as a result of its release from prosthetic devices, used in oral rehabilitation. Metal salt solutions were used in previous studies of cytotoxicity of dental alloys such the ones performed by Schmakz et al. (1998) and Milheiro et al. (2016).

In addition, the effect of the pH and of the presence of chlorhexidine digluconate as carrier, on the transport of these salts have also been analysed, permitting us to obtain a better understanding of the mechanisms behind the mass transport by diffusion, and its relevant role on reduction of the toxicity of the oral cavity.

1.4.3. Taylor dispersion technique

The experimental procedure of this technique will be widely described in the following chapters, and, consequently, a brief summary is here presented. At the start of each run, a small volume of solution (approximately 0.063 mL) is injected into a laminar carrier stream of slightly different composition. Radial diffusion and axial convection shape the injected concentration pulse into a Gaussian distribution as it flows through a long capillary tube (Figure 1.14).

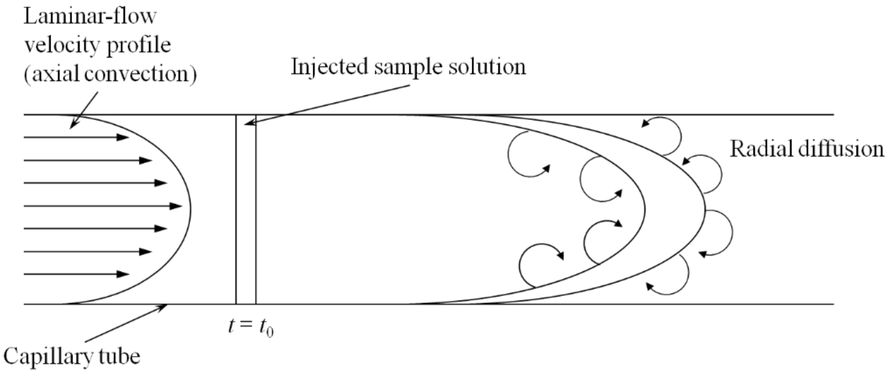


Figure 1.14. Schematic representation of the Taylor dispersion technique.
Image reproduced from Callendar & Leaist (2006a).

The length of the teflon dispersion tube used in the present study was measured directly by stretching the tube in a large hall and using two high quality theodolites and appropriate mirrors to accurately focus on the tube ends. This technique gave a tube length of 3.2799 (±0.0001) $\times 10^4$ mm, in agreement with less-precise control measurements using a good-quality measuring tape. The radius of the tube, 0.5570 (± 0.00003) mm, was calculated from the tube volume obtained by accurately weighing (resolution 0.1 mg) the tube when empty and when filled with distilled water of known density.

A flow rate of 0.17 mL min^{-1} is maintained by a metering pump (Gilson model Minipuls 3) to give retention times of about 1.1×10^4 s. The dispersion tube and the injection valve are kept at 298.15 K and 303.15 K (± 0.01 K) in an air thermostat.

Dispersion of the injected samples is monitored using a differential refractometer (Waters model 2410) at the outlet of the dispersion tube. Detector voltages, $V(t)$, are measured at accurately 5 s intervals with a digital voltmeter (Agilent 34401 A) with an IEEE interface (A. C. F. Ribeiro, 2020).

Sample injection and the dispersion measurements are conveniently automated using HPLC equipment (Figure 1.15) (Alizadeh et al., 1980a; Callendar & Leaist, 2006a; Loh, 1997a).

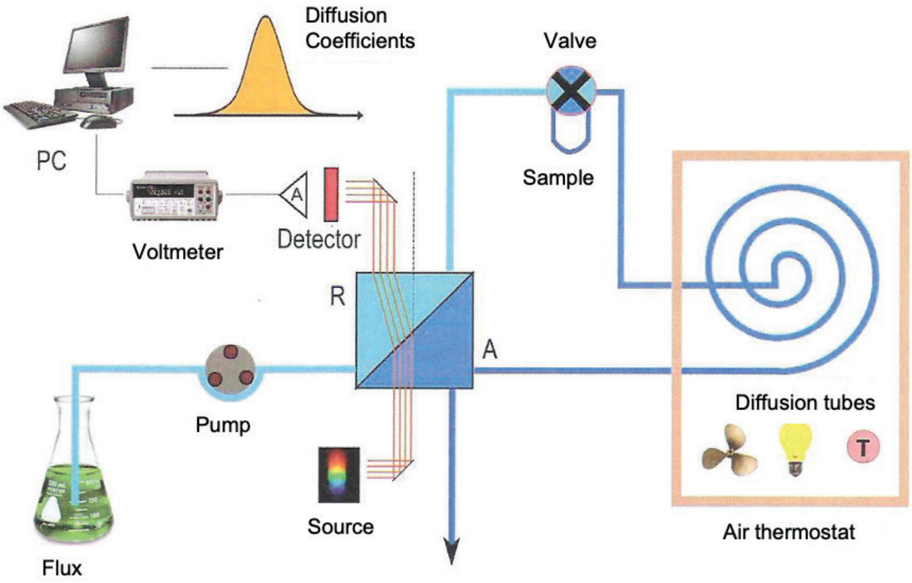


Figure 1.15. Schematic representation of the Taylor dispersion technique.
Image courtesy of the Intermolecular Diffusion Laboratory-Coimbra Chemistry Center.

This technique ends up being of considerable interest from bionanotechnology, including pharmacology and toxicology (Taladriz-Blanco et al., 2020) supplying important data related to the transport of ionic systems resulting from the deterioration of dental materials in the oral cavity, contributing to a better understanding of this phenomenon.

In Chapters 3 e 4 of the present work, from an experimental model based on the Taylor dispersion technique, we have studied the mutual diffusion coefficients of some salts in aqueous solutions (such as $CoCl_2$, $CrCl_3$, $AlCl_3$ and NH_4VO_3), because these metal ions can exist in the oral cavity, as a result of its release from prosthetic devices, used in oral rehabilitation.

In addition, the effect of the pH and of the presence of chlorhexidine digluconate as carrier, on the transport of these salts have also been analysed, permitting us to obtain a better understanding of the mechanisms behind the mass transport by diffusion, and its relevant role on reduction of the toxicity of the oral cavity.

Based on what was previously said and reviewed, we established for this thesis the following objectives:

Objectives:

1 Evaluate the tribological properties of Ti-6Al-4V and amalgam alloys subject to wear in different media.

2 Compare the surface roughness of dental alloys Ti-6Al-4V and amalgam, before and after being subjected to wear simultaneously or isolated, in different solutions (artificial saliva and fluoridated artificial saliva).

3 Compare mass loss of dental alloys Ti-6Al-4V and amalgam, before and after being subjected to wear simultaneously or isolated, in different solutions (artificial saliva and fluoridated artificial saliva).

4 Compare the volume loss of dental alloys Ti-6Al-4V and amalgam, before and after being subjected to wear simultaneously or isolated, in different solutions (artificial saliva and fluoridated artificial saliva).

5 Characterize the mutual diffusion of ionic systems produced in the oral cavity, resulting from the tribocorrosion of alloys used in Prosthodontics, such as Al^{3+}, V^{4+}, Cr^{3+} and Co^{2+}, in several medium and at different pH.

6 Determine the ability of metallic ions (Al^{3+}, Cr^{3+} and Co^{2+}), produced by tribocorrosion, to react and form complexes with pharmacological molecules like digluconate chlorohexidine present in mouthwashes, in order to facilitate their elimination from the oral cavity.

2

Chapter 2

Evaluation of tribological properties of Ti-6Al-4V alloy subject to wear

2.1 Introduction

Metal alloys used in the oral cavity for restorative or prosthetic purposes are submitted to a wet environment where the saliva can act like an electrolyte solution that promotes electrochemical reactions between different alloys. Therefore, metal restorations permanently held in saliva can undergo progressive degradation due to corrosion (Toumelin-Chemla et al., 1996). Up to this moment, however, there is little evidence whether the existence of multiple metallic restorations in the same medium enhances wear. Nonetheless, empirical clinical observations frequently detect signs of wear in the dental restorations, and this phenomenon could be intensified by the coexistence of different metallic alloys in the oral cavity such as amalgam, cobalt-chromium and titanium alloys.

The expansion of dental implants and implant-supported prostheses increased the development and optimization of metallic alloys (Starosvetsky & Gotman, 2001), namely titanium alloys, such as Ti-6Al-4V. This alloy has higher strength than commercially pure grade IV titanium and can be used to manufacture implant components (namely abutments) and the frameworks of fixed and removable prostheses (tooth and implant-supported).

Ti-6Al-4V biomedical alloys, are subjected, in the oral cavity, to a process called tribocorrosion, which is the combination of corrosion and wear action, culminating in an irreversible degradation of the material, in which wear probably acts like an initiating mechanism (Dini et al., 2020; Licausi et al., 2013; Mathew et al., 2009; Rocha et al., 2013). Adequate wear resistance is a requirement of a dental alloy used in oral rehabilitation to ensure the durability of the restorations. On the contrary, a high wear rate may be related to the elongation of antagonists, tilting and movement of teeth and other disfunctions (Heintze, 2006).

Although titanium and it´s alloys are considered corrosion resistant, they are susceptible to acidic environments and to the presence of fluoride (Donachie, 2000; Nakagawa et al., 1999). According to the literature, ion fluoride existent in toothpastes, mouth washes and fluoridated acidic prophylactic gels may be harmful to the corrosion resistance of materials for dental use. The fluoride ion can disrupt the protective oxide layer existent in the surface of Ti alloys, which has been considered responsible for the corrosion resistance of the mentioned alloys (H.-H. Huang, 2002; Kaneko et al., 2003; Könönen et al., 1995; Licausi et al., 2013).

Several studies evaluate the effect of fluoride in the corrosion behaviour of titanium isolated or coupled with amalgam but under static conditions (H.-H. Huang, 2003; Johansson & Bergman, 1995; Reclaru & Meyer, 1998; Toumelin-Chemla et al., 1996). Other authors also evaluated tribocorrosion, particularly stress corrosion (Könönen et al., 1995), fretting corrosion (Sivakumar et al., 2011; Vieira et al., 2006), fatigue (C. B. Correa et al., 2009; Guilherme et al., 2005; Okazaki et al., 1998; Zavanelli et al., 2004) and elastic tensile strain (H.-H. Huang, 2002) but on dental alloys isolated.

Despite the existence of previous studies, the literature is sparse regarding the evaluation of different alloys in the same medium under dynamic wear testing conditions, with and without fluoride.

Objectives: the purpose of the present study was to evaluate the sliding wear of two dental alloys coexisting in a simulated temperature-controlled cell containing artificial saliva, with and without fluoride.

We hypothesized that due to galvanic phenomenon, simultaneous presence of amalgam and Ti-6Al-4V samples in a wet medium leads to higher wear and surface roughness in amalgam and lower wear and surface roughness in Ti-6Al-4V, compared to the samples isolated.

Additionally, we hypothesized that fluoridation of the wet medium would contribute to an increase in wear of Ti-6Al-4V samples due do the disruption of the superficial oxide layer, regardless of the presence of the amalgam.

2.2. Materials and methods

2.2.1. Preparation of test materials

To carry out this study, 10mm x 3mm disc-shaped samples of two metallic alloys with application in Dentistry were prepared: a titanium alloy (Ti-6Al-4V) and a spherical silver amalgam alloy with the following composition in percent weight (wt.%): Ag- 60 wt.%, Sn- 28 wt.% and Cu- 12 wt.%.

Ti-6Al-4V samples (n=30) were obtained by machining metal rods (Titanium/Aluminium/Vanadium Ti-6Al-4V Rod, Goodfellow Cambridge Limited®; Lot LS278237SJP). Samples were then mechanically polished for 1 minute using abrasive sandpaper grit 1000 (Struers Inc., Cleveland, OH, USA) at 150 rpm and 10N pressure.

Amalgam samples (n=30) were prepared using self-activating capsules (Tytin, Kerr Manufacturing Co. Romulum MI, USA; Lot 3-3069), as indicated by the manufacturer. The capsule was placed and secured in the amalgamator and triturated for 8 seconds. The mix was immediately condensed after trituration according to Specification No. 1 ANSI/ADA into a 10mm x 3mm cylindric mold. After setting the samples were polished for 15 seconds using abrasive sandpaper grit 2500 (Struers Inc., Cleveland, OH, USA) at 300 rpm and 5N pressure.

The polished samples were enclosed in an acrylic resin cylinder and subjected to a mass loss study and then stored in water for 14 days at 37° C. After storage, samples were cleaned using distilled water in an ultrasonic cleaner and stored for another 7 days at room temperature.

2.2.2. Initial characterization of the samples

Before being subjected to the tribological experiments, the samples were analyzed with regard to initial mass, surface roughness and Vickers hardness.

2.2.2.1. Initial mass

All samples were weighed before the wear tests using an analytical electronic scale with a 0.0001g sensitivity (Denver Instrument Company® Model AA-200Ds).

2.2.2.2. Vickers hardness measurement

Vickers hardness test is based on the resistance that the material offers to the penetration of a diamond pyramid with a square base and an angle between faces of 136°, under a given load. The samples were subjected to forces of 1, 2, 5, 10N and 20N, with the computerized equipment model HMV-2, Shimazdu® that allows the acquisition, recording and processing of data for the evaluation of hardness. Five repetitions were performed for each force. This test followed ISO 6507 standards.

2.2.2.3. Surface roughness

The surface roughness of the samples was determined in Perthometer Mahr model Perthen S4P using a filter LT (0.800*5) 5600 Mn. The amplitude parameters used were arithmetic average height (Ra) and ten-point height (Rz).

Ra is defined as the average absolute deviation of the roughness irregularities from the mean line over one sampling length (Gadelmawla et al., 2002).

Rz is more sensitive to occasional high peaks or deep valleys than Ra. The International ISO system defines this parameter as the difference in height between the average of the five highest peaks and the five lowest valleys along the assessment length of the profile (Gadelmawla et al., 2002).

2.2.3. Tribocorrosion experiments

The samples were tested in couples: Ti-6Al-4V + Ti-6Al-4V (G1); amalgam + amalgam (G2) and Ti-6Al-4V + amalgam (G3), and subjected to a wear test.

The wear was carried out in an alternative sliding equipment with customizable stroke, speed, and load (Figure 2.1), designed in the Pedro Nunes Institute, Coimbra, Portugal, which simulates the conditions of use usually given to these materials in the oral cavity. The wear equipment operates by applying a vertical load onto the specimen using a counter-body, sliding horizontally, and then repeating the cycle. The counter-body type was made of a chemically inert ceramic material (95% Alumina spheres (Al_2O_3), ¼'', Helmut Kreutz, DE) (Lambrechts et al., 2006), with hardness similar to dental enamel; the contact is a punctual type using a counter-plane sphere. The parameters for this test were as follows: load of 15N and 2400 cycles at 0.7Hz.

During the tests, the samples were immersed in an electrolyte solution kept in circulation at 310,15 K, simulating the conditions found in the oral cavity. Two electrolyte solutions were considered: artificial saliva, and artificial fluoridated saliva (Sodium Fluoride (NaF) 225ppm).

This test allows the evaluation of the amount of wear in each sample and the type of interaction between the two metal alloys when tested simultaneously.

After the tests, the samples were washed with distilled water, ultrasonically cleaned for 5 minutes and dried, before final characterization.

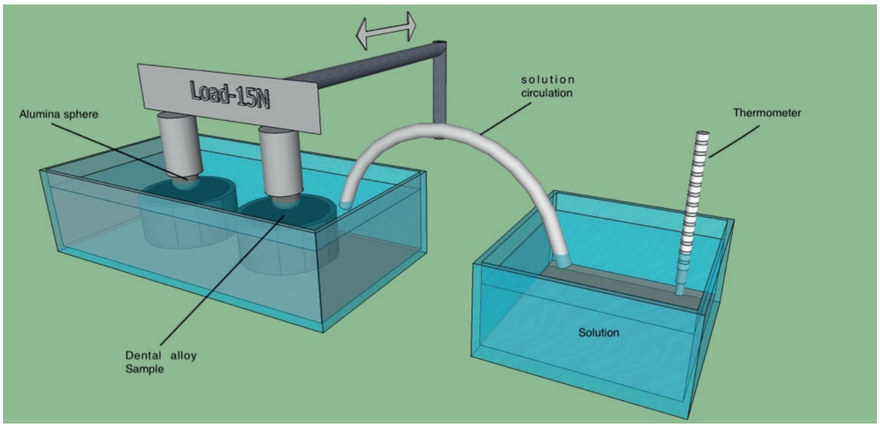


Figure 2.1. Representation of the wear testing equipment.

2.2.4. Final characterization of the samples

After the tribocorrosion experiments, all samples were subject to weighing in the same equipment described in section 2.2.2.1, the wear track profile was acquired by Perthometer Perthen S4P, the same equipment used in section 2.2.2.3, in order to evaluate the final surface roughness and the volume of the wear tracks.

The wear track morphology was also observed by scanning electron microscopy in a microscope JEOL® JSM-5310 with a voltage of 20 KéV.

2.2.5. Statistical analysis

All statistical analysis were carried out on SPSS Statistics Version 27.0 (IBM, USA). Data on the variation of mass, wear track volume and surface roughness variation (Ra and Rz) of the samples were analyzed using a three-way factorial design, considering the independent variables material, electrolyte and cylinder combination (pairs of cylinders tested simultaneously). Data was checked for normality and homoscedasticity and for three-way and simple two-way interactions.

Simple comparisons (pre-post and between levels of the independent variables) were performed with non-parametric tests (Kruskal-Wallis and Mann-Whitney U for independent groups, Wilcoxon for paired data), followed by post-hoc Bonferroni correction where applicable. The significance level was set to 0.05.

2.3. Results

2.3.1. Specimen mass variation

The mean and median values of the registered initial and final mass of the Ti-6Al-4V and amalgam samples tested in artificial saliva (AS) and fluoridated saliva (FS) are detailed in Table 2.1.

Table 2.1. Mean and median values of the registered initial and final mass of the Ti-6Al-4V and amalgam samples tested in artificial saliva (AS) and fluoridated artificial saliva (FS).

				T0		T1		∆T0-T1	
			N	Mean (SD), g	Median (Min-Max), g	Mean (SD), g	Median (Min-Max), g	Mean (SE), g	p-value
AS	G1	Ti-6Al-4V	10	2.451 ± 0.235	2.448 (2.232 - 3.025)	2.450 ± 0.235	2.447 (2.231 - 3.024)	0.00082 (0.0003)	0.025*
	G2	Amalgam	10	3.605 ± 0.443	3.621 (2.676 - 4.407)	3.602 ± 0.443	3.617 (2.672 - 4.404)	0.00307 (0.0003)	<0.001*
	G3	Ti-6Al-4V	5	3.485 ± 0.464	3.601 (2.676 - 3.815)	3.482 ± 0.464	3.600 (2.672 - 3.812)	0.00304 (0.0004)	0.002*
		Amalgam	5	3.323 ± 0.594	3.255 (2.674 - 4.216)	3.321 ± 0.593	3.251 (2.674 - 4.212)	0.00244 (0.0008)	0.035*
FS	G1	Ti-6Al-4V	10	2.418 ± 0.277	2.480 (2.052 - 2.841)	2.415 ± 0.276	2.477 (2.049 - 2.836)	0.00356 (0.0002)	<0.001*
	G2	Amalgam	10	3.664 ± 0.451	3.745 (2.974 - 4.182)	3.658 ± 0.451	3.741 (2.969 - 4.175)	0.00562 (0.0003)	<0.001*
	G3	Ti-6Al-4V	5	2.423 ± 0.264	2.396 (2.051 - 2.689)	2.416 ± 0.266	2.380 (2.044 - 2.683)	0.00720 (0.0025)	0.045*
		Amalgam	5	3.356 ± 0.646	3.540 (2.311 - 4.063)	3.355 ± 0.650	3.533 (2.304 - 4.069)	0.0012 (0.0032)	0.732

Wearing induced a statistically significant mass loss for all groups except for the amalgam samples in G3 (simultaneous testing of Ti-6Al-4V and amalgam specimens) when submerged in fluoridated saliva (p=0.732).

There were statistically significant differences in mass loss between G1 and G2 regardless of the electrolyte solution (artificial or fluoridated saliva, p=0.004 and p=0.026 respectively).

However, there were no statistically significant differences between TiAlV samples (G1 and G3, p>0.05) or amalgam samples (G2 and G3, p=0.205) when submerged in artificial saliva.

Three-way ANOVA detected a statistically significant three-way interaction between cylinder material, cylinder combination in testing setup (Ti-6Al-4V-Ti-6Al-4V or Amalgam-Amalgam or Ti-6Al-4V-Amalgam) and type of electrolyte solution: F(1, 52)= 5.40, p= 0.024. There was a statistically significant simple two-way interaction between material and type of electrolyte solution for cylinder combination Ti-6Al-4V-Amalgam, F(1, 52)= 8.56, p= 0.01, but not for cylinders of the same material, F(1, 52) = 0.012, p>0.05. Such findings are graphically replicated in figure 2.2 in which is possible to notice that the fluoridation of saliva promotes a similar increase in mass loss for the samples of Ti-6Al-4V and amalgam when tested separately (full thickness lines representing G1 and G2) but not when tested simultaneously (dashed lines representing Ti-6Al-4V and amalgam samples of G3). In this case, saliva fluoridation leads to higher mass loss of the Ti-6Al-4V samples (approximately twice of G1) and lower mass loss of the amalgam samples (4 to 5 times lower than that of G2).

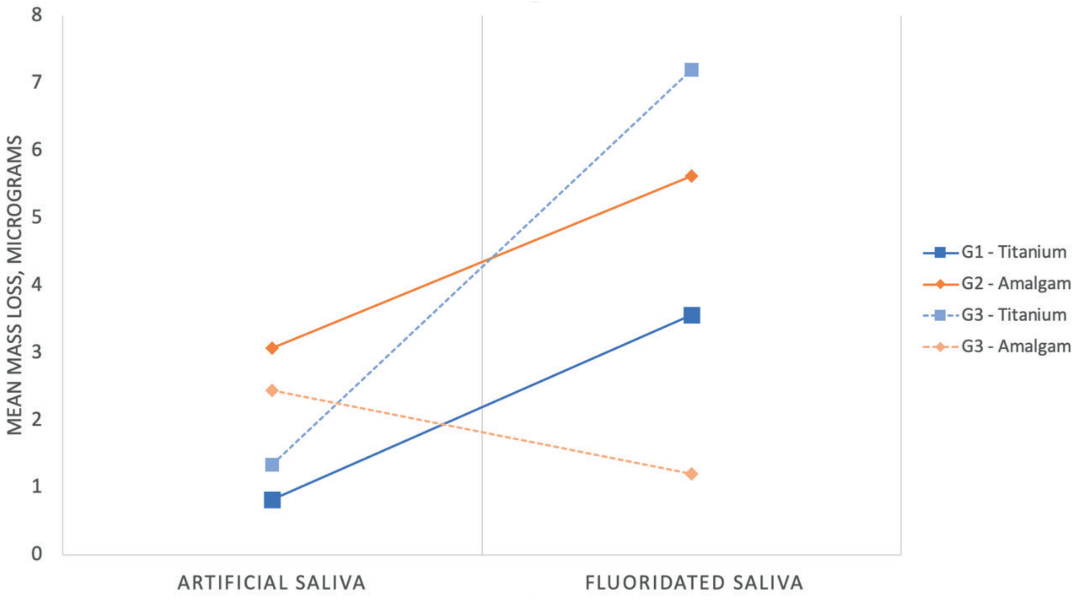


Figure 2.2. Mean mass loss (micrograms) of G1, G2 and G3 samples tested in artificial saliva and fluoridated saliva. Notice the cylinder combination – electrolyte solution interaction.

2.3.2. Vickers hardness

The hardness values for each dental alloy are shown in Figure 2.3.

The Vickers test revealed that Ti-6Al-4V samples present hardness superior to 3 GPa regardless of the applied load (up to 20 N). Under the same conditions, amalgam samples present lower test values, approximately 2 GPa.

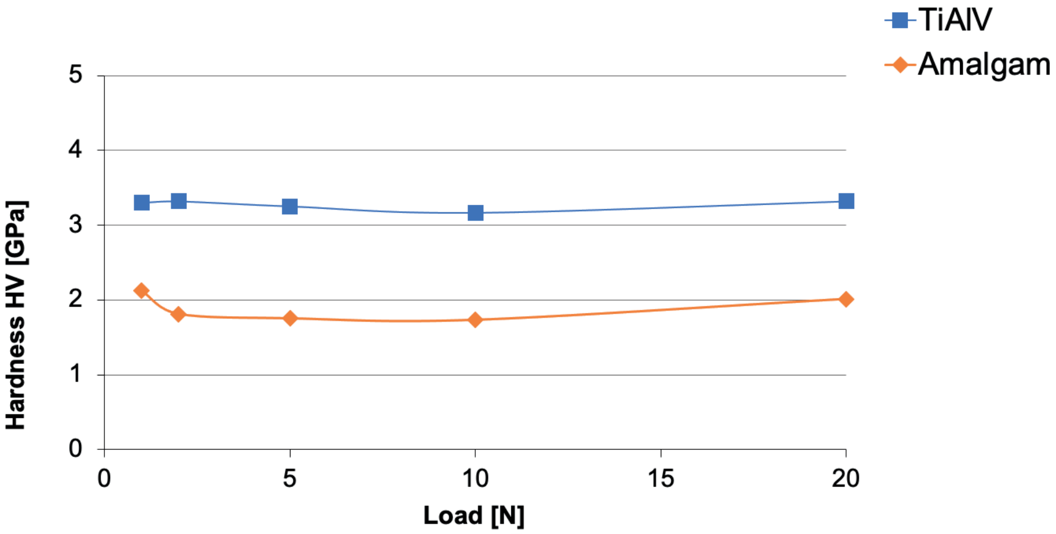


Figure 2.3. Vickers hardness test for Ti-6Al-4V and amalgam under loads up to 20N.

2.3.3. Surface roughness

Prior to the wear test (T0), all groups presented similar arithmetic average surface roughness (Ra), with exception to the Ti-6Al-4V samples of G3 to be tested in fluoridated saliva that presented lower Ra values. The wearing test induced an increase of the Ra values of all amalgam samples, statistically significant for G2 in saliva (p=0.001) and G3 in fluoridated saliva (0.010). After the wear test (T1), Ra values of titanium samples remained relatively similar to those of pre-test (T0) despite the slight increase of Ti-6Al-4V samples of G3 tested in fluoridated saliva.

Table 2.2. Mean values of the registered initial and final Ra and Rz to Ti-6Al-4V and amalgam samples tested in artificial saliva and fluoridated artificial saliva.

				Ra		
				T0	T1	
			N	Mean (SD), µm	Mean (SD), µm	p-value
ARTIFICIAL SALIVA	G1 (Ti-6Al-4V)		10	0.43 ± 0.07	0.49 ± 0.12	0.431
	G2 (Amalgam)		10	0.49 ± 0.05	0.59 ± 0.09	**0.001***
	G3	Ti-6Al-4V	5	0.47 ± 0.05	0.51 ± 0.25	0.689
		Amalgam	5	0.49 ± 0.05	0.53 ± 0.07	0.118
FLUORIDATED SALIVA	G1 (Ti-6Al-4V)		10	0.43 ± 0.08	0.44 ± 0.09	0.659
	G2 (Amalgam)		10	0.47 ± 0.05	0.54 ± 0.11	0.146
	G3	Ti-6Al-4V	5	0.38 ± 0.06	0.43 ± 0.03	**0.032***
		Amalgam	5	0.50 ± 0.05	0.68 ± 0.07	**0.010***
	p-value			**0.042***	**0.024***	
				Rz		
				T0	T1	
			N	Mean (SD), µm	Mean (SD), µm	p-value
ARTIFICIAL SALIVA	G1 (Ti-6Al-4V)		10	4.64 ± 0.69	4.84 ± 1.09	0.679
	G2 (Amalgam)		10	5.09 ± 0.64	6.21 ± 0.99	**0.009***
	G3	Ti-6Al-4V	5	5.00 ± 0.41	5.75 ± 2.81	0.580
		Amalgam	5	5.13 ± 1.18	5.61 ± 0.65	0.261
FLUORIDATED SALIVA	G1 (Ti-6Al-4V)		10	4.69 ± 0.87	4.80 ± 0.63	0.728
	G2 (Amalgam)		10	4.98 ± 0.65	5.49 ± 0.82	0.118
	G3	Ti-6Al-4V	5	4.69 ± 0.38	4.50 ± 0.48	0.345
		Amalgam	5	4.68 ± 0.36	7.38 ± 1.68	**0.023***
	p-value			0.426	**0.019***	

At T0 there were no statistically significant differences in the average maximum height of the profile (Rz) (p=0.426). After the wear test (T1), there was an increase in Rz of all amalgam samples, as detailed in table 2.2 and graphically represented in figure 2.4. At this moment, amalgam samples of G3 tested in fluoridated saliva presented statistically higher Rz than their counterpart Ti-6Al-4V samples (p=0.036).

Ti6-Al4-V samples of G1 presented almost null Rz variation regardless of the electrolyte solution (Figure 2.4). However, Ti6-Al4-V samples of G3 presented a slight increase of Rz in artificial saliva whereas there was a reduction of the maximum height of the profile for Ti6-Al4-V samples of G3 tested in fluoridated saliva.

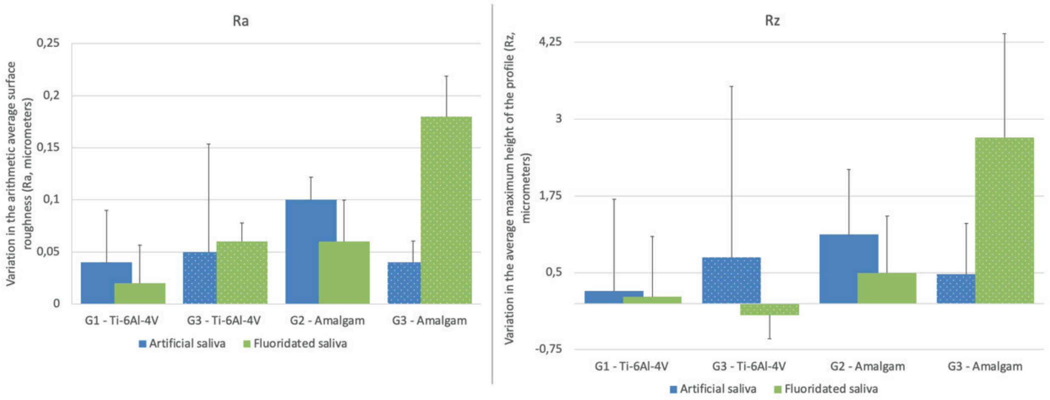


Figure 2.4. Distribution of Ra and Rz variation (micrometers) of all samples from T0 to T1 clustered by type of solution.

2.3.4. Volume of the wear tracks

The mean volume of the wear tracks of each group is presented in Table 2.3.

Table 2.3. Volume loss associated with the wear tracks measured by profilometry, mm^3.

Group	Material	Wear volume in artificial saliva Mean ± SD	Wear volume in fluoridated saliva Mean ± SD	p-value†
G1	TiAlV	0.0753 ± 0.0152 ^{a,c}	0.0655 ± 0.0233 ^{a,c}	0.315
G2	Amalgam	0.0061 ± 0.0097 ^{a,b}	0.0050 ± 0.0092 ^{a,b}	0.190
G3	TiAlV	0.0740 ± 0.0116 ^{b,d}	0.0730 ± 0.0097 ^{b,d}	>0.05
G3	Amalgam	0.0100 ± 0.0081 ^{c,d}	0.0020 ± 0.0011 ^{c,d}	0.151
p-value‡		<0.001	<0.001	

Similar superscript letters indicate groups that differ at the 0.05 level.

Three-way ANOVA was unable to detect a three-way interaction between material, type of electrolyte solution and cylinder combination in the volume of the wear track: F(1, 52)= 1.05, p= 0.311. Also, no two-way interactions were identified between any pair of variables (p>0.05 for all interactions). Only material presented a statistically significant simple main effect (F(1,52)= 310.68, p<0.001), indicating that Ti-6Al-4V samples presented, on average, 0.07 mm^3 (95% CI: [0.06 – 0.07]) higher wear track volume.

The absence of simple main effects for electrolyte solution and any type of interactions indicated that there were no statistically significant differences between samples of the same material in different electrolyte solutions, i.e., Ti-6Al-4V in artificial saliva presented comparable volume loss to Ti-6Al-4V in fluoridated artificial saliva (p=0.315) and a similar situation occurred with amalgam in artificial and fluoridated artificial saliva (p=0.190). The simultaneous presence of two different materials had no impact in the volume of the wear track for both Ti-6Al-4V and Amalgam (p>0.05 and p=0.151, respectively).

2.3.5. SEM observation of the wear tracks

Representative images of the scanning electron microscopy observation of the different samples after the wearing tests are shown in figures 2.5 to 2.17.

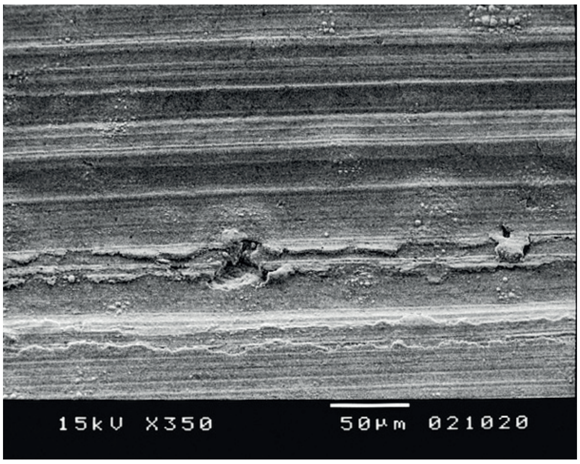


Figure 2.5. Amalgam sample tested with Ti-6Al-4V in artificial saliva 350X

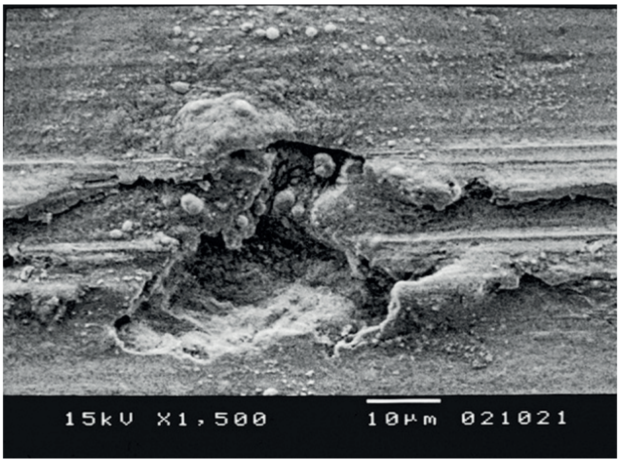


Figure 2.6. Amalgam sample tested with Ti-6Al-4V in artificial saliva 1500X.

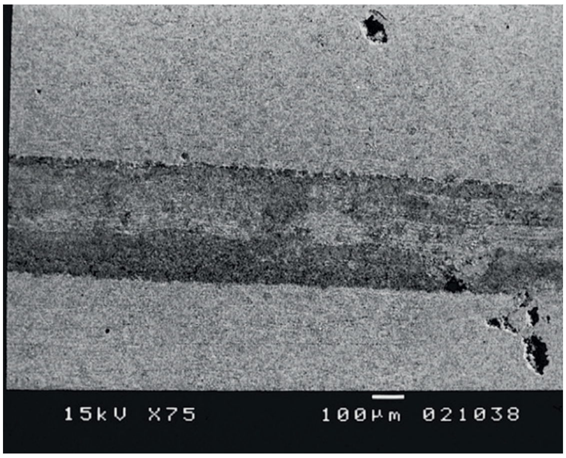


Figure 2.7. Amalgam sample tested with Ti-6Al-4V in fluoridated artificial saliva 75X.

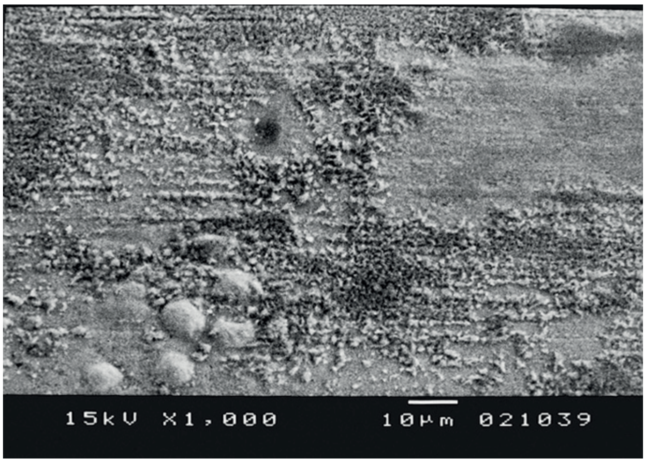


Figure 2.8. Amalgam sample tested with Ti-6Al-4V in fluoridated artificial saliva 1000X.

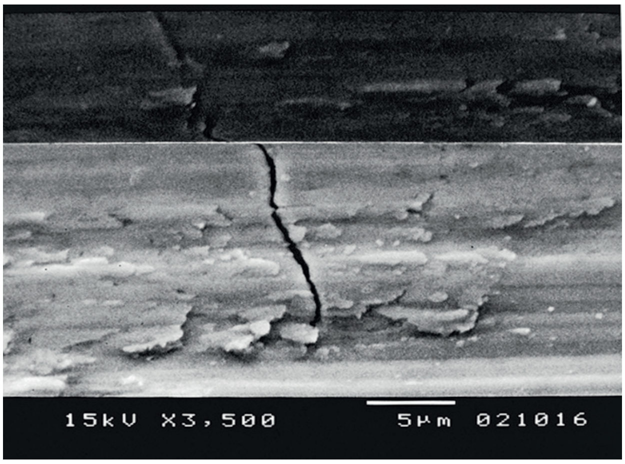


Figure 2.9. Ti-6Al-4V sample tested with amalgam in artificial saliva 3500X.

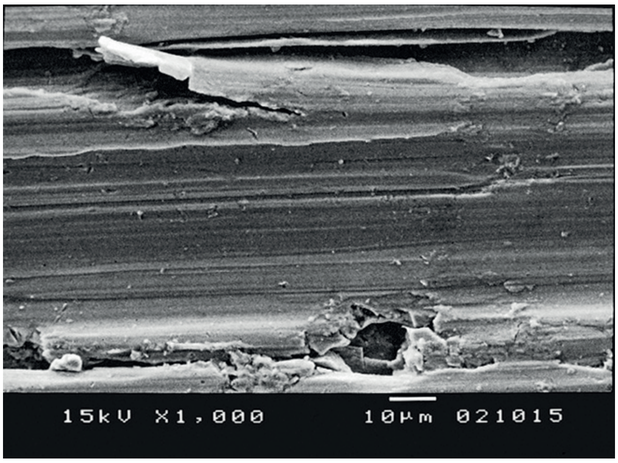


Figure 2.10. Ti-6Al-4V sample tested with amalgam in artificial saliva 1000X.

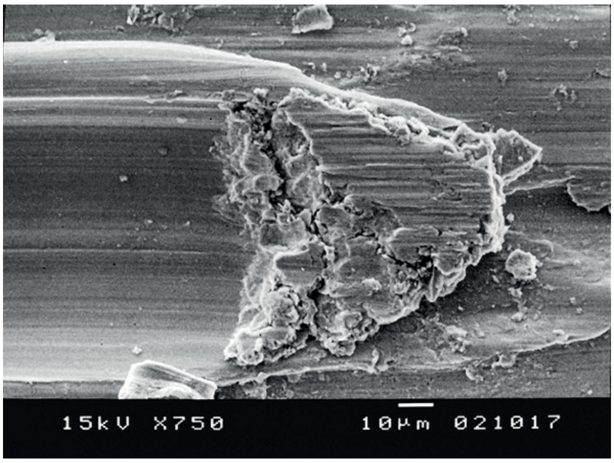


Figure 2.11. Ti-6Al-4V sample tested with amalgam in artificial saliva 750X.

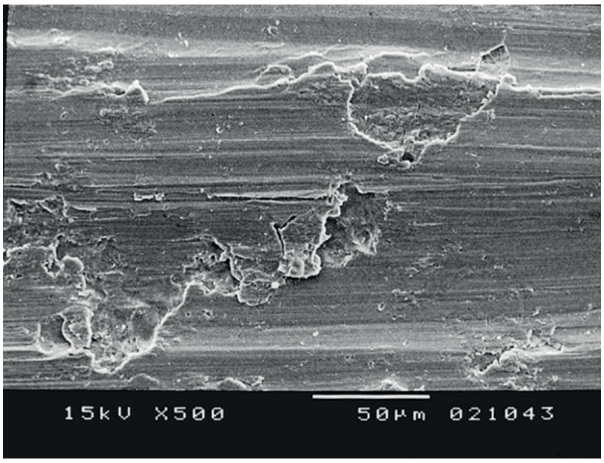


Figure 2.12. Ti-6Al-4V sample tested with amalgam in fluoridated artificial saliva 500X.

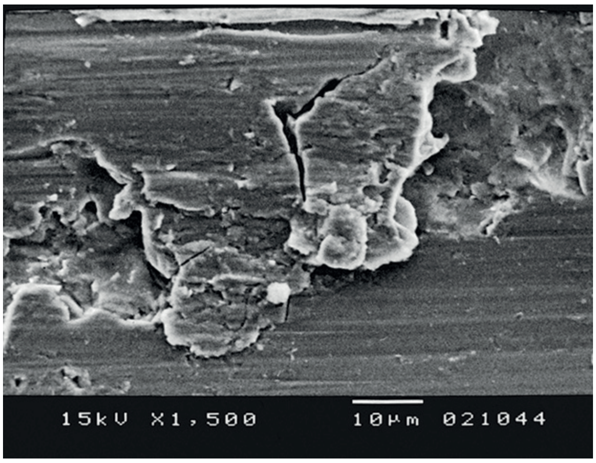


Figure 2.13. Ti-6Al-4V sample tested with amalgam in fluoridated artificial saliva 1500X.

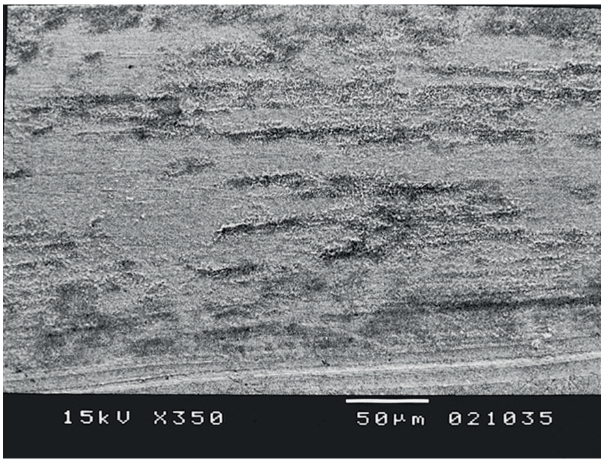


Figure 2.14. Amalgam sample tested with amalgam in fluoridated artificial saliva 350X.

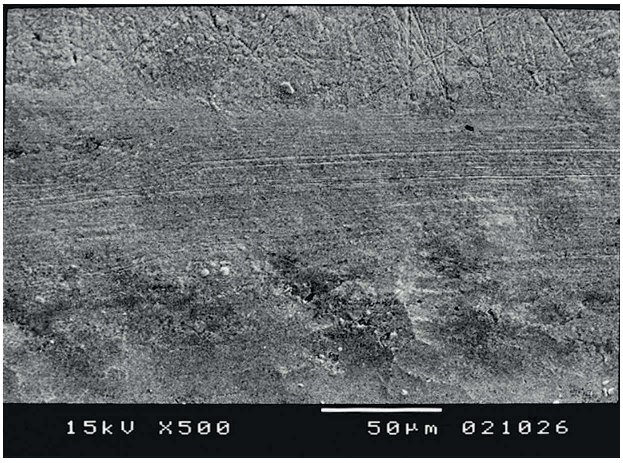


Figure 2.15. Amalgam sample tested with amalgam in artificial saliva 500X.

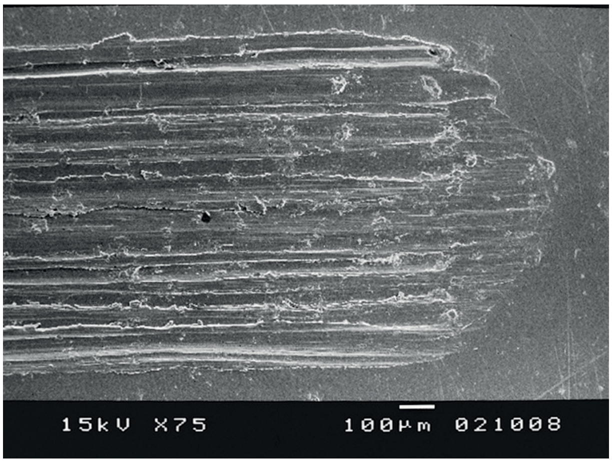


Figure 2.16. Ti-6Al-4V sample tested with Ti-6Al-4V in artificial saliva 75X.

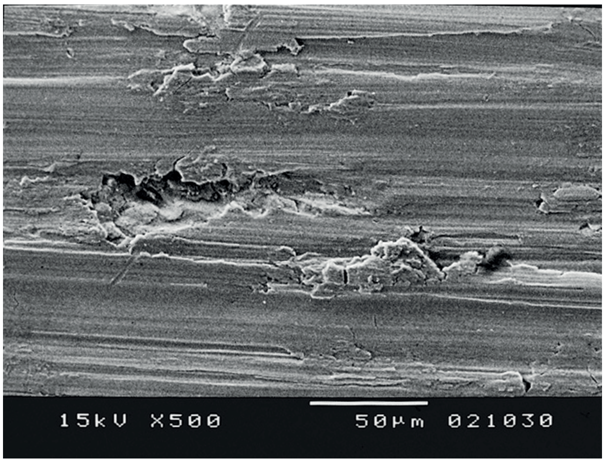


Figure 2.17. Ti-6Al-4V sample tested with Ti-6Al-4V in fluoridated artificial saliva 500X.

In figures 2.5 and 2.6, amalgam samples tested simultaneously with Ti-6Al-4V (G3) in artificial saliva show uniform wear tracks, without significant material destruction. However, some pores are visible. When these two materials are tested simultaneously in fluoridated saliva (figures 2.7 and 2.8), it is possible to observe cylindrical particles deposited on the surface of the amalgam samples, even though no significant destruction can be reported for the wear tracks. Simultaneously the Ti-6Al-4V samples of the same group show great destruction caused by wear, occasionally with micro-cracks in the bottom of the track perpendicular to the sliding direction (Figure 2.9). It is also possible to observe detachment of metal lamellas on the periphery of the wear tracks (Figure 2.10) and accumulation of debris particles in front of the counter body (Figure 2.11.) That crumbling material that drags in front of the counter body, works as a third body, and sometimes facilitates wear as a lubricant.

When the two materials are tested simultaneously in fluoridated saliva, the Ti-6Al-4V samples (figures 2.12 and 2.13) show visible tips, material deposit on the bottom of the wear track and an extensive ploughing.

Figures 2.14 and 2.15 present the wear tracks of amalgam samples (G2) tested in fluoridated and artificial saliva, respectively. The wear tracks of these samples show no morphological differences, nor material pullout, although it is possible to see in the surface the same cylindrical particles that are observed in the figure 2.8.

Ti-6Al-4V samples of G1 tested in artificial saliva shown in figure 2.16 present material deformation aligned in lamellae, small microfractures in a repetitive process, extensive ploughing, large deformation and very strong abrasion, but no great crumbling. The dragged material shapes a track and not the counter body.

The SEM observation of the Ti-6Al-4V samples of G2 tested in fluoridated saliva in figure 2.17 reveal a spallation and extensive ploughing on wear track, demonstrating a strong abrasive wear action of the alumina ball.

2.4. Discussion

Dental alloys corrosion measured by electrochemical methods has been extensively tested in recent years, however, in our literature review, we found that there is a gap in tribological studies from TI-6Al-4V and the most studies refer to titanium isolated, i.e., without the presence of other metal alloys. Heaving in mind that are many patients who have different metal alloys in the oral cavity and corrosion is often exacerbated by mechanical processes such as wear, a sliding equipment was developed that allows us to simulate not only the coexistence of two alloys, but also to subject them to the action of forces immersed in an electrolyte. In previous studies, Taher (2003) reported that amalgam alloy resulted in higher galvanic interactions when coupled with a Ti implants. Bumgardner & Johansson (1996) also indicated that coupling those two dental alloys may undergo the release of galvanic corrosion products with potential to become toxic. Although the use of amalgam has fallen into disuse, we still find many patients with teeth restored with this material. So, in the present study we evaluated wearing of two different metallic alloys, amalgam and Ti-6Al-4V, submerged in a wet medium, with and without fluoride.

Main findings indicated that in artificial saliva there is no significant difference in mean mass loss of Ti-6Al-4V samples tested isolated or in conjunction with amalgam. However, fluoridation of the wet medium led to an increase of the mass loss in all

Ti-6Al-4V samples, what could be explained by the delamination of the oxide film over the surface of Ti-6Al-4V samples due to the presence of fluoride ions (Sivakumar et al., 2011). It is important to highlight that when Ti-6Al-4V is tested simultaneously with amalgam samples, mass loss is twice that of Ti-6Al-4V tested isolated.

Even though in this study mass loss was considered a surrogate variable for the wear assessment, others variables like surface roughness and volume of the wear track were assessed. According to DeLong the preferred parameter for measuring wear is volume(DeLong, 2006).

We obtained the wear volume measuring the wear crater by profilometry, taking a measurement that crosses the wear track; and multiplying this value by the length of the wear track. In the present study we unable to find differences between the wear crater of Ti-6Al-4V samples regardless the presence of amalgam, or type of electrolyte solution. Notwithstanding that, Ti-6Al-4V presented on average 0,07 mm^3 higher volume loss than amalgam, this result is in accordance with the reports of Atar(2013), that studied the sliding wear performance of Ti-6Al-4V, 316 Stainless steel and CoCr isolated in dry conditions, and demonstrated that the Ti6-Al-4V has the worst wear resistance when compared to the other studied alloys, these data were correlated with the low shear strength of titanium and high tendency to scuffing of the HCC structure, contrariwise dental amalgam is known for its high resistance to occlusal wear.

In the present study, volume wear values obtained were not in agreement with those obtained by the measurement of mass loss. The reason for this disagreement might be due to the fact that wear track volume calculation was based on a single sampling of the cross-section of the crater. As shown in the SEM images, the wear crater may have different depths along its route, therefore the associated wear volume differs and this variation was not considered in the calculation. Further cross- sectional areas of the wear track should be measured so overcome this limitation.

With regard to the SEM images, in Ti-6Al-4V an extensive shear deformation and signals of abrasive wear were observed, we found also some areas of particle detachments and delaminated wear compatible with adhesive wear, to confirm this type of adhesive wear, it would be necessary to analyze the surface of the counter bodies, but our results are in line with the studies of Atar, Fu *et al.* and Meddah *et al.* (Atar, 2013; Fu et al., 2000; Meddah et al., 2020), even though the conditions of these studies are different.

In Ti-6Al-4V samples the wear mechanism forms with the worn material accumulated in surrounded areas of the wear track, and in particularly in front of the counter body, acting as a third body that enhanced the surface degradation.

From the results obtained, we verified that there was in the Ti-6Al-4V samples an extensive carving in the wear tracks as well as a delamination mechanism, which corroborated by some other studies (Fu et al., 2000; C. S. Lee et al., 2003)that report that despite the high resistance of titanium to corrosion, it is little wear resistant. From the among of damage mechanism seen in Ti-6Al-4V, micro-cracks observed in the wear track, they act as crack initiation sites, may coalesce and lead to a premature fatigue.

In the amalgam samples, we observed grooves parallel to the sliding direction compatible with abrasive wear mechanism, it is possible to observe in amalgam samples, cylindrical particles that we suppose to correspond to oxides; according to Landolt et al. (2004) during the sliding contact, metallic wear particles are torn from the first body , those particles create a metallic third body, and some may undergo electrochemical oxidation, leading to formation of solid oxides, so it would be of interest in future investigations to analyze the surface chemical structure of these particles. in the amalgam samples, we observed grooves parallel to the sliding direction compatible with abrasive wear mechanism, it is possible to observe in amalgam samples, cylindrical particles that we suppose to correspond to oxides; according to Landolt et al. (2004) during the sliding contact, metallic wear particles are torn from the first body , those particles create a metallic third body, and some may undergo electrochemical oxidation, leading to formation of solid oxides , so it would be of interest in future investigations to analyze the surface chemical structure of these particles. Additionally, as previously described, when we tested the 2 alloys simultaneously, in a fluoridated medium the mass of amalgam samples increased, which leads us to suppose that some debris and oxides released from the Ti-6Al-4V, are deposited in amalgam samples.

While for all the amalgam samples the roughness parameters analyzed (Ra and Rz) increased in both media, in the Ti-6Al-4V alloy, when tested simultaneously with amalgam, the Rz value in artificial saliva and the Ra value in fluoridated saliva increased slightly, with a contrasting decrease in the value of Rz. These results may be explained through the development of galvanic currents, and by the release and deposition of oxides resulting from this phenomenon, as well as by the wear to which they were subjected.

Regarding Vickers hardness, we consider that it is stable with the loads used, since it does not suffer large variations, for both the alloys studied.

The hardness of the metal alloy must be enough to resist occlusal forces without wearing out the opposing teeth. According to El-Bediwi et al. (2009) alloys with a Vickers hardness <125kg/mm^2 are susceptible to wear, while with a hardness greater than 340kg/mm^2 there is a risk of wearing out the opposing teeth, by the results obtained both Ti-6Al-4V and amalgam has surface hardness within this range of values.

According to the Archard´s Law the volume of material loss should be proportional to load and sliding distance, and inversely proportional to the hardness value of the material (Archard, 1953), Martin el al. (2010) investigated the tribocorrosion behavior of Ti-6Al-4V and established a connection between a lower hardness with high mechanical wear.

In the present study it was not possible to correlate the hardness of Ti-6Al-4V and wear, what is consistent with the findings of Chan et al. (2007): despite the higher surface hardness of Ti-6Al-4V than amalgam, it suffered more pronounced mechanical wear.

It should be noted that in this tribological study, due to equipment limitations, the applied load was lower than that recommended by Heintze for this type of simulation. The author states that the applied load should be between 20 and 120N, that is similar to that of the biting force in molars (Heintze, 2006). Nevertheless, 15N was the load chosen by some other authors (de Gee et al., 1986; Hockin et al., 1999; Sunnegårdh-Grönberg et al., 2002) , and under 15N load, severe material damage of the Ti-6Al-4V samples was observed, being higher than that of the amalgam samples.

We cannot fail to consider the limitations of the tribological experiments, which are essentially centered on the fact that we used a prototype not yet validated as a wear test equipment, which makes the comparative analysis with other similar studies in the literature difficult due to the variability of parameters between them. Nevertheless, important findings can be withdrawn from this study.

Due to the large difference in galvanic potentials between amalgam and Ti-6Al-4V, it is reasonable that the amalgam suffers greater galvanic corrosion than the more noble alloy, Ti-6Al-4V. As would be expected, in artificial saliva the Ti-6Al-4V does not suffer alterations when tested simultaneously with amalgam. However, this premise does not happen in the presence of fluor, in this medium Ti-6Al-4V suffers a greater loss of mass, and a decrease in Rz, which makes us suppose that there is a synergistic effect of fluoride and amalgam.

2.5. Conclusions

Within the limits of this study the following conclusions were drawn:

The surface roughness and mass loss findings in both alloys correspond to the SEM observations.

According to the results of this study in artificial saliva, when Ti-6Al-4V and amalgam are tested simultaneously, there seems to be no effect on Ti-6Al-4V for all the tested parameters.

However, in a fluoridated medium, when Ti-6Al-4V and amalgam are tested simultaneously there is higher mass loss in Ti-6Al-4V and on the contrary amalgam samples do not suffer any mass variation. In these conditions, surface roughness (Rz) of Ti-6Al-4V reduces whereas their counterpart amalgam increases significantly.

In fact, apparently Ti-6Al-4V alloy seems to suffer the synergistic action of fluoride and amalgam.

The use of Ti-6Al-4V alloys should be avoided in areas subject to wear and where they may come into contact with fluor-containing substances, especially if dental amalgam is also present.

Chapter 3

3

The behaviour of aluminium and vanadium ions in artificial saliva and the impact of the chlorhexidine digluconate on its removal - an experimental diffusion model

The results from this chapter were partially published under:

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3.1. Introduction

Titanium alloys have been used for decades in the manufacture of dental prostheses and implants due to their adequate mechanical properties, biocompatibility and good longevity, however, due to the fact that they are inserted in the oral cavity and surrounded by saliva, they can be subject to corrosion, resulting in the release of undesired wear particles and ions(Abey et al., 2014; B. C. Costa et al., 2019; el Sawy & Shaarawy, 2014).

Trace metal elements in human body are sometimes originated from biomaterials implanted in the body, although they are usually taken from food and water (Hanawa, 2004).

The role of these ions released by the corrosion of prosthetic devices in the human body is a reason of concern (Arregui et al., 2021a; Durgalakshmi et al., 2013b; Kunrath et al., 2021). So, the study of transport properties of these ions, once they are released into the body, is of utmost importance to understand their toxicological potential. Particle debris released by orthopedic and dental implants is often related with failure of treatments (B. C. Costa et al., 2019; Keegan et al., 2007; A. R. Ribeiro et al., 2016).

In this study we gave special attention to the aluminium and vanadium ions, because they are one of the components of the Ti-6Al-4V alloys, used in several biomedical applications namely in the fabrication of dental implants, and other prosthetics components (abutments, healing components and supra-structures) as well as in orthopedics prostheses, but present low shear strength and low wear resistance (Elias et al., 2008).

Ti-6Al-4V alloys are characterized by their high resistance to corrosion, due to the passive layer of oxides that forms on their surface, however, under certain conditions this passive layer can be destroyed and corrosion may occur(Arregui et al., 2021a; Licausi et al., 2013).

Among these conditions, we can highlight the mediums with acidic pH or the presence of fluoride, which is part of the composition of most mouth rinses or tooth pastes used in the prevention of dental caries, and such fluoride ions may attack the superficial oxide layer of the titanium alloys leading to a decrease in its protective capacity (Delgado-Ruiz & Romanos, 2018; H.-H. Huang, 2002; Licausi et al., 2013; Zavanelli et al., 2004).

Additionally, Ti-6Al-4V alloys are very susceptible to the mechanical action caused by fretting and sliding wear as found by Wang. *et al.* and Jin *et al.* (Jin et al., 2016; S. Wang et al., 2015).

In previous studies we have been able to quantify, by using atomic absorption spectroscopy the ions released after subjecting Ti-6Al-4V alloys to tribological tests (A. Ribeiro et al., 2012).

Although not completely clear and predictable, the potential cytotoxicity of these ions are susceptible to cause long-term health effects (B. C. Costa et al., 2019; Dias Corpa Tardelli et al., 2020), has been already demonstrated that Ti-6Al-4V alloys may release aluminium and vanadium even in short periods of time, both of which are capable of causing biological problems (Ananthanarayanan et al., 2016; B. C. Costa et al., 2019; Delgado-Ruiz & Romanos, 2018; A. Ribeiro et al., 2012).

Kasai et al. have reported, in a retrospective study, that approximately one third of patients with titanium alloy spinal implants exhibited abnormal serum or hair metal concentrations at a mean time of 5.1 years after surgery. Titanium or aluminium may travel to distant organs after dissolution of metals from the spinal implants (Kasai et al., 2003).

Aluminium has been related to bone fragility (Klein, 2019; Mjöberg et al., 1997), Alzheimer’s disease (Mjöberg et al., 1997; Willhite et al., 2014) and also Parkinson´s disease (Bolt & Hengstler, 2008), according to Willhite, high levels of Al^{3+}can induce cytotoxicity by oxidative damage (Willhite et al., 2014), which is corroborated by Kermani *at al.*. These authors related Al_2O_3 nanoparticles with structural damage and cellular apoptosis (Kermani et al., 2018).

Inorganic vanadium compounds are described in the literature as carcinogenic, immunotoxic and neurotoxic (Zwolak, 2014), however most of the existent studies are in vitro; furthermore, other authors suggest that vanadium interferes in the hemopoietic system in rats (Adachi et al., 2000); a significant decrease in the fibroblasts cell viability is shown in another in vitro study (B. C. Costa et al., 2019). Due to his suspected strong cellular toxicity, tissue accumulation can cause serious damage (B.-H. Lee et al., 2002; Park et al., 2013).

It should be noted that even though relatively low concentrations of ions may be released from Ti-6Al-4V implants in vivo, the continuous contact with peri-implant cells for long periods of time may represent a potentially hazardous situation displaying systemic and local effects (Arregui et al., 2021a; Kunrath et al., 2021). At long term interactions between peri-implant tissues and ion release might lead to the liberation of inflammatory mediators from macrophages, that lead to bone resorption and may culminate with the implant failure (Azzi & Szpunar, 2007; Kunrath et al., 2021; Rocha et al., 2013).

For the above, it is of paramount importance to better understand the conduct of these ions released by Ti-6Al-4V prostheses frameworks and by Ti-6Al-4V supra-structures in peri-implant region.

We created an experimental model based on the Taylor dispersion technique, which will help us understand from a chemical point of view the diffusion properties of the released particles and the influence of different media potentially present in the oral cavity by diffusion.

Taking into account that the oral cavity undergoes several pH variations resulting from the diet and/or pathologies such as gastroesophageal reflux(Abey et al., 2014; M. Edgar et al., 2012; Koike & Fujii, 2001; Kunrath et al., 2021), a different pH range was considered in this work.

Thereby, the main aim of this study was the analysis of the release of aluminium and vanadium in the artificial saliva models, in the context of chemical research, by assessing diffusion behaviour in several medium at different pH; also, and understand chlorhexidine digluconate, present in mouthwashes, can react with released free metal ions present in the oral cavity and act as a carrier, with the purpose of eliminating them in the act of mouth washing, decreasing their toxic potential to the human body.

3.2. Materials and Methods

3.2.1. Materials

Aluminium chloride (Sigma-Aldrich, pro analysis, purity (mass fraction) > 0.99) and ammonium vanadate (Riedel-de-Haen, Seelze, Germany, *pro analysi* > 97%), sodium fluoride, lactic acid and chlorhexidine digluconate were used without further purification (Table 3.1). Saliva artificial was prepared according the composition indicated in Table 3.1. The composition of Eludril Extra® with 0.2 % chlorhexidine digluconate is also showed in same Table.

Solutions for the diffusion measurements were prepared using Millipore-Q water (specific resistance = 1.82 × 10^5 Ω m, at 25.00 °C). All solutions were freshly prepared at 25.00 °C before each experiment. The weighing was done using a Radwag AS 220C2 balance with readability of 10^{-5} g in the lower range.

Table 3.1. Sample description

Chemical name	Source	CAS Number	Mass fraction Purity
$AlCl3.6H_2O$	Merck	7784-13-6	>0.99$^{a)}$
NH_4VO_3	Merck	7803-55-6	≥0.99$^{a)}$
NaF	Sigma-Aldrich	7681-49-4	>0.99$^{a)}$
Lactic acid	Sigma-Aldrich	50-21-5	>0.85 wt%$^{a)}$
Artificial saliva$^{b)}$			
Eludril$^{c)}$			
Digluconate Chlorhexidine digluconatec)			
H_2O	Millipore-Q water (1.82 ×10^5 Ω m at 298.15 K)	7732-18-5	

$^{a)}$As stated by the supplier. $^{b)}$Artificial saliva was prepared according the following composition: potassium chloride (KCl): 20 mmol dm^{-3}; sodium bicarbonate ($NaHCO_3$): 17.9 mmol dm^{-3}, sodium phosphate ($NaH_2PO_4.H_2O$): 3.6 mmol dm^{-3}, potassium thiocyanate (KSCN): 5.1 mmol dm^{-3} and lactic acid: 0.10 mmol dm^{-3}. $^{c)}$Chlorhexidine digluconate used in this work was Eludril Extra® 0.2 % of chlorhexidine digluconate, with the following constituents: chlorhexidine digluconate, glycerin and propylene glycol.

3.2.2. Taylor dispersion method: Some concepts and its summary description

There are two approaches to describe the isothermal diffusion: the thermodynamics of irreversible processes and Fick´s laws (Harned & Owen, 1964; Loh, 1997a; Tyrrell & Harris, 1984). In the first case, the gradient of chemical potential in the real solution is the true virtual force producing this irreversible phenomenon. However, in ideal solutions, that force can be quantified by the gradient of the concentration at constant temperature, and this parameter, in binary systems, may be defined in terms of the concentration gradient by a phenomenological relationship, known as Fick´s first law (3.1).

$$J = -D\nabla C \tag{3.1}$$

J and ∇C are the molar flux and the gradient in the concentration of solute.

Diffusion in a ternary solution (that is, two solutes and water) is described by the diffusion equations (eqs. 3.2 and 3.3)

$$J_1 = -D_{11}\nabla C_1 - D_{12}\nabla C_2 \tag{3.2}$$

$$J_2 = -D_{21}\nabla C_1 - D_{22}\nabla C_2 \tag{3.3}$$

J_1 and J_2 are the molar fluxes of component 1 and component 2 driven by the concentration gradients ∇C_1 and ∇C_2 of each solute 1 and 2, respectively. Main diffusion coefficients D_{11} and D_{22} give the flux of each solute driven by its own concentration gradient. Cross-diffusion coefficients D_{12} and D_{21} give the coupled flux of each solute driven by a concentration gradient in the other solute. A positive D_{ab} cross-coefficient ($a \neq b$) indicates co-current coupled transport of solute a from regions of higher to lower concentrations of solute b. On the other hand, a negative D_{ab} coefficient indicates counter-current coupled transport of solute a from regions of lower to higher concentration of solute b.

The Taylor dispersion method, which has been used to obtain mutual diffusion coefficients for a wide variety of aqueous binary and ternary systems, is described in great detail in papers (Callendar & Leaist, 2006a; A. C. F. Ribeiro et al., 2010). Basically, it consists on the dispersion of small amounts of solution injected into laminar carrier streams of water or solution of different composition, flowing through a long capillary tube with length and radius of 3.2799 (±0.0001) $\times 10^4$ mm and 0.5570 (± 0.00003) mm, respectively (Loh, 1997a; Musilová et al., 2021a; Price, 1988a)

At the start of each run, a 6-port Teflon injection valve (Rheodyne, model 5020) is used to introduce 0.063 mL of solution into the laminar carrier stream of slightly different composition. Using a metering pump (Gilson model Minipuls 3), a flow rate of 0.17 mL min^{-1} is maintained by to give retention times of about 1.1×10^4 s. The injection valve and the dispersion tube are kept at 298.15 K (± 0.01 K) in an air thermostat.

Dispersion of the injected samples is monitored using a differential refractometer (Waters model 2410) at the outlet of the dispersion tube. Detector voltages, $V(t)$, are measured at accurately 5 s intervals with a digital voltmeter (Agilent 34401 A) with an IEEE interface.

Binary diffusion coefficients are evaluated by fitting the dispersion equation

$$V(t) = V_0 + V_1 t + V_{\max} (t_R/t)^{1/2} \exp[-12D(t - t_R)^2/r^2 t] \tag{3.4}$$

to the detector voltages. The additional fitting parameters are the mean sample retention time t_R, peak height $V_{\max}$, baseline voltage V_0, and baseline slope V_1.

Using this technique, our group has also been focused on the study of the diffusion behavior of the salts $AlCl_3$ or NH_4VO_3 at tracer concentrations the systems in different media: artificial saliva without and with other components (lactic acid, sodium fluoride and the mixture of both), and chlorhexidine digluconate pure or present in commercial mixture. In fact, these systems are actually ternary systems, but, from experimental conditions, we may consider these systems as pseudo-binary ones, and, consequently, take the measured parameters as binary diffusion coefficients, D, of these salts at tracer concentrations in these media. For that, the dispersion profiles were prepared by injecting artificial saliva with $AlCl_3$ or NH_4VO_3 0.001 mol dm^{-3} into carrier streams of artificial saliva (or in chlorhexidine digluconate). The respective diffusion coefficients were evaluated by fitting the dispersion equation (eq. 4).

Extensions of the Taylor technique have been used to measure ternary mutual diffusion coefficients (D_{ab}) for multicomponent solutions. These D_{ab} coefficients, defined by eqs 3.2 and 3.3, were evaluated by fitting the ternary dispersion equation (eqs. 3.5.and 3.6) to two or more replicate pairs of peaks for each carrier-stream.

$$V(t) = V_0 + V_1 t + V_{\max} (t_R/t)^{1/2} \left[W_1 \exp\left(-\frac{12D_1(t-t_R)^2}{r^2 t} \right) + (1-W_1)\exp\left(-\frac{12D_2(t-t_R)^2}{r^2 t} \right) \right] \tag{3.5}$$

$$V(t) = V_0 + V_1 t + V_{\max} (t_R/t)^{1/2} \left[W_1 \exp\left(-\frac{12D_1(t-t_R)^2}{r^2 t} \right) + (1-W_1)\exp\left(-\frac{12D_2(t-t_R)^2}{r^2 t} \right) \right] \tag{3.6}$$

Two pairs of refractive –index profiles, D_1 and D_2, are the eigenvalues of the matrix of the ternary D_{ab} coefficients. In these particular experiments, small volumes of ΔV of solution, of composition $\bar{c}_1+\overline{\Delta c}_1$ and $\bar{c}_2+\overline{\Delta c}_2$ are injected into carrier solutions of composition, $\bar{c}_1$ and $\bar{c}_2$, at time t = 0.

In present work, the effect of chlorhexidine digluconate (CHDG) on the diffusion of aluminium ions was investigated by using this method to measure the ternary mutual diffusion coefficients (D_{ab}) of aqueous $AlCl_3$ (Component 1) + chlorhexidine digluconate (Component 2) solutions and using coupled Fick equations (eqs. 3.4 and 3.5). Relative to vanadium ions, it was not possible to realize these experiments due to the low solubility of this salt in solutions containing this component.

3.2.3. pH measurements

The pH measurements of solutions were carried out with a Radiometer pH meter PHM 240 with an Ingold U457-K7pH conjugated electrode. The electrode was calibrated immediately before each experimental set of fresh solutions using IUPAC-recommended pH 4, 7 and 10 buffers. From pH meter calibration, a zero pH of 6.400 ± 0.030 and sensitivity higher than 98.7 % were obtained. To perform these measurements at pH 2.3 and 7.9 the intended values of the pH was adjusted by the addition of acid lactic. All solutions were freshly prepared at 298.15 K and degassed by sonication for about 60 min before each experiment.

3.2.4. Molecular dynamics simulations

The interpretation of the diffusion coefficients measured for the Al^{3+} was attempted by employing molecular dynamics (MD) simulations to acquire further information about the interaction of this ion with gluconate in aqueous solution. The MD simulations were performed with the 2019 version of the GROMACS package (Abraham et al., 2015; Lindahl et al., 2018 and the OPLS-AA force field (J. Wang et al., 2004) to establish the interactions among the main species; the OPLS-AA parameters for Al^{3+} were taken from the work of Faro et al. (Faro et al., 2010).

The preparation of the system for the simulations has followed a set of well-established steps: (i) the OpenBabel program (O'Boyle et al., 2011) was employed to obtain a 3D structure of the gluconate ion, which was optimized at the RHF/6-31G* level with the GAMESS package (Schmidt et al., 1993); (ii) for the optimized geometry, the partial charges (see Table S1 of the Supplementary Information) were obtained by using the RESP method, which is implemented in the RED code (Dupradeau et al., 2010); (iii) the topology of gluconate to be used in GROMACS was generated through the LigParGen server (Dodda et al., 2017); (iv) one Al^{3+} and three gluconate ions (GLU1, GLU2 and GLU3) were placed, far apart, in a cubic simulation box (whose dimensions were adjusted to obtain a gluconate concentration of 0.008 M) and, then, solvated with TIP4P-2005 water molecules (Abascal & Vega, 2005); (v) finally, the system was subjected to energy minimization in order to reduce repulsions and, then, equilibrated at a temperature of 298.15 K and pressure of 1 bar by performing two consecutive simulations of 250 ps, respectively, within the NVT and NPT ensembles. The average values of temperature and pressure were kept constant by employing the velocity-rescaling thermostat (Bussi et al., 2007), with a coupling time of 0.1 ps and the Parrinello-Rahman barostat (Parrinello & Rahman, 1981), with a coupling time of 2 ps, respectively.

By departing from the equilibrated configuration, we have carried out a 100 ns MD simulation within the NVT ensemble to investigate the possibility of forming an Al^{3+}-gluconate complex. For the integration of the equations of motion, we have employed the leapfrog algorithm and a time step of 2 fs. In addition, bond constrains were imposed by applying the linear constraint solver (LINKS) scheme (Hess et al., 1997), which is available in GROMACS. In turn, periodic boundary conditions were employed along with a cut-off of 1.2 nm for both Coulomb and van der Waals interactions, and the long-range electrostatic energy was calculated by using the particle-mesh Ewald method (Darden et al., 1993; Essmann et al., 1995).

3.3. Results

Table 3.2 shows our results of diffusion coefficients for $AlCl_3$ and NH_4VO_3 at tracer concentrations in different media, that is, artificial saliva without and with other components (lactic acid, sodium fluoride and the mixture of both), by injecting artificial saliva with $AlCl_3$ or NH_4VO_3 0.001 mol dm^{-3} into carrier streams of artificial saliva (or in chlorhexidine digluconate). Having in mind the previous studies about the corrosion of the dental alloys in these media(A. Ribeiro et al., 2012), it was only measured these transport coefficients of these salts at tracer concentrations.

Table 3.2. Tracer diffusion coefficients, D^0_{IT}, of $AlCl_3$ and NH_4VO_3 in saliva artificial at different pHs and the respective standard deviations of the mean[a], S_D, at 298.15 K

Salts	$D^0_{IT} \pm S_D$ [b] /($10^{-9} m^2 s^{-1}$) (water) pH=6.5	$D^0_{IT} \pm S_D$ /($10^{-9} m^2 s^{-1}$) (Artificial saliva pH = 7.5)	$D^0_{IT} \pm S_D$ /($10^{-9} m^2 s^{-1}$) (Artificial saliva with NaF pH = 7.9)	$D^0_{IT} \pm S_D$ /($10^{-9} m^2 s^{-1}$) (Artificial saliva pH = 2.3)	$D^0_{IT} \pm S_D$ /($10^{-9} m^2 s^{-1}$) (Artificial saliva with NaF and pH = 2.3)
$AlCl_3$	1.208±0.005	1.216±0.021	1.199±0.010	0.683±0.011	1.184±0.017
NH_4VO_3	1.352±0.010	0.907±0.018	0.929±0.015	0.635±0.009	0.657±0.009

[a] Averaged result for n= 3 experiments and obtained by injecting aqueous solutions $AlCl_3$ or NH_4VO_3 0.001 mol dm^{-3} into carrier artificial saliva at different pH, and without and with NaF. Standard uncertainties u are $u_r(c)$ = 0.03; $u(T)$ = 0.01 K and $u(P)$ = 2.03 kPa. [b] Averaged result for n= 3 experiments and obtained by injecting aqueous solutions $AlCl_3$ or NH_4VO_3 0.001 mol dm^{-3} into carrier water.

Table 3.3 shows our results of diffusion coefficients for $AlCl_3$ at tracer concentrations in chlorhexidine digluconate pure 0.2% (pH=5.3), and in commercial mixture containing 0.2% at pH 5.6. In both situations, it can be seen that the diffusion coefficients obtained (D^0_{1T}) are lower than those obtained in artificial saliva, regardless of the pH of the medium, although a more accentuated difference is observed when using the chlorhexidine digluconate in commercial mixture flux as carrier.

Table 3.3. Tracer diffusion coefficients of $AlCl_3$ (component 1) and NH_4VO_3 (component 1) in chlorhexidine digluconate (CHDG) at different pH (D^0_{1T}) and the respective standard deviations of the mean[a], at 298.15 K

Salts	$D^0_{1T} \pm S_D$ /(10^{-9}m² s⁻¹) (Chlorhexidine digluconate pure 0.2 %) pH= 5.3	$D^0_{1T} \pm S_D$ /(10^{-9}m² s⁻¹) (Chlorhexidine digluconate 0.2% in commercial mixture)[b] pH = 5.6
$AlCl_3$	1.105±0.014	0.629±0.014
NH_4VO_3	[c]	[c]

[a] Averaged result for n= 3 experiments.

[b] In present work, Eludril Extra was the moisture mentioned.

[c] Very cloudy dissolution immediately from mixing, and consequently, it was not possible to measure D^0_{1T}.

Relative to the ammonium vanadate, it was not possible to obtain results, due to low solubility of this salt in those solutions.

Table 3.4 shows our results of the average (D_{11}, D_{12}, D_{21}, D_{22}) values of diffusion coefficients for the ternary aqueous system {$AlCl_3$ (C_1)} + CHDG pure (C_2)} solutions determined for each carrier solution composition by fitting Equation (3.5) to five replicate pairs of dispersion profiles. Main diffusion coefficients D_{11} and D_{22} which give the molar fluxes of the $AlCl_3$ (1) and CHDG (2) components, respectively, driven by their own concentration gradients were generally reproducible within ± 0.015×10^{-9} m² s⁻¹. Cross-diffusion coefficients D_{12} and D_{21}, describing the coupled diffusion of $AlCl_3$ and CHDG; they were reproducible within ± 0.050×10^{-9} m² s⁻¹.

Table 3.4. Ternary mutual diffusion coefficients (D_{11}, D_{12}, D_{21}, D_{22}) of aqueous $AlCl_3$ (C_1) +CHDG (C_2) solutions at 298.15 K

C_1[a]	C_2[a]	$D_{11} \pm S_D$[b]	$D_{12} \pm S_D$[b]	$D_{21} \pm S_D$[b]	$D_{22} \pm S_D$[b]	D_{12}/D_{22}	D_{21}/D_{11}
0.001	0.000	1.260±0.002	−0.250±0.005	0.008±0.003	0.640±0.003	−0.390	0.006
0.000	0.004[c]	1.236±0.010	−0.036±0.030	−0.448±0.01	0.679±0.010	−0.053	−0.563

[a] concentrations in units of mol dm⁻³;

[b] Experimental diffusion coefficients and standard deviation, S_D, in units of 10^{-9} m² s⁻¹.

[c] C_2= 0.004 mol dm⁻³ is approximately 0.2 % in CHDG m/v.

3.4. Discussion

3.4.1. Tracer diffusion of $AlCl_3$ and NH_4VO_3 in artificial saliva at different pH

From Table 3.2, it can be observed that while the tracer diffusion coefficients of $AlCl_3$ in artificial saliva at two pH values (7 and 7.9), in artificial saliva containing sodium fluoride and pH = 2.3, and in water are very close, being the differences almost zero, within the method's uncertainty (< 2 %); however, the diffusion of this salt in artificial saliva at pH = 2.3 decrease significantly when compared with the value obtained in water (49 %). Regarding the presence of sodium fluoride, it can be observed that this component is responsible for a significant increasing of the diffusion coefficients of this salt in saliva (pH 2.3). Concerning the behavior of diffusion of NH_4VO_3, an accentuated decrease of this parameter in all media was observed, at most 51%, when compared with those obtained in water. The decrease in these D^0 values when compared with the corresponding one in water indicates the presence of salting-in effects for NH_4VO_3. These vanadate anions (VO_3^-) that consist of a complex mixture of different oligomers with different states of protonation (A. C. F. Ribeiro et al., 2006), will suffer more frictional resistance to motion through the fluid and, consequently, their diffusion coefficients in these media become lower, and they can be retained in oral cavity which can cause severe disturbances associated with the potential toxicity of those ions. Relative to the presence of sodium fluoride, it can be observed that this component is responsible for a light decreasing of the diffusion coefficients of both salts in artificial saliva at pH 7.9 and 2.3.

However, at low pH value (i.e., pH 2.3), it is observed a significant decrease of D for $AlCl_3$ and NH_4VO_3. Despite the possible occurrence of complexes as, for example, $[Al(OH)_2Cl_2]^-$ or $[Al(OH)Cl_3]^-$ in the case of aluminium salts (Urabe et al., 2007), this behaviour may be rationalized by an electrostatic mechanism. It is known that in acidic solutions, H_3O^+ is a predominant species; this ion is characterized by a fast mobility in comparison with others. Thus, a strong electric field will be generated by a concentration gradient in H_3O^+, slowing down these ions and driving large counter-current fluxes of Al^{3+} and NH_4^+ in aqueous solutions. This is supported by negative diffusion coefficients (i.e., a salting-in effect is occurring).

3.4.2. Diffusion coefficients of $AlCl_3$ in chlorhexidine digluconate solutions

Table 3.3 shows the pseudo diffusion coefficients of $AlCl_3$ in chlorhexidine digluconate solutions. From this Table 3.3, it was concluded that the diffusion coefficients of $AlCl_3$ in chlorhexidine digluconate solutions (present in commercial mixture, and prepared from of the pure sample) are lower than values obtained in water. From these facts, it can be concluded that this salt also suffers more resistance to its motion which can be related to its ionic character in these mixtures.

Ternary diffusion coefficients, D_{11}, D_{12}, D_{21} and D_{22} measured at tracer concentration for both components (chlorhexidine digluconate and $AlCl_3$) are summarized in Table 3.4. These D values are the average ones obtained from four replicate dispersion profiles.

In the first situation, $C_2 / C_1 \rightarrow 0$ (chlorhexidine digluconate -free solutions), it is observed that the limiting value of the main coefficient of chlorhexidine digluconate, D_{22}, tends to approach the diffusion coefficient value at infinitesimal concentration, D^0_{CLO}, in aqueous solutions (i.e., $D^0 = 0.762 \times 10^{-9}$ m^2 s^{-1}). Under these conditions, the salt concentration gradient cannot produce a coupled flow of chlorhexidine and, consequently, cross-coefficients D_{21} should be zero. In fact, it can be seen in Table 3.4 that D_{11} value is considerably larger than the D_{22} value, and D_{21} value is approximately zero within the precision of the measurements. However, D_{12} value is negative, suggesting that there is a significant coupled diffusion. In the other words, the presence of chlorhexidine digluconate at trace concentration affects the behaviour of diffusion of this salt by the presence of the counter-current coupled transport of $AlCl_3$ from regions of lower to higher concentration of solute chlorhexidine digluconate. In addition, despite the D_{22} is close to the binary diffusion coefficients of chlorhexidine digluconate at infinitesimal concentration ($D_2^0 = 0.635\times 10^{-9}$ m^2 s^{-1}) (Fangaia et al., 2021), the main coefficients D_{11} is not identical to the binary diffusion coefficients of aqueous $AlCl_3$ measured previously by the same technique at 0.001 moldm^{-3} (that is, $D_1 = 1.144\times 10^{-9}$ m^2 s^{-1}) (A. C. F. Ribeiro et al., 2007). This observed significant deviation (10%) reveals the presence of strong interactions between these components. Indeed, the values of the ratio D_{12}/D_{22} show that a mole of diffusing chlorhexidine digluconate can counter-transports 0.4 mol of this salt (or Al^{3+} ions).

In second case, $C_1 / C_2 \rightarrow 0$ ($AlCl_3$ free solutions), whereas D_{12} is almost zero and D_{11} is approximately equal to the binary diffusion coefficient of this component at infinitesimal concentration ($D_1^0 = 1.225\times 10^{-9}$ m^2 s^{-1}), D_{21} value is negative and significant. Since $D_{21}<0$, it is expected D_{22} value to be considerably larger than the binary D_2 value at 0.004 moldm^{-3} (that is, $D_2 = 0.602 \times 10^{-9}$ m^2 s^{-1}). The ratio D_{21}/D_{11} show that a mole of $AlCl_3$ can counter-transports 0.6 mol of the chlorhexidine digluconate.

From these observations, we can gather that there is evidence of strong interactions between these two components. This assumes special importance because, according Hanawa (Hanawa, 2004), free cations have greater probability of combining with biomolecules, and become toxic, due to their strong interaction with chlorhexidine digluconate, it is possible to reduce his potential toxicity.

Support for this effect of chlorhexidine digluconate was further assessed by the found values for other, where they conclude that there are inclusion complexes between Al^{3+} and gluconate (Amin & Refat, 2013; Buckó et al., 2020a; Cardiano et al., 2017; Lakatos et al., 2008). For that, these association reactions between Al^{3+} and hydroxycarboxylates are expected to yield stable mononuclear complexes where metal ion-binding takes place via the COO^{-} and the (deprotonated) OH groups in acidic to mildly basic medium, while condensation reactions start to dominate at high pH (> 11).

In addition, support for this effect of chlorhexidine digluconate was further assessed by the values predicted resulting by applying a more detailed treatment of the diffusion with inclusion complexes, and using reasonable assumptions. That is, succinctly, assuming the formation of a 1:1 complex between Al^{3+} and chlorhexidine digluconate(Pallagi et al., 2013). The computed values for these parameters are reported in Table 3.3.

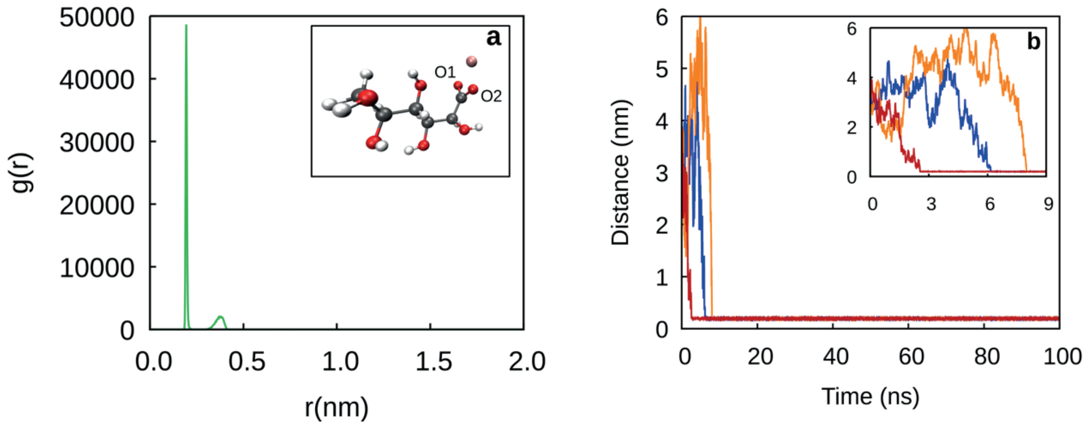

Figure 3.1. (a) Radial Distribution Function of Al3+ around the O1 and O2 oxygen atoms of the three gluconate ions, and (b) average distance between aluminium ion and O1 and O2 oxygen atoms of GLU1 (red), GLU2 (blue), GLU3 (orange). The insert in panel a labels the O1 and O2 oxygen atoms of the gluconate ion, while the insert in panel b is a zoom of the plotted curves for the first 9 ns.

In order to get insight at the molecular level of the interaction between Al^{3+} and the gluconate ions in aqueous solution, we have performed molecular dynamics simulations; the details of these simulations were described in Section 3.2.4.

Figure 3.1 shows the average radial distribution function (RDF) between the aluminium ion and the O1 and O2 oxygen atoms of the three gluconate ions (panel a). The prominent peak of the RDF observed in Figure 1a at approximately 0.2 nm is a clear evidence of the formation of a stable Al^{3+}-gluconate complex. As shown in Figure 1b, the complex begins to be formed at about 2.5 ns, when the O1 and O2 atoms of GLU1 approaches close to Al^{3+}; a snapshot illustrating the geometry of the complex is displayed in Figure 3.2a. Then, after 6.5 ns of simulation time, a second gluconate ion (GLU2) joins the Al^{3+}-GLU1 complex (cf. structure in Figure 3.2b). Finally, the third gluconate ion (GLU3) also approaches de aluminium at about 8 ns of simulation, and a complex with three gluconate ions is formed (cf. structure in Figure 3.2c). Moreover, it is apparent from Figure 3.1b that such complex does not dissociates during the remaining of the simulation that lasts for 100 ns.

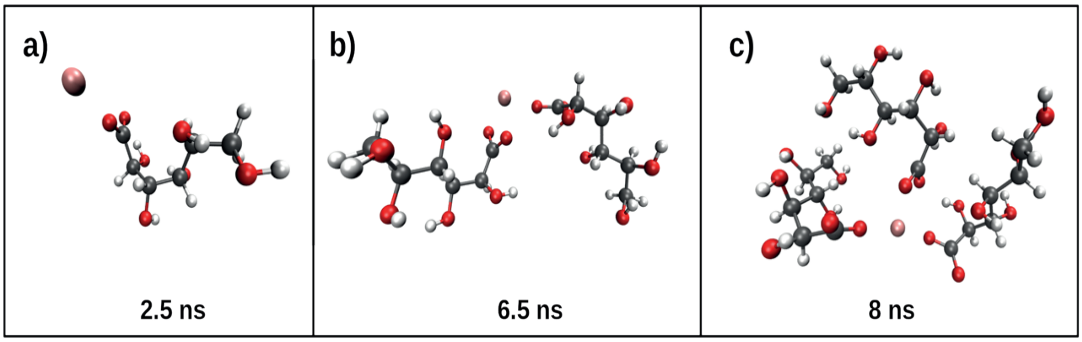

Figure 3.2. Representation of the interaction between the three gluconate ions and the aluminium ion. Colors for atoms: hydrogen (white); carbon(grey); oxygen(red); aluminium (pink).

It is worth noting that the present MD results, which clearly point to the formation of a stable Al^{3+}-gluconate complex, may explain the decrease of the diffusion coefficient of $AlCl_3$ in the presence of gluconate ions observed in the experimental measurements reported in Section 3.3.

By the data obtained, we can assume that it may be very useful to advise rinsing with mouthwashes with chlorhexidine gluconate after meals when the prosthetic devices are subject to tribocorrosion and when the oral cavity is subject to a very acidic pH, such as in patients with gastroesophageal reflux or after acidic food intake.

3.5. Conclusions

The Taylor dispersion method can provide diffusion coefficients with an inaccuracy of ±1% to ±2%. For this particular system ($AlCl_3$(component 1) + Chlorhexidine Digluconate (component 2), keeping in mind that the limiting values of cross-coefficients D_{12} are negative, and there is significant difference between main diffusion D_{11} and the binary diffusion D_1 we can conclude that, for finite and tracer concentration of $AlCl_3$ and chlorhexidine digluconate respectively, the solutes are interacting. Indeed, the MD simulation performed in the present work clearly indicates that the gluconate ions strongly interact with the Al^{3+} ion, thus leading to the formation of a stable Al^{3+}-gluconate complex. This can explain the decrease of the diffusion coefficient of $AlCl_3$ in the presence of gluconate ions observed in the present experimental results. Hence, we can consider that chlorhexidine digluconate can be an important therapeutic agent acting as a carrier in the elimination of aluminium ions from the oral cavity.

According to our results we can also conclude that aluminium ions will suffer less frictional resistance to motion through the fluid and, consequently, their diffusion coefficients in these media becomes higher in all media except in artificial saliva pH-2.3 in which coefficients diffusion decrease significantly.

As for vanadium ions coefficients diffusion decrease in all medium, which may indicate that these ions remain longer in the areas where they are released in the oral cavity.

This experimental model provides important transport data by measuring the diffusion coefficients for aqueous solutions of metal ions.

Chapter 4

4

Effect of cobalt and chromium ions on the chlorhexidine digluconate as seen by intermolecular diffusion

The results from this chapter were published under:

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4.1. Introduction

The use of metal alloys in the manufacture of dental prostheses has been a common practice for decades. Several dental alloys have been used in the fabrication of these devices such as titanium alloys, cobalt-chromium alloys, chromium-nickel alloys, etc. Although these alloys are biocompatible and relatively resistant to corrosion, when exposed to certain media (e.g., acid environments (Arregui et al., 2021b; Kassapidou et al., 2020)), and also as a consequence of wear (G. Wang & Zreiqat, 2010) resulting from chewing, the release of metal ions in the oral cavity is a matter of concern. If these metal ions are constantly diffusing into body fluids, their concentration in the body will increase and, after a certain latency period, it can reach a toxic threshold value that can cause significant harmful effects on the body (by combining with biomolecules such as enzymes and proteins) leading to health problems (Asri et al., 2017; Hanawa, 2019).

The chromium released during the degradation of the Co-Cr alloys is essentially Cr(III), but it can be oxidized to Cr(VI) at the cellular level. Cr(VI) is mutagenic and carcinogenic, being its potential biological effects controversial (Reclaru et al., 2020b). In any case, metals such as chromium and cobalt, which may have potential or demonstrated oncogenic effects in humans, must be subject to strict regulations for the protection of humans (Mulware, 2013b).

Given that the alloys used in prosthetic restorations may produce ionic species, as a result of corrosion, and from the possible ingestion of those ions (which have a potential toxicity depending on their concentration) by individuals, it is important to analyse the diffusion behavior of those ions in the oral cavity.

In the present manuscript the effect of chlorhexidine digluconate ($C_{34}H_{54}Cl_2N_{10}O_{14}$, Figure. 4.1) (CHDG), which are present in many mouthwashes, and a corresponding commercial formulation, on the diffusion of divalent cobalt and trivalent chromium ions is evaluated by measuring intermolecular diffusion coefficients using the Taylor dispersion method (Callendar & Leaist, 2006b; Loh, 1997b; Taylor, 1953).

It is known that a considerable decrease in salivary pH occurs after drinking acidic beverages whose pH is around 2 (W. M. Edgar & O'Mullane, 1996; Suito et al., 2013)., and several studies are related to the behavior of metal alloys under physiological conditions using a pH=2.3 (Arregui et al., 2021b; Puskar et al., 2014a).

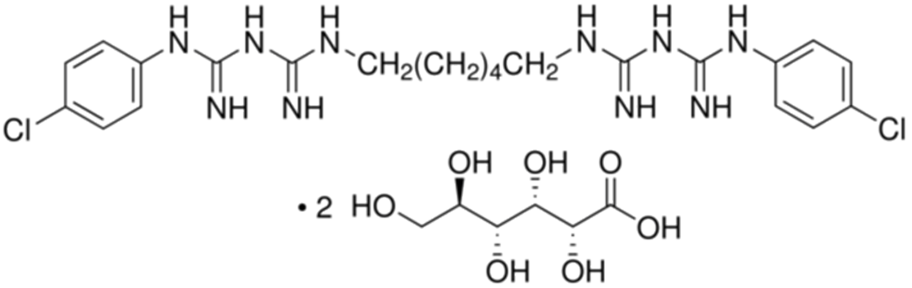

Figure 4.1. Chlorhexidine digluconate structure

In the first part of this study, tracer diffusion coefficients, *D*, or apparent diffusion coefficients for aqueous solutions of cobalt (II) chloride and chromium (III) chloride at 0.001 mol dm^{-3}, in artificial saliva at pH: 8.3, 7.0 and 2.3, and in artificial saliva with sodium fluoride at pH=7.0 are reported. This study is complemented by the evaluation of the diffusion behaviour of the chlorhexidine digluconate (as pure compound or contained in commercial formulation), in aqueous solution in the absence of presence of metal ions salts. UV-visible spectroscopy is also used to assess the effect of chlorhexidine digluconate on Co(II) and Cr(III).

4.2. Materials and Methods

4.2.1. Materials

Cobalt(II) chloride hexahydrate (Sigma-Aldrich, pro analysis, purity (mass fraction) > 0.99, Lyon, France), chromium(III) chloride hexahydrate (Riedel-de-Haen, pro analysis > 97%, Seelze, Germany) and chlorhexidine digluconate solution (20% in water, Sigma-Aldrich Lyon, France) were used as received without further purification. (Table 4.6)

The solutions needed for diffusion measurements were prepared in calibrated volumetric glass flasks, using as solvents, ultrapure water (Millipore, Germany, Milli-Q Advantage A10, specific resistance = 1.82 x 10^5 Ω m, at 298.15 K), artificial saliva, prepared according the composition indicated in Table 6, and a commercially available Extra Eludril ® from Pierre Fabre Oral Care with 0.2 % chlorhexidine digluconate.

The weighing was done using a Radwag AS 220C2 balance with readability of 10^{-5} g in the lower range.

The pH measurements of solutions were carried out with a Radiometer pH meter PHM 240 with an Ingold U457-K7pH conjugated electrode; pH was measured in fresh solutions, and the electrode was calibrated immediately before each experimental set of solutions using IUPAC-recommended pH 4, 7 and 10 buffers. From pH meter calibration a zero pH of 6.897 ± 0.030 and sensitivity higher than 98.7 % were obtained. To perform these measurements at pH 2.3 and 7.0, the intended values of the pH was adjusted by the addition of acid lactic. All solutions were freshly prepared at 298.15 K and degassed by sonication for about 60 min before each experiment.

Table 4.1. Sample description

Chemical	Source	CAS number	Mass fraction purity
$CoCl_2.6H_2O$	Sigma-Aldrich	7791-13-1	>0.98[a)]
$CrCl_3.6H_2O$	Riedel-de-Haen, Seelze	10060-12-5	>0.98[a)]
NaF	Sigma-Aldrich		>0.99[a)]
Lactic acid	Sigma-Aldrich		>0.85 wt%[a)]
Artificial saliva[b)]			
Chlorhexidine digluconate[c)]	Sigma-Aldrich	18472-51-0	20% in water
H_2O	Millipore-Q water (1.82 ×10^5 Ω m at 298.15 K)	7732-18-5	

[a)] As stated by the supplier. [b)] Artificial saliva was prepared according the following composition (Horasawa et al., 1999; Marek, 1997): potassium chloride (KCl, 20 mmol/L), sodium hydrogenocarbonate ($NaHCO_3$, 17.9 mmol/L), sodium phosphate ($NaH_2PO_4.H_2O$, 3.6 mmol/L), potassium thiocyanate (KSCN, 5.1 mmol/L) and lactic acid (0.10 mmol/L). [c)] In this work a commercial formulation containing 0.2 % chlorhexidine digluconate was also used.

Solutions for the diffusion measurements were prepared using Millipore-Q water (specific resistance = 1.82 × 10^5 Ω m, at 298.15 K). All solutions were freshly prepared at 298.15 K before each experiment.

4.2.2. Taylor method

4.2.2.1. Tracer diffusion coefficients

We consider the system containing cobalt chloride (or chromium chloride) in artificial saliva, a pseudo binary system. That is, a system with two components, Co(II) (or Cr(III)) and Cl^- ions, assuming the artificial saliva (with and without lactic acid and sodium fluoride components) as a mixed solvent.

The diffusion coefficient, *D,* in these pseudo-binary systems, may be defined in terms of the concentration gradient by the phenomenological relationship of Fick´s first law (eq.4.1).

$$J = -D\nabla C \tag{4.1}$$

where J and ∇C are the molar flux and the gradient in the concentration of solute, respectively.

In addition, considering that the ionic strength of the artificial saliva is significantly higher than the salt concentration under study ($CoCl_2$ 0.001 mol dm^{-3} or $CrCl_3$ 0.001 mol dm^{-3}) (that is, approximately 0.075 mol dm^{-3}), ensuring the occurrence of tracer diffusion, and that the composition of saliva in the injection and carrier solutions are equal. This is also confirmed by the detector signal resembling a single normal distribution with variance $r^2t_R/24D_T$, and not two overlapping normal distributions (Callendar & Leaist, 2006b). We may consider the system as pseudo-binary and consequently take the measured parameters as the tracer diffusion coefficients of the $CoCl_2$ (or $CrCl_3$) in the artificial saliva.

The Taylor dispersion method is based on the dispersion of small amounts of solution injected into laminar carrier streams of water or solution of different composition, flowing through a long capillary tube (Figure 4.1). Once the detailed description of this method can be found in the literature (e.g., (Alizadeh et al., 1980b; Barthel et al., 1996; Callendar & Leaist, 2006b; Loh, 1997b; Lopes & Nieto de Castro, 1992)), only a few more relevant points are highlighted here.

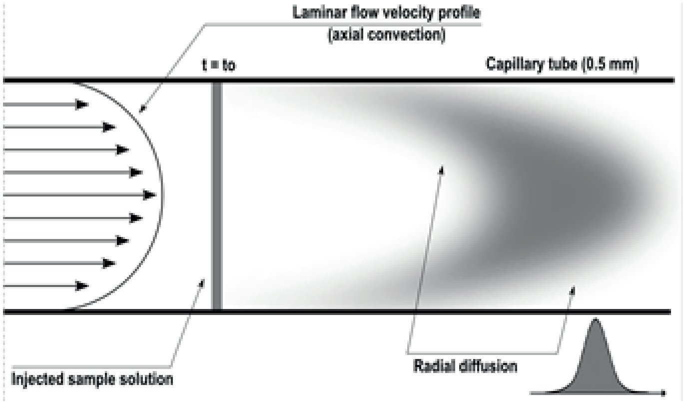

Figure 4.1. Schematic representation of the dispersion technique

The length of the dispersion tube (PTFE) used in the present study was measured directly by stretching the tube in a reservoir and using two high quality theodolytes and appropriate mirrors to accurately focus on the tube ends. This technique gave a tube length of 3.2799 (±0.0001) $\times 10^4$ mm, in agreement with less-precise control measurements using a good-quality measuring tape. The radius of the tube, 0.5570 (± 0.00003) mm, was calculated from the tube volume obtained by accurately weighing (resolution 0.1 mg) the tube when empty and when filled with distilled water of known density. The tube is mounted on a rigid cylindrical support in side-by-side coils of 200 mm radius.

At the start of each run, a 6-port Teflon injection valve (Rheodyne, model 5020) is used to introduce 0.063 mL of solution into the laminar carrier stream of slightly different composition. A flow rate of 0.17 mL min^{-1} is maintained by a metering pump (Gilson model Minipuls 3) to give retention times of about 1.1×10^4 s. The dispersion tube and the injection valve are kept at 298.15 K (± 0.01 K) in an air thermostat.

Dispersion of the injected samples is monitored using a differential refractometer (Waters model 2410) at the outlet of the dispersion tube. Detector voltages, $V(t)$, are measured at precisely 5 s intervals with a digital voltmeter (Agilent 34401 A) with an IEEE interface. Binary diffusion coefficients are evaluated by fitting the dispersion equation

$$V(t) = V_0 + V_1 t + V_{\max} (t_R/t)^{1/2} \exp[-12D(t - t_R)^2/r^2 t] \tag{4.2}$$

to the detector voltages. The additional fitting parameters are the mean sample retention time t_R, peak height $V_{\max}$, baseline voltage V_0, and baseline slope V_1.

4.2.2.2. Diffusion of chlorhexidine digluconate

The diffusion behaviour of aqueous solutions of chlorhexidine digluconate at different concentrations without added salts was analysed. The dispersion profiles were prepared by injecting water into carrier streams of chlorhexidine digluconate 0.2 %, and by injecting chlorhexidine digluconate of composition 0.2% and 0.04% (m/v) into water. Although chlorhexidine digluconate 0.2% was accompanied by other substances, the resulting aqueous systems were assumed as pseudo-binaries. The respective diffusion coefficients were measured for the same procedure described in the preceding section 4.2.1, and evaluated by fitting the dispersion equation (eq. 4.2).

The effect of chlorhexidine digluconate on the diffusion of cobalt and chromium ions was investigated by using Taylor dispersion to measure the ternary mutual diffusion coefficients (D_{ik}) of aqueous $CoCl_2$ (or $CrCl_3$)(C_1) + chlorhexidine digluconate(C_2) solutions and using coupled Fick equations (eqs. 4.3, 4.4) (Deng & Leaist, 1991a; Price, 1988b).

$$J_1 = -D_{11}\nabla C_1 - D_{12}\nabla C_2 \tag{4.3}$$

$$J_2 = -D_{21}\nabla C_1 - D_{22}\nabla C_2 \tag{4.4}$$

where J_1 and J_2 the molar fluxes of $CoCl_2$ (or $CrCl_3$) (component 1) and chlorhexidine digluconate (component 2) driven by the concentration gradients ∇C_1 and ∇C_2 of each solute 1 and 2, respectively. D_{11} and D_{22} are the main- whilst D_{12} and D_{21} cross-diffusion coefficients. For more details see, for example, (Musilová et al., 2021b).

In the present work, pseudo ternary dispersion profiles were prepared by injecting $CoCl_2$ (or $CrCl_3$) (component 1) + chlorhexidine digluconate (component 2) solution samples of composition $\overline{C}_1+\overline{\Delta C}_1$, $\overline{C}_2$ into carrier streams of composition, $\overline{C}_2$, and, by injecting $CoCl_2$ (or $CrCl_3$) (component 1) + chlorhexidine digluconate (component 2) solution samples of composition $\overline{C}_2$, $\overline{C}_2+\overline{\Delta C}_2$ into carrier streams of composition $\overline{C}_1$. Coupled diffusion produces ternary dispersion profiles (eqs. 4.5-4.7)

$$V = \bar{V} + V_1 + V_{\max}\sqrt{\frac{t_{\mathrm{R}}}{t}}\ \frac{(a + b\alpha_1)\sqrt{D_1}e^{-12D_1(t-t_{\mathrm{R}})^2/r^2t} + (1 - a - b\alpha_1)\sqrt{D_2}e^{-12D_2(t-t_{\mathrm{R}})^2/r^2t}}{(a + b\alpha_1)\sqrt{D_1} \quad + \quad (1 - a - b\alpha_1)\sqrt{D_2}} \tag{4.5}$$

$$\alpha_1 = \frac{R_1\Delta C_1}{R_1\Delta C_1 + R_2\Delta C_2} \tag{4.6}$$

where D_1 and D_2 are the eigenvalues of the matrix of ternary D_{ik} coefficients (eqs. 4.7, 4.8) and α_1 is the fraction of the initial refractive index difference due to$CoCl_2$ (or $CrCl_3$). R_1 and R_2 are the detector sensitivities for $CoCl_2$ (or $CrCl_3$) (1) and chlorhexidine digluconate (2): $R_1 = \partial V/\partial C_1$ and $R_2 = \partial V/\partial C_2$.

$$D_1 = \left\{D_{11} + D_{22} + (D_{11} - D_{22})\sqrt{1 + \left[4\,D_{12}\,D_{21}/(D_{11} - D_{22})^2\right]}\right\}/2 \tag{4.7}$$

$$D_2 = \left\{D_{11} + D_{22} - (D_{11} - D_{22})\sqrt{1 + \left[4\,D_{12}\,D_{21}/(D_{11} - D_{22})^2\right]}\right\}/2 \tag{4.8}$$

Ternary mutual diffusion coefficients were calculated from D_1, D_2, a, b fitting parameters and the relative detector sensitivity R_2/R_1 using

$$D_{11} = D_1 + \frac{a(1-a-b)}{b}(D_1 - D_2) \tag{4.9}$$

$$D_{12} = \frac{R_2}{R_1}\,\frac{a(1-a)}{b}(D_1 - D_2) \tag{4.10}$$

$$D_{21} = \frac{R_1}{R_2}\,\frac{(a+b)(1-a-b)}{b}(D_2 - D_1) \tag{4.11}$$

$$D_{22} = D_2 + \frac{a(1-a-b)}{b}(D_2 - D_1) \tag{4.12}$$

a and b parameters in these equations (4.9-4.12) are described by equations:

$$a = \frac{D_{11} - D_1 - (R_1/R_2)D_{12}}{D_2 - D_1} \tag{4.13}$$

$$b = \frac{D_{22} - D_{11} + (R_1/R_2)D_{12} - (R_2/R_1)D_{21}}{D_2 - D_1} \tag{4.14}$$

4.2.3. UV-VIS Spectroscopy measurements

Electronic absorption spectroscopy of solutions of Cr(III) and Co(II), in the concentration range: 10 - 50 mM, in water and in a mixture containing Clorhexidine digluconate, was carried out by using a Shimadzu UV-2600i UV-VIS spectrophotometer.

4.3. Results and Discussion

Table 4.2 shows the tracer diffusion coefficients, D^0_I, for aqueous solutions of cobalt (II) chloride and chromium (III) chloride in water and in CHDG-containing solutions. It can be seen that the D^0_I value for $CoCl_2$ matches with that previously reported in literature (A. C. F. Ribeiro et al., 2002) and measured by using the open-ended capillary cell. Although the tracer diffusion coefficients are similar for both salts, and for the moisture as well, at pH 6.4, the predominant cationic species are Co^{2+} (Baes Jr. & Mesmer, 1976) and $Cr_3(OH)_4^{5+}$ (Stuenzi & Marty, 1983). It can also be seen that, in general, the tracer diffusion coefficients decrease by decreasing the solution pH. In fact, the presence of CHDG either as pure or in a commercial formulation leads to a slight increase in the acidity of the media.

Table 4.2. Tracer diffusion coefficients, D^0_I, of $CoCl_2$ (0.001 mol dm^{-3}), $CrCl_3$ (0.001 mol dm^{-3}) and mixture of $CoCl_2$ and $CrCl_3$ in different aqueous media and the respective standard deviations of the mean[a)], S_D, at T = 298.15 K and P = 101.3 kPa.

	D^0_I ($\pm S_D$)/(10^{-9} m^2 s^{-1})			
	pH	**$CoCl_2$**	**$CrCl_3$**	**$CoCl_2$/$CrCl_3$**
water	6.4	1.295 (±0.010)	1.272 (±0.014)	1.189 (±0.015)
0.2% CHDG	5.0	0.666 (±0.015)	0.656 (±0.015)	-
CHDG commercial formulation	5.7	0.709 (±0.020)	0.718 (±0.018)	0.915 (±0.022)

[a)] Averaged result for n = 3 experiments. Standard uncertainties u are $u_r(C)$ = 0.03; $u(T)$ = 0.01 K and $u(P)$ = 2.03 kPa.

Table 4.3 shows the tracer diffusion coefficients of the same salts and mixture of salts in artificial saliva (AS). In order to evaluate the effect of ionic strength and pH on the tracer diffusion coefficients we have used sodium fluoride and lactic acid, to control both parameters, respectively. The former has been chosen once is an important component of several oral mouth rinses used for preventing dental caries (Jafari et al., 2019; Rugg-Gunn, 2013), whilst the latter has been used to simulate the variation of pH occurring in the oral cavity (Mabilleau et al., 2006; Takemoto et al., 2005).

Table 4.3. Tracer diffusion coefficients, D^0_{I}, of $CoCl_2$, $CrCl_3$ and mixture of $CoCl_2$ and $CrCl_3$ in different artificial saliva (AS) media, at T = 298.15 K and P = 101.3 kPa.

D^0_{I} (± S_D)/(10^{-9} m^2 s^{-1})				
	pH	**$CoCl_2$**	**$CrCl_3$**	**$CoCl_2/CrCl_3$**
AS	8.3	1.823 (±0.024)	1.789 (±0.040)	1.890 (±0.045)
AS	7.0	1.860 (±0.010)	1.808 (±0.020)	1.909 (±0.014)
AS + lactic acid	2.3	0.921 (±0.030)	0.908 (±0.012)	0.917 (±0.010)
AS + NaF	7.0	1.701 (±0.029)	2.065 (±0.019)	2.315 (±0.020)
AS + lactic acid + NaF	2.3	0.899 (±0.031)	0.782 (±0.030)	0.826 (±0.028)

[a] Averaged result for n = 3 experiments. Standard uncertainties u are $u_r(C)$ = 0.03; $u(T)$ = 0.01 K and $u(P)$ = 2.03 kPa.

Table 4.4 shows the average experimental diffusion coefficients of solutions of chlorhexidine digluconate (D_{CHDG}). These values are compared with those obtained by using a CHDG-based commercial formulation ($D_{CHDG\text{-}cf}$) (see Table 4.6). It should be noted that information on apparent diffusion coefficients of CHDG into a commercial mixture is of significant importance for understanding the behaviour of this compound in practical dental applications. The reproducibility of these diffusion coefficients is similar to those observed for other systems; i.e. within ± 2% (Musilová et al. 2021).

Table 4.4. Diffusion coefficients for aqueous chlorhexidine digluconate solutions, D_{CHDG}, at different concentrations, C, at temperature T = 298.15 K and pressure P = 101.3 kPa[a].

C/ (mol dm^{-3})	D_{CHDG} /(10^{-9} m^2 s^{-1})	$D_{CHDG,cf}$ /(10^{-9} m^2 s^{-1})
0.000	0.635±0.010	0.762±0.016[b]
0.001	0.617 ±0.009	0.740±0.012
0.004	0.602 ±0.008	0.677±0.013

The standard uncertainties are $u_r(C)$ = 0.03, $u(T)$ = 0.01 K, $u(P)$ = 2.03 kPa and $u(D)$ = 0.01 x 10^{-9} m^2 s^{-1}.

Tracer diffusion coefficients of $CoCl_2$ and $CrCl_3$ and the mixture of them increase significantly in artificial saliva and in artificial saliva with NaF pH = 7.0, when compared with those obtained in water. The deviations between the tracer diffusion coefficient values (D^0_{I}) in these media and the limiting diffusion coefficients of these salts in water (D^0) (A. C. F. Ribeiro et al., 2005b, 2012), at the same temperature, are positive ($\Delta D^0/D^0$ = 44 %, 62 % and 95%, for $CoCl_2$ and $CrCl_3$, and the mixture of them, respectively). The increase in these D^0 values when compared with D^0 value in water indicates the presence of salting-out effects. These ions, as its chlorides, will suffer less frictional resistance to motion through the fluid and, consequently, their diffusion coefficients in these media becomes higher, and can flow faster inside living tissues, which can cause severe disturbances associated with these heavy metal ions.

However, in acidic media (artificial saliva pH = 2.3 with and without NaF), tracer diffusion coefficients values are much smaller when compared with D^0 values in water. This fact may be explained on the basis of an electrostatic mechanism. Considering that in acid solutions, H_3O^+ is one of the predominant species, due to its large mobility, a strong electric field will be generated by a concentration gradient in H^+, slowing down these H^+ ions drive large counter-current fluxes of Co^{2+} and Cr^{3+} in aqueous solutions and consequently, their values of $D< 0$ (salting-in). For Cr(III) solutions an alteration in the structure of the solution as due to modification of predominant species, as a function of pH, is also expected.

Table 4.4 shows the average experimental diffusion coefficients of chlorhexidine digluconate in water and in commercial formulation (cf). It is observed that the diffusion coefficients of CHDG at cf are higher than in water. In the case of salts, it has been found that pH is slightly lower than water and that has an effect in the tracer diffusion coefficients. Another possible explanation comes out from the occurrence of higher ionic strength in the cf and thus inducing less electrostatic interactions between the diffusing species (Kittner & Klapp, 2007). It can also be noticed that, in both cases, diffusion coefficients decrease by increasing the concentration; however, the effect of concentration in the diffusion of CHDG in the cf is more significant, when compared with water.

These results show that, although the interpretation of diffusion coefficients is not straightforward, they can provide relevant information on the effect of different conditions on the mobility of relevant ions. That can be of utmost importance for practical purposes. Keeping that in mind, ternary mutual diffusion coefficients for the systems {($CoCl_2$) (1) + CHDG (2)} and {($CrCl_3$) (1) + CHDG (2) } in water and in a commercial formulation (cf), were measured at tracer concentrations, and data reported in Tables 4.5 and 4.6, respectively.

Table 4.5. Tracer ternary diffusion coefficients (D_{11}, D_{12}, D_{21}, D_{22}) of aqueous $CoCl_2$ (or $CrCl_3$) (C_1=1×10^{-3} mol dm^{-3}) + CHDG (C_2=0) solutions and at T = 298.15 K and P = 101.3 kPa.

	$D_{11}\pm S_D$	$D_{12}\pm S_D$	$D_{21}\pm S_D$	$D_{22}\pm S_D$	D_{12}/D_{22}
$CoCl_2$	1.325±0.018	−0.105±0.030	−0.020±0.020	0.780±0.010	−0.135
$CrCl_3$	1.310±0.020	−0.205±0.030	−0.050±0.010	0.736±0.010	−0.279

Diffusion coefficients and standard deviation, S_D, in units of 10^{-9} m^2 s^{-1}. The standard uncertainties are $u_r(C)$= 0.03, $u(D)$ = 0.01 x 10^{-9} m^2 s^{-1}, $u(T)$ = 0.01 K and $u(P)$ = 2.03 kPa.

Table 4.6. Tracer ternary diffusion coefficients (D_{11}, D_{12}, D_{21}, D_{22}) of aqueous $CoCl_2$ (or $CrCl_3$) (C_1=1×10^{-3} mol dm^{-3}) + (CHDG,*cf*) (C_2 =0) solutions and at T = 298.15 K and P = 101.3 kPa.

	$D_{11} \pm S_D$	$D_{12} \pm S_D$	$D_{21} \pm S_D$	$D_{22} \pm S_D$	D_{12}/D_{22}	D_{21}/D_{11}
$CoCl_2$	1.193±0.018	−0.150±0.030	−0.003±0.020	0.809±0.010	−0.060	−0.003
$CrCl_3$	1.309±0.020	−1.080±0.030	−0.002±0.010	0.819±0.010	−1.201	−0.001

Diffusion coefficients and standard deviation, S_D, in units of 10^{-9} m^2 s^{-1}. The standard uncertainties are $u_r(C)$= 0.03, $u(D)$ = 0.01 x 10^{-9} m^2 s^{-1}, $u(T)$ = 0.01 K and $u(P)$ = 2.03 kPa.

Tracer diffusion coefficients values for chlorhexidine digluconate (at X_2 = 0) are reported in Tables 4.5 and 4.6. It should be noticed that the main diffusion coefficients D_{11} and D_{22} were generally reproducible within ± 0.02 x 10^{-9} m^2 s^{-1}, whilst the cross-coefficients were reproducible within ± 0.05 x 10^{-9} m^2 s^{-1}.

The D_{11} values are considerably larger than the D_{22} values, and, in the case of $CrCl_3$, they are higher than the binary diffusion coefficient of this salt measured previously by the same technique (A. C. F. Ribeiro et al., 2005b, 2012).

It can also be seen that coupled diffusion is significant. Indeed, D_{21}/D_{11} ratios indicate that a mole of diffusing salt can counter-transports up to 0.6 mol of chlorhexidine digluconate, whereas the values of the ratio D_{12}/D_{22} show that a mole of diffusing chlorhexidine can counter-transports up to 1.2 mol of salt ($CrCl_3$).

From the significant negative cross diffusion coefficients for the system $CrCl_3$ and chlorhexidine, indicating counter-current coupled flows of this salt and chlorhexidine digluconate, we can infer that there is evidence of strong interactions between these two components. This mechanism also accounts for the large negative values of cross diffusion as well as the increased diffusivity of the $CrCl_3$ and $CoCl_2$ components in aqueous chlorhexidine digluconate. (Tables 4.5 and 4.6). The presence of complexes ions between Co(II) and Cr(III) ions and chlorhexidine digluconate. may explain the obtaining of negative D_{12} values. That is, in the region of the solution where the concentration of chlorhexidine digluconate is highest, there will be a more pronounced decrease of a large amount of the free Co^{2+} and Cr^{3+} ions resulting from the formation of those complex ions. Hence, the appearance of a gradient of these ions Co(II) (or Cr(III)) with signal opposite to the gradient of cobalt chloride (or chromium chloride).

Support for this effect of chlorhexidine digluconate on chromium ions was further assessed by UV-visible spectroscopy. Figure 4.2a shows the spectra of aqueous solutions of $CrCl_3.6H_2O$. The spectra show characteristics of maximum absorbances at 417 and 520 nm (Scattergood, 2020). These spectra are similar to those obtained for Cr(III) in the presence of CHDG (1 mM). However, an increase in the absorbance was found at both wavelengths: 417and 590 nm (Figures 4.2b), accompanied by a maximum 0.2 and 0.05 red shift displacement. No alteration in the spectra of Co(II) in the absence and presence of CHDG is observed (not shown). This can be justified by changes in the conjugated system of ligand molecules. The Cr(III) water exchange rate, from the first hydration shell, is very low (Baes Jr. & Mesmer, 1976), which confers some inert features for ligand substitution (D. Costa et al., 2012); consequently, it can be hypothesized that a strong interaction with some component of CHDG, acting as ligand, takes place. Concomitantly, Cr(III) has high charge density when compared with Co(II) (the ionic radii of Co(II) and Cr(III) are 0.74 and 0.61 nm, respectively (Jiménez et al., 2021)). The digluconate shows a high affinity towards metal ions (as, for example, calcium and aluminium) and, consequently, it could be the referred ligand (Buckó et al., 2020b; Sawyer, 1964).

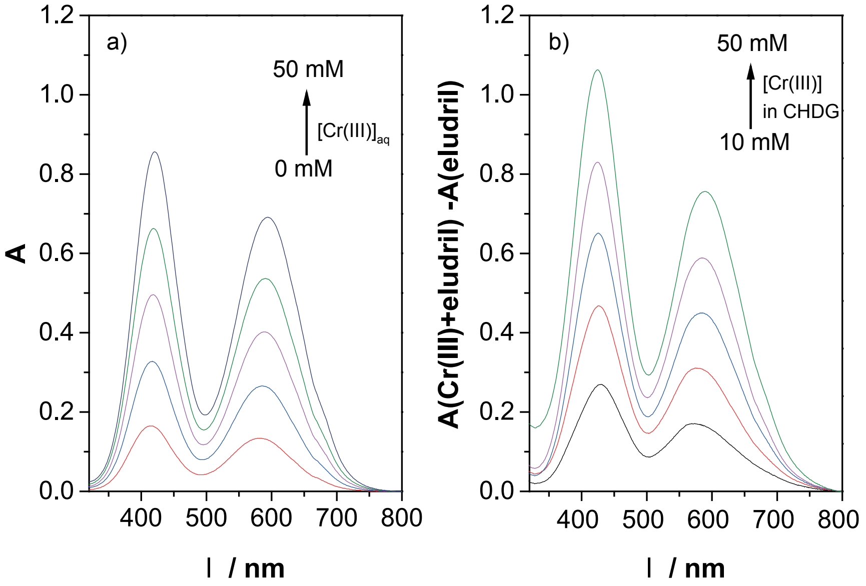

Figure 4.2. UV-vis spectra of aqueous solution of $CrCl_3$ (a) and solutions of $CrCl_3$ in chlorhexidine digluconate (b, 0.004 mol dm^{-3}).

4.5. Conclusions

Binary and ternary diffusion coefficients of cobalt chloride and chromium chloride, and chlorohexidine digluconate, alone and in mix solutions, respectively, were measured by the Taylor dispersion technique. In artificial saliva at pH = 2.3, containing lactic acid and sodium fluoride, a decreasing of the diffusion coefficients of these salts from 10 to 40 %, approximately, was observed when compared with those obtained in water. In the presence of saliva, a salting-in effect affecting the metal ion salts are observed.

However, in artificial saliva at pH = 7 and pH = 8.0, the diffusion coefficients of these salts increase significantly (at most 60 %), which indicates the presence of salting-out effects. These ions will suffer less frictional resistance to motion through the fluid and, consequently, their diffusion coefficients in these media becomes higher, can flow faster inside living tissues, causing severe disturbances associated with these heavy metal ions.

The interactions between the metal salts and CHDG is also observed, essentially for Cr(III), by UV-vis spectroscopy.

The experimental results suggest that interactions between metal ions and CHDG might be justified by the occurrence of metal-digluconate interactions and are stronger for Cr(III), probably due to its high charge density.

We can conclude that the chlorhexidine digluconate may be used as a controlled heavy metal chromium and cobalt capture system, and therefore contribute to reduce the toxicity levels in the oral cavity.

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Chapter 5

Conclusions and future perspectives

5.1. Conclusions

Ti-6Al-4V is widely used in biomedical devices, in dental and orthopedic prostheses, because of their excellent mechanical properties, biocompatibility and corrosion resistance. However, despite possessing a passive film, it is susceptible to mechanical depassivation and fretting corrosion. Furthermore, fluoride-containing solutions that can also cause the breakdown of that protective layer.

In our study, Ti-6Al-4V was subjected to sliding wear isolated or with dental amalgam and tested simultaneously, in artificial saliva with and without fluoride. With the limitations of an in vitro study, the following conclusions could be obtained:

- In artificial saliva, when Ti-6Al-4V and amalgam were tested simultaneously, we didn´t find any effect on Ti-6Al-4V regarding all the tested parameters.
- However, in a fluoridated medium, when both materials were tested simultaneously, there was a higher mass loss in the Ti-6Al-4V alloy and amalgam samples do not suffer any mass variation.
- In these conditions, surface roughness (Rz) of Ti-6Al-4V reduces, whereas their counterpart amalgam increases significantly.
- In fact, apparently Ti-6Al-4V alloy seems to suffer the synergistic action of fluoride and amalgam.

The use of Ti-6Al-4V alloys should be avoided in areas subject to wear and where they may come into contact with fluor-containing substances, especially if dental amalgams are also present. Furthermore, it seems to us to be advisable the replacement of amalgam restorations when Ti-6Al-4V is present in prosthetic rehabilitations. Besides, it is mandatory that the clinician knows the constitution of the alloys present in the prostheses used in the rehabilitation of their patients.

Considering that Ti-6Al-4V has a low wear resistance as we saw previously, with consequent ion metal release to the medium; and that aluminium and vanadium are known to be cytotoxic, it is quite important to understand the behavior of those ions in different conditions related to the oral cavity, like artificial saliva and fluoridated saliva, at different pH, and with digluconate chlorhexidine.

Mutual diffusion coefficients were measured by the Taylor dispersion technique, and according to our results, we can conclude that:

- Aluminium ions suffered less frictional resistance to motion through the fluid and, consequently, their diffusion coefficients in these media becomes higher in all media except in artificial saliva pH-2.3 in which coefficients diffusion decrease significantly.
- As for vanadium ions coefficients diffusion decrease in all medium, which may indicate that these ions remain longer in the areas where they are released in the oral cavity.

The effect of chlorhexidine digluconate, on aluminium chloride, was also studied by using the Taylor dispersion method and molecular dynamics simulations.

For the particular aqueous system ($AlCl_3$(component 1) + Chlorhexidine Digluconate (component 2), the limiting values of cross-coefficients D_{12} are negative, and there is a significant difference between main diffusion D_{11} and the binary diffusion D_1 we can conclude that:

- For finite and tracer concentrations of $AlCl_3$ and chlorhexidine digluconate respectively, the solutes are interacting.

This conclusion is fully supported by the present MDS, which clearly indicate a strong interaction between gluconate ions and Al^{3+}, thus leading to the formation of a stable Al^{3+}-gluconate complex. This can explain the decrease of the diffusion coefficient of $AlCl_3$ in the presence of gluconate ions observed in the present experimental results. Hence, we can consider that chlorhexidine digluconate can be an important therapeutic agent acting as a carrier in the elimination of aluminium ions from the oral cavity.

Due to the fact that Co-Cr alloys are widely used in the fabrication of removable and fixed prostheses, and with the growing concern about the toxic potential of cobalt, we used the same experimental model employed with aluminium and vanadium, based on the Taylor dispersion technique, for the two major constituents of the Co-Cr alloys: cobalt and chromium.

- In artificial saliva at pH = 2.3, containing lactic acid and sodium fluoride, a decrease of the diffusion coefficients of these salts from 10% to 40 %, approximately, was observed when compared with those obtained in water. In the presence of artificial saliva, a salting-in effect affecting the metal ion salts was observed.
- However, in artificial saliva at pH = 7.0 and pH = 8.0, the diffusion coefficients of these salts increase significantly (at most 60 %), which indicates the presence of salting-out effects. These ions suffered less frictional resistance to motion through the fluid and, consequently, their diffusion coefficients in these media become higher and can flow faster inside living tissues, causing severe disturbances associated with these heavy metal ions.
- Interactions between the metal salts and chlorhexidine digluconate were also observed, essentially for chromium, by UV-Vis spectroscopy.

The experimental results suggested that interactions between those metal ions and chlorhexidine digluconate might be justified by the occurrence of metal-digluconate interactions and were stronger for chromium, probably due to its high charge density.

We stated that:

Chlorhexidine digluconate may be used as a controlled captures system for chromium, cobalt and aluminium metal ions, acting as a carrier in the elimination of their elimination from the oral cavity.

To the best of our knowledge, this technique has never been used for the purpose of this research study, namely using artificial saliva and chlorhexidine digluconate as solutions.

We consider that this experimental model provides important transport data by measuring the diffusion coefficients of metal ions, with a great impact in reducing their toxicological potential, with an inherent benefit to patients' health.

5.2. Future perspectives

Despite the numerous studies concerning dental alloys, the available information between in vivo and in vitro studies is often unclear and even contradictory; so, it is mandatory the prosecution of research in this field.

It would be quite important to focus on the tribological properties of dental alloys and the role of the released metal ions.

Within the new technologies for the fabrication of metallic structures in prosthetic dentistry, one of our next objectives is to compare the tribological properties of the titanium and Co-Cr alloys obtained by different manufacturing methods, such as casting, milling and selective laser melting. We think that in the literature this subject is not yet sufficiently studied and fully clarified.

Regarding to the metal ions released, it is essential to consider their impact both at local and systemic level. Therefore, it is essential to carry out these studies with other types of simulated physiological fluids, such as phosphate buffer saline (PBS) or Hank´s solutions, and with more metal ions also present in dental alloys.

Due to the fruitful and intense collaboration between the Institute of Implantology and Prosthodontics of the Faculty of Medicine, the Centre for Innovation and Research in Oral Sciences and the Centre of Chemistry of Coimbra, our joint works will continue in two important fields:

- Molecular dynamics simulations, having in mind to describe the dynamical behavior of dissolved metal ions in physiologic fluids and predicting corrosion rates.
- Mutual diffusions studies, looking for the reduction of the toxicological effect of the ions released by dental alloys. For this, we will analyze the removal of metal ions from the oral cavity by other pharmacological molecules commonly used as carriers, such as ß-cyclodextrins, hyaluronic acid and resorcinarenes, the first two are already constituents of some mouthwashes, but with other clinical purposes. The potential interaction of metal ions with these molecules is, in our opinion, of utmost importance.

We believe that this line of research will bring fundamental data of high clinical interest to the field of Dentistry and in particular to oral rehabilitation, trying to minimize deleterious local and systemic effects of the particles released due to tribocorrosion.

Additionally, one of our most important goals is to promote in vivo and clinical studies in this area, as it is of vital significance the identification and quantification of these ions, either at serological or salivary levels, in patients with prosthetic rehabilitation, knowing that some of them are passive of bioaccumulation.

We consider this topic very challenging and with added value, in the final purpose of achieving prosthetic oral rehabilitation with greater longevity and while maintaining the systemic health of our patients.

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Chapter 6

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Appendices

Appendix I. List of Tables

Appendix II. List of Figures

Appendix III. Equipment's Used

Figure III.1. Micro Vickers Tester HMV-2 Shimadzu, Japan. Hosted in Pedro Nunes Institute, Testing, Wear and Materials Laboratory (LED&MAT). Image reproduced from: https://www.shimadzu.com/.

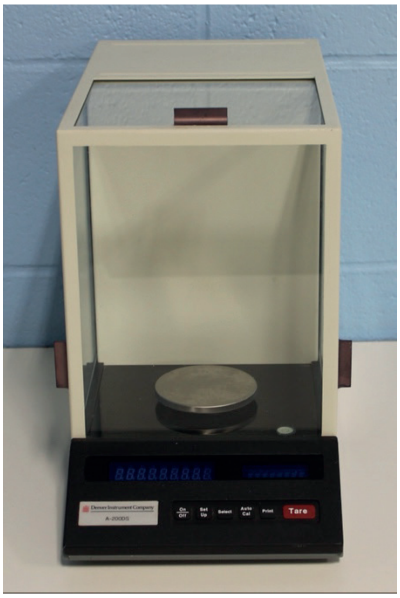


Figure III.2. Denver Instrument A-200DS Analytical Balance, USA. Hosted in Pedro Nunes Institute, Testing, Wear and Materials Laboratory (LED&MAT).

Figure III.3. Perthen S4P Rugosimeter 2D with mechanical probe and laser, Mahr, Germany. Hosted in Pedro Nunes Institute, Testing, Wear and Materials Laboratory (LED&MAT).

Figure III.4. Two-body alternative sliding equipment with parameterizable stroke, speed and applied load, designed by Pedro Nunes Institute, Testing, Wear and Materials Laboratory (LED&MAT).

Figures III.5. Scanning Electron microscope JEOL® JSM-5310, Japan, Hosted in Pedro Nunes Institute, Testing, Wear and Materials Laboratory (LED&MAT). Image reproduced from https://www.ipn.pt/laboratorio/LEDMAT/meios.

Figure III.6. Precision scale AND, A&D Instruments Ltd. Oxford, UK. Hosted in Coimbra Chemistry Centre.

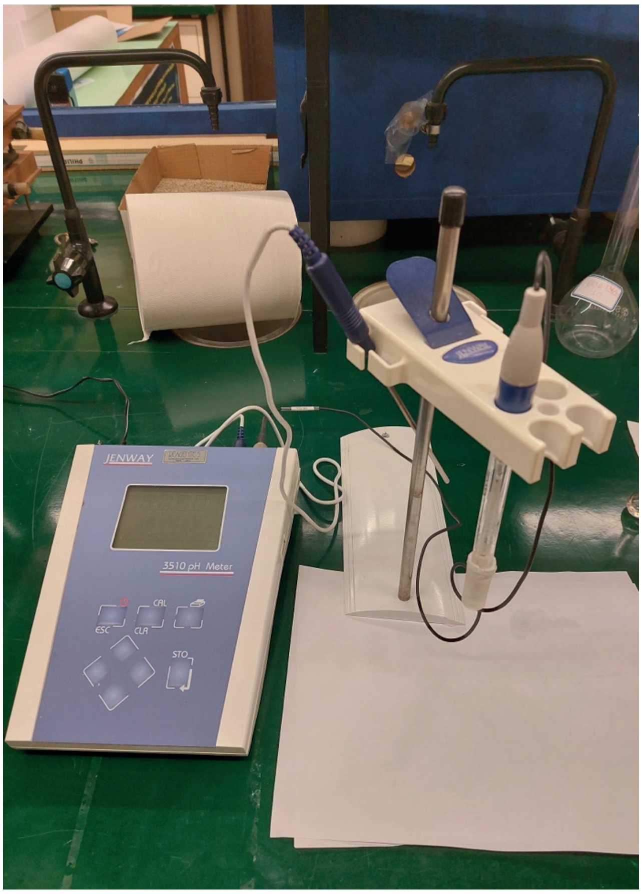


Figure III.7. pH meter 3510, Jenway, UK. Hosted in Coimbra Chemistry Centre.

Figure III.8. Ultrasonic cleaning unit SONOREX SUPER, without heating, Bandelin, Germany. Hosted in Coimbra Chemistry Centre.

Figure III.9. (top and bottom) Equipment used in Taylor Dispersion Technique. Hosted in Coimbra Chemistry Centre.

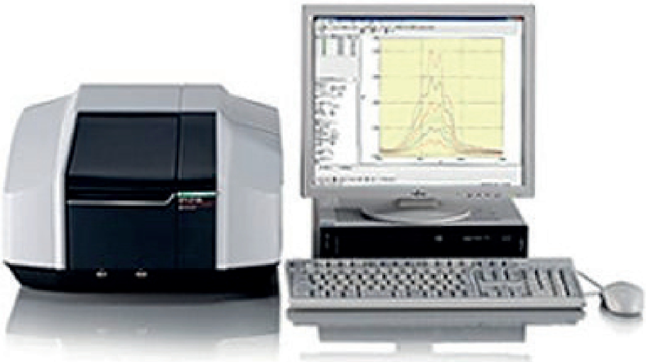
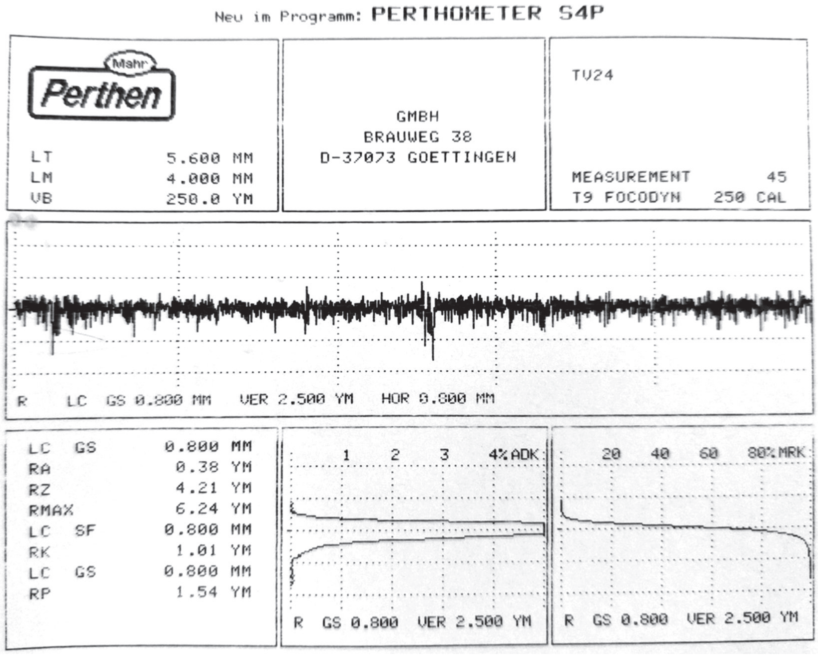


Figure III.10. Shimadzu UV-2600i UV-Vis spectrophotometer, Japan. Hosted in Coimbra Chemistry Centre. Image reproduced from: https://www.shimadzu.com/.

Apendix IV. Images obtained in the Perthometer Mahr model Perthen S4P for roughness analysis (an example for each dental alloy).



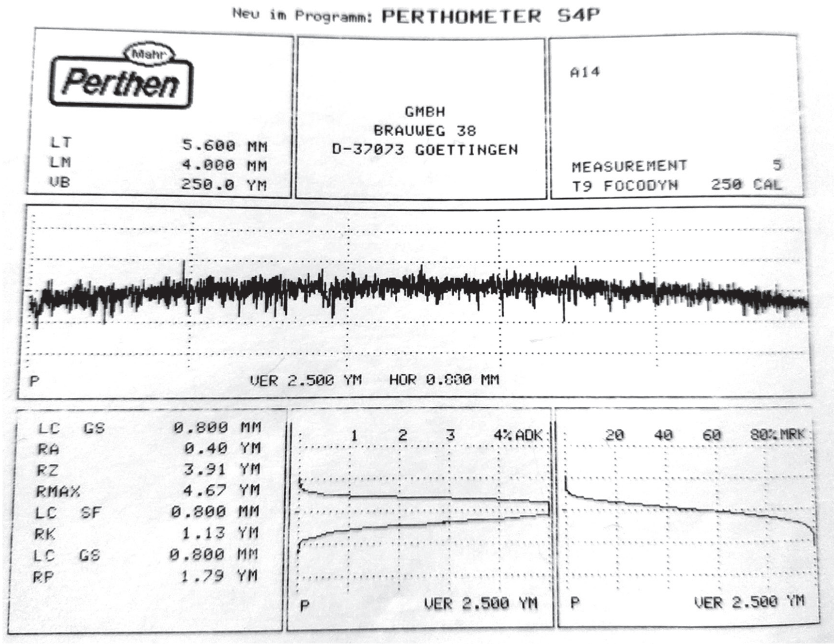
```
Neu im Programm: PERTHOMETER S4P

Mahr
Perthen                  GMBH                   TV24
                         BRAUWEG 38
LT      5.600 MM         D-37073 GOETTINGEN
LM      4.000 MM                                MEASUREMENT        45
VB      250.0 YM                                T9 FOCODYN    250 CAL

R    LC   GS 0.800 MM    VER 2.500 YM    HOR 0.800 MM

LC   GS     0.800 MM      1    2    3    4%ADK      20   40   60   80%MRK
RA           0.38 YM
RZ           4.21 YM
RMAX         6.24 YM
LC   SF     0.800 MM
RK           1.01 YM
LC   GS     0.800 MM
RP           1.54 YM
                          R  GS 0.800  VER 2.500 YM   R  GS 0.800  VER 2.500 YM
```

Figure IV.1. Roughness analysis of sample Ti-6Al-4V number 24



```
Neu im Programm: PERTHOMETER S4P

Mahr
Perthen                  GMBH                   A14
                         BRAUWEG 38
LT      5.600 MM         D-37073 GOETTINGEN
LM      4.000 MM                                MEASUREMENT         5
VB      250.0 YM                                T9 FOCODYN    250 CAL

P                   VER 2.500 YM    HOR 0.800 MM

LC   GS     0.800 MM      1    2    3    4%ADK      20   40   60   80%MRK
RA           0.40 YM
RZ           3.91 YM
RMAX         4.67 YM
LC   SF     0.800 MM
RK           1.13 YM
LC   GS     0.800 MM
RP           1.79 YM
                          P         VER 2.500 YM    P         VER 2.500 YM
```

Figure IV.2. Roughness analysis of sample Dental Amalgam number 14