

# Evaluation of the corrosion resistance of ear piercing studs in a culture medium by electrochemical impedance spectroscopy

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## Abstract

In this investigation, electrochemical impedance spectroscopy (EIS) was used to compare the corrosion resistance presented by two gold-coated commercial studs used for ear piercing, and produced using nickel containing substrate alloys, with that exhibited by a laboratory-made titanium stud in a cell culture medium. The aim was to evaluate Ti as a potential material for the manufacture of ear piercing studs. Scanning electron microscopy examination of the coating defects on the two commercial studs, both before and after their exposure to the culture medium, have shown the establishment of a corrosion process. Instrumental neutron activation analysis results of the culture medium after 10 days of contact with the commercial studs have shown the presence of nickel among the alloying elements leached out from the surfaces of the studs in spite of the high corrosion resistance exhibited by one of them. EIS has been demonstrated to be a sensitive technique, not only to rank the corrosion resistance of the studs, but also to detect some features of the corrosion process, like pore blocking and oxide layer structure. From the results it was concluded that Ti is a candidate material for ear piercing studs due not only to its high corrosion resistance, but also to its non-cytotoxicity and the absence of nickel in the stud composition.

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**Keywords:** Corrosion; Ear piercing studs; Ni containing alloys; Titanium; Electrochemical impedance spectroscopy

## 1. Introduction

Stainless steels, particularly AISI 316L, are widely used as substrates for gold-coated jewellery and ear studs. These materials have adequate physical and mechanical properties and can be moulded into a variety of shapes and sizes. Other metals, such as nickel containing copper–zinc alloys, have also been used as the substrate material of gold-coated jewellery [1]. One of the main limitations to the clinical use of these substrate materials is their tendency to corrode in the presence of chloride ions present in body fluids [2]. To avoid contact between the substrate and body fluids

while the pierced earlobes are healing, the coating on these studs should be adherent and defect free. However, industrial coating processes seldom produce defect free coatings, and corrosion of the substrate at these sites causes metal ions to be released.

Previous results have shown that nickel and zinc leached out from the substrate into a cell culture medium (CCM) in which the studs were immersed [3]. The former metal is the main cause of allergic contact dermatitis caused by Ni<sup>2+</sup> ions binding to carrier proteins, forming a nickel–protein complex that activates immune reactions [4]. Nickel sensitisation can result in this manner, from using such studs for piercing ears [5–9].

The high incidence of contact allergy to nickel is due not only to the sensitisation capability, but also to frequent exposure to various nickel products. It is well known that skin reactions do not occur if the amount

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released into the body fluids does not exceed the elicitation concentration of the chemical in the person [5].

Nickel contact dermatitis is a common type of allergic contact eczema closely related to ear piercing. It is hence important to consider other materials as substrate material for ear piercing studs.

Since the beginning of the 1960s Ti has been investigated as a material for surgical implants [10], and it is now largely used in this field. Among the main properties exhibited by this metal are its biocompatibility and high corrosion resistance, this latter property due to the presence of an oxide layer. A great number of electrochemical investigations have been performed with Ti and its alloys in media which mimic human fluids [10–12], but only a few investigations used the medium adopted in this work [13].

This paper reports the results on the corrosion resistance of (i) two commercial gold-coated ear piercing studs, one with a copper–zinc based alloy substrate (Pf) and the other with a stainless steel substrate (St) and (ii) a titanium stud, in a medium for cell culture, as a function of time. The corrosion performance was determined by electrochemical impedance spectroscopy (EIS). Titanium was evaluated because of its biocompatibility. Despite the high cost of Ti, the amount of material used in the fabrication of studs is small, thus making it a potential material, even economically. Scanning electron microscope (SEM) analyses of the stud surfaces were performed before and after their exposure to the CCM. Instrumental neutron activation analysis (INAA) was used to determine the CCM composition after 10 days of contact with the studs.

## 2. Experimental

Three types of specimens were investigated: two commercial ear-piercing studs (gold-coated austenitic stainless steel (St) and gold-coated copper–zinc alloy containing Ni (Pf)) and a laboratory-made titanium (Ti) stud. The compositions of the materials used as substrates for the gold-coated and for the titanium studs were determined by INAA. The results are presented in Table 1. In these analyses, the compositions of the studs were determined after removing the gold coating since the high gamma activity of  $^{198}\text{Au}$  causes interference in the measurements of other radioisotopes. The gold-free substrates were obtained by removing the gold coatings with a solution of hydrochloric acid and then they were washed using distilled water. In this procedure the ear piercing studs were taken out of the acid solution when the yellow colour of the gold was no longer present. Fig. 1 shows one of the studs used in this study where the area exposed to CCM is indicated.

### 2.1. Electrochemical impedance spectroscopy

The corrosion performance of the three types of studs was investigated by means of EIS in a CCM (Minimum Eagle Medium), designated as CCM. EIS was carried out using a Solartron Model SI 1255 frequency response analyser coupled to a Princeton Applied Research (PARC) Model 273A potentiostat/galvanostat and controlled by electrochemical impedance software PAR model 398. The measurements were carried out on a periodic basis during 10 days of immersion of the studs in the CCM. Experiments were performed at room temperature, and in aerated conditions. All diagrams were obtained in potentiostatic mode and at the corrosion potential,  $E_{\text{corr}}$ , with a voltage perturbation amplitude of 10 mV in the frequency range from 50 kHz to 3 mHz, with 10 points per decade. A three-electrode cell arrangement was used for the EIS tests, with a graphite rod, a saturated calomel electrode, and cold resin mounted studs as counter, reference and working electrodes, respectively.

Before the EIS experiments, the studs were mounted in an epoxy resin leaving only their stem exposed to the culture medium, since it is this part which contacts the body fluids during puncture healing. The specimens were immersed in the culture medium for 10 days, time sufficient for healing of the punctures caused by ear piercing.

### 2.2. Scanning electron microscopy and energy dispersive spectroscopy

The surface characteristics of the studs were examined in a SEM and analysed by energy dispersive spectroscopy (EDS), before and after 10 days of immersion in the culture medium. The surfaces of the studs were scanned for defects in the coating, which could expose the substrate, and for the presence of corrosion products.

### 2.3. Chemical analysis

Twelve pairs of each kind of stud were exposed to MEM–FCS (Minimal Eagle Medium supplemented with 10% fetal calf serum (FCS) and 1% penicillin–streptomycin solution) at 37 °C for 10 days. After this period, part of the culture medium was taken for cytotoxicity assay and another part for chemical analysis. The latter was carried out by INAA, according to a procedure described in a previous paper [3]. A blank of the culture medium was also analysed.

### 2.4. Cytotoxicity test

The cytotoxicity assay was carried out according to Rogero et al. [1] and the International Standards

Table 1  
Elemental composition of ear piercing studs obtained by instrumental neutron activation analysis

Element	Substrate of Pf ear piercing studs	Substrate of St ear piercing studs	Ti studs
As, wt.%	0.00081 ± 0.00005	0.00574 ± 0.00008	0.00177 ± 0.00003
Co, wt.%	0.00274 ± 0.00004	0.2203 ± 0.0011	≤ 0.0001
Cr, wt.%	0.0070 ± 0.0005	16.1 ± 0.2	0.0096 ± 0.0001
Cu, wt.%	36.5 ± 1.2	0.35 ± 0.01	≤ 0.04
Fe, wt.%	9.0 ± 0.2	67.9 ± 0.2	≤ 0.04
Mn, wt.%	2.34 ± 0.07	1.81 ± 0.02	0.0007 ± 0.0002
Mo, wt.%	≤ 0.2 <sup>a</sup>	0.394 ± 0.002	≤ 0.0003
Ni, wt.%	6.80 ± 0.07	7.86 ± 0.07	0.009 ± 0.001
Ti, wt.%	≤ 0.23	≤ 0.23	97.4 ± 6.4
V, wt.%	≤ 0.0077	0.0987 ± 0.0026	0.0033 ± 0.0003
Zn, wt.%	36.4 ± 3.3	≤ 0.7	≤ 0.002

<sup>a</sup> For the elements not detected, the detection limit values were evaluated according to Currie [14].

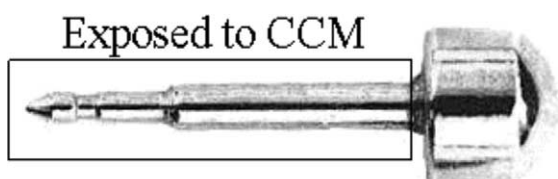


Fig. 1. Photograph of an ear piercing stud showing the area exposed to the test medium.

Organization (ISO) [15]. Chinese hamster ovary cell cultures (ATCC CHO K1) were used in this investigation. Phenol solution (0.02%) and high purity titanium were used as the positive and the negative control, respectively. The surface area of titanium exposed to MEM–FCS was 60 cm<sup>2</sup> and the volume of MEM–FCS used was 60 ml.

### 3. Results and discussion

Impedance diagrams were obtained periodically during the 10 days exposure period of the three studs to the culture medium. Fig. 2 shows the diagrams obtained after only one day of immersion. The responses suggest the existence of two time constants for the Pf and Ti studs, whereas only one time constant is present in the St stud diagram. From the EIS results presented in Fig. 2 it is evident that there are significant differences in the electrochemical behaviour of the three types of studs.

#### 3.1. Pf stud

The Nyquist plot corresponding to the Pf stud presented in Fig. 2(a) shows the presence of two capacitive loops. The high frequency (HF) one is very depressed and offset from the real axis, departing from typical R–C behaviour. This is likely to be due to the porosity in the gold coating with the corrosion products precipitated therein, as discussed below.

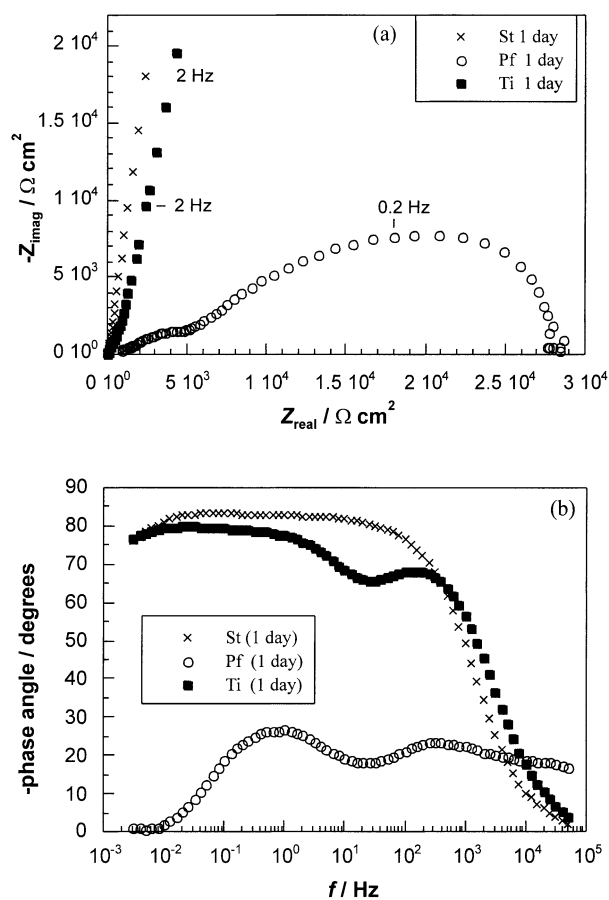


Fig. 2. Impedance spectra of St, Pf and Ti studs after 1 day of immersion in the culture medium (a) Nyquist plots, (b) Bode-phase plots.

Fraturet et al. [16], modelling the behaviour of the cast iron/drinking water system have reported the existence of a depressed capacitive loop in the HF range. This was attributed to a compact microporous layer of green rust, originating from a process controlled by diffusion of the reagents. Fig. 3 presents an SEM micrograph of a Pf electrode after 10 days exposure to the CCM where

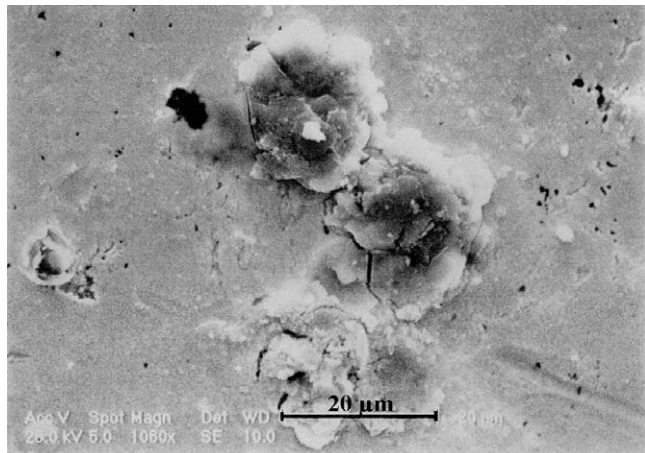


Fig. 3. Cracked corrosion products on surface leading to exposure of the substrate.

porosity can be seen in the coating, together with heavy corrosion products. So, even though, the system studied here is very different from that reported by Frateur et al. [16], the similarity between the structures of both top layers, and between the loop shapes, justifies the analogies used to explain the origin of the HF loop.

The HF loop is followed by a short, medium frequency (MF) diffusion controlled region, probably due to electrolyte penetration in the precipitate filled coating pores and by another capacitive loop at low frequency (LF). This latter loop presents some dispersion in the very LF region, which was ascribed to the onset of active corrosion at the electrolyte | Pf alloy interface. Diagrams obtained after 2 days of exposure of the Pf stud to the electrolyte, not presented here, showed a slight decrease of the LF loop diameter, indicating a diminution in the corrosion resistance of the substrate.

Fig. 4 presents Bode (a) and Nyquist (b) diagrams for a Pf stud after 1, 3, 4, 6 and 10 days of exposure to the culture medium. In this figure, an increase in the impedance modulus between the first and the sixth day is visible, followed by a decrease between the sixth and the tenth day. Nyquist diagrams, Fig. 4(b), also clearly show a 45° diffusion controlled region in the MF range. Observation of the Bode phase angle diagram, Fig. 4(a), shows a complex evolution of the interfacial phenomena in these electrodes.

Bode diagrams presented in Fig. 4(a) show that upon increasing the immersion period from 1 to 3 days, the peak in the LF range of the Bode-phase plot broadened and the capacitive characteristic of this time constant is also increased. This can be related to the precipitation of corrosion products inside the pores, blocking them and, somehow, hindering the corrosion process, with a consequent increase of the impedance modulus. After the fourth day of immersion, this time constant is clearly separated into two; with this feature becoming more clearly defined the longer the immersion time. Never-

theless, as the two time constants become more separated, there is a shift of the maximum phase angle to higher frequencies, indicating the onset of a faster process. Moreover, the diminution of the capacitive characteristics of the LF phase angle for 10 days of immersion points towards a well established corrosion process for longer immersion periods.

As already stated, the medium to LF behaviour of the impedance was attributed to diffusion phenomena inside the precipitate filled pores, together with the corrosion process therein. In Fig. 3 the presence of a cracked corrosion product inside the pores is evident. The joint analysis of Figs. 3 and 4 can help to picture a mechanism to explain the observed impedance behaviour of the Pf studs, which is presented below.

As soon as the studs are immersed in the CCM the corrosion process starts through the coating defects. However, precipitation of corrosion products taking place inside the pores causes a progressive blockage of these defects hindering the corrosion process inside them, explaining thus the increase in impedance values verified between the first and the sixth day of immersion, as well as the displacement to lower frequency of the LF phase angle peak. However, at the same time, hydration and/or cracking of voluminous corrosion

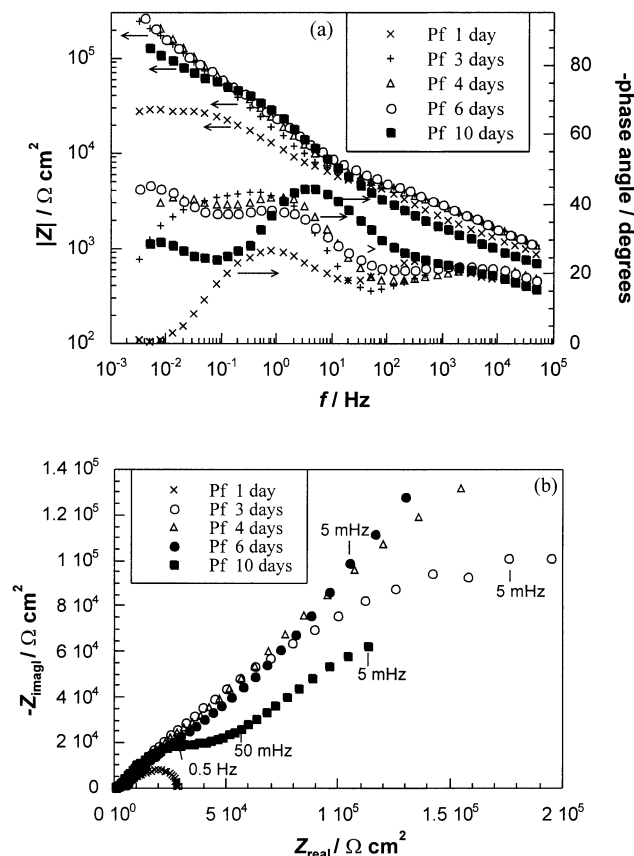


Fig. 4. (a) Bode (phase angle and impedance modulus) and (b) Nyquist plots of Pf stud after 1, 3, 4, 6 and 10 days of test.

products inside the pores facilitates diffusion phenomena and displaces the MF time constant, attributed to this phenomenon, to higher frequencies.

Upon increasing the exposure time further, from 6 to 10 days, the diminution of the phase angle associated with the LF time constant, together with the displacement of its maximum to higher frequency, indicates an increase in the corrosion activity at the substrate | electrolyte interface beneath the corrosion products. Moreover, the phase angle associated with the diffusion phenomenon is further displaced to higher frequency, indicating faster diffusion processes, which in turn would enhance the corrosion reaction. The exposure of new areas of the substrate to the electrolyte, for instance, caused by cracks in the coating and/or corrosion products, as shown in Fig. 3, could be responsible for this overall behaviour.

The changes verified in the HF part of the impedance diagrams, attributed to the precipitates formed within the pores, further confirm the mechanism proposed above. Observation of Fig. 4 also shows that from the first to the sixth day of immersion there is an increase in the HF phase angle, indicating a more effective blocking effect of the corrosion products precipitated within the pores. On the other hand, between the sixth and the tenth day of immersion this phase angle decreases slightly and this feature can be attributed to the appearance of heavy cracks in the precipitates, as verified in Fig. 3, allowing the establishment of faster corrosion processes, as already discussed.

### 3.2. St stud

Impedance spectra of an St stud for different exposure periods are shown in Fig. 5. The wide peak with a maximum near  $90^\circ$  suggests the presence of a compact coating layer on the surface acting as a barrier. No significant changes in the impedance of the St stud occurred over the entire frequency range, during the 10 days of the test. The slight change in the LF range probably corresponds to a small increase in the corrosion resistance, from 1 to 3 days, due to the formation of adherent corrosion products at small defects/pores in the coating. For longer exposure periods the impedance response did not change significantly, and a high corrosion resistance was maintained until the end of the test. It is important to remember that the substrate of the St stud was stainless steel which itself has good corrosion resistance in the test medium. Moreover, SEM images of these stud surfaces have shown the presence of almost no defects in the coating. So the capacitive behaviour observed during the whole frequency range can be ascribed either to the gold coating alone or to the gold coating in parallel with the St substrates, in the former case, defects would play no role in the impedance response. The results of chemical analysis by INAA,

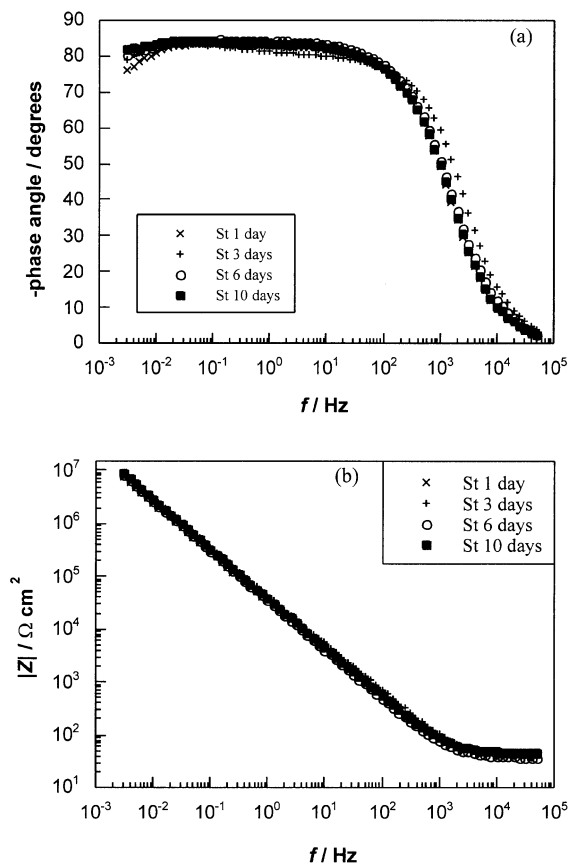


Fig. 5. Bode plots corresponding to an St stud at increasing exposure times. (a) Phase angle and (b) impedance modulus plots.

which detected Fe ions in the CCM, supports the parallel model.

### 3.3. Ti stud

The effect of increasing immersion time on the impedance response of Ti studs is shown in Fig. 6. Two time constants are evident in all the diagrams. The LF limit of the impedance modulus decreases from 1 to 6 days, and then seems to stabilise for longer immersion periods.

For Ti studs, the major changes in the impedance response occur in the HF range, with the phase angle maximum decreasing with exposure time to the culture medium. It has been published in the literature that Ti oxide is composed of a two-layer structure, one outer porous layer on the top of an inner barrier layer [11,12]. In Fig. 6, the LF time constant is probably due to the latter, while the HF time constant can be ascribed to the former layer. The small decrease in the LF limit of the impedance modulus can be attributed to an increase in the number of defects in the structure of the oxide barrier layer due to the corrosion of the substrate. On the other hand, the evolution of the HF phase angle, which decreases with immersion time, can be ascribed to

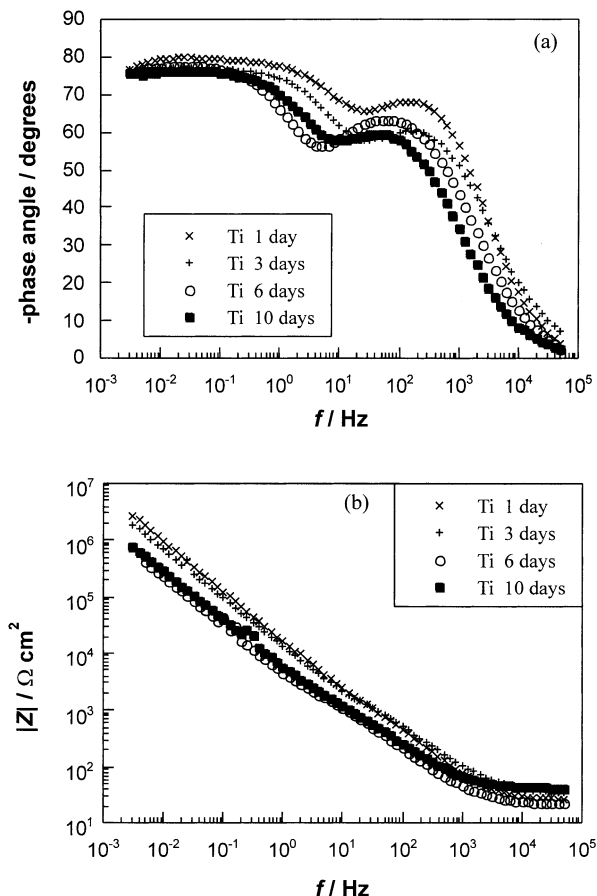


Fig. 6. Bode-phase plots of a Ti stud showing the effect of increasing time of immersion in the culture medium.

the thickening of the porous outer layer as will be discussed in the following paragraphs.

In the diagrams presented in Fig. 6, no sign of pore blocking due to precipitation of corrosion products was noticed, since this probably would cause an increase in the LF limit of the impedance [12]. So, due to this fact, the HF time constant is more likely to be associated with the depth penetration of the perturbation sign inside the pores, as proposed by Song et al. [17]. Similar HF behaviour was reported by Saliba-Silva [18] and Aoki et al. [19], for the corrosion behaviour of phosphate-coated magnets [18] and for pure aluminium [19], which were also explained using the Song model [17].

Similar Bode plots diagrams to those presented in Fig. 6, with two time constants, were reported by Mansfeld and Kendig [20] for anodised aluminium in 0.5 M NaCl. In their work, Mansfeld and Kendig [20] verified an increase in the HF capacitive loop phase angle due to the sealing of the pores with oxide/hydroxide. Pan et al. [12] have also reported the sealing of the pores in the outer layer of Ti oxide after long-term exposure (more than 15 days) to a phosphate buffered solution in the presence of  $\text{H}_2\text{O}_2$ , which proved to be aggressive to the metal. Using XPS, these authors [12] have found the

presence of some hydrates/precipitates inside the porous oxide film. For the Ti studs investigated in this work, these explanations are not likely, since neither no sealing procedure was adopted, nor were heavy corrosion products detected at the end of the exposure period, as was proved by SEM analysis. Moreover, the decrease of the HF phase angle with immersion time verified in the experimental results is not compatible with these two phenomena, so the observed HF behaviour can be more probably attributed to the thickening of the outer porous layer.

For Ti studs, during the whole period, no dc-limit was observed in the Nyquist diagrams, confirming, thus, the high corrosion resistance of the material.

Fig. 7 compares the impedance spectra of the three types of studs after 10 days of immersion in the CCM. The trend observed after 1 day of immersion was maintained during the whole time span of the investigation, and seems to rank the materials for increasing corrosion resistance as follows: Pf, Ti and St.

#### 3.4. Results of chemical analysis by INAA

The results of chemical analysis, as determined by INAA, of the CCM after 10 days of immersion of each stud, as well as that of a blank analysis are presented in Table 2.

For the St substrate, Fe and Ni were detected; indicating that these elements had leached into the culture medium due to corrosion of the substrate, however, the amount of the former element was higher. Ni was also detected in the culture medium where the Pf studs were exposed, and, under normal conditions, these studs could cause nickel sensitisation; nevertheless the element found in the highest concentration for Pf studs was Zn. Cobalt was found in all experimental samples in very low concentrations. Concentration of this element was only slightly higher than that found in the blanks.

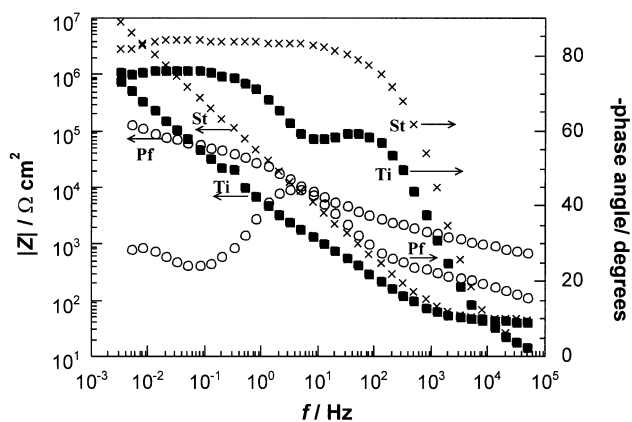


Fig. 7. Bode plots of the three types of stud tested after 10 days of exposure.

Table 2  
Concentration of different elements in the cell culture media and in the blank

Element	Blank	Extract after exposure of studs		
		Pf	St	Ti
Co, $\mu\text{g/ml}$	$0.0121 \pm 1.2$	$0.0616 \pm 0.0005$	$0.099 \pm 0.0010$	$0.0538 \pm 0.0021$
Cr, $\mu\text{g/ml}$	$0.72 \pm 0.02$	$0.73 \pm 0.04$	$0.73 \pm 0.03$	$0.75 \pm 0.03$
Fe, $\mu\text{g/ml}$	$0.60 \pm 0.09$	$0.61 \pm 0.08$	$4.03 \pm 0.31$	$0.43 \pm 0.11$
Ni, $\mu\text{g/ml}$	ND <sup>a</sup>	$0.96 \pm 0.09$	$0.66 \pm 0.06$	ND
Zn, $\mu\text{g/ml}$	$0.58 \pm 0.05$	$3.84 \pm 0.41$	$0.61 \pm 0.01$	$0.75 \pm 0.10$

<sup>a</sup> ND, not detected.

In the culture medium where Ti studs were exposed, the elements detected were of the same order of magnitude as that in the blanks, except for Ti, reflecting a slight corrosion of this stud, as already supposed by the results of impedance measurements.

Copper was not analysed in the solutions by INAA, due to the interference of Na, present in high concentration in these samples. The high activity of <sup>24</sup>Na masks the photopeak of <sup>64</sup>Cu in the gamma spectrum. For all the analyses, Cr levels were close to that observed in the blank solution.

### 3.5. SEM and EDS analysis

SEM examination of the surfaces of Pf studs before the corrosion test revealed many defects in the coating, Fig. 8(a), and after 10 days of immersion, corrosion products were seen at these sites, Fig. 8(b). EDS analysis of the corroded area revealed significant amounts of phosphorus and zinc, due to precipitation of zinc phosphate at defects in the coating. Zinc forms insoluble complexes with phosphates [21], and this latter species are present in the culture medium. The precipitation of zinc phosphates inside the pores/defects would be responsible for the increase in the impedance values verified for these studs during the first days of immersion.

Other ionic species and elements from the culture medium, such as chlorides, calcium and sodium, were also detected in the corroded area of Pf studs. The presence of aggressive ions such as chlorides, in these areas, suggests that these ions may have initiated corrosion. Leaching of zinc into the culture medium near the substrate interface, with the formation of insoluble phosphates that block the defects, possibly followed this process.

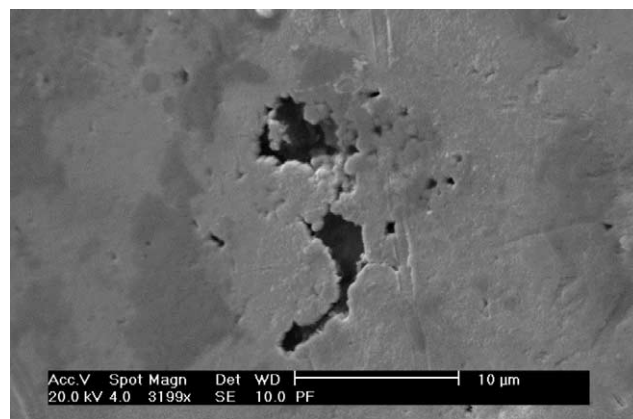
The surfaces of the St studs, observed by SEM, revealed fewer defects before exposure to the culture medium. In addition, they were almost unaffected after 10 days of exposure, confirming the high corrosion performance indicated by the EIS results. Oxides were seen on the Ti stud surface, in regions affected by the

machining process. However, most of the Ti surface had a metallic shine even after 10 days of immersion.

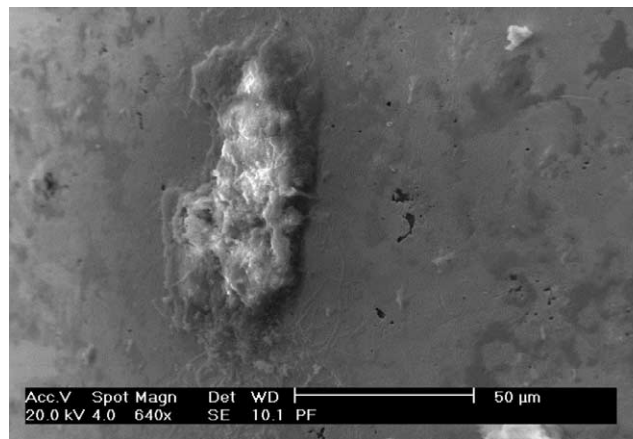
### 3.6. Cytotoxicity test

The cytotoxic potential can be quantitatively expressed as IC<sub>50(%)</sub> (cytotoxicity index), determined by plotting the percentage of the colony number in relation to CHO cell control and the concentration of the extract. IC<sub>50(%)</sub> is the concentration of the extract, which suppresses colony formation to 50% of the control value. St and Pf commercial studs showed cytotoxicity, presenting IC<sub>50(%)</sub> = 78 and 44, respectively. On the other hand, the extract obtained from the Ti studs was not cytotoxic.

The concentration of Ni in CCM was one of the highest, among the different elements leached from the PF stud. A cytotoxicity test was therefore carried out, to determine whether Ni at such levels was cytotoxic and it was not found to be so, even though nickel is known to have toxic effects in cell cultures and in tissues [2].



(a)



(b)

Fig. 8. (a) SEM of a Pf stud surface showing defects in the coating, (b) SEM of corrosion products at defects in the gold coating.

#### 4. Conclusions

The corrosion behaviour of two commercial gold-coated studs and of a laboratory-made Ti stud was compared using EIS. For the commercial studs the results revealed a strong dependence of their electrochemical behaviour on the quality of the gold coating, as well as on the substrate material, with St (gold-coated stainless steel) studs presenting a much better performance than Pf (gold-coated copper based alloy) studs. Ti studs presented good corrosion behaviour, even though their performance was inferior to that presented by St studs.

Analysis of impedance diagram shapes together with SEM observation led us to propose a physical model to explain the corrosion behaviour of the materials. For Pf studs the complex impedance behaviour observed was attributed to the precipitation of corrosion products inside the coating pores, which, in their turn, impose a diffusion control to the species participating at the electrochemical reactions. On the other hand, for Ti studs, the thickening of the outer oxide porous layer can be envisaged as responsible for the observed impedance response. No modification of impedance response with time was verified for St studs due to the coating quality and also to the good corrosion resistance of stainless steel to the test media.

Results of chemical analysis by INAA have shown that Pf stud had higher cytotoxicity and more Ni was leached into the medium in which they were immersed, relative to the St stud. Despite the lower Ni content in the extracts when St studs were immersed, its presence indicates that the gold coating process needs careful control to avoid defects in the coating.

Cytotoxicity results for Ti studs were negative, and, as so far, Ti is not known to cause allergic reactions, it is therefore a candidate material for making piercing studs.

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