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Corrosion Analysis of AISI 430 Stainless Steel in the presence of *Escherichia coli* and *Staphylococcus aureus*

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Abstract

AISI 430 stainless steel is an attractive material to be used in the healthcare industry, particularly as a sensor due to its low cost, corrosion resistance, as well as being Ni-free. AISI 430 was evaluated in an artificial sweat solution with the presence of *Escherichia coli*, and *Staphylococcus aureus*. Surface microbial analyses did not reveal colonization of bacteria on metallic surfaces, even when bacteria adhesion was investigated in a Mueller-Hinton solution. However, by electrochemical techniques, the AISI 430 surfaces demonstrated clear signs of corrosion mainly in a sterile medium after two weeks of exposure.

Key-words: *Escherichia coli*; *Staphylococcus aureus*; Stainless Steel; AISI 430; Corrosion.

1. Introduction

AISI 430 is ferritic stainless steel, which has been utilized in both the food [1] and medical industry [2] since it is relatively cheaper when compared to austenitic alloys as AISI 304 [3], as well as having an appropriated corrosion resistance and high sensitivity to chlorides [3]. Moreover, it is also essentially Ni-free and does not produce allergies [4], making it a valuable material for use in contact with the skin. Therefore, its features are appropriately used on door handles, handrails, medical devices [2,5,6], and in particular, as a sensor for early diagnosis for type 2 diabetes by electrochemical skin conductance (ESC). This is a simple electrophysiological method used in the functional evaluation of the sweat gland that probes sudomotor/small fiber dysfunction [4]. In this case, small fiber nerves lacking myelinated axons produce a durable shift in the ionic balance and pH value of sweat conducts [3,7,8]. Indeed, it has been reported that the pH value of the sweat can vary from 4.5 to 7.0 [9–11] due to the damage in the glands [7], sweat-rate [12], and glucose concentration [13].

In previous investigations, stainless steels have been analyzed as new biocompatible sensors [3,4,14]. However, the behavior of stainless steels, such AISI 430 surface, has never been adequately determined neither under sweat conditions nor with bacterial adhesion. So far what is known is that *Escherichia coli* is frequently present in human hands and feet [15], while *Staphylococcus aureus* is typically transmitted during hospital stays, since they are able to adhere to surfaces due to cell adhesion molecules being closely located around the outer part of the bacterial cell wall; they may even adhere to several types of surfaces, that include medical equipment [16]. The latter is substantially important due to the damage that bacterial colonization can cause, for instance, biocorrosion (MIC) [17,18].

The main goal of this investigation is to analyze the influence of *Escherichia coli* and *Staphylococcus aureus* bacteria on the corrosion behavior of AISI 430 surface after exposure to acidic and neutral artificial sweat solutions through the use of surface analysis and electrochemical techniques, specifically the bacterial growth on the surface and its effect on the corrosion rate of the steel.

2. Experimental

2.1 Sample and solutions

The material studied was a stainless steel AISI 430 (Good Fellow) plate with the following chemical composition (in wt.%): 0.12 max. % C, 1 max. % Mn, 0.004 max. P, 0.030 max. % S, 1 max. % Si, 16-18% Cr, 0.50 max. % Ni, balance Fe. The AISI 430 samples were used as received, which have been polished according to ASTM A480 N°8 to achieve mirror surface, in order to minimize the surface imperfection effects on the electrochemical response. Before exposure, the samples were cleaned using bi-distilled water, and dried under airflow.

In this work, the artificial sweat solution (ASS) was prepared under ISO-3160-2 protocol, which contains (in g · L⁻¹): 20 sodium chloride, 17.5 ammonium chloride, 4.76 acetic acid, 15 2 hydroxy-propanoic acid. The solution was made using distilled water, with the pH being set at 4.7 and 6.5 by using 0.1M sodium hydroxide solution. Notice that the acid solution corresponds to the case when the peripheral neuropathy is early developed in patients with diabetes [19], producing a durable shift in the ionic balance and pH value of sweat conducts [3,7–11]. In this condition, glands [7], sweat-rate [12], and glucose concentration [13] can be strongly damaged, therefore, **analyzing the metal surfaces that can be in contact with the sweat is** very interesting from a corrosion point of view. Additionally, the ASS solution was inoculated with an active inoculum of *Escherichia coli* BW25113 and *Staphylococcus aureus* BAA977, separately, which are frequently found on the human skin.

2.2 Microbiological characterization

E. coli and *S. aureus* were grown in a nutritive Mueller-Hinton medium containing (mg · L⁻¹): 2 beef extract, 17.5 casein acid hydrolysate, 1.5 starch, 17.0 agar, along with 1000 mL of distilled water at 37 °C overnight. Bacterial growth was controlled by direct Petri dish counting and 96-well plate growth curves under shaking conditions in a Multiskan FC (Thermo) equipment. Bacterial growth was measured at 600 nm. Tests for bacteria adhesion

were performed by dipping a sample of 1 cm² of AISI 430 into a stock of *E. coli* and *S. aureus* (10⁶ cells per milliliter) in different solutions, using Mueller-Hinton (MH) broth as a positive control and an artificial sweat solution (ASS) without a nutritional agent, with 0.5 wt.% glucose (G), 1.0 wt.% glucose, 0.5 wt.% lactose (L), 1.0 wt.% lactose, respectively, at pH 4.7. Additionally, the artificial sweat solution was set at pH: 7.0, 6.5, 6.0, and 5.5.

The metallic samples were incubated at 37 °C during 24 hours in the presence of each type of bacteria, under convective conditions. A 0.01 M sodium cacodylate/0.15 M NaCl buffer with a 7.0 pH was used to rinse the samples twice, with them later being fixed with 1% glutaraldehyde at 23± 1 °C for 2 hours. Samples were then dehydrated by using ascending percentages of ethanol (30%, 50%, 70%, 80%, 90%, and 100%), and later coated with a thin film of Au, all done before being observed by FE-SEM [20].

2.3 Surface analysis

The surface of AISI 430 samples was cleaned with bi-distilled water and alcohol before to be characterized by field-emission scanning electron microscopy (FE-SEM) model QUANTA 250 FEG that was equipped with an energy dispersion spectrometer. This was carried out prior to and after 14 days of exposure. Additionally, the chemical compositions of samples were analyzed in depth, before and after exposure to sterile and inoculated artificial sweat solution, by using a Glow-Discharge Optical Emission Spectroscopy (GD-OES) model Spectrums Analytik GmbH GDA 750 HR, with a 2.5 mm diameter anode that operates in DC excitation mode. The analysis was carried out in an Ar atmosphere (5.0 quality) with an average discharge pressure of 5 x 10⁻² hPa, by using the following excitation parameters: 1000 V, 12 mA and 0.1 µm·min⁻¹ sputtering rate [21]. All samples were measured in triplicate. In this context, an aliquot of 50 µL of each bacteria solution, standardized to 10⁶ CFU·mL⁻¹, was deposited on the AISI 430 surface and incubated for 24 h at 37 °C. The metal samples were then stained with a live/dead® kit (marking live bacteria in green and dead bacteria in red) and left alone for 15 minutes. Next, samples were washed by applying a 0.85 wt.% NaCl solution in order to remove the stain surplus. Moreover, the

1 samples were placed on the slide, wrapped with a coverslip, and as well as sealed by tape to
2 allow later observation in a Carl Zeiss LSM 700 confocal microscope.

3 4 2.4 Electrochemical measurements

5
6 Electrochemical techniques were performed utilizing a potentiostat/galvanostat (Bio-Logic,
7 VSP) and a three-electrode electrochemical cell with a Pt grid and a saturated calomel
8 electrode (SCE) employed as the counter and reference electrodes, respectively. AISI 430
9 samples were utilized as the working electrode with a geometrical area of 12 cm², which
10 were immersed during 14 days to ASS sterile and inoculated, at 37 °C. Chronopotentiometry
11 at open circuit was performed, allowing electrochemical impedance spectroscopy (EIS)
12 measurements after reaching a stable open circuit potential (OCP). A frequency range from
13 10 kHz to 10 mHz was scanned, with seven points per decade, and impedance data were
14 collected using a 10 mV peak-to-peak sinusoidal voltage at $E = \text{OCP}$. The EIS data was then
15 fitted using SIMAD (LISE UPR 15 CNRS, France) after performing Kramer-Krönig tests, in
16 order to determine the frequency range to analyze [22,23]. Linear sweep voltammetry (LSV)
17 was also performed at $E = \text{OCP} \pm 10 \text{ mV}$ vs. SCE by using a sweep rate of $0.05 \text{ mV} \cdot \text{s}^{-1}$. OCP,
18 EIS, and LSV were collected after 1, 3, 7, and 14 days of exposure. All samples were
19 electrochemically measured in duplicate.

20 21 **3. Results and discussion**

22 23 3.1 Microbial growth in simulated sweat solution

24
25 A microbial growth analysis of *E. coli* and *S. aureus* on AISI 430 was carried out in both
26 Müller-Hinton medium (MH) and artificial sweat solution (ASS). Figure 1 shows a dramatic
27 increase in the optical density of *S. aureus* and *E. coli* in the Müller-Hinton medium when
28 exposed for short times, followed by a plateau close to 0.35 and 0.4 after a 10 h exposure,
29 respectively, and a rapid rise after 18 h, reaching a maximum growth near 0.55 after 22 h.
30 However, bacteria did not grow in inoculated ASS, even when glucose and lactose were
31 added as nutritional agents (see Figure S1 in supplementary material). Indeed, the optical

density only increased slightly after 13 h of exposure in an ASS with a neutral pH value. It should be mentioned that bacterial adhesion and colonization correspond to the earlier stages in a biofilm formation, and even though those processes do not take place, the metabiological activity may provoke biocorrosion [24,25][26].

Figure 1: Growth kinetic curves of (a) *S. aureus* and (b) *E. coli* in (■)MH medium at pH 7.4 and ASS solutions at pH (□) 7.0, (▲) 6.5, (▼) 6.0, (●) 5.5, (○) 4.7.

3.2 Surface analysis

Figure 2 shows confocal images of the AISI 430 surface after 24 hours of exposure to *E. coli* and *S. aureus* in different growth conditions. For both bacterial strains, no adhesion or biofilm formation was observed on the AISI 430 surface. FE-SEM images after 24 hours of exposure to various growth solutions for *E. coli*. Even in the presence of a MH nutrient, both microorganisms were rarely detected, as also seen in Figures S2 and S3. The high chromium content in the AISI 430 steel alloy, and its high toxicity, could explain the extremely low colonization of the microorganism on these types of surfaces [27]. The low presence of bacteria on the metal surface can indicate that the effect of microbially influenced corrosion (MIC) could be ruled out with this type of alloy, which will be later discussed.

Figure 2: Confocal images of AISI 430 after 24 h exposure to (a,b) MH at pH 7.4 and (c-f) ASS media inoculated with *S. aureus* and *E. coli* at pH (c,d) 4.7 and (e,f) 6.5.

FE-SEM images of AISI 430 prior and after 1 day of exposure to ASS sterilized and inoculated at pH 4.7, are presented in Figure 3, revealing some polished defects (aligned lines) and imperfection before exposure (see Figure 3(a)), and some damage related to exposure mainly in inoculated media (see Figure 3(b-d)), which can be associated with the release of the metabolic products, although only some bacteria could be observed due to the cleaning surface treatment carried out after exposure.

Figure 3. FE-SEM images of AISI 430 (a) prior to and after 14 days of exposure to ASS (b) sterilized and inoculated with (c) *S. aureus* and (d) *E. coli* at pH 4.7.

It has been reported that the adhesion and colonization of *E. coli* on metal surfaces produce changes in the interface due to its metabolic products. For instance, Baeza [28] reported how the catalase activity of *E. coli* modifies the oxygen uptake after the colonization on stainless steel. The enzymatic action of catalase promotes an increase in the cathodic current and,

consequently, an increase in the overall corrosion process. It has also been found that the *S. aureus* colonization can produce several metabolic products, where the main one is polysaccharide intercellular adhesin (PIA) [29]. This type of compound acts as an active barrier for the metal [30]. However, after the biofilm establishment, *S. aureus* produces exoenzymes and surfactants, such as protease that corresponds to a type of hydrolase [31], and the hydrolase can promote the oxygen concentration at the metal interface, influencing the local pH, and therefore, a modification of the corrosion rate. On the other hand, FE-SEM analysis also revealed that the worst surface damage occurred with the destruction of passive oxide film after 14 days exposed in sterilized medium at pH 4.7, as shown in Figure 4. Similar surface damage has been reported previously by Sekine *et al.* [32] and Pistorius [33], who studied the same stainless steel but exposed to an acid solution with pH=2 and 2.5, respectively.

Figure 4. FE-SEM images of AISI 430 after 14 days of exposure to ASS sterilized at pH 4.7. (a) 1000x and (b) 5000x

Figure 5 shows the composition profile of AISI 430 determined by GD-OES prior to and after exposure to a sterilized as well as inoculated media at pH 4.7 for 14 days. The composition profile reveals a depletion of the alloying elements until 200 s of abrasion, which increases until 400 s in the inoculated media with *E. coli* and *S. aureus*, and a rise of oxygen content toward the surface. Figure 5(a) insert shows that the oxide layer response was observed until 2.5 s before exposure, decreasing until 1.5 s after 14 days of exposure to the

sterilized solution; therefore, the oxide could be present, but with greater roughness and possibly a different chemical composition that can also influence its protective properties. The inserts in figures c,d for inoculated media show a rising of alloying elements after 15 s, which were five times greater than in the sterilized solution. This can be associated with an oxide thickness increase, as previously reported [34,35]. Furthermore, the oxide film is composed of a bi-layer, an outer film with a Fe-enrichment at the surface, formed by FeOOH and Fe₃O₄, as well as an inner layer with a Cr-enrichment that can be formed by Cr₂O₃ and Cr(OH)₃, as was previously described by Luo *et al.* [36] and Wonneberger *et al.* [37].

Figure 5. Composition profile by GD-OES of AISI 430 (a) prior to and after 14 days of exposure to ASS (b) sterilized and inoculated with (c) *S. aureus* and (d) *E. coli* to at pH 4.7.

3.3. Electrochemical behavior

It is worth mentioning first that the validity of the impedance diagrams was evaluated using Kramers-Kronig relations that showed residuals of real and imaginary parts of the impedance increased mainly in higher and lower frequency domains [38]. Therefore, the frequency range of the impedance data analyzed generally varied between 10^4 to 10^{-2} Hz, except for inoculated media with *S. aureus* at pH 4.7. Figures 6 & 7 show the impedance response for AISI 430 after being exposed to both the sterilized and inoculated ASS with *S. aureus* and *E. coli* at pH 4.7 and pH 6.5, respectively. For all the evaluated solutions, a capacitive behavior was observed, which can be associated with the oxide film formed on the surface, but also with the charge transfer resistance (R_{ct}) related to the cathodic reaction that can be in parallel with the double layer capacitance, therefore, two-time constant could be superposed in the frequency domain studied, as was previously described by Euch *et al.* [39,40]. It should be noted that the characteristic frequency (f_c) is shifted during exposure to higher frequencies in sterilized solution and inoculated medium with *S. aureus*. This suggests that the corrosion process took place [41]. However, f_c was slightly shifted to lower frequencies in inoculated medium with *E. coli*. Additionally, Figures 6 and 7 (b,d,f) show the Bode plots that were corrected by the electrolyte resistance (R_e), as described by Orazem *et al.* [42], revealing a plateau possibly due to a constant phase element behavior, however, the phase was slightly influenced mainly at high frequency (10^2 - 10^4 Hz). This can be associated with the non-uniform primary current distribution in the electrode periphery, as was described by Gharbi *et al.* [43]. But also, this can be also attributed to the difficulty in estimating the electrolyte resistance (R_e) in some solutions. On the other hand, after being immersed in a sterilized solution with pH 4.7 during 14 days, the sample showed corrosion signs, whose constant-time was solely associated with the charge transfer resistance in parallel to the double layer capacitance.

Figures 6 (b) also show in the low-frequency range, the impedance modulus ($|Z|_{LF}$) showed a drastic drop in the sterilized medium after exposure, from $10^5 \Omega \text{ cm}^2$ to $10^2 \Omega \text{ cm}^2$, suggesting the development of the corrosion process with a break on the passive layer and an increase of surface roughness. While in the inoculated medium, the $|Z|_{LF}$ decreased in the presence of *S. aureus* but significantly less than in the sterilized solution, indicating that its metabolic products could act as a barrier to metal release [30]. Conversely, in the presence

of *E. coli* after 14 days, the drop of $|Z|_{LF}$ was stronger than in the presence of the *S. aureus* in both pHs. It could be due to the fact that *E. coli* develops microorganism induced corrosion as demonstrated by Baeza *et al.* and Nan *et al.* [28,44]. Table 1 shows the variation of the $|Z|_{LF}$ for all evaluated systems. At pH 4.7, the phase angle ($-\theta$) decreased significantly from 82° to 45° in the sterile medium, corroborating that the dissolution of the oxide took place. In the inoculated media, $-\theta$ decreased during exposure, yet the fall being less evident. A similar trend exhibited the impedance response of AISI 430 after exposure at pH 6.5, but less significant, as shown in Figure 7.

Figure 6. Nyquist diagrams and **corrected** Bode plots of AISI 430 after (□) 1 and (★) 14 days of exposure to ASS (a,b) sterile and inoculated with (c,d) *S. aureus* and (e,f) *E. coli* at pH 4.7.

Figure 7. Nyquist diagrams and **corrected** Bode plots of AISI 430 after (□) 1 and (★) 14 days of exposure to ASS (a,b) sterile and inoculated with (c,d) *S. aureus* and (e,f) *E. coli* at pH 6.5.

The variation of the imaginary part (Z_{imag}) versus frequency suggests that the experimental impedance data can be related to a constant phase element (CPE) behavior, as shown in Figure S4 in the supplementary material [38], revealing a linear domain at MF and HF, with a slope (α) lower than -1 [38], as can be seen in Table S1 in the supplementary material. The CPE behavior can be associated with the oxide film formed on the metal surface, but also can be related to the charge transfer resistance due to the cathodic reaction at MF domain. In

order to better understand this behavior. Figure 8 shows the normalized resistivity (ρ/ρ_0) as a function of the position (γ/δ) or ξ [23,45] for all systems evaluated, where the resistivity ρ in the oxide/solution and metal/oxide interfaces are represented as ρ_δ and ρ_0 , respectively, while the oxide thickness is δ . It should be noted that ρ/ρ_0 showed a quick drop after 1 day for all media and after 14 days in Ph 4.7, but revealed a more homogeneous oxide layer mainly after 14 days in sterilized solution.

Figure 8. Normalized resistivity as a function of dimensionless position (γ/δ) of AISI 430 after (a,b) 1 and (c,d) 14 days of exposure to ASS (—) sterilized and inoculated media with (•••) *S. aureus* and (---) *E. coli* (a,c) pH 4.7 and (b,d) pH 6.5.

The impedance response related to an oxide film has been described by [45,46]:

$$Z_{oxide} = \int_0^\delta \frac{\rho(\gamma)}{1+j\omega\rho(\gamma)\varepsilon(\gamma)\varepsilon_0} d\gamma \quad (1)$$

Under the assumption of a uniform permittivity, the impedance of the oxide can be obtained as follow:

$$Z_{oxide} = \int_0^\delta \frac{\rho(\gamma)}{1+j\omega\rho(\gamma)\varepsilon\varepsilon_0} d\gamma \quad (2)$$

To better understand the impedance response, CPE parameters related to film properties were estimated using the power-law model (PLM) conforming to:

$$Z(\omega) = g \frac{\delta\rho_\delta^{1-\alpha}}{(\rho_0^{-1}+j\omega\varepsilon\varepsilon_0)^\alpha} \quad (3)$$

where α corresponds to a CPE parameter, which can also be estimated from the slope of the Log-Log Z_{imag} vs. frequency plots (see Figure S5), as was previously described. The vacuum permittivity and the dielectric constant of the oxide layer are represented as ε_0 , which is equal to $8.8542 \times 10^{-14} \text{ F} \cdot \text{cm}^{-1}$, and ε . Additionally, g is a numerical coefficient close to 1 when α is close to 1, given as:

$$g = 1 + 2.88 \gamma^{-2.375} \quad (4)$$

where γ is estimated from the following relation: $\alpha = (\gamma - 1)/\gamma$, and if the $(\rho_0\varepsilon\varepsilon_0)^{-1} < \omega < (\rho_\delta\varepsilon\varepsilon_0)^{-1}$. It should be mentioned that the ρ_0 and ρ_δ were determined in the frequency range where the CPE behavior was observed, taking the lower and upper limits, respectively, which varied between 60-10,000 Hz. Moreover, Q corresponds to a CPE parameter, estimated as:

$$Q = \frac{(\varepsilon\varepsilon_0)^\alpha}{g\delta\rho_\delta^{1-\alpha}} \quad (5)$$

The capacitance of the oxide film was estimated as follow:

$$C_{oxide,PLM} = gQ(\rho_\delta\varepsilon\varepsilon_0)^{1-\alpha} \quad (6)$$

where $C_{oxide,PLM}$ is the capacitance of the oxide film [23,45]. Table 1 summarizes the PLM parameters obtained for all evaluated systems. In the sterile solution, R_e was influenced by the pH, the lowest being at pH 4.7 rather than at pH 6.5, suggesting a higher metal ion concentration at the electrolyte/oxide interface, with the more acidic pH possibly being due

to a corrosion process. The R_e was modified in the presence of *E. coli* and *S. aureus* media, being close to $30 \Omega \cdot \text{cm}^2$. As was reported above, Figure S4 revealed two slopes identified with different values for α . Therefore, two Q coefficients were determined, which can be associated with the double layer capacitance with the oxide layer. In both cases, the Q coefficients increased slightly at pH 4.7, and any trend was observed at pH 6.5. It should be noted that the Q coefficient at HF range risen in inoculated medium with *E. coli* between 7 and 14 days of exposure, which can be associated with a modification of the passive film properties [47], that was named as Q_{PLM} in this work. Wan *et al.*[48] investigated SS 316L exposed to *E. coli*, showing that it had negatively influenced the corrosion process, which was observed after 7 days of exposure. This was attributed to the metabolic products generated and exo-polymeric substances (EPC), which could modify the metal/electrolyte chemical interface. On the other hand, α did not show a clear trend in the acidic and neutral pHs, at MF and HF domains, nor with the presence of bacteria. Nonetheless, it should be mentioned that α value was larger at MF than at HF range, which can reveal the frequency distribution over the oxide surface possibly due to its roughness and heterogeneity. From PLM [38] the thickness of the oxide was estimated, revealing a decrease in the sterile solution and in the inoculated media, which was more significant in sterilized solution at pH 4.7, as shown in Table 1. Those values are in accordance with the drop of the $|Z|_{LF}$.

Complex capacitance was estimated, as was previously described by Benoit *et al.* [49–51] and Barrès *et al.* [49–51], who used the following equations:

$$C_{Imag} = \frac{-Z_{Real}}{2\pi f \times (|Z|)^2} \quad (7)$$

$$C_{Real} = \frac{Z_{imag}}{2\pi f \times (|Z|)^2} \quad (8)$$

where Z_{Real} , Z_{Imag} symbolize the real and imaginary parts of the impedance, $|Z|$ being the impedance modulus that must be corrected by the R_e [51]. Figure 9 shows the infinitive complex capacitance ($C_{complex}$) of AISI 430 after exposure to ASS sterile and inoculated conditions at pH 4.7 and 6.5, which were obtained by using the extrapolation of Cole-Cole plots at HF range, as was previously described [52] (not shown here). In the sterile solution, the $C_{complex}$ was strongly modified overtime at pH 4.7 and pH 6.5. In inoculated media, the

complex capacitances were not influenced by *S. aureus*, but the complex capacitances were affected by *E. coli*, in particular, after 7 days of exposure. From the C_{complex} , the oxide thickness was also estimated, as was previously described by Tran [46], Benoit [47], and Barrès [48]:

Table 1. Impedance parameters for AISI 430 exposed to ASS estimated graphically. The oxide thickness was estimated by using a PLM with an average of $\rho_{\delta} = 3.7 \times 10^{-6} \Omega \text{cm}$

t day	Media	R_e $\Omega \cdot \text{cm}^2$	CPE _{dl}		$C_{dl} \times 10^{-6}$ $\mu\text{F} \cdot \text{cm}^{-2}$	$ Z _{LF} \times 10^3$ $\Omega \cdot \text{cm}^2$	CPE _{PLM}		δ_{PLM}	$\delta_{Cole\ Cole}$
			α	$Q \times 10^{-5}$ $\text{F} \cdot \text{cm}^{-2} \cdot \text{s}^{(\alpha-1)}$			α	$Q \times 10^{-5}$ $\text{F} \cdot \text{cm}^{-2} \cdot \text{s}^{(\alpha-1)}$		
1	Sterilized pH 4.7	4.7	0.91	2.2	8.9	120	0.84	3.2	1.7	2.9
3		6.1	0.87	3.2	9.2	28	0.80	5.2	2.3	2.5
7		6.9	0.90	13.2	60.6	3.3	0.72	32.4	0.5	0.3
1	Sterilized pH 6.5	21.2	0.90	3.9	17.7	110	0.84	5.8	1.2	1.2
3		21.4	0.88	4.8	18.7	56	0.84	6.1	1.3	1.5
7		42.0	0.90	4.3	21.2	28	0.87	4.5	0.9	0.9
14		53.0	0.91	3.0	16.0	190	0.88	3.6	1.0	1.1
1	Inoculated with <i>S. aureus</i> pH 4.7	36.0	0.90	3.4	16.1	42	0.80	5.9	2.0	2.8
3		18.1	0.87	3.6	11.3	58	0.82	4.9	2.0	2.1
7		25.3	0.86	3.5	11.5	12	0.82	4.6	2.2	2.3
14		23.9	0.88	3.3	12.3	6.3	0.82	4.9	2.0	0.8
1	Inoculated with <i>S. aureus</i> pH 6.5	54.1	0.91	3.5	19.1	190	0.83	5.4	1.7	1.8
3		38.8	0.90	2.5	16.6	144	0.82	5.9	1.7	2.1
7		49.8	0.90	3.6	17.8	41	0.83	5.9	1.5	1.7
14		43.7	0.89	4.0	18.1	86	0.79	8.5	1.6	2.0
1	Inoculated with <i>E. coli</i> pH 4.7	31.9	0.91	3.2	16.3	49	0.84	5.4	1.6	1.8
3		23.6	0.91	3.2	15.6	42	0.85	4.6	1.7	1.7
7		26.2	0.88	4.5	17.4	11	0.84	5.8	1.5	1.5
14		23.2	0.92	12.0	71.9	6.9	0.71	35.6	1.6	0.9
1	Inoculated with <i>E. coli</i> pH 6.5	54.2	0.91	3.6	18.7	191	0.82	6.2	1.6	1.8
3		18.1	0.87	3.6	11.5	58	0.83	4.6	1.9	2.1
7		28.6	0.68	2.4	23.9	3.7	0.67	25.6	1.8	5.3
14		15.2	0.76	2.3	22.7	17	0.73	19.5	1.3	1.5

$$C = \frac{\epsilon \epsilon_0}{\delta} \quad (9)$$

In this case, ϵ_0 and ϵ are also the vacuum permittivity and the dielectric constant for the oxide film [4,49–51], with a $\epsilon = 15.6$ value [53] since, as previously reported for AISI 304, the value should be close for AISI 430 [49–51]. Figures 9 (b,d) shows that the oxide thickness decreased overtime in the sterilized at pH 4.7, while in the neutral pH, it increased slightly from 3 to 8 nm. Notice that the error bars were obtained from measurements made in duplicate samples to show the reproducibility. The thickness was not influenced in inoculated media at pH 4.7, but surprisingly, it was affected in neutral pH. Therefore, it seems that the

bacteria did not accelerate the corrosion process, even in an acidic medium. It should be mentioned that the values obtained through C_{complex} were slightly higher than the values obtained by PLM (see Table 1), being similar to that reported previously for stainless steel [34,35]. This can be associated with the capacitive contribution of the complex ohmic impedance, but also due to the R_e estimation, since a low error in its value can strongly influence the infinitive complex capacitances and the oxide thickness. For instance, an error around 5% in the R_e can mean an error around 20% of the capacitances estimation, as was previously described by Liao *et al.* [23].

Figure 9. (a,c) Infinitive complex capacitance estimated from high-frequency limit of the real part Cole-Cole plots and (b,d) oxide thickness of AISI 430 after exposure to ASS in (□) sterilized and inoculated media with (○) *S. aureus* and (△) *E. coli* as a function of time. (a,b) pH 4.7 and (c,d) pH 6.5.

Furthermore, the corrosion potential (E_{corr}), anodic current (i_a), and polarization resistance (R_p) of AISI 430 were estimated from the LSV (see Figure S6 in the supplementary material) according to ASTM G102. Table 2 shows that in the sterilized acidic medium, E_{corr} was shifted towards more negative values, i_a increased, and R_p decreased drastically. This is in agreement with what was reported by Hamidah *et al.* [54], who studied the corrosion behavior of AISI 304 stainless steel at different NaCl concentrations by using gravimetric measurements. However, those parameters were not significantly influenced in the neutral sterilized solution. Therefore, if the AISI 430 is exposed to a corrosive solution, as can be considered in the ASS at pH 4.7, it is expected that a corrosion process of this material could take place after 7 days of continuous exposure. In the inoculated media with a neutral pH, E_{corr} and i_a did not show a clear trend, while R_p increased in the presence *S. aureus*, but decreased significantly in the presence of *E. coli*. In the inoculated media with acidic pH, the electrochemical parameters did not show a trend with exposure.

Table 2. LSV parameters of AISI 430 after exposure to ASS at pH 4.7 and 6.5

t (day)	Sterilized at pH 4.7			Sterilized at pH 6.5		
	E_{corr} (mV vs. SCE)	$i_a \times 10^{-4}$ at $E=E_{ocp}+10\text{mV}$ (mA·cm ⁻²)	R_p (Ω·cm ²)	E_{corr} (mV vs. SCE)	$i_a \times 10^{-4}$ at $E=E_{ocp}+10\text{mV}$ (mA·cm ⁻²)	R_p (Ω·cm ²)
1	-403.8	0.1	9090.9	-465.4	1.3	8842.1
3	-535.3	0.5	2105.2	-527.7	0.6	13927.6
7	-642.3	4.6	246.9	-406.9	6.6	1287.0
14	-654.8	120.9	11.1	-455.3	5.3	1234.5
	<i>S. aureus</i> at pH 4.7			<i>S. aureus</i> at pH 6.5		
1	-285.8	3.6	1023.2	-356.0	0.4	2083.3
3	-316.2	5.0	64102.5	-530.0	0.6	13210.0
7	-317.8	2.3	2127.7	-355.3	0.7	11363.6
14	-137.6	3.6	270.3	-334.9	0.8	10183.3
	<i>E. coli</i> at pH 4.7			<i>E. coli</i> at pH 6.5		
1	-324.4	1.1	12820.5	-104.7	0.2	33222.6
3	-641.1	2.6	2923	-22.7	0.04	27777.8
7	-655.3	7.4	970.8	-442.2	6.7	1574.8
14	-316.3	1.5	5714.3	-273.3	4.8	1789.5

4. Conclusion

The behavior of AISI 430 after being exposed to mimic electrolytic sweat solutions used in Sudoscan™ technology, was analyzed by electrochemical measurements and surface analysis. AISI 430 can be considered as a suitable material that can be employed in massive spaces.

Kinetics analyses reveal that *Escherichia coli* and *Staphylococcus aureus* did not grow in the inoculated artificial sweat solution, as was observed in Mueller-Hinton medium, even with a differentiating pH between 4.7 and 6.5. Additionally, scanning electron microscopy analysis did not show colonization of such bacteria over the metallic surface. Electrochemical measurements show that the corrosion effect caused by *S. aureus* can be discarded, however, *E. coli* should be considered.

Therefore, AISI 430 has excellent performance as a sensor, even in an aggressive medium, *i.e.* for people with acidic sweat. Nevertheless, the electrochemical impedance response for this material reveals a short lifespan. Indeed, the corrosion rate increases dramatically as a function of exposure time, particularly after 7 days.

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- ☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Authorship contributions

Please indicate the specific contributions made by each author. The name of each author must appear at least once in each of the three categories below.

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