ABSTRACT

The importance of biomaterials is increased steadily with the augmentation of population and aging. In this research, casein, a protein present in milk, used to modify the 316L stainless steel (316L SS) alloy surface, used in medical application, against corrosion in the corrosive body fluid. Due to the presence of hetero-atoms in its moiety, casein has the ability to form a protective thin layer. Self-assembled monolayers (SAMs) technique is a common tool providing a well ordered thin film on the metallic surface. Casein was assumed to form SAMs on the 316L SS surface and the corrosion inhibition efficiency was examined electrochemically. The results proved the formation of SAMs and displayed that casein reduce the corrosion rate and serves as a mixed type inhibitor. The inhibition efficiency is increased by increasing the casein’s concentration and immersion time in the casein’s solution. A maximum inhibition efficiency obtained at 1000 ppm with immersion time equals 120 minutes. Further modification is undertaken to increase the inhibition efficiency.

Key words:
Biomaterial, corrosion inhibition, SAMs, natural protein, casein, 316L stainless steel, SBF

1. INTRODUCTION

The primary feature of biomaterials is that they are used in contact with the living body. Biomaterial can be defined as a biocompatible material, natural or synthetic, that used to replace or repair parts of an organ or tissue in the human body [1-3]. Biomaterials must display some requirements in their design such: biologically non-toxic, excellent biocompatibility, good mechanical properties, osseo-integration and high corrosion and wear resistance [1, 4-5].

Medical grade stainless steel 316L widely used as a metallic implant due to ease availability, lower cost, accepted biocompatibility and its corrosion resistivity is very high [1, 3, 6].

The formation of (SAMs) on the metal surface is one of the simplest method used to reduced the corrosion rate of metal by blocking the active sites present in the surface of metal [7-14].

Caseins are soluble milk proteins exist as micelles of four different types of protein; each one of them contains different types of amino acid besides minerals such as phosphorous and calcium [15-16]. These proteins have many usages such as manufacture of plastics and paints, and it was also used in food industries [17-19]. In surface science, few investigators have been inspected the effect of casein on the
corrosion of metals [20-22]. Lately, the effect of casein on the rate of 
corrosion of mild steel in 0.1 M HCl was done by T. Rabizaden et al., 
2019 [22].

In this work, casein is supposed to form 
SAMs on the surface of stainless steel 316L. These SAMs are investigated 
electrochemically by electrochemical impedance techniques and 
potentiodynamic polarization.

2. EXPERIMENTAL METHODS

2.1. Preparation of the Working Electrode

A plate of Stainless steel 316L, with known 
chemical composition [23-24] was cutted into 
small samples. The surfaces of these samples 
were polished with emery papers have different 
degrees (320, 400, 500, 600 and 1200) to 
mirror finish. Only 2 × 1 cm² was exposed to 
electrolyte while the remaining area covered 
with Teflon tap and a coat material. The 
samples were then cleaned with acetone and 
washe several times with double distilled 
water then dried at ambient temperature before 
each measurement.

2.2. Self-assembled Monolayers Formation

The working electrodes were immersed for 
different time intervals (60, 90 and 120 min) in 
solutions of 0.1 M NaHCO₃ containing several 
concentrations (100, 300, 500, 700 and 1000 
ppm) from casein at room temperature.

2.3. Electrochemical Measurements

The measurements performed by using 
three-electrode cell which are saturated 
Calomel electrode (reference electrode), 
platinum electrode (counter electrode) and 
blank and treated samples of 316L stainless 
steel which used as working electrodes. The 
tests were done in 100 mL solution of SBF at 
37 °C as electrolyte solution that was intended 
by to Kokubo [25].

2.3.1. Potentiodynamic Polarization 
Measurements

The electrodes immersed in simulated body 
fluid solution at open circuit potential to attain 
steady state before measurements. Potentiodynamic 
polarization studies performed in scan rate of 0.5 mVs⁻¹ with a 
potential range from -250 to 250 mV. We used 
Potentiostat/Galvanostat (model PGZ 301, 
Voltalab, Radiometer Analytical – France) controlled with VoltaMaster software Version 
4.0 for these measurements. The inhibition 
efficiency of casein (η%) can be calculated 
using the following equation [9, 13-14]:

\[
\eta = \left( \frac{I_1 - I_2}{I_1} \right) \times 100
\]  

(1)

where \( I_1 \) is the corrosion current density in the 
absence of inhibitor and \( I_2 \) is the corrosion 
current density in the presence of inhibitor.

The surface coverage \( \theta \) can also calculate 
from equation [14, 26-28]:

\[
\theta = \left( \frac{\%}{\text{100}} \right)
\]  

(2)

2.3.2. Electrochemical Impedance Studies 
(EIS)

The measurements of electrochemical 
impedance were done in range of frequency 
from 0.1 to \( 10^5 \) Hz with amplitude 10 mV. A 
Potentiostat (model AutoLab 87070) is used for 
performed these measurements. EIS data were 
analyzed using a (FRA) impedance module 
which represented by Nyquist plot. The 
experimental data was analyzed and interpreted 
based on the equivalent circuits.

The following equation is used to 
calculate inhibition efficiency (η%) of the 
casein [9, 26, 29]:

\[
\eta = \left( \frac{R_{ct}^{\text{untreated}}}{R_{ct}^{\text{treated}}} \right) \times 100
\]  

(3)

where \( R_{ct} \) is the values of charge transfer 
resistances of the untreated electrode and \( R_{ct}^{\text{treated}} \) 
is the values of charge transfer resistances of the 
treated electrode.

3. RESULTS AND DISCUSSION

Corrosion behavior for bare and altered 
316L SS in SBF were studied and analyzed by 
electrochemical techniques

3.1. Potentiodynamic Polarization (Tafel) 
Measurements

The Tafel polarization curves for blank and 
immersed 316L Stainless steel electrodes in 
various concentrations of casein’s solutions at 
different immersion time in SBF at 37°C shown in figure 1.

In Figure 1, the curves exhibited that the 
values of \( E_{corr} \) obtained in the case of the 
treated electrodes are slightly moved to positive
value than that of blank electrode proposed forming casein’s SAMs and indicating its effect on the stainless steel performance. The electrochemical parameters derived from the polarization curves are putted in Table 1. The inhibition efficiency ($\eta \%$) and surface coverage ($\theta$) of electrodes are putted also in Table 1.

It was observed from table 1 that the values of corrosion potential $E_{corr}$ are shifted to positive values in case of covered electrodes suggesting the effect of casein on the corrosion reaction [11, 24, 26, 30]. In this study, the differences between $E_{corr}$ of blank and treated electrodes are less than $\pm$ 85 mV. Thus, casein can be served as a mixed type inhibitor [9, 13, 24, 30]. It is also clear that the corrosion current density ($I_{corr}$) of untreated electrode higher than that of treated ones and polarization resistance ($R_p$) is lower. Additionally, with the increase of the concentration of the casein’s solutions the values of ($I_{corr}$) are decreased consecutively; the inhibition efficiency is increased. Moreover, as the immersion time of working electrodes in casein’s solutions increased the corrosion rate is decreased resulting in maximum inhibition efficiency after 120 minutes immersion. This may be attributed to the formation of dense backed SAMs that require higher concentration and more immersion time to be achieved [8-9, 24, 30-31].

**Figure 1:** Tafel curves for blank and immersed 316L Stainless steel in various concentrations of casein’s solutions for: (a) 60 (b) 90 and (c) 120 minutes, respectively.

**Table 1:** Electrochemical parameters for blank and immersed 316L Stainless steel specimens in various concentrations of casein’s solution at different immersion time.

<table>
<thead>
<tr>
<th>Time min</th>
<th>Conc. ppm</th>
<th>$E_{corr}$ mV</th>
<th>$R_p$ k$\Omega$/cm$^2$</th>
<th>$I_{corr}$ $\mu$A/cm$^2$</th>
<th>$\beta_\alpha$ mV</th>
<th>$\beta_\beta$ mV</th>
<th>Corrosion rate (m/Y)</th>
<th>$\theta$</th>
<th>$\eta %$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Blank</td>
<td>-242</td>
<td>24.47</td>
<td>3.232</td>
<td>121</td>
<td>-231</td>
<td>37.92</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>100</td>
<td>100 ppm</td>
<td>-206</td>
<td>29.82</td>
<td>2.053</td>
<td>147</td>
<td>-160</td>
<td>24.07</td>
<td>0.3648</td>
<td>36.48</td>
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<tr>
<td>300</td>
<td>300 ppm</td>
<td>-212</td>
<td>32.92</td>
<td>2.043</td>
<td>156</td>
<td>-148</td>
<td>23.95</td>
<td>0.6379</td>
<td>36.79</td>
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<tr>
<td>500</td>
<td>500 ppm</td>
<td>-220</td>
<td>34.55</td>
<td>1.933</td>
<td>167</td>
<td>-176</td>
<td>22.67</td>
<td>0.4019</td>
<td>40.19</td>
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<tr>
<td>700</td>
<td>700 ppm</td>
<td>-211</td>
<td>34.69</td>
<td>1.849</td>
<td>149</td>
<td>-141</td>
<td>21.68</td>
<td>0.4278</td>
<td>42.78</td>
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<tr>
<td>1000</td>
<td>1000 ppm</td>
<td>-202</td>
<td>36.64</td>
<td>1.805</td>
<td>152</td>
<td>-145</td>
<td>21.16</td>
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<td>90</td>
<td>100 ppm</td>
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<td>29.96</td>
<td>2.012</td>
<td>140</td>
<td>-127</td>
<td>23.59</td>
<td>0.3776</td>
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<tr>
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<td>32.20</td>
<td>1.939</td>
<td>169</td>
<td>-176</td>
<td>22.73</td>
<td>0.4002</td>
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<tr>
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<td>1.899</td>
<td>140</td>
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<tr>
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<td>1.799</td>
<td>144</td>
<td>-146</td>
<td>21.09</td>
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<tr>
<td>120</td>
<td>100 ppm</td>
<td>-205</td>
<td>36.95</td>
<td>1.842</td>
<td>141</td>
<td>-150</td>
<td>21.60</td>
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<tr>
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<td>38.57</td>
<td>1.796</td>
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<td>-140</td>
<td>21.06</td>
<td>0.4442</td>
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<tr>
<td>500</td>
<td>500 ppm</td>
<td>-197</td>
<td>40.91</td>
<td>1.779</td>
<td>138</td>
<td>-128</td>
<td>20.86</td>
<td>0.4588</td>
<td>44.96</td>
</tr>
<tr>
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<td>700 ppm</td>
<td>-203</td>
<td>41.41</td>
<td>1.749</td>
<td>147</td>
<td>-136</td>
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<td>0.4588</td>
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<tr>
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<td>1000 ppm</td>
<td>-199</td>
<td>44.37</td>
<td>1.640</td>
<td>139</td>
<td>-177</td>
<td>19.23</td>
<td>0.4924</td>
<td>49.24</td>
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</table>
3.2. Electrochemical impedance spectroscopy (EIS)

Figures 2 show the Nyquist plots for untreated and covered 316L Stainless steel electrodes with various concentrations of casein’s solution at different immersion time in SBF at 37 ± 0.2 °C.

The obtained Nyquist plot of the blank electrode shows a depress semicircle indicating the charge transfer process [13, 23-24, 27, 31]. The imperfection of the semicircle assigned to harshness and non identity of the metal surface [31-32]. Additionally, arcs of the treated electrodes with casein have large diameters than that of the blank electrode. When concentrations of casein’s solution increase increasing the probability of the interaction of its molecules with the active sites present in the surface of metal driving to more adsorption thus diameter of the semicircles of treated samples is increased [9, 13, 22, 26]. Moreover, by increasing the time of immersion of working electrode in casein’s solutions the diameter of the obtained semicircle is increased this might be rationalize to the formation of more complete, regulate and denser SAMs on surface of the metal with increasing immersion time that increasing impedance of the metal surface consequently, reducing the corrosion rate [9, 26, 30, 31, 33, 34]. The equivalent electrical circuits used to analyze the EIS spectra for untreated and treated samples with casein’s solution are shown in figure 3 [35-36].

where, \( Q \) is the constant phase element (CPE), \( R_s \) is the solution resistance, which used instead of the double-layer capacitor [9, 22, 37], \( W \) is the Warburg element that indicating diffusion process, \( R_{ct} \) is the charge transfer resistance, \( R_f \) is the resistance of the formed film (SAMs) and \( C_f \) is the capacitance of the formed film (SAMs).

The constant phase element (CPE) is mathematically expressed as [27, 31, 37]:

\[
Z_{CPE} = Y_0^{-1}(|Jw|)^{-n}
\]  

(4)

where \( w \) is the angular frequency, \( Y_0 \) is the modulus of the CPE, \( n \) is the surface parameter and \( J \) is the imaginary root .

The fitted impedance parameters are presented in Table 2. The inhibition efficiency \( (\eta) \) was also putted in the same table.

As shown from Table 2, the charge transfer resistances \( (R_{ct}) \) in the case of treated samples with casein are higher than that of blank one. Additionally, the charge transfer resistance is increased by increasing the concentration of casein’s solutions. Furthermore, with increasing the immersion time of working electrode in casein’s solutions, the charge transfer resistances are increased. This could be attributed to the complete formation of regular and dense SAMs of casein on surface of the metal as mentioned before.

Figure 2: Nyquist plots for blank and immersed 316L SS electrodes in various concentrations of casein’s solutions for: (a) 60 (b) 90 and (c) 120 minutes, respectively.
4. CONCLUSION

The self-assembled monolayers (SAMs) of casein which formed on stainless steel 316L surface was investigated electrochemically by electrochemical impedance spectroscopy and potentiodynamic polarization technique in simulated body fluid and the following conclusions could be taken from the study:

- The modification of surface of stainless steel with casein solutions improved its surface against the corrosive body fluid.
- Casein serves as a mixed type inhibitor.
- As the concentration of the casein’s solution is increased, the corrosion rate decreased.
- Additionally, by increasing the immersion time of the stainless steel in casein’s solutions the inhibition efficiency of casein is increased.
- The inhibition efficiency of casein is found to be 50% subsequently, further modification is undertaken to increase the inhibition efficiency.

5. REFERENCES


تثبيط معدل تآكل الصلب المقاوم للصدأ من نوع L316 في سوائل جسم الإنسان باستخدام بروتين اللبن الكازين

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في هذا البحث تم استخدام الكازين وهو بروتين طبيعي موجود في الحليب لتعديل سطح سبانك الفولاذ المقاوم للصدأ من نوع 316L SS المستخدمة في التطبيقات الطبية ضد تآكل في سوائل الجسم المسببة للتأكل. نظرًا لوجود ذرات غير متجانسة في جزيئ الكازين فإن الكازين لديه القدرة على الامتصاص على السطح المعدني لتشكيل طبقة رقيقة واقية تسمى (SAMs). هذه الطبقة هي غشاء توفر تكوين طبقة رقيقة مرتبة جيدًا على السطح المعدني. تم افترض أن الكازين يشكل SAMs على سطح سبانك 316L SS وتم فحص كفاءة تثبيت التآكل كهربيًا في سوائل الجسم عند 37 درجة مئوية. أثبتت النتائج تكوين SAMs وأظهرت أن الكازين يقلل من معدل التآكل ويقل كثافة التآكل النوع المختلف. تزداد كفاءة التثبيت بزيادة تركيز الكازين ووقت الغمر في محلول الكازين. أقصى كفاءة تثبيت تم الحصول عليها عند تركيز 1000 جزء في المليون مع وقت غمر يساوي 120 دقيقة. في الدراسات المستقبلية سوف يتم إجراء مزيد من التعديل على جزيئ الكازين لزيادة كفاءة التثبيت.