Multifunctional inhibitor mixture for reducing bacteria growth and corrosion on marine grade steel

Rainier A. Catubig^a, Agnes Michalczyk^b, Wayne C. Neil^c, Grant M^cAdam^c, John Forsyth^{ab}, Mahdi Ghorbani^a, Ruhamah Yunis^a, M. Leigh Ackland^b, Maria Forsyth^a, and Anthony E. Somers^{a*}

^a Institute for Frontier Materials, Deakin University, Burwood VIC 3125 Australia

^b Life and Environmental Sciences, Deakin University, Burwood VIC 3125 Australia

^c Defence Science and Technology Group, Fishermans Bend VIC 3207 Australia

*Corresponding author. Tel: (+61 3) 9246 8728, Email: anthony.somers@deakin.edu.au

Key words: Steel, corrosion, seawater, anti-microbial, inhibitor, rare-earth, profilometry

Abstract

High strength steel in marine environments suffers from severe corrosion susceptibility and the presence of bacteria can exacerbate the effect, accelerating degradation via microbiologically influenced corrosion (MIC). Here we propose a novel approach to MIC inhibition by designing a system capable of limiting the effects of both bacteria growth and corrosion. The combination of a newly synthesised compound, cetrimonium 4-hydroxycinnamate, with lanthanum 4-hydroxycinnamate was the only system tested to date that could both inhibit abiotic corrosion in artificial seawater and minimise bacteria consortium densities over an exposure period of 24 hours. This success was proposed to be due to their compatibility in the testing environment. Furthermore, we confirmed from cytotoxicity testing that Cet-4OHCin demonstrated similarly limited toxicity towards human cells as the commercially available cetrimonium bromide, a known safe additive to cosmetic products. Thus, this new system shows promise as a safe and effective multifunctional inhibitor to reduce the effects of MIC.

1 INTRODUCTION

The susceptibility of high strength steel to corrosion in marine environments due to chloride ions is well established (Frankel 1998; Wranglen 1974). On some steel alloys, localised corrosion attack can initiate adjacent to cathodic inclusions embedded in the alloy matrix (Szklańska-Smiałowska 1972). Corrosion attack on steel can be exacerbated in the presence of microbiological colonies, falling under the effects referred to as microbiologically influenced corrosion (MIC) (Kakooei et al. 2012; Loto 2017). A common mechanism for accelerated corrosion attack due to MIC involves the formation of a biofilm to protect the numerous individual bacteria strains within from the external environment (Videla & Herrera 2005). The bacteria can change the potential distribution on the steel surface, such as through the formation of anaerobic areas, which can result in localised corrosion attack on the steel. Considering that MIC accounts for approximately 20% of all corrosion costs, it is a significant issue to address in addition to degradation in the absence of microbes (referred to as abiotic corrosion) (Jia et al. 2019; Loto 2017; Xu et al. 2017).

A simple method of controlling MIC is by eliminating bacteria with effective biocides before it has the chance to form biofilms (Loto 2017). In the past various biocides have been incorporated into coating systems to limit microbial adhesion, including copper, tin, mercury and arsenic. However, such systems were found to be toxic to the environment (de Carvalho 2018). In a similar way, abiotic corrosion was effectively diminished with chromate-based inhibitors, which are carcinogenic and also environmentally toxic. Due to the economic and

environmental issues associated with MIC, we are in dire need of “greener” more environmentally friendly inhibitor alternatives (Wang et al. 2017).

To effectively combat MIC, both microbial and abiotic corrosion effects need to be limited and we hypothesise that either a single, multifunctional inhibitor, or, more likely, a mixture of two inhibitors, is needed to eliminate bacteria growth and mitigate corrosion. Our previous work reported the mixture of lanthanum 4-hydroxycinnamate (La(4OHCin)_3) and imidazolinium 4-hydroxycinnamate (IMI-4OHCin), referred to as La+IMI, provided more significant abiotic corrosion inhibition on the HY80 high strength steel in artificial seawater than La(4OHCin)_3 tested separately (Catubig et al. 2020). La(4OHCin)_3 is known to inhibit corrosion to a similar level as sodium dichromate but at a quarter of the concentration, (Blin et al. 2004), suggesting the La+IMI synergistically works together to provide a much more efficient corrosion inhibitor compared to sodium dichromate and La(4OHCin)_3 . In the current work, we exposed cultures of a single species of bacteria and a consortium of planktonic bacteria to the La+IMI mixture to verify the bactericidal properties of the inhibitors, in addition to their already confirmed abiotic corrosion properties.

Cetrimonium nalidixate (CetNal) has previously demonstrated significant antimicrobial properties to single bacteria strains on coated mild steel in 0.01 M NaCl solution (Seter et al. 2013). However, in artificial seawater all systems containing CetNal accelerated corrosion attack on HY80 steel (Catubig et al. 2020). We include this compound in the current study to confirm the antimicrobial properties on a consortium of planktonic bacteria in addition to individual bacteria strains.

This study investigates the potential of each inhibitor and mixture as a biocide by means of anti-microbial testing and investigates their cytotoxicity. Additionally the data obtained for each inhibitor system tested thus far has prompted the synthesis of a new compound, cetrimonium 4-hydroxycinnamate (Cet-4OHCin). As the mechanism proposed for rare earth carboxylates is that the carboxylate group initially attaches to the surface to protect against corrosion, (Somers et al. 2016) we propose that having the same cinnamate cation on both a corrosion inhibiting compound and an anti-microbial compound will result in enhanced protection. Thus a carboxylate was substituted for the nalidixate anion in CetNal to give Cet-4OHCin with the aim of retaining biocide properties whilst also improving the protection against corrosion.

This work focuses on elimination of planktonic bacteria by inhibitors prior to formation of the biofilm. Additionally, the initial processes of corrosion inhibition were also examined to better understand the mechanisms in which the Cet-4OHCin inhibitor and its mixtures provide protection. For these reasons the exposure periods were purposely limited to 24 hours. Longer exposure times would have hindered examinations due to the presence of excessive biofilms or corrosion product. This study will be followed up by a subsequent study on sessile consortia within the biofilm and MIC studies over longer times for promising systems identified here.

2 MATERIALS AND METHODS

2.1 Material Properties

All abiotic corrosion experiments were performed on the high strength steel alloy, 80HLES, with the composition shown in Table S1, determined by inductively coupled plasma atomic emission spectroscopy.

Abiotic corrosion experiments were performed under standard laboratory conditions (23 ± 1 °C) at neutral pH in artificial seawater (details of artificial seawater presented in Section 2.5). Prior to encasing into cold mounted epoxy, samples were cleaned by immersion in concentrated hydrochloric acid 32% (Chem-Supply, $\geq 31.5\%$ w/w) for 5 minutes, thoroughly rinsed with deionised water and dried with argon gas. The epoxy mounted alloy surface was polished to P1200 grit silicon carbide polishing paper with deionised water, rinsed with deionised water, dried with argon gas and stored in a desiccator for 1 hour prior to testing.

2.2 Synthesis of inhibitors

Sodium hydroxide (LR, chem-supply Australia), *trans*-4-hydroxycinnamic acid or *p*-Coumaric acid (97%, Sigma Aldrich Australia), silver nitrate (LR, chem-supply Australia), cetrimonium bromide ($\geq 99\%$, Sigma Aldrich Australia), acetonitrile (HPLC, chem-supply Australia), lanthanum(III) chloride heptahydrate (≥ 99 , Sigma Aldrich Australia) and nalidixic acid ($\geq 98\%$, Sigma Aldrich Australia) were used as received. Distilled water was used in synthesis and to make solutions where required. The synthesis of La(4OHCin)₃, IMI-4OHCin and CetNal were performed by previously reported procedures (Chong et al. 2015; Deacon et al. 2009; Seter et al. 2012b).

¹H NMR spectra were collected on Bruker Avance III instrument operating at 400 MHz in CD₃OD by referencing the solvent peak. Mass spectrometry was performed on an Agilent 1200 series HPLC system. All samples were dried for at least 72 hours on a Schlenk line. All samples were sent to The Campbell Microanalytical Laboratory, New Zealand for elemental analysis.

Synthesis of Cet-4OHCin

The synthetic scheme for the synthesis of cetrimonium *trans*-4-hydroxycinnamate is presented in Figure S1. Sodium *trans*-4-hydroxycinnamate was synthesized according to Deacon *et al.* Sodium *trans*-4-hydroxycinnamate (1.506 g, 8.1 mmol) was dissolved in 10 mL of distilled water and silver nitrate (1.38g, 8.1 mmol) was dissolved in 10 mL of distilled water (Deacon et al. 2009). Upon mixing above clear solutions, a white precipitate formed instantaneously. The solution with white precipitate was left to stir at room temperature for an hour in dark. The white precipitates are washed with water (5×20 mL) to obtain silver *trans*-4-hydroxycinnamate.

Freshly prepared silver *trans*-4-hydroxycinnmate (2.19 g, 8.1 mol) and cetrimonium bromide (1.9786 g, 5.4 mmol) were added to acetonitrile solution (20 mL), a yellow precipitate of AgBr formed instantaneously. The solution was left to stir at 85 °C in dark for a week. The solution was filtered by using filter paper and later filtered over celite to remove silver bromide and unreacted silver *trans*-4-hydroxycinnmate completely. The organic solvent was removed *in vacuo* and later dried for 48 hours *in vacuo* again to get Cet-4OHCin (1.6 g,

66%). ^1H NMR (400 MHz, CD_3OD); 7.40 (m, $J = 16$ Hz, $J = 8.2$ Hz, ring, $\text{C}=\text{CH}-\text{COO}$, 3H), 6.69 (d, $J = 8.25$ Hz, ring, 2H), 3.35 (d, $J = 16$ Hz, $\text{HC}=\text{C}-\text{COO}$, 1H), 3.30 (m, NCH_2 , 2H), 3.11 (s, $\text{N}(\text{CH}_3)_3$, 9H), 1.78 (m, NCH_2CH_2 , 2H), 1.34 (m, CH_2 , 26H), 0.92 (t, $J = 7$ Hz, CH_2CH_3 , 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD); 173.98 (COO), 158.53 (ring carbon attached to OH), 140.30 (ring C), 128.81 (ring C, $\text{C}-\text{C}(\text{OH})-\text{C}$), 127.18 ($\text{C}=\text{CH}-\text{COO}$), 120.99 ($\text{C}=\text{C}-\text{COO}$), 115.19 (ring C), 66.50 (NCH_2), 52.07 ($\text{N}(\text{CH}_3)_3$), 31.67 (CH_2), 29.38 (CH_2), 29.32 (CH_2), 29.23 (CH_2), 29.14 (CH_2), 29.07 (CH_2), 28.83 (CH_2), 25.97 (CH_2), 22.53 (CH_2), 22.33 (CH_2), 13.04 (CH_3) ppm. ES^+ m/z 284.5 ($(\text{CH}_3)_3\text{N}(\text{C}_{16}\text{H}_{33})^+$, ES^- m/z 163.0 ($\text{trans-4-hydroxycinnamate}^-$). Anal. Calculated for $\text{C}_{28}\text{H}_{53}\text{N}_1\text{O}_5$; C, 70.09; H, 11.04; N, 2.89. Found: C, 70.09; H, 11.09; N, 2.68.

The residual silver and bromide content were determined by an Ionode IJ-Ag or IJ-Br selective electrode after calibration with 10 and 100 ppm solutions of the silver (from silver nitrate) or bromide ion (potassium bromide). Bromide content measured by ion selective electrode (ISE) was 600 ppm. Silver content measured by ISE was below the detection limit of the electrode.

Due to the relatively low solubility of some inhibitors, each was sonicated separately in analytical grade ethanol (Chem-Supply, $\geq 96.5\%$) until fully dispersed and then added into the artificial seawater solution. The final solutions were heated and stirred at approximately 50°C for 3 hours to allow for the ethanol to evaporate while not being too hot to cause the degradation of the inhibitors. The solution concentrations and chemical structures are presented in Table 1. A precipitate would form immediately after mixing CetNal with $\text{La}(\text{4OHcin})_3$ together into solution. The steel coupons were tested in a vertical position to avoid any precipitate resting on the steel surface and potentially influence inhibition performance.

Table 1: The abbreviations, concentrations and chemical structure of each inhibitor solution tested. The anti-microbial testing used dilutions of these concentrations. The molecular weights (W_M) are also included.

Inhibitor	Abbreviation, max solubility tested	Chemical structure
No inhibitor (artificial seawater only)	Uninhibited - 0 mM	-
Lanthanum <i>trans</i> -4-hydroxycinnamate	La(4OHCin) ₃ - 1.1 mM (W_M = 718.4 g/mol)
Cetrimonium Nalidixate	CetNal - 4 mM (W_M = 516.8 g/mol)
Imidazolinium <i>trans</i> -4-hydroxycinnamate	IMI-4OHCin - 10 mM (W_M = 248.3 g/mol)
Lanthanum <i>trans</i> -4-hydroxycinnamate + Cetrimonium Nalidixate	La+CetNal 1.1 mM : 4 mM
Lanthanum <i>trans</i> -4-hydroxycinnamate + Imidazolinium <i>trans</i> -4-hydroxycinnamate	La+IMI 1.1 mM : 10 mM
Cetrimonium <i>trans</i> -4-hydroxycinnamate	Cet-4OHCin – 1.1 mM (W_M = 467.7 g/mol)
Lanthanum <i>trans</i> -4-hydroxycinnamate + Cetrimonium <i>trans</i> -4-hydroxycinnamate	La+Cet 1.1 mM : 1.1 mM

2.3 Antimicrobial assay of inhibitors

The bacterial species: *Shewanella chilikensis*, *Pseudomonas balearica* and *Proteus mirabilis* (CDC PR14) were used in this study due to their known association with biofilm formation on steel and their potential association with MIC (Kip & van Veen 2015). The silt bacterial consortium was obtained from Port Adelaide River silt in South Australia, where it was associated with an aerobic, corroding environment. The *Escherichia coli* strain D-037, while not commonly associated with MIC, was used as a control organism. Bacterial strains were inoculated from fresh Tryptone Soya Agar plates into Tryptone Soya Broth (TSB) (ThermoFisher Scientific, Australia) and grown aerobically overnight at 30°C with shaking at 200 rpm. Each bacterial culture was then diluted in TSB to 0.5 on the

McFarland Standard (OD_{600nm} = 0.1 - 0.15) and used for inhibitor testing as described below.

A high-throughput method for testing the inhibitors was adapted from the 'Methods for the Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition (Clinical and Laboratory Standards Institute, 2012) utilised by Seter *et al.* Briefly, inhibitors were tested in 96-well plates, where the first column was used as TSB blank, second one as a pure inhibitor blank, followed by the inhibitors that were serially diluted in TSB by a factor of 2, starting from column 3 until column 11 (Seter *et al.* 2012b). Column 12 contained 'no inhibitor' control for bacterial growth. The same volume (100 µl) of diluted bacterial stock was added into columns 3-12 and plates were incubated for 24 hours at 30°C with shaking at 150 rpm. All samples were run in quadruplicate. Optical density readings (OD_{600nm}) were collected using a POLARstar Omega (BMG Labtech, Australia) microplate reader and MARS data analysis software (BMG Labtech, Australia).

Data were firstly analysed by subtracting the averaged TSB blank readings, followed by subtraction of the average of the inhibitor blank reading then recalculated to reflect the concentration of the inhibitors in each diluted sample. The transformed data was then normalised to provide the bacterial survival percentage by dividing each of the sample results by the average from the 'no inhibitor' control, that represented 100% growth.

2.4 Immersion abiotic corrosion testing

Coupons (10 mm × 10 mm) were immersed in separate 80 ml solutions for 30 minutes and 24 hours with and without each inhibitor. The relatively large volume was used to ensure full immersion was achieved throughout the immersion periods and to exceed the recommended solution-to-specimen area ratio of 0.2 ml/mm² according to ASTM G31 72 (reapproved 2004) "Standard practice for laboratory immersion corrosion testing of metals" Clause 8.9.2 (ASTM 2004). Solutions for abiotic corrosion tests were all exposed to artificial seawater prepared with analytical grade NaCl (Chem-Supply, ≥99.7%), MgCl₂ (Sigma, ≥98.0%), MgSO₄ (Merck, ≥96.0%), CaCl₂ (Sigma, ≥96.0%) and KCl (Univar, ≥99.8%) as shown in Table S2. Artificial seawater was used so that the composition of the electrolyte was known and constant throughout all testing and consistent with previous testing. Upon removal the coupons were subsequently gently rinsed with deionised water and dried with argon gas.

2.5 SEM/EDS on abiotic full immersion samples

Each sample was carefully removed from its epoxy and mounted onto an aluminium stub using double-sided adhesive carbon tape for analysis. Surface characterisation was performed with a JEOL JSM-IT300-LV scanning electron microscope (SEM) with a 50 mm² X-Max energy dispersive X-ray spectroscopy (EDS) detector.

2.6 Electrochemical testing on abiotic solutions

All polarisation experiments were performed with a standard three-electrode polarisation cell, using 80HLES as the working electrode, a titanium mesh (approximately 700 mm² in occupied space of the mesh) as the counter electrode and silver-silver chloride (Ag/AgCl) as the reference electrode. A glass Luggin capillary containing the test solution held the

Ag/AgCl reference electrode and enabled the tip of the Luggin (and by extension the reference electrode) to an approximate distance of 2D from the steel sample, whereby D is the diameter of the Luggin tip (2.5 mm) (Barnartt 1952). The surface area of the working electrode was 10 mm x 10 mm. Electrochemical testing was performed using a BioLogic 16 Channel-VMP3 (variable multichannel potentiostat) with the EC-lab software version 11.03.

Linear polarisation tests were performed at 30 minutes and every 4 hours thereafter (for 24 hour tests). A potential was applied to the steel from -10 to +10 mV relative to the open circuit potential (OCP) at a scan rate of 0.167 mV/s.

Potentiodynamic polarisation scans were performed after 30 minutes and 24 hours on separate samples. The scans were conducted from -150 to +250 mV relative to OCP, at a scan rate of 0.167 mV/s. The uninhibited sample was scanned from -60 to +250 mV instead since polarising to more negative potentials without an inhibitor resulted in significant changes in the measured corrosion potential as compared to the OCP prior to the test. This was thought to be due to changes in the environment near the working electrode when polarising further than -60 mV relative to OCP leading to inconsistent results. This effect was not seen for the inhibited solutions. The solution volume was 300 ml and was left open to air over the duration of the experiments. A minimum of triplicate tests was performed for each condition.

2.7 Removal of corrosion product and optical profilometry on immersion samples

Once the corrosion product and inhibitor deposits were examined with SEM/EDS, they were removed chemically to characterise the localised corrosion statistics for each sample. The corrosion product was removed by immersing each sample for 3 minutes in a solution of analytical grade HCl (25 ml), deionised water (25 ml) and analytical grade hexamethylene tetramine (0.175 g, Sigma, ≥99.0%). This was adopted from ASTM G1-03 (reapproved 2017) "Standard practice for preparing, cleaning and evaluating test specimens" designation C.3.5 (ASTM 2017). Following corrosion product removal each sample was rinsed with deionised water and dried with argon gas.

Three dimensional surface profilometry was conducted with a Bruker Contour GT-K1 3D optical profilometer running Vision 64 software. The samples were analysed with the optical profilometer within 1 hour of corrosion product removal.

2.8 Inhibitor cytotoxicity test

HaCaT (immortalised human keratinocytes) and HuTu80 (human duodenum adenocarcinoma) were used to test cytotoxicity of the inhibitors, as they represent a biologically relevant model of skin and intestine for studies of chemical product safety in human health, an alternative to *in vivo* tests (Kreja & Seidel 2002; Lopez-Garcia et al. 2014).

HaCaT or HuTu80 cells were cultured to 85% confluency in 75 cm² culture flasks in culture media: Dulbecco's Modified Eagle medium (DMEM) (Life Technology, Australia) with 10% Fetal bovine serum (FBS) for HaCaT cells and Roswell Park Memorial Institute medium (RPMI) (Life Technology, Australia) with 10% FBS for HuTu80 cells. Cells were trypsinized, counted and seeded at 5×10³/well in 96-well plates in 200 µl of growth medium and incubated for 24 hours at 37°C with 5% CO₂. A range of inhibitor concentrations was added to the cells and they were incubated at predetermined time intervals from 15 minutes to

5 days. At the end of incubation, 50 µl of MTT (3(4,5-dimethylthiazol-2-yl) 2,5- diphenyltetrazolium bromide) (Sigma-Aldrich, Australia) solution (5 mg/ml) was added to each well and plates were incubated at 37°C with 5% CO₂ for 2 hrs. Media were then removed and 150 µl of dimethyl sulfoxide (Sigma-Aldrich, Australia) was pipetted into each well. Plates were agitated for 20 min on a shaker and absorbances (OD550 nm) were measured on a POLARstar Omega (BMG Labtech, Australia) microplate reader with MARS data analysis software (BMG Labtech, Australia). Data were firstly analysed by subtracting the averaged cell culture media readings, followed by normalisation to 'no inhibitor' control. This will be referred to the cell density.

3 RESULTS

3.1 Bacterial testing of inhibitors in solution

The significant reductions in abiotic corrosion attack by the mixture La+IMI from our previous work and its potential surfactant properties made it the ideal system to investigate against MIC (Catubig et al. 2020). The CetNal inhibitor was also included as it has been reported to have a strong effect on bacteria (Seter et al. 2013) and will likely give a good anti-microbial baseline compared to the other inhibitors.

For evaluating the anti-microbial properties of the compounds and their mixtures, each inhibitor was dissolved in solution containing the Port silt (collected at a location from the Port River in Adelaide, South Australia) that is known to contain a mixture of bacteria strains. Since the microbial tests are purely to observe the effect on the bacteria density in the presence of the various inhibitors, no steel was present.

Figure 2 presents the bacteria density curve (%) from the mixed Port silt culture as a function of inhibitor concentration for La(4OHCin)₃, IMI-4OHCin, CetNal and their respective combined inhibitor mixtures. Lower percentages correlate to a reduction in the bacteria density. The concentrations presented for La(4OHCin)₃ was much smaller than the CetNal or IMI-4OHCin systems because it had a much smaller saturation limit. The concentrations for the La+CetNal and La+IMI mixtures correspond to the saturation concentrations of CetNal and IMI-4OHCin respectively.

Figure 1: Bacteria density curve for the different inhibitors exposed to the mixed bacteria culture. For the mixtures, the concentration corresponds to IMI-4OHCin for La+IMI and CetNal for La+CetNal. The individual inhibitors are solid lines while the mixtures are dashed lines.

La(4OHCin)₃ was predominantly added for abiotic corrosion inhibition and not expected to provide any anti-microbial properties. The lack of effect on the bacteria is shown when bacteria density was only reduced to approximately 60% at the highest concentration tested (almost 300 μM) after 24 hours.

In contrast, even at very small concentrations (< 100 μM), CetNal limited the bacteria density irrespective of the presence of La(4OHCin)₃, demonstrating its effectiveness as an anti-microbial. Unfortunately, the mixture La+CetNal accelerated abiotic corrosion, as previously demonstrated, therefore it is not a viable option for this project (Catubig et al. 2020). We present it here as an example of an efficient anti-microbial compound as compared with La(4OHCin)₃, IMI-4OHCin and the La+IMI mixture.

La+IMI, which showed the most effective abiotic inhibition from previous work, had almost no effect on the bacteria density, having less reduction in density compared to La(4OHCin)₃ alone (Figure 2) (Catubig et al. 2020). Thus, it too cannot combat both abiotic corrosion and MIC simultaneously.

As the initial inhibitor mixtures were only effective in minimising either abiotic corrosion or bacteria density but not both, the new compound, Cet-4OHCin, was synthesised. It was hypothesised that the cetrimonium cation will retain its anti-microbial properties while the 4OHCin⁻ anion will allow for corrosion inhibitive speciation, either on its own or to complement the inhibition mechanism of La(4OHCin)₃.

Initially this new Cet-4OHCin compound was tested against several single strain bacteria as well as the Port silt (the bacteria density curves are presented in Figure 3). *Shewanella chilikensis*, *E. coli* and the mixed bacteria Port silt, were very sensitive to the presence of Cet-4OHCin, minimising bacteria density to less than 10% between concentrations of 10-20 μM. *Proteus mirabilis* and *Pseudomonas balearica* showed a more gradual decrease as concentration increased.

Figure 2: Bacteria density curves for a) Cet-4OHCin in the presence of individual bacteria strains and the mixed bacteria culture from the Port silt and b) the mixture La+Cet in the presence of individual bacteria strains.

The inset on the top right corner of Figure 3a compares the Port silt microbial consortium density of CetNal and Cet-4OHCin. The similar density percentages demonstrated that the anti-microbial properties of CetNal were retained when the nalidixate anion was replaced with the 4OHCin⁻ anion.

The mixture of Cet-4OHCin and La(4OHCin)₃, referred to as La+Cet, was exposed to the same individual bacteria strains (shown in Figure 3b). At concentrations smaller than 40 μM (of each inhibitor) for the individual bacteria strains and 8.8 μM for the Port silt, the bacteria density remained relatively constant around 100% which is indicative of no significant bacteria death. Beyond these respective concentrations, the bacteria density dramatically drops to less than 8%, at least for the Port silt, *E. Coli* and *Shewanella chilikensis*. This indicates the presence of a minimum inhibitor concentration to cause noticeable reductions in the bacteria density and the reduced sensitivity of the La+Cet towards the Port silt, *E. Coli* and *Shewanella chilikensis* bacteria strains compared to Cet-4OHCin alone in solution without La(4OHCin)₃.

This study showed a significant reduction in planktonic bacteria density by inhibitors after 24 hours. This time was selected on the basis that the bacteria should be killed prior to them establishing a biofilm. Bacterial growth reduction by CetNal seen in the anti-microbial tests (Figure 1) strongly supports the observations by Seter *et al.* (2013). A similarly strong anti-microbial effect was observed with the new Cet-4OHCin compound (as seen in the inset of Figure 2a). This indicates the substitution of nalidixate for the 4OHCin⁻ anion had little effect on its anti-microbial effect, and that the anti-microbial functionality resides mainly with the cetrimonium cation. The minor reduction in sensitivity of the La+Cet mixture compared to Cet-4OHCin towards each bacteria strain became negligible beyond 70 μM at which point the bacteria density are converging towards zero (at least for the *Shewanella chilikensis* strain and **silt consortium** shown in Figure 2b).

IMI-4OHCin was proposed as a possible MIC inhibitor and was reported to provide significant abiotic corrosion inhibition at acidic (pH 2) through to relatively alkaline (pH 8) conditions on mild steel (Bajpai & Tyagi 2006; Chong *et al.* 2015; Chong *et al.* 2016). Furthermore, due to its similarity to imidazolium compounds, which are known to have anti-microbial properties, we hypothesised that IMI-4OHCin would also provide some level of anti-microbial effects. However, this is typical for structures with longer alkyl chains of 6-8,

(Docherty & Kulpa 2005) which is much longer than with the IMI-4OHCin and likely explains the lack of anti-microbial properties observed in Figure 1.

3.2 Observing corrosion inhibition behaviour with electrochemistry

Beyond the anti-microbial properties demonstrated by the new compound Cet-4OHCin and the La+Cet mixture, it is important to determine if these inhibitor systems can also limit abiotic corrosion. Figure 5 presents the potentiodynamic polarisation curves for 80HLES steel after 24 hours immersion in the artificial seawater solution. The average corrosion current density (i_{corr}) values and the corrosion potential (E_{corr}) are shown in Table 4.

Figure 3: Potentiodynamic polarisation curves for each inhibitor after a 24 hour immersion period.

Table 2: Average E_{corr} and i_{corr} values for each inhibitor after 30 minutes and 24 hours OCP.

Sample	Average E_{corr} vs Ag/AgCl (mV)		Average i_{corr} ($\mu\text{A}/\text{cm}^2$)	
	30 minutes OCP	24 hours OCP	30 minutes OCP	24 hours OCP
Uninhibited	-470 ± 2	-623 ± 14	3.41 ± 1.07	3.46 ± 0.21
La(4OHCin) ₃	-405 ± 11	-541 ± 15	1.14 ± 0.12	0.28 ± 0.07
Cet-4OHCin	-420 ± 8	-505 ± 102	3.92 ± 1.12	3.67 ± 0.08
La+Cet	-406 ± 12	-385 ± 2	0.68 ± 0.43	0.47 ± 0.00

All inhibitors were predominantly anodic relative to the uninhibited samples, as indicated by reductions in anodic current densities and anodic shifts in their respective corrosion potentials, as seen in Figure 3 and Table 2. The most significant reduction in anodic current densities was with the La+Cet mixture with over 2 orders of magnitude reduction at approximately -380 mV vs Ag/AgCl. Additionally only the La+Cet mixture showed a region between -250 mV to -150 mV which is characteristic of a passive region like behaviour, with an inflection characteristic of a pitting potential at approximately -150 mV vs Ag/AgCl.

It is also interesting to note that the i_{corr} for La(4OHCin)₃ was initially higher than La+Cet after 30 minutes but was lower after 24 hours. This may suggest the La+Cet mixture initially forms a more protective film after 30 minutes, however, over time the La(4OHCin)₃ becomes comparatively more protective. As previously reported, La(4OHCin)₃ behaved as a mixed inhibitor by also reducing cathodic current densities over 24 hours (Blin et al. 2004). The i_{corr}

of Cet-4OHCin tested alone was similar to the uninhibited sample indicative of limited inhibition for this HY80 steel in artificial seawater.

Considering this dependence of inhibition effectiveness with time (ie. 30 minutes vs 24 hours), the polarisation resistance (R_p) was measured throughout a 24-hour period using a non-destructive technique. This resistance is indicative of corrosion inhibition. Linear Polarisation Resistance (LPR) was conducted at 30 minutes and 4 hourly intervals thereafter during the 24 hour exposure and is shown in Figure S2. Similar to the average i_{corr} data, the LPR measurements for the uninhibited and the Cet-4OHCin inhibitor were almost coincident throughout the exposure period. Given R_p is inversely proportional to i_{corr} , this is to be expected.

The R_p for the La(4OHCin)_3 inhibitor steadily increased over 12 hours, then plateaued up to 24 hours. In contrast the La+Cet mixture showed a higher R_p at 30 minutes, but over the 24 hour period the R_p fluctuated around that of the La(4OHCin)_3 . The scatter in the data is evident in the error bars on Figure S1 and may be indicative of a less stable protective film compared to La(4OHCin)_3 on its own.

When exposed to artificial seawater, Cet-4OHCin alone still performed poorly as an abiotic corrosion inhibitor in this aggressive environment. This demonstrates the observation of Kalinowska *et al.* and Seter *et al* (Kalinowska et al. 2007; Seter et al. 2015) that having the 4OHCin⁻ anion incorporated into an inhibitor does not guarantee an effective inhibitor.

However, there was a marked improvement in abiotic corrosion inhibition with the La+Cet mixture compared to the La+CetNal mixture. Upon mixing La(4OHCin)_3 with CetNal (to form the mixture La+CetNal), a precipitate would immediately form (likely to be a lanthanum-nalidixate salt) and it is presumed that the remaining ions may form Cet-4OHCin and remain dissolved in solution. We now know from this current work that Cet-4OHCin is a poor corrosion inhibitor when dissolved in solution by itself and so we have a rational explanation for accelerated corrosion attack for the La+CetNal mixture, as it likely only consists of Cet-4OHCin in solution.

In contrast, both La+IMI and La+Cet mixtures did not result in precipitation upon mixing and additionally yielded significant abiotic corrosion inhibition. This suggests that preserving the general structure of the inhibitor (in this case the La(4OHCin)_3) should be one of the priorities when mixing multiple inhibitors to maintain a high level of abiotic corrosion inhibition. One way this can be achieved is by mixing inhibitors with identical anions, as with the 4OHCin⁻ anion in the current work. Since there is only one type of anion present then there is limited possibility of new compounds forming and precipitating out.

3.3 Surface characterisation with SEM/EDS

Characterisation of 80HLES surfaces immersed in the solutions for 30 minutes and 24 hours was conducted to compare their appearance and correlate with the electrochemical data and overall corrosion inhibition. Representative SEM micrographs are presented in Figure 4, with 24 hour EDS data shown in Figure S3.

After 30 minutes immersion, Figure 4c shows corrosion product deposition as well as localised attack on the steel surface in the presence of the Cet-4OHCin inhibitor alone was

evident (Figure 8c). This was visibly similar to the uninhibited sample surface (Figure 4a). However, in the presence of either $\text{La}(\text{4OHCin})_3$ (4b) or the La+Cet mixture (4d), the steel surface was relatively pristine, as shown by the lack of locally attacked sites, lack of corrosion product deposits and visibility of original polishing marks. These results support the observations from the electrochemical data, whereby Cet-4OHCin alone in solution offered little change in i_{corr} compared to the uninhibited control whilst the $\text{La}(\text{4OHCin})_3$ and La+Cet systems both provided significant inhibition.

Figure 4: Secondary electron micrographs of each sample immersed after 30 minutes and 24 hours.

Immersion for 24 hours in the uninhibited solution resulted in greater corrosion product deposits on the general surface that increased significantly around locally attacked sites

(Figure 4a). EDS in Figure S3 shows increased sulphur present at these sites, suggesting localised corrosion is initiated at sulphide inclusions.

The La(4OHCin)_3 sample surface had isolated deposits and EDS showed the presence of increased amount of La present at these (Figure S3b), most likely as mixed oxide/hydroxide and La(4OHCin)_x species (Forsyth et al. 2011). The surfaces for samples exposed to either the La+Cet mixture or the La(4OHCin)_3 were similar in appearance, however more fine deposits can be seen in Figure 4d for the mixture. Again these deposits contain higher amounts of La and so are likely to be a reaction of the inhibitor with the surface as opposed to common corrosion product. In both cases there is an increased presence of sulphur at these deposits, suggesting that these La deposits predominantly form in the vicinity of localised corrosion occurring at sulphide inclusions. Such areas are cathodic compared to the bulk and become active sites of localised corrosion. The presence of La at these sites is consistent with the mechanism previously proposed and the 24 hour polarisation presented here, whereby the La part of the molecule predominantly acts as a cathodic inhibitor (Forsyth et al. 2011). The increased number of deposits in the mixture may be indicative of increased surface activity eg. corrosion initiation which is quickly stifled as a result of inhibitor compound being deposited. This may also explain the less stable electrochemical response of the La+Cet as compared to the La(4OHCin)_3 seen in the polarisation resistance (Figure S2).

While the samples immersed in the La(4OHCin)_3 and La+Cet solutions had a consistent level of deposition over the entire steel surface, those immersed in the uninhibited solution and the Cet-4OHCin solution resulted in non-uniform corrosion. Optical images of the whole surface for both samples after 24 hours are shown in Figure S4. The more heavily deposited regions can be observed as rust-coloured areas on the uninhibited sample in Figure S4a and a dark grey colour on the Cet-4OHCin (Figure S4b). Away from these heavily deposited regions, representative SEM micrographs for the uninhibited (Figure S4c) and the Cet-4OHCin (Figure S4d) show significantly less corrosion product as compared to their counterparts in the heavily deposited regions shown in Figure 4a and c.

3.4 Localised attack examination with optical profilometry

To quantify the penetration of corrosion into the steel, corrosion product was chemically removed from samples immersed for 30 minutes and 24 hours and the underlying corroded steel was examined with optical profilometry to measure damage depths and diameters at locally corroded sites. The large deposits of corrosion product tended to act as “caps” for underlying localised corrosion. While the surface of the steel was not in a passive state and thus classical pitting did not occur, the removal of the corrosion product confirmed the presence of pit-like attack beneath the corrosion product. With this in mind, it was assumed that every site undergoing localised corrosion were cone shaped. This allowed the approximation for the volume of corroded material using the maximum corroded depth (average of the 10 deepest sites), corroded diameter (average of the 10 widest sites) and the average number of corroded sites per squared micrometre.

Figure 5 presents the approximate corroded volume results for each inhibitor after 30 minute and 24 hour immersion periods. The visual difference observed across the samples for both the uninhibited and Cet-4OHCin (as per Figure S4), appears to only influence the surface deposit. This is assumed since subsequent to the removal of these surface deposit, the differences in the maximum corroded depth, width or number of sites between the two zones in both cases were insignificant. Therefore the average values from the zones that appeared rusted and less rusted were combined for Figure 5 below.

Figure 5: Average corroded volume for each inhibitor after 30 minutes (left) and 24 hour (right) immersion periods.

After 30 minutes, the corroded volume of the Cet-4OHCin sample was greater than the uninhibited sample. Both of which were greater in corroded volume of La(4OHCin)₃ and La+Cet.

After 24 hours, the corroded volume has substantially increased for the uninhibited and Cet-4OHCin, with the latter having the largest volume, indicating accelerated localised corrosion compared to the uninhibited sample. While remaining low compared to the control, the La+Cet yielded higher volumes than the La(4OHCin)₃, which correlate to the i_{corr} values in Table 2. These results are mostly consistent with the electrochemistry, with the increased localised corrosion in the case of Cet-4OHCin after 24 hours being the exception.

3.5 Skin and intestine cell viability of Cet-4OHCin and Cet-Br

While Cet-4OHCin has been shown here to be an effective anti-microbial, it is also important to investigate its toxicity to humans. As an initial indication of toxicity the effect of Cet-4OHCin on various human cell cultures was compared to the well-established antimicrobial, cetrimonium bromide (Cet-Br). Cet-Br is a known safe additive in numerous cosmetic products and is a starting material for synthesising Cet-4OHCin (Becker et al. 2012).

Cytotoxicity tests were conducted on the cultured skin and intestine cell models at predetermined time intervals from 15 minutes to 3 hours exposure, in the presence of La(4OHCin)₃, Cet-4OHCin, the La+Cet mixture and Cet-Br. Skin and intestine cells estimate the potential effect on skin exposure and ingestion. The absorption of Cet-Br into skin and intestine has been reported at 0.59% and 10% respectively (Becker et al. 2012). To

approximate this, we present the results for skin and intestine cells exposed to inhibitor concentrations of 1 μM and 10 μM respectively in Figure 6.

The high cell density percentages in the presence of $\text{La}(\text{4OHCin})_3$ demonstrates its relatively low toxicity expected towards humans at least through skin or ingestion. Increased sensitivity is observed for both Cet-4OHCin and Cet-Br, however, the reductions in cell % can still be considered to be relatively small.

Interestingly the cells are not as sensitive to the La+Cet mixture compared to Cet-4OHCin alone, implying reduced sensitivity of the mixture towards the human cells compared to Cet-4OHCin tested alone. Given that the results for the Cet-4OHCin containing samples were very similar or better than that of the Cet-Br it is an excellent indication that it is safe for human contact.

Figure 6: The skin and intestine cell viability after 15 minutes exposure in the presence of 0.5 mM concentration of either Cet-4OHCin or Cet-Br in solution.

4 DISCUSSION

4.1 Corrosion inhibition of the La+Cet mixture compared to $\text{La}(\text{4OHCin})_3$ alone

As shown from the electrochemical and optical profilometry data, 30 minute test results indicated La+Cet was initially abiotically more effective than $\text{La}(\text{4OHCin})_3$ alone, with a considerably lower i_{corr} (Table 2) and similar average localised corrosion volume (Figure 5). By 24 hours of exposure however, while the La+Cet mixture was able to maintain its low i_{corr} , the localised corrosion volume increased slightly, whereas the $\text{La}(\text{4OHCin})_3$ exhibited a lower i_{corr} and maintained low localised corrosion volumes. Considering the La+Cet mixture is predominantly an anodic inhibitor, within the first 30 minutes of immersion there is a higher concentration of inhibitor in solution to deposit and inhibit corrosion attack on the steel, resulting in the lower i_{corr} of La+Cet relative to $\text{La}(\text{4OHCin})_3$. Since initial deposition onto the steel for the $\text{La}(\text{4OHCin})_3$ inhibitors has been proposed to occur via the carboxyl functional group, an increased amount of this anion present is likely the reason for this improved performance after 30 minutes (Somers et al. 2018). This suggests that Cet-

4OHCin improves the robustness of the deposited film within the first 30 minutes of immersion through increased film deposition due to the higher local concentration of 4OHCin⁻ anions. Since the La(4OHCin)₃ is at its solubility limit, adding the Cet-4OHCin is a way of getting more of the 4OHCin⁻ anion into solution and subsequently onto the surface. By being able to by-pass the solubility limit in this way along with not being detrimental to the chemical speciation (as mentioned in Section 4.2), an improved level of inhibition compared to the La+CetNal mixture has been enabled.

The process whereby the carboxyl functional group attaches to the metal surface would also bring the La³⁺ and the Cet⁺ cations towards the steel surface, however, as more Cet⁺ cations complex into the film over the steel, steric hinderance and competition to other inhibitor species, such as La, may occur. This may effectively limit the deposition of the inhibitor, resulting in reduced protection over time. Forêt *et al.* reported a possible steric obstruction effect from multiple nitrogen atoms limiting film formation of amines while steric hindrance has also been shown to reduce inhibiting ability in the nitrogen containing piperidin-4-one derivatives (Forêt *et al.* 2008; Thirumalairaj & Jaganathan 2016). However when in a mixture (such as the La+IMI system), better corrosion inhibition was observed when the inhibitors had matching chain lengths (Shiao *et al.* 1998). This may explain why the previously tested La+IMI (Catubig *et al.* 2020) outperformed the La+Cet system, both in i_{corr} and corroded volume, the imidazolinium cation having a similar chain length to the 4OHCin⁻ anion, unlike the cetrimonium cation. Additionally, it has previously been shown that the La present in La(4OHCin)₃ takes time to deposit and reduce cathodic reactions, (Forsyth *et al.* 2014) which would explain the increased protection over 24 hours. In the La+Cet mixture however, the cetrimonium cation present may compete with the La, reducing its ability to affect the cathodic reaction.

Nonetheless the level of inhibition with the La+Cet mixture remained similar to La(4OHCin)₃ alone over 24 hours and reduced bacteria growth to validate the potential of this mixture as a MIC inhibitor. No other inhibitor combination in this work was able to achieve such definitive multifunctionality. This makes the non-toxic, more environmentally friendly La+Cet system a more attractive inhibitor mixture than traditional carcinogenic chromate-based inhibitors.

4.2 Cytotoxicity of Cet-4OHCin versus Cet-Br

Cytotoxicity is a simple and relatively inexpensive method to estimate toxicity of a chemical towards specific human cells. It can act as a precursor for expensive animal testing and has been shown to correlate to toxicity in the form of LD₅₀ values (Frank & Monica 1998). We can expect Cet-4OHCin will be relatively safe for exposure to the cell lines tested in this work due to the similar cell density between Cet-4OHCin and Cet-Br (Figure 6).

Additionally, exposing the inhibitors directly to bare human cells means we cannot account for the increased resistance provided in an *in vivo* situation. *In vitro* data for dermal penetration is expected to be overestimated compared to *in vivo* (van Ravenzwaay & Leibold 2004). We therefore expect the reductions in cell density in our work (Figure 11) to be overestimated, suggesting minimal reductions and thus minimal sensitivity towards human skin cells. These factors may explain why Cet-Br is considered safe in cosmetic products (between 1-30 g/L) even though cell density was reduced by just over 30% on skin cells in

Figure 11 (Becker et al. 2012). We assume the intestinal cells will behave in a similar fashion and sensitivity towards the inhibitors will be less under *in vivo* conditions.

The $\text{La}(\text{4OHCin})_3$ inhibitor demonstrated complete none toxicity towards skin cells and very limited sensitivity towards intestinal cells. In addition to the low sensitivity to bacteria, this provides evidence that $\text{La}(\text{4OHCin})_3$ is non-toxic and environmentally friendly, further acting as a strong alternative to the toxic chromate inhibitors.

Interestingly the La+Cet mixture yielded similar cell densities as $\text{La}(\text{4OHCin})_3$ alone in solution, indicating the presence of $\text{La}(\text{4OHCin})_3$ in solution can nullify the sensitivity of Cet-4OHCin towards human cells whilst still providing significant reductions in bacteria density and abiotic corrosion. This makes the La+Cet mixture a very strong candidate as an environmentally friendly MIC inhibitor for high strength steel while simultaneously being relatively safe to human skin and intestine exposure (van Ravenzwaay & Leibold 2004)

5 CONCLUSIONS

The electrochemical data for the La+Cet mixture demonstrated the significant inhibition of both abiotic corrosion to a level similar to $\text{La}(\text{4OHCin})_3$, as well as the ability to reduce bacteria densities of single strains and a consortium. This is unlike the La+CetNal mixture which accelerated abiotic corrosion and the La+IMI which had an insignificant effect on microbial densities (Catubig et al. 2020).

A compatible mixture of ionic inhibitors was achieved by using the same cinnamate anion.

This mixture of Cet-4OHCin and $\text{La}(\text{4OHCin})_3$ demonstrated significant abiotic corrosion inhibition and bacteria density reductions, making it a strong candidate as an MIC inhibitor system for 80HLES.

The Cet-4OHCin compound and its mixture with $\text{La}(\text{4OHCin})_3$ retained relatively low sensitivity towards skin and intestinal cells, making it a safer and more attractive alternative than other more hazardous corrosion inhibitor materials.

6 ACKNOWLEDGEMENTS

DST Group is credited for the project funding as well as supplying the steel substrates. The Deakin University Electron Microscopy Facilities are acknowledged for use of their SEM/EDS instruments. A.E. Somers is grateful to Deakin University for funding his Alfred Deakin Post-Doctoral Fellowship. A.E. Somers and M. Forsyth are grateful for funding from the Australian Research Council through a Discovery Project, DP180101465.

7 REFERENCES

ASTM 2004, *G31-72 (reproved 2004) Standard practice for laboratory immersion corrosion testing of metals*, ASTM International, West Conshohocken, PA.

ASTM 2017, *G1-03 (Reapproved 2017) Standard practice for preparing, cleaning, and evaluating corrosion test specimens*, ASTM International.

Bajpai, D & Tyagi, VK, 2006, 'Fatty Imidazolines: Chemistry, Synthesis, Properties and Their Industrial Applications', *Journal of Oleo Science*, vol. 55, no. 7, pp. 319-29.

Barnartt, S, 1952, 'Primary current distribution around capillary tips used in the measurement of electrolytic polarization', *Journal of the Electrochemical Society*, vol. 99, no. 12, pp. 549-53.

Becker, LC, Bergfeld, WF, Belsito, DV, Hill, RA, Klaassen, CD, Liebler, D, Marks, JG, Shank, RC, Slaga, TJ, Snyder, PW & Andersen, FA, 2012, 'Safety Assessment of Trimoniums as Used in Cosmetics', *International Journal of Toxicology*, vol. 31, pp. 296S-341S.

Blin, F, Leary, SG, Wilson, K, Deacon, GB, Junk, PC & Forsyth, M, 2004, 'Corrosion mitigation of mild steel by new rare earth cinnamate compounds', *Journal of Applied Electrochemistry*, vol. 34, no. 6, pp. 591-9.

Catubig, RA, Neil, WC, McAdam, G, Yunis, R, Forsyth, M & Somers, AE, 2020, 'Multifunctional Inhibitor Mixtures for Abating Corrosion on HY80 Steel under Marine Environments', *Journal of the Electrochemical Society*, vol. 167, no. 2, p. 021503.

Chong, AL, Forsyth, M & Macfarlane, DR, 2015, 'Novel imidazolinium ionic liquids and organic salts', *Electrochimica Acta*, vol. 159, pp. 219-26.

Chong, AL, Mardel, JI, MacFarlane, DR, Forsyth, M & Somers, AE, 2016, 'Synergistic Corrosion Inhibition of Mild Steel in Aqueous Chloride Solutions by an Imidazolinium Carboxylate Salt', *Acs Sustainable Chemistry & Engineering*, vol. 4, no. 3, pp. 1746-55.

de Carvalho, C, 2018, 'Marine Bio films: A Successful Microbial Strategy With Economic Implications', *Frontiers in Marine Science*, vol. 5.

Deacon, GB, Forsyth, M, Junk, PC, Leary, SG & Lee, WW, 2009, 'Synthesis and Characterisation of Rare Earth Complexes Supported by para-Substituted Cinnamate Ligands', *Zeitschrift Fur Anorganische Und Allgemeine Chemie*, vol. 635, no. 6-7, pp. 833-9.

Docherty, KM & Kulpa, CF, 2005, 'Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids', *Green Chemistry*, vol. 7, no. 4, pp. 185-9.

Forêt, C, Stoianovici, G, Chaussec, G, Bache, Ad, Kolk, Cz & Hater, WD 2008, 'Study of efficiency and stability of film forming amines (FFA) for the corrosion protection of the carbon steel in water circuits', in *EUROCORR*, Edinburgh, United Kingdom.

Forsyth, M, Seter, M, Hinton, B, Deacon, GB & Junk, P, 2011, 'New 'Green' Corrosion Inhibitors Based on Rare Earth Compounds', *Australian Journal of Chemistry*, vol. 64, no. 6, pp. 812-9.

Forsyth, M, Seter, M, Tan, MY & Hinton, B, 2014, 'Recent developments in corrosion inhibitors based on rare earth metal compounds', *Corrosion Engineering Science and Technology*, vol. 49, no. 2, pp. 130-5.

Frank, AB & Monica, C, 1998, 'Acute Cytotoxicity Testing with Cultured Human Lung and Dermal Cells', *In Vitro Cellular & Developmental Biology. Animal*, vol. 34, no. 8, pp. 631-5.

Frankel, GS, 1998, 'Pitting corrosion of metals - A review of the critical factors', *Journal of the Electrochemical Society*, vol. 145, no. 6, pp. 2186-98.

Harvey, TG, Hardin, SG, Hughes, AE, Muster, TH, White, PA, Markley, TA, Corrigan, PA, Mardel, J, Garcia, SJ, Mol, JMC & Glenn, AM, 2011, 'The effect of inhibitor structure on the corrosion of AA2024 and AA7075', *Corrosion Science*, vol. 53, no. 6, pp. 2184-90.

Jia, R, Unsal, T, Xu, DK, Lekbach, Y & Gu, TY, 2019, 'Microbiologically influenced corrosion and current mitigation strategies: A state of the art review', *International Biodeterioration & Biodegradation*, vol. 137, pp. 42-58.

Kakooei, S, Ismail, MC & Ariwahjoedi, B, 2012, 'Mechanisms of microbiologically influenced corrosion: A review', *World Applied Sciences Journal*, vol. 17, no. 4, pp. 524-31.

Kalinowska, M, Swisocka, R & Lewandowski, W, 2007, 'The spectroscopic (FT-IR, FT-Raman and ¹H, ¹³C NMR) and theoretical studies of cinnamic acid and alkali metal cinnamates', *Journal of Molecular Structure*, vol. 834-836, no. SPEC. ISS., pp. 572-80.

Kip, N & van Veen, JA, 2015, 'The dual role of microbes in corrosion', *Isme Journal*, vol. 9, no. 3, pp. 542-51.

Kreja, L & Seidel, HJ, 2002, 'On the cytotoxicity of some microbial volatile organic compounds as studied in the human lung cell line A549', *Chemosphere*, vol. 49, no. 1, pp. 105-10.

Lopez-Garcia, J, Lehocky, M, Humpolicek, P & Saha, P, 2014, 'HaCaT Keratinocytes Response on Antimicrobial Atelocollagen Substrates: Extent of Cytotoxicity, Cell Viability and Proliferation', *Journal of Functional Biomaterials*, vol. 5, no. 2, pp. 43-57.

Loto, CA, 2017, 'Microbiological corrosion: mechanism, control and impact-a review', *International Journal of Advanced Manufacturing Technology*, vol. 92, no. 9-12, pp. 4241-52.

Seter, M, Girard, G, Hinton, B & Forsyth, M 2012a, 'Rare earth cinnamates and their corrosion inhibition mechanisms for AS1020 steel', in *ACA Corrosion & Prevention 2012: Proceedings of the Annual Australasian Corrosion Association Conference*, Melbourne, VIC AUS, pp. 1-11.

Seter, M, Girard, GMA, Lee, WW, Deacon, G, Junk, P, Hinton, B & Forsyth, M, 2015, 'The influence of organic structure and rare earth metal cation on the corrosion efficiency observed on AS1020 steel compared with La(4OHCin)₃', *AIMS Materials Science*, vol. 2, no. 1, pp. 1-15.

Seter, M, Thomson, MJ, Chong, A, MacFarlane, DR & Forsyth, M, 2013, 'Cetrimonium Nalidixate as a Multifunctional Inhibitor to Combat Biofilm Formation and Microbiologically Influenced Corrosion', *Australian Journal of Chemistry*, vol. 66, no. 8, pp. 921-9.

Seter, M, Thomson, MJ, Stoimenovski, J, MacFarlane, DR & Forsyth, M, 2012b, 'Dual active ionic liquids and organic salts for inhibition of microbially influenced corrosion', *Chemical Communications*, vol. 48, no. 48, pp. 5983-5.

Shiao, SY, Chhabra, V, Patist, A, Free, ML, Huibers, PDT, Gregory, A, Patel, S & Shah, DO, 1998, 'Chain length compatibility effects in mixed surfactant systems for technological applications', *Advances in Colloid and Interface Science*, vol. 74, no. 1, pp. 1-29.

Somers, AE, Deacon, GB, Hinton, BRW, MacFarlane, DR, Junk, PC, Tan, MYJ & Forsyth, M, 2016, 'Recent Developments in Environment-Friendly Corrosion Inhibitors for Mild Steel', *Journal of the Indian Institute of Science*, vol. 96, no. 4, pp. 285-92.

Somers, AE, Hinton, BRW, de Bruin-Dickason, C, Deacon, GB, Junk, PC & Forsyth, M, 2018, 'New, environmentally friendly, rare earth carboxylate corrosion inhibitors for mild steel', *Corrosion Science*, vol. 139, pp. 430-7.

Szklarska-Smialowska, Z, 1972, 'Influence of sulfide inclusions on the pitting corrosion of steels', *Corrosion (Houston)*, vol. 28, no. 10, pp. 388-96.

Thirumalairaj, B & Jaganathan, M, 2016, 'Corrosion protection of mild steel by a new binary inhibitor system in hydrochloric acid solution', *Egyptian Journal of Petroleum*, vol. 25, no. 3, pp. 423-32.

van Ravenzwaay, B & Leibold, E, 2004, 'A comparison between in vitro rat and human and in vivo rat skin absorption studies', *Human & Experimental Toxicology*, vol. 23, no. 9, pp. 421-30.

Videla, HA & Herrera, LK, 2005, 'Microbiologically influenced corrosion: looking to the future', *International Microbiology*, vol. 8, no. 3, pp. 169-80.

Wang, YF, Su, H, Gu, YL, Song, X & Zhao, JS, 2017, 'Carcinogenicity of chromium and chemoprevention: a brief update', *Oncotargets and Therapy*, vol. 10, pp. 4065-79.

Wranglen, G, 1974, 'Pitting and sulphide inclusions in steel', *Corrosion Science*, vol. 14, no. 5, pp. 331-49.

Xu, D, Jia, R, Li, Y & Gu, T, 2017, 'Advances in the treatment of problematic industrial biofilms', *World Journal of Microbiology & Biotechnology*, vol. 33, no. 5, pp. 1-10.