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Full Paper

Cetrimonium Nalidixate as a Multifunctional Inhibitor to Combat Biofilm Formation and Microbiologically Influenced Corrosion

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Microbial infection of surfaces and the formation of biofilms is a pervasive problem that appears in diverse fields from medical implants to corrosion of marine structures. We show here, for the first time, the multifunctional inhibitory effects of an environmentally friendly organic salt, cetrimonium nalidixate, a dual active compound based on concepts emerging from the active ionic liquids field. This salt when incorporated into a polyurethane coating leads to complete inhibition of microbiologically influenced corrosion in the presence of several bacteria strains commonly found in marine environments.

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Introduction

The exposure of material surfaces to everyday environments creates the potential for infection of the surface by a variety of bacterial species. The intrinsic roughness on the micrometer scale of many metal and ceramic surfaces provides an ideal host for the establishment of bacterial colonies. The impact of this surface infection can be very significant in a very wide range of contexts, such as damage and corrosion of structures exposed to marine environments.

Microbiologically influenced corrosion (MIC) is directly related to the undesirable accumulation of microorganisms on a metal substrate which can lead to dramatic damage and rapid metal dissolution.[1–6] MIC is responsible for at least 20 % of all corroding infrastructure costing billions of dollars in terms of replacement and repair.[7,8] Biofilm formation on metals can begin immediately after the metal is exposed to an aqueous environment in the presence of bacteria, as they adhere, colonise, and excrete polysaccharides to protect themselves under this biofilm, meanwhile creating an environment (e.g. acidic or anoxic) which is extremely corrosive.[9,10]

Microbial colonisation has the ability to facilitate either one of the anodic or cathodic reactions of the corrosion process and thus increase corrosion rate.[11,12] For example, stimulation of the anodic reaction by acidic metabolites or the cathodic reaction by microbial production of a cathodic reactant such as hydrogen sulfide, significantly enhances corrosion and can lead to dramatic metal loss and hence failure of structures in a fraction of the intended lifetime.

While coatings incorporating tributyltin compounds are very effective they have been banned from use in the marine environment due to the adverse effects they create. Copper-based solutions have gained increased attention; however, while less toxic to the environment than tributyltin-based coatings, copper-containing paints are also an environmental concern.[13]

As biofilm formation requires as its first step, adhesion of the microorganisms on a given surface, attempts to modify the surface tension of coatings has received much attention. This is usually in combination with a leachable biocide incorporated into such coatings. For example paints employing a cross linked copolymer with a hydrolysable group built into the backbone[14] as well as silicone-based release paints which provide a very smooth and slippery surface[15] have been recently described. An alternative approach, which has shown promise in combating MIC, involves the attachment of antimicrobial units to polymer coating via copolymerisation or grafting of these groups onto more conventional polymer systems. However, over long-term exposure conditions, penetration of aggressive species through the coating may still lead to failure. On the other hand, designing environmentally friendly antimicrobial compounds which can be used as pigments in more traditional coating systems or indeed in novel surface pretreatments[16] could offer a simple and inexpensive solution to combat biofilm formation, having implications for both marine infrastructure and medical implants.

Ionic liquids have shown promising outcomes as ‘active’ compounds for pharmaceutical applications and as antimicrobials.[17–19] Together with several other unique properties, the ability of such compounds to be designed with pre-determined features could potentially lead to the design of a broad range of compounds to combat microbiologically influenced corrosion and biofilm formation. Indeed we have recently shown that synergistic effects are observed when two active ions are

combined to prepare a dual active compound.[20] Herein we report the use of an environmentally friendly dual active organic salt, cetrimonium nalidixate, as an inhibitor of corrosion and biofilm formation. The individual components of this dual active compound, comprised of cetrimonium bromide (LD_{50} oral – rat – 410 mg kg^{-1}) and nalidixic acid (LD_{50} oral – rat – 2040 mg kg^{-1}), are commonly used in the pharmaceutical industry as antibiotics and antiseptics and have relatively low lethal toxicity as see from the LD_{50} data and other detailed toxicological testing.[20–22] Indeed the cetrimonium bromide compound is found in common cosmetics and has been shown to have no adverse effects[23] This novel, combined form of these active species carries the bio-activity of the constituent ions, but also allows modulation of the solubility properties such that blending into a polymer coating film with controlled release into the aqueous environment becomes possible. In previous work,[20] we investigated the efficiency of several dual active compounds and their individual constituents on inhibiting microbial growth, and showed that the cetrimonium nalidixate compound had excellent inhibition properties across a panel of different bacteria and that there was a synergy between these two constituents. Cetrimonium bromide itself is a high melting point compound and was not able to be tested due to very low solubility in the medium used. On the other hand, nalidixic acid performed well for some of the bacterial strains, but was less consistent than the dual active compound and also showed poor solubility at higher concentrations in the bacterial testing studies. Thus we focus on this dual active compound in terms of its behaviour in preventing biofilm formation when present on a metal surface or in a coating. In order to understand the efficiency of this compound, and any synergy that may arise with respect to simple corrosion inhibition (i.e. in the absence of bacteria), we also investigate the corrosion inhibition properties of the compound and the individual components using electrochemical methods and surface characterisation techniques.

The panel of bacteria, *Desulfovibrio desulfuricans*, *Citrobacter freundii*, *Enterobacter cloacae* and *Vibrio alginolyticus*, were all chosen based on their participation in microbiologically influenced corrosion and also in human implant infections. The broad spectrum of physical properties offered by these bacteria gives us the opportunity to analyse the applicability of the new multifunctional inhibitor.[24–32]

Results and Discussion

Prevention of Biofilm Formation Using Cetrimonium Nalidixate

Fig. 1 shows optical images of mild steel coupons after exposure for 10 days in a bacteria rich medium. These images show the behaviour of control specimens (untreated bare steel) in the absence or presence of bacteria, the pre-treated specimens where the steel is simply dipped into the medium containing the antimicrobial, and specimens where the metal was coated with a polyurethane containing 10 wt-% antimicrobial. As expected, the control samples, where the environmental conditions were optimal for general corrosion, exhibit a reddish iron oxide deposit on their surface. However, for the control samples containing bacteria in the solution, a slimy black deposit is also found, as a result of an extracellular polymeric substance (EPS) accumulating onto the surface of the steel.

When the samples were treated with cetrimonium nalidixate via a simple 'dip coating', remarkably different results were seen for the experiment containing the *D. desulfuricans*; as seen in Fig. 1 the coupon shows that no biofilm has settled onto the steel surface and there appears to be negligible corrosion.

The coupons which incorporate the cetrimonium nalidixate in a polyurethane coating highlight the remarkable effectiveness

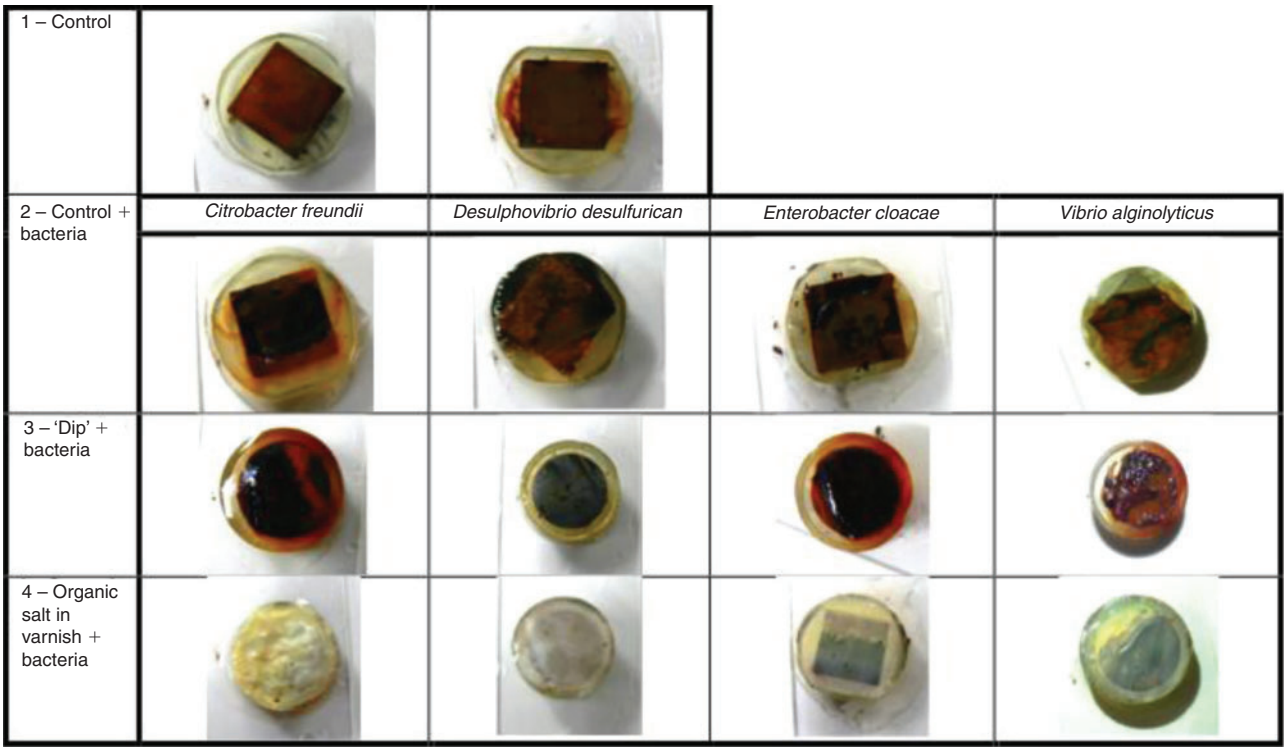

Fig. 1. Optical images obtained after 10 days immersion of treated and non-treated mild steel coupons in solutions containing bacteria.

of this compound against microbiologically influenced corrosion and biofilm formation, as can be seen in Fig. 1 where no bacterial biofilm has accumulated and very little corrosion is evident.

The biofilm layer was removed from the steel substrate using ethanol and the specimen dried before being analysed using 5 kV at 1000× magnification to obtain the SEM images seen in Fig. 2. As expected the surface of the control samples are indicative of general corrosion, showing a rough topography. As we focus on the control coupons exposed to the bacteria we see highly attacked surfaces as evidenced by the surface roughness and pitting corrosion. These features are all typical of microbiologically influenced corrosion.[33]

The optical images also indicate a dramatic effect of the cetrimonium nalidixate when applied as a 'dip' following exposure to *D. desulfuricans.* The SEM image shown in Fig. 2 also supports this; the surface is exceptionally smooth with only the artificial scribe shown as a crevice. Furthermore, despite the biofilm accumulation on the coupons exposed to the other three bacteria, in each case there is an absence of localised corrosion (i.e. pitting) observed in the control specimens indicating that even in these cases the cetrimonium nalidixate is having an effect on microbiologically influenced corrosion.

Of particular note are the images obtained for the polyurethane coated specimens when cetrimonium nalidixate is incorporated as a pigment. In all cases the artificial scribe can be seen clearly and the surfaces are relatively smooth, with the exception of the coated steel coupon exposed to *E. cloacae* where some areas of localised attack is evident near the scribe.

The corrosion attack was quantified using surface roughness measurements from optical profilometry. Profile images are shown in Fig. 3 for the control plus coupons exposed to *D. desulfuricans*. The surface roughness measurements are tabulated in Table 1.

Determination of the Corrosion Inhibition Mechanism of Cetrimonium Nalidixate

The immersion data discussed above suggests that the cetrimonium nalidixate not only has an influence on MIC and biofilm formation, but also may be a corrosion inhibitor in its own right. Therefore simple potentiodynamic polarisation experiments were used to investigate the corrosion inhibition efficacy in chloride containing solutions and to determine the corrosion inhibition mechanism of this compound. The polarisation curves in Fig. 4 demonstrates a study of the parent compounds compared with the cetrimonium nalidixate at concentrations of 0.1 mM in 0.01 M NaCl solution and Fig. 5 shows the effect of concentration for cetrimonium nalidixate; both sets of results suggest that the cetrimonium nalidixate is behaving as an anodic inhibitor. It also shows that the cathodic currents are reasonably independent of the solution used implying that the inhibitor compound has minimal cathodic effects.

It can be seen in Fig. 4 that all solutions, including the parent compounds of cetrimonium nalidixate, are having an effect on the anodic reaction. However, it is clear that the combination of cetrimonium bromide and sodium nalidixate can result in synergistic inhibition of mild steel substrates. Comparison of the values, shown in Table 2, implies that at 0.1 mM, the cetrimonium bromide is not acting as a successful inhibitor compared with the control 0.01 M NaCl solution. However, both the sodium nalidixate and cetrimonium nalidixate displayed a reduction in current density (i_{corr}), to a similar extent. This suggests that at this concentration the inhibiting effect of the multifunctional inhibitor is derived predominantly from the nalidixate anions. While there do not appear to be any

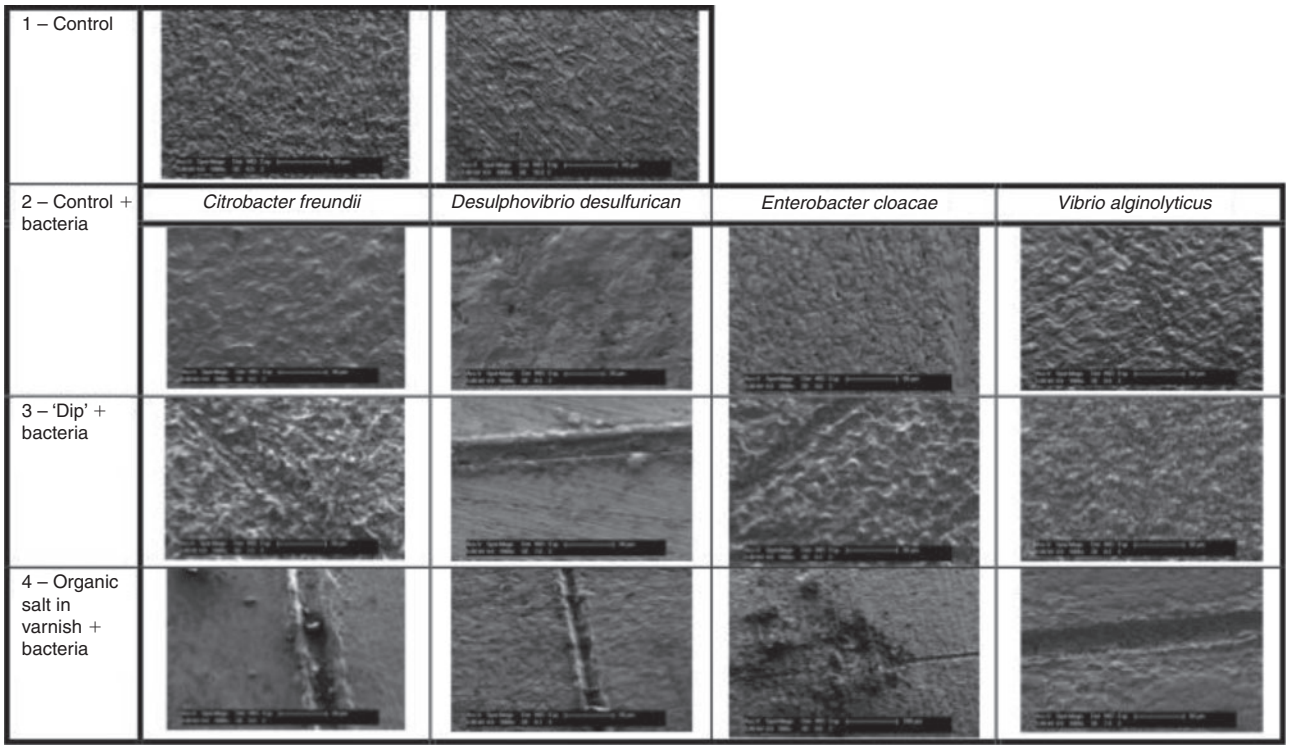

Fig. 2. SEM images of mild steel coupons after biofilm removal.

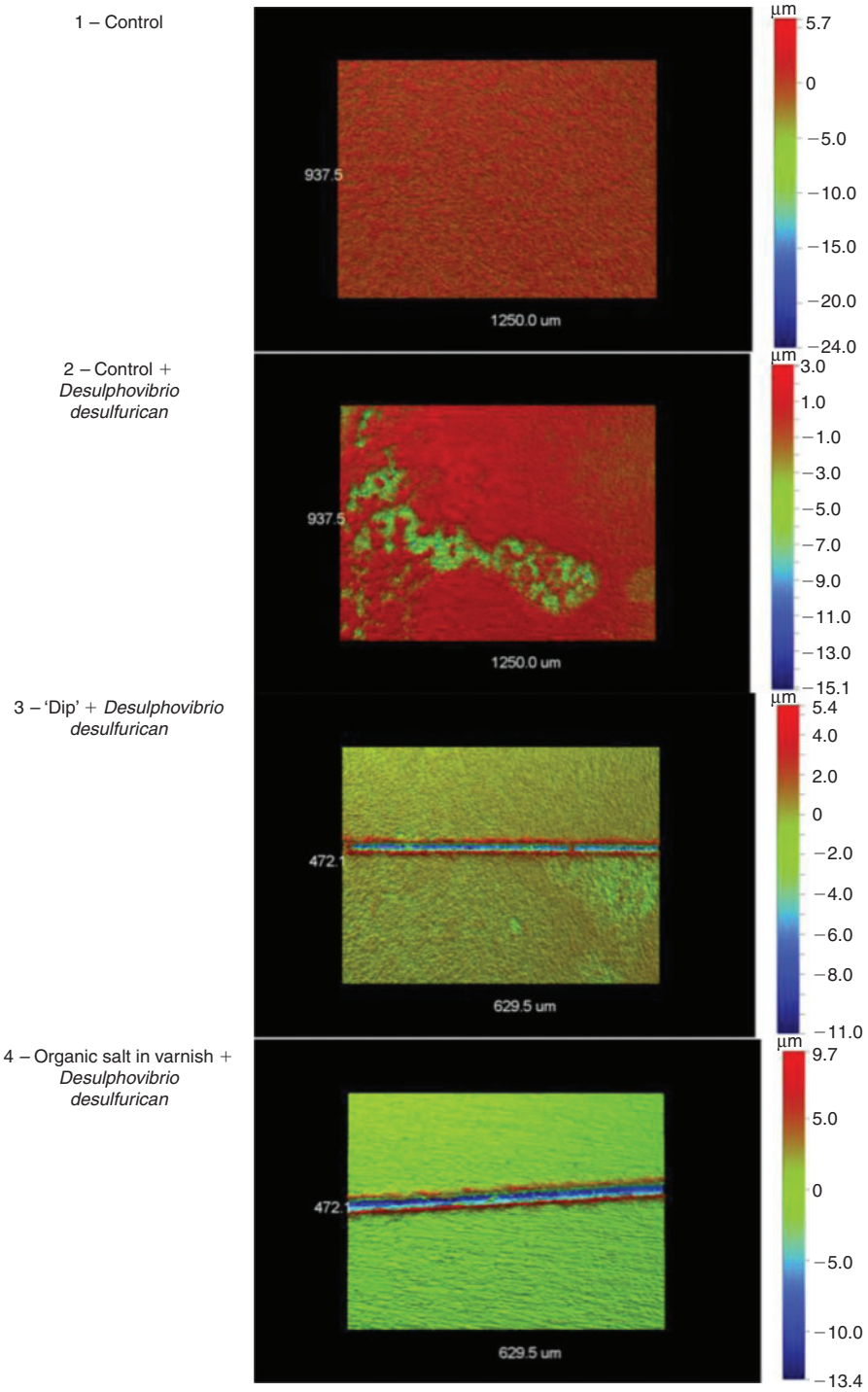

Fig. 3. Profilometry images of mild steel coupons after biofilm removal.

particularly high synergistic effects from the combination of the two active ions in terms of the corrosion current density, i_{corr}, it can be noted that the cetrimonium nalidixate polarisation curves do have a more noble value for the corrosion potential, E_{corr}, than the sodium nalidixate curves, suggesting a stronger influence on the anodic reaction.

The effect of the concentration of cetrimonium nalidixate was also investigated (Fig. 5) and the data are presented in Table 3. This data show that as the concentration increased, there was a noticeable decrease in i_{corr}, and the E_{corr} shifted to more positive potentials. This indicates an improvement in the degree of inhibition via increased suppression of the anodic reaction. It is important to note that the scatter between the duplicate results seems to vary, and is most noticeable at the lowest inhibitor concentrations. However, this does not reflect the precision of the polarisation measuring technique alone; intrinsic fluctuations in the corrosion rate as a function of time are most likely the principal contributors to the uncertainty that is apparent in the data. This is especially true at low concentrations where stochastic variation in corrosion events over the surface becomes significant. As the concentration of inhibitor increases, this becomes less of a problem, as demonstrated

Table 1. Surface roughness, R_a, of the mild steel coupons after biofilm removal

Surface roughness, R_a [μm]	1 – Control	2 – Control + bacteria	3 – 'Dip' + bacteria	4 – Organic salt in varnish + bacteria
	0.91			
C. freundii		1.12	1.05	0.32
D. desulfuricans		1.17	0.40	0.65
E. cloacae		1.87	0.90	0.60
V. alginolyticus		1.22	0.91	0.92

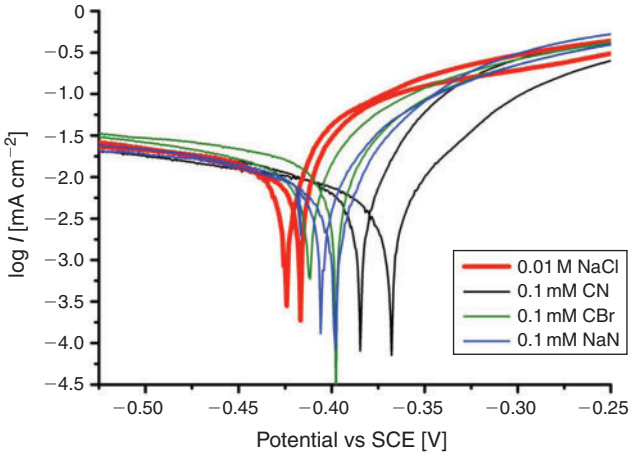

Fig. 4. Polarisation curves for cetrimonium nalidixate (CN) and parent compounds, cetrimonium bromide (CBr) and sodium nalidixate (NaN), at 0.1 mM concentration in a 0.01 M NaCl aqueous solution compared with a control of 0.01 M NaCl solution.

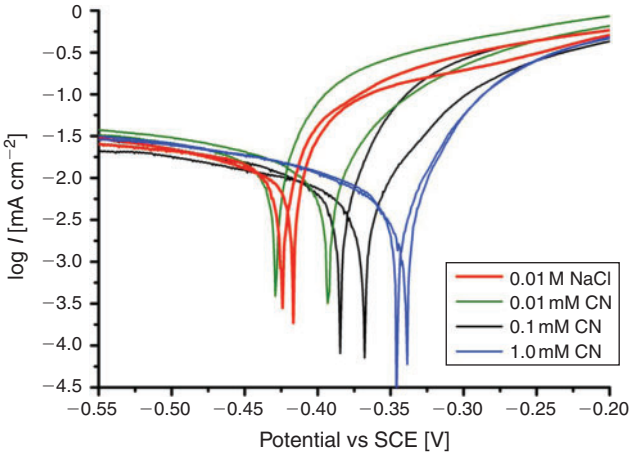

Fig. 5. Comparison of cetrimonium nalidixate (CN) at varying concentrations.

by the minimised scatter seen for the 1.0 mM cetrimonium nalidixate measurements.

Surface Characterisation Using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR spectroscopy was used to analyse the surface of steel after exposure to a chloride contaminated aqueous solution containing the cetrimonium nalidixate. The mild steel coupons

Table 2. E_{corr} and i_{corr} for 0.1 mM cetrimonium nalidixate and parent compounds

Solution	Open circuit potential [V]	E_{corr} [V]	i_{corr} [μA cm^{-2}]
0.01 M NaCl (control)	−547	−417	2.4
	−548	−424	2.6
0.1 mM cetrimonium bromide	−555	−412	2.6
	−554	−397	2.1
0.1 mM sodium nalidixate	−556	−406	1.6
	−585	−398	1.4
0.1 mM cetrimonium nalidixate	−545	−385	1.6
	−534	−368	1.1

Table 3. E_{corr} and i_{corr} data for cetrimonium nalidixate at different concentrations

Cetrimonium nalidixate concentration [mM]	Open circuit potential [V]	E_{corr} [V]	i_{corr} [μA cm^{-2}]
0 (control)	−547	−417	2.4
	−548	−424	2.6
0.01	−555	−412	2.6
	−554	−397	2.1
0.1	−545	−385	1.6
	−534	−368	1.1
1.0	−497	−339	1.1
	−510	−346	0.9

(AS1020) were immersed in a solution of 1.0 mM cetrimonium nalidixate in 0.01 M NaCl for 24 h before being analysed. An imaging FTIR microscope was used to obtain a 32 × 32 spectral array of the surface of the coupon over a 300 μm^2 area.

It is very clear from Fig. 6 that the coupon surface is not uniform. The colour image mimics the photograph of the surface, with the darker, more corroded patches in the image showing up the most intense on the array. In some cases, as in Fig. 6b, this is merely due to a high baseline; such behaviour can be explained by the roughness of the surface in these areas due to light scattering. On the corroded portion in the central region of the scan (Fig. 6c), however, there are clear peaks in the spectra in the range 1700–1200 cm^{-1}, which are indicative of organic species being present on the surface. On the less corroded areas of the coupon (Fig. 6d), these peaks were not observed. This does not exclude the possibility of there being inhibitor attached to the surface in these regions, but rather indicates that such a protective layer may be too thin to be detected with this technique.

Single point detection of one of the corroded patches on the coupon was used to obtain a baseline-corrected spectrum of the inhibitor on the surface. Comparison to an ATR-FTIR spectrum of powdered cetrimonium nalidixate shows clear similarities between the peaks from 1700–1200 cm^{-1}, although there are several significant differences, in particular the absence of bands between 2900–2800 cm^{-1} and below 1500 cm^{-1} which correspond to the cetrimonium cation (Fig. 7). A direct comparison of the spectra (Fig. 8) shows a lack of all the significant cetrimonium bromide peaks. This indicates that the cetrimonium ions are not present on the corroded region of the coupons, although they may still be depositing elsewhere on the surface.

Of particular interest are the bands that correspond to the carboxylate stretching modes, which are amongst the strongest IR absorptions (see Table 4). These can be assigned to the peaks at 1621 cm^{-1} (asymmetric, ν_{as}(COO-)) and 1260 cm^{-1}

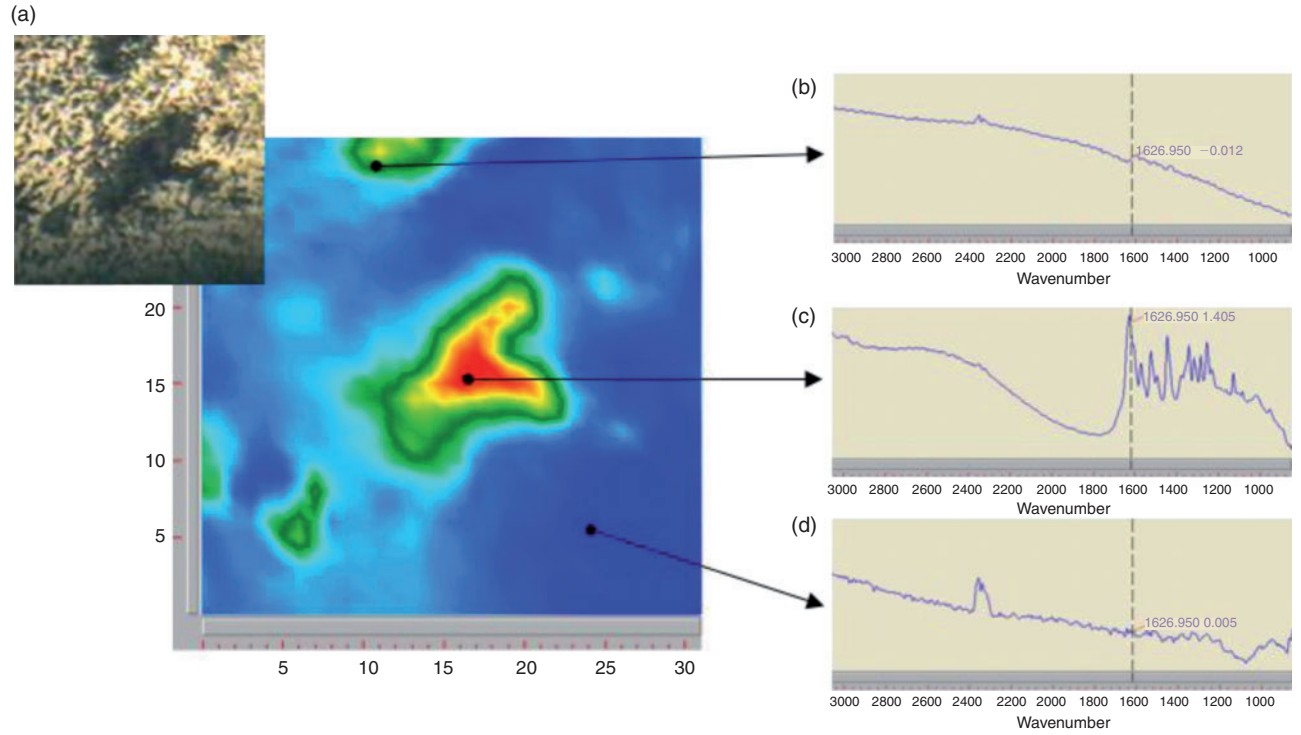

Fig. 6. Colour image based on absorbance of spectra at 1627 cm^{-1}. (a) Microscope image of scanned area. (b) Absorbance reading due to high baseline. (c) Organic material on the corroded surface. (d) Minimal detection on the less corroded areas.

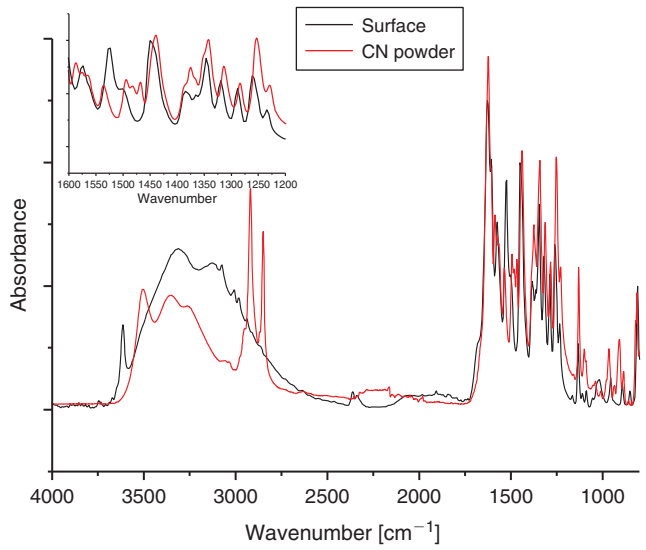

Fig. 7. A comparison of the ATR-FTIR spectra from the surface of the coupon and cetrimonium nalidixate (CN).

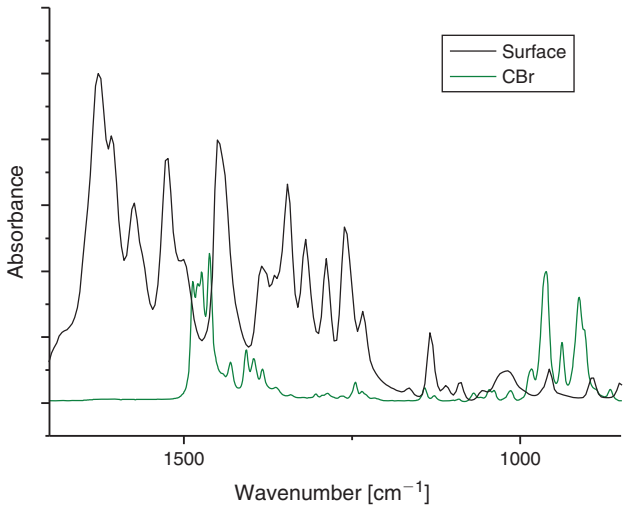

Fig. 8. ATR-FTIR spectra of the coupon surface and cetrimonium bromide (CBr).

(symmetric, ν_s(COO-)). It can be seen that the corresponding bands in the cetrimonium nalidixate spectrum sit further apart, with ν_{as}(COO-) occurring at a higher wavenumber and ν_s(COO-) at a lower one. Comparison of this separation ($\Delta\nu$) with the surface IR can eliminate the systematic differences between the two instruments. It can be seen that there is an increase of 10 cm^{-1} between sodium nalidixate and cetrimonium nalidixate (Fig. 9), which can be explained by the difference in the ionic nature of the cations where the carboxylate groups bind to a single sodium ion compared with the charge diffused quaternary ammonium on the cetrimonium.

Table 4. Summary of carboxylate bands observed in IR spectra of the nalidixate compounds and the steel surface

Sample	$\nu_{as}(COO^-)$ [cm^{-1}]	$\nu_s(COO^-)$ [cm^{-1}]	($\Delta\nu$) [cm^{-1}]
Sodium nalidixate	1621	1260	361
Cetrimonium nalidixate	1624	1253	371
Steel surface	1627	1261	366

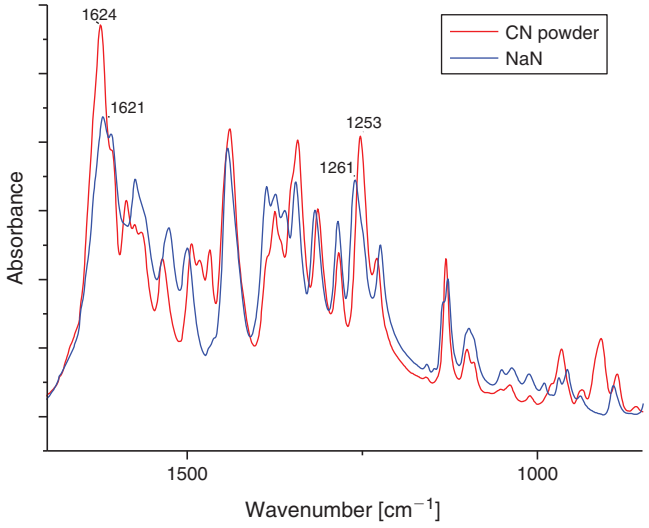

Fig. 9. IR spectra of cetrimonium nalidixate (CN) and sodium nalidixate (NaN), showing shifts in the carboxylate bands.

The peak separation in the surface IR spectrum was in between the others. The increase compared with the sodium salt is consistent with findings by Blin et al.[34] where the coordination to iron on the steel surface corresponds to an increase in Δν. This suggests that in the corroded areas, the nalidixate forms iron complexes, while the cetrimonium cation does not remain attached to the surface. This hypothesis requires further investigation and could be confirmed more conclusively by preparing an iron nalidixate compound for analysis and comparison.

Cetrimonium Nalidixate as a Multifunctional Inhibitor

In this study we have shown that a dual active, organic salt has multifunctional features that dramatically reduce the effects caused by microbiologically influenced corrosion by inhibiting both biofilm formation and the initiation and propagation of corrosion. The optical images taken after 10 days of exposure to harsh environments illustrate the severe level of biofilm accumulation under standard conditions, even after such a short period of time, and demonstrate the level of effectiveness of this new multifunctional inhibitor.

Cetrimonium bromide, the precursor to cetrimonium nalidixate, is a quaternary ammonium salt that has powerful antiseptic properties for sterilising surgical equipment and cleaning wounds etc. Surfaces that are functionalised with quaternary ammonium salts have high positive charge densities, which exert a strong electrostatic interaction with negatively charged bacterial membranes. Once adsorbed onto the bacterial cell the quaternary ammonium cation moiety diffuses through the cell wall and disrupts the cytoplasmic membrane to release several constituents, such as potassium, that cause cell death[35,36]. Quaternary ammonium salts retain their biocidal properties when they are attached to a substrate via a non-hydrolysable link. This means that surfaces coated with quaternary ammonium salt-containing polymers preserve their ability to kill microorganisms over extended periods of time.[37] An important structural feature of quaternary ammonium salt biocides is the presence of at least one long hydrocarbon lipophilic substituent at the nitrogen. Hydrocarbon chains from C_8H_{17} to $C_{19}H_{39}$ are effective, with a maximal activity around $C_{14}H_{29}$.[38]

Nalidixic acid, also a parent ion to cetrimonium nalidixate, belongs to the quinolone family of antibiotics and is effective against gram-positive and gram-negative bacteria. Depending on the concentration, nalidixic acid can be bacteriostatic, acting to inhibit the growth and reproduction of the bacteria or at higher concentrations behaves in a bactericidal manner, meaning it kills the bacterium rather than inhibiting its growth. Furthermore, nalidixic acid contains a carboxylate functional group which is well known to exhibit excellent corrosion inhibition properties; examples include salicylic acid and cinnamic acid-based compounds[34,39–43].

Recently, we have shown that this novel combination of antibacterial ions in a single compound displays enhanced antimicrobial activity with respect to the broad panel of bacteria chosen.[20] In this work we have demonstrated that, when utilised as a coating it also shows extraordinary effectiveness in combating microbiologically influenced corrosion and biofilm formation. It is hypothesised that the mechanism of action is attributed to several physical and chemical properties of the organic salt, however solubility plays an important role, in particular when the organic salt is incorporated as a pigment in a coating. The low aqueous solubility of the organic salt studied here assists in the slow release of the active ions from the coating thereby prolonging the lifetime of the coating.

Conclusions

In this preliminary work we have shown that the multifunctional inhibitor, cetrimonium nalidixate, prevents biofilm formation and initiation of pitting corrosion. The benefit of slow release inhibitors is that they can be incorporated into products such as marine based paints for ship hulls and other marine hardware and infrastructure. Furthermore, based on the results seen in this investigation, for the first time we have shown that the organic salt in liquid form can be adsorbed onto the surface of the metal and deter the settlement of bacteria.

The potentiodynamic polarisation tests proved useful in identifying the effectiveness of cetrimonium nalidixate at also inhibiting general aqueous corrosion. It was found that the salt acts as an anodic inhibitor and at a concentration of 0.1 mM this effect is predominantly due to the nalidixate ions. Increasing the inhibitor concentration led to further suppression of the anodic reaction and hence a decrease in the overall corrosion rate.

Experimental Methodology

General

Cetrimonium nalidixate was prepared according to already published methodology[20] and the bacterial strains used in this study are those identified in the literature as being associated with microbial biofilms on steel in the marine environment. *Citrobacter freundii,*[31,32] *Enterobacter cloacae,*[31,32] *Vibrio alginolyticus,*[31,32] and *Desulfovibrio desulfuricans*[31,32] were purchased from the ATCC (Cryosite Distribution Pty Ltd, Granville, NSW, Australia) as pure freeze-dried stock strains. The strains were recovered as instructed by the supplier and genomic information is also available for all of these candidates. Frozen stocks of each strain were stored in 20 % sterile glycerol in Mueller-Hinton broth (MHB) (Oxoid, Thermo Scientific, Melbourne, Vic., Australia) at −80°C until use.

Bacterial Preparation

Each bacterial strain was diluted from fresh Mueller-Hinton agar plate stocks to 0.5 on the McFarland Standard (OD_{600nm} = 0.1–0.15) and cultured aerobically overnight in MHB, or in modified Barr's medium for the *D. desulfuricans,* under

anaerobic conditions (<1 % O_2, 9–13 % CO_2) for 4 days. On the day of each experiment, bacterial strains were first diluted from broth cultures in the appropriate medium, then cultured at 37°C with shaking at 210 rpm until bacterial growth reached mid-logarithmic phase. These strains were then diluted to $OD_{600nm} = 0.1–0.15$ in MHB. Modified Baar's medium for sulfate reducers was made as directed on the ATCC datasheet for the *Desulfovibrio desulfuricans*.[31,32]

Steel Preparation and Test Solutions

Commercial mild steel alloy (0.26 % C, 0.45 % Mn, 0.14 % Cr, 0.19 % Si, 0.18 % Al, 0.35 % other, and balance Fe) was machined to 10 mm^3 specimens and mounted in epoxy resin leaving one face exposed. The steel coupons required for the immersion corrosion tests were abraded with silicon carbide paper to P1200 grit from P320 grit. Coupons were then rinsed with de-ionised water, and finally ethanol. The working electrodes for use in the potentiodynamic polarisation studies were abraded with silicon carbide paper to a P4000 grit finish from P320 grit.

The test solutions were prepared by dissolving the appropriate weights of cetrimonium nalidixate, cetrimonium bromide, and sodium nalidixate in 1 L to make concentrations of 0.01, 0.1, and 1.0 mM in 0.01 M sodium chloride solution. The solutions were exposed to a temperature-controlled laboratory atmosphere at ~22°C.

Biofilm Study

The antimicrobial compounds were applied in two different ways:

i) *As a pigment in a polyurethane coating*. Coating mixture: 10 wt-% cetrimonium nalidixate was mixed into a polyurethane based varnish (Feast WATSON Satinproof). This was ground together with a mortar and pestle. The mixture was then applied onto the polished steel substrates until an even thin film was achieved. The coating of the control sample consisted of only the polyurethane varnish component. The coatings were allowed to cure for at least 72 h before experimentation.

ii) *As a 'dip' and adsorbed onto the surface*. A small drop of the cetrimonium nalidixate as a solution was placed onto the surface of the polished steel sample and allowed to air dry for 24 h before commencement of experiment.

Using a scalpel blade, a 10 mm artificial defect was made at the centre of each coupon consisting of a coating. Immersion of the steel substrates were placed into sterile 12 well plates with the exposed side on the steel on the vertical, and the substrates were left for 10 days in a nutrient-based solution in the presence of the bacteria (bacteria absent for the control samples) at a controlled temperature of 25°C. Experiments for *D. desulfuricans* were conducted under anaerobic conditions.

Potentiodynamic Polarisation

All polarisation experiments were carried out open-to-air using 100 mL of inhibitor solution in a standard three-electrode system consisting of a saturated calomel reference electrode, a titanium mesh counter electrode, and the mild steel working electrode. The cell assembly was located in a Faraday cage to prevent electrical interference. The exposed area of the working electrode was 0.78 cm^2. After immersion the open circuit potential (OCP) was monitored for 12 h, and then the potentiodynamic polarisation experiment was carried out at a scanning rate of 0.1667 mV s^{-1}. The scans started at 200 mV more negative than E_{corr} and continued through E_{corr} for a range of 1.0 V in the positive direction. Polarisation curves were replicated in at least triplicate to assess reproducibility; the duplicates shown in the graphs represent the extent of the scatter obtained. All electrochemical measurements were conducted using a Biologic VMP multichannel potentiostat with EC-Laboratory v 10.12 software used for analysis. The current density (i_{corr}) values were calculated using Tafel extrapolation methods.

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