

“Quebracho” tannin derivative and boosters biocides for new antifouling formulations

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Abstract The growth of fouling communities on ships' hulls causes economic losses due to increased fuel consumption and to the deterioration of the metallic substrate by corrosion. Antifouling paints are formulated to avoid the settlement of these organisms and may contain biocides as active compounds. The objective of this research was to evaluate the antifouling performance of paints formulated with a “quebracho” tannin derivative (zinc “tannate”) and “boosters” (secondary biocides). The “boosters” used in this study were bismuth lactate, a zeolite exchanged with silver cations, and the same zeolite modified with silver chloride. Bioassays with “boosters” solutions were carried out employing *Artemia persimilis*. Soluble matrix antifouling paints were formulated and their action was assessed in a natural sea water environment. Results showed that the bismuth lactate resulted in a coating with almost triple service life of the antifouling paints with zinc “tannate.” Paints formulated with the silver composites behaved slightly better than the control paint.

Keywords Coatings, Tannin, Biocides, Antifouling paints, Composite materials

Introduction

From ancient times, different types of coatings have been used to prevent the settlement of marine organisms on ships' hulls. The growth of the biofouling community on ships' hulls causes economic losses due to the increased fuel consumption required to keep the cruising speed.

They may also deteriorate the metallic substrate by corrosion. Antifouling paints are formulated to avoid the settlement of these fouling organisms, and most of them contain biocides in their formulation. Copper and copper oxides are commonly employed as biocides, but their use is being restricted because recent reports have shown high levels of copper in marine waters.¹ Apart from copper, other metals with antifungal and antibacterial properties, such as zinc and silver, have been widely used in advanced coating technology.²

More recently, natural products are being employed as possible replacements for conventional metallic biocides.³ In this context, it was demonstrated that tannins possess a narcotic effect on *Balanus amphitrite* (barnacle) nauplii; therefore, they became materials of interest in the field of antifouling coatings.⁴ Tannins are polyphenols produced naturally by different plants which use the former as a defense against bacteria, fungi, etc.^{5,6} Their influential action rests upon three different but complementary mechanisms: (a) the ability of tannin to subtract metallic ions involved in microorganisms' vital processes, (b) the inhibition of the enzymatic activity, and (c) the influence on the oxidative phosphorylation at the membrane level.⁷ Tannin extracts are not used directly in paints because they are highly soluble in water. However, the employment of less-soluble compounds, obtained by coagulation with metallic cations, such as aluminum, copper, and zinc, was reported in the literature.⁴ Aluminum tannate was found to last for 1 month after being incorporated into a gel, while a paint formulated with copper “tannate” presented no macrofouling after 12 months.^{4,8} The antifouling efficiency diminished when copper was substituted by zinc in the corresponding “tannate”,⁹ but service life could be extended if suitable boosters are selected.

“Boosters,” or secondary biocides, enhance antifoulants' action, the concentration of which in the paint film may be reduced. The impact of these “boosters”

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on the environment is still a matter of discussion because some of them are chloride derivatives of organic compounds.^{10,11} Compounds with recognized antibacterial or antifungal activities which have been used previously in the industry, in medical therapy, etc., are also candidate substances to be employed as secondary antifoulants.^{2,12–14}

The antifouling efficiency of a paint containing zinc “tannate,” WW rosin (water white variety commonly used in antifouling paints), and styrene acrylate copolymer (SAC) as the binders proved effective for a period up to 3 months in natural sea water (NSW).⁹ The aim of the present investigation was to extend the service life of this paint by incorporating new secondary biocides or “boosters” having less environmental impact than those commonly employed.^{12,15} Three “boosters”—bismuth lactate, silver-exchanged zeolite, and silver chloride supported on zeolites—were selected because of their antimicrobial characteristics. These compounds had previously been employed in the food industry and medical applications.^{16–21}

Materials and methods

Pigment and “boosters” preparation and characterization

The antifouling pigment was obtained from “Quebracho colorado” (*Schinopsis balansae*) tannin. “Quebracho colorado” is a characteristic argentine tree which contains high amounts of polyphenols, easily extractable by hot water. In order to obtain the corresponding “tannate,” the tannin was dispersed in distilled water, and 0.65 M zinc nitrate was added to the suspension with continuous stirring. The pH was adjusted to 4 with the addition of 0.5 M sodium hydroxide after the addition of zinc nitrate, the pH being controlled periodically.⁹ The zinc “tannate” (TZn4) was separated from the supernatant by filtration, washed three times with distilled water, centrifuged, and dried at room temperature under an air current and, finally, in an oven at $50 \pm 5^\circ\text{C}$.

The amount of tannin in TZn4 was obtained by indirect gravimetry, heating the solid at $100 \pm 5^\circ\text{C}$ to eliminate water. On further heating to $800\text{--}900^\circ\text{C}$, zinc was converted to zinc oxide and weighed to estimate the zinc content in TZn4. The density of the pigment, needed for paint formulation, was determined according to a standard specification (ASTM D 1475).

The solubility of TZn4 in artificial sea water (ASW) was obtained by dispersing 2 g of the pigment in 100 mL of ASW at $20 \pm 2^\circ\text{C}$ for 24 h. The ASW was prepared according to ASTM D 1141. The solid was separated by filtration; both tannin, as total polyphenols (TP), and zinc were determined in the filtrate. The concentration of tannin was obtained spectrophotometrically at 750 nm using the Folin-Denis reagent (FDr).^{22–24} Zinc was determined by atomic absorption spectroscopy.

Bismuth lactate and bismuth oxichloride were reagent grade chemicals purchased from Merck. The

amount of bismuth in the saturated solution was determined spectrophotometrically at 465 nm complexing it with thiourea in nitric acid medium.²⁵ The concentration of lactate in the saturated solution was determined by UV-Vis spectrophotometer at 215 nm, employing sodium lactate solutions to build up the calibrations curve.

The X-ray diffraction pattern of the zeolitic rock was recorded employing a PAN analytic X'Pert PRO equipment with Cu anodes and a graphite monochromator. The exchanged zeolites were prepared as follows: 50 g of the zeolitic rock was ground and washed twice with distilled water and boiled for 1 h in HNO_3 0.2 M to eliminate iron. The complete elimination of iron was accomplished by means of a magnetic stirrer. Then, the zeolite powder was washed again with distilled water and exchanged for 24 h with NaNO_3 2 M to put the zeolite in the Na^+ form. Finally, the sodium-exchanged zeolite was brought into contact with 1 M AgNO_3 for 1 day to exchange Na^+ with Ag^+ (ZAg). The exchanged zeolite was separated by centrifugation, washed twice with distilled water, and dried at $50 \pm 5^\circ\text{C}$ in a stove. The ZAg was dispersed in 0.1 M NaCl, previous to centrifugation, to obtain the zeolite modified with AgCl (ZAgCl). Afterwards, it was centrifuged and washed twice with distilled water.²⁶

The amount of silver exchanged by ZAg was determined by titration with KSCN, employing the Volhard's method.²⁷ Previously, ZAg was back exchanged with 10% NaCH_3COOH for 2 h to replace all the silver ions with sodium. After being in contact for 24 h, the supernatant was separated by vacuum filtration, and the solid precipitate was washed five times with distilled water. The supernatant and the washing solutions were placed in a volumetric flask, and 50 mL of this solution were titrated with KSCN 0.0488 N employing a 1.25 M $\text{Fe}(\text{NO}_3)_3$ nitric solution as indicator.

A given amount of ZAgCl was brought into contact with a $\text{NH}_4\text{NO}_3/\text{NH}_3$ 2 M/2 M solution and stirred for 2 h, just to dissolve silver chloride by complexation. After 24 h, the supernatant was separated as before, washed five times, and then placed in a volumetric flask. As AgCl precipitates in acidic medium, the titration was done with a 0.0184 M KCN solution according to Liebig's method with the modification introduced by Denigès to detect the end point.²⁸ The excess of CN^- was titrated with AgNO_3 0.05 N solution employing 1 mL of 10% IK as indicator.

All the determinations were done in duplicate employing two different samples in the case of the exchanged zeolites.

Bioassays

The biocidal action of the different compounds and “composites” employed in this research was assessed by screening bioassay to provide adequate information for the development of antifouling coatings.²⁹ *Artemia* (brine shrimp) larvae are used extensively as test

organisms in research studies and applied toxicology because of the commercial availability of dried cysts from which live material can be hatched easily.^{30,31} Toxicity tests with *Artemia* nauplii are quick and inexpensive; they are also found to possess good predictive potential. These biological assays constitute an alternative to others with crustacean larvae, principally those from barnacles (*B. amphitrite*, for example).^{32,33}

The “booster” concentration which kills 50% of the *Artemia* nauplii within 24 h (LC₅₀) was determined experimentally using a short-term toxicity test.³⁰ *Artemia persimilis* eggs commercially obtained were hatched in ASW (ASTM D1141-98) at 24 ± 2°C for 24 h under gentle aeration with an aquarium pump.³⁰ Finally, after 48 h, a homogenous population of instar II–III nauplii was obtained and used for the bioassays.

Zinc tannate and Boosters-saturated solutions in ASW were prepared at 25°C for further dilutions to determine LC₅₀.

Several dilutions of the zinc tannate-saturated solution in ASW were prepared in such a way that they finally contained 10, 50, 100, 120, and 200 mg/L of TP. The corresponding zinc cation concentrations were 0.1, 0.4, 1.0, and 1.6 mg/L, respectively.

From the saturated solution of bismuth lactate in ASW, adequate dilutions were made to obtain different concentrations of lactate in ASW: 950, 930, 900, 750, 400, and 200 mg/L. The corresponding Bi(III) concentrations were: 0.10, 0.09, 0.08, 0.04, 0.02, and 0.01 mg/L, respectively. In addition, lactate and bismuth ions were tested separately to determine the bioactivity of each species. A sodium lactate solution in ASW was prepared by neutralizing a 10,000 mg/L lactic acid solution with 1 M NaOH. This solution was diluted with ASW to obtain different concentrations of sodium lactate: 2500, 1000, 500, 250, 100, and 50 mg/L. The final pH value obtained for these solutions was between 7 and 8. On the other hand, an oxichloride bismuth saturated solution in ASW was prepared as a source of Bi(III) to determine LC₅₀. Aliquots of 50, 25, 12.5, and 6.25 mL were each diluted to 100 mL with ASW.

The saturated solutions obtained by dispersing ZAg or ZAgCl in ASW were diluted as follows: 25, 12.5, and 6.25 mL up to 100 mL of ASW. The saturated solutions were also tested to determine LC₅₀.

The biological assays to determine LC₅₀ for the different “boosters” were carried out by placing 10 nauplii in different vessels with 10 mL of the corresponding solution. A vessel with ASW was used as control. Three replicates were set up for each concentration and for the control. After an incubation period of 24 h, dead larvae were counted and LC₅₀ values were calculated by Probit analysis.³⁴

Formulation, preparation, and application of the antifouling paints

The paints were prepared employing WW rosin, provided by Cicloquímica, as film-forming material.

Table 1: Paint composition expressed as percentage of solids by volume and by weight

Components	Composition (vol%)	Composition (wt%)
TZn4	34.1	36.5
Chalk	8.7	17.6
WW rosin	32.3	26.1
Styrene acrylate copolymer	16.9	13.0
Plasticizer	6.6	5.8
Pine oil	0.5	0.3
Castor oil	0.9	0.7

Table 2: Boosters incorporated into the paints

Paints	A	B	C	D
Secondary biocide (booster)	–	Bismuth lactate	ZAg	ZAgCl

The SAC, purchased from Arquimex, was added as co-binder, with the ratio of WW rosin/SAC being 1.9/1, by volume. The plasticizer was chlorinated paraffin with 42% of chloride (Cereclor 42); the ratio SAC/plasticizer was 2.6/1, by volume.

Rosin was dissolved in xylene employing a high speed disperser, while SAC was dispersed in xylene together with the plasticizer. The pigments (chalk and TZn4) were dispersed in a fraction of the solvent in a ball mill jar. Then, both vehicles and pine oil were incorporated into the jar with the pigments and dispersed for 24 h. Finally, castor oil gel was added.⁹ Paint composition can be seen in Table 1. Pine and castor oil were obtained from a local supplier, Cicloquímica.

From the base formulation reported in Table 1, four paints were prepared by adding different boosters (Table 2). Paint A only contained TZn4, while the other paints (B–D) contained TZn4 and a “booster.” These “boosters” were bismuth lactate (paint B), ZAg (paint C), and ZAgCl (paint D); their contents were 2%, by weight, with respect to the total pigment concentration. Control paints were also elaborated with the same formula as paints A–D, but zinc “tannate” was replaced in volume by TiO₂.

Tests in sea water environment

Paints were applied by brush on 8 × 12 cm sand-blasted acrylic panels to match a total dry film thickness of 200 ± 5 µm; 24 hours passed between the application of each coat to allow the system to dry adequately. Not more than 48 h elapsed before immersion of the painted panels in NSW. Panels were attached to aluminum holders and immersed at a depth of 50–60 cm in Mar del Plata harbor (38°08'S

Table 3: Settlement degree assessment

Settlement degree	Efficiency (%)
0, no settlement	100
1, little	80
2, limited	60
3, common	40
4, abundant	20
5, completely covered	0

and 57°31'W) during the high biological activity season. Biological and hydrological conditions of this harbor had been studied previously.³⁵ Besides bacteria and diatoms, other species like barnacles, serpulidae, ascidians, bryozoans, algae, and polychaetes are the frequent fouling organisms encountered on hard substrates in Mar del Plata harbor.³⁵

The panels were observed with the naked eye periodically and evaluated according to a six-degree scale (Table 3).^{9,36–38} The fouling settlement (FS) 0–1 implied no settlement or little settlement of soft foulers onto the panel. An increasing rating means an increased fouled area and a decreased efficiency of the antifouling paint. A rating higher than 1 implies that hard foulers like barnacles and serpulidae were attached onto the paints. Only panels qualified as 0 or 1 are considered satisfactory.

Results and discussion

Pigment and “boosters” characterization

The analysis of TZn4 showed that it contained 86.70% of tannin and 0.53% of zinc, the rest being water (12.77%). The density of TZn4 was 1.43 g/mL. The concentration of TP in the saturated solution in ASW was 3.33 g/L, while that of zinc cation was 27 mg/L. The metal content of the antifouling paints tested in this research was strikingly lower than that of commercial paints which may contain 20–40% of metals.³⁹ The addition of boosters (~2%) did not increase metal content significantly.

The zeolitic rock employed in this investigation contained 15–30% of clinoptilolite, the rest being quartz (15–30%), calcite (15–30%), plagioclass (5–15%), and clay (5–15%). The amount of silver ion in exchanged ZAg was 25.1 meq/100 g of ground rock, while the modified ZAgCl contained 75.6 meq/100 g.

Bioassays

After an incubation period of 24 h, LC₅₀ of zinc “tannate” was found to consist of 117 ± 24 mg/L of TP and 0.97 mg/L of zinc cation.

LC₅₀ of bismuth lactate was found to consist of 920 ± 28 mg/L of lactate and 0.09 ± 0.01 mg/L of

bismuth ion. None of the sodium lactate solutions was lethal to nauplii, and the results were similar to the control. The bioassay carried out with bismuth oxichloride solutions showed that the LC₅₀ was achieved only with the saturated solution. This would indicate the greater biocidal efficiency of bismuth lactate than those of both lactate and bismuth ions.

When bismuth lactate dissolves, bismuth ion undergoes an intense hydrolysis:



where L represents the lactate anion (CH₃CH(OH)COO⁻). The hydrolysis of Bi(III) occurs as follows³⁹:



The solubility product constant is $K_{\text{sp}} = K^{-1} = 2.09 \times 10^{-7}$.

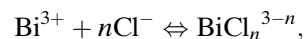
The lactate anion also hydrolyzes as follows:



$$K_h = \frac{K_w}{K_a} = \frac{10^{-14}}{1.4 \times 10^{-4}} = 7 \times 10^{-11}$$

The equilibrium constant for the formation of the precipitate is much higher than the hydrolysis constant, K_h , for lactate. Therefore, it was concluded that the formation of BiOCl is greatly favored with the concomitant diminution of the pH of the medium.

Other reactions usually could take place in sea water, but owing to the very high chloride concentration and the values of the equilibrium constants, those with chloride may be considered the most important ones. The precipitation of BiOCl would explain the low pH, 3.3, registered in the saturated solution of the bismuth lactate in ASW. The solubility of bismuth may increase by chloride complexation⁴⁰:



$$\beta_n = \frac{[\text{BiCl}_n^{3-n}]}{[\text{Bi(III)}][\text{Cl}^-]^n} = 2.5 \times 10^7 \quad \text{where } n = 1 - 6$$

The logarithms of the accumulative or gross constants (β) are $\beta_1 = 10^2$, $\beta_2 = 4.04$, $\beta_3 = 5.3$, $\beta_4 = 7.47$, $\beta_5 = 8.04$, and $\beta_6 = 7.36$.⁴⁰ The solubility (S) of bismuth in a chloride medium depends on its value and on the chloride concentration.

$$S = [\text{Bi(III)}] + \left[\sum_{n=1}^{n=6} \text{BiCl}_n^{3-n} \right]$$

$$S = [\text{Bi(III)}] + \sum_{n=1}^{n=6} \beta_n [\text{Bi(III)}] [\text{Cl}^-]^n$$

$$S = [\text{Bi(III)}] + \left(1 + \sum_{n=1}^{n=6} \beta_n [\text{Cl}^-]^n \right)$$

$$S = \frac{K_{sp}[H^+]^2}{[Cl^-]} \left(1 + \sum_{n=1}^{n=6} \beta_n [Cl^-]^n \right)$$

The solubility of bismuth lactate in the saturated solution in ASW at pH 3.3, calculated in this way, was 9.15×10^{-7} M (0.19 ppm) and may decrease as the pH increases. The experimental solubility was found to be 1.95 ppm.

The concentration of Bi(III) in the saturated solution of bismuth oxychloride in ASW may be calculated taking into account the adequate equilibrium:



$$\log K_{sp} = -30.75$$

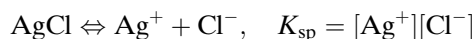
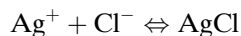
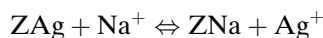
$$K_{sp} = [Bi^{3+}][Cl^-][OH^-]^2$$

The concentration of Bi(III) was found to be 7.98×10^{-15} M, too low to be measured spectrophotometrically.

These results would indicate that there are two factors which determine bismuth lactate activity. On the one hand, the acidification of the medium, due to the hydrolysis of Bi(III), with direct consequences on the survival of marine organisms. On the other hand, Bi(III) biocidal activity was proven in the bioassay with bismuth oxychloride saturated solution which did not show any pH changes. The concentration of Bi(III) in the bismuth lactate solution increased sensibly with respect to the oxychloride and at the same time the pH is decreased, thus enhancing the biocidal action of bismuth lactate.

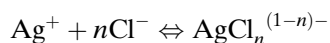
In spite of the reported action of silver on bacterial growth,^{14,16,18,41} bioassays with modified zeolites showed no lethal effect at any concentration. This fact may be attributed to the low equilibrium concentration of silver in ASW.

The ionic equilibrium of ZAg in ASW may be described according to the following equations:



The solubility product constant (K_{sp}) of silver chloride was reported to be equal to 1.78×10^{-10} .

The solubility of silver chloride changes in sea water because of the presence of chloride governed by the following complexation reactions:



where $n = 1-4$.

The accumulative equilibrium constants (β) may be defined by the following equation:

$$\beta_n = \frac{[AgCl_n^{(1-n)-}]}{[Ag^+][Cl^-]}$$

The logarithms of these equilibrium constants are as follows⁴⁰:

$$\beta_1 = 3.44, \beta_2 = 5.3, \beta_3 = 5.49, \beta_4 = 5.4.$$

The solubility (S) of silver chloride in sea water is commonly calculated according to the following equations:

$$S = [Ag^+] + [AgCl] + [AgCl_2^-] + [AgCl_3^{2-}] + [AgCl_4^{3-}] = [Ag^+] + K_1 [Ag^+][Cl^-] + \beta_2 [Ag^+][Cl^-]^2 + \beta_3 [Ag^+][Cl^-]^3 + \beta_4 [Ag^+][Cl^-]^4$$

$$S = [Ag^+] \left(1 + K_1 [Cl^-] + \beta_2 [Cl^-]^2 + \beta_3 [Cl^-]^3 + \beta_4 [Cl^-]^4 \right)$$

$$\text{where } Ag^+ = \frac{K_{sp}}{[Cl^-]}$$

According to the preceding equations, the solubility of silver chloride in the sea water is 4.51×10^{-5} M (6.4 ppm), considering that the concentration of chloride in ASW was 0.56 M. This value is a little higher than that in pure water (1.33×10^{-5} M) because the chloride began to dissolve the silver chloride. Evidently, these low values of silver in solution are not enough to inhibit the growth of the bacterial film before macrofouling settlement. This is why the paints containing silver-modified zeolites failed after 2 months of exposure as will be seen in the next paragraph.

Tests in sea water environment

This assay was conducted in triplicate. Figures 1 and 2 show representative records obtained after 3, 6, and 9 months of exposure of the tested paints in NSW.

Figure 1 depicts the result obtained with the paint A. After 3 months, the FS was 5, thus indicating that the paint failed completely. Ascidiens and bryozoans were attached to the panel in a greater proportion (Fig. 1). The paint film was in good condition, but the efficiency of the paint was unacceptable. The control paint A, with TiO₂ instead of TZn4, obtained a similar FS qualification (Fig. 2).

Paint B showed the best results: the FS was 1 after 6 months of immersion, and 1–2 after 9 months (Fig. 1). However, after 10 months, the paint failed completely; serpulidae, barnacles, bryozoans, and ascidiens were found to be attached to the panels' surface. After three months of immersion, the panels coated with the zeolites containing paints (Paints C and D, Fig. 1) showed a much better performance than the control; however,







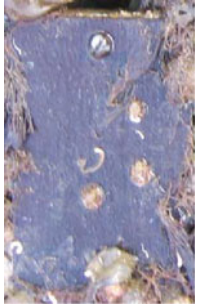





Paints	3 months	6 months	9 months
A			
FS	5	5	5
B			
FS	1	1	1–2
C			
FS	1–2	3	5
D			
FS	2	4	5

Fig. 1: Panels with experimental paints (A, B, C, and D) immersed in sea water environment and their fouling settlement (FS). All the formulations included TZn4 as the principal biocide

some slime, bryozoans, barnacles, and polychaetes were found to be attached to them. The FS ratings of these paints were 1–2 and 2 for paints C and D, respectively.

The antifouling performance of the paint pigmented with zinc “tannate” did not differ significantly from the control paint containing titanium dioxide. However, paints’













Control paints	3 months	6 months	9 months
A			
FS	5	5	5
B			
FS	3	4-5	5
C			
FS	3	4-5	5
D			
FS	3-4	4-5	5

Fig. 2: Control panels of paints A, B, C and D immersed in sea water environment and their fouling settlement (FS). All formulations contained TiO₂ in place of TZN4 biocide

antifouling performance improved when boosters were incorporated into the paint formulation. There seemed to be a synergism between the boosters and zinc “tannate”

because the coating performance was better when both compounds were employed together. This finding was particularly noticeable in the case of bismuth lactate.

Conclusions

- The study of ionic equilibrium in sea water could help to identify the bioactive species and explain the antifouling action of biocides.
- Low quantities of “booster” can modify the behavior of marine antifouling paint.
- The paints with the exchanged zeolite were more efficient than the blank, but the zeolite did not extend the paint’s useful life significantly.
- Antifouling paint with zinc “tannate” and the “booster” bismuth lactate showed a higher efficiency up to 9 months of exposure to NSW.
- Bismuth lactate was proven to be active in the bioassays, bismuth being the more active species, but its most striking effect could be attributed to the decrease of the pH of ASW effected by bismuth ion hydrolysis.

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