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Procedia Materials Science 1 (2012) 259 – 265

Procedia Materials Science

www.elsevier.com/locate/procedia

11th International Congress on Metallurgy & Materials SAM/CONAMET 2011.

Caesalpinia spinosa tannin derivatives for antifouling formulations

N. Bellotti[a]*, B. del Amo[a], R. Romagnoli[a,b,]

[a] *Centro de Investigación y Desarrollo en Tecnología de Pinturas CICBA-CONICET, Calle 52 e/ 121 y 122, B1900AYB, La Plata, Argentina*

[b]*Facultad de Ciencias Exactas - Universidad Nacional del la Plata,La Plata, Argentina.*

Abstract

AF coatings prevent the undesirable accumulation of a wide variety of marine organisms (biofouling) on ship hulls can lead to significant increased costs, principally by increased fuel consumption. The bioactive compounds (biocides) used in antifouling coatings, like tributyltin (TBT), are banned for their toxic action towards non-target marine organisms. As a consequence, the development of alternative AF coatings with new natural products as biocides inhibit is a key factor. In this approach, tara *(Caesalpinia spinosa*) tannin (TT) derivatives are being proposed as a promising solution.

The aim of this research work was, primarily, to explore the possible application of a natural and abundant product, such as the TT, in the preparation of AF coatings. So, two TT derivatives were obtained at different temperatures (20ºC and 60ºC) as zinc "tannate" and characterized to be employed as bioactive compounds in AF coatings. Previous to the immersion in natural environments, the dissolution of TT from coatings in artificial sea water was studied. Antifouling paints with a soluble matrix were formulated and their action was assessed in natural sea water environment (Mar del Plata harbor).

Keywords: antifouling coatings, tara tannin, biocide, leaching rate.

* Corresponding author. Tel.: (0221) 483-1141/44; fax: 54-221-427 1537
E-mail address: pinturashigienicas@cidepint.gov.ar.

doi:10.1016/j.mspro.2012.06.035

1. Introduction

The accumulation on hard substrates of a wide variety of marine organisms (algae, barnacles, mussels, polychaetes, ascidians, bryozoans, etc.) is known as biofouling. One of the main consequences of fouling is the increased fuel consumption to maintain the cruising speed as consequence of the increased friction of the vessel hull with water. It is estimated that the increased friction could be as high as 70% for ships without antifouling (AF) protection Schultz, 2007. Taking into account the importance of the shipping industry and the international trade, economical losses caused by fouling, according to projections for 2020, could originate a leakage of U$S 150 billion per year all around the world Hellio, 2009.

One of the most effective compounds for AF coatings is tributyltin (TBT) but International Maritime Organisation (IMO) banned its use on ships because it accumulated in the environment and resulted toxic to non-target marine organisms. It was established that vessels should eliminate completely organotin compounds in AF systems from September 2008 Almeida, 2007. The must commonly used replacement for TBT is cuprous oxide; however, there is concern about the high concentration of this metal in coastal areas and the potential damage that it could cause to marine organisms Yebra et al., 2004.

The replacement of these biocides by more environment friendly ones is a matter of great interest in this sense tannins have been proposed as a promising solution Clare, 1998; Stupak et al., 2003; Chambers, 2006. Tannins are polyphenols produced by different plants as part of their defense system against pathogenic agents Laks, 1987. As it was demonstrated that they possess a narcotic effect on nauplii of *Balanus amphitrite*, they became of interest in the field of AF coatings Clare, 1998. Because their higher solubility in water, tannins extracts, are not used directly in coatings. Recent research has shown that quebracho tannin coagulated with metallic cations, such as aluminium, copper and zinc, to form the corresponding "tannates" was less soluble than the original tannin Stupak et al., 2003; Pérez et al., 2007; Bellotti et al., 2010.

A different kind of tannin extract, tara tannin (TT), obtained from the fruits pods of tara tree (*Caesalpinia spinosa*) was used in this research. *Caesalpinia spinosa* is a small thorny tree whose distribution covers various arid areas of South America with high amount of tannin (40-60 wt %) in their fruits pods with an average yield of 20-40 kg per tree per year Hasalam, 1966; Gálvez, 1997. TT has different industrial applications such as leather tanning. It is also employed in the food industry to clarify wines, substitute malt and give body to beer. The TT is mainly of the hydrolyzable type and consists of basic units of gallic acid linked by ester unions to quinic acid Hasalam, 1966; Gálvez, 1997.

The aim of this research work was assess the possible application of two TT derivatives obtained at different temperatures (20ºC and 60ºC) as zinc "tannate" as bioactive compounds in AF coatings. Therefore preliminary screening was carried out in laboratory and, then, in natural sea water environment in static conditions. The derivative was characterized in relation to its bioactivity with larvae of *Artemia*. AF coatings with a soluble matrix and the "tannate" as biocide were formulated. The tannin leaching rate of these coatings in artificial sea water (ASW) was determined by spectrophotometry using the Folin-Denis reagent (FDr). The ASW was prepared according to ASTM D 1141. Their biocidal activity was assessed in natural sea water (NSW) in Mar del Plata harbor in Argentina (38º08´17´´S-57º31´18´´W).

2. Materials and Methods

2.1. Precipitation curves

The variables for precipitating TT with zinc cation were optimized through different suspensions containing the corresponding precipitation curve. In order to obtain this curve, 1.000g of TT, dispersed in distilled water for 10 minutes, and then, 0.50, 1.25, 2.50 and 5.00ml, respectively, of 0.65 M zinc nitrate were prepared. Distilled water was added to match a final volume of 25.0 ml. After the addition of zinc nitrate, the suspensions were stirred for 5 minutes and the pH adjusted to 4.0 using 0.5M NaOH. The suspensions were stirred during 1 hour more, the pH was adjusted again and the systems were allowed to settle down for 24 hours to accomplished complete flocculation. Finally, an additional pH adjustment was carried out under stirring and, then, the solids were separated by centrifugation and dried at 50 ± 5ºC until constant weight.

2.2. Preparation of zinc "tannate"

Zinc "tannate" was prepared taking into account the suitable ratio of reactants according to the results obtained from the analysis of the precipitation curve.

In this sense, 150.0g of TT was dispersed in 3.0L of distilled water and 750 ml of 1M zinc nitrate was added under constant stirring to precipitate TZn at 20ºC (TZn20). Immediately, pH was adjusted to 4.0 using a 0.5M NaOH and the suspension was stirred during 1hour. The suspension was kept overnight without stirring and pH was adjusted once more before separating the solids from the supernatants. The solids were washed with distilled water by decantation, centrifuged, dried at room temperature with the aid of an air current and, finally, in a stove at 50 ± 5ºC. The same procedure was used to obtain the other derivative but increasing the temperature to 60° C (TZn60).

2.3. Characterization

The amount of water in TZn20 and TZn60 were determined by indirect gravimetric, heating the solid at 105 ± 5ºC. Zinc was determined as zinc oxide by a gravimetric procedure heating the solids at 900ºC. The amount of tannin was calculated as the difference between the mass of dried TZn and the zinc oxide content. The density of the pigment, needed for coating formulation, was determined according to a standardized procedure (ASTM D 1475).

The solubility of the TZn in ASW was determined as described in a previously published paper Bellotti, 2010. The concentration of tannin was obtained as total polyphenols (TP) spectrophotometrically at 750 nm using FDr (Ferreira et al., 2004; Erdemoğlu et al., 2005). This wavelength corresponds to the maximum of the so called molybdenum blue compound obtained due to the reducing ability of tannin Killeffer et al., 1952.

2.4. Bioassay

The concentration which kills 50% of the *Artemia* nauplii population within 24 hours (LC_{50}) was determined using a short term toxicity test Persoone et al., 1983.

Artemia persimilis eggs, commercially available, were hatched in ASW, at 22 ± 2ºC during 24 hours, under gentle aeration with an aquarium pump Persoone et al., 1987. After 48 hours a homogenous population of *Artemia* larvae were obtained and used for the test. TZn20 and TZn60 saturated solutions were diluted adequately with ASW to prepare different solutions containing 2.5, 12.5, 35.0, 50.0, 125 ppm and 5, 25, 50, 70 y 100 ppm of TP respectively. The respective diluted TZn solutions (10 ml) were placed in vessels with ten larvae in each one. Controls without TZn were made. Copper sulphate was used as positive control being the concentrations of the testing solutions 1.0, 10.0, 50.0, 100 and 200 ppm respectively. Three replicates were set up for each concentration, including the controls. After an incubation period of 24 h, dead larvae were counted and LC_{50} values were calculated by Probit analysis Castillo, 2004.

2.5. Formulation and preparation of coatings

Two Coatings 1 and 2 with each derivative, TZn20 and TZn60 respectively, were elaborated in a ball mill jar (3.3l). WW rosin was the resin used to formulate the coatings and oleic acid was employed as plasticizer (Tabla 1). The solvents used were xylene and white spirit (4/1). Castor oil gel was added in a load of 2% by weight of the total coating. Castor oil gel (15 % by weight) was previously activated with xylene, using a shear stress at 40–45ºC, until a stable colloidal structure was obtained and dispersed for 24 hours.

Table 1. Composition of coatings as % of solids by volume

Biocide	Chalk	WW rosin	Oleic acid	Solvent
14.9	9.9	24.9	5.4	44.9

2.6. Determination of leached polyphenols and zinc ions in ASW

Coatings were applied on 8 x 8 cm sandblasted acrylic panels. Three or four coats of coating were applied and allowed to dry 24 hours between each application. The total dry film thickness was 120 ± 5 µm. Not more than 48 hours elapsed for the painted panels to be submerged in plastic container containing 150 ml of ASW. The leached "tannate" was determined on a 2 ml aliquot using the FDr. The original level of the liquid in the containers was restored periodically with distilled water and the pH was adjusted to 8.2. The concentration of zinc cation was determined by atomic absorption spectroscopy.

2.7. Essays in NSW

Sandblasted acrylic panels (8 x 12 cm) were coated as described before to a total dry film thickness of 180 ± 5 µm. No more than 48 hours elapsed before the painted panels were immersed in NSW at Mar del Plata harbor Bastida, 1971.

Panels were immersed 50-60cm deep and biofouling resistance was evaluated monthly according to ASTM D6990-05. The range used for the fouling rating (FR) is from 0 to 100. The FR for coating system free of adherent biofouling settlement is recorded as 100. The total sum of percentage of area covered by macrofouling is deducted from 100. A coating free of macrofouling settlement but with adherent slime (microorganisms such as bacteria, fungi, diatoms and protozoa) is recorded with a FR = 99, irrespective of the percent area covered by the slime. Therefore, fouling rating reflects non-fouled area. Distance smaller than 1cm from the edge of the panels were not considered. Uncoated panels were used as control and immersed in the same conditions as the painted ones.

3. Results and Discussion

3.1. Preparation and characterization of zinc "tannate"

The analysis of the precipitation curve revealed that the flocculation of tannin could be achieved with zinc cation. So, it was decided to carry out this precipitation employing 0.940-1.000g of $Zn(NO_3)_2$ per g of tannin.

The chemical composition of TZn20 and TZn60 are shown in Table 2. The density of TZn20 and TZn60 were 2.18 and 1.72 g/cm^3 respectively.

Table 2. TT derivatives composition (%by weight)

Components	TZn20	TZn60
Tannin	69.00	68.05
Zinc	18.50	18.87
Lost at 100ºC	12.50	13.08

The concentrations of TP in TT, TZn20 and TZn60 saturated solution in ASW were found to be equal to 8546, 524 and 545 ppm respectively. These decreases in derivatives solubility respect to TT make them more suitable to be incorporated into antifouling formulation.

3.2. Bioassays

After an incubation period of 24 h, LC_{50} was found to be 42 ± 10 mg/l for TZn20, 37 ± 9 mg/l for TZn60 and 7 ± 2.0 mg/l for copper sulphate (positive control), respectively. These results showed that TT derivatives are active against *Artemia persimili* larvae; however its toxicity is much lower than that of copper sulphate.

3.3. Determination of leached polyphenols as a function of time

Results of the leaching experiments are shown in Fig. 1a and 1b. Coating 2, formulated with TZn60 leached lesser amounts of tannin than coating 1 (Fig. 1a). The amount of zinc leached at the end of the assay from coating 1 was the double that the coating 2 (Fig. 1b).

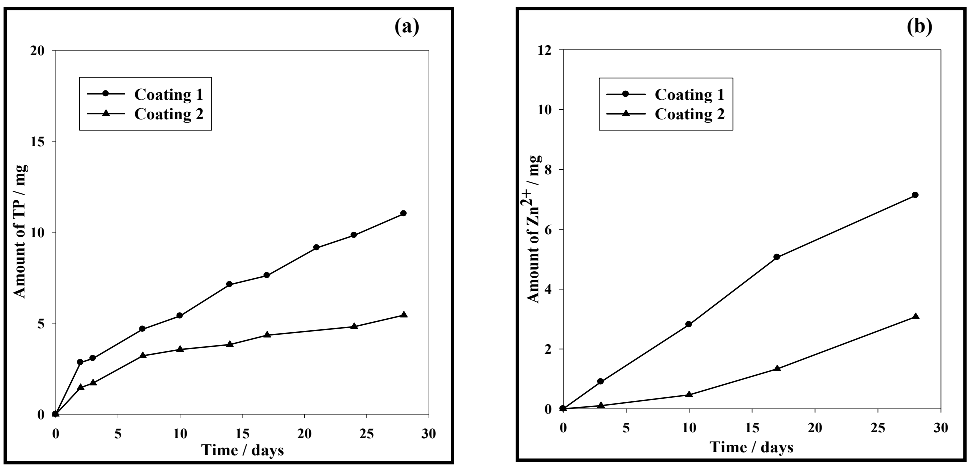

Fig. 1. Amount of (a) tannin and (b) zinc ions leached from the coating in ASW.

3.4. Essays in NSW

This assay was conducted for triplicate in Fig. 2a - d, is shown a representative panel for each case. All painted panels performed better than the control. After five months, coating 1 presented a FR 100 with an incipient erosion of the film, the control panels FR was 0, see Fig. 2a and 2b. After eight month panels with coating 1 had lost 80% of the coating but without signs of macrofouling on the panels. Panels with coating 2 showed a FR = 99, after eight months of immersion, see Fig. 2c. The control panels presented a high fouling settlement (FR = 0) and were completely covered with ascidians, algae, serpulids, barnacles and bryozoans.

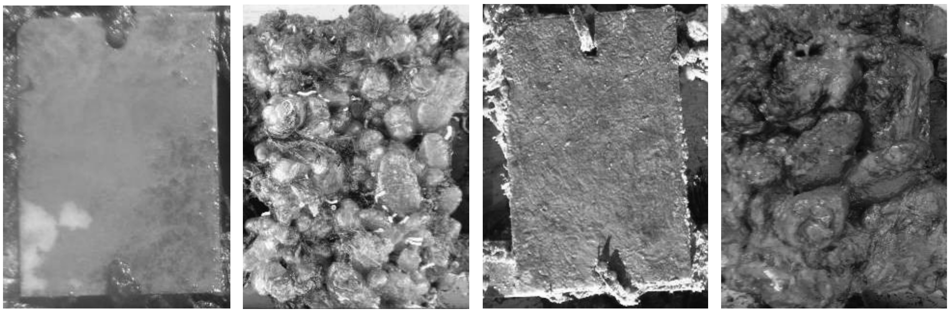

Fig. 2. Panels coated with coatings 1 and 2 immersed in NSW: a) coating 1 (5 months); b) control (5 months); c) coating 2 (8 months); d) control (8 months).

At 12 months of immersion, the panels with coating 2 had lost 80% of the paint film; therefore, the coating with TZn60 showed to have a longer service life than the coating with TZn20. Possibly an increase in temperature promotes the hydrolysis of ester unions in this kind of tannin generating a greater number of active functional groups. This TZn60 characteristic could affect its retention in the film producing a lower leaching rate to the environment and, therefore, increasing the life of the coating.

4. Conclusion

The LC_{50} showed that both tannin derivatives were effective against the larvae of *Artemia*, which was corroborated in the immersion tests in NSW by presenting both coating a FR higher than the controls. The leaching of tannin in ASW from the coatings showed that it depends of the derivative used. The longer life and integrity of the coating containing TZn60 would be related to the increase in the number of active functional groups due to the increase in temperature to obtain it. This improved results decreasing TP leached from the coating in ASW.

Acknowledgments

The authors wish to thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CIC), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and the Universidad Nacional de La Plata (UNLP) for the support to do this research.

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