August 6, 2008

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Biocidal performance of acrylated glyphosate in a model photopolymerizable coating formulation†‡§

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Received 9th June 2008, Accepted 21st July 2008

First published as an Advance Article on the web 6th August 2008

DOI: 10.1039/b809659g

Acrylated glyphosate was blended into a model polyolacrylate formulation and copolymerized. The resulting copolymer retains herbicidal activity similar to that of the monomer as indicated by the results of biological tests. No release of biocide from the coating was observed. The potential value of these biologically active acrylic formulations as biofouling compositions has been demonstrated by field trials.

Introduction

Biofouling, the undesired attachment of microorganisms, plants and invertebrates to an underwater surface, represents a major problem for seagoing vessels.^{1,2} A thick layer of marine organisms that rapidly forms on a ship compromises the speed and manoeuvrability of the vessel which, in turn, increases fuel consumption and results in elevated release of harmful emissions. Biofouling can also enhance metal corrosion³ by seawater as well as promote undesired transport of marine organisms from one ecosystem to another.

The prevention of biofouling has been a subject of intense research for hundreds of years.² Several sequential steps are proposed causal to marine fouling.^{4,5} Initially, surfaces of submerged objects serve as substrates for thin films of adsorbed organic compounds. Bacteria (including cyanobacteria) can colonize on the surface in hours followed by single cell diatoms that secrete sticky extracellular polymeric substances leading to the formation of a microbial biofilm. The presence of such adhesive residues promotes further attachment of marine microorganisms including larger marine invertebrates (*i.e.* barnacles, sponges, *etc.*) and these grow concomitantly with macroalgae such as *Enteromorpha*.

Modern antifouling coating compositions fall into three main categories: contact leaching biocidal coatings,⁶ self-polishing biocidal coatings,⁷ and biocide-free foul release coatings.⁸ The latter are the most promising. However, even the latest foul-release

formulations lack efficiency, are expensive, and work only when vessels are in motion.⁹ A Convention adopted in 2001 by the International Maritime Organization will prohibit, upon ratification by member states, all use of toxic tin-based paint formulations by January 1, 2008.¹⁰ Copper-containing compositions may also soon be banned. The disadvantage of the leading alternative biocidal coatings is their short service lifetime. The biocide is rapidly depleted because it is generally mechanically blended into the polymer coating matrix.

The present study investigates the antifouling potential of photopolymerizable model polyolacrylate coating formulations containing a new type of a biocide: *N*-acryloyl-*N*-(phosphonomethyl)glycine (**1**), a glyphosatic herbicide containing an acrylic functionality.

The glyphosate (**2**), commercially known as Roundup[®], is a non-selective systematic phosphonate herbicide. It is an effective inhibitor of the shikimic acid pathway in microorganisms and plants. The target of glyphosate action is 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase, an enzyme responsible for the synthesis of essential aromatic amino acids.^{11,12} Because EPSP synthase does not exist in mammals and other marine animals, compounds containing the glyphosate functionality could be attractive biocides for antifouling applications. Another important feature is ready degradation of glyphosate by some microorganisms thereby preventing accumulation in the environment.¹³ Overall, these characteristics may yield a more environmentally sound approach to the development of antifouling coatings.

We have recently demonstrated biological activity of **1**.¹⁴ Because **1** acts as a herbicide, its presence in a coating may impede biofilm formation by preventing attachment of photosynthetic cyanobacteria and diatoms. This, in turn, is expected to prevent the attachment of larger invertebrates. In addition, an acrylic functionality permits chemical incorporation of the biocide into

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† This paper was published as part of the themed issue in honour of Nicholas Turro.

‡ Contribution # 630 from the Center for Photochemical Sciences.

§ Electronic supplementary information (ESI) available: Additional details of copolymerization experiments and compositions of biological growth media. See DOI: 10.1039/b809659g

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a coating ensuring better control of release rates. This, in turn, is expected to prolong the service lifetime of the coating.

Experimental

Materials

The acrylated derivative of glyphosate, *N*-acryloyl-*N*-(phosphonomethyl) glycine (AA), was synthesized according to the procedure described previously.¹⁴ All other acrylic monomers and epoxy acrylic oligomers were obtained from either Sartomer or UCB Chemicals and used as received. Darocure 1173 and Irgacure 819 were obtained from CIBA. 2-Ethylhexyl-4-(dimethylamino)benzoate (ODAB, First Chemical Corporation, 98%) and isopropylthioxanthone (ITX, New Sun Chemical Co. Ltd., 98%) were used as received. Cultures of diatoms *Phaeodactylum tricornutum* Bohlin and *Navicula pelliculosa* (Breb. et Kuetzing) Hilse were obtained from The Provasoli-Guillard National Center for Culture of Marine Phytoplankton.

Photocopolymerization experiments

Photocopolymerization was carried out by irradiation of *ca.* 17% solutions of AA and 17% of 2-hydroxyethyl acrylate (HEA) in D₂O in presence of 1.5% wt. of Darocure 1173. Solutions were irradiated in borosilicate glass NMR tubes. The extent of polymerization was monitored by ¹H NMR by observing the disappearance of the signals for vinyl hydrogens of AA and HEA. ¹H and ¹³C NMR spectra were recorded using Bruker Avance 300 MHz nuclear magnetic resonance spectrometer. Sample irradiations were carried out using either an array of 395 nm LEDs from Clearstone Inc. equipped with CF-1000 power supply or with a Rayonet photochemical reactor equipped with the 350 nm lamps.

Photopolymer pellet preparation

Model acrylic resin (designated as 459S2) containing several epoxy acrylic oligomers (57%), acrylic monomeric diluents (43%) and a corresponding amount of herbicide was used to make rectangular copolymer pellets (15 × 10 × 1.5 mm). A designated quantity of herbicide was dispersed or partially dissolved in 459S2 resin using ultrasonication and heat (60 °C, overnight). Photoinitiator (ITX, 1.5% wt.), ODAB synergist (5% wt.) and co-photoinitiator (Irgacure 819, 0.1% wt.) were added to formulations. Formulations were poured into an open top Teflon mold and irradiated under N₂ by a 395 nm LED light source (5 min) to produce photopolymer pellet.

Herbicide release experiments

Round copolymer pellets (*d* = 5 mm, *h* = 2 mm) were formed from a 459S2 resin and a proper amount of herbicide. Liquid formulations (1 g) were loaded with 2.4%, 3.2%, and 6% wt. of acrylated glyphosate or 1.6%, 2.4% and 5% wt. of glyphosate. To each sample, 1.5% wt. of Irgacure 819 was added. The formulations were poured into a plastic mold and irradiated at 350 nm (Rayonet) under N₂ for 5 min. The resulting pellets were placed in 1 ml of D₂O and kept on a shaker (100 rpm) for 20 days. The amount of herbicide released was periodically monitored by observing

the ¹H NMR of pellets washings. Peak intensities from standard glyphosate and *N*-acryloyl-*N*-(phosphonomethyl) glycine D₂O solutions (containing herbicide amounts identical to the loads in the pellets) were used to normalize the amount of herbicide released.

Biological activity experiments

Two different diatom cultures, *Phaeodactylum tricornutum* and *Navicula pelliculosa* were used in the biological activity tests. Polymer pellets loaded with different concentrations (0.1, 0.15, and 0.3M) of glyphosate and acrylated glyphosate were placed on Petri plates loaded with a layer of f/2-containing agar medium (1.5% wt., 10 ml, see electronic supplementary information, ESI[†]) and a layer of f/2-containing low melting agarose medium (0.8% wt., previously seeded with diatoms (6 × 10⁶ and 2 × 10⁷ cell mL⁻¹, respectively). Following propagation over 5 days under continuous cool fluorescent light (40 micromol photons m⁻² s⁻¹), the average radii of inhibition zones were measured. Experiments for each herbicide concentration were repeated at least two times. Pellets comprised of model resin 459S2 without glyphosate additives were used as controls.

Field trials: periphyton biofouling assessment in an artificial stream facility

Field tests were conducted during July and August, 2006 at the University of Michigan Biological Station Experimental Stream Laboratory located near Douglas Lake, Pellston, Michigan. Resin 459S2 containing 0.15M of a designated herbicide was coated on terracotta ceramic tiles (3 × 6 inches) pretreated with silanes for improved adhesion and photocured by a H-bulb system from Fusion Inc. The coating thickness was 12.5 μm (0.5 ml). Bare tiles and tiles coated with 459S2 containing no additive were used as controls. Nine tiles for each series were placed into separate flow channels with a constant water flow of 3 L min⁻¹ for each channel. Each flow channel utilized water pumped from the East Branch of the Maple River containing natural freshwater biofouling organisms. Each flow channel was covered with a mesh screen to reduce direct light exposure and to ensure more favorable growth conditions. Tiles were left in the flow channels for 10 days.

Chlorophyll (chl) biomass attached to the surface of each tile was determined after the tiles had been washed three times with tap water to remove mud and other loosely attached species. The coated surface of the tile was submerged into a plastic container containing 25 mL of 90 : 10 acetone : saturated aqueous MgCl₂. The container itself was placed into an ultrasonic bath and the entire system sonicated for 30 s to facilitate chl extraction. The UV-vis spectrum of each extract was measured on a Shimadzu Multispec-1500 diode-array spectrophotometer using a 1 cm quartz cell. The amount of chl was determined using the following equation:¹⁵

$$[\text{Chl}](\mu\text{g tile}^{-1}) = \{[11.87(A_{663-750}) - 1.54(A_{645-750}) - 0.08(A_{630-750})] \times 25 \text{ mL}\} / l$$

where $A_{\lambda_1-\lambda_2}$ is the difference in absorbance at two designated wavelengths and *l* is the spectroscopic cell pathlength. Two independent field trials were conducted.

Results and discussion

Copolymerization experiments

In our previous report,¹⁴ we showed that both the monomer and homopolymer of *N*-acryloyl-*N*-(phosphonomethyl) glycine, AA, exhibited biological activity. In this work, AA was copolymerized with 2-hydroxyethyl acrylate (HEA). AA conversion was complete in a short period of time upon 350 nm irradiation of a D₂O solution. When a mixture of AA and HEA was subjected to a similar irradiation, the monomer conversion observed for copolymerization was as fast as in the case of a solo AA (Fig. 1).

Fig. 1 Results of copolymerization experiments for *N*-acryloyl-*N*-(phosphonomethyl) glycine (AA) and 2-hydroxyethyl acrylate (HEA).

Additional copolymerization experiments were conducted. To the mixtures listed in Table S1,§ 1.5% w/w of Darocur 1173 was added. The resulting mixtures were spin-coated on glass slides followed by irradiation at 350 nm. All resulting films adhered to the slides. The combination of AA with HEA resulted in tougher and less brittle films. Quantitative measurements of the film properties, however, were not pursued.

Biological activity of model coatings

To test the biological activity of AA incorporated into the acrylic formulation, we adapted as a model the acrylic matrix (less all pigments) from a commercial anticorrosion marine paint.¹⁶ Two diatom strains were used in these experiments because of their known roles as precursors for marine biofouling.¹⁷ Similar observations were made for both diatom strains tested (Table 1). When the acrylic resin contained no herbicide, inhibition of diatom growth was minimal. Incorporation of similar molar amounts of non-functionalized glyphosate and acrylated glyphosate into a model resin resulted in either substantial growth inhibition or complete bleaching in the case of high herbicide concentrations. This was observed for both non-functionalized and functionalized glyphosates despite their different arrangements within the coating. The former was a (poly)acrylic matrix with glyphosate particles trapped in the pores while the latter was a copolymer where acrylated glyphosate was chemically incorporated into the polymer backbone.

These structural differences were reflected in the rates of release of the active component from the coating (Fig. 2). While about 94% of loaded non-functionalized glyphosate was depleted from the coating after 18 days of shaking in D₂O, no release of AA (or products of AA decomposition) was detected by NMR over the same period. In the case of AA copolymer, the glyphosate functionality is chemically incorporated into a polymer preventing its release. However, the resulting polymer still demonstrates significant biological activity. This highlights the excellent antifouling and herbicidal potential of AA containing coatings.

Fig. 2 (a) ¹H NMR of D₂O washings of model coating formulation pellets containing 0.3 M of AA (lower spectrum) and 0.3 M of glyphosate (upper spectrum) after 18 days of shaking. Horizontal axes: field shift in ppm relative to tetramethylsilane reference, vertical axes: arbitrary NMR signal intensity. (b) Normalized quantity of active component released from the coating as a function of testing time.

Field trials: periphyton biofouling assessment in an artificial stream facility

Absorption spectra of acetone-extracted pigments (Fig. 3) show the dominant pigment present to be chl *a* (peaks at 435 and 663 nm). Transformation of the data to yield a second derivative spectrum also indicated the presence of absorbing species at 468 and 645 nm, suggestive of chl *b*, and 455 and 630 nm, indicative of chl *c*. These data suggest that the major taxa adhering to the tiles were likely cyanobacteria (containing only chl *a*), green algae (containing both chl *a* and *b*), and diatoms (containing chl *a* and *c*). The presence of diatoms as a major member of the stream facility biota has been described previously.¹⁸

Table 1 Average radii of the inhibition zones measured around the polymer pellets containing the designated herbicide^a

Strain name (diatom)	Herbicide in polymer pellet	Concentration in pellet/M (% mass)	Diameter of inhibition zone/mm
<i>Navicula</i>	None	0.0 (0.0)	10 ± 1
	AA	0.15 (3.2)	20 ± 2
	AA	0.3 (6.0)	Bleaching
	Glyphosate	0.15 (2.4)	22.5 ± 2
	Glyphosate	0.3 (5.0)	Bleaching ^b
<i>Phaeodactylum</i>	None	0.0 (0.0)	0 (no inhibition)
	AA	0.1 (2.4)	23 ± 2
	AA	0.3 (6.0)	Bleaching ^b
	Glyphosate	0.1 (1.6)	17.5 ± 2
	Glyphosate	0.3 (5.0)	Bleaching ^b

^a Data are from triplicate assays on 100 mm agar plates. ^b No growth detected on a 100 mm plate.

Fig. 3 Typical UV-vis spectrum obtained after the chlorophyll extraction (λ represents wavelength). Inset: differences in the amount of chlorophyll extracted for various samples.

Total chl extracted from bare uncoated tiles established a baseline for attachment with $11.1 \pm 2.87 \mu\text{g tile}^{-1}$ (Fig. 3, inset). Blank tiles coated with resin containing no herbicide and tiles containing unfunctionalized glyphosate yielded periphyton chl biomass of $9.35 \pm 1.07 \mu\text{g tile}^{-1}$ and $11.9 \pm 2.56 \mu\text{g tile}^{-1}$, respectively (one way analysis of variance, $P > 0.05$, $df = 17$). The lack of inhibition by unfunctionalized glyphosate can be justified using our release data. Glyphosate, when chemically unbound, rapidly leaches out of the coating. Further, this leaching is expected to create porosity on a coated surface promoting attachment of microorganisms.¹⁹ Blank tiles display some inhibition owing to toxicity of acrylic coating components. Finally, the composition containing AA embedded into the polymer backbone yielded the least amount of attached biomatter ($7.92 \pm 1.12 \mu\text{g tile}^{-1}$), roughly two-thirds of the periphyton chl measured from tiles containing unfunctionalized glyphosate (unpaired two-tailed t -test, $P < 0.05$) and thus, supporting the good potential of AA as a biocide.

In summary, a new polymerizable derivative of glyphosate was blended into a polyolacrylate formulation and copolymerized. The copolymer resulting retained the herbicidal activity of the monomer. These biologically active acrylic formulations have potential as anti-biofouling coatings.

Acknowledgements

The authors acknowledge the Office of Naval Research for funding this project (Grant # N00014-04-1-0406). Ohio Department of Development and Bowling Green State University are also acknowledged for funding this project through the Wright Photo-science Laboratory. Photopolymerizations of the coated tiles were carried out in collaboration with our colleagues at Spectra Group Ltd., Millbury, OH. Facilities and personnel of the University of Michigan Biological Station are acknowledged. We thank Prof. Paul Moore for help with setting up the field trials.

Notes and References

- 1 D. Rittschof, *Biofouling*, 2000, **15**, 119.
- 2 (a) D. M. Yerba, S. Kill and K. Dam-Johansen, *Prog. Org. Coat.*, 2004, **50**, 75–104; (b) V. J. D. Rascio, *Corros. Rev.*, 2000, **18**, 133–154.
- 3 H. A. Vadela and L. K. Herrera, *Int. Microbiol.*, 2005, **8**(3), 169.
- 4 Marine BioFoulNet <http://www.biosciences.bham.ac.uk/external/biofoulnet>.
- 5 (a) M. E. Callow, *Chem. Industry*, 1990, **5**, 123; (b) M. E. Callow and J. E. Callow, *Biologist*, 2002, **49**, 1–5.
- 6 (a) S. N. Ghiya, *Paintindia*, 1987, 19–30; (b) V. J. D. Rascio, C. A. Giudice and B. Del Amo, *Corros. Rev.*, 1988, **8**, 87–153.
- 7 (a) I. Omae, *Appl. Organomet. Chem.*, 2003, **17**, 81–105; (b) S. Küll, C. E. Weinell, M. S. Pedersen, K. Dam-Jaohansen and S. A. Codolar, *J. Coat. Technol.*, 2002, **74**, 45–54; (c) International Marine Coatings home page: <http://www.international-marine.com>.
- 8 (a) R. F. Brady, *Prog. Org. Coat.*, 2001, **43**, 188–192; (b) A. F. Thunemann and R. H. Kublickas, *J. Mater. Chem.*, 2001, **11**, 381–384.
- 9 (a) M. Ryle, *The Motor Ship*, 1999, 34; (b) C. D. Anderson, IBC UK Conferences Limited, United Kingdom, 1998, pp. 1–12.
- 10 (a) M. A. Champ, *Sci. Total Environ.*, 2000, **258**, 21–71; (b) M. A. Champ, Proceedings of the 24th UJNR (US/Japan) Marine Facilities Meeting, Hawaii, Nov. 7–8, 2001.
- 11 R. Bentley, *Biochem. Mol. Biol.*, 1990, **25**, 307.
- 12 J. G. Levin and D. B. Sprinson, *J. Biol. Chem.*, 1964, **239**, 1142.
- 13 (a) N. G. Ternan, J. W. McGrath, G. McMullan and J. P. Quinn, *World J. Microbiol. Biotechnol.*, 1998, **14**, 635–647; (b) E. Mallat and D. Barcello, *J. Chromatogr., Sect. A*, 1998, **823**, 129.
- 14 A. Bogdanova, V. Piunova, D. Berger, A. V. Fedorov and D. C. Neckers, *Biomacromolecules*, 2007, **8**, 439.
- 15 ESS Method 150.1, Environmental Science Section, Wisconsin State Lab of Hygiene, webpage: <http://www.epa.gov/glnpo/lmmb/methods/methd150.pdf>.
- 16 A. Mejiritski, T. Marino, D. Martin, D. J. Berger, A. V. Fedorov, K. C. Anyaogu, A. A. Ermoshkin and D. C. Neckers, *Rad. Tech. Rep.*, 2006, 11–20.

-
- 17 (a) K. Sitaraman, N. Wang, C. K. Ober, J. A. Finlay, M. E. Callow, J. A. Callow, A. Hexemer, K. E. Sohn, E. J. Kramer and D. A. Fischer, *Biomacromolecules*, 2006, **7**, 1449; (b) J. A. Finlay, M. Callow, L. Ista, G. P. Lopez and J. A. Callow, *Integr. Comp. Biol.*, 2002, **42**, 1116; (c) M. E. Callow, in *Biofilm: Recent Advances in Their Study and Control*, ed. L. V. Evans, Harwood Academic Publishers, Amsterdam, 2000, p. 189.
- 18 (a) R. S. Stelzer and G. A. Lamberti, *Limnol. Oceanogr.*, 2001, **46**, 356–367; (b) R. S. Stelzer and G. A. Lamberti, *Ecology*, 2002, **83**, 1039–1051.
- 19 (a) M. E. Callow, A. R. Jennings, A. B. Brennan, C. E. Seegert, A. Gibson, L. Wilson, L. A. Feinberg, R. Baney and J. A. Callow, *Biofouling*, 2002, **18**, 237; (b) L. Hoipkemer-Wilson, J. F. Schumaker, M. L. Carman, A. L. Gibson, L. A. Feinberg, M. E. Callow, J. A. Finlay, J. A. Callow and A. B. Brennan, *Biofouling*, 2004, **20**, 53.