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The effects of acrylamide polyelectrolytes on aquatic organisms: Relating toxicity to chain architecture

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HIGHLIGHTS

- The effects of polyelectrolytes on aquatic biota depend on chain architecture.
- Branched polymers are less toxic due to lower coverage of the biological surfaces.
- Chain architecture is a critical design variable to optimise environmental safety.
- Improved biofouling bivalve control may rely on chain architecture manipulation.

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ABSTRACT

Understanding the inherent toxicity of water-soluble synthetic polyelectrolytes is critical for adequate risk management as well as enhancing product design when biological activity is a key performance index (e.g. for application in biofouling bivalves' control). The toxicity of two cationic acrylamide copolymers with different chain branching degree was evaluated. Standard ecotoxicity tests were conducted with microalgae and daphnids. The susceptibility of *Corbicula fluminea*, as a biofouling bivalve, was also evaluated. The effect of polyelectrolyte on the test media viscosity and the polymer chain size distributions under the experimental conditions were also examined. The susceptibility of the microalgae to both polymers was similar. As the complexity and size of the test organisms increased, differences in toxicity due to different chain architecture were noticeable. The more branched polymer was significantly less toxic to both daphnids and the bivalves, which could be linked to the distinctive features of its bimodal size chain distribution. This architecture resulted in both more compact globular molecules and the formation of aggregates, which reduce the polymer interaction with the biological surfaces. The results of this study promote the incorporation of environmental considerations in polyelectrolyte development and contribute to the design of improved solutions for controlling biofouling bivalves.

1. Introduction

Water-soluble synthetic polyelectrolytes are routinely employed in a number of fields, including mining, drinking water treatment, papermaking, personal care products manufacturing (Cary et al., 1987; Rowland et al., 2000; de Rosemond and Liber, 2004; Cumming, 2008; Harford et al., 2010).

Despite the common use of these chemicals, their ecotoxicity has not been extensively investigated (Liber et al., 2005; Harford et al., 2010). Often their pollutant potential is somewhat disregarded because low controlled discharges into the environment are generally expected, and there is a tendency for natural waters to further mitigate polyelectrolyte toxicity through adsorption phenomena (Cary et al., 1987; Goodrich et al., 1991; Bolto and Gregory, 2007; Cumming, 2008). Although this might be true in most cases, under some circumstances polyelectrolytes may pose significant ecological concerns. For instance, de Rosemond and Liber (2004) identified a cationic polyquaternary ammonium compound as the main toxic component of a diamond mine effluent. In some specific applications, as mining, the rates of use and discharge are such that only a very small fraction of the polyelectrolyte released needs to be in unbounded state to potentially affect the biota in the receiving waters (Liber et al., 2005). Additionally,

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polyelectrolyte accidental release or uncontrolled overdosing imply increased hazard (Rowland et al., 2000; ARC, 2004; Cumming et al., 2010). Therefore, the potential environmental risk posed by these substances should always be acknowledged. Understanding their inherent ecotoxicity is crucial for adequate management of such risk.

The functionality, for example flocculant action, of polyelectrolytes may be maximised by manipulating not only the chemistry but also parameters such as polymer charge density, molecular weight and chain architecture (Rasteiro et al., 2010). Comprehending how these variables also affect ecotoxicity enables environmental considerations to be incorporated into the first steps of the polyelectrolyte development process (Hamilton et al., 1994), instead of being postponed to later stages when the product is close to get into the market. Furthermore, this type of information may assist the optimisation of the overall product performance, related to the specific application context, through the maximisation of polymer functionality and the minimisation of environmental impacts. A particular situation where molecular parameters may be exploited for optimising the overall product performance is the multi-objective molecular design of polyelectrolytes for the industrial control of biofouling bivalves, such as the Asian clam *Corbicula fluminea* and the zebra mussel *Dreissena polymorpha*. Polyelectrolytes have proved effective to mitigate these pests, and many are particularly promising for application in the highly regulated drinking water treatment industry because they are licensed for dosing in potable water and may also be used in the plant for other treatment purposes (Costa et al., 2011b). In this context, optimal overall polyelectrolyte performance would involve maximal pest control activity and operational functionality (for example as a flocculant) and ideally minimal impacts on non-target species.

Some attention has been devoted to the effects of polymer chemistry, molecular weight and charge density on polyelectrolyte ecotoxicity (Goodrich et al., 1991; Hall and Mirenda, 1991; Jop et al., 1998). While polymer chain architecture is a useful design variable, able to significantly affect operational functionality (Rasteiro et al., 2010), little is known about the way this parameter influences the impacts on aquatic biota and the importance of further exploring this theme has been acknowledged (Cumming, 2008).

In this study the ecotoxicity of two cationic acrylamide/dimethylaminoethylacrylate methyl chloride copolymers (AM-co-DMAEA) with different chain branching degree, synthesised by inverse-emulsion polymerisation, was investigated. The green microalgae *Pseudokirchneriella subcapitata* and the cladoceran zooplankter *Daphnia magna*, both standard test species for ecotoxicological evaluation, as well as the Asian clam *C. fluminea*, representing biofouling bivalves, whose industrial control may be achieved through polyelectrolytes, were used as model organisms. For aquatic organisms, it is reasonable to envisage that polyelectrolyte effects result from both indirect mechanical impairment, related to changes in the medium viscosity, and physiological disturbance, due to the direct action of the polymer molecules on the organisms. For this reason, the viscosity of solutions containing the polyelectrolytes in the test media and the polymer chain size distributions under the experimental conditions were also measured in an attempt to provide a physiochemical basis for the interpretation of the toxicity data. The former assisted the assessment of the mechanical impairment contribution to polyelectrolyte toxicity while the latter provided an insight into the interaction of the polymers with the biological surfaces.

The outcome of this work may be relevant not only for risk management as part of polyelectrolyte development process, but also for the design of improved solutions for controlling biofouling bivalves.

2. Material and methods

2.1. Polymer synthesis and characteristics

The AM-co-DMAEA were synthesised through an inverse-emulsion polymerisation process (Barajas et al., 2004; Rasteiro et al., 2010; Palomino et al., 2012). The two model flocculants had similar composition and charge density (approximately 42 wt% acrylamide) as well as molecular weight (approximately 1.3×10^6 g mol^{-1}, measured by analytical ultracentrifugation in a Beckman Coulter OPTIMA XL-I analytical ultracentrifuge and calculated by the modified Flory–Mandelkern–Scheraga equation (Mandelkern and Flory, 1952)). One (hereinafter designated as E2+) was a slightly branched polymer, containing, on average, one branch per chain. The other (thereafter referred to as E2++++) was characterised by highly branched architecture, with the individual chains containing, on average, four branches. Reference values of the polymers' intrinsic viscosity, determined in a 0.05 M NaCl aqueous solution (Bourdillon et al., 2006), reflected the different chain branching degree: 1164 mL g^{-1} for E2+ and 977 mL g^{-1} for E2++++.

2.2. Ecotoxicological evaluation

2.2.1. Bioassays with the green microalgae P. subcapitata

P. subcapitata was maintained in the laboratory as a non-axenic batch culture in Woods Hole MBL medium, at 20 ± 2 °C, under permanent illumination. New cultures were started by inoculating microalgae harvested during the exponential growth phase in fresh medium.

Microalgae growth inhibition tests were conducted by adapting the appropriate OECD guideline (OECD, 2011) to the use of 24-well microplates (Geis et al., 2000). The microalgae were exposed to 12 concentrations (defined as a geometric series of ratio 1.75 in the range 0.318–150 mg L^{-1}) of E2+ and E2++++, tested in triplicate. Each treatment (1 mL of algal suspension in Woods Hole MBL medium per microplate well) consisted of 900 μL of test solution plus 100 μL of algal inoculum, the latter adjusted to provide each well with an initial cell density of 10^4 cells mL^{-1}. The cell density of the original inoculum was determined microscopically using a Neubauer haemocytometer. Each microplate held the replicates for two test concentrations plus two replicates of control treatments, where no chemicals were dosed. The microplates were incubated for 72 h at 20 ± 2 °C, under continuous illumination. The algal suspension in each well was thoroughly mixed by repetitive pipetting twice a day to prevent cell clumping. At the end of the test, the microalgae density in the wells was quantified by measuring the absorbance at 440 nm using a Jenway 6505 UV/Vis spectrophotometer, based on a previously determined calibration curve. The microalgae production in each individual treatment (yield) was calculated as the difference between the cell densities at the end and the beginning of the test. The inhibition in yield (I_Y) was then expressed as $I_Y = (Y_C - Y_T)/Y_C \times 100$, where Y_C and Y_T represent the mean value of yield for the controls and the yield in each replicated treatment, respectively.

As a complementary analysis to aid the interpretation of the bioassay results, the zeta potential of the microalgae, an indication of the cells' charge surface, was determined in a Zetasizer Nano ZS, model number ZEN3600, using filtered (0.2 μm pore size) Woods Hole MBL medium as dispersing agent.

2.2.2. Bioassays with the cladoceran D. magna

The neonates used in the study were obtained from monoclonal bulk cultures of *D. magna* (clone A *sensu* Baird et al., 1989a). The cultures were maintained in synthetic ASTM hard water (ASTM, 1980), supplemented with a standard organic additive (Baird