METHACRYLATE COPOLYMERS FOR ANTI-BIOFOULING COATINGS: A CHEMICAL APPROACH

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ABSTRACT

The project discussed here aims at the development of materials for anti-biofouling coatings in freshwater applications, in particular for aluminum and glass surfaces. To create such coatings by a chemical approach new copolymers containing three or more comonomers were prepared. Each of the comonomers adds a specific function to the final material. Incorporation of metal ion complexing monomer units allows the formation of complexes with ions working as biocide and should result in a drastic reduction of the necessary biocide concentration in coatings because complexation of ions takes place only at the outer surface of the material and thus, the total concentration tends to be negligible. Moreover, generation of strong complexes should prevent leaching of the metal ions and improve the durability of the biocide effect. Here, results concerning synthesis, physical characterization, preparation of coatings on glass and the effect of these coatings on biofouling as probed with bacteria and algae in freshwater is presented. A considerable reduction of biofouling by bacteria (*Staphylococcus aureus*) and algae (type *Achnanthidium spec.*) could be detected.

INTRODUCTION

Biofouling is a serious problem which disturbs almost everywhere it appears. The term summarizes all biological processes and phenomena occurring on the surface of materials in contact with different environments. Examples of these different types of fouling are marine biofouling in aquatic environment, freshwater fouling in sweet water environment, membrane fouling in water treatment processes, biofouling of (bio)materials in body fluids or during medical treatments (e.g. at dialysis membranes), to name just a few. The complex processes of biofouling involve colonization of structures that are either living or artificial (Briand, 2009). Depending on the environment, different foulers play a role which significantly influences the resulting biofouling film. Each of the various kinds of fouling needs to be solved by a special strategy and general solutions are almost impossible.

The present work is directed at developing coatings able to prevent freshwater biofouling, in order to limit the variety of parameters and to understand basic relations between chemistry of the surfaces and biofouling. These polymer materials are intended to be used as coatings for constructions in contact with freshwater, e.g. on boats, as protecting layer in aquaria, shower cubicles and bathrooms, but also in heat exchangers. Biofouling in heat exchangers yields the formation of biofilms which reduce the heat transfer coefficient, heat transfer rates and thus, efficiency of the heat exchanger drastically (Müller-Steinhagen, 2010). Glass surfaces require coatings which are transparent with good adhesion; heat exchanger surfaces require adhesion to the substrate, high performance against biofouling and high durability. Strategies to reduce heat exchanger fouling include biocide treatment of the water used, impregnation of the parts of the heat exchanger system, cleaning of the exchanger parts, and others (IKSR, 2002). Currently, there is no main pathway known to prevent biofouling completely, and therefore further studies, in particular with respect to structure-action relationships, are required.

The work presented here is focused on the creation of materials applicable as impregnation coatings. The *chemical* approach includes synthesis of new copolymers consisting of different comonomers which are randomly distributed along the polymer chains and insert different functions into the material. The concept follows work about fouling release surfaces by the group of C. K. Ober et al. performed within the EU Ambio project (Krishnan, 2008, Marabotti, 2009). Here, fouling is in a first step prevented by anti-adhesive surfaces with fluorinated parts. If fouling however occurs, foulers were destroyed by biocides and removed/released by soft parts of the coatings. Combination can be achieved either by incorporation into copolymers, block copolymers or by physical mixing in blends.

The polymeric system examined here is based on methacrylate chemistry, allowing versatile replacement of monomers by others. Several attemps have been reported to prevent biofouling by means of methacrylic or acrylic polymers with quaternary units, respectively (Lu, 2007, Charnley, 2011).

The project covers a broad experimental field from synthesis and characterization of the new copolymers to determination of physical properties, preparation of coatings and their wetting behavior, finally performing biofouling investigations. Thus, systematic variations of the chemical structure are possible and the direct influence of the chemistry on biofouling processes can be elucidated.

CHEMICAL CONCEPT

The important basic structure of the system under study is poly(methylmethacrylate) (PMMA), providing mechanical stability, durability, hardness to the material. PMMA coatings alone, however, could be removed by shear stresses in water from the substrate. Thus, they need to be stabilized, which we do here by introduction of cross-linking. Cross-linking allows only to swell the coating and not to be detached.

The ability to cross-link is added by 4-benzoylphenyl methacrylate (BPMA) monomer which can connect polymer chains (cross-link) by reactions initiated by irradiation with UV-light. The comonomer is inserted in a concentration of 10 mol% in maximum.

Beside these two comonomers, more functions are needed to design an optimal anti-biofouling polymer material. Copolymerization with a metal ion-complexing monomer should allow chemical incorporation of biocide-acting metal ions not as free ions, but as a complex. Thereby, the required concentration of metal-based biocide (we used as model copper acetate) could be significantly lowered compared to standard formulations. The metal is not incorporated by mixing with the coating base as in standard coatings, but fixed in a stable complex. Thus, the stability against leaching should be enhanced. In this work, 2-acetoacetoxy ethyl methacrylate (AAMA) was employed as complexing unit. The ability to form complexes was examined using UV-VIS spectroscopy on the monomer because complexation of the polymers would generate insoluble precipitates due to physical cross-linking and do not allow to prepare films.

Incorporation of semifluorinated methacrylates, such as 1H,1H,2H,2H-perfluorodecyl methacrylate (sfMA-H2F8) adds very hydrophobic properties to the material. In addition, the semifluorinated polymethacrylate is soft (reflected by the low glass transition temperature, T_g). Both properties should cause fouling release, i.e. rejection of death bacteria after action of the biocide, in combination with a certain suppression of fouler attachment by hydrophobicity.

The chemical structure of a typical copolymer example is shown in Fig. 1.

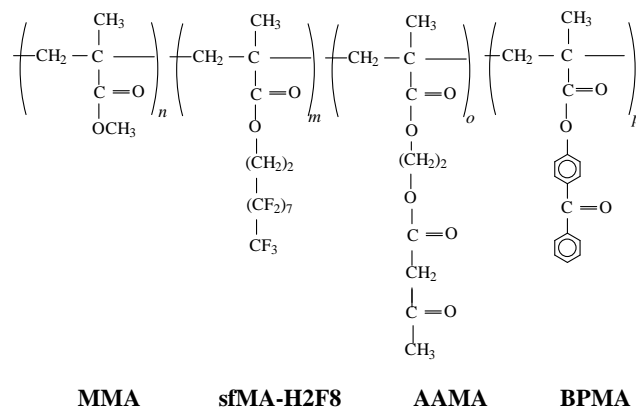**MMA   sfMA-H2F8   AAMA   BPMA**

Fig. 1 Chemical structure of the copolymers developed to prevent freshwater biofouling.

METHODOLOGY

After synthesis and characterization of the polymers, thin films on test surfaces (glass slides or aluminum specimens) were prepared by spin-coating. The films were dried and cross-linked by UV-irradiation. The wetting behavior was investigated on these films. Complexation with copper ions directly on the surface of the film was performed by the so-called solvent annealing procedure (Li et al., 2007) with copper(II)acetate dissolved in tetrahydrofurane. After thorough washing and drying the copolymer films were tested for their anti-biofouling properties with different classes of foulers representative for freshwater fouling: *Staphylococcus (S.) aureus* as representative for bacteria, and *Achnanthidium spec.* (diatom algae) as representative for algae were employed.

EXPERIMENTAL

Materials

1H,1H,2H,2H-perfluorodecyl methacrylate, methyl methacrylate (both ABCR) and 2-acetoacetoxy ethyl methacrylate (Sigma Aldrich) were used after purification over alumina. 4-hydroxybenzophenone and methacryloyl chloride (Sigma Aldrich) were used as received. 2,2'-Azobisisobutyronitrile (AIBN) (Sigma Aldrich) was recrystallized twice from diethyl ether and stored at 4 °C. Chloroform, ethanol, acetone, tetrahydrofurane, diethyl ether, n-hexane, 1,3-bis(trifluoromethyl) benzene (BFMB) and methyl ethyl ketone (Sigma Aldrich) were used without further purifications. Copper(II)acetate was received from ABCR.

Synthesis of copolymers

All polymers were synthesized by free radical polymerization. A typical synthesis included AAMA, MMA, sfMA-H2F8, BPMA (for compositions see Table 1) and AIBN (3 mol% with respect to the total concentration of monomers) in solution of BFMB. All constituents of the

mixture were introduced in a Schlenk tube, sealed under nitrogen and degassed by several freeze-thaw pump cycles. The polymerization reaction was carried out under nitrogen and mechanical stirring for 6 hours at 70 °C. After that time, the polymer was precipitated from chloroform or BFMB solution into the tenfold amount of methanol, filtered off and dried under vacuum at 50 °C (yield > 85%). The molar masses achieved were high enough to ensure appropriate polymer coating properties.

The detailed analytical results are reported elsewhere (Starke, 2013). Table 1 summarizes the composition of copolymer samples selected for the present study. The samples are chosen to outline basic trends.

Table 1 Chemical composition of the copolymers studied.

Poly-mer	MMA	AAMA	sfMAH2F8	BPMA	T_g	Adv. CA
	(mol%)	(mol%)	(mol%)	(mol%)	(°C)	(°)
1	100	0	0	0	116.0	78
2	10	0	80	10	67.8	126
3	10	10	80	0	67.4	126
4	80	10	0	10	104.2	84

T_g: Glass transition temperature.
CA: Advancing contact angle measured with water as probe.

Preparation of coatings

Glass slides (76 mm x 26 mm x 1 mm) were cleaned subsequently with acetone, Caro's acid, and bi-distilled water, then dried under reduced pressure in a vacuum oven at 50 °C. Aluminum specimens were cleaned by subsequent washing with acetone, ethanol and water. The films were prepared by spin coating using a POLOS wafer spinner BL-type spin-coater (APT GmbH, Germany) with spinning speed of 2000 rpm for 30 s from a 2 wt.-% polymer solution in chloroform or BFMB, respectively. The coated substrates were dried in vacuum at 80 °C for 1 h. After drying, the polymer films were cross-linked by UV-irradiation with an UV-lamp at 365 nm at room temperature for 45 min.

For the solvent annealing step performed to initiate copper complexation, the coated slides were treated with copper(II)acetate solution in THF according to Li et al. (2007) after treatment thoroughly rinsed with bi-distilled water and dried at 80 °C for 1 h in vacuum.

UV/VIS spectroscopy

Titration experiments of the copper salts with AAMA monomer with a starting volume of 1 mL for 0.4 cm standard quartz cuvettes were carried out at room temperature (22 ± 2 °C) in a sealed quartz cuvette (quartz glass Suprasil, HELLMA, Germany) by stepwise addition of appropriate amounts of concentrated AAMA solution in ethanol into the solution of the appropriate copper(II) salt through the serum cap by using a gas-tight micro liter syringe.

Differential scanning calorimetry

The polymer samples were dried in a vacuum oven under reduced pressure at 60 °C for several hours to remove water prior to investigation in order to avoid failures in the glass transition temperature determination. Modulated differential scanning calorimetry was performed with a DSC Q 100 (TA Instruments) under nitrogen. The samples were measured in the temperature range from -80 °C – 150 °C with 2 Kmin⁻¹ heating/cooling rate. The modulation of the heating rate was ± 0.31 K/40 s. The glass transition temperature T_g was determined using the half-step method.

Contact angle measurements

Dynamic contact angle measurements were performed using an OCA 30 contact angle goniometer (DATA Physics) with bi-distilled water as probe liquid using the sessile drop method. Advancing contact angles θ_a were determined using tangents on the drop. The dynamic advancing contact angle was measured on polymer coated glass slides on at least three positions.

Biological assay

The coated test specimens were incubated for 24 h at 36 °C in a suspension of *Staphyloccocus aureus* within a growth chamber covered by a membrane. After rinsing the membrane and the sample substrates with isotonic saline solution, the diluted solutions were applied on nutrient agar plates and further incubated for 24 h at 36 °C. The grown colonies were counted under a microscope.

To assess the growth of diatom algae (*Achnanthidium species)* on the coated surfaces, the slides were incubated for 1 week at 20 °C in an aquarium with strong diatom concentration. After that time, the slides were rinsed and the attached cells were counted under a microscope.

RESULTS AND DISCUSSION

Complexation experiments of AAMA monomer with copper(II)acetate studied by UV/VIS spectroscopy

UV/VIS experiments were carried out to get information about the formation of complexes between AAMA monomer and copper(II) ions. As shown in Figure 2, complexation of both copper(II)sulfate and chloride with the monomer AAMA worked successfully, as expected from literature (Ummathur, 2009).

The difference in the increase of the absorbance at 283 nm between the two copper salts (copper(II) sulfate and chloride) indicates that $CuSO_4$ forms the more stable complex with AAMA. The observed slope of the curve is higher than that for the copper(II) chloride.

If the complex would not have been formed a curve as in the absence of copper(II) (i.e., a line with the slope of the blue curve) would be observed.
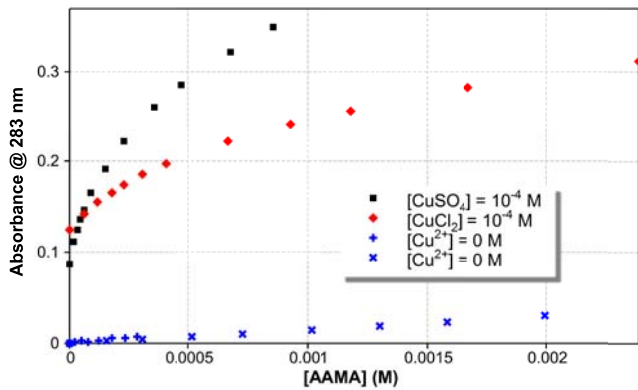
Fig. 2 Absorbance at 283 nm during titration of copper(II) salt solutions with AAMA in ethanol.

Wetting behavior of the copolymer films with water

The results of dynamic contact angle measurements with water are summarized in Table 1. Generally, the coatings result in surfaces more hydrophobic than glass and metals. The high advancing contact angles θ_a of 126° of the copolymers with the semifluorinated monomer sfMA-H2F8 reflect the strong hydrophobicity effect of these units, as already recognized for other semifluorinated poylmers (Pospiech, 2003). Polymer 1 (PMMA) resembles the well-known value for PMMA, polymer 4 reflects a comparable wetting behavior. These two polymers are more hydrophilic and polar in nature.

Biofouling results

Effect of the polymer chemistry on attachment and growth of S. aureus

The biocide and fouling release activity towards *S. aureus* were investigated using spin-coated copolymer films on glass slides. It has to be pointed out that reproducibility of different experiments is complicated by the fact that each bacteria culture contains different total amounts of *S. aureus*. This explains deviating results in two experiments.

The results obtained are shown in Figure 3. Even on a pure PMMA surface, attachment and growth of *S. aureus* is reduced compared to the glass control. Incorporation of the semifluorinated comonomer sfMA-H2F8 in polymer 2 reduces the fouling further. Additional incorporation of the complexing comonomer AAMA in polymer 3 and polymer 4 together with complexation with copper results in the largest suppression of *S. aureus* growth by almost one order of magnitude.
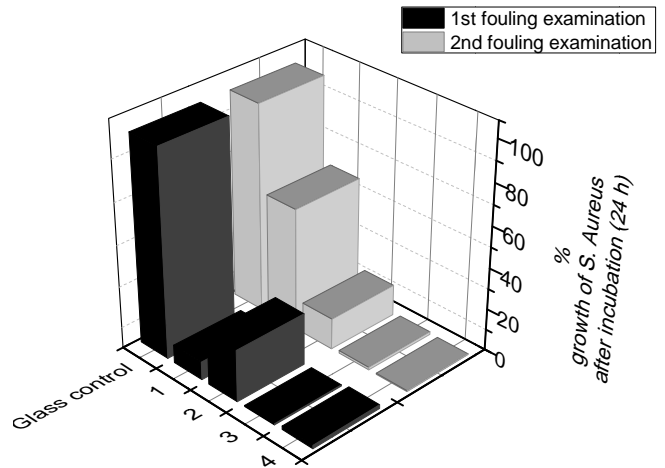

Fig. 3 Effect of coating chemistry on the adsorption and growth of *S. aureus*.

Attachment and growth of brown algae and diatom algae on copolymer films on glass

Figure 4 illustrates what happens when the test glass slides without coating are exposed to the experiment. Significant encrustation is observed in both cases.
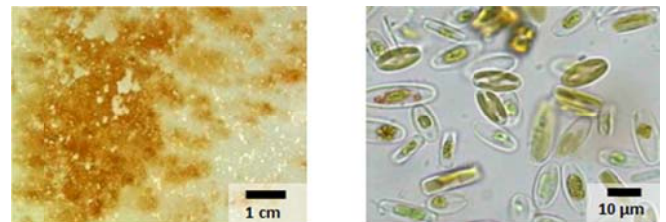

Fig. 4 Microscopic images of biofouling films on glass specimens (left: layer of brown algae; right: settlement of *Achnanthidium spec.*).

To determine the effect of the copolymer films on diatom algae (*Achnanthidium spec.*) growth the coated glass slides were incubated for a defined time in an aquarium with strong diatom presence. The results are given in Figure 5.

The populations of *Achnanthidium spec.* growth well on glass, PMMA-covered glass and the glasses coated with PMMA-rich copolymers with a molar concentration of 80 mol% MMA. In contrast, copolymers 2 and 3 with low molar concentration of MMA, but 80 mol% semifluorinated comonomer appear to be more efficient in their biocide and fouling release function, independent of the presence of copper complexes. This can only be understood as action of the soft, hydrophobic, semifluorinated copolymer segments.
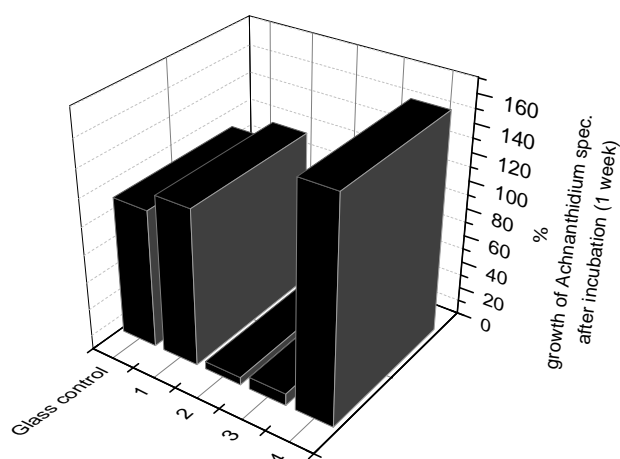
Fig. 5 Biofouling results: effect of coating chemistry on the growth of *Achnanthidium spec.*

CONCLUSIONS

Tailored PMMA-based copolymers were designed, synthesized and characterized in order to provide new materials with biocide and fouling release properties.

The settlement and growth bacteria and algae on polymer-covered glass specimens was examined. Complexation of the AAMA monomer with Cu^{2+} was successfully proven by UV/VIS measurements. Mechanically stable films could be prepared employing the cross-linking by UV irradiation. A clear reduction of the growth of *S. aureus* and *Achnanthidium spec.* (diatom algae) in freshwater aquaria could be observed.

In a next step, the copper complexes used so far will be replaced by zirconium complexes.

Future work will chemically focus on the exchange of AAMA by phosphonic acid-containing and imidazolium-containing monomers as biocides. Furthermore, biofouling examination of polymer-coated stainless steel surfaces will provide information if the polymer materials may be of use for coatings of heat exchanger parts.

ACKNOWLEDGEMENTS

NOMENCLATURE

AAMA	2-acetoacetoxyethyl methacrylate
BFMB	1,3-bis(trifluoromethyl) benzene
BPMA	4-benzoylphenyl methacrylate
CA	contact angle
DSC	differential scanning calorimetry
MMA	methyl methacrylate
PMMA	poly(methyl methacrylate)
S. aureus	*Staphylococcus aureus*
sfMA-H2F8	1H,1H,2H,2H-perfluorodecyl methycrylate

Subscript

A	θ_a	advancing contact angle
g	T_g	glass transition temperature (onset of macro-Brownian segmental motions in the amorphous phase of polymers)

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