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Polymeric Antimicrobial Coatings Based on Quaternary Ammonium Compounds

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Abstract: Biocidal coatings that are based on quaternized ammonium copolymers were developed after blending and crosslinking and studied as a function of the ratio of reactive groups and the type of biocidal groups, after curing at room temperature or 120 °C. For this purpose, two series of copolymers with complementary reactive groups, poly(4-vinylbenzyl chloride-co-acrylic acid), P(VBC-co-AAx), and poly(sodium 4-styrenesulfonate-co-glycidyl methacrylate), P(SSNa-co-GMAx), were synthesized via free radical copolymerization and further modified resulting in covalently bound (4-vinylbenzyl dimethylhexadecylammonium chloride, VBCHAM) and electrostatically attached (hexadecyltrimethylammonium 4-styrene sulfonate, $SSAmC_{16}$) units. The crosslinking reaction between the carboxylic group of acrylic acid (AA) and the epoxide group of glycidyl methacrylate (GMA) of these copolymers led to the stabilization of the coatings through reactive blending. The so developed coatings were cured at room temperature and 120 °C, and then immersed in ultra-pure water and aqueous NaCl solutions at various concentrations for a time period up to three months. Visual inspection of the integrity of the materials coated onto glass slides, gravimetry, scanning electron microscopy (SEM) characterization, as well as the determination of total organic carbon (TOC) and total nitrogen (TN) of the solutions, were used to investigate the parameters affecting the release of the materials from the coatings based on these systems. The results revealed that curing temperature, complementary reactive groups' content, and type of antimicrobial species control the release levels and the nature of releasable species of these environmentally-friendly antimicrobial coatings.

Keywords: antimicrobial action; quaternary ammonium groups; acrylic acid; glycidyl methacrylate; crosslinking reaction; coating

1. Introduction

Research on antimicrobial polymeric materials and surfaces is intensive [1–7], since such materials are important for diverse applications, like health care/biomedical devices, food packaging, agriculture/aquaculture, marine biofouling, etc. [8–11]. Among the numerous examples of antimicrobial polymers that were evaluated for such potential applications [12–14], polymers based on quaternary ammonium groups [15–18] are possibly the most widely studied, concerning synthetic biocidal polymeric materials [19].

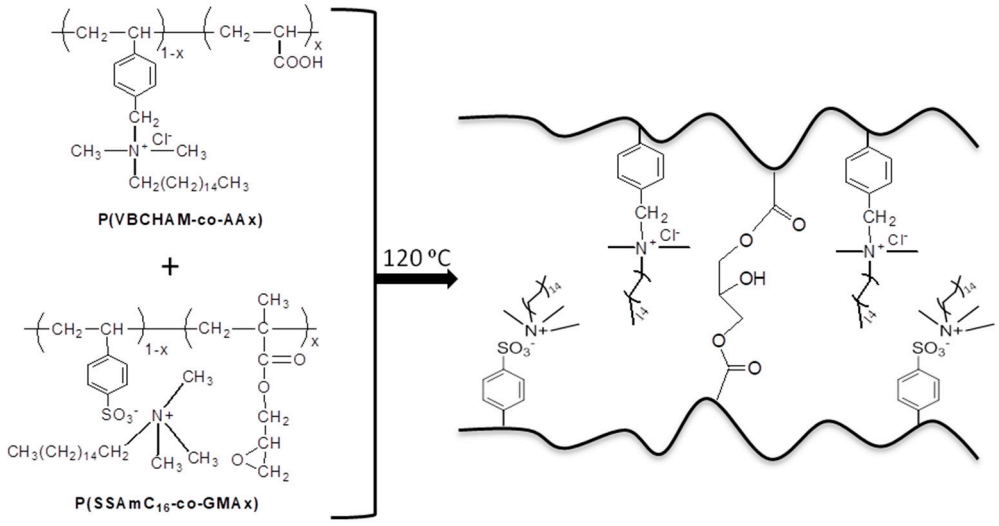
A critical issue regarding infection-related health is the formation of bacterial biofilms on surfaces. To overcome this problem, an urgent need for the development of coatings that are able to prevent

biofilm formation or eliminate already-constructed biofilms is well established [20–22]. Efficient antimicrobial coatings can prevent bacteria adhesion or kill the bacteria before or after contact with the surface, although many strategies include both mechanisms [23–26].

To better tune biocidal activity and duration, research nowadays focuses on dual-action biocidal materials and surfaces, i.e., materials with a simultaneous contact-killing and released-based biocidal action. The contact-killing action is usually based on quaternary ammonium groups that are covalently incorporated into a polymeric chain or polymeric substrate [27–29], whereas the released-based biocidal action is often introduced through the use of biocidal agents capable of leaching from the polymeric surfaces or materials, such as metallic/inorganic nanoparticles, antibiotics, or others [30].

Having this in mind, our research group is focusing on the potential use of quaternary ammonium groups with contact-based action, released-based action, as well as the combination of both actions. Thus, hexadecyltrimethylammonium (cetyl trimethylammonium) cations (AmC_{16}) electrostatically bound onto an anionic poly(styrene sulfonate) (PSS) backbone have been evaluated as potential released-based polymeric biocidal materials, while polymers of 4-vinylbenzyl chloride (VBC) modified with *N*,*N*-dimethylhexadecylamine (HAM) have been developed to achieve the contact-based action [31]. Moreover, random or block copolymers of the respective units, hexadecyltrimethylammonium styrene sulfonate ($SSAmC_{16}$) and 4-vinylbenzyl dimethylhexadecylammonium chloride (VBCHAM), have been designed for the combination of both actions [32].

Recently, we have shown that we can take advantage of the reactive blending concept, in order to prepare self-standing crosslinked membranes containing both released-based hexadecyltrimethylammonium groups and contact-based VBCHAM biocidal units [33]. Thus, polymeric precursors of the two biocidal species with complementary chemical functions, e.g., carboxylic groups of acrylic acid (AA) units and epoxide groups of glycidyl methacrylate (GMA) units, were initially synthesized. Blends of these copolymers, P($SSAmC_{16}$-co-GMAx) and P(VBCHAM-co-AAx) were then formed, and were cured at solid state at the desired temperature, leading to the final crosslinked membranes (Scheme 1), as a consequence of the reaction between the carboxylic and the epoxide groups. These membranes presented strong antimicrobial activity against *S. aureus* and *P. Aeruginosa*, while when applied as coatings on aquaculture nets exhibited high antifouling action as compared to blank net [33]. An interesting observation in that work was that the observed release in salt solution was maintained in much lower levels than the release in pure water, offering an additional advantage for potential antifouling applications in sea water.



Scheme 1. Reaction between P(VBCHAM-co-AAx) and P($SSAmC_{16}$-co-GMAx) copolymers after curing at 120 °C.

Motivated by the aforementioned encouraging initial findings, our aim in the present work is to investigate in more detail this methodology by using different copolymers' composition and blending ratio, in order to get a deeper understanding on the structural factors affecting the release behavior. Such knowledge is a prerequisite in order to optimize the efficacy and duration of the antifouling action of these novel polymeric biocidal coatings.

2. Materials and Methods

2.1. Materials

The monomers glycidyl methacrylate (GMA), sodium 4-styrene sulfonate (SSNa), acrylic acid (AA) and 4-vinylbenzyl chloride (VBC), the initiator azobisisobutyronitrile (AIBN), the surfactant hexadecyltrimethylammonium (cetyl trimethylammonium) bromide (AmC_{16}), the amine *N*,*N*-dimethylhexadecylamine (HAM), the salt NaCl; as well as deuterium oxide (D_2O), and deuterated chloroform ($CDCl_3$) were purchased from Aldrich, and were used as received. The solvents *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), chloroform ($CHCl_3$), hexane and acetone were purchased from Fischer and used as received. Micro glass slides with size $\pm$ 26 mm $\times$ 76 mm were acquired from Sigma-Aldrich. Ultra-pure water was obtained by means of an SG apparatus water purification unit.

2.2. Synthesis of the Precursors

The copolymers poly(4-vinylbenzyl chloride-co-acrylic acid) and poly(sodium 4-styrenesulfonate-co-glycidyl methacrylate) were synthesized through free radical polymerization in $CHCl_3$ at 70 °C and DMF/pure H_2O (50/50) or DMSO at 80 °C, respectively, using AIBN as initiator. These copolymers (Table 1) will be denoted as P(VBC-co-AAx) and P(SSNa-co-GMAx), where x is the mole fraction of AA and GMA units in the copolymer, as determined by the ^{1}H NMR characterization in $CDCl_3$ and D_2O, respectively. The experimental procedures for the synthesis and characterization of copolymers have been described previously [31,33].

Table 1. Characterization results for the P(VBCHAM-co-AAx) and P($SSAmC_{16}$-co-GMAx) complementary antimicrobial copolymers.

Precursors	Feed Composition % (mol AA or GMA)	^{1}H NMR Composition % (mol AA or GMA)	M_w	PDI	Antimicrobial Copolymers
P(VBC-co-AA7)	10	7	22,000	1.7	P(VBCHAM-co-AA7)
P(VBC-co-AA20)	20	20	28,200	2.7	P(VBCHAM-co-AA20)
P(SSNa-co-GMA6)	5	6	27,800	1.9	P($SSAmC_{16}$-co-GMA6)
P(SSNa-co-GMA20)	15	20	12,200	1.8	P($SSAmC_{16}$-co-GMA20)

2.3. Introduction of Antimicrobial Species

2.3.1. Covalently Bound Antimicrobial Groups

The quaternization process of P(VBC-co-AAx) copolymers has been described previously [31]. Briefly, the copolymers were dissolved in $CHCl_3$ and quaternized with an excess of *N*,*N*-dimethylhexadecylamine (HAM) at 60 °C for 48 h. The quaternized polymers, denoted P(VBCHAM-co-AAx), were recovered by precipitation in acetone, thoroughly washed with hexane, and dried in a vacuum oven at 60 °C for 24 h.

2.3.2. Electrostatically Attached Antimicrobial Groups

The introduction of electrostatically attached quaternary ammonium cations on the P(SSNa-co-GMAx) copolymers or PSSNa homopolymer was achieved through an ion exchange reaction in aqueous solution between the sodium cations of SSNa units and an excess of quaternary hexadecyltrimethylammonium

cations (AmC_{16}). The final products, denoted P($SSAmC_{16}$-co-GMAx), were obtained through filtration, washed thoroughly with ultra-pure water and dried in a vacuum oven at 60 °C for 24 h. The experimental procedures are described in detail elsewhere [33,34].

2.4. Preparation of Antimicrobial Coatings

Mother solutions of series of the two copolymers P(VBCHAM-co-AAx) and P($SSAmC_{16}$-co-GMAx) were prepared in $CHCl_3$ at a 10% (*w*/*v*) concentration and left overnight at room temperature under mild stirring. Afterwards, the mother solutions of the complementary copolymers were mixed at various compositions. The compositions of P(VBCHAM-co-AAx) and P($SSAmC_{16}$-co-GMAx) were expressed as weight/weight ratio (*w*/*w*) and set at the desired values (20/80, 40/60, 85/15) (Table 2). Subsequently, an appropriate volume of each mixture (1 mL) was incorporated onto the surface of micro glass slides and was left at room temperature for 24 h until complete solvent evaporation. Then, the coated slides were cured at 120 °C. For comparison reasons, uncured coated slides were used.

Table 2. Composition and curing conditions of the P(VBCHAM-co-AAx) and P($SSAmC_{16}$-co-GMAx) complementary antimicrobial copolymers coated onto glass slides.

Complementary Copolymers		Composition, % *w*/*w*	Curing Temperature and Time of Curing	Polymeric Coating
P($SSAmC_{16}$-co-GMA20)	P(VBCHAM-co-AA7)	40/60	RT (1 day) 120 °C (1 day)	C1-RT C1-120
P($SSAmC_{16}$-co-GMA6)	P(VBCHAM-co-AA20)	40/60	RT (1 day) 120 °C (1 day)	C2-RT C2-120
P($SSAmC_{16}$-co-GMA20)	P(VBCHAM-co-AA20)	40/60	RT (1 day) 120 °C (1 day)	C3-RT C3-120
		85/15	120 °C (1 day)	C4-120
		20/80	120 °C (1 day)	C5-120

Note: RT, Room temperature.

2.5. Immersion of Coatings in Ultra-Pure Water and Aqueous NaCl Solutions

The uncured and cured coated glass slides were immersed in ultra-pure water and aqueous NaCl solutions at 1 M, 0.5 M, and 0.25 M concentrations for significant time periods up to three months (1 day, 7 days, 30 days, 60 days). The volume was set at 130 mL, until complete coverage of the coated glass slides' surface was achieved. Finally, the immersed coated glass slides were taken out at the specific time period, immersed in ultra-pure water for the removal of NaCl when necessary and dried at room temperature for two days.

2.6. Characterization Techniques

2.6.1. Scanning Electron Microscopy (SEM) Examination

Scanning electron microscopy (SEM, Zeiss SUPRA 35VP instrument equipped with an Energy-dispersive X-ray spectroscopy, EDS, detector, Carl Zeiss AG, Oberkochen, Germany) was performed in order to investigate the polymeric coatings' surface morphologies.

2.6.2. Release Studies

The coated glass slides were immersed in ultra-pure water or aqueous NaCl solutions and left for different time intervals up to three months. The glass slides were removed at the specific time period, washed (in the case of NaCl solutions) and dried. The soluble fraction % (*w*/*w*) of the membranes in ultra-pure water or aqueous NaCl solutions was evaluated gravimetrically from the equation soluble fraction % $(w/w) = \frac{|w-w_0|}{w_0}$%, where W_0 and W are the measured weights of the coatings before and after the immersion, respectively.

2.6.3. Total Organic Carbon (TOC) and Total Nitrogen (TN) Measurements

Simultaneous analyses of TOC and TN were carried out using a Shimadzu TOC analyzer (TOC-VCSH) coupled to a chemiluminescence detector (TNM-1 TN unit). TOC analysis was performed using the Combustion-Infrared method. The principle of this method is that a microportion of the sample is injected into a heated reaction chamber packed with an oxidative catalyst, Pt/Al_2O_3. The organic and inorganic carbon is oxidized to CO_2, and is measured by means of a nondispersive infrared analyzer (NDIR analyzer). TN analysis was performed using the Pyrolysis-Chemiluminescence detection method. Oxidative pyrolysis converts chemically bound nitrogen to nitric oxide (NO). Nitric oxide is contacted with ozone (O_3) to produce metastable nitrogen dioxide (NO_2*). As NO_2* decays, the emitted light is detected by a photomultiplier tube.

3. Results and Discussion

The selection of the copolymers that will be used as the blends components was made in such a way that the initial biocidal activity was assured. More specifically, the copolymers with high content of electrostatically attached ammonium groups were used (see Table 1) that were previously tested and showed high biocidal activity in the range of 5–6 logarithmic reduction for all microbial that were tested [31]. The respective copolymers with covalently bound ammonium groups P(VBCHAM-co-AAx) have shown high activity against *E. faecalis* and *P. aureginosa* [31]. Additionally, all of the random copolymers [31] and coatings [33] having both types of ammonium groups in a comparable ratio have shown extremely high biocidal activity, as shown in the previous publications [31,33].

The main goal of the present work is a deeper understanding of the factors affecting the release rate of crosslinked antimicrobial polymeric coatings with dual contact-based and release-based antimicrobial activity. For this purpose, as was shown previously [33], the complementary reactive antimicrobial polymers P(VBCHAM-co-AAx) and P($SSAmC_{16}$-co-GMAx) were used for the coating development and stabilization. More specifically, new series of copolymers with AA and GMA groups contents from 6% up to 20% (see Table 1) were synthesized. The respective solutions in $CHCl_3$ of blends of the above copolymers were stirred for adequate time until complete homogenization. Then, they were casted on glass slides and the coated glass slides, after treatment either at room temperature (uncured) or at 120 °C (cured), were tested in respect to their release behavior by immersion in aqueous NaCl solutions or ultra-pure water for a period of time up to three months.

In order to understand the behavior, the initial solubility of the copolymers with the highest amount of the reactive groups e.g., P($SSAmC_{16}$-co-GMA20) and P(VBCHAM-co-AA20), was tested applying the same coating and release procedure in aqueous NaCl 1 M solution and ultra-pure water for 30 days. The results are shown in Figure 1 in terms of soluble fraction % (*w*/*w*) and total organic carbon (TOC) evolution. As expected, despite the presence of the hydrophilic AA groups, the solubility of hydrophobic P(VBCHAM-co-AA20) is marginal in water and salt solution, as a consequence of the hydrophobic character of VBCHAM units. On the other hand, the copolymer P($SSAmC_{16}$-co-GMA20) is readily soluble in salt solution, since it is well-known that the addition of electrolytes weakens the interactions between polyelectrolytes and oppositely charged surfactants [35]. Interestingly, at the low polymer concentration that was applied for these studies, this copolymer is also soluble in pure water. This behavior has been also observed for the complexation of CTAB with copolymers of SSNa with methyl methacrylate (MMA) [36] and possibly originates from the copolymer structure of the polyelectrolyte, leading to the disruption of the synergistic character of the polyelectrolyte/surfactant complexation. As an additional step in this initial study, the release of a $PSSAmC_{16}$/P(VBCHAM-co-AA20) 40/60 *w*/*w* was also followed under similar conditions. The homopolymer $PSSAmC_{16}$ was used in this case, instead of the copolymer, in order to avoid any crosslinking possibility even at room temperature. As seen, a significant fraction of the blend, comparable to the $PSSAmC_{16}$ content of the blend, is solubilised in pure water. Moreover, the soluble fraction in salt solution is very low and comparable to that of P(VBCHAM-co-AA20), besides the high solubility of $PSSAmC_{16}$ in aqueous NaCl 1 M solution. The investigation of these intriguing solubility

characteristics, already observed earlier with similar crosslinked membranes [33], is one of the main goals of the present work.

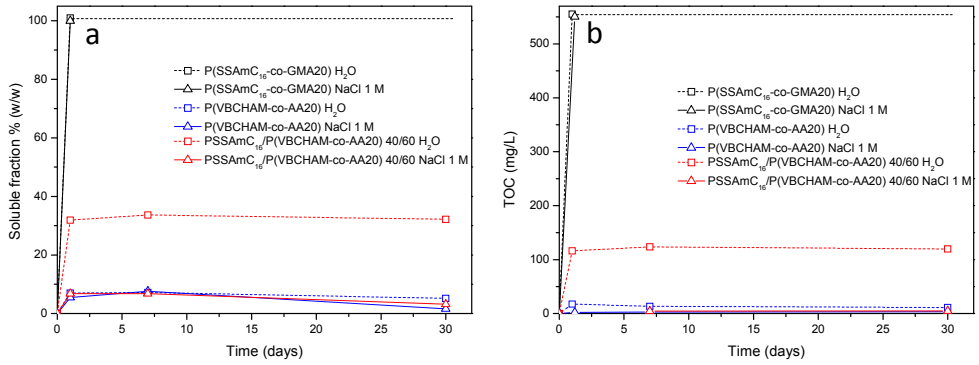


Figure 1. (**a**) Evolution of the soluble fraction % (*w*/*w*) after immersion of P(SSAmC$_{16}$-co-GMA20), P(VBCHAM-co-AA20) and PSSAmC$_{16}$/P(VBCHAM-co-AA20) 40/60 *w*/*w* polymeric coatings in ultra-pure water and aqueous NaCl 1 M solution for different time periods; and, (**b**) Evolution of the total organic carbon (TOC) values of the solutions for the same studies.

Three systems of the complementary antimicrobial polymers were initially investigated (C1-C3). In these systems, the ratio of the complementary reactive units varied significantly, whereas the content of the two types of biocidal units, i.e., releasable SSAmC$_{16}$ groups or immobilized quaternary ammonium VBCHAM units, was rather constant, since they were prepared at a fixed mixing weight ratio (40/60, see Table 2). The polymeric coatings presented different mechanical integrity after their immersion, depending on the curing temperature and the salinity of the aqueous solution. A characteristic example is depicted in Figure 2 for the C3 polymeric coatings, i.e., the uncured (C3-RT) and cured (C3-120) P(SSAmC$_{16}$-co-GMA20)/P(VBCHAM-co-AA20) 40/60 *w*/*w* polymeric coatings. It is evident that in ultra-pure water the C3-RT disintegrate after a few minutes (Figure 2a), whereas the C3-120 detach while preserving its integrity (Figure 2b). This behavior is mostly attributed to the crosslinking effect at 120 °C. On the other hand, both C3-RT and C3-120 polymeric coatings remain intact in the aqueous NaCl 1 M solution (Figure 2c,d).

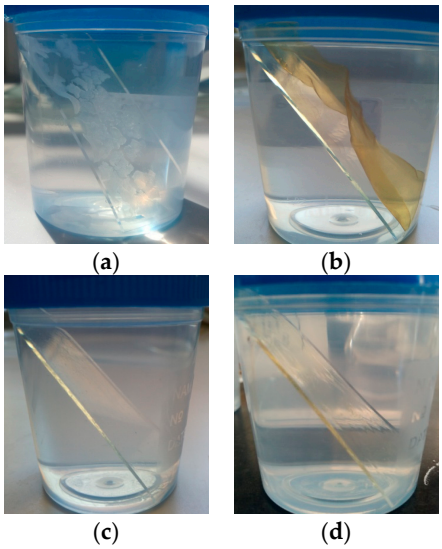


Figure 2. Photos of (**a**,**c**) Uncured C3-RT or (**b**,**d**) Cured C3-120 polymeric coatings, after immersion in ultra-pure water (**a**,**b**) or aqueous NaCl 1 M solution (**c**,**d**) for 7 days.

The release of leachable species of the C1-C3 polymeric coatings in pure water or aqueous NaCl 1 M solution was also followed as a function of time. The evolution of the soluble fraction, determined gravimetrically from the weight change of the coatings, is presented in Figure 3. The observed release trends were also verified from the TOC and TN values determined in the solutions for the same periods (Figures S1 and S2). The dashed lines correspond to the P(SSAmC$_{16}$-co-GMAx) content and represent the upper release limit when considering full solubility for these copolymers and marginal solubility for the complementary P(VBCHAM-co-AAx) copolymers. As seen, both for cured and uncured coatings, the release in water is higher than in NaCl 1 M solution, while the soluble fraction levels do not differentiate substantially with the change of treatment temperature. In addition, the soluble fraction levels are not significantly affected by the chemical composition of the complementary copolymers, i.e., the content of reactive units.

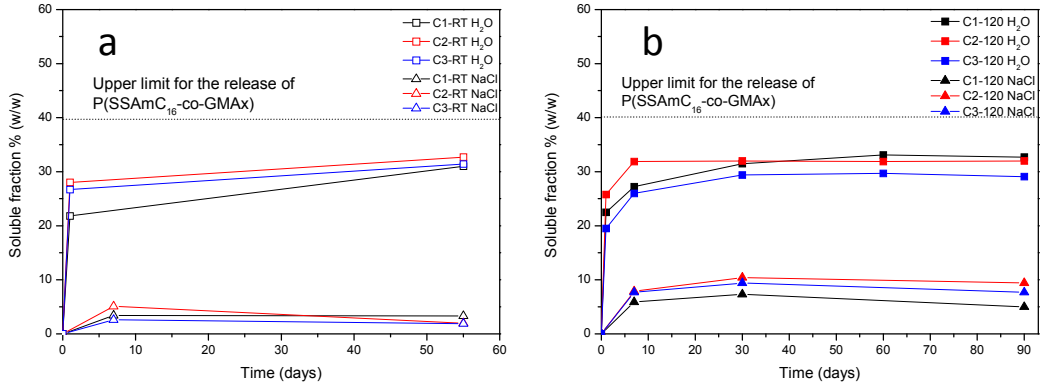


Figure 3. Evolution of the soluble fraction % (*w*/*w*) after immersion in ultra-pure water and aqueous NaCl 1 M solution for different time periods. (**a**) Uncured C1-RT, C2-RT, C3-RT polymeric coatings, (**b**) Cured C1-120, C2-120, C3-120 polymeric coatings.

These observations, in combination with the behavior presented in Figure 1 for the PSSAmC$_{16}$/P(VBCHAM-co-AA20) coating, indicate that the release of leachable species is not controlled by the possible crosslinking reaction between the complementary reactive units, but it is the result of non-covalent interactions between the two complementary copolymers. However, the crosslinking reaction is a crucial factor, concerning the mechanical integrity of the coatings: the visual inspection of coatings shows a clear difference between the cured and uncured coatings, with the coatings being developed at 120 °C to be more robust. For that reason, the release study for this type of coatings that was performed for three months showed a slow release after the first 10 days, but the quality of the coatings remained unchanged for the whole period of testing.

In order to understand these behaviors in terms of the releseable species [37], the C/N molar ratio was calculated from the TOC/TN ratio and plotted versus time, as shown in Figure 4. The C/N molar ratio is releated either to the cetyl trimethylammonium ions (AmC$_{16}$) or to the whole polymeric chain. As a consequence, the expected values are 19 for the cetyl units (dotted line) and about 27–28 for the whole polymeric chains of both complemetary polymers (dashed area). As seen in this Figure, the release comes in most cases from the liberation of the cetyl trimethylammonium ions (C/N molar ratios about 16–20). The release of these cations in the case of coatings cured at 120 °C is expected in aqueous NaCl 1 M solutions, since they can be ion exchanged with the Na^+ cations of the salt, while the polymeric chains cannot be released as they are crosslinked. However, the explanation for the release of cetyl trimethylammonium cations is not straightforward when the same coatings are treated with ultra-pure water. Possibly, in these systems, an ion rearrangement takes place, allowing for the internal complexation of the positive VBCHAM and negative styrene sulfonate ions immobilized onto the different polymeric backbones of the coating. This helps the release of cetyl trimethylammonium cations (from SSAmC$_{16}$ units) with Cl^- anions (from VBCHAM units) as counterions. In the case of

the coatings cured at room temperature, the crosslinking conditions are very mild, and crosslinking, if any, is expected to be very low. In fact, in these systems the main trends of the C/N molar ratios are quite similar to those observed for the PSSAmC$_{16}$/P(VBCHAM-co-AA20) 40/60 *w*/*w* coating (Figure S3). Thus, in the case of this coating and the coatings cured at room temperature, the ion rearrangement described previously could also take place in ultra-pure water, leading to the release of AmC$_{16}$ cations, as evidenced by the observed values of C/N molar ratios (~18–20). In contrast, when these coatings are treated with aqueous NaCl 1 M solutions, high C/N molar ratios are observed (~25–27). In these cases, the release (though low) is made from the whole polymeric chain (PSSAmC$_{16}$ in Figure S3 and P(SSAmC$_{16}$-co-GMAx) in Figure 4). Apparently, when crosslinking cannot be applied (PSSAmC$_{16}$) or the crosslinking conditions are very mild (coatings treated at room temperature), the respective polymers (PSSAmC$_{16}$ or P(SSAmC$_{16}$-co-GMAx)) turn to water-soluble in salt solution, as discussed in Figure 1. In fact, the solubilization of the whole polymeric chain seems to be gradual for the P(SSAmC$_{16}$-co-GMAx)/P(VBCHAM-co-AAx) coatings, since the high C/N molar ratios are observed at large releasing times, whereas these ratios are of the order of 19 for shorter releasing times. Alhough more detailed studies are needed, this observation possibly indicates the potentiality to develop self-eroding biocidal coatings using such materials.

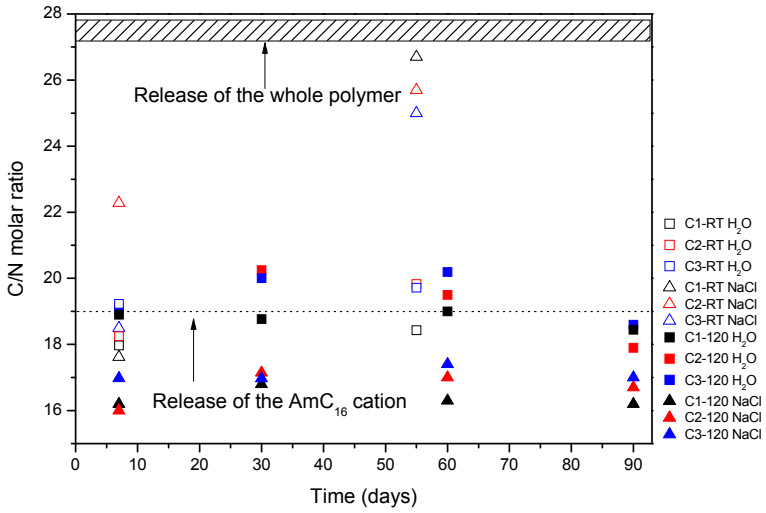


Figure 4. Evolution of the C/N molar ratio, determined from the TOC/ total nitrogen (TN) studies, after immersion in ultra-pure water and NaCl 1 M for different time periods of uncured C1-RT, C2-RT, C3-RT polymeric coatings and cured C1-120, C2-120, C3-120 polymeric coatings.

The influence of the blend composition and more specifically the ratio between the electrostatically and covalently bound quaternary ammonium biocidal species of the initial copolymers on the release properties was examined using the system P(SSAmC$_{16}$-co-GMA20)/P(VBCHAM-co-AA20), after curing at 120 °C. The release characteristics of three coatings with weight compositions 85/15 (C4-120), 40/60 (C3-120), and 20/80 (C5-120) were evaluated for 30 days (Figure 5). The system C5-120 with the lower content of the exchangeable ammonium groups shows the same behavior as before with higher release rate in water than in NaCl 1 M. In this case, the soluble fraction is lower when compared to C3-120, as a consequence of the lower content in P(SSAmC$_{16}$-co-GMA20). On the contrary, for the system C4-120 with high P(SSAmC$_{16}$-co-GMA20) content, a significant acceleration of the release rate was found and the selectivity observed between water and NaCl 1 M was vanished. In fact, this system shows the same release rate in both of the solutions used in this study, but the released fraction at 30 days is almost half of the P(SSAmC$_{16}$-co-GMA20) content.

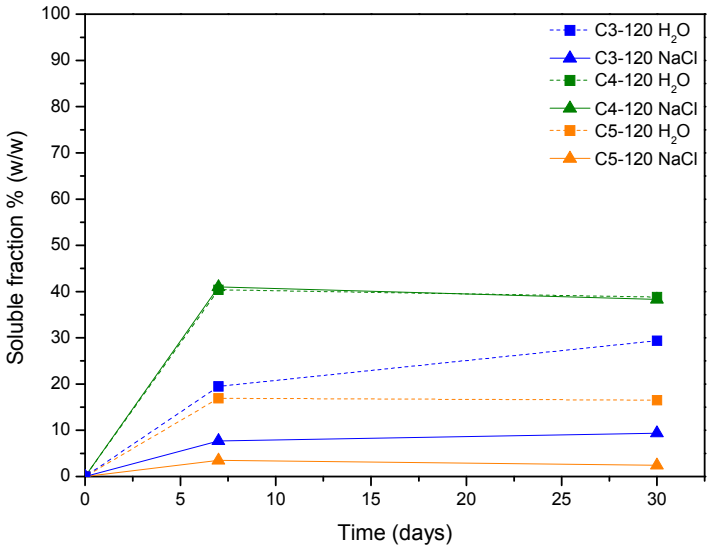


Figure 5. Evolution of soluble fraction % (*w*/*w*) after immersion in ultra-pure water and aqueous NaCl 1 M solution of cured C3-120, C4-120, C5-120 polymeric coatings.

The influence of the blend composition is more clearly depicted in Figure 6, where the soluble fraction % (*w*/*w*) after seven days testing in ultra-pure water or aqueous NaCl 1 M solution is plotted as a function of the P(VBCHAM-co-AA20) content of the P(SSAmC$_{16}$-co-GMA20)/P(VBCHAM-co-AA20) coatings. The respective TOC and TN values are also plotted, for reasons of comparison. As seen, the trends of all techniques used are in agreement. Moreover, the results show that the released material decreases with the increase of the P(VBCHAM-co-AA20) content, supporting the previous discussions that this material originates from the P(SSAmC$_{16}$-co-GMA20) copolymer.

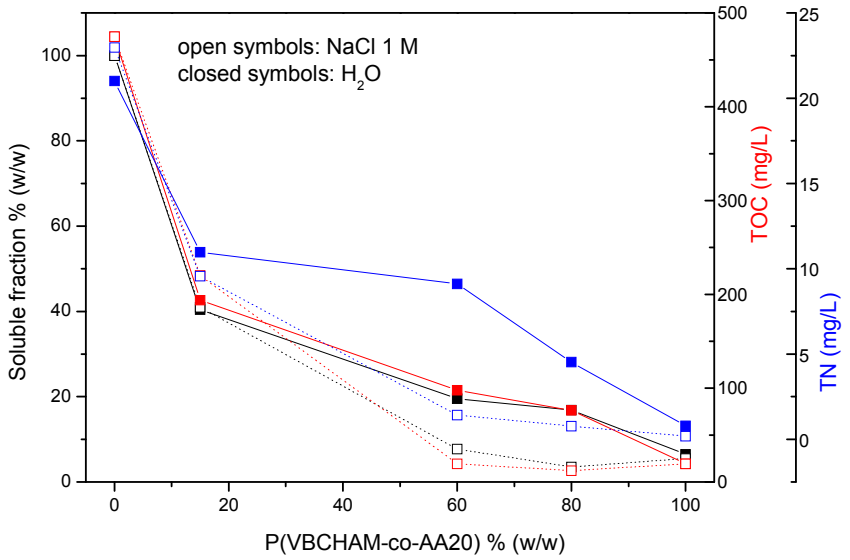


Figure 6. Influence of the P(VBCHAM-co-AA20) % (*w*/*w*) content of P(SSAmC$_{16}$-co-GMA20)/P(VBCHAM-co-AA20) 40/60 *w*/*w* polymeric coatings on the soluble fraction (%) and the TOC, TN values of the solutions after immersion of the coatings for 7 days in ultra-pure water and aqueous NaCl 1 M solutions. Apart from pure copolymers, all other coatings were cured at 120 °C.

The C/N molar ratio for the C3-C5 coatings, cured at 120 °C after seven days immersion in ultra-pure water or salt solution is shown in Figure 7a as a function of the P(VBCHAM-co-AA20) % (*w*/*w*) content of the coatings. Similar results are also obtained for 30 days immersion, as shown in Figure 7b. As seen, for the rich in P(VBCHAM-co-AA20) coatings (C3-120 and C5-120), the values of the C/N molar ratio are in the region 16–20, suggesting the release of AmC_{16} cations in these cases. Due to the high content of reactive units of the copolymers, crosslinking reaction is expected to take place in a large extent. Moreover, most P(SSAmC_{16}-co-GMA20) chains are expected to be crosslinked for these coatings and release of the whole polymeric chain is not possible. On the other hand, for the C4-120 coating containing P(SSAmC_{16}-co-GMA20) in a large excess, it is possible that a part of these chains are not crosslinked. As a consequence, in salt solution they turn to water-soluble and they can be dissolved, leading to the observation of a C/N molar ratio of ~27. Finally, regardless of blend composition, a C/N molar ratio of ~20 is observed for all of the coatings in ultra-pure water, suggesting the release of cetyl trimethylammonium cations, as discussed earlier.

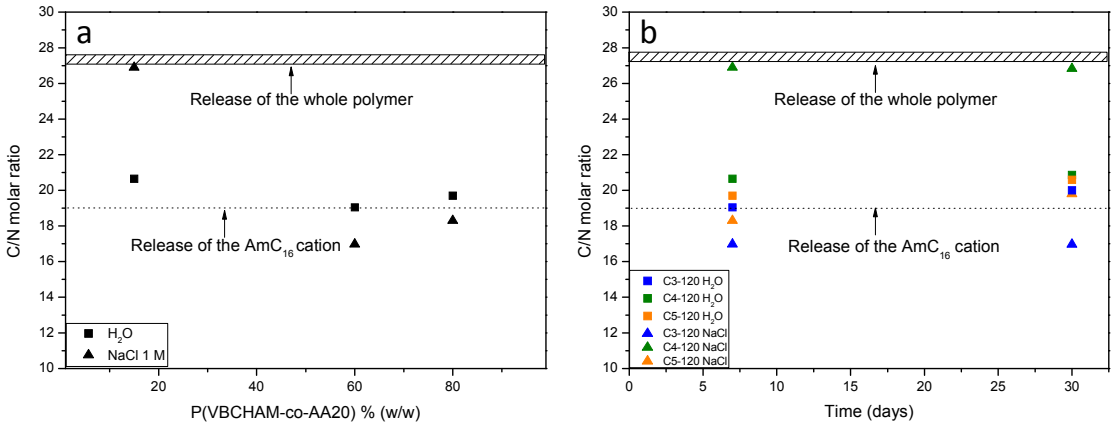


Figure 7. (**a**) Influence of the P(VBCHAM-co-AA20) % (*w*/*w*) content of the cured P(SSAmC_{16}-co-GMA20)/P(VBCHAM-co-AA20) 40/60 *w*/*w* polymeric coatings on the C/N molar ratio determined from the TOC and TN values of the solutions after immersion of the coatings for seven days in ultra-pure water and aqueous NaCl 1 M solutions, (**b**) Evolution of the C/N molar ratio, determined from the TOC/TN studies, after immersion in ultra-pure water and NaCl 1 M for different time periods of cured C3-120, C4-120, C5-120 polymeric coatings.

Since the salinity of the aqueous solution is a decisive parameter controlling the release levels of the coatings that are developed in the present study, the influence of NaCl concentration was also investigated, using the C3-120 coating. It is reminded that only the AmC_{16} cations may be released from this coating, see Figure 4. The evolution of the soluble fraction in ultra-pure water and aqueous NaCl solutions of varying concentrations (0.25 M, 0.50 M and 1 M) is shown in Figure 8a. Moreover, the variation of the soluble fraction with the NaCl concentration after treatment for 7 days are depicted in Figure 8b, and are compared with the respective TOC variation. It is clear that for NaCl concentrations higher than 0.5 M the release levels are low and do not depend significantly on the salt concentration. However, as the salt concentration decreases below 0.5 M, the release levels progressively increase to attain the maximum release fraction observed in ultra-pure water.

The morphology of the coatings in terms of SEM examination of the self-supported membranes was also studied. Thus, the initial membranes of the C3 system (cured and uncured) together with the respective membranes after treatment for 60 days are depicted in Figure 9. As it is observed, the initial membranes differ depending on their initial thermal treatment. The uncured C3–RT sample shows a particulate structure, while the cured C3-120, due to the higher temperature during thermal treatment, shows a more homogeneous structure. In both cases, the samples after 60 days in aqueous solutions show a more homogeneous structure, as revealed by the cross section images shown in Figure 9. This is

mainly due to the swelling of the membrane, as it is seen by the thickness increase from about 20 μm to 40 μm for the C3-120 sample. Thus, the release of the active groups cannot be visualized by SEM, since their competition to swelling vanishes the ability to show any difference.

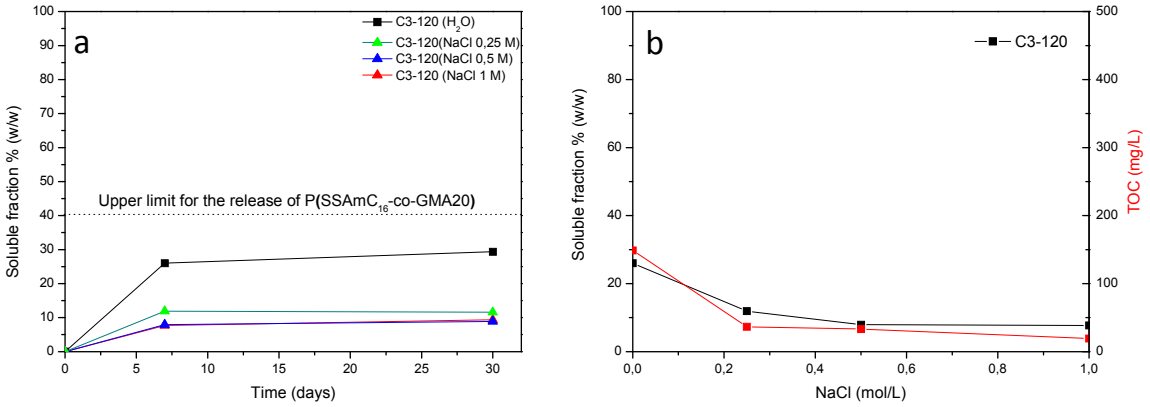


Figure 8. (**a**) Soluble fraction % (w/w) after immersion in ultra-pure water and NaCl 0.25 M, 0.5 M, 1 M for different time periods of cured C3-120 polymeric coatings; (**b**) Soluble fraction % (w/w) with the NaCl concentration after treatment for seven days when compared with the respective TOC variation.

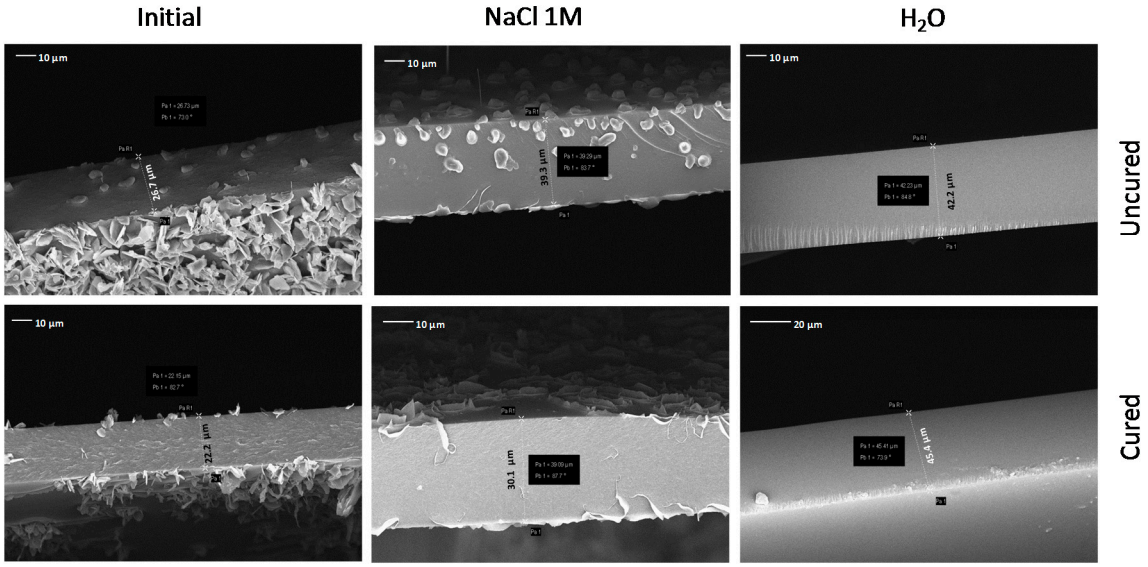


Figure 9. Scanning electron microscopy (SEM) images of uncured C3-RT and cured C3-120 polymeric coatings before and after immersion in ultra-pure water and NaCl 1 M for 60 days.

As a closing remark, it should be noticed that the synthetic protocols of the coatings and characterization protocols concerning the release in aqueous solutions of varying salinity were established in the present work, using covalently attached and releasable hexadecylammonium moieties. Nevertheless, respective units with alternative alkyl chains, for instance, dodecyl chains can also be applied to modulate biocidal activity and its interrelation to the release rate.

4. Conclusions

Different coatings based on covalently and electrostatically bound ammonium groups at various ratios were developed by combination and crosslinking of the respective copolymers bearing the proper active biocidal groups together with reactive acrylic acid and glycidyl methacrylate moieties. The stabilization of the coating was performed by curing at 120 °C, and the release of the active groups

was studied for periods up to three months in ultra-pure water and NaCl 1 M solutions in comparison to the uncured analogues. The rate of the release, as well as the type of the releasable species, was monitored by a combination of analytic techniques, like gravimetric and TOC, TN analysis. The ratio of TOC/TN was used as a decisive factor for the type of the releasable species. Depending on the mobile and immobilized ammonium groups' content and the curing temperature, coatings with controllable release behavior were developed. More interestingly, the release rate in NaCl 1 M solutions was lower than the respective in ultra-pure water, mainly because the internal complexation of immobilized oppositely charged species results in the release of AmC_{16} groups, even in ultra-pure water. This phenomenon together with the low release rate in NaCl 1 M solutions makes these biocidal coatings potentially useful candidates both for freshwater and sea water applications.

Supplementary Materials: The following are available online at www.mdpi.com/2079-6412/8/1/8/s1, Figure S1: Evolution of the TOC values of the solutions, after immersion in ultra-pure water and aqueous NaCl 1 M solution for different time periods of: (a) Uncured C1-RT, C2-RT, C3-RT polymeric coatings and (b) Cured C1-120, C2-120, C3-120 polymeric coatings, Figure S2: Evolution of the TN values of the solutions, after immersion in ultra-pure water and aqueous NaCl 1 M solution for different time periods of: (a) Uncured C1-RT, C2-RT, C3-RT polymeric coatings and (b) Cured C1-120, C2-120, C3-120 polymeric coatings, Figure S3: Evolution of the C/N molar ratio, determined from the TOC/TN studies, after immersion in ultra-pure water and NaCl 1 M for different time periods of uncured polymeric coatings $PSSAmC_{16}$/P(VBCHAM-co-AA20) 40/60 *w*/*w*.

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Conflicts of Interest: The authors declare no conflict of interest.

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