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Synthesis of antimicrobial block copolymers bearing immobilized bacteriostatic groups†

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Antimicrobial block copolymers bearing covalently bonded quaternized ammonium groups were synthesized through atom transfer radical polymerization (ATRP) using hydrophobic macro-initiators based on methyl methacrylate (MMA) and *tert*-butyl acrylate (tBA) for further polymerization of the cationic monomer 4-vinylbenzyl dimethylhexadecylammonium chloride (VBCHAM). Subsequent hydrolysis of the *tert*-butyl acrylate (tBA) units led to the formation of hydrophilic acrylic acid (AA) blocks. Moreover, a new class of antimicrobial block copolymers were designed containing VBCHAM and cetyltrimethylammonium 4-styrene sulfonate ($SSAmC_{16}$) units, combining two types of biocide incorporation into one system, operating through both contact-based and release-based mechanisms. The bacteriostatic efficacy of the novel materials was evaluated against Gram-negative (*P. aeruginosa* and *E. coli*) and Gram-positive (*S. aureus* and *E. faecalis*) bacteria and the obtained results are compared with those of the respective random copolymers in an attempt to examine the influence of the detailed polymeric structure on the bacteriostatic properties.

1 Introduction

Antimicrobial polymers[1–6] have attracted the interest of the scientific community over the past few years, due to their high efficacy in preventing and controlling the microbial infection. The use of polymeric antimicrobial agents appeared as an attractive alternative towards conventional antimicrobial agents. Their advantages, such as no volatility, chemical stability, no toxicity, the difficulty in permeation through the human or animal skin and the prolong lifetime,[7] have increased the interest from both academic and industrial points of view. Their use is of great importance in various fields,[8–14] especially in hospital surfaces, surgery equipment, dental restoration, water purification, soil sterilization, drugs, textiles, food packaging, and antifouling paints. Depending on the binding of the antimicrobial agents onto polymers, the latter can be classified into two categories: those with immobilized antimicrobial groups,[15,16] where the antimicrobial agent is covalently attached to the polymeric backbone, and those with controlled release type antimicrobial groups, where the antimicrobial agent is electrostatically bound to the polymer through electrostatic interactions[17,18] or coupled as the end group to an inactive polymer.[19,20]

A class of polymers that are widely used, are the cationic polymers with incorporated antimicrobial quaternary ammonium groups.[21–29] The proposed mechanism of their action is well known for years and involves adsorption onto the negatively charged group of the bacterial cell, diffusion through the cell wall, binding and disruption of the cytoplasmic membrane, release of K^+ ions and constituents of the cytoplasmic membrane and, eventually, cell death.[30]

To obtain well-defined charged copolymers with the desired architecture,[31] controlled radical polymerization (CRP) techniques,[32] such as Cu(0)-mediated reversible-deactivation radical polymerization,[33,34] nitroxide-mediated polymerization (NMP),[35] reversible addition fragmentation chain transfer polymerization (RAFT)[36] and atom transfer radical polymerization (ATRP),[37–40] have been employed. The introduction of the ionic groups can be achieved either pre- or post-polymerization. Although it is difficult to obtain complete functionalization, the post-quaternization process[41] has been widely used, because it offers the advantage of high efficiency in polymerization. On the other hand, in the pre-quaternization process,[42,43] the monomeric stability may be a limiting factor. Especially in ATRP, this method can potentially lead to low conversions due to many possible disadvantages, like complexation of the copper[44,45] or replacement of the amine

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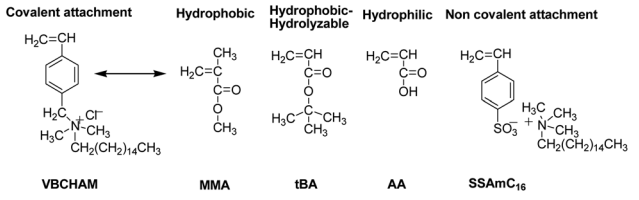
ligand by the ammonium groups, creation of an unfavorable equilibrium between the activating and deactivating copper species, degradative transfer with amines[46] or quaternization of terminal alkyl halides.[47] For these reasons, there is a limited number of publications regarding pre-quaternization block copolymer synthesis by means of ATRP.

As it was proved in our previous studies, the bacteriostatic efficacy of immobilized ammonium groups is highly dependent on the type of polymeric structure in which these groups are bound.[17] This finding, supported with the growing interest in ionic polymers designed for different applications,[32] prompted us to attempt the preparation of block copolymers containing one or both charged blocks. The guiding tool for the block copolymers' formation was our previous efforts to synthesize different random copolymers combining immobilized ammonium salts based on 4-vinylbenzyl chloride (VBC) derivatives, with hydrophobic monomers like methyl methacrylate (MMA), hydrophilic units like sodium 4-styrene sulfonate (SSNa) and acrylic acid (AA)[17,48] and ionically linked quaternary ammonium salts based on SSNa. What should be emphasized here is that differences in bacteriostatic efficacy were noticed between the poly(4-vinylbenzyl dimethylhexadecylammonium chloride) (PVBCHAM) homopolymers and specific copolymers based on the active 4-vinylbenzyl dimethylhexadecylammonium chloride (VBCHAM) units.[17] Additionally, due to selected morphologies that block copolymers offer against random copolymers, their use drastically increases the possibilities for incorporation of the active copolymer in different polymeric matrices.

Therefore, in the present work, we report on the synthesis of 4-vinylbenzyl dimethylhexadecylammonium chloride (VBCHAM) diblock copolymers with MMA, tBA, AA and $SSAmC_{16}$ blocks *via* ATRP.[49] In this way, we were able to combine a unit bearing covalently attached biocidal groups (VBCHAM) with hydrophobic units (MMA, tBA), a hydrophilic unit (AA) and a unit with electrostatically attached biocidal moieties ($SSAmC_{16}$). In the last case two different biocides' incorporations were achieved in the same system to operate through both contact-based and release-based mechanisms. To the best of our knowledge, there is no report in the literature for the synthesis of block copolymers with both contact-based and release-based biocidal groups incorporated into one system, making this class of polymers indispensable materials for potential biocidal use. The structures of the monomeric units are depicted in Scheme 1.



Scheme 1 Chemical structure of the monomers used in this work.

2 Experimental and methods

2.1 Materials

The monomers, sodium 4-styrene sulfonate (SSNa) and 4-vinylbenzyl chloride (VBC), were purchased from Aldrich. Methyl methacrylate (MMA) and *tert*-butyl acrylate (tBA) were passed through a basic alumina column to remove the polymerization inhibitors prior to use. All the other monomers were used as received. The catalyst copper(I) bromide (CuBr), the ligands 2,2′-bipyridine (bpy), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA), the initiator methyl 2-chloropropionate (2-MCP), as well as deuterated dimethylsulfoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$) are products of Aldrich and used as received. The initiator 4-[3,5-bis(carboxyl)-phenoxymethyl]benzyl bromide (Bis COOH-Init) was synthesized according to the literature.[50,51] The solvents dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), dry DMF, anisole, 1,4-dioxane, chloroform ($CHCl_3$), ethyl acetate, methanol, acetone and diethyl ether, as well as concentrated hydrochloric acid (HCl, 37%) were purchased from Fischer and used as received. The reagents *N*,*N*-dimethylhexadecylamine (HAM) and cetyltrimethylammonium bromide (AmC_{16}) were purchased from Aldrich and Acros Organics, respectively. Ultrapure water was obtained by means of an SG apparatus water purification unit.

2.2 Instruments and measurements

^{1}H NMR spectra were recorded on a Bruker Advance DPX 400 spectrometer, using DMSO-d_6 and $CDCl_3$ as solvents containing TMS as the internal standard. ATR-FTIR spectra were recorded on a Platinum ATR Bruker. Size Exclusion Chromatography (SEC) measurements were carried out at 25 °C with a flow rate of 1 mL min^{-1}, using a Polymer Lab chromatographer equipped with two Ultra Styragel linear columns (10^4, 500 Å) and a UV detector. Polystyrene standards were used for the calibration and the eluent was $CHCl_3$. TEM experiments were carried out using a JEM 2100 microscope operating at 200 kV. For TEM investigation, diluted chloroform, ethanol or methanol solutions/dispersions of the diblock copolymers were used.

2.3 Quaternization of monomers

The experimental procedure for the synthesis of 4-vinylbenzyl dimethylhexadecylammonium chloride (VBCHAM) and cetyltrimethylammonium 4-styrene sulfonate ($SSAmC_{16}$) has been described in previous studies.[48,52] It is worth mentioning that quaternized 4-vinylbenzyl chloride was used as the monomer for the polymerization, due to the ability of the CH_2Cl group of 4-vinylbenzyl chloride (VBC) to act as the ATRP initiator.[53,54]

2.4 Synthesis of poly(methyl methacrylate)-*b*-poly(4-vinylbenzyl dimethylhexadecylammonium chloride) copolymers (PMMA-*b*-PVBCHAM*x*)

2.4.1 PMMA macro-initiator[55] synthesis using methyl 2-chloropropionate (2-MCP). A 50 mL round bottom flask,

equipped with a reflux condenser and a magnetic stirrer, was flamed under vacuum and filled with argon. Then, 235.2 μL PMDETA (1.1 mmol), 161.6 mg CuBr (1.1 mmol), 15.0 mL degassed DMF, 12.0 mL MMA (112.7 mmol) and 128.4 μL (1.1 mmol) 2-MCP were added into the flask. After the addition of each substance, the system was degassed and flushed with argon. The mixture was stirred and heated at 110 °C for 24 h. The next day, the solution was cooled and precipitated in a triple distilled water/methanol mixture (50%/50% v/v). The product was filtered and dried under vacuum at 80 °C overnight. The same procedure was followed for the PMMA synthesis with 4-[3,5-bis(carboxyl)-phenoxymethyl]benzyl bromide (Bis COOH-Init) as the initiator. The macro-initiators will be denoted as PMMA(1) with 2-MCP initiator and PMMA (2) with Bis COOH-Init. The results are summarized in Table 1.

2.4.2 PMMA-*b*-PVBCHAM*x* synthesis. A 50 mL round bottom flask, equipped with a reflux condenser and a magnetic stirrer, was flamed under vacuum and filled with argon. Then, 300.0 mg PMMA(1) macro-initiator and 3.4743 g VBCHAM (9.0 mmol) monomer were dissolved in 40 mL degassed anisole after stirring. The addition of 30.2 mg bpy (0.2 mmol) and 13.9 mg CuBr (0.1 mmol) was followed. After the addition of each substance, the system was degassed and flushed with argon. The mixture was stirred and heated at 110 °C for 24 h. The next day, the solvent was evaporated under vacuum and the solid was washed several times with triple distilled water, acetone and a small amount of methanol. The product was dried under vacuum at 60 °C overnight. The same procedure was followed using PMMA(2) as the macro-initiator, using DMSO as the solvent. The block copolymers will be denoted as PMMA(1)-*b*-PVBCHAM*x* and PMMA(2)-*b*-PVBCHAM*x* according to the macro-initiators used. The results are summarized in Table 1.

2.5 Synthesis of poly(*tert*-butyl acrylate)-*b*-poly(4-vinylbenzyl dimethylhexadecylammonium chloride) copolymers (PtBA-*b*-PVBCHAM*x*)

2.5.1 PtBA macro-initiator synthesis[56] using 2-MCP. A 50 mL round bottom flask, equipped with a reflux condenser and a magnetic stirrer, was flamed under vacuum and filled with argon. Subsequently, 9.5 mL degassed tBA (65.4 mmol), 273.3 μL PMDETA (1.2 mmol), 93.8 mg CuBr (0.6 mmol), and 37.3 μL (0.3 mmol) 2-MCP were placed in the flask. After the addition of each substance, the system was degassed and flushed with argon. The mixture was stirred and heated at 80 °C or 110 °C for 24 h. The next day, the solution was cooled and precipitated in triple distilled water/methanol in percentage (20%/80% v/v). The product was filtered and dried under vacuum at 80 °C overnight. The macro-initiators will be denoted as PtBA(1a) and PtBA(1b) depending on the temperature of the reaction. The results are summarized in Table 2.

2.5.2 PtBA-*b*-PVBCHAM*x* synthesis. A 100 mL round bottom flask, equipped with a reflux condenser and a magnetic stirrer, was flamed under vacuum and filled with argon. Then, 200.0 mg PtBA macro-initiator and 2.0 g VBCHAM (5.1 mmol) monomer were dissolved in 55.0 mL degassed

Table 1 ^{1}H NMR characterization results of PMMA macro-initiators and PMMA-*b*-PVBCHAM*x* diblock copolymers

Macro-initiator	Initiator	Reaction conditions	M_n (estimated from ^{1}H NMR)	Yield[a] (%)
PMMA(1)	2-MCP	CuBr/PMDETA, DMF, 110 °C	6500	40
PMMA(2)	Bis COOH-Init	CuBr/PMDETA, DMF, 110 °C	3500	35

Diblock copolymer	Reaction conditions	Feed composition (mol% VBCHAM)	^{1}H NMR composition (mol% VBCHAM)	Yield (%)
PMMA(1)-*b*-PVBCHAM75	CuBr/bpy, anisole, 110 °C	50	75	20
PMMA(2)-*b*-PVBCHAM65	CuBr/bpy, DMSO, 110 °C	75	65	40

[a] The yield percentage of all polymers presented in this work was calculated after purification of each product.

Table 2 ^{1}H NMR characterization results of PtBA macro-initiators and PtBA-*b*-PVBCHAM*x* diblock copolymers

Macro-initiator	Initiator	Reaction conditions	M_n (estimated from ^{1}H NMR)	Yield[a] (%)
PtBA(1a)	2-MCP	CuBr/PMDETA, 80 °C (bulk)	6500	25
PtBA(1b)	2-MCP	CuBr/PMDETA, 110 °C (bulk)	11 000	35

Diblock copolymer	Reaction conditions	Feed composition (mol% VBCHAM)	^{1}H NMR composition (mol% VBCHAM)	Yield (%)
PtBA(1a)-*b*-PVBCHAM65	CuBr/bpy anisole, 80 °C	95	65	20
PtBA(1b)-*b*-PVBCHAM55	CuBr/bpy, anisole, 80 °C	75	55	30
PtBA(1b)-*b*-PVBCHAM48	CuBr/bpy, anisole, 80 °C	50	48	23

[a] The yield percentage of all polymers presented in this work was calculated after purification of each product.

anisole after stirring. The addition of 11.4 mg bpy (0.08 mmol) and 5.2 mg CuBr (0.04 mmol) followed. After the addition of each substance, the system was degassed and flushed with argon. The mixture was stirred and heated at 80 °C for 24 h. The next day, the solvent was evaporated under vacuum and the solid was washed several times with triple distilled water and acetone. The product was dried under vacuum at 60 °C overnight. The block copolymers will be denoted as PtBA(1a)-*b*-PVBCHAM*x* and PtBA(1b)-*b*-PVBCHAM*x* depending on the macro-initiators used. The results are summarized in Table 2.

2.6 Synthesis of poly(acrylic acid)-*b*-poly(4-vinylbenzyl dimethylhexadecylammonium chloride) copolymers (PAA-*b*-PVBCHAM*x*)

In a 50 mL round bottom flask, equipped with a reflux condenser and a magnetic stirrer, 242.0 mg PtBA(1a)-*b*-PVBCHAM65 (0.8 mmol tBA) were dissolved in 6.0 mL 1,4-dioxane under stirring. Then, 150.0 μL (1.8 mmol) of concentrated HCl were added. The mixture was stirred and heated at 110 °C for 24 h. The next day, the solvent was evaporated under vacuum and the solid was washed several times with ethyl acetate, acetone and triple distilled water. The product was dried under vacuum at 60 °C overnight.

2.7 Synthesis of poly(4-vinylbenzyl dimethylhexadecylammonium chloride)-*b*-poly(cetyltrimethylammonium 4-styrene sulfonate) copolymers (PVCBHAM*x*-*b*-PSSAmC$_{16}$)

2.7.1 PVBCHAM macro-initiator synthesis using 2-MCP. A 50 mL round bottom flask, equipped with a reflux condenser and a magnetic stirrer, was flamed under vacuum and filled with argon. Then, 31.4 mg CuBr (0.2 mmol), 68.4 mg bpy (0.4 mmol), 12.5 μL (0.1 mmol) 2-MCP, 4.2357 g VBCHAM (11.0 mmol) and 25.0 mL degassed anisole were added into the flask. After the addition of each substance, the system was degassed and flushed with argon. The mixture was stirred and heated at 80 °C for 24 h. The next day, the solvent was evaporated under vacuum and the solid was washed several times with triple distilled water and acetone. The product was dried under vacuum at 40 °C overnight. The same procedure was followed using Bis COOH-Init in anisole or DMSO at 110 °C. The macro-initiators will be denoted as PVBCHAM(1), PVBCHAM (2a) and PVBCHAM(2b) depending on the initiator and the reaction conditions. The results are summarized in Table 3.

2.7.2 PVBCHAM*x*-*b*-PSSAmC$_{16}$ synthesis. A 50 mL round bottom flask, equipped with a reflux condenser and a magnetic stirrer, was flamed under vacuum and filled with argon. Then, 300.0 mg of the PVBCHAM(1) macro-initiator were added in 25.0 mL degassed anisole or DMSO and 121.0 mg SSAmC$_{16}$ (0.3 mmol) monomer, 15.7 mg bpy (0.1 mmol) and 7.2 mg CuBr (0.05 mmol) were also added into the flask. After the addition of each substance, the system was degassed and flushed with argon. The mixture was stirred and heated at 110 °C for 24 h. The next day, the solvent was evaporated under vacuum and the solid was washed several times with ethyl acetate, diethyl ether and acetone. The product was dried under vacuum at 60 °C overnight. The block copolymers will be denoted as PVBCHAM(1)*x*-*b*-PSSAmC$_{16}$ and PVBCHAM(2a)*x*-*b*-PSSAmC$_{16}$ depending on the macro-initiators used. The results are summarized in Table 3.

2.8 Synthesis of poly(cetyltrimethylammonium 4-styrene sulfonate)-*b*-poly(4-vinylbenzyl dimethylhexadecylammonium chloride) copolymers (PSSAmC$_{16}$-*b*-PVCBHAM*x* copolymers)

2.8.1 PSSAmC$_{16}$ macro-initiator synthesis using 4-[3,5-bis-(carboxyl)-phenoxymethyl]benzyl bromide (Bis COOH-Init) PSSNa precursor synthesis. A 50 mL round bottom flask, equipped with a reflux condenser and a magnetic stirrer, was flamed under vacuum and filled with argon. Subsequently, 27.8 mg CuBr (0.2 mmol), 30.3 mg bpy (0.2 mmol), 70.8 mg (0.2 mmol) Bis COOH-Init, 4.0 g SSNa (19.4 mmol) and 35.0 mL degassed DMSO were added into the flask. After the addition of each substance, the system was degassed and flushed with argon. The mixture was stirred and heated at 110 °C or 90 °C for 24 h. The next day, the solution was cooled and precipitated in acetone. The mixture was stirred for 24 h, filtered and dried under vacuum at 60 °C overnight. Then, the solid was dissolved in triple distilled water, purified through dialysis tubing and dried under vacuum at 60 °C overnight. The macro-initiators will be denoted as PSSNa(1) and PSSNa(2) depending on the temperature of the reaction. The results are summarized in Table 4.

Table 3 ^{1}H NMR characterization results of the successful polymerization of PVBCHAM macro-initiators and PVBCHAM*x*-*b*-PSSAmC$_{16}$ diblock copolymers

Macro-initiator	Initiator	Reaction conditions	M_n (estimated from ^{1}H NMR)	Yield (%)
PVBCHAM(1)	2-MCP	CuBr/bpy, anisole, 80 °C	5000	40
PVBCHAM(2a)	Bis COOH-Init	CuBr/bpy, DMSO, 110 °C	3000	20
PVBCHAM(2b)	Bis COOH-Init	CuBr/bpy, anisole, 110 °C	5000	30

Diblock copolymer	Reaction conditions	Feed composition (mol% VBCHAM)	^{1}H NMR composition (mol% VBCHAM)	Yield (%)
PVBCHAM(1)10-*b*-PSSAmC$_{16}$	CuBr/bpy, anisole, 110 °C	75	10[a]	45
PVBCHAM(2a)60-*b*-PSSAmC$_{16}$	CuBr/bpy, DMSO, 110 °C	85	60	25

[a] Low ^{1}H NMR percentage of the VBCHAM unit is attributed to problems in solubility of the macro-initiator PVBCHAM during the reaction.

Table 4 ^{1}H NMR characterization results of the successful polymerization of PSSNa precursors, PSSAmC$_{16}$ macro-initiators and PSSAmC$_{16}$-*b*-PVBCHAM*x* diblock copolymers

Precursor	Initiator	Reaction conditions	M_n (estimated from ^{1}H NMR)	Yield[a] (%)
PSSNa(1)	Bis COOH-Init	CuBr/bpy, DMSO, 110 °C	3000	17
PSSNa(2)	Bis COOH-Init	CuBr/bpy, DMSO, 90 °C	9000	12

Macro-initiator	—	Reaction conditions	—	Yield (%)
PSSAmC$_{16}$(1)	—	AmC$_{16}$, H_2O, 25 °C	—	80

Diblock copolymer	Reaction conditions	Feed composition (mol% VBCHAM)	^{1}H NMR composition (mol% VBCHAM)	Yield (%)
PSSAmC$_{16}$(1a)-*b*-PVBCHAM50	CuBr/bpy, DMFd, 110 °C	75	50	13
PSSAmC$_{16}$(1b)-*b*-PVBCHAM25	CuBr/bpy, DMSO, 80 °C	25	25	20

[a] The yield percentage of all polymers presented in this work was calculated after purification of each product.

2.8.2 PSSAmC$_{16}$ macro-initiator synthesis. In a 50 mL round bottom flask, equipped with a reflux condenser and a magnetic stirrer, 400.0 mg PSSNa(1) precursor (1.9 mmol) were dissolved in 4.0 mL triple distilled water after stirring. In a 50 mL Erlenmeyer flask, equipped with a reflux condenser and a magnetic stirrer, 800.0 mg of AmC$_{16}$ (2.2 mmol) were dissolved in 8.0 mL triple distilled water and added dropwise into the round bottom flask. The solvent of the resulting turbid solution was evaporated and the solid was washed with triple distilled water and dried under vacuum at 80 °C overnight. The results are summarized in Table 4.

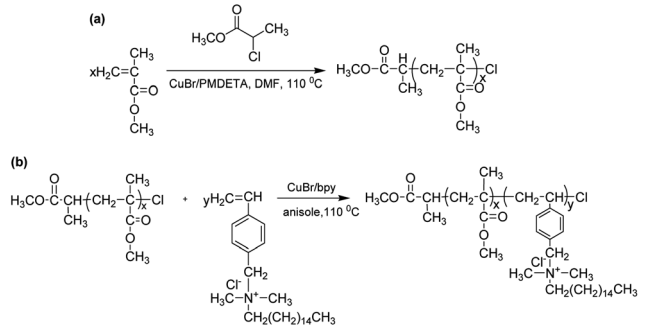
2.8.3 PSSAmC$_{16}$-*b*-PVCBHAM*x* synthesis. A 50 mL round bottom flask, equipped with a reflux condenser and a magnetic stirrer, was flamed under vacuum and filled with argon. Then, 200.0 mg PSSAmC$_{16}$(1) macro-initiator and 120.0 mg VBCHAM (0.3 mmol) monomer were dissolved in 5.0 mL degassed dry DMF or DMSO after stirring. In the next step, 41.8 mg bpy (0.2 mmol) and 19.2 mg CuBr (0.1 mmol) were added into the flask. After the addition of each substance, the system was degassed and flushed with argon. The mixture was stirred and heated at 110 °C or 80 °C for 24 h. The next day, the solution was cooled and precipitated in ethyl acetate. The mixture was stirred for 24 h, filtered and dried under vacuum at 80 °C overnight. The solid was washed with small amounts of chloroform and dried under vacuum at 60 °C overnight. The block copolymers will be denoted as PSSAmC$_{16}$(1a)-*b*-PVCBHAM*x* and PSSAmC$_{16}$(1b)-*b*-PVCBHAM*x* depending on the reaction conditions. The results are summarized in Table 4.

3 Results and discussion

In our previous studies, it has been shown that polymeric materials with immobilized quaternized ammonium groups presented different bacteriostatic activities depending on their structure.[17] More specifically, poly(4-vinylbenzyl dimethylhexadecylammonium chloride) (PVBCHAM) was inactive as a homopolymer while its acrylic acid random copolymer was highly active.[17] Since the utmost goal in the design of bacteriostatic or biofouling materials is the gradual release of the active species, block copolymers are attractive materials for such applications. So, in this work, by taking advantage of the results obtained previously,[17] concerning the proper combination of the different bacteriostatic groups (both contact and release types in the random copolymer structure), we aimed to design and develop the respective block copolymers. In a further step, a comparison of the bacteriostatic activity between random and block copolymers having the same subunits was attempted.

3.1 Synthesis and characterization of PMMA-*b*-PVBCHAM*x* copolymers

The synthetic approach for the preparation of diblock copolymers with hydrophobic units of MMA and VBCHAM bearing covalently attached bacteriostatic moieties is depicted in Scheme 2. In the first step, the PMMA(1) macro-initiator was synthesized *via* ATRP (using 2-MCP as the initiator) (Scheme 2a) and was further used for the polymerization of VBCHAM (Scheme 2b).



Scheme 2 Synthesis of (a) PMMA(1) macro-initiators (using 2-MCP) and (b) PMMA(1)-*b*-PVBCHAM*x* diblock copolymers.

Apart from the 2-MCP initiator, 4-[3,5-bis(carboxyl)-phenoxymethyl]benzyl bromide (Bis COOH-Init) was also used in the ATRP, since the two free carboxyl groups that this initiator contains may further react with other functional groups, like amines or epoxy rings. This may lead to the potential use of macro-initiators and diblock copolymers in the synthesis of more complex architectures. Surprisingly, as it was demonstrated for the polymerization of styrene,[57,58] the efficiency of carboxylic acid initiators with remote halogens is high, whereas low initiator efficiency is obtained for α-halocarboxylic acids. The latter is attributed to an intramolecular cyclization reaction between α-halocarboxylic acids and olefins forming γ-butyrolactones under atom transfer radical addition (ATRA) conditions.[59] Furthermore, as it has been previously reported for the ATRP of polyacrylamide with chloroacetic acid,[60] also in the case of VBCHAM polymerization, the use of acidic conditions may protect the polymerization from the unexpected complexation between copper and quaternary ammonium monomers. The synthesis of PMMA macro-initiators with Bis COOH-Init and the subsequent polymerization of VBCHAM are described in Scheme 3.

The chemical structure of PMMA-*b*-PVBCHAM*x* copolymers was verified through ATR-FTIR spectroscopy which is presented in ESI-1.†

The determination of the chemical composition of the copolymers was achieved through ^{1}H NMR spectroscopy in $CDCl_3$, as shown in Fig. 1. The presence of VBCHAM was confirmed by the peaks at 0.9 and 1.2 ppm, corresponding to the protons of the methyl (l) and methylene (k′) groups of the hexadecyl unit, while the remaining methylene group (k) appeared at approximately 1.4 ppm. The peaks observed at 3.2 and 3.6 ppm attributed to the protons of the methyl (h) and methylene (i) groups of the hexadecyl unit linked with the nitrogen atom. Moreover, the peak at 4.9 ppm assigned to the methylene protons of the benzyl group (g) linked with the nitrogen atom (2H, $-CH_2N^+$), whereas the protons of the copolymer backbone (a, b, c) appeared between 1.5–2.2 ppm. Finally, the broad peaks at 6–8 ppm were assigned to the protons of the aromatic rings (f, f′). In addition, the presence of PMMA was confirmed by the peaks at 0.9 and 3.6 ppm assigned to the α-methyl group (d) and the methyl ester group (e) of MMA, respectively. The composition of the PMMA-*b*-PVBCHAM*x* copolymers was determined by the comparison of the peaks at 0.9 ppm (MMA unit) and 4.9 ppm (VBCHAM unit). It is worth mentioning that there is no displacement of the peak at 4.9 ppm of PVBCHAM compared to PMMA-*b*-PVBCHAM65, proving the block architecture of the copolymer.

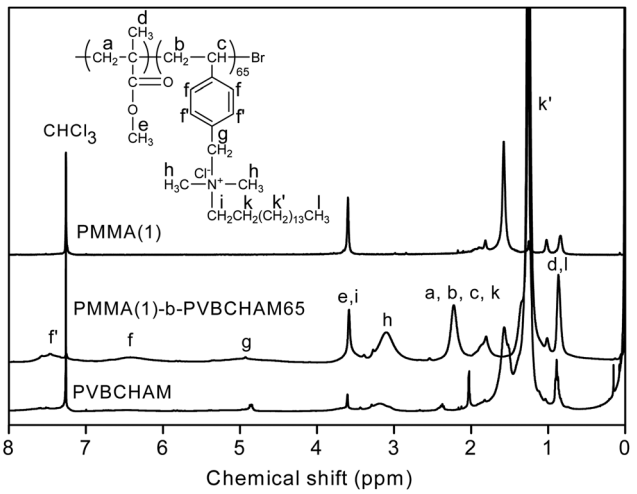
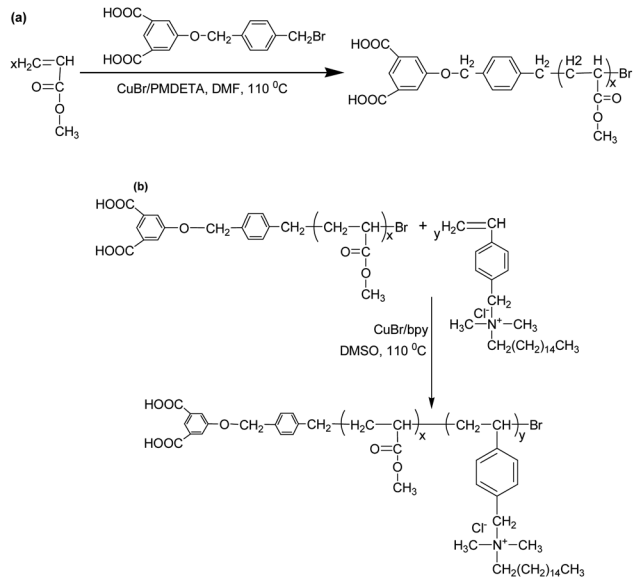


Fig. 1 ^{1}H NMR spectra of the diblock copolymer PMMA(1)-*b*-PVBCHAM65, the macro-initiator PMMA(1) and the homopolymer PVBCHAM.

The results from ^{1}H NMR characterization for the successful polymerization of the macro-initiators PMMA and the diblock copolymers PMMA-*b*-PVBCHAM*x*, are presented in Table 1.

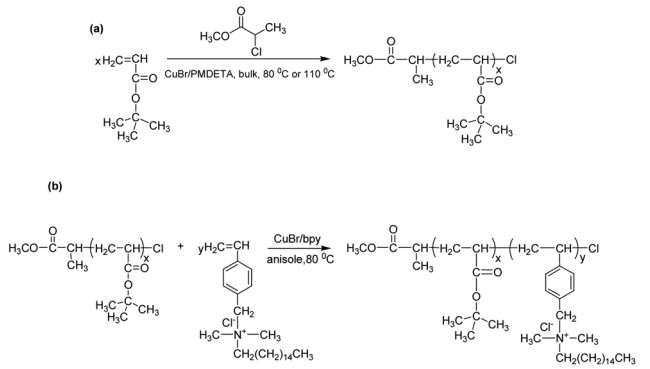
In conclusion, PMMA macro-initiators were easily synthesized using both Bis COOH-Init and 2-MCP with 35–40% yields, and M_n values in the range 3500–6500, as estimated from ^{1}H NMR. The subsequent polymerization of VBCHAM was successful in few attempts with relatively good agreement of ^{1}H NMR with feed compositions and yields around 20 and 40%, indicating the difficulties in terms of ATRP polymerization, solubility and demanding purification of this quaternary ammonium unit.



Scheme 3 Synthesis of (a) PMMA(2) macro-initiators (using Bis COOH-Init) and (b) PMMA(2)-*b*-PVBCHAM*x* diblock copolymers.

3.2 Synthesis and characterization of PtBA-*b*-PVBCHAM*x* diblock copolymers

The synthesis of PtBA-*b*-PVBCHAM copolymers was accomplished after the synthesis of the tBA macro-initiator and the subsequent polymerization of VBCHAM *via* ATRP. The synthetic processes are shown in Scheme 4.



Scheme 4 Synthesis of (a) PtBA macro-initiators and (b) PtBA-*b*-PVBCHAM*x* diblock copolymers.

^{1}H NMR characterization was used to confirm the successful polymerization of the PtBA-*b*-PVBCHAM*x* polymers. As was observed in Fig. 2 for the copolymer PtBA(1a)-*b*-PVBCHAM65, the protons of the –CH_3 groups (e) of tBA and the copolymer backbone (a, b, c, d) appeared at 1.7 ppm and 1.5–1.9 ppm, respectively. It is worth mentioning that the peak of the –CH_3 groups in the PtBA spectra was overlapped from the water traces of $CDCl_3$ at approximately 1.6 ppm. The peaks assigned to the protons of VBCHAM have been described above. The composition of the PtBA-*b*-PVBCHAM*x* copolymers was determined by the comparison of the peaks at 1.2–1.7 ppm (tBA and VBCHAM units) and 6.5–7.5 ppm (VBCHAM unit).

The results from ^{1}H NMR for the successful polymerization of the macro-initiators PtBA and the diblock copolymers PtBA-*b*-PVBCHAM*x* are presented in Table 2. As it may be observed, PtBA macro-initiators were easily synthesized using 2-MCP with yields up to 35% and M_n values ranging between 6500 and 11 000, as estimated from ^{1}H NMR. Despite the low yield obtained with this macro-initiator, the M_n, M_w and the polydispersity of PtBA(1b), estimated from SEC, were 10 379, 11 638 and 1.12 respectively, indicating also in this case the controlled polymerization of the monomer. The low conversion may be attributed to the extensive purification of the final product to assure complete removal of the unreacted monomers. An example of the spectra obtained from SEC is shown in ESI-2.†

The subsequent polymerization of VBCHAM was successful in most cases with relatively good agreement between ^{1}H NMR and feed compositions and yields between 20 and 30%, due to the difficulties mentioned above.

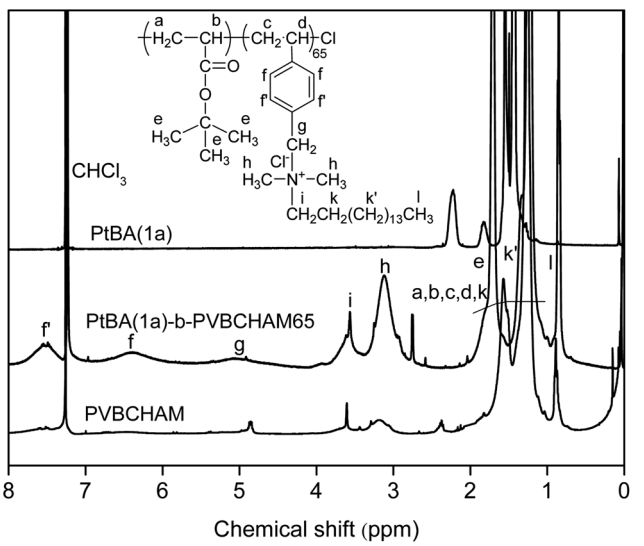


Fig. 2 ^{1}H NMR spectra of the diblock copolymer PtBA(1a)-*b*-PVBCHAM65, the macro-initiator PtBA(1a) and the homopolymer PVBCHAM.

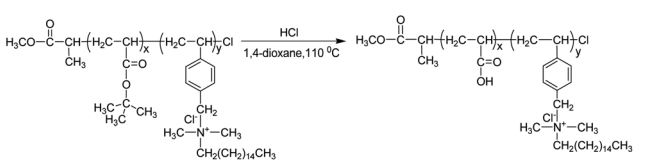
3.3 Synthesis and characterization of PAA-*b*-PVBCHAM*x* diblock copolymers

In order to obtain copolymers of VBCHAM with hydrophilic units, the ester bond of the *tert*-butyl acrylate group was hydrolyzed (with hydrochloric acid in 1,4-dioxane at 110 °C) to form the acrylic acid group, resulting in PAA-*b*-PVBCHAM*x* copolymers (Scheme 5). ATRP of acrylic acid was not attempted, because, as previously reported, strong interactions between this acidic monomer and the catalytic complex leads to a loss of control during the polymerization process.[61] The selection of AA as the hydrophilic co-monomer of VBCHAM, stems from the fact that, in a previous study, the corresponding random copolymers with a low AA content exhibited high bacteriostatic action against the tested microorganisms (*S. aureus*, *E. coli*, *E. faecalis* and *P. aeruginosa*).[17]

The success of the reaction was verified through ^{1}H NMR spectroscopy depicted in Fig. 3. What was observed was disappearance of the peak at 1.7 ppm, indicating the hydrolysis reaction. Moreover, concerning the protons of the copolymer backbone (a, b, c, d) a peak at 1.8 ppm occurred. The new peak at 1.6 ppm was assigned to water traces of $CDCl_3$.

3.4 Synthesis and characterization of VBCHAM and $SSAmC_{16}$ diblock copolymers

For the synthesis of $SSAmC_{16}$ and VBCHAM diblock copolymers, two different synthetic routes were followed. In the first case, PVBCHAM macro-initiators were initially prepared *via* ATRP followed by the polymerization of $SSAmC_{16}$. In the second synthetic route, due to difficulties in the direct polymerization of $SSAmC_{16}$ *via* ATRP, $PSSAmC_{16}$ macro-initiators were synthesized after ATRP of the SSNa monomer followed by the reaction with AmC_{16}. Subsequently, VBCHAM



Scheme 5 Synthesis of PAA-*b*-PVBCHAM*x* diblock copolymers through hydrolysis.

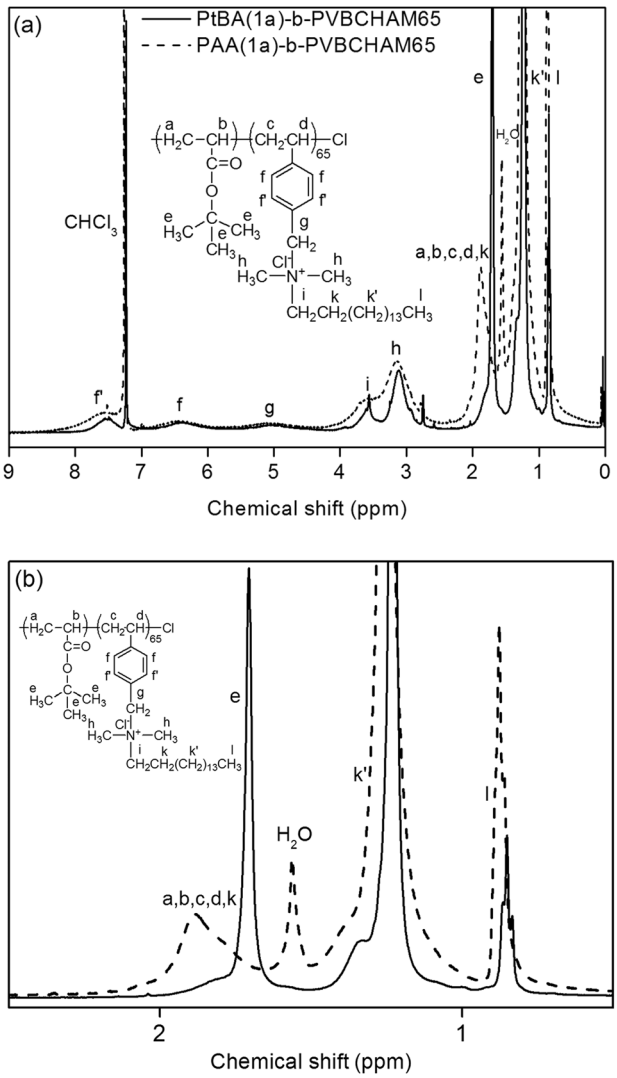
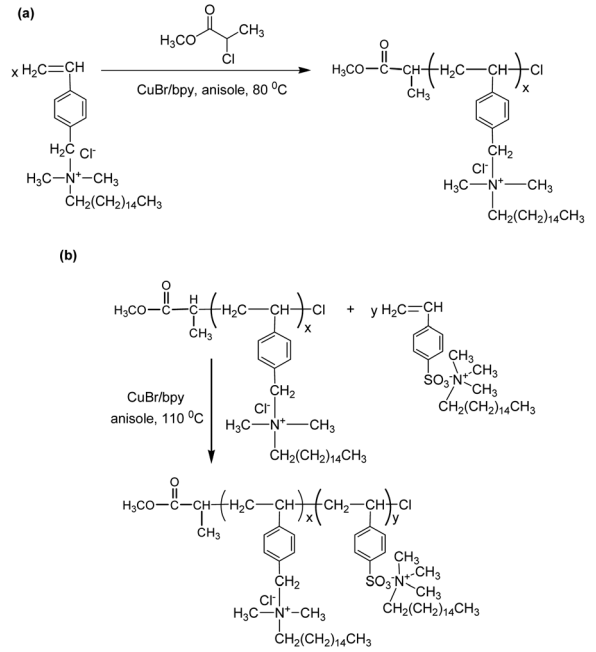


Fig. 3 (a) ^{1}H NMR spectra of the diblock copolymer PtBA(1a)-*b*-PVBCHAM65 and the hydrolyzed PAA(1a)-*b*-PVBCHAM65. (b) Magnification of the spectral region 0.5–2.5 ppm.



Scheme 6 Synthesis of PVBCHAM(1) macro-initiators (using 2-MCP) and PVCBHAM(1)*x*-*b*-PSSAmC$_{16}$ diblock copolymers (1st synthetic procedure).

As it was observed, PVBCHAM macro-initiators were synthesized using both Bis COOH-Init and 2-MCP with 20–30% and 40% yields, respectively and M_n values around 3000–5000. The molecular weight could not be estimated quantitatively from SEC, due to the interaction of VBCHAM with the column fillers, despite the fact that a qualitative indication for the diblock copolymer formation can be obtained by SEC. In this case the VBCHAM initiator and the soluble in chloroform fraction of the copolymer were characterized by SEC and the results are shown in Fig. 4. The comparison of SEC between the macro-initiator PVBCHAM and diblock copolymer was polymerized, using PSSAmC$_{16}$ as the macro-initiator. Comparing the two synthetic routes, better results appeared in the first case, concerning the reaction yield and the success of the polymerization, as reflected from the ^{1}H NMR and ATR-FTIR characterization; the latter is presented in ESI-3.† It should be noticed that such complex structures present serious solubility problems, since the different blocks are soluble in completely different solvents and the overall block copolymers are hardly soluble in some cases. This behavior makes both the synthesis and characterization processes very difficult. The synthetic procedures of the PVBCHAM(1) macro-initiator (2-MCP) and PVCBHAM(1)*x*-*b*-PSSAmC$_{16}$ are illustrated in Scheme 6 (1st synthetic procedure). The results from ^{1}H NMR characterization for the successful polymerization of the macro-initiators PVBCHAM and the diblock copolymers PVBCHAM*x*-*b*-PSSAmC$_{16}$, are presented in Table 3.

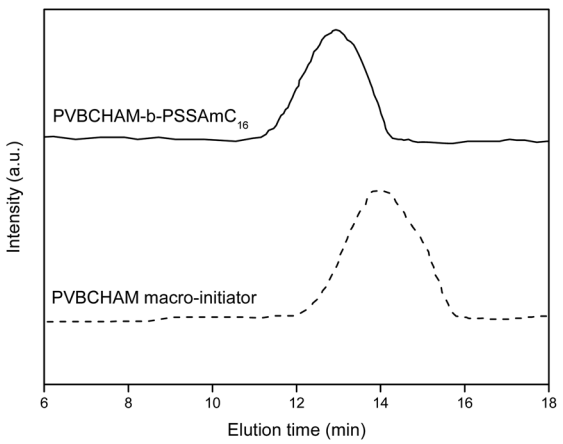


Fig. 4 Size exclusion chromatography for the diblock copolymer PVBCHAM-*b*-PSSAmC$_{16}$ and the macro-initiator PVBCHAM using $CHCl_3$ as the eluent.

PVBCHAM-*b*-PSSAmC_{16} clearly indicated the increase in the molecular weight obtained. Additional attempts for SEC measurements using DMF/water were also made but the results were not found satisfactory.

In the ^{1}H NMR spectra of the PVBCHAM(1)10-*b*-PSSAmC_{16} copolymer in $CDCl_3$, reported in Fig. 5(a), the peak at 0.9 ppm is assigned to the protons of the methyl group (l) and the large peak at 1.2 ppm to the protons of the methylene groups (k′) of the hexadecyl units, respectively. The wide peak at 1.8 ppm is assigned to the protons of the polymer backbone (a, b, c, d) and the remaining methylene groups (k) of the hexadecyl unit. Moreover, the peaks observed at 3.2 and 3.7 ppm attributed to the protons of the methyl (h) and methylene (i) groups of the hexadecyl unit linked with the nitrogen atom, respectively. The aromatic protons (f′, e′, f, e) were found at 6–8 ppm. The existence of the VBCHAM unit was verified by the peak at 4.9 ppm, assigned to the methylene protons of the benzyl group (g) linked with the nitrogen atom (2H, CH_2N^+).

In the second synthetic route, the PSSNa(1) precursor was synthesized in DMSO at 110 °C using CuBr and bpy as the catalyst/base system and Bis COOH-Init was further reacted with AmC_{16} leading to the synthesis of the PSSAmC_{16} macro-initiator (Scheme 7a). The newly formed polymer, as mentioned above, acted as the macro-initiator for the synthesis of PSSAmC_{16}-*b*-PVCBHAM*x* copolymers using CuBr and bpy as the catalyst/base system (Scheme 7b).

The results from ^{1}H NMR characterization for the successful polymerization of the precursors PSSNa, the macro-initiators PSSAmC_{16} and the diblock copolymers PSSAmC_{16}-*b*-PVBCHAM*x*, are presented in Table 4. As it was observed, PSSNa (precursor of the PSSAmC_{16} macro-initiator) was synthesized with Bis COOH-Init with yields around 12% and 17% and M_n value around 3000–9000. The polymerization of VBCHAM was also successful, showing good agreement between the ^{1}H NMR and the feed composition with yields between 13 and 20%.

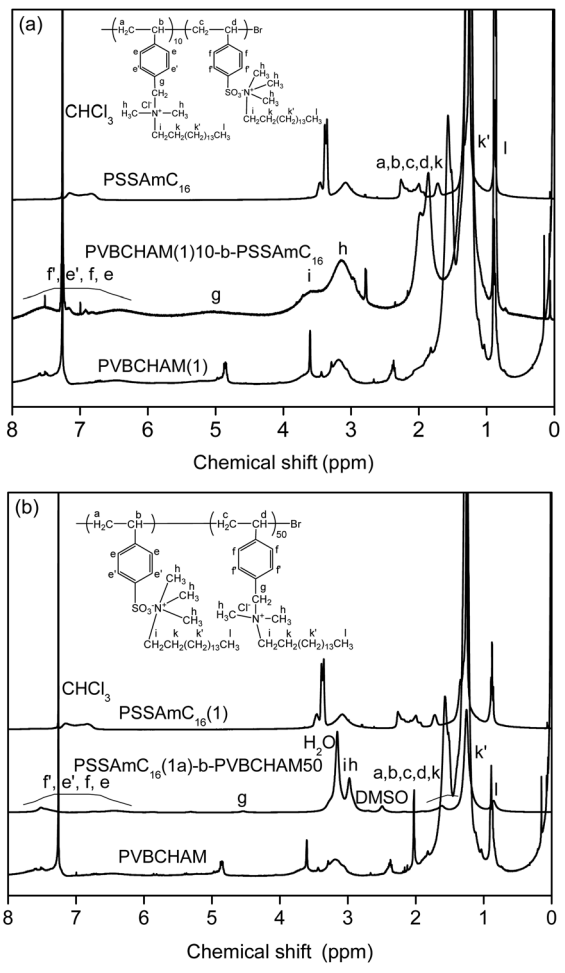
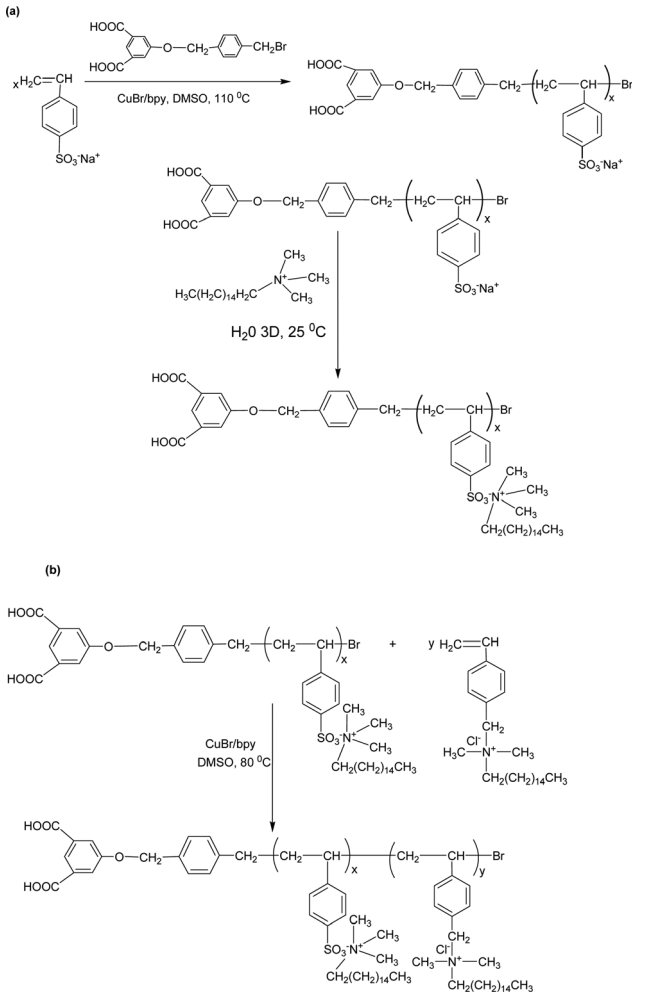


Fig. 5 ^{1}H NMR spectra of (a) the diblock copolymer PVBCHAM(1)10-*b*-PSSAmC_{16}, the macro-initiator PVBCHAM(1) and the homopolymer PSSAmC_{16} and (b) the diblock copolymer PSSAmC_{16}(1a)-*b*-PVBCHAM50, the macro-initiator PSSAmC_{16}(1) and the homopolymer PVBCHAM.



Scheme 7 Synthesis of (a) PSSAmC_{16}(1) macro-initiators (using Bis COOH-Init) and (b) PSSAmC_{16}(1b)-*b*-PVCBHAM*x* diblock copolymers (2nd synthetic procedure).

The chemical structure of the copolymers was verified with ATR-FTIR (ESI-3†) and ^{1}H NMR (Fig. 5(b)). The peaks in both characterization techniques have been described above. The only difference was the shift of the peak observed at 4.9 ppm to 4.6 ppm (2H, CH_2N^+) in the ^{1}H NMR spectra, probably due to the change in the deuterated solvent used to dissolve the polymer (DMSO-d_6 instead of $CDCl_3$).

In order to confirm the successful purification of the isolated copolymers and the total removal of the macro-initiators, solubility studies were also conducted between the macro-initiators and the final block copolymers in the same solvent. The main issue here was the selection of a solvent that would dissolve the macro-initiators, but would not dissolve or dissolve partially the respective diblock copolymers. In support of this claim, the solubility study of the system PVBCHAM macro-initiators and PVBCHAM-*b*-PSSAmC_{16} diblock copolymers (which is the most difficult studied system in the present work) was conducted in chloroform. More specifically, the macro-initiators PVBCHAM were easily soluble in chloroform while the copolymers were insoluble or marginally soluble only after heating.

Based on the above described synthetic efforts, significant conclusions have been drawn regarding the synthesis and characterization of such block copolymers. To be more precise, the synthesis of the charged block copolymers was not an easy process. Several issues, concerning the solubility of monomers, the incompatibility of the different blocks and the ATRP of the charged quaternary ammonium groups, have been faced, especially for the block copolymers of VBCHAM and SSAmC_{16}. However, the synthesis of this kind of copolymer, bearing simultaneously covalently attached and electrostatically bound bacteriostatic groups, combined with the well-organized structures that block copolymers offer (see below), is a critical issue for future applications. Most important, our previous experience has proved that the bacteriostatic activity was maintained even when the respective random copolymers were embedded in a polysulfone matrix. These kinds of functional antibacterial composites, operating through both contact-based and release-based mechanisms,[62–66] are of major importance, since they may improve their specific properties and achieve better performance in various fields, with emphasis on nosocomial infections and sea water applications.

3.5 TEM characterization

In order to initially prove the self-organization ability of the diblock copolymers developed in this work, the systems PMMA-*b*-PVBCHAM65, PtBA-*b*-PVBCHAM55 and PVBCHAM10-*b*-PSSAmC_{16} were characterized by TEM. Solutions from different solvents like chloroform, ethanol and methanol were used and as it was expected, the organization ability of the copolymers differed depending on the solvent used. In our case, more interesting features were obtained from solutions in ethanol 95% or methanol. As shown in Fig. 6 for the PMMA-*b*-PVBCHAM65 diblock copolymers from methanol solutions, spherical micelles[27] in the size of nanometers were

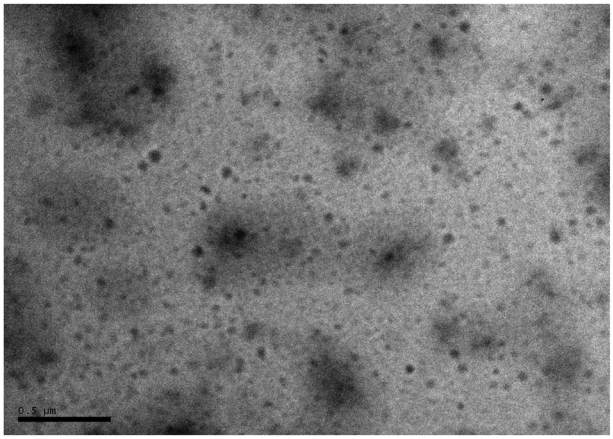


Fig. 6 TEM image of the diblock copolymer PMMA-*b*-PVBCHAM65.

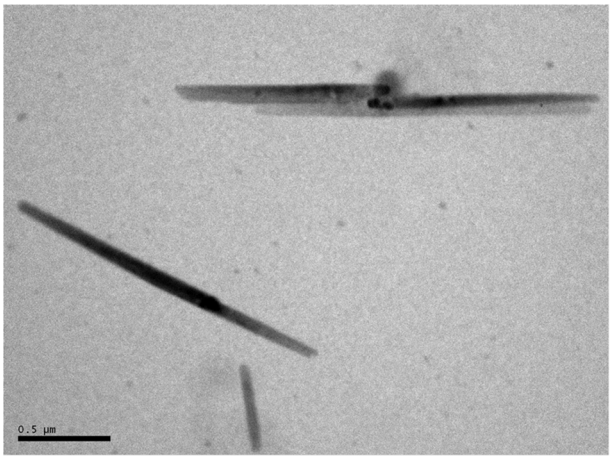


Fig. 7 TEM image of the diblock copolymer PtBA-*b*-PVBCHAM55.

formed, as revealed from TEM. The PtBA-*b*-PVBCHAM55 diblock copolymer, self-assembled mostly into long narrow fibrillar aggregates, as shown by TEM images presented in Fig. 7, derived from ethanol solution. The PVBCHAM10-*b*-PSSAmC_{16} from methanol solutions was organized to form a three-dimensional morphology with a tendency for hierarchical structures, as shown in Fig. 8. This different morphological structure in this kind of copolymer is probably attributed to the partial dissociation of the PSSAmC_{16} blocks due to the protic solvent used during sample preparation for TEM characterization. A more systematic study of the organization ability of these complex structures is in progress.

3.6 Antimicrobial tests

As the final point, in order to have a complete and thorough aspect of the aforementioned synthesized block copolymers, their antimicrobial activity was evaluated against Gram-negative (*P. aeruginosa* and *E. coli*) and Gram-positive (*S. aureus* and *E. faecalis*) bacteria. The antimicrobial effect was measured as

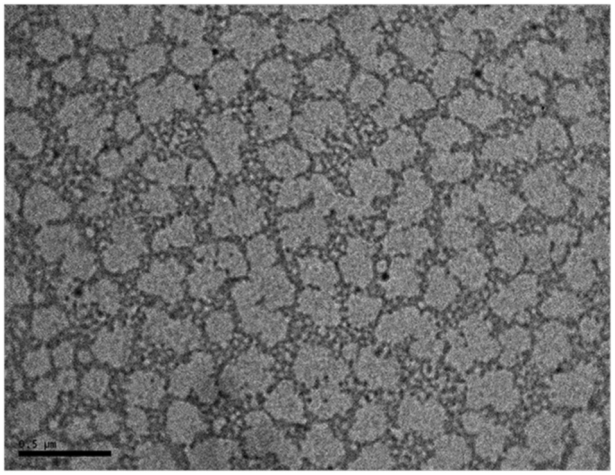


Fig. 8 TEM image of the diblock copolymer PVBCHAM10-*b*-PSSAmC$_{16}$.

a function of log reduction of the bacterial cells after 24 h contact with each material at 22 °C.

It is worth mentioning that, in previous studies, we have tested the antimicrobial activity of a wide range of random copolymers, including P(AA-*co*-VBCHAM*x*) and P(SSAmC$_{16}$-*co*-VBCHAM*x*), at various monomeric ratios.[17] Thus, we quote a comparative study of both random and block copolymers concerning their bacteriostatic activity against various bacteria.

In this line, the antibacterial effect of the copolymers of the AA–VBCHAM category are graphically presented in Fig. 9, after 24 h of contact with *S. aureus*, *E. faecalis*, *P. aeruginosa* and *E. coli*, at 22 °C. More specifically, the random copolymers P(AA-*co*-VBCHAM88) with 88% VBCHAM content and P(AA-*co*-VBCHAM20) with 20% VBCHAM content were compared with the block copolymer PAA-*b*-PVBCHAM65 with 65% VBCHAM content, in terms of their bacteriostatic activity. In fact, the random copolymer with 20% VBCHAM content exhibited lower antimicrobial activity than PAA-*b*-PVBCHAM65 against *S. aureus*, *E. faecalis* and *E. coli* after 24 h of contact at 22 °C. The increase of the VBCHAM content up to 88% in the random copolymer led to higher antimicrobial activities against all the tested microorganisms. The high bacteriostatic activity of this copolymer against *P. aeruginosa* and *E. faecalis* with log reduction of 2.5 and 6.2, respectively is definitely remarkable. The block copolymer showed the highest antimicrobial activity against *E. coli* bacteria. The aforementioned results, concerning the bacteriostatic activity of random and block copolymers of the AA–VBCHAM category, showed a logical coherence since the antimicrobial activity was increased with the increase of the VBCHAM unit, irrespective of the polymer architecture.

However, another aspect that needed to be tested was the requirement of the hydrolysis reaction of the PtBA-*b*-PVBCHAM65 block copolymer. Thus, by comparing PAA-*b*-PVBCHAM65 with its precursor PtBA-*b*-PVBCHAM65, in terms of its antimicrobial activity, significant results were obtained. In fact, as illustrated in Fig. 10, the antimicrobial activity of the materials PtBA-*b*-PVBCHAM65 and PAA-*b*-PVBCHAM65, against *S. aureus* and *P. aeruginosa*, showed that the diblock copolymer with the covalently attached bacteriostatic group VBCHAM and the hydrophobic unit tBA (PtBA-*b*-PVBCHAM65) did not present any bacteriostatic activity. However, the change of the hydrophobic tBA unit to the hydrophilic acrylic acid unit (PAA-*b*-PVBCHAM65) led to a slight increase in the antimicrobial effect only against *S. aureus*. From the above-mentioned observations it is now clear that the antimicrobial activity of the specific copolymers, depends not only on the high content of the unit bearing covalently attached quaternary ammonium groups but also on the combination of this unit with the hydrophilic AA moiety. This behavior along with the solubility of the copolymers during the process seemed to be the driving force for the determination of polymer antimicrobial activity.

In a further step, the bacteriostatic activity of the diblock copolymer bearing both covalently and electrostatically attached bacteriostatic units (PSSAmC$_{16}$-*b*-PVBCHAM50) was also tested and compared with our previous findings for the respective random copolymers. Based on our experience, a high antimicrobial activity of this category of copolymers

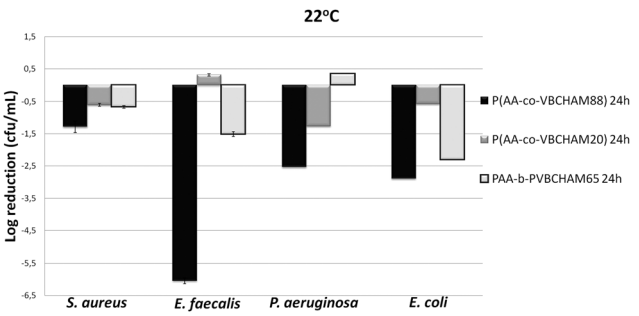


Fig. 9 Antibacterial effect of random copolymers P(AA-*co*-VBCHAM88) and P(AA-*co*-VBCHAM20) and the diblock copolymer PAA-*b*-PVBCHAM65, after 24 h of contact with *S. aureus*, *E. faecalis*, *P. aeruginosa* and *E. coli* at a temperature of 22 °C. Each bar represents the log reduction from 3 independent experiments done in duplicate (mean ± standard deviation).

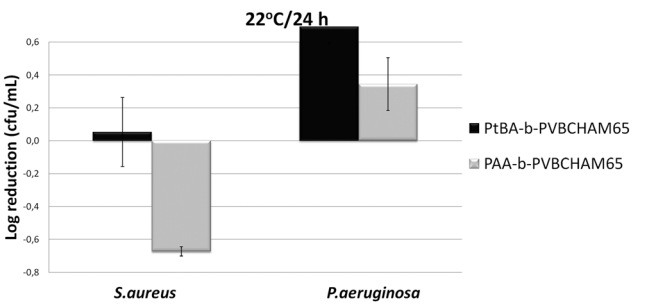


Fig. 10 Antibacterial effect of diblock copolymers PtBA-*b*-PVBCHAM65 and PAA-*b*-PVBCHAM65 after 24 h of contact with *S. aureus* and *P. aeruginosa*, at a temperature of 22 °C. Each bar represents the log reduction from 3 independent experiments done in duplicates (mean ± standard deviation).

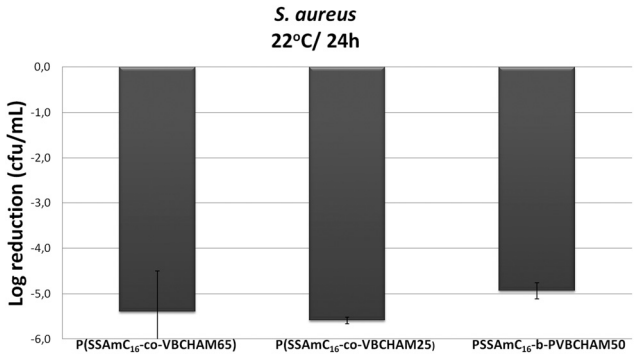


Fig. 11 Antibacterial effect of random copolymers P(SSAmC$_{16}$-*co*-VBCHAM65) and P(SSAmC$_{16}$-*co*-VBCHAM25) and the diblock copolymer PSSAmC$_{16}$-*b*-PVBCHAM50, after 24 h of contact with *S. aureus*, at a temperature of 22 °C. Each bar represents the log reduction from 3 independent experiments done in duplicates (mean ± standard deviation).

appeared against *S. aureus*. Thus, a comparative study of the random copolymers P(SSAmC$_{16}$-*co*-VBCHAM65), with 65% VBCHAM, P(SSAmC$_{16}$-*co*-VBCHAM25), with 25% VBCHAM and the block copolymer PSSAmC$_{16}$-*b*-PVBCHAM50, with 50% VBCHAM, against *S. aureus* at 22 °C after 24 h of contact, is presented in Fig. 11. As it may be observed, the high values of antimicrobial activity were also maintained in the case of the diblock copolymer. The abovementioned results might open new ways to the use and application of these materials, because it is demonstrated that well-defined and self-organized structures with antimicrobial properties can be prepared.

4 Conclusions

In the present work, diblock copolymers bearing charged blocks and more specifically, PMMA-*b*-PVBCHAM*x*, PtBA-*b*-PVBCHAM*x*, PAA-*b*-PVBCHAM*x*, PVBCHAM*x*-*b*-PSSAmC$_{16}$ and PSSAmC$_{16}$-*b*-PVBCHAM*x* copolymers were successfully synthesized in various monomer percentages through ATRP and characterized with ^{1}H NMR, ATR-FTIR spectroscopy, SEC chromatography and solubility tests. PMMA and PtBA macroinitiators were easily synthesized exhibiting a low PDI, as expected for the ATRP process. PMMA-*b*-PVBCHAM*x* and PtBA-*b*-PVBCHAM*x* copolymers were obtained in rather low yield, with relatively good agreement of ^{1}H NMR composition with the feed composition. Furthermore, the hydrolysis of tBA units led to the synthesis of PAA-*b*-PVBCHAM*x* copolymers. In an additional experimental procedure, ATRP initiators with bis COOH groups were used, in order to synthesize more complex structures.

For the synthesis of VBCHAM and SSAmC$_{16}$ diblock copolymers, two different synthetic protocols were followed leading to the synthesis of PVBCHAM*x*-*b*-PSSAmC$_{16}$ and PSSAmC$_{16}$-*b*-PVBCHAM*x* copolymers. The synthetic attempts were successful in general, with rather low isolated yields mainly due to difficulties in removal of the unreacted monomers. A major issue in this class of copolymers was the limited solubility of the different blocks in the used deuterated ^{1}H NMR solvents, leading in some cases to misinterpretation of the composition. Nevertheless, the synthesis of PVBCHAM*x*-*b*-PSSAmC$_{16}$ copolymers seemed to be the most promising synthetic procedure for this innovative class of copolymers, which combine both contact-based and release-based biocidal groups in the same system. The isolated block copolymers showed self-organizational features in selected solvents as proved by TEM.

The bacteriostatic activity of the novel block copolymers was investigated and compared with that of the respective random copolymers from our previous studies. The results indicated that the high content of the VBCHAM unit (bearing covalently attached quaternary ammonium groups) in combination with the hydrophilic AA units, led to high bacteriostatic activity for this class of copolymers. In the case of PSSAmC$_{16}$-*b*-PVBCHAM50, the diblock copolymer bearing both covalently and electrostatically attached bacteriostatic units, exhibited high antimicrobial activity against the bacteria *S. aureus* at 22 °C after 24 h of contact.

These results are of great importance since well-defined and self-organized structures combining high values of antimicrobial activity consist of a new class of materials that may be incorporated in different polymeric matrices targeting specific biocidal applications.

Acknowledgements

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