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Dual effect of coumarin benzimidazolium ionic salt covalently bonded on a silica network

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Abstract: Synthesis of the ionic liquid 1-allyl-3-(4-methyl-7,8-dihydroxy-2H-chromen-2-one) benzimidazolium chloride followed by the conversion of ammonium salts on the imidazole ring hooked to the coumarin group was modified by postmodification of the silica matrix where 3-chloropropyltrimethoxysilane was used as not only a surface modification agent, but also as an ATRP halogen source. The synergetic effect of charged ammonium groups as well as hydroxyl groups present on the coumarin was investigated in terms of ATRP polymerization. The results confirmed that brush polymers show outstanding antibacterial effects against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Key words: Ionic liquid, ATRP, silica, antibacterial polymer, benzimidazolium derivatives, coumarin, quaternary ammonium compounds (QACs)

1. Introduction

With the growing world population, there is an increase in both the number and use of social places such as schools, hospitals, shopping centers, and airports. This increase also causes a rapid rise in the spread of microbial contaminations. Microbial contamination of water purification systems, medical products, food packaging and storage systems, hospital and dental furniture, textiles, etc. creates serious problems.[1−3] Improvement in living and hygiene standards of people make it necessary to take some precautions against microbial contamination in daily life. For example, liquid or gaseous disinfectants such as chlorine are used for disinfection of water and living spaces like houses, hospitals, and schools. Even if these disinfectants are used in appropriate amounts, they can cause environmental problems.[4,5] In order to overcome these problems, use of water insoluble polymeric materials with antibacterial activity becomes more important. Antibacterial polymers do not only solve the problems stated above but also provide continuous sterile surfaces on materials.[6−12] Antibacterial polymeric materials have an advantage over commonly used small molecules such as chemical stability, lack of vaporization, long-term antibacterial effect, and low diffusivity from skin.[13,14] The most commonly used polymer among the antimicrobial polymers is the one that contains quaternary ammonium compounds (QACs) like benzimidazole derivatives.[15−25] The antimicrobial effect of QACs comes from the electrostatic interaction of positively charged antibacterial polymer with the negatively charged wall of bacteria cells, destroying the cells by adsorption and penetration of active groups from cell walls.[26−28] Coumarin derivative materials also show antibacterial properties against many microorganisms similar to QACs.[29−35]

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In the present study, 1-allyl-3-(4-methyl-7,8-dihydroxy-2H-chromen-2-one) benzimidazolium chloride was synthesized and covalently linked to 3-chloropropylytrimethoxysilane (CLS) via ATRP polymerization in which CLS was used as halogen source for ATRP polymerization.[36] CLS is attached to the silica matrix before polymerization; thus surface polymerization is performed at the surface and so the silica and the resulting materials have not only QACs groups but also coumarin derivatives. The dual antibacterial effect of QACs as well as coumarin was tested and synergetic effects in solution and surfaces were reported.

2. Results and discussion

2.1. FTIR characterization

The spectra of the intermediates are shown in Figure 1. Spectrum DA-01 is that of silica particles not activated and spectrum DA-02 is that activated silica with piranha solution; O–H stretching bands at 3630 and 3550 cm^{-1} and a bending band at 800 cm^{-1} are clearly shown in the DA-02 spectrum while they are not clear in DA-01. Spectrum DA-03 is that of CLS. In the immobilized intermediate CLS spectrum (DA-04) C–H stretching bands at 2930–2850 cm^{-1} and C–H bending bands between 1500 and 1400 cm^{-1} can be observed. In the 1-allyl-3-(4-methyl-7,8-dihydroxy-2H-chromen-2-one) benzimidazolium chloride (Figure 2, DA-05), bands at 3276 and 1728 cm^{-1} can also be observed. These bands are assigned to N–H and N–C stretching modes, respectively. The C=O group in the coumarin ring for DA-05 and DA-06 gives a band at about 1660 cm^{-1} after removal of DMF. The infrared spectra of aromatic groups grafted on the silica surfaces are shown in Figure 2, DA-06, and the main bands of the spectra are shown. It was observed that imidazole groups grafted onto the silica surface can be identified by their characteristic bands distinguishable from those of the linear chain intermediate.

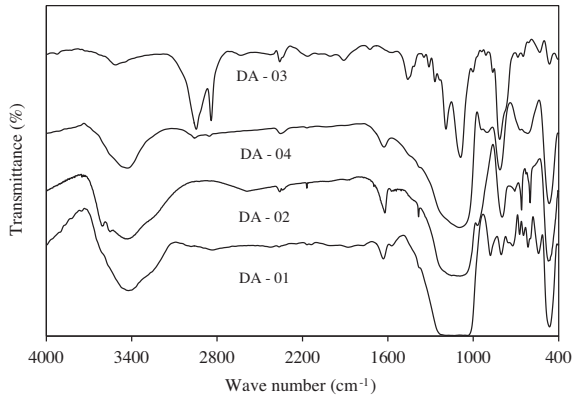


Figure 1. FTIR characterization of modified silica (DA-04).

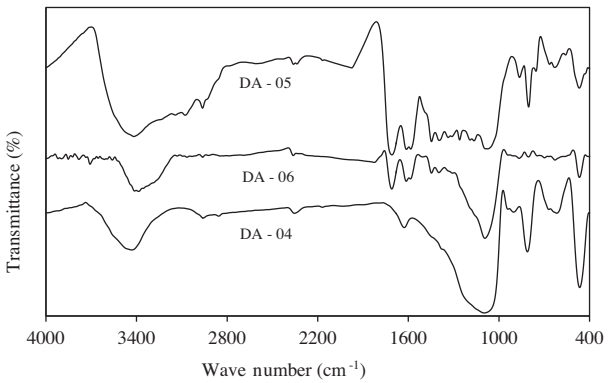


Figure 2. FTIR characterization of materials DA-04 to DA-06.

2.2. Thermal analysis

Thermal gravimetric analysis (TGA) is a useful procedure for the characterization of the organic grafting process. This technique allows the study of interaction strength between the attached organic groups and the surface with increasing temperature. However, TGA is a limited technique since it is difficult to differentiate between organic and water mass loss, the latter being produced by dehydroxylation of silanol, which takes place with increasing temperature. It has been reported that, in some cases, the organic thermal decomposition occurs in more than one step. Thus, the TGA technique could not be used for the identification of the desorbed organic groups in a specific step.

The TGA curves of DA-04 to DA-06 are shown in Figure 3. The mass loss of DA-04 below 100 °C is due to the loss of moisture and adsorbed water. On the other hand, the mass loss in the temperature range of 250–340 °C is ascribed to the pyrolysis of labile oxygen containing functional groups. The thermal stability of DA-06 is higher than that of pure DA-05. This is attributed to the earlier degradation of organic moieties covalently attached to the surfaces of silica. It is also noteworthy that the thermal stability of DA-06 above 400 °C is much better than that of DA-04. This is attributed to the attachment. Differential scanning calorimetry (DSC) thermograms are found to be in good agreement with the TGA.

DSC thermograms of the particles are given in Figure 4. All the particles have DSC and TGA curves of the same shape. The weight loss below 250 °C is due to water evaporation, corresponding to an exothermic DSC peak at around 300 °C. A further weight decrease over 300 °C is related to organic group decomposition and silanol group condensation (dehydroxylation), which corresponds to a weak and broad exothermic DSC peak at around 250–500 °C.

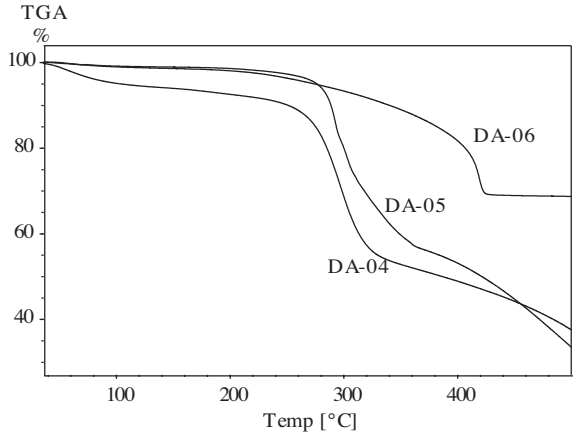


Figure 3. TGA characterization of the materials.

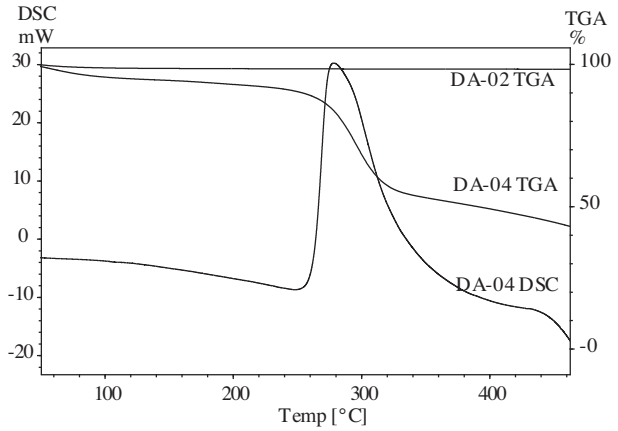


Figure 4. DSC and TGA thermograms of the materials.

Figures 5a and 5b demonstrate scanning electron microscope (SEM) images of the activated silica particles before (DA-02) and after (DA-04) modification with CLS. Figure 5c represents an antibacterial hybrid polymeric material (DA-06). The changes in surface morphology clearly indicate that antibacterial hybrid polymeric materials were synthesized successfully.

2.3. Antibacterial effects

The bacterial strains used throughout the experiments were *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29923, microorganisms that play a major role in biofilm formation. The activity of polycationic agents such as imidazolium salts is presumed to be through the positively charged polymers on the negatively charged bacterial cell surface, resulting in increased cell permeability and disruption of the cell membranes. This mode of action may have caused total inhibition of bacterial growth.

The colony-forming units per milliliter (CFU/mL) values were converted to logarithmic (base 10) values and stated as log (viable cell number)$^{-1}$ values. These values were determined as a measure of the number of bacterial cells that are viable (i.e. alive and capable of growth) after the treatment with DA-05 and DA-06 for 0 and 3 h. The control log (viable cell number)$^{-1}$ values of the same bacteria are also given in the Table and in Figure 6.

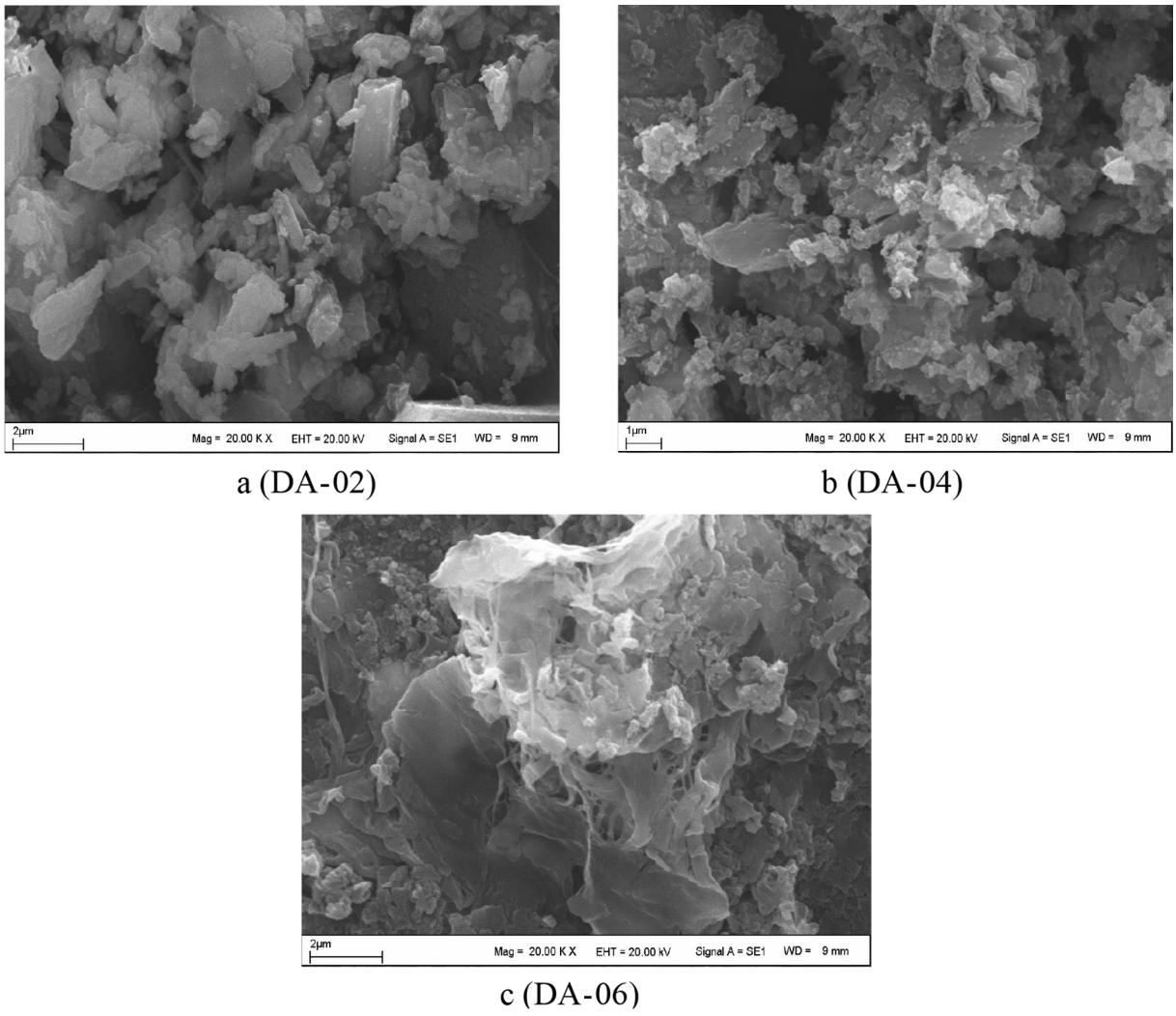


Figure 5. SEM images of DA-02 (a), DA-04 (b), and DA-06 (c).

Table. Removal log (viable cell number)$^{-1}$ for *E. coli, P. aeruginosa*, and *S. aureus* after the treatment with DA-05 and DA-06 for 0 and 3 h.

Bacteria	Log (viable cell number)$^{-1}$ of control		Log (viable cell number)$^{-1}$ of DA-05		Log (viable cell number)$^{-1}$ of DA-06	
	After 0 h	After 3 h	After 0 h	After 3 h	After 0 h	After 3 h
E. coli	9.57 ± 0.14	9.57 ± 0.14	9.51 ± 0.12	0 ± 0.00	9.49 ± 0.21	0 ± 0.00
S. aureus	9.34 ± 0.29	9.34 ± 0.29	9.26 ± 0.23	0 ± 0.00	9.30 ± 0.14	0 ± 0.00
P. aeruginosa	9.49 ± 0.30	9.49 ± 0.30	9.41 ± 0.10	0 ± 0.00	9.41 ± 0.07	0 ± 0.00

Pseudomonas aeruginosa carried the highest negative charge, while *Escherichia coli* was second. Moreover, the negative charge on the cell surface of the gram negative bacteria was higher than that on the gram positive bacterium. The data of inhibition capacity of the poly (1-allyl-3-(4-methyl-7,8-dihydroxy-2H-chromen-2-one) benzimidazolium chloride modified silica hybrid materials (DA-06) against the tested bacteria were collected and treated by regression analysis.

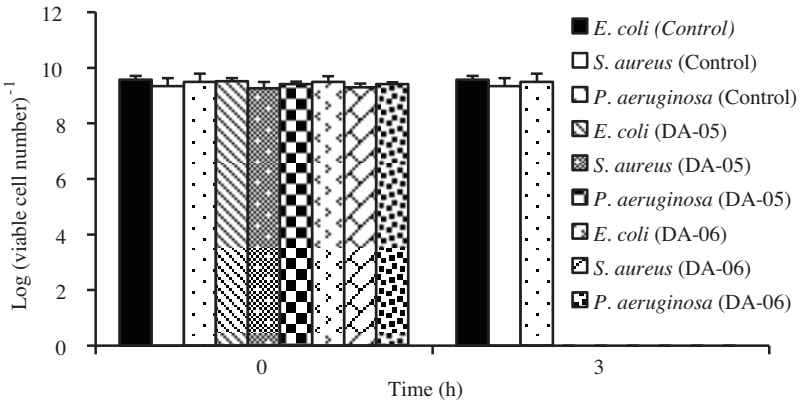


Figure 6. Regression analysis of the bacteria tested.

In conclusion, ATRP techniques were used to prepare brush polymer on a silica matrix that showed dual properties. Although monomers containing allyl group did not give a high molecular weight polymer due to α-methylenic hydrogen, quaternary ammonium salts containing an allyl group produced low molecular weight products or oligomers. The radical initiators formed by abstraction of α-methylenic protons that terminated the chain formation by reaction with the other radical species. To explain the formation of oligomers due to the insoluble nature of the DA-06, TGA and FTIR results were used. Functional monomers were chosen to manipulate the resulting material in which modified silica was used as an ATRP source where a monomer used an antibacterial agent. It can be clearly stated that poly (1-allyl-3-(4-methyl-7,8-dihydroxy-2H-chromen-2-one) benzimidazolium chloride) incorporated at the surface of silica exhibited a strong antibacterial effect due to the charged species and hydroxyl group on the coumarin group.

3. Experimental

3.1. Materials

All the chemicals used in this work were supplied by Sigma Aldrich and they were used after appropriate purification. All reactions for the preparation of benzimidazolium salt and modification of silica surface with CLS and ATRP polymerization were carried out under argon with flame dried glassware using standard Schlenk techniques.

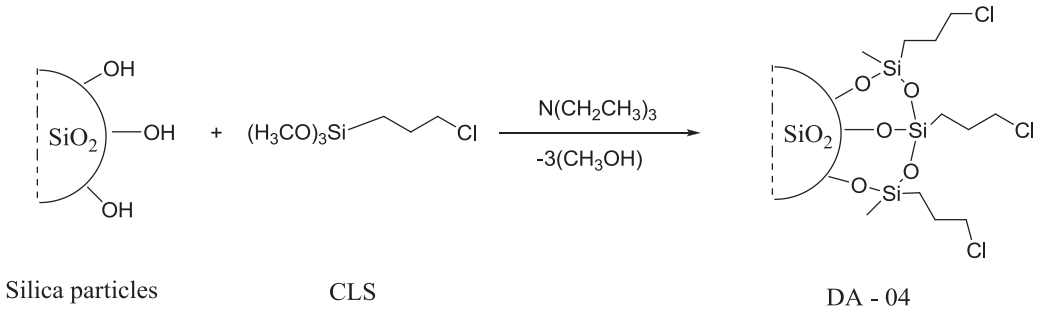
3.2. Characterization

FTIR spectra were recorded on an ATR unit in the range 400–4000 cm^{-1} with a Bruker AC300P FTIR spectrometer. The DSC and TGA were performed on a Shimadzu Network System 60 instrument at heating rate of 10 °C/min in air. ^{1}H NMR and ^{13}C NMR were recorded using a Bruker AC300P FTIR spectrometer operating at 300.13 MHz (1H) and 75.47 MHz (13C). Chemical shifts are given in ppm relative to TMS; coupling constants (J) are in Hz. Elemental analyses were performed by İnönü University Scientific and Technological Research Center. SEM images were obtained on a Nova NanoSEM 600.

3.3. Surface functionalization of silica (DA-04)

To activate the silica sphere surfaces, the silica particles were boiled with piranha solution (3 parts of 30% H_2O_2 and 7 parts of concentrated H_2SO_4) for at least 30 min, rinsed with methanol and then with distilled water, dried in a vacuum oven at 100 °C, and kept in a glove box until use.[37]

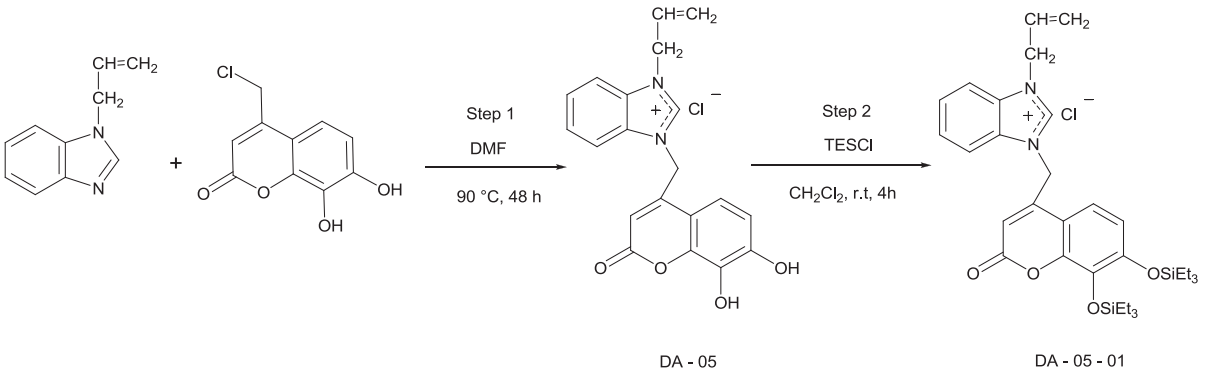
As shown in Scheme 1, 10 g of the activated silica particles were dispersed in 100 mL of dry toluene and 10 mL of CLS solution. In order to avoid gel formation the methanol to water ratio was fixed at 3:10 and aliquot parts were added and stirred for 24 h under reflux conditions. Surface-modified silica particles were extracted in methanol in a Soxhlet apparatus for 12 h and dried as described in our previous paper.[38]



Scheme 1. Surface functionalization of silica (DA-04).

3.4. Synthesis of benzimidazolium compounds (QACs) (DA-05)

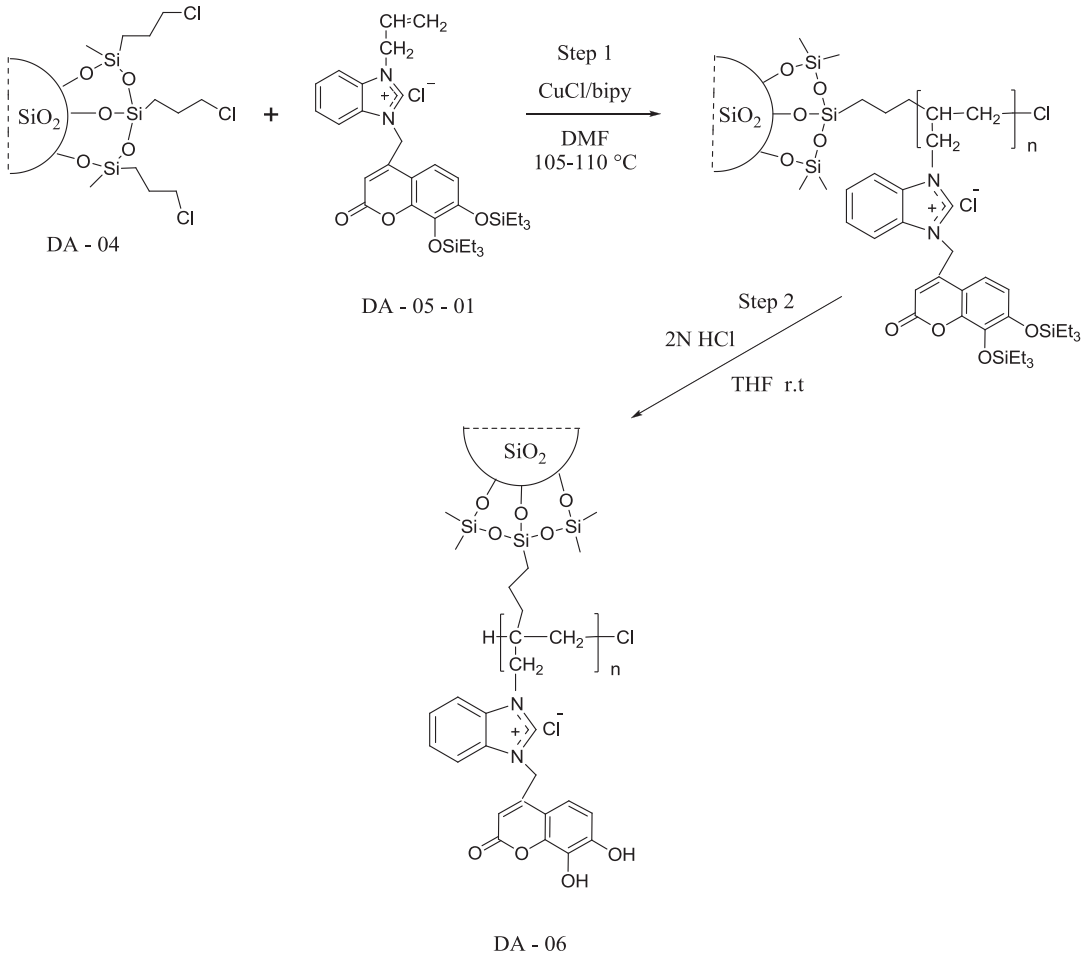
Synthesis of 1-allyl-3-(4-methyl-7,8-dihydroxy-2H-chromen-2-one) benzimidazolium chloride was prepared and characterized as reported earlier as shown in Scheme 1 step 1.[34] In a classical ionic liquid experiment 10 mmol of 1-allylimidazole was solved in dry DMF (5 mL) and 10 mmol of 4-chloromethyl-7,8-dihydroxy-2H-chromen-2-one was added and the mixture was heated for 48 h at 90 °C. The precipitant was obtained by using diethyl ether and collected by filtration, washed with acetone and ethanol, and dried under nitrogen (DA-05). After this, DA-05 was silylated for protection of OH groups as shown in Scheme 2 step 2.[39]



Scheme 2. Synthesis of 1-allyl-3-(4-methyl-7,8-dihydroxy-2H-chromen-2-one) benzimidazolium chloride (DA-05).

3.5. Surface polymerization (DA-06)

Surface polymerization was performed to obtain polymeric benzimidazolium salts by ATRP where 1 g of surface functionalized silica (DA-04), 5 g of silyl protected OH groups 1-allyl-3-(4-methyl-7,8-dihydroxy-2H-chromen-2-one) benzimidazolium chloride (DA-05-01), 0.3 g of bipyridine, and 0.1 g of CuCl were added to a Schlenk tube in 10 mL of DMF. The reaction was carried out under nitrogen atmosphere for 24 h at 105–110 °C. Surface polymerization was completed after 24 h and the hybrid material was rinsed with methanol and water, dried in a vacuum oven and then dispersed in THF and hydrolyzed with HCl to obtain reactive OH groups[39] as shown in Scheme 3 step 2.



Scheme 3. Synthesis of hybrid polymeric benzimidazolium salt (DA-06).

3.6. Antibacterial tests

Escherichia coli ATCC 35218 (gram-negative), *Pseudomonas aeruginosa* ATCC 27853 (gram-negative), and *Staphylococcus aureus* ATCC 29923 (gram-positive) were used in the study. One loopful of each bacterium was inoculated into 10 mL of nutrient broth (Merck) and incubated at 37 °C for 24 h. Then, 6.5 mL of the liquid cultures of these bacteria were pipetted into sterile glass tubes and the bacterial cells were harvested by centrifugation (at 6000 rpm for 5 min) and washed twice with sterilized water. After these applications, the collected bacterial cells were resuspended in 6.5 mL of sterilized water. The cell density of the culture suspensions was determined as 3.72×10^9, 3.10×10^9, and 2.19×10^9 viable cells mL^{-1} for *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively.

The surface spread plate technique was used for measuring the viable cell counts of the bacteria.[23] First, 0.1-mL samples were taken from the flasks at each sampling time (0 h as control and 3 h) under aseptic conditions and after the serial dilutions (10^7-fold) with normal saline solution (0.90% w/v of NaCl), 0.1 mL of diluted samples were pipetted and spread onto triplicate sterile nutrient agar plates. The inoculated plates were incubated in a static incubator at 37 °C for 24 h and then the numbers of colonies (viable cells) were counted manually. Next, 100 mg of DA-06 was transferred into sterile 100-mL flasks containing 19 mL of sterilized water and then the flasks were autoclaved at 121 °C for 20 min. After the autoclaving, 1 mL of the cell suspension

was added to the flasks, followed by agitation at 200 rpm at 37 °C for 3 h. At the end of the contact of the materials with the bacterial cells by agitation, the suspensions were allowed to stand (about 3 min) for settling the materials. The counting numbers were stated as the mean colony-forming units (CFU) per milliliter. The same antibacterial test procedures were repeated for DA-05. All experiments were performed in triplicate and repeated twice.

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