Bulgarian Chemical Communications, Volume 54, Issue 4 (pp. 332-336) 2022 *DOI: 10.34049/bcc.54.4.NC01*

Design, preparation and antibacterial activity of light-activated polymer coatings

N. Philipova [1], D. Ganchev [2], I. Lalov[3], R. Bryaskova [1*]

[1]*Department of Polymer Engineering, University of Chemical Technology and Metallurgy, Sofia, Bulgaria*
[2]*Department of Machine Elements and Non-Metal Constructions, Technical University, Sofia, Bulgaria*
[3]*Department of Biotechnology, University of Chemical Technology and Metallurgy, Sofia, Bulgaria*

Received: September 14, 2022; Revised: November 23, 2022

Here, we report on the preparation of light-activated polymer coatings obtained *via* a simple dip-coating procedure on a stainless steel (SS) substrate by using the following components: 1) P(mDOPA)-co-P(DMAEMA$^+$) polycationic copolymer; 2) Pox(mDOPA)/PAH) nanogel which possesses quinone groups and 3) a photosensitizing agent on the base of porphyrin IX (PPIX - ED). Energy dispersive X-ray analysis with SEM (EDX-SEM) was used to analyse the chemical composition and distribution of elements of the coatings. The antibacterial activity against G. negative *E. coli* and G. positive *B. subtilis* was established by a modified disc diffusion method.

Keywords: light-activated coatings; photosensitizers; antibacterial activity

INTRODUCTION

Several research groups, as well as healthcare industry are oriented toward the design and preparation of effective and long-lasting antibacterial coatings. This is a requirement to prevent the initial bacterial attachment to different surfaces in the hospital, which are responsible for a number of nosocomial infections. The common methods for disinfection in the practice include physical or chemical disinfection treatment [1, 2]. However, these procedures are not effective enough and recontamination of the surfaces occurs very rapidly. Therefore, other more effective technologies have been developed in order to prevent the spread of infections. Most of them are based on the release of active biocidal agents from the coating as silver, copper, or zinc, which are able to kill microorganisms on the top of the coated surfaces [3, 4]. The anti-adhesive coatings are other antibacterial products which reduce bacterial attachment to surfaces [5]. Among them, super-hydrophobic surfaces attract interest since they may delay or even prevent microbial attachment to a surface [6, 7]. The other technology includes the preparation of contact-active surfaces that exhibit antimicrobial activity without releasing biocidal substances [8]. Presently, attention is paid to so-called light-activated surfaces. They require special molecules known as photosensitizers, which absorb light in the visible part of the spectrum and transfer the absorbed energy *via* its triplet state to adjacent molecules, in particular, molecular oxygen, thus leading to the generation of reactive oxygen species (ROS) [9]. Two types of antimicrobial coatings can produce reactive oxygen species: i) a coating consisting of an embedded or grafted photosensitizer [10-13], and ii) a titanium dioxide based photocatalyst coating [14, 15]. The main advantage of these light-activated coatings is the lack of developing microbe resistance with time [10]. Recently, we reported on the preparation of bio-inspired photoactivated antibacterial coatings on stainless steel (SS) with covalently attached photosensitizer of the 9-aminoacridine-3 type, which possess good photobactericidal activity against G. negative *E. coli* [13]. Therefore, here we report on the preparation and antibacterial activity of light-activated coatings based on protoporphyrin IX and bio-inspired nanogel based on Pox(mDOPA).

EXPERIMENTAL

Materials

Protoporphyrin IX (PPIX) (Sigma-Aldrich), N-hydroxysuccinimide (NHS), 1-(3-dimethylamino-propyl)-3-ethyl carbodiimide hydrochloride, 98+% (EDC) and poly (allylamine hydrochloride) (PAH) (Alfa Aesar) were used without further purification. Stainless steel 1.2 (SS) was used as a substrate. Poly (N-methacryloyl 3,4-dihydroxy-L-phenylalanine methyl ester)-b-poly (2-methacryloxyethyltrimethyl ammonium chloride) (P(mDOPA)-*co*-P(DMAEMA+) copolymer [16], poly (N-methacryloyl 3,4-dihydroxy-L-phenylalanine methyl ester) (P(mDOPA)) [17], oxidized poly (N-methacryloyl 3,4-dihydroxy-L-phenylalanine methyl ester)-/poly(allylamine) (Pox(mDOPA)/ PAH)) cross-linked nanogel [18] were prepared as reported in [16-18].

* To whom all correspondence should be sent:
E-mail: rbryaskova@uctm.edu

Synthesis of (Pox(mDOPA)/PAH) cross-linked nanogel

Pox(mDOPA)/PAH-based nanogels were prepared according to a procedure reported in [17]. In brief, P(mDOPA) (10 mg) was dissolved in distilled water (10 mL) and NaOH (0.1 M) was slowly added in order to increase the pH of the medium to oxidize the catechol groups of P(mDOPA) and the solution was stirred for 12 h at room temperature. Then, an aqueous PAH solution (3 mL; 1 g L^{-1}) at pH 10 was slowly added to the Pox(mDOPA) solution and stirred for one hour at room temperature.

Synthesis of amino-modified protoporphyrin IX (PPIX-ED)

Amino-modified protoporphyrin IX (PPIX-ED) was prepared according to the procedure reported in [19]. In brief, PPIX (200 mg, 0.355 mmol) was dissolved in 20 ml DMF (pre-purged with N_2) at room temperature. To this solution, ethylene diamine (40 mg, 0.666 mmol), NHS (40 mg, 0.348 mmol), and EDC (190 mg, 0.991 mmol) were added under stirring at room temperature. After 30 minutes, a fine precipitate was obtained. The resulting mixture was stirred for 24 h at room temperature and after that, the precipitate was collected, washed with 50 mL of diethyl ether, and left to dry.

Preparation of the light-activated polymer coatings on SS substrate

Stainless steel samples with different sizes were cut out from the as-received 1 mm thick SS, 1.0 × 1.0 cm. They were cleaned and degreased by washing for 2 minutes with ethanol and acetone, respectively and purged by nitrogen. The substrate was first dipped in an aqueous solution of P(DOPA)-*co*-P($DMAEMA^+$) (2 g L^{-1}, pH 7) for 15 min, then rinsed with deionized water for 5 min, followed by dipping into an aqueous solution of Pox(mDOPA)/PAH nanogel (1 g L^{-1}) for 15 min and rinsed with deionized water for 5 min. The substrate was dipped in a solution of amino-modified PPIX (1 g L^{-1}, pH 10) for 15 min followed by rinsing with deionized water for 5 min.

Characterizations

ATR FT-IR spectra were recorded using Agilent Cary 600 equipment. Transmission electron microscopy (TEM) observations were carried out with a HR STEM JEOL JEM 2100 instrument. Dynamic light scattering (DLS) measurements were performed using a Brookhaven instrument (NanoBrook 90Plus) with ZetaPlus particle sizing software Version 5.23. SEM-EDX spectra were recorded on SEM Lyra, Tescan with Quantax EDS detector – Bruker. The inhibition zones of the antibacterial test were measured using Image-Pro Plus software.

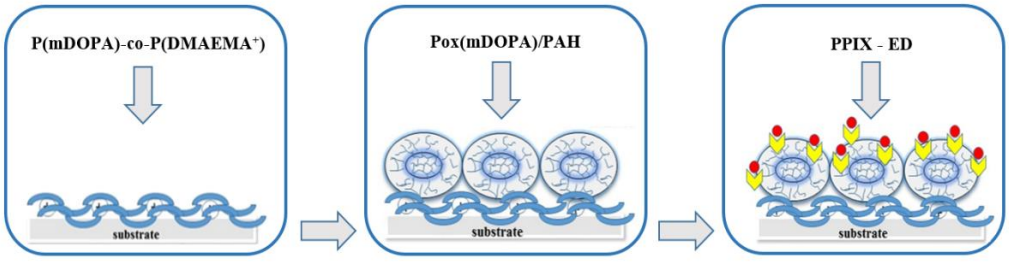
Antibacterial activity

The antibacterial activity of P(mDOPA)-*co*-P($DMAEMA^+$)/ Pox(mDOPA)/ PAH/ PPIX-ED coatings on SS substrate was tested against Gram-negative bacterium *Escherichia coli* (*E. coli*) and Gram-positive bacterium *Bacillus subtilis* (*B. subtilis*) using a modified agar disk-diffusion method (DDM). In this procedure, agar plates were inoculated with 0.2 ml of standardized inoculum (107 cells.ml^{-1}) of the test microorganism. Then, films (previously sterilized by UV irradiation) deposited onto 1×1 cm SS substrate were placed on the agar surface. The Petri dishes were illuminated using a 300 W spotlight and incubated under suitable conditions (30°C for *B. subtilis* and 37°C for *E. coli*) for 24 hours. Then the diameters of inhibition growth zones were measured using Image-Pro Plus software.

RESULTS AND DISCUSSION

Preparation and surface characteristics of the light-activated antibacterial polymer coating on stainless steel

Light-activated antibacterial coatings were prepared by a simple dip-coating procedure, according to Scheme 1.



Scheme 1. Strategy for preparation of light-activated antibacterial coatings on stainless steel.

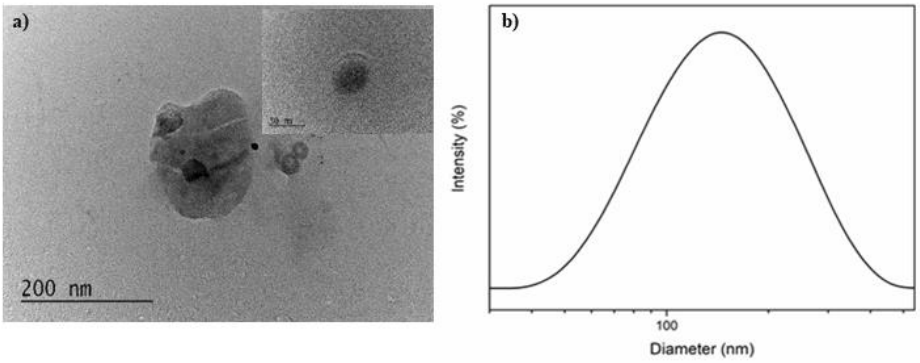
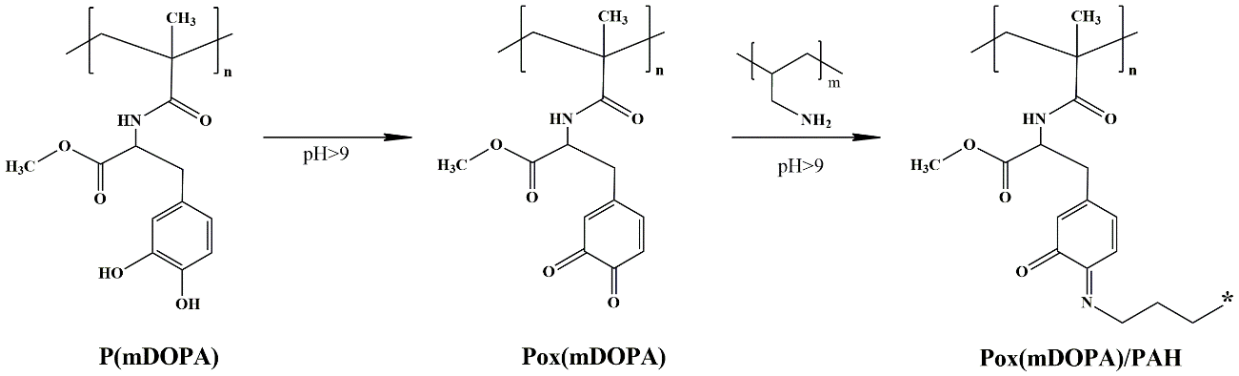


Figure 1. a) TEM of Pox(mDOPA)/PAH nanogels, b) DLS analysis of Pox(mDOPA)/PAH nanogels



Scheme 2. Preparation of Pox(mDOPA)/PAH nanogels

Scheme 3. Synthesis of amino-modified protoporphyrin IX

Initially, the substrate is immersed into an aqueous solution of polycationic P(mDOPA)-*co*-P(DMAEMA$^+$) copolymer (2g L^{-1}, pH 7) at room temperature. It is well known that DOPA-functionalized polycation copolymers can strongly anchor to the surface by DOPA/metal interactions [20]. The next layer is then built by dipping the substrate into an aqueous solution of Pox(mDOPA)/PAH nanogel (1g L^{-1}, pH > 9), which can be further modified with an appropriate amino-modified photosensitizer by the well-known amino/quinone reaction. For this purpose, Pox(mDOPA)/PAH nanogels were prepared according to the procedure reported by Detrembleur *et al.* [17] by the addition of a PAH solution (1 g L^{-1}) to Pox(mDOPA) aqueous solution (Scheme 2) which results in the formation of nanogel particles.

The preparation of Pox(mDOPA)/PAH nanogel was proven by TEM and DLS analysis. The TEM images demonstrated the formation of spherical Pox(mDOPA)/PAH nanogel particles with an average size of 50 nm (Figure 1a). DLS analysis showed that the nanogel particles have an average hydrodynamic diameter of 120 ± 10 nm at an index of polydispersity of 0.25 (Figure 1b). The last step consists of deposition of an amino-modified protoporphyrin IX (PPIX-ED) (1 g L^{-1}), which is known for its high photoactivity and antibacterial properties [21]. The synthesis of PPIX-ED was performed according to Scheme 3.

The modification of protoporphyrin IX was proven by ATR-FTIR spectroscopy. The ATR-FTIR spectrum confirms the chemical modification of PPIX by displaying all characteristic peaks at 3300

cm^{-1} (*v*NH); 2911 cm^{-1} (*v(s)*CH_2); 2855 cm^{-1} (*v(as)*CH_2); 1628 cm^{-1}(amide I band) and 1533 cm^{-1} (amide II band) for *v*C=O of –CONH– groups.

The energy dispersive X-ray analysis with SEM (EDX-SEM) was used to analyze the chemical composition of the coatings, as well as the distribution and concentration of the elements at the surface and in the vicinity of the surface (Figure 2). The overall elemental mapping of the P(mDOPA)-co-P(DMAEMA$^+$)/ Pox(mDOPA)/ PAN /PPIX-ED coatings on SS demonstrated uniform distribution of the main elements which was derived from the obtained coating as follows: carbon (53.9 at. %), oxygen (2.79 at. %) and nitrogen (4.2 at. %) (Figure 2a). In comparison, in the overall elemental mapping of the pristine SS substrate, all elements characteristic for the SS substrate as iron (67.2 at. %), chromium (16.08 at. %), and nickel (9.15 at. %) were detected (Figure 2b). These results demonstrated the successful preparation of the light-activated polymer coatings.

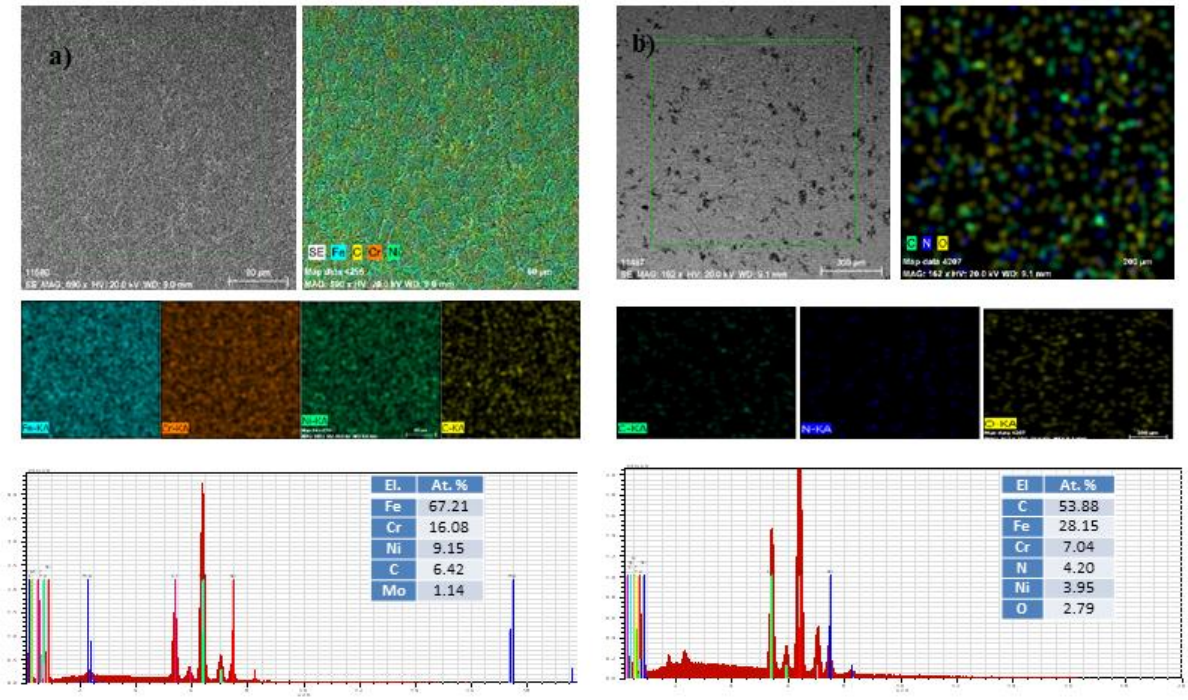


Figure 2. SEM-EDX mapping of a) neat SS substrate and b) P(mDOPA)-co-P(DMAEMA+)/Pox(mDOPA)/PAH/PPIX-ED coating on SS substrate.

Table 2. Antibacterial activity of the light-activated coatings on SS steel

Sample	*B. subtilis* Inhibition zone (mm)	*E. coli* Inhibition zone (mm)
P(mDOPA)-co- P(DMAEMA$^+$) /Pox(mDOPA)/PAH/PPIX-ED	12.6	16.5

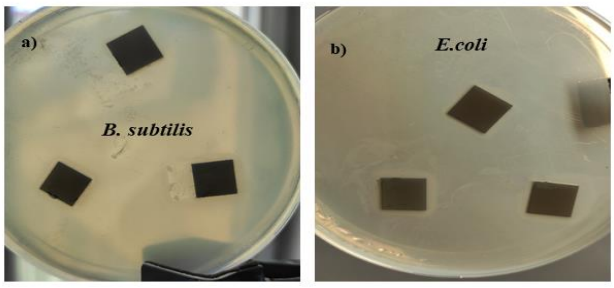


Figure 3. Antibacterial activity of P(mDOPA)-co-P(DMAEMA$^+$) /Pox(mDOPA)/PAH/PPIX-ED against a) *B. subtilis* and b) *E. coli* by DDM.

Antibacterial activity of light-activated antibacterial coating

The antibacterial activity of the obtained light-activated coatings was demonstrated against Gram-negative *E. coli* and Gram-positive *B. subtilis* strains using DDM. It was found in both cases that the light-activated coatings possess strong bactericidal activity demonstrated by an inhibition zone in the range of 12-16 mm (Table 2, Figure 3 a, b). However, the bactericidal activity against G. negative *E. coli* was higher in comparison to G. positive *B. subtilis*, which is probably due to the difference in the membrane structures of the G. negative *E. coli* and G. positive *B. subtilis* strains, the peptidoglycan layer in the former being thicker.

CONCLUSION

In this study, light-activated antibacterial coatings were successfully prepared on a SS substrate using an amino-modified photosensitizing agent on the base of porphyrins. The EDX-SEM analysis indicates the deposition of the coatings on the SS substrate by the presence of all chemical elements arising from the components used. The established strong antibacterial activity of the coatings against G. negative *E. coli* and G. positive *B. subtilis* under light irradiation demonstrated their potential use in medical and biomedical fields.

Acknowledgements: *The authors gratefully acknowledge the financial support from the National Science Fund of Bulgaria (project no. KP-06-H29/5).*

REFERENCES

1. W. A. Rutala, D. J. Weber, *Am. J. Infect. Control*., **47S**, A3 (2019).
2. W. A. Rutala, D. J. Weber, *Am. J. Infect. Contol*., **41S**, 2 (2013).
3. M. Ahonen, A. Kahru, A. Ivask, K. Kasemets, S. Kõljalg, P. Mantecca, I. Vinković Vrček, M. M. Keinänen-Toivola, F. Crijns, *Int. J Environ. Res. Public Health*, **14**, 366 (2017).
4. K. Vasilev, *Coatings*, **9**, 654 (2019).
5. C. Adlhart, J. Verran, N.F. Azevedo, H. Olmez, M. M. Keinänen-Toivola, I. Gouveia, L. F. Melo, F. Crijns, *J. Hosp. Infect*., **99**, 239 (2018).
6. X. Zhang, L. Wang, E. Levänen, *RSC Adv*., **3**, 12003 (2013).
7. H. Zhu, Z. Guo, W. Liu, *Chem Commun (Camb).*, **50**, 3900 (2014).
8. C. Krumm, J. C. Tiller, *Nachrichten aus der Chemie*, **62**, 984e7 (2014).
9. R. Bonnett, *Chem. Soc. Rev*., 24, 19 (1995).
10. K. Page, M. Wilson, I. P. Parkin, *Mater. Chem*.**, 19**, 3819 **(**2009).
11. A. J. T. Naik, S. Ismail, Ch. Kay, M. Wilson, I. P. Parkin, *Materials Chemistry and Physics*, **129**, 446 (2011).
12. M. J. Bovis, S. Noimark, J. H. Woodhams, C. W. M. Kay, J. Weiner, W. J. Peveler, A. Correia, M. Wilson, E. Allan, I. P. Parkin, *RSC Adv.*, **5**, 54830 (2015).
13. R. Bryaskova, N. Philipova, N. Georgiev, I. Lalov, V. Bojinov, C. Detrembleur, *Journal of Applied Polymer Science*, **138,** 50769 (2021).
14. G. W. Park, M. Cho, E. L. Cates, D. Lee, B. T. Oh, J. Vinje, et al. *J. Photochem. Photobiol. B,* **140**, 315 (2014).
15. J. Bogdan, J. Zarzynska, J. Plawinska-Czarnak. *Nanoscale Res. Lett*., **10**, 1023 (2015).
16. E. Faure, P. Lecomte, S. Lenoir, C. Vreuls, C. Van De Weerdt, C. Archambeau, J. Martial, C. Jerome, A.-S. Duwez, C. Detrembleur, *J. Mater. Chem*., **21**, 7917 (2011).
17. E. Faure, C. Falentin, T. S. Lanero, C. Vreuls, G. Zocchi, C. Van de Weerdt, J. Martial, C. Jérôme, A.-S. Duwez, C. Detrembleur, *Adv. Funct. Mater*., **22**, 5271 (2012).
18. E. Faure, C. Falentin-Daudré, C. Jérôme, J. Lyskawa, D. Fournier, P. Woisel, C. Detrembleur, *Prog. Polym. Sci*., **38**, 236 (2013).
19. J. Sherrill, S. Michielsen, I. Stojiljkovic, *Journal of Polymer Science: Part A: Polymer Chemistry*, **41**, 41(2003).
20. H. Lee, N. F. Scherer, P. B. Messersmith, *Proc. Natl. Acad. Sci. U.S.A*., **103**, 12999 (2006).
21. E. R. Reis, K. Metze, E. M. D. Nicola, J. H. Nicola, I. E. Borissevitch, *Journal of Luminescence*, **137**, 32 (2013).