ORGANIC BACTERIOSTATIC MATERIAL

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Abstract

The use of antibiotics to treat bacterial infections is becoming less and less effective year by year due to the increasing resistance of bacteria. The microbial evolutionarily acquired resistance to antibiotics increases the threat to man's life due to difficulties regarding effective therapies to fight infections. Therefore, apart from treatment, it is necessary to introduce appropriate prophylaxis which limits the multiplication of bacterial colonies on everyday use objects. Due to the antibiotic resistance phenomenon, it is important to find a new material with antibacterial properties for FDM 3D printing in medical applications.

The work contains research on a new chemical compound used as an additive to thermoplastics. The rhodamine derivative was synthesized via the 4-diphenylaminobenzaldehyde reaction with 1.3-indendione in a boiling mixture of EtOH/H_2SO_4. The obtained chemical compound was used as a bacteriostatic modifier of the polycarbonate (PC) properties, as such a modification enables application e.g. for medical device housings or for surfaces frequently touched by people.

The modifier and the commercially available polymer were compounded with a high-temperature screw extruder and a filament for FDM 3D printer was created. The modified polymer revealed antibacterial properties relative to Escherichia coli and good thermal stability during the processing.

Keywords: *bacteriostatic, rhodamine, polymer, Escherichia Coli*

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Introduction

In this study, we present the method of obtaining a new bacteriostatic material and its practical applications. Commercially available polymers, in most cases, are not biodegradable. Some of those plastics can biodegrade over time but it is a phenomenon of the irrelevant scale. Low biodegradation potential is connected with low susceptibility to degradation by fungi, bacteria, or mites and high resistance to most of the solvents. The aforementioned properties should provide conditions inhibiting the development of microbial colonies due to low nourishment availability. However, in everyday situations, most materials are covered with contaminants, such as tallow, food debris, or dust, which create a friendly environment for bacteria. In most cases, this phenomenon does not have a major impact on the material applicability, but in medical and personal hygiene products (syringes, toothbrushes, shower cabins) as well as other personal items (headphones, mobile phone cases) bacteriostatic properties become a very desirable feature.

Due to the increasing resistance of bacteria, the use of antibiotics in the fight against microbial infections is becoming less and less effective year by year [1-3]. The evolutionarily acquired resistance to antibiotics increases the threat to man's life due to difficulties regarding effective infection treatment. Therefore, it is necessary to introduce appropriate prophylaxis which limits the multiplication of bacterial colonies on everyday use objects.

Currently, the most commonly used methods of limiting bacterial growth on the materials surface are sterilization and creating a protective surface that contains nano additives. As a result of sterilization, bacterial colonies are removed, but this effect usually does not last as there is no additional long-term antimicrobic protection. In case of contamination of a newly sterilized surface, it quickly becomes a potential friendly environment for the bacteria growth. A new way to decrease the bacteria population is protection based on nanoparticles. Nowadays, nanosilica and nanosilver are in the spotlight as potential antibacterial substances [4-5]. However, there are still some apprehensions concerning the safety of using nanomaterials [6] and this solution is far from perfect.

Unfortunately, microbes have an evolutionary ability to adapt to new disinfectants. Therefore, it is reasonable to continuously look for new methods to limit their proliferation. Rhodamine B and its derivatives can be a great alternative to nanomaterials as well as thermal or chemical sterilization [7]. Rhodamine B is a red-purple compound with the chemical formula $C_{28}H_{31}ClN_2O_3$ and one of the most important water-soluble organic dyes (FIG. 1).

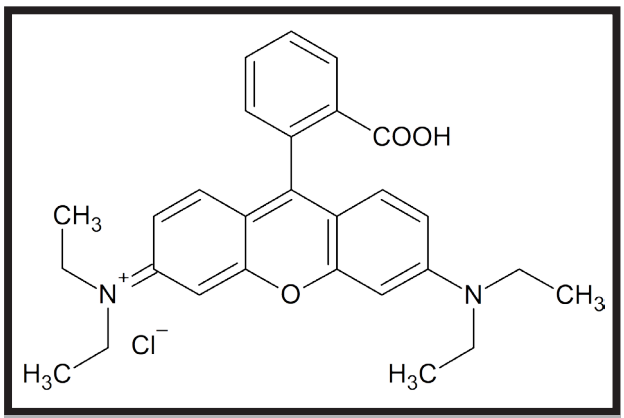

FIG. 1. Rhodamine B structure.

It is a very useful compound with properties supporting its application as a chromogenic reagent. Rhodamine B can also be used as a fluorescent sensor, e.g. it to detect glutathione [8], Cu^{2+} ions and 1-methionine. Rhodamine B and its derivatives have also found many applications in the chemical industry, for instance in the production of a polyvinyl chloride membrane that is able to detect mercury ions [9]. In this research, we synthesized a new derivative of rhodamine B endowed with bacteriostatic properties. However, unlike rhodamine B, the obtained compound is insoluble in water. Due to this advantageous property, a plastic additive is not susceptible to the compound washout. What is more, there is no increase in the hydrophilic properties of plastic, which is likely to occur while using water-soluble compounds as additives.

Materials and Methods

Pure ethanol (CAS number 64-17-5), 4- (diphenylamino) benzaldehyde 97% (CAS number 4181-05-9) and 1,3-indandion 97% (CAS number 606-23- 5) was purchased at Sigma Aldrich. Sulphuric acid solution 95% a.a. (CAS number 7664-93-9) was purchased from Linegal Chemicals. Makrolon® 2407 polycarbonate was purchased from Covestro.

The synthesis scheme of the rhodamine derivative is shown in FIG. 2. In a 250 mL three-necked flask, under an inert atmosphere, 20 mL of anhydrous ethanol was mixed with a droplet of sulphuric acid to obtain an acidic ionic reaction medium. Then 2.00 g (7.33 mM) of 4- (diphenylamino)benzaldehyde and 0.52 g (3.5 mM) of 1.3-indandione were added to the mixture. The resulting suspension was purged with argon 5.0 for 15 min. After that the reaction system was equipped with a magnetic dipole and brought to reflux under an inert gas atmosphere, being stirred vigorously for 24 h. The mixture turned brightly red. Then it was cooled to room temperature and the desired product was isolated using the SiO_2 column chromatography. A mixture of hexane and methylene chloride in gradient concentration was used as a mobile phase. The product was vacuum dried to solid weight and recrystallized from chloroform.

The filament for the 3D FDM printing was obtained in a homogenizer equipped with a Teflon mechanical stirrer. There the previously obtained 700 g of Makrolon® 2407 polycarbonate in the form of granules and 0.2 g of the dried bacteriostatic rhodamine modifier were mixed until the homogeneous polymers surface was covered by the rhodamine derivative. The surface-modified granulate was dried for 24 h at 100°C. In the next step, a 2.7 mm diameter string was made at temperatures: 180, 200, 210 and 225°C in subsequent heating zones within a four-zone single-screw extruder. Plates for analysis of microbiological interactions were made of the obtained material with the 3D FDM technology, using the proprietary FDM system with a heated 600 W table. During the process, the temperature of 285°C was maintained on the print-head and 165°C - on the table.

The evaluation of the antibacterial activity of the modified polycarbonate surface was carried out in accordance with the standard method [10]. The bacterial strain: Escherichia coli (ATCC® 25922) was used as the reference material. The inoculum volume at $6*10^5$ cells/mL was 0.4 mL.

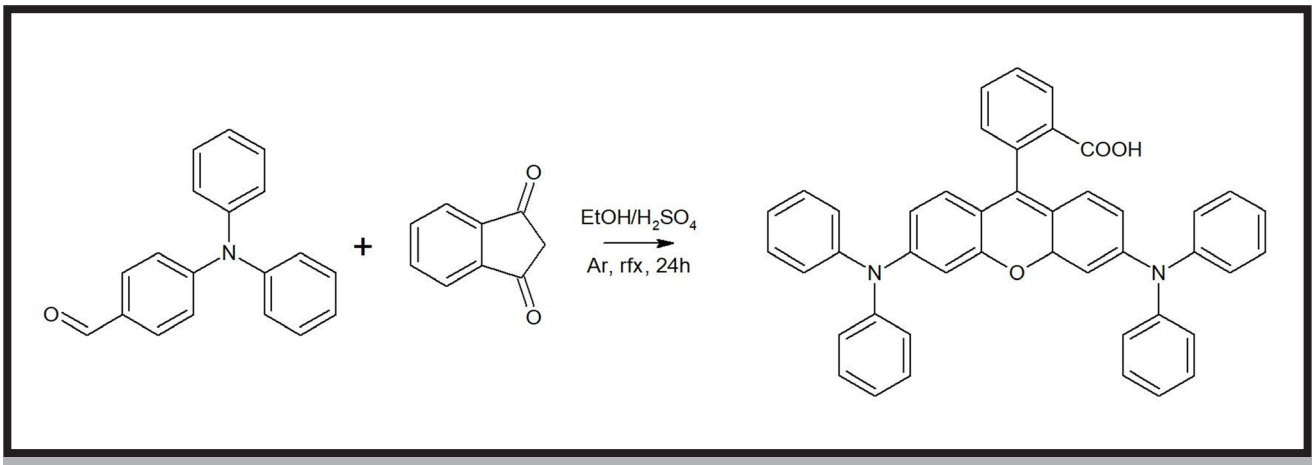

FIG. 2. Synthesis of rhodamine derivative - [9-(2-carboxyphenyl)-6-diphenylamino-3-xanthenylidene]-diphenylammonium.

TABLE 1. The antibacterial activity test parameters.

Parameter	Value
Control sample length x width x thickness	50x50x2.4 mm
Test sample length x width x thickness	50x50x2.4 mm
Cover film	Polypropylene foil 40x40x0.05 mm
Bacterial species and strain	Escherichia Coli, ATCC 25922
Inkulum volume	0.4 mL
Bacterial cell concentration	$6*10^5$ cells/mL
Volume and type of neutralizer	10 mL, SCDLP broth
Study time	72 h

All the culture media and solutions were prepared according to the standard method [10]. The parameters of the antibacterial activity are given in TABLE 1. The coefficient of antibacterial activity(R) of the tested material was determined according to the formula (1):

$$R = (U_t-U_0)-(A_t-U_0)=U_t-A_t$$

where: U_0 is the average logarithm of the number of live bacteria recovered from the untreated samples after plating, number of cells/cm^2; U_t means the decimal logarithm of the number of live bacteria recovered from untreated samples after 24 h, number of cells/cm^2; and A_t is the average logarithm of the live bacteria number recovered from the samples treated after 24 h, the number of cells/cm^2.

The MALDI –ToF analysis was carried out to determine the structure of the obtained rhodamine derivative. The spectra were obtained on AXIMA Performance instrument using graphite as a matrix material for those analyses.

The FTIR tests were carried out using the Shimadzu IR Tracer spectrophotometer equipped with the ATR multi-reflection device. The sample was applied directly to the ATR. The measurement parameters were 100 scans for each sample.

Results and Discussions

The new compound was synthesized through the reaction mechanism shown in FIG. 3.

The structure of the obtained additive was confirmed with the MALDI – ToF analysis (FIG. 4). On the mass spectra, the rhodamine derivative was recorded as an adduct with the potassium ion. In this case, the intensity did not determine the compound amount, but only the ionization method did.

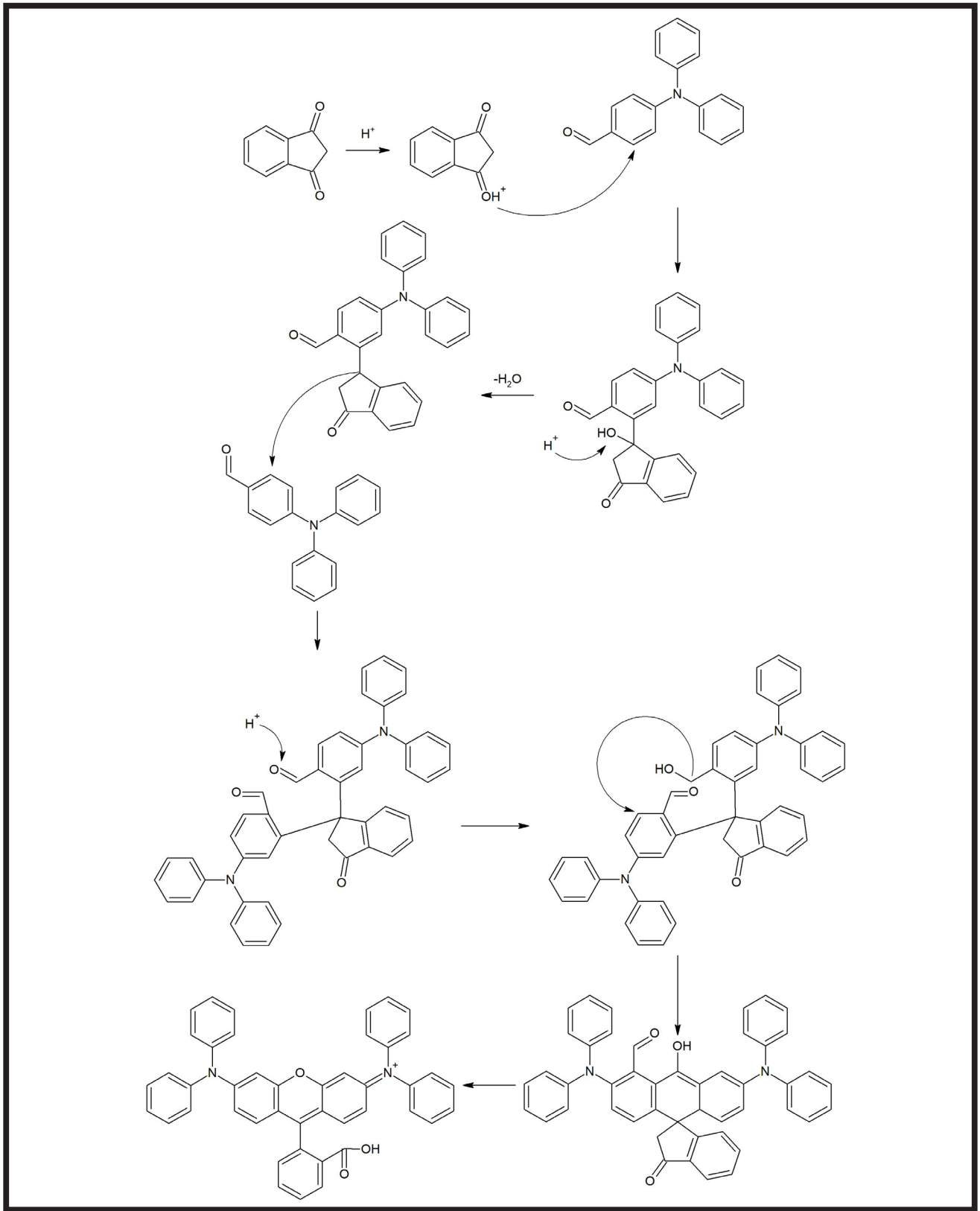

FIG. 3. The proposed reaction mechanism.

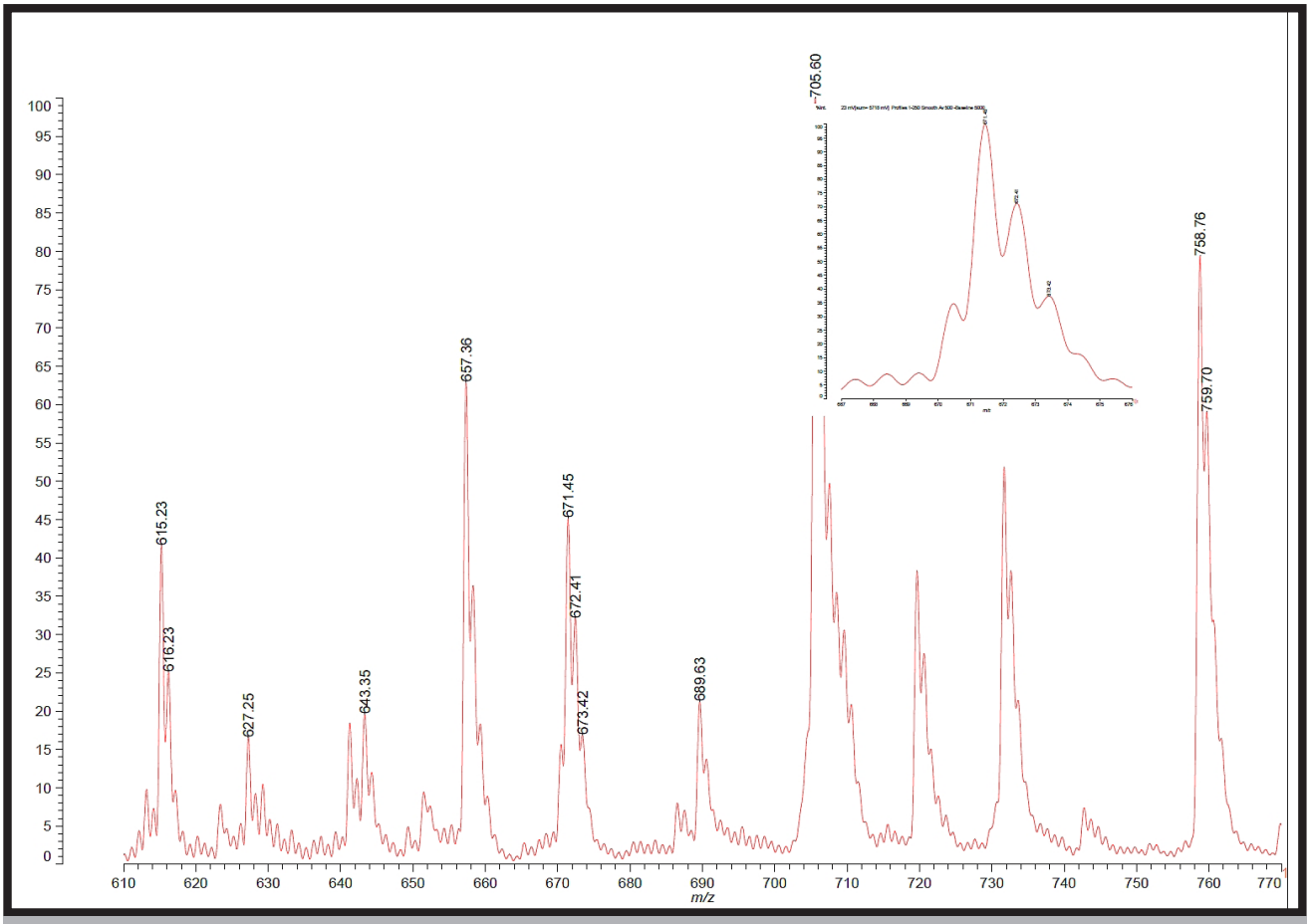

FIG. 4. The MALDI -ToF spectra of rhodamine derivative - additive.

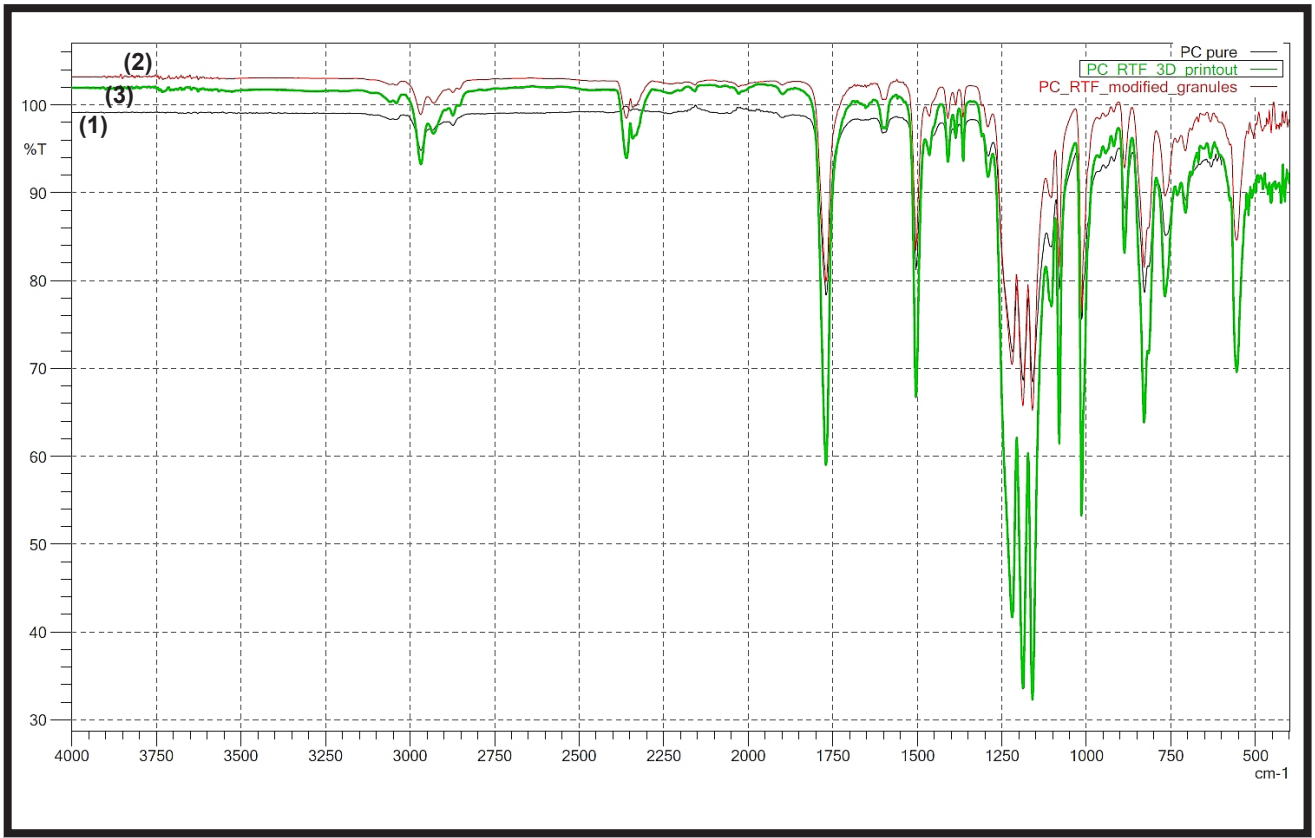

FIG. 5. Evolution of the IR spectra during thermal processing of the composite compared with pure polycarbonate. PC – black (1), modified granules – red (3), modified material printout from 3D printer – green (2).

Therefore, some impurities and residues of the reaction mixture had a higher intensity than the registered adduct of the rhodamine B derivative with potassium ion.

The obtained additive was used as a polycarbonate modifier and for 3D printing.

Comparing the obtained IR spectra for the pure material (PC), the modified granules and the sample after the thermal treatment – the 3D printing (FIG. 5.), the appearance of new bands was not noticed. Therefore, it may be concluded that the thermal treatment did not adversely affect the material.

It was observed that the polymers potential to form the biofilm on the product's surface decreased. The test results are presented in TABLE 2. The determined coefficient of antibacterial activity R indicated the bacteriostatic properties of the material in relation to the reference strain *E. coli* specified in the standard.

TABLE 2. The antibacterial activity test results.

Parameter	Value
U_0	4.21
U_t	4.91
A_t	3.46
R	1.45

Conclusions

The novel potentially bacteriostatic plastic modifier was synthesized. The obtained chemical compound proved that the rhodamine derivative may be used as an additive in plastics in the processing of high-temperature polycarbonate. Based on the applied low concentration and the high temperatures required for polycarbonate processing, it is assumed that this additive is also applicable for modifications of other thermoplastics. Moreover, the insolubility in water increases its potential as an addition to plastics. The authors are planning to perform a series of tests on the developed prototype material using 3D printing. Further work is also recommended to determine the mechanism of antibacterial activity of the obtained rhodamine derivative. The structure of the rhodamine B derivative was confirmed spectroscopically (FIG. 3). Even if further analysis should be carried out, it was already revealed that the examined material can be used to create surfaces limiting the bacterial biofilm formation.

The comparative analysis results proved all the tested materials to be suitable for filaments in the FDM 3D printing. The compatibility between polycarbonate and its additive was also demonstrated. No surface changes, inclusions or agglomeration of the additive were observed during the material preparation and processing.

Future studies of this material are planned in order to define the mechanism of antibacterial activity more accurately.

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