Pyrazole-based photoinitiator with antibacterial activity

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Abstract: 1-(naphthalen-2-yl)-2-(1H-pyrazol-1-yl) ethanone (MPPY) was synthesized. The structure was characterized by spectral analysis (^{1}H-NMR, FT-IR). The molar absorptivity of MPPY is high enough, similarly to acetonaphthone derivatives, to be used as an efficient type I photoinitiator. The influence of MPPY concentration and the addition of the co-initiator *N*-methyldiethanolamine (MDEA) on methyl methacrylate (MMA) polymerization were also investigated. Additionally, MPPY showed good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (diffusion method).

Keywords: photopolymerization, methyl methacrylate, antibacterial properties.

Fotoinicjator na bazie pirazolu o działaniu przeciwbakteryjnym

Streszczenie: Zsyntetyzowano 1-(naftalen-2-ylo)-2-(1H-pirazol-1-ylo)etanon (MPPY). Strukturę scharakteryzowano metodą analizy spektralnej (^{1}H-NMR, FT-IR). Molowa absorpcja MPPY jest na tyle wysoka, że można go stosować, podobnie jak pochodne acetonaftonu, jako skuteczny fotoinicjator typu I. Zbadano również wpływ stężenia MPPY oraz dodatku koinicjatora *N*-metylodietanoloaminy (MDEA) na polimeryzację metakrylanu metylu (MMA). Dodatkowo MPPY wykazywał aktywność przeciwbakteryjną w stosunku do *Escherichia coli* i *Staphylococcus aureus* (metoda dyfuzji).

Słowa kluczowe: fotopolimeryzacja, metakrylan metylu, właściwości antybakteryjne.

In recent years, the science and technology of photopolymerization has gained scientifical and industrial importance in many applications [1–4]. These applications are holographic recording, coatings, adhesives, and printing inks on various materials [5, 6]. The term radiation curing can be explained as the transformation of a liquid into a solid or the change in the physical properties of a polymer caused using radiation. Ultraviolet and electron beam radiation was often used to cause excitation, but nowadays continuous wave lasers and light emitting diodes' (LED) are used for this purpose [7]. The principle of this technology is to irradiate a photoinitiator with a radiation of a certain wavelength or high-energy electron beam to start a reaction of generating reactive species (free radicals, cations, or anions) that will convert the multifunctional monomer into a cross-linked structure. With the development of free radical photoinitiators with high quantum efficiencies, these industrial applications have developed more rapidly [8, 9].

Most research has focused on type 1 initiators that undergo α-cleavage upon excitation and produce two radicals [10, 11]. Among type 1 photoinitiators, reagents like benzylketals, hydroxylalkylphenones, benzoin ethers, α-aminoketones and acylphosphine oxides can be found [12, 13]. In type 2 photoinitiators, triplet excited states react with hydrogen donors to form an initiator radical. Type 2 initiators are slower than type 1 initiators, because the initiation step is based on bimolecular reaction [14–16]. The choice of photoinitiators in UV curing systems is especially important because the rate of polymerization and/or crosslinking depends on the photoinitiator. In addition, the flexibility, hardness, and chemical resistance properties of coatings vary depending on the degree of hardening in the system [17, 18].

Pyrazole derivatives are one of the most important groups of heterocyclic compounds in pharmacology [19]. Various pyrazoles are known to exhibit a wide range of pharmacological effects. New compounds with hypoglycemic, analgesic, anti-inflammatory, antimicrobial, anticonvulsant, antidepressant, antibacterial, antioxidant, antiviral, insecticidal and antitumor activities are being developed with these structures. For this reason, these compounds are considered important by researchers due to their biological activities [20–22].

In the presented study, pyrazole based 1-(naphthalen-2-yl)-2-(1H-pyrazol-1-yl) ethanone (MPPY) was synthesized and characterized as a new photoinitiator in the light of α-amino acetonaphthone derivatives [23–26]. In addition, photochemical and antibacterial properties of MPPY were investigated for biomedical applications [27–29].

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EXPERIMENTAL PART

Materials

2-Bromo-2′-acetonaphthone (99%), pyrazole (98%), *N*-methyl diethanolamine (MDEA, 99%), *N*,*N*′-dimethyl formamide (DMF, 99%), methanol (99.8%), toluene (99.7%), ethyl acetate (EA, 99.5%) was supplied from Sigma-Aldrich (Burlington, MA, USA) and used without purification. Triethylamine (99.5%), chloroform ($CHCl_3$, 99.4%), ethyl alcohol (ETH, 98%), dimethyl sulfoxide (DMSO, 99.5%) and dichloromethane (DCM, 99.9) were obtained from Merck (Darmstadt, Germany) and used as received. Methyl methacrylate (MMA, 99%) is supplied from Alfa Afsar (Haverhill, MA, USA) and purified by passing through a column of alumina to remove the inhibitor and dried over $CaCl_2$.

Synthesis of 1-(naphthalen-2-yl)-2-(1H-pyrazol-1-yl) ethanone (MPPY)

0.02 M of 2-bromo-2′-acetonaphthone (1.5 g) was dissolved in 10 mL of dry toluene in a two-necked round-bottomed flask. Pyrazole (0.44 g) in a mixture of 5 mL of toluene and 1 mL triethylamine was added dropwise to the reaction medium. After the addition, the reaction mixture was left 72 h for stirring at room temperature under N_2 atmosphere. The solution was filtered, and excess toluene was removed by using a rotary-evaporator. Yellow MPPY crystals are dried and characterized by spectrometric methods. Synthesis reaction is indicated in Scheme 1.

Methods

Spectroscopic methods

^{1}H-NMR spectrum was recorded on a Bruker Biospin AG 500 MHz (Billerica, MA, USA) instrument with $CDCl_3$ as solvent and tetramethyl silane (TMS) as the internal standard. The infrared spectrum was taken on a Nicolet 6700 FT-IR (Thermo Fisher Scientific, Waltham, MA, USA) spectrophotometer. Photopolymerization and photobleaching experiments were performed using a photoreactor consisting of 8-watt 6 UVA (254 nm) and 6 UVC (365 nm) lamps.

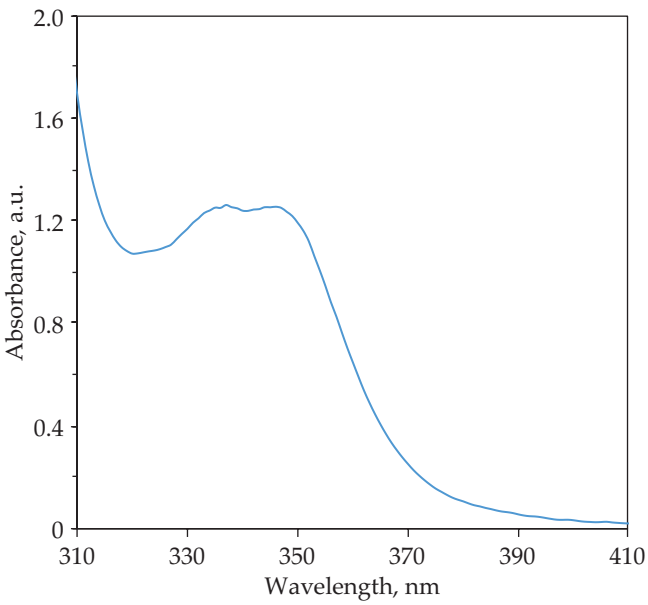


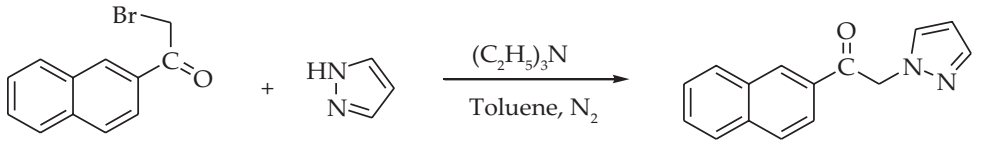
Fig. 1. MPPY UV-absorption spectrum in dichloromethane

UV-absorption spectrum of MPPY

Absorption spectra in the UV-visible region were obtained with an Agilent Technologies 8453 (Agilent Technologies, Santa Clara, CA, USA) spectrophotometer UV-absorption spectrum of MPPY was measured in dichloromethane and the molar absorptivity coefficients were calculated as, 1646 L/(mol × cm) at 335 nm and 1573 L/(mol × cm) at 344 nm (Fig. 1). Molar absorptivity coefficient values of MPPY are similar to other synthesized acetonaphthone derivatives [23–26].

Photoinitiated polymerization

Appropriate solutions of initiator and monomer were prepared in 5-, 10- and 20 mM concentrations of MPPY in 9.35 M of MMA monomer. Samples were put into Pyrex tubes (i.d = 9 mm) and then irradiated in photoreactor in air and N_2 atmosphere. After the irradiation, the resulting PMMA was precipitated in an excess of methyl alcohol and dried in vacuum. Other photopolymerization processes were performed by using *N*-methyl diethanolamine as co-initiator. Conversion percentages (conv. %) and polymerization rate values (Rp) were determined gravimetrically and calculated according to Equations 1 and 2.



1-(naphthalen-2-yl)-2-(1*H*-pyrazol-1-yl) ethanone (MPPY)

Scheme 1. Synthesis of MPPY

^{1}H-NMR (250 MHz, $CDCl_3$) δ 8.44 (s, 1H, Ar-H), 8.01-7.69 (m, 4H, Ar-H), 7.65-7.36 (m, 4H, Ar-H), 6.35 (s, 1H, Ar-H), 5.78 (s, 2H, -CH_2). FT-IR (ATR): ν = 3050 (aromatic C-H), 2968 (aliphatic C-H), 1679 (C=O), 1627 (C=N), 1515 (aromatic C=C), 1396 (C-N) cm^{-1}.

T a b l e 1. Photoinitiated bulk polymerization of MMA at 25°C

MPPY conc., mmol/L	MDEA, mmol/L	Conv., %	$Rp \times 10^5$ mol/(mL × min)
5	–	0.43	1.11
10	–	6.84	18.0
20	–	5.02	13.1
10	–	9.62[a)]	25.0
10	0.34	10.9	28.3
10	0.34	15.6[a)]	40.6

[a)]Under N_2 atm.

$$\text{Conv.}\,(\%) = \frac{W}{m \cdot 1000} \quad (1)$$

where: w – weight of polymer (g), m – weight of monomer (g).

$$R_p = \frac{W \cdot 1000}{M \cdot V \cdot t} \quad (2)$$

where: M – molar mass of monomer (g), w – weight of polymer (g), V – volume of solution (mL), t – polymerization time (min).

Antibacterial assessment

The antibacterial activity of MPPY was applied on *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC25923) by standard well diffusion method [30]. Inoculate culture of bacteria in Log phase (10^8 cfu/mL) were used against MacFarland's standard. The microorganisms were both incubated in nutrient broth then the active cultures were seeded on nutrient agar. Approximately 6 mm diameter of three wells were drilled and filled with 30 µL of MPPY solution (10 mg/mL in DMSO), antibiotic disc (streptomycin) and DMSO (50%). Inhibition zone diameters were measured for each tested organism after 16–18 h of incubation at 37°C and the results were evaluated. All experiments were made in triplicate.

RESULTS AND DISCUSSION

Photoinitiated polymerization of MMA

Bulk polymerizations were carried out for 60 min at different concentrations of MPPY photoinitiator in MMA monomer in the presence of air and N_2 by using photoreactor as the radiation source. Emission spectrum of photoreactor consisting of 6 UVA (254 nm) and 6 UVC (365 nm) lamps is compatible with the absorption spectrum of the photoinitiator. Light intensity of irradiation is measured as 25 W/m^2 (UVA) and results were indicated in Table 1.

When the polymerization results are examined, formation of polymer was observed even at low initiator concentrations in air (5 mmol/L; conv: 0.43%). Various concentrations of the initiator were used to determine the optimum photoinitiator concentration (10 mmol/L). The results show that increase in MPPY concentration increase conv. % and Rp values. However further increase of photoinitiator concentration led to decrease in conv. % due to filter effect [31]. It is known from previous studies that the tertiary amines added to the formulations will contribute to the conversion rate of polymerization by forming initiator particles while helping to reduce the oxygen in the environment [22, 23]. To evaluate the effectiveness of radicals in polymerization, photopolymerization of the samples to which NMDEA was added was carried out under nitrogen atmosphere. The results demonstrate that, by reacting with oxygen, NMDEA reduces the retarding effect of oxygen.

Solvent effects on absorption properties of MPPY

When the absorption spectra taken in different solvents are examined, it is observed that the positions, shapes, and intensities of the absorption bands are different. Spectral shifts are due to solute-solute and solute-solvent interactions [32]. The study of the absorption spectra of photoinitiators in solvents of different polarities explains the elucidation of the structure of different absorption transitions resulting from light absorption.

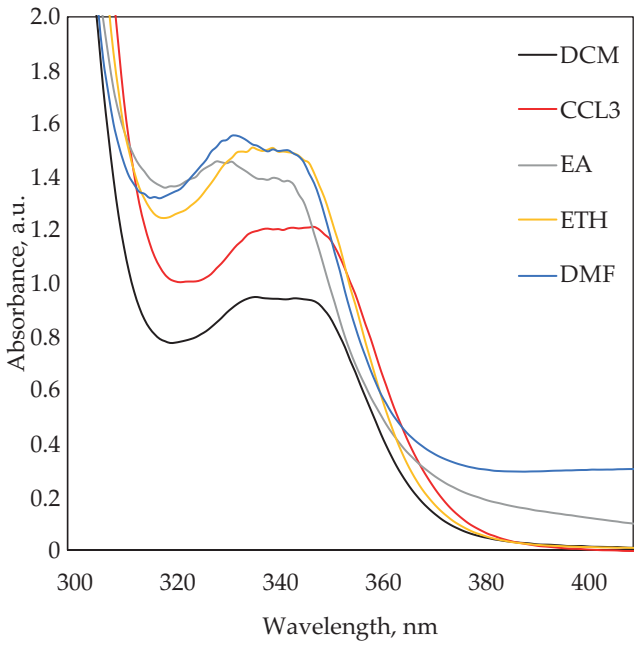


Fig. 2. UV-absorption spectra of MPPY (0.85 mM) in various solvents

The effect of solvent polarity on absorption properties can be explained based on different polarities in the ground and excited states [33].

In this study, the changes in the absorption spectra of the samples prepared in DCM, $CHCl_3$, EA, ETH and DMF solvents at equal concentrations were examined and the results are given in Fig. 2.

From Fig. 2, it is monitored that the n→π* absorption band is near 340 nm and dichloromethane is the best solvent for MPPY. Indeed, dielectric constants of solvents that are used in this study are shown in Table 2. It is observed that the increase in solvent polarity has a hyperchromic effect on UV spectra (OD_{DMF}> OD_{ETH}> OD_{EA}> OD_{CHCl3}> OD_{DCM}).

T a b l e 2. Dielectric constants of solvents

Solvent	Dielectric constant F/m
Dichloromethane	3.1
Chloroform	4.8
Ethyl acetate	6.0
Ethanol	24
Dimethyl formamide	38

Photobleaching of MPPY

In photoinitiated polymerization reactions, the initiator undergoes photolysis to produce ions or radicals. The rapid photobleaching of the photoinitiator under UV light is significant as it means it has a high initiation efficiency and a high polymerization rate. Therefore, the photolysis reaction was monitored by recording the decrease in UV absorbance of the initiator with irradiation. For this reason, a solution of MPPY with a concentration of 1 mM was prepared in dichloromethane, and irradiations were carried out in air with photoreactor at 15 minutes intervals. As seen in Fig.3 that the absorption peak of the initiator at 344 nm was disappeared after 80 minutes of irradiation.

Antibacterial activity of MPPY

The antibacterial efficiency of the synthesized MPPY was studied against *S. aureus* and *E. coli*. The antibiotic disc was employed as positive control. Table 2 shows the zone diameters (mm) of the wells of MPPY, DMSO (negative control) and streptomycin (positive control). The sample has strong inhibition against *E. coli* (18.5 mm) and *S. aureus* (20 mm). The results indicated that sample represented considerable antibacterial activity against both types of bacteria. However, the diameter of zone inhibition towards *S. aureus* was greater than that of *E. coli*; this can be referred to the outer membrane of gram-negative stain that is composed of proteins, lipids and polysaccharides that may block external entries which does not found in gram negative stain [34]. The antibacterial efficiency of MPPY may be attributed by its binding on cell membrane and inhibit the active transport

T a b l e 2. Diameters of MPPY, DMSO and streptomycin well zones

Bacteria	Zone diameter, mm		
	MPPY	DMSO	Positive control (streptomycin)
E. coli	18	8	22
S. aureus	20	9	24

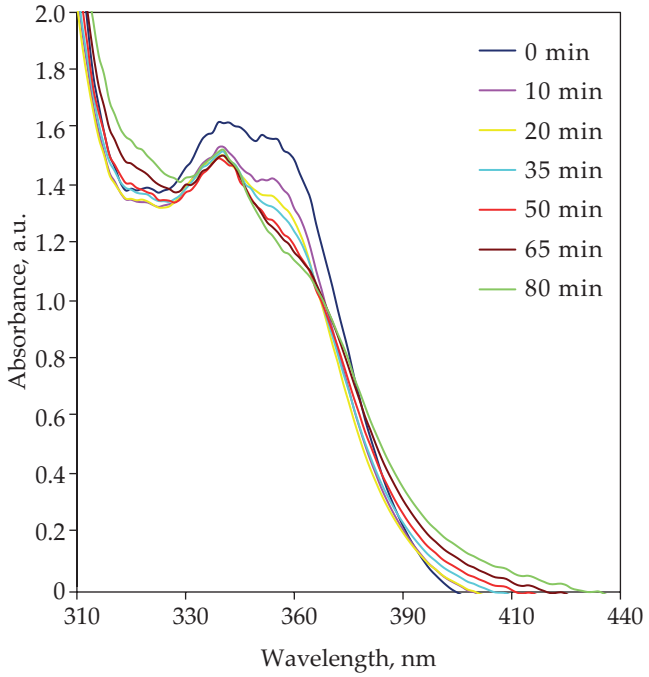


Fig. 3. Photolysis of MPPY (1 mM) in dichloromethane

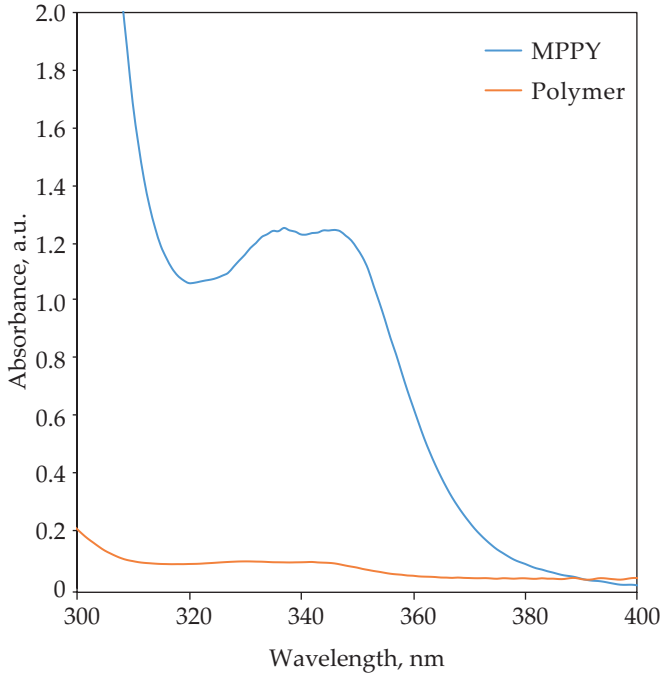


Fig. 4. UV-absorption of MPPY (0.85 mM) and PMMA in dichloromethane

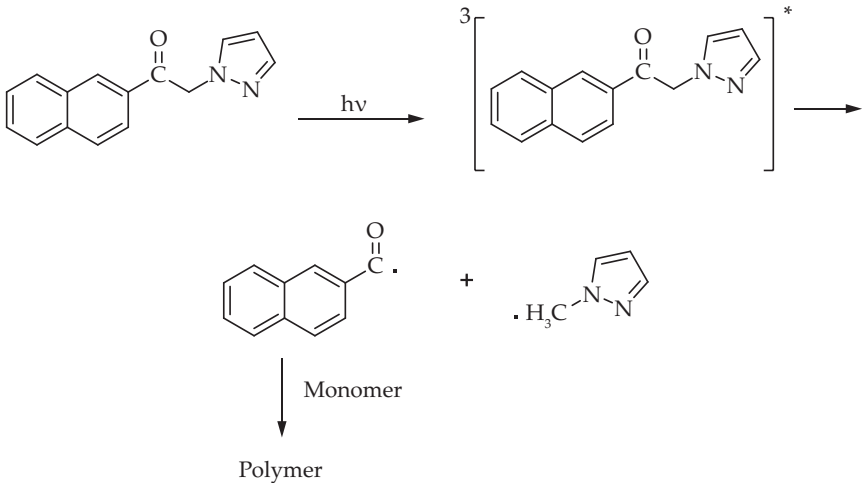


Fig. 5. Proposed MPPY photoinitiation mechanism

process which results in bacterial death [35]. The other mechanism considers MPPY binding to microbial DNA to inhibit the mRNA and protein synthesis, by entering the cell nucleus. It is known that pyrazole-based compounds disrupt the structure of membranes, enter the bacterial cells, and damage biochemical processes [36].

Proposed photoinitiation mechanism of MPPY

Absorption spectrum of the resulting polymer was examined to elucidate the mechanism of the photopolymerization process of the photoinitiator. For this reason, PMMA obtained in air by using a $2x10^{-2}$ M concentration of MPPY was dissolved in dichloromethane and then purified by precipitation in methanol. Absorption spectrum of the polymer measured in dichloromethane compared to absorption spectrum of MPPY photoinitiator were indicated in Fig. 4.

When the absorption spectrum of the PMMA obtained because of polymerization is taken, an information was obtained that the naphtoyl chromophore group of photoinitiator was attached to the polymer. The reason for the poor absorption is that the molecular weight of the polymer is high, and the absorption of the chromophore group is weakened (Fig. 4.).

According to the mechanism, after irradiation with UV-visible light, MPPY becomes excited state. Afterwards, free radicals are generated and naphthoyl radical initiates the polymerization reaction in the presence of methyl methacrylate monomer (Fig. 5).

CONCLUSIONS

A new photoinitiator, 1-(naphthalen-2-yl)-2-(1H-pyrazol-1-yl) ethanone (MPPY), was synthesized and characterized. MPPY has a broad absorption band in the UV-vis region, with a maximum of 335 and 340 nm. High molar absorptivity values and photolysis of MPPY in air in 80 min allow it to be used as a photoinitiator. Photoinitiated polymerization of MMA monomer was carried out in air and N_2 atmosphere. The obtained results indicate that MPPY initiates the polymerization of the MMA monomer according to the Norrish type 1 reaction. The strong antibacterial activity of MPPY results from the inhibition of bacterial growth and the destruction of their cell wall. Future research will be conducted towards a detailed analysis of the structure of the MPPY photoinitiator and its application in antibacterial coatings.

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Authors contribution
M.A.Y. – investigation, visualization, methodology, validation, writing-original draft.; S.K.D. – conceptualization, methodology, supervision, writing-original draft, writing-review and editing, project administration. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest
The authors declare there is no conflict of interest.

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