Macromolecular Rapid Communications
www.mrc-journal.de

UV Light-Driven Nitric Oxide Release from Porous Nitrogen Heterocyclic Polymers

*Sharon T. Gregg, René-Ponce Nze, Qingchun Yuan, Su He, Tianchao Xie, and Bo Xiao**

In this study, porous polymers with nitrogen heterocyclic core structures are synthesized through the condensation of enaminonitrile and terephthalaldehyde monomers. These polymers are used as a platform to store bioactive nitric oxide (NO) and control its release. NO loading is achieved by nitrosating the polymers with acidified nitrite, a process that also imparts photoresponsivity to the polymers. Polymer composition and porosity affect NO storage and release. It is observed that under UV light at 365 nm in a PBS solution, the polymers (NO@DHP-POP) can release NO in a manner fully controlled by UV lighting. Under experimental conditions, these porous polymers release NO at a rate of ≈10.0–50.0 µmol g^{-1} over 60 min. These findings demonstrate the potential of these polymers for integrating NO delivery into phototherapy applications.

1. Introduction

Porous organic polymers (POPs) offer significant advantages over inorganic and hybrid polymers such as activated carbons, zeolites, and metal–organic frameworks (MOFs). They excel in structural diversity, allowing for easier tuning of porosity and functionality. Their structural versatility enables a variety of applications, including CO_2 capture, gas separation, fuel gas (CH_4, H_2) storage, catalysis, and biomedical applications under mild temperature operation conditions.[1–11] A variety of typical crystalline or amorphous porous POP materials, e.g. covalent–organic frameworks (COF), covalent–triazine frameworks (CTF), polymers of intrinsic microporosity (PIM), conjugated microporous polymers (CMP), porous aromatic frameworks (PAF), and hyper-crosslinked polymers (HCP),[12–21] have been synthesized through various routes such as dehydration, condensation, or cross-linking reactions. The inherent porosity of POPs plays a beneficial role in accommodating the interesting molecules and enhancing their interaction with POPs to maximize POPs' effectiveness, particularly in drug delivery for therapeutic applications. To meet the requirements for specific applications, the POPs are usually post-modified through the functionalization of anchor sites or the unreacted terminal sites present in POPs; inserting of the interesting molecules (or metal ions) into the pores; modification of the POP's surface to change the hydrophobic/hydrophilic property and the affinity to the interesting molecules; and formulation of POPs into composites.[22] The inheritance of monomer functional groups in POPs makes postmodification easier to implement. These strategies have been used to transform POPs into biomaterials. For example, the covalent link of glucose oxidase (GOX) enzyme to the carboxyl functionalized COF for detecting the concentration of glucose for the control of diabetes;[23] introduction of polyethene-glycol modified curcumin derivatives (PEG-CCM) to the amine-functionalized COF to improve COF water dispersibility and increase the accumulation of antitumor agent doxorubicin (DOX) on tumor site;[24] formulating polydopamine (PDA)/COF core/shell nanoparticles that response pH–photo dual stimuli to release the loaded active pharmaceutical ingredients (APIs) for multimodal imaging-guided tumor photothermal-chemotherapy;[25] incorporation of photoactive moiety and a highly reversible proton acceptor into the skeleton of a covalent triazine polymer (CTP) to photocatalyze hydrogen peroxide production,[12] and so on. To date, there remains significant research interest in developing cost-effective methods for synthesizing porous organic polymers and optimizing their properties for drug delivery and other therapeutic applications.

Previous studies successfully demonstrated the condensation reaction of enaminonitrile compound with terephthalaldehyde to yield 1,4-bis(4-(3,5-dicyano-2,6-dipyridyl) dihydropyridyl) benzene molecules.[26,27] Subsequent assembly of these molecules via hydrogen bonds and π–π stacking interactions can form an organic framework. This discovery inspired us to develop new porous organic polymers using monomers with two identical substituent groups (e.g., di-enaminonitrile groups and di-aldehyde groups) for polymerization. Accordingly, **Figure 1**

S. T. Gregg, R.-P. Nze, S. He, T. Xie, B. Xiao
School of Chemistry and Chemical Engineering
Queen's University of Belfast
David Keir Building, Stranmillis Road, Belfast BT9 5AG, UK
E-mail: b.xiao@qub.ac.uk
Q. Yuan
Chemical Engineering and Applied Chemistry
Aston University
Birmingham B4 7ET, UK

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/marc.202400142

DOI: 10.1002/marc.202400142

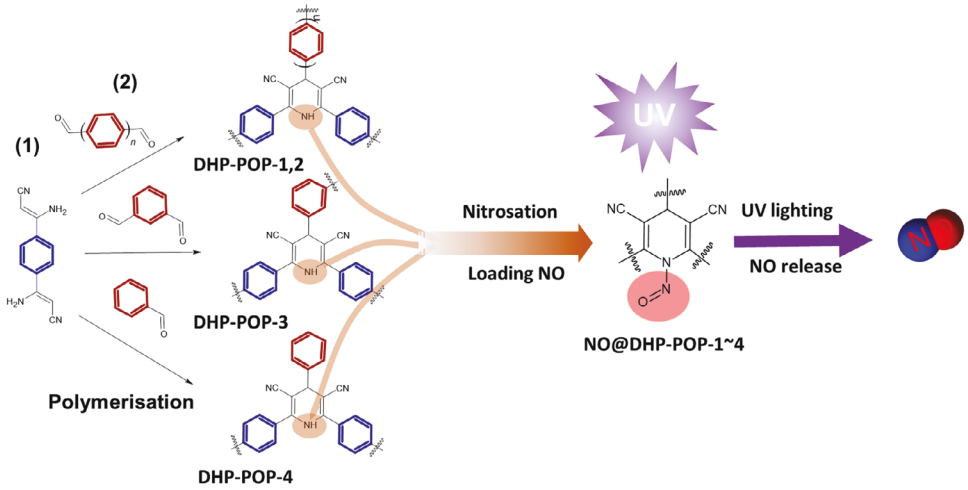

Figure 1. Condensation of enaminonitrile **(1)** with various terephthalaldehydes **(2)** to assemble polymers POPs (DHP-POP-1–4), followed by nitrosation of POPs to load NO. UV light triggers the release of NO from the polymers by cleaving N—NO bond.

illustrates the scheme for polymer synthesis, NO loading until it is released.

The new POPs were synthesized through condensation of monomers, e.g., enaminonitrile (**1**) with different terephthalaldehydes (**2**). The yielded polymers were coded as DHP-POP-*x* (*x*: 1–4). These resulting POPs contain nitrogen heterocyclic ring dihydropyridine (DHP) moieties. It is worth noting that the DHP derivatives exhibit various medicinal activities such as antitumor, antitubercular, and anti-inflammatory activities.[28] The subsequent step is to postmodify the DHP-POP-*x* by nitrosation reaction to incorporate nitroso groups (>N—NO) into DHP-POP-1–4, in this manner NO was charged in the POPs. The corresponding POPs were coded as NO@DHP-POP-1–4. As anticipated, these nitrosated POPs exhibit light responsiveness and can release NO under light irradiation, a characteristic that holds promising implications for diverse applications in responsive materials and biological systems. It is well known that bioactive NO, as a messenger molecule in the body, plays a crucial role in mediating various physiological and pathological processes. For example, it serves as a modulator, orchestrating vasodilation, inflammation, and immune responses during infection.[29] It can also hinder platelet adhesion and aggregation and promote the healing of diabetic wounds. In particular, its antiviral effectiveness was observed in recent years against diverse virus families, including the coronavirus SARS-CoV-2.[30] The precise and efficient delivery of NO remains a topic of intense research focus.

2. Synthesis of Dihydropyridyl Polymers and Characterization

By following the route illustrated in Figure 1, the polymers DHP-POP-1–4 were synthesized from the condensation of 3,3′-(1,4-phenylene)bis(3-aminoprop-2-enenitrile) with benzene-1,4-dicarbaldehyde, [1,1′-biphenyl]–4,4′-dicarbaldehyde, benzene-1,3-dicarbaldehyde, and benzaldehyde, respectively. The FTIR spectra of the derived polymers are shown in **Figure 2**a, verifying the dihydropyridine moieties as the core structure of the DHP-POP polymers as illustrated in Figure 1, in which the nitrile moieties —CN is identified by a typical sharp absorption peak at ~2210 cm^{-1}. The stretching vibration of —C≡C— conjugated with aryl occurs at ~1640 cm^{-1}. The secondary amine N—H stretching vibration is observed at ~3450 cm^{-1}. Further identification of the local structure was conducted by the solid-state NMR (^{13}C and ^{15}N). In **Figure 3**, ^{13}C NMR peak designation is denoted by numbering carbon 1–5 on the partial structure, where the lowest number corresponds to the lowest chemical shift. The peak at 42.7 ppm corresponds to the aliphatic sp^3 carbon C1, confirming the presence of the dihydropyridine ring in the structure, rather than an alternative aromatic pyridyl alternative; the peaks at 85 and 134 ppm are ascribed to the sp^2 carbons C2 and C4, respectively; the peak at 118 ppm is from the C3 in —CN, and the peak of 149 ppm from aromatic C5. Peaks at 129.5 and 49.7 ppm correspond to the strong aromatic C—H signal and residual solvent. By comparing to ^{15}N NMR correlation tables, the ^{15}N NMR spectra clearly show a peak at −254.7 ppm, ascribed to the N—H in the dihydropyridine group, and the peak appearing at −210.4 ppm corresponds to the nitrogen in the nitrile group, this is feint in most spectra due to the effect of shielding and the remoteness of protons. Both FTIR and ssNMR spectra decode the local structures as expected, indicating the desirable DHP-POP polymers have been obtained through the route designed in Figure 1. Thus, it was expected that the polymerisation would follow a similar reaction in the synthesis of 1,4-bis(4-(3,5-dicyano-2,6-dipyridyl)dihydropyridyl)benzene molecules, in which the lateral nitrile functional groups are cojoined by dihydropyridyl moieties.[27] The derived polymers are quite chemically stable, no decomposition was detected in water, benzene, toluene, *n*-hexane, methanol, ethanol, acetonitrile, ethyl acetate, diethyl ether, dichloromethane, chloroform, THF, DMF, and DMSO at ambient temperature. Thermogravimetric analysis (TGA) (Figures S6–S9, Supporting Information) confirms that this series of polymers can endure temperatures of up to ~350 °C, suggesting they possess adequate stability for subsequent modification if required.

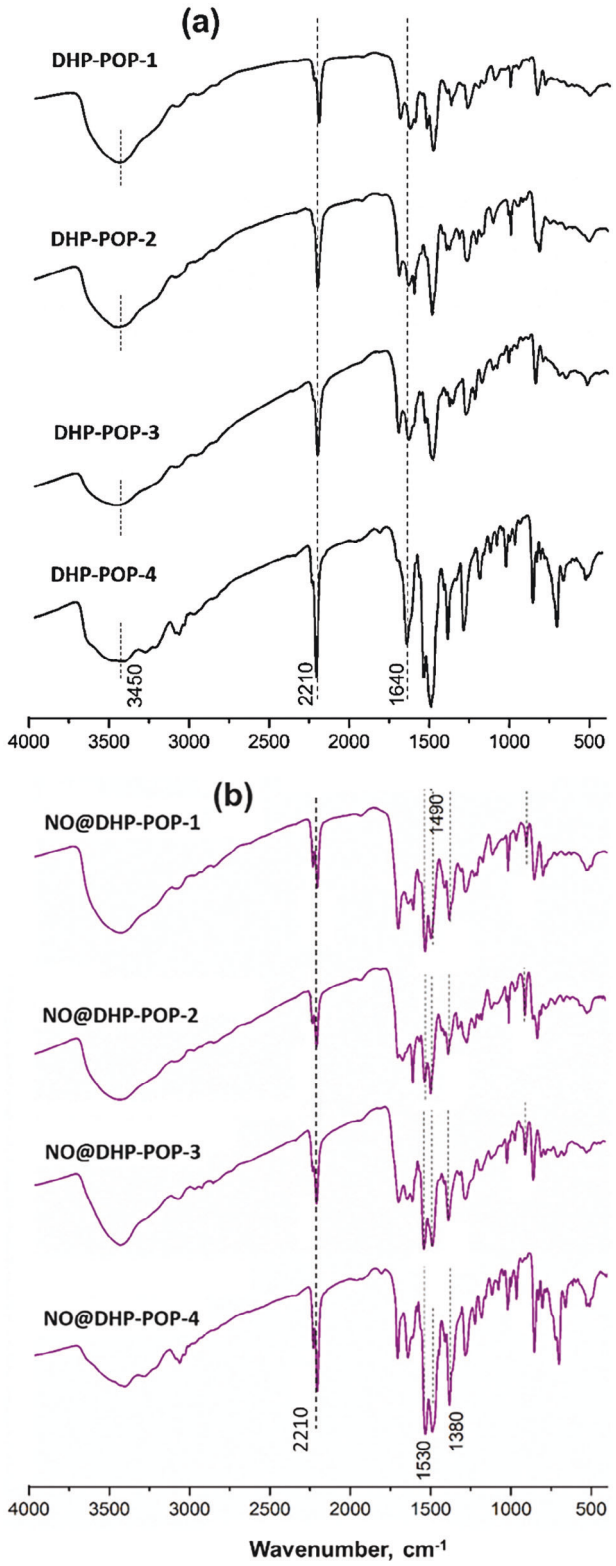

Figure 2. FTIR spectra of polymers (DHP-POP-1–4): a) before NO loading; b) after NO loading through nitrosation of polymers.

3. Nitrosation of Polymers by Acidified Sodium Nitrite to Load NO

The subsequent nitrosation process transforms the secondary amine groups >NH within the DHP-POP into *N*-nitrosamine (>N—NO) groups, thus completing the NO loading in the polymers. This process was accomplished by treatment of the polymers with acidic $NaNO_2$. The complex polymer structure may cause the >NH group to behave differently compared to simple molecules containing a dihydropyridine ring, such as felodipine and amlodipine, thus complicating the nitrosation mechanism and affecting the formation of N-nitroso groups. The formed N-nitroso groups are recognized by the FTIR spectra. In Figure 2b, the N═O stretching vibration gives strong peaks at 1530 cm^{-1}, C—N stretching vibration at 1380 cm^{-1}, and 906 cm^{-1} is correlated to N—N stretching vibration.[29] Similar to the DHP-POPs, the derived NO@DHP-POPs are relatively stable in the PBS solution, and no nitrosamine release was observed. The inclusion of NO species impacts the porosity of DHP-POP, as evidenced by the N_2 adsorption/desorption isotherms (Figures S4 and S5, Supporting Information), the BET surface areas are reduced by ~75–95% from previous ~96–512 $m^2\ g^{-1}$. In this scenario, noticeable hysteresis loops have been observed in the isotherms before and after NO bonding to polymers, deviating from conventional isotherm classifications, which may be attributed to the deformation of pore geometry induced by N_2 molecule adsorption. This characteristic has been extensively observed during the gas adsorption over other porous POP polymers,[30] highly advantageous for API delivery, yet the mechanism has not been accurately interpreted due to the intricate nature of polymer structures. The incorporated NO species exhibit thermal release within the range of ~250–350 °C (Figures S6–S9, Supporting Information), and their thermal stability remains relatively consistent across the various DHP-POP structures investigated in this study.

4. Ultraviolet (UV) Light Driving NO Release from the Polymers

After the nitrosation reaction, the polymers DHP-POP have been converted into bioactive NO donors. In contrast to other NO donors, for example, the coordinate polymers (or MOFs) and zeolites where the NO molecules are coordinated with metal open sites as well as *N*-diazeniumdiolate-based NO donors are moisture-responsive,[31] the polymers NO@DHP-POP exhibit light responsiveness. To verify this, in this study the NO@DHP-POP polymers were dispersed in the PBS solution, followed by UVA light irradiation at a wavelength of ~365 nm and an intensity of ~15 mW cm^{-2}. Under the experimental conditions, the photolysis of NO@DHP-POP cleaves the bonded NO species to release NO into the PBS solution where the released NO forms the NO_2^- species which can readily react with the Griess reagent to form the azo dye (λ_{max} = 540 nm). This reaction is quite sensitive and can be quantitatively detected. In plasma or other physiological fluids or buffers, NO is oxidized almost completely to nitrite, where it remains stable for several hours.[32] The Griess method was adopted in this study to evaluate the NO release from the polymers. The positive results were obtained as shown in **Figure 4**, convincing that NO has been charged into

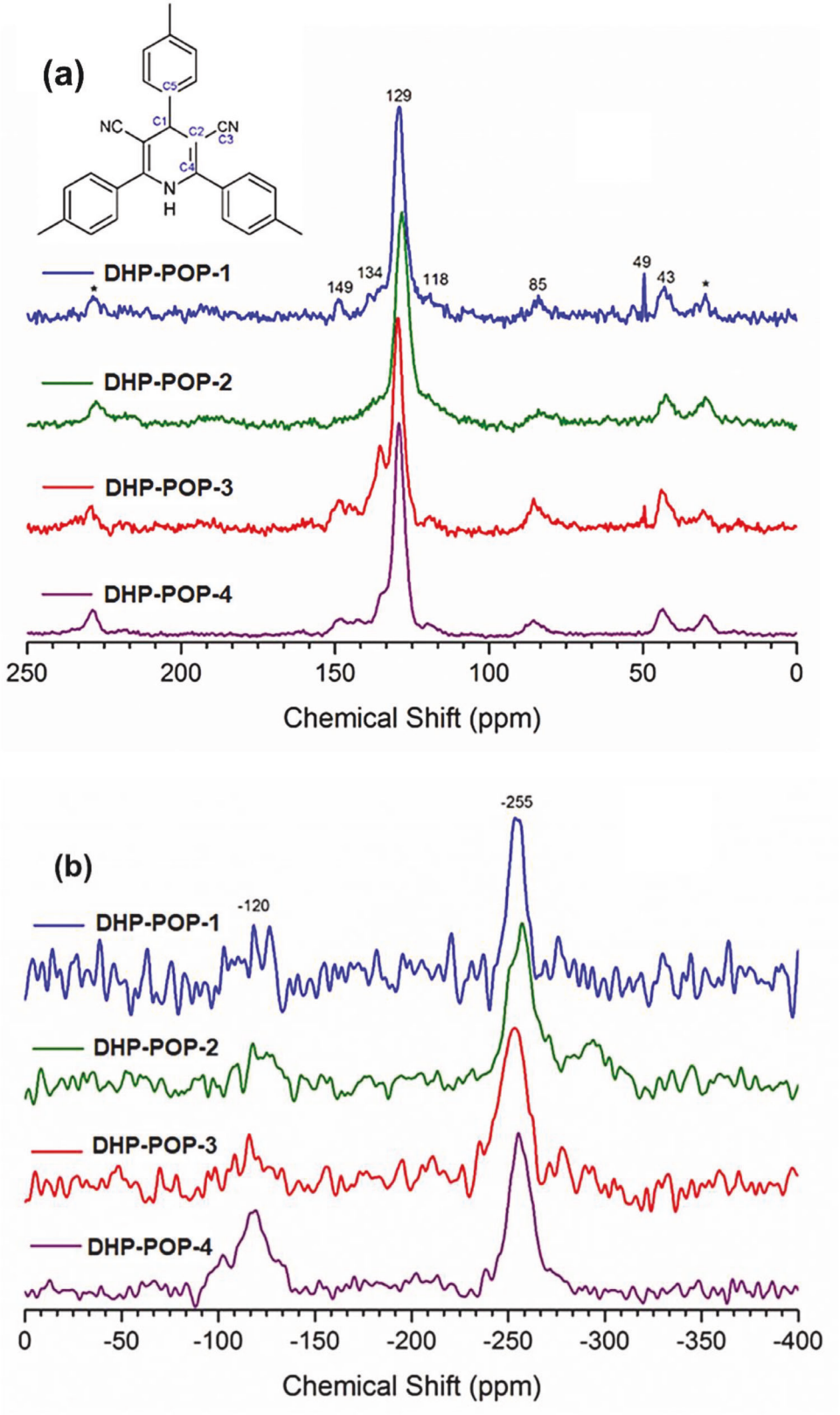

Figure 3. Solid-state NMR spectra of polymers DHP-POP-1–4: a) ^{13}C and b) ^{15}N (*spinning sidebands).

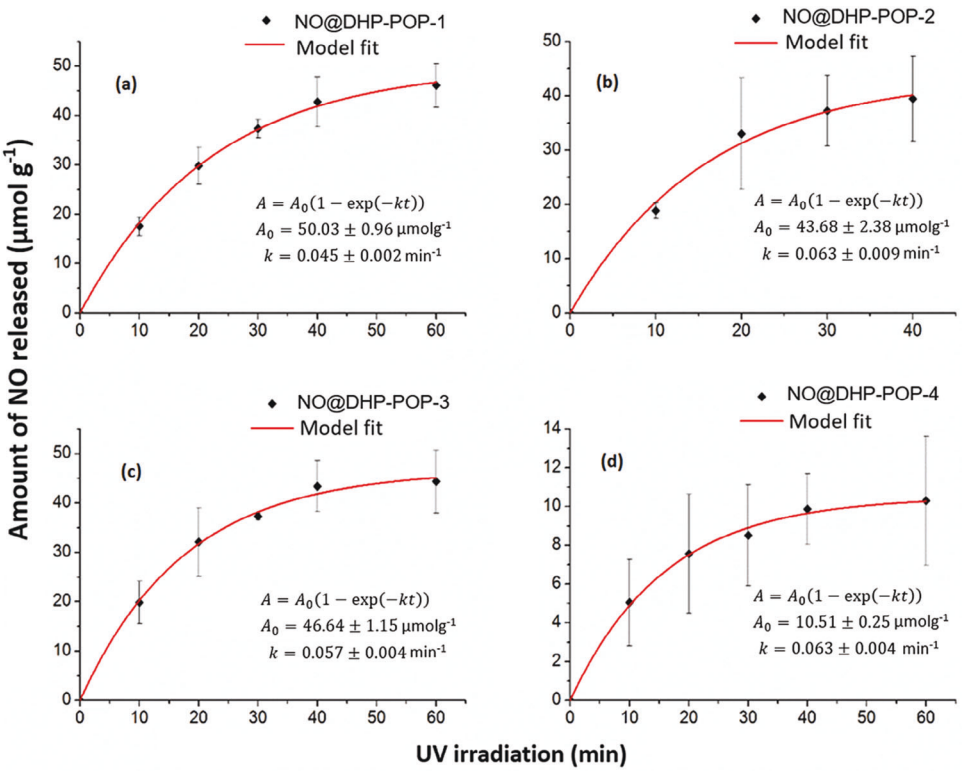

Figure 4. NO release profiles from the polymers a) NO@DHP-POP-1, b) NO@DHP-POP-2, c) NO@DHP-POP-3, and d) NO@DHP-POP-4 under UVA light irradiation at ~365 nm.

the derived polymers, and the DHP-POP polymer-based method for bioactive NO delivery is a feasible.

The accumulated amount of NO released over time is shown in Figure 4, obtained by lighting the polymers NO@DHP-POPs at an interval of ~10–20 min. It was observed that without UV light irradiation, the NO concentration in the PBS solution remained unchanged, indicating that NO release from the polymers was precisely controlled by switching the light on and off. This is distinct from the mechanism of water displacement reaction in NO release from MOFs and diazeniumdiolate-based NO delivery materials.[33] This observation largely rules out the possibility of NO_2^- species adsorbed in the polymer pores interfering with the Griess test. Analysis of the NO release profiles in Figure 4 showed that NO release can be fitted by the apparent first-order kinetics ($A = A_0\ (1 - \exp(-kt))$). The rate constant k varies within a narrow range of ~$(0.75–1.05) \times 10^{-3}$ s^{-1}, implying that photolysis of NO species in polymers is not significantly affected by the polymer structures. Compared to the others, the polymer DHP-POP-4, made from linear chains with lower porosity, may not have as many exposed secondary amines to bind NO. Consequently, it stores a smaller amount of NO to be released (Table S2, Supporting Information). In general, these polymers can store and release approximately $(10.5–50.0) \times 10^{-3}$ mmol g^{-1} of NO in PBS solution under UVA irradiation.

5. Summary

In summary, through the condensation reaction of enaminonitrile and terephthalaldehyde, a series of polymers with nitrogen heterocylic moieties have been successfully synthesized. Their porous nature to much extent allows the nitrosation of these polymers, to complete the transformation of polymers DHP-POPs into the polymeric NO donors for light-driven NO delivery. The NO release is completely controlled by UV light lighting. It would be possible to use this type of porous polymer as the bimodal vector for delivering different APIs simultaneously. Further work is still required focusing on polymer modification to improve or optimize NO storage and release. It was noted that the long-wavelength UVA (340–400 nm) phototherapy has been reported to be effective in treating atopic dermatitis, localized scleroderma and T-cell-derived skin diseases.[34] In future medical practice, there is potential to combine light-driven NO delivery with phototherapy.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This research program was funded by the National Research Foundation (EPSRC, EP/M027295/1), the Ph.D. studentship for Sharon from the Department for Employment and Learning (DEL), and the QUB and Aston University internal funding.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords

light irradiation, nitric oxide, nitrosation, photoresponsiveness, porous polymer

Received: March 12, 2024
Revised: June 7, 2024
Published online:

[1] M. M. Abdelnaby, T. A. Saleh, M. Zeama, M. A. Abdalla, H. M. Ahmed, M. A. Habib, *ACS Omega* **2022**, *7*, 14535.
[2] J. Wang, S. Xiong, J. Tao, C. Liu, J. Tang, C. Pan, X. Jian, G. Yu, *Sep. Purif. Technol.* **2020**, *248*, 117044.
[3] K. S. Song, P. W. Fritz, A. Coskun, *Chem. Soc. Rev.* **2022**, *51*, 9831.
[4] H. A. Patel, S. H. Je, J. Park, D. P. Chen, Y. Jung, C. T. Yavuz, A. Coskun, *Nat. Commun.* **2013**, *4*, 1357.
[5] L. Zou, Y. Sun, S. Che, X. Yang, X. Wang, M. Bosch, Q. Wang, H. Li, M. Smith, S. Yuan, Z. Perry, H.-C. Zhou, *Adv. Mater.* **2017**, *29*, 1700229.
[6] S. Bracco, D. Piga, I. Bassanetti, J. Perego, A. Comotti, P. Sozzani, *J. Mater. Chem. A* **2017**, *5*, 10328.
[7] J. Du, H. Ouyang, B. Tan, *Chem. Asian J.* **2021**, *16*, 3833.
[8] Z. Xu, L. Hu, J. Ming, X. Cui, M. Zhang, J. Dou, W. Zhang, B. Zhou, *Microporous Mesoporous Mater.* **2020**, *303*, 110259.
[9] M. Liu, C. Yao, C. Liu, Y. Xu, *Sci. Rep.* **2018**, *8*, 14071.
[10] P. Bhanja, S. Mishra, K. Manna, K. Das Saha, A. Bhaumik, *ACS Omega* **2018**, *3*, 529.
[11] Y. Zhu, P. Xu, X. Zhang, D. Wu, *Chem. Soc. Rev.* **2022**, *51*, 1377.
[12] S. Wang, Z. Xie, D. Zhu, S. Fu, Y. Wu, H. Yu, C. Lu, P. Zhou, M. Bonn, H. I. Wang, Q. Liao, H. Xu, X. Chen, C. Gu, *Nat. Commun.* **2023**, *14*, 6891.
[13] Y. Su, Z. Wang, A. Legrand, T. Aoyama, N. Ma, W. Wang, K. Otake, K. Urayama, S. Horike, S. Kitagawa, S. Furukawa, C. Gu, *J. Am. Chem. Soc.* **2022**, *144*, 6861.
[14] Y. Su, B. Li, H. Xu, C. Lu, S. Wang, B. Chen, Z. Wang, W. Wang, K. Otake, S. Kitagawa, L. Huang, C. Gu, *J. Am. Chem. Soc.* **2022**, *144*, 18218.
[15] H. M. El-Kaderi, J. R. Hunt, J. L. Mendoza-Cortés, A. P. Côté, R. E. Taylor, M. O'Keeffe, O. M. Yaghi, *Science* **2007**, *316*, 268.
[16] S. Dey, A. Bhunia, D. Esquivel, C. Janiak, *J. Mater. Chem.* **2016**, *A 4*, 6259.
[17] B. S. Ghanem, K. J. Msayib, N. B. McKeown, K. D. M. Harris, Z. Pan, P. M. Budd, A. Butler, J. Selbie, D. Book, A. Walton, *Chem. Commun.* **2007**, *1*, 67.
[18] J.-X. Jiang, F. Su, A. Trewin, C. D. Wood, N. L. Campbell, H. Niu, C. Dickinson, A. Y. Ganin, M. J. Rosseinsky, Y. Z. Khimyak, A. I. Cooper, *Angew. Chem., Int. Ed.* **2007**, *46*, 8574.
[19] T. Ben, H. Ren, S. Ma, D. Cao, J. Lan, X. Jing, W. Wang, J. Xu, F. Deng, J. M. Simmons, S. Qiu, G. Zhu, *Angew. Chem., Int. Ed.* **2009**, *48*, 9457.
[20] J. Huang, S. R. Turner, *Polym. Rev.* **2018**, *58*, 1.
[21] W. Lu, D. Yuan, D. Zhao, C. I. Schilling, O. Plietzsch, T. Muller, S. Bräse, J. Guenther, J. Blümel, R. Krishna, Z. Li, H.-C. Zhou, *Chem. Mater.* **2010**, *22*, 5964.
[22] J. H. Kim, D. W. Kang, H. Yun, M. Kang, N. Singh, J. S. Kim, C. S. Hong, *Chem. Soc. Rev.* **2022**, *51*, 43.
[23] J.-Y. Yue, X.-L. Ding, L. Wang, R. Yang, J.-S. Bi, Y.-W. Song, P. Yang, Y. Ma, B. Tang, *Mater. Chem. Front.* **2021**, *5*, 3859.
[24] G. Zhang, X. Li, Q. Liao, Y. Liu, K. Xi, W. Huang, X. Jia, *Nat. Commun.* **2018**, *9*, 2785.
[25] P. Gao, R. Wei, X. Liu, Y. Chen, T. Wu, M. Shi, M. Wang, N. Li, B. Tang, *Chem. Commun.* **2021**, *57*, 5646.
[26] Y. Yamaguchi, I. Katsuyama, K. Funabiki, M. Matsui, K. Shibata, *J. Heterocycl. Chem.* **1998**, *35*, 805.
[27] W. Yang, A. Greenaway, X. Lin, R. Matsuda, A. J. Blake, C. Wilson, W. Lewis, P. Hubberstey, S. Kitagawa, N. R. Champness, M. Schröder, *J. Chem* **2010**, *132*, 14457.
[28] K. K. Rajbongshi, B. Dam, B. K. Patel, in *N-Heterocycles: Synthesis and Biological Evaluation* (Eds: K. L. Ameta, R. Kant, A. Penoni, A. Maspero, L. Scapinello), Springer Nature Singapore, Singapore **2022**, pp. 1–49.
[29] N. B. Colthup, L. H. Daly, S. E. Wiberley, in *Introduction to Infrared and Raman Spectroscopy*, 3rd ed. (Eds: N. B. Colthup, L. H. Daly, S. E. Wiberley), Academic, San Diego, CA **1990**, pp. 261–288.
[30] J. Jeromenok, J. Weber, *Langmuir* **2013**, *29*, 12982.
[31] S. T. Gregg, Q. Yuan, R. E. Morris, B. Xiao, *Mater. Today Commun.* **2017**, *12*, 95.
[32] M. Schlaak, S. Schwind, T. Wetzig, J. Maschke, R. Treudler, N. Basara, T. Lange, J. C. Simon, D. Niederwieser, H. K. Al-Ali, *Bone Marrow Transplant.* **2010**, *45*, 1741.
[33] N. S. Bryan, M. B. Grisham, *Biol. Med.* **2007**, *43*, 645.
[34] S. T. Emmerling, J. Maschita, B. V. Lotsch, *J. Am. Chem. Soc.* **2023**, *145*, 7800.

