HHS Public Access

Author manuscript

ACS Appl Mater Interfaces. Author manuscript; available in PMC 2019 January 07.

Published in final edited form as:

ACS Appl Mater Interfaces. 2017 October 18; 9(41): 35628–35641. doi:10.1021/acsami.7b11846.

Nitric Oxide Generation from Endogenous Substrates Using Metal–Organic Frameworks: Inclusion within Poly(vinyl alcohol) Membranes To Investigate Reactivity and Therapeutic Potential

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Abstract

Cu-BTTri (H_3BTTri = 1,3,5-tris[1*H* 1,2,3-triazol-5-yl]benzene) is a water-stable, copper-based metal− organic framework (MOF) that exhibits the ability to generate therapeutic nitric oxide (NO) from S-nitrosothiols (RSNOs) available within the bloodstream. Immobilization of Cu-BTTri within a polymeric membrane may allow for localized NO generation at the blood−material interface. This work demonstrates that Cu-BTTri can be incorporated within hydrophilic membranes prepared from poly(vinyl alcohol) (PVA), a polymer that has been examined for numerous biomedical applications. Following immobilization, the ability of the MOF to produce NO from the endogenous RSNO *S*-nitrosoglutathione (GSNO) is not significantly inhibited. Poly(vinyl alcohol) membranes containing dispersions of Cu-BTTri were tested for their ability to promote NO release from a 10 μM initial GSNO concentration at pH 7.4 and 37 °C, and NO production was observed at levels associated with antithrombotic therapeutic effects without significant copper leaching (<1%). Over 3.5 ± 0.4 h, 10 wt % Cu-BTTri/PVA membranes converted 97 ± 6% of GSNO into NO, with a maximum NO flux of 0.20 ± 0.02 nmol·cm^{-2}·min^{-1}. Furthermore, it was observed for the first time that Cu-BTTri is capable of inducing NO production from GSNO under aerobic conditions. At pH 6.0, the NO-forming reaction of 10 wt % Cu-BTTri/PVA membrane was accelerated by 22%, while an opposite effect was observed in the case of aqueous copper(II) chloride. Reduced temperature (20 °C) and the presence of the thiol-blocking reagent *N*-ethylmaleimide (NEM) impair the NO-forming reaction of Cu-BTTri/PVA with GSNO, with both conditions resulting in a decreased NO yield of 16 ± 1% over 3.5 h. Collectively, these findings suggest that Cu-BTTri/PVA membranes may have therapeutic utility through their ability to generate NO from endogenous substrates. Moreover, this work provides a more comprehensive analysis of the parameters that influence Cu-BTTri efficacy, permitting optimization for potential medical applications.

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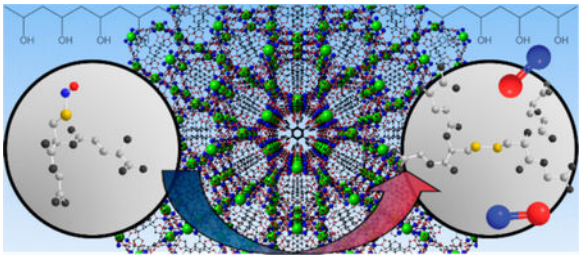
Notes

The authors declare the following competing financial interest(s): Prof. Melissa M. Reynolds is an equity holder in Diazamed, Inc. which has exclusively licensed the MOF platform from CSU Ventures. The other authors have no competing interests.

Graphical Abstract



Keywords

water-stable metal−organic frameworks; S-nitrosothiols; poly(vinyl alcohol); nitric oxide; biomaterials

1. INTRODUCTION

Over the past two decades, there has been considerable growth in the proposed use of metal −organic frameworks (MOFs) for a variety of wide-ranging applications. These unique materials are characterized by ordered networks formed from metal nodes and organic linkers and may display one, two, or three-dimensional architectures. The remarkable degree of porosity exhibited by many MOFs confers correspondingly large surface areas and pore volumes that have led to their implementation in gas storage and separation.[1] Furthermore, the density of potential active sites and the ability to controllably alter the physical and chemical properties within the framework through variation or modification of the metal ion or organic constituents have facilitated their use in heterogeneous catalysis.[1] More recently, the utility of MOFs in biomedicine has been recognized, including use in drug delivery and antibacterial applications.[2,3] This biomedical potential has been united with the well-characterized gas storage properties of MOFs in the form of nitric oxide (NO)-releasing frameworks.[4] These efforts were prompted by the broad therapeutic action of NO, which is produced endogenously as a crucial signaling molecule and exhibits potent antithrombotic, antimicrobial, and wound-healing effects.[5] As examples, the adsorption and subsequent release of NO have been demonstrated with iron-based MIL-88 and copper-based Cu-BTC (HKUST-1).[6,7] This function has been expanded with the postsynthetic modification of Cu-BTC to include NO-forming *N*-diazeniumdiolate functional groups.[8] An alternative approach has utilized copper-based MOFs such as copper benzene-1,3,5-tricarbox-ylate (Cu-BTC, or HKUST-1) to promote the release of NO from S-nitrosothiols (RSNOs), compounds that naturally occur in blood as a component of mammalian biochemistry.[9,10] Because RSNOs decompose to form NO and disulfide ($2RSNO \rightarrow 2NO + RSSR$) upon exposure to environmental triggers such as heat, light, and certain transition metal ions (e.g., copper), the controlled recruitment of these natural compounds for therapeutic applications has been the target of substantial prior research.[11] The established ability of particular copper-based MOFs to initiate NO formation from RSNOs permits the development of polymeric biomaterials that also exhibit this property.

However, biological application of this phenomenon requires the immobilization of copper-based MOFs within polymer matrices while retaining the NO-generating therapeutic activity. As an example, it has been demonstrated that the water-stable, triazolate-bridged MOF Cu-BTTri (H_3BTTri = 1,3,5-tris[1*H*-1,2,3-triazol-5-yl]benzene) (Figure 1a) can be dispersed within polymers such as plasticized poly(vinyl chloride) (PVC) and chitosan (a polysaccharide primarily consisting of glucosamine repeating units) to produce processable formulations that retain the NO-generating properties of the MOF in the presence of RSNOs.[12,13]

Predictably, it was observed that the physical properties of the polymer exert a considerable influence on the ability of the MOF to interact with aqueous-phase RSNOs in vitro. Limited diffusion of the dissolved RSNO into hydrophobic polymer formulations resulted in an *eightfold reduction* in NO generation relative to an aqueous suspension of Cu-BTTri powder (i.e., not blended within a polymer).[14] In comparison, a 10 wt % Cu-BTTri/chitosan formulation induced a 65-fold increase in NO generation over the baseline thermal decomposition of *S*-nitrosoglutathione (GSNO) (Figure 1b), the most abundant small-molecule RSNO present in blood.[13] In this earlier example, no statistically significant difference in Cu-BTTri performance was observed following incorporation within the polymer.

From these prior results, it is clear that polymers exhibiting substantial water uptake facilitate the interaction of aqueous-phase RSNOs with blended MOFs. For this reason, the use of copper-based MOFs to promote NO release from RSNOs may be best achieved through selection of hydrophilic polymer systems that permit optimization of this process for blood-contacting, therapeutic applications. In this respect, poly(vinyl alcohol) (PVA) (Figure 1c) represents a uniquely suitable candidate due to its hydrophilicity, hemocompatibility, and broad use in biomedicine. This polymer is a linear, water-soluble derivative of poly(vinyl acetate) produced through hydrolysis of ester groups. Cross-linking of PVA through various physical or chemical means produces insoluble materials that have been investigated for a wide range of medical applications, including drug delivery and tissue engineering.[15] Moreover, various NO-releasing, PVA-based materials have been used to fabricate wound dressings as well as to promote vasodilation to treat cutaneous endothelial dysfunction associated with cardiovascular disease.[16–18]

Herein, we report the synthesis and characterization of hybrid materials prepared from water-stable Cu-BTTri and PVA for the generation of NO from GSNO. The MOF was blended into aqueous solutions of PVA at 1, 5, and 10 wt % relative to the polymer, and the PVA host material was subsequently cross-linked by exposure to glutaraldehyde (GA) under mildly acidic conditions. The water-swollen membranes prepared through this procedure were examined for their ability to promote NO release from GSNO under varying conditions and compared to the performance of aqueous suspensions of Cu-BTTri powder. While PVA has been frequently used as a biomaterial, previous efforts involving the combination of PVA with MOFs or zeolites have generally targeted nonmedical applications such as chemical separation or water treat-ment.[19,20] This work describes the first material combining a water-stable MOF with PVA for potential therapeutic applications and demonstrates that physiologically relevant levels of NO release can be induced from GSNO. Furthermore, the

unique properties of Cu-BTTri/PVA membranes permit the controlled study of the NO-forming interaction between GSNO and Cu-BTTri in a manner that has not been previously possible. As a highly hydrophilic material, cross-linked PVA allows the comparatively rapid transport of GSNO to active sites within the polymer matrix. Unlike chitosan, PVA does not exhibit an independent ability to induce the decomposition of GSNO, thereby constraining observed effects to the influence of Cu-BTTri alone. Taken together, the attributes of Cu-BTTri/PVA membranes indicated the possibility for therapeutic use and provided the opportunity to evaluate critical performance parameters needed to facilitate the adaptation of copper-based MOFs to medical applications.

2. SYNTHESIS AND CHARACTERIZATION OF CU-BTTRI/POLY(VINYL ALCOHOL) MEMBRANES

S-Nitrosothiols occur naturally in human blood at concentrations that have been reported to range from nanomolar to micromolar, depending on analytical methodology, natural variation, and donor morbidity.[21] This fact has prompted the development of therapeutic materials that are intended to induce the NO-forming decomposition of biological RSNOs when placed in contact with flowing blood. These materials have included polymer-immobilized organoselenium and tellurium catalysts as well as various systems based on immobilized copper complexes, nanoparticles, and MOFs.[12–14,22–25] The use of MOFs to induce the generation of NO from endogenous substrates could permit sustained production without adsorption of finite quantities of gaseous NO or incorporation of NO-forming functional groups. This concept was initially limited by the susceptibility of Cu-BTC to water-induced degradation, which does not permit extended exposure to physiological media such as blood.[26] It was later discovered that the water-stable, triazolate-bridged framework Cu-BTTri (originally proposed by Demessence et al. for CO_2 capture) similarly promotes NO release from RSNO substrates.[14,27] The MOF was found to remain crystalline after exposure to both boiling water and dilute hydrochloric acid and retained its ability to induce NO release from RSNOs following immersion in whole blood. Moreover, Cu-BTTri does not exhibit the significant level of copper ion leaching observed from certain copper complexes used to catalyze NO release from RSNOs.[12–14,26,28] This retention of MOF function permits the development of polymer-based materials with the ability to produce therapeutic levels of NO directly from blood. For example, Cu-BTTri has been blended with biomedically relevant polymers such as plasticized PVC, where it demonstrated the ability to promote NO release from RSNOs such as *S*-nitrosocysteine and *S*-nitroso-N-acetylpenicillamine (SNAP).[12,14] However, the ability of aqueous-phase, biological RSNOs to interact with blended Cu-BTTri (or other copper-based agents) is limited by the poor water uptake of hydrophobic polymers like PVC. In comparison, the promotion of NO release from an RSNO exposed to blended Cu-BTTri occurred more rapidly when the MOF was incorporated within chitosan, a hydrophilic polysaccharide.[13] Despite this improved performance, the potential blood-contacting applications for this material were constrained by the inherent hemostatic attributes of chitosan.[29] As additional complications, the basic glucosamine repeating units of chitosan are capable of reacting with acidic functional groups that occur as part of the structure of physiological RSNOs and may also strongly bind transition metal ions that are independently capable of initiating RSNO decomposition.[30,31]

As such, it is clear that MOF-based materials combining the water permeability of chitosan with the structural simplicity of PVC may permit NO generation from RSNOs and examination of the parameters influencing this behavior without interference from the polymer matrix.

Membrane Preparation.

Poly(vinyl alcohol) is a highly hydrophilic linear polymer derived from hydrolysis of poly(vinyl acetate). The Cu-BTTri blends prepared from hydrophilic PVA represent promising alternatives to previous systems due to the existing use of PVA in numerous blood-contacting applications, its lack of hemolytic properties, and the potential for considerable water permeability and transport of GSNO.[32] Moreover, the absence of acidic or basic functional groups (e.g., the primary amine groups of chitosan) and the general structural simplicity of PVA allow it to function as a comparatively inert host for the MOF. Implementation of this concept is particularly beneficial in the case of studies intended to elucidate the NO-forming behavior of Cu-BTTri under varying conditions. For example, incorporation within a polymer matrix permits straightforward isolation and recharacterization of the immobilized MOF following experiments without requiring the separation of fine MOF particle suspensions. Because PVA has been frequently examined as a nontoxic biomaterial, there has been notable prior interest in the utilization of PVA or PVA-based materials as NO delivery platforms. For example, Masters et al. modified PVA hydrogels with *N*-diazeniumdiolate NO donor groups to prepare wound dressings, while the vasodilatory effect of NO was exploited by Marcilli and de Oliveira in the development of NO-releasing PVA films for the treatment of microvascular skin disorders.[16,17,33,34] In addition to these examples of NO-releasing derivatives, physically cross-linked PVA films were used to uptake and subsequently release GSNO itself as a therapeutic. Since PVA exhibits solubility in water, physical or chemical cross-linking techniques are frequently used to produce stable materials for use in biomedicine.[15] Cross-linking by reaction with dialdehydes such as GA occurs through the formation of acetal linkages between GA and the 1,3-diol units of independent PVA chains.[35] This type of cross-linking process permits the synthesis of water-insoluble membranes that encapsulate crystalline Cu-BTTri. The MOF was synthesized following the original protocol published by Demessence et al. and was characterized by powder X-ray diffraction (pXRD) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) prior to use (Figure 2a and b).[27] The MOF was then blended into aqueous solutions of PVA in the presence of GA and acetic acid at 1, 5, and 10 wt % loading relative to the polymer. It was observed that the direct addition of Cu-BTTri powder to aqueous PVA resulted in substantial MOF particle agglomeration that reduced the visual uniformity of the mixture, potentially resulting from physical incompatibility between the MOF and polymer solution. This phenomenon was limited by adding Cu-BTTri as an aqueous suspension, which was gradually mixed with the polymer phase to promote a more uniform dispersion. Similar effects in organic solvents have been noted by multiple prior authors and may be attributable to the formation of a polymer coating on the MOF that inhibits agglomeration.[36,37] While strong acid catalysts (e.g., sulfuric or hydrochloric acid) are commonly used to produce highly cross-linked PVA, the sensitivity of Cu-BTTri to harshly acidic conditions prompted the use of acetic acid as a milder alternative. After 2 days of curing at ambient temperature, followed by 2 h at 80 °C,

membranes were immersed in Millipore water for 1 day to remove residual acetic acid and GA. This immersion was followed by treatment with a 0.1% w/v aqueous suspension of Chelex 100 resin to capture trace metal ions (chiefly iron or copper) that may diffuse from membranes into the surrounding aqueous medium. The membranes were immersed in Millipore water for an additional 2 days, punched into 15 mm diameter circles (water-swollen), and then air-dried prior to characterization.

Membrane Characterization.

Characterization of the 10 wt % Cu-BTTri/PVA membranes by pXRD revealed the expected diffraction pattern of Cu-BTTri overlapping with a broad feature originating from semicrystalline PVA, supporting retention of the crystalline structure of the MOF following incorporation within the polymer matrix (Figure 2a).[27] At lower loading of 1 and 5 wt % Cu-BTTri, key diffraction peaks remain defined (Figure S1). Control membranes prepared from PVA alone exhibit characteristic IR absorption bands at 3600−3000 (O−H stretching), 2937, 2916, 2850 (CH stretching), 1709 (acetate C=O stretching), 1655 (H_2O), 1418 (CH_2 bending), 1377 (CH_2 rocking), 1327, 1238 (CH rocking), 1086 (CO stretching), 916, and 830 cm^{-1}.[38] The incorporation of 10 wt % Cu-BTTri is accompanied by the appearance of bands at 1616 (C=C stretching) and 775 cm^{-1} (C−H out-of-plane bending) associated with the triazolate-bridged MOF (Figure 2b). At 5 wt % Cu-BTTri, these absorption bands are less pronounced but remain present (Figure S2). At a lower concentration of 1 wt % Cu-BTTri, the absorption bands of the MOF are no longer clearly resolved (Figure S2). In all cases, no peaks directly assignable to GA are detectable. This outcome was predictable due to the comparatively mild cross-linking conditions, which are unlikely to result in extensive acetal formation and incorporation of GA within the PVA matrix.

However, the presence of cross-linking was qualitatively demon-strated by heating samples of GA cross-linked Cu-BTTri/PVA, cross-linked PVA, and non-cross-linked PVA in Millipore water. The PVA membrane prepared without exposure to GA readily redissolves in water, while cross-linked Cu-BTTri/PVA and PVA do not exhibit solubility at temperatures as high as 90 °C. Cross-linking under mild conditions is not an unexpected outcome since it has been previously observed that a degree of GA-mediated cross-linking occurs in the total absence of catalytic acid.[39] While PVA is capable of chelating copper ions through a variety of proposed binding mechanisms (particularly under strongly alkaline conditions), there is no spectroscopic evidence supporting the possibility that similar interactions occur between Cu-BTTri and PVA.[40,41] In general, both pXRD patterns and IR spectra of Cu-BTTri/PVA membranes are additive with respect to the spectra of individual constituents and do not indicate a detectable change in crystallinity or chemical structure of Cu-BTTri or PVA as a consequence of membrane preparation.

The thermal properties of 10 wt % Cu-BTTri/PVA membranes were examined by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). For TGA, studies were performed on the PVA control membranes and Cu-BTTri powder as well as the 10 wt % Cu-BTTri/PVA membranes taken both before and after the materials were studied in NO release experiments. Samples were analyzed as prepared with the respective thermograms shown in Figure S3a. The thermal transitions observed for both the PVA and

Cu-BTTri starting materials were consistent with the literature. Dehydration of the PVA control began at ~65 °C with membrane decomposition (colorless to light brown color change) beginning at ~225 °C.[42,43] The MOF showed significant dehydration at room temperature followed by desolvation beginning at ~145 °C with onset of decomposition observed at ~295 °C.[27] For 10% Cu-BTTri/PVA membranes, incorporation of the MOF as a polymeric dopant did not significantly alter the thermal stability of the synthesized membranes. For the initial membrane, dehydration was observed at the same temperature as the PVA control with an onset of membrane decomposition (purple to tan/light brown color change) observed at ~245 °C. After the membrane was subjected to NO release studies, the thermal behavior of the membrane showed no differences compared to the initial membrane (with the exception of a slightly lowered onset of dehydration). Further studies examining the starting materials and membranes after dehydration at 90−95 °C for 24 h (Figure S3b) showed no significant differences in the thermal behavior of the materials (with only Cu-BTTri starting material showing less desolvation shifted to higher temperatures, beginning around 205 °C).

For DSC, PVA control membranes were assessed as well as the synthesized 10 wt % Cu-BTTri/PVA membrane both before and after the membranes were subjected to NO release experiments. Samples were placed under vacuum at 65 °C for 3 h prior to DSC analysis. For the PVA control membrane, two reversible thermal transitions were observed: an endothermic glassy transition (T_g) recovered at 56.5 °C and an endothermic melting transition (T_m) recovered at 201.5 °C with an enthalpy of 42.86 $J{\cdot}g^{-1}$ (Figure S4). Thermal transitions for PVA are well-known to shift in temperature with respect to differences in molecular weight.[42,44] Thermal transitions of the 10 wt % Cu-BTTri/PVA membrane matched strongly with the PVA control: an endothermic T_g recovered at 56.8 °C and an endothermic T_m recovered at 196.1 °C with an enthalpy of 27.55 $J{\cdot}g^{-1}$. The slight depression in the T_m of the MOF-doped membrane is attributed to an eutectic effect as the MOF particles represent solid impurities as compared to the neat PVA membrane. Thus, the additional disorder and microstates within the doped polymer matrix allow melting to occur at slightly lower temperatures. Following NO release experiments, the 10 wt % Cu-BTTri/PVA membrane showed no significant differences in the observed thermal transitions seen for the initial membrane.

Incorporation of Cu-BTTri within the PVA matrix was demonstrated by scanning electron microscopy (SEM) imaging, which revealed the anticipated octahedral morphology of individual MOF crystallites (Figure 3a−f). Because the membranes were cured in poly(tetrafluoroethylene) (PTFE) molds, the sides of the membranes were expected to exhibit a degree of nonequivalence resulting from physical differences between the air and PTFE interfaces and gradual settling of the MOF suspension. As compared in Figures 3c and 3e, the apparent surface distribution of Cu-BTTri crystallites is relatively consistent between the air and PTFE interfaces of 10 wt % Cu-BTTri/ PVA membrane. However, images acquired of 1 and 5 wt % Cu-BTTri/PVA membranes depict more pronounced differences in Cu-BTTri surface distribution as the overall concentration of the MOF decreases (Figure S5). This outcome is most directly attributable to settling of Cu-BTTri crystallites/ agglomerates prior to evaporation of water. To further characterize the materials following incorporation of the copper-based MOF Cu-BTTri, the overall surface distribution of copper

was imaged by energy-dispersive X-ray spectroscopy (EDX). Figures 3d and 3f show concentration of copper in regions of the 10 wt % Cu-BTTri/PVA membrane that contain Cu-BTTri particles for both the air and PTFE interfaces. This analysis was also performed for 1 and 5 wt % Cu-BTTri/PVA membranes, which display a similar concentration of copper at the location of MOF crystallites (Figure S5). Additionally, cross-sectional SEM imaging of the 10 wt % Cu-BTTri/PVA membrane indicated a dry thickness of approximately 56 μm with EDX confirming dispersion of copper throughout the interior of the membrane (Figure S5). Taken together, these results demonstrated successful incorporation of Cu-BTTri with improved uniformity between the air and PTFE interfaces at higher MOF loading. Although MOF particle agglomeration was observed in all images, the typical size of individual crystallites was relatively uniform at approximately 1 μm in diameter. The outcome of blending Cu-BTTri into the PVA solution as an aqueous suspension was evident in the qualitatively improved uniformity of the MOF dispersion compared to prior work.

3. NITRIC OXIDE GENERATION FROM S-NITROSOGLUTATHIONE

Nitric oxide exhibits antithrombotic properties that are crucial in the maintenance of the healthy vascular endothelium and is also produced by the immune system as an antimicrobial agent.[5,45] As a diatomic radical that readily reacts with O_2 and biomolecules such as hemoglobin, NO exhibits a physiological half-life ranging from seconds to milliseconds, permitting localized action in a biological environment.[46] In biomolecules, the *S*-nitrosation of thiol groups (such as those present in cysteine residues) produces natural RSNOs.[47] In principle, the use of copper-based MOFs such as Cu-BTTri permits the continuous generation of NO directly from RSNO substrates in blood, obviating the use of exogenous NO sources that are often limited by finite storage capacity. The possibility of generating NO from endogenous RSNOs is particularly attractive in the case of blood-contacting medical devices, such as extracorporeal circuits, venous catheters, and hemodialysis membranes, where the natural antithrombotic function of NO may inhibit the formation of thrombi that are associated with artificial polymeric surfaces.[5,11] Similarly, the ability to produce NO in this fashion may facilitate the development of wound dressings that accelerate the healing process in the presence of perfusing blood. While it has been determined that RSNOs are present in blood at concentrations as high as the micromolar range, these compounds commonly occur in the form of macromolecules such as *S*-nitrosoalbumin (approximately 67 kDaA) that are unlikely to rapidly interact with blended Cu-BTTri.[48] In contrast, GSNO (336 Da) is widely reported to be the most abundant low molecular weight RSNO available in blood.[47] For this reason, GSNO was utilized in NO release experiments as the most relevant endogenous RSNO. Although there has been controversy concerning the actual physiological level of RSNOs in blood, a concentration of 10 μM GSNO was selected due to the use of similar micromolar-range concentrations in prior work.[13,24,28,49] Measurement of NO release was carried out using Sievers chemiluminescence-based NO analyzers, which rely on the gas-phase reaction of NO with ozone to form excited state nitrogen dioxide ($NO + O_3 \rightarrow NO_2^* + O_2$). The return of excited state NO_2 to the ground state is accompanied by the emission of light, which is transduced and used to continuously measure the production of NO as a function of time.

Chemiluminescence-based analysis is highly selective and permits detection of NO from solutions containing as little as 1 pmol of RSNO.[50] Using this technique, the ability of Cu-BTTri/PVA membranes to promote NO release under varying conditions was examined.

Performance at Physiological pH and Temperature.

To assess the ability of 10 wt % Cu-BTTri/PVA membranes to generate NO from GSNO at physiological pH and temperature, membranes were submerged in pH 7.4 phosphate-buffered saline (PBS) at 37 °C, and this mixture was deoxygenated by sparging with N_2. Prior to use, PBS was treated with Chelex 100 resin to remove trace metal ions and was subsequently filtered and adjusted to the appropriate pH with dilute hydrochloric acid. Following deoxygenation, an aqueous solution of GSNO was injected to produce an initial GSNO concentration of 10 μM. The reaction was then shielded from ambient light and allowed to progress until NO release returned to baseline levels (<1 ppb). During this period, N_2 was continuously bubbled through the solution to remove NO as it formed, and a flow of N_2 was used to transport NO from the headspace of the analysis vessel into the NO analyzer. The NO yield was calculated using the quantity of recovered NO determined by chemiluminescence and the theoretical amount of NO based on the quantity of added GSNO. After an average of 3.5 ± 0.4 h (mean ± SD), 97 ± 6% of theoretical NO was released and quantified by chemiluminescence (Table 1). The complete consumption of the initial quantity of GSNO produces the dramatic decline in NO release observed in the representative NO release profile plotted in Figure 4a. In comparison, control experiments using cross-linked PVA membrane (without Cu-BTTri) resulted in an NO yield of 2.5 ± 0.1% over the same duration, a result that was representative of the baseline decomposition rate of GSNO under prevailing experimental conditions. This finding was confirmed by evaluating the decomposition of GSNO alone (without added membrane), which resulted in an NO recovery of 2.7 ± 0.3%. These results indicate that the presence of 10 wt % Cu-BTTri/PVA membrane induces a 36-fold increase in the rate of NO generation from GSNO. Furthermore, the absence of any statistically significant elevation in NO release in the presence of PVA controls indicates that the performance of the 10 wt % Cu-BTTri/PVA membranes is wholly attributable to inclusion of the MOF. The essentially quantitative recovery of NO and the previous observation that oxidized glutathione is formed during the reaction indicate that the products likely correspond to those produced by thermal, light-induced, or copper ion-catalyzed decomposition ($2RSNO \rightarrow 2NO + RSSR$).[13]

The pXRD patterns of the 10 wt % Cu-BTTri/PVA membrane and Cu-BTTri powder after NO release experiments showed retention of the characteristic peaks associated with Cu-BTTri, providing qualitative support for the lack of MOF degradation (Figure S6 and S7). Following each experiment, inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to determine the amount of trace copper in the buffer solution, which was limited to 0.34 ± 0.08% of the theoretical quantity present in 10 wt % Cu-BTTri membrane. Because copper may produce toxicological concerns at sufficient levels, this lack of appreciable copper leaching supports, at minimum, the use of Cu-BTTri/PVA membranes in short-term blood-contacting applications. To evaluate the potential for copper leaching under harsher conditions, samples of 10 wt % Cu-BTTri/PVA were immersed in pH 7.4 PBS at

37 °C for 24 h at a 1000-fold higher, nonphysiological GSNO concentration of 10 mM. At the end of this exposure period, ICP-AES was used to determine a quantity of copper in the buffer solution equivalent to 2.5 ± 0.2% of the theoretical amount present in the membrane. In contrast, no leaching was observed in PBS alone under otherwise identical conditions. This result indicates that the MOF exhibits a remarkable degree of stability even at a GSNO concentration that vastly exceeds potential physiological levels. Furthermore, this resistance to leaching compares favorably to previously reported polyurethane-bound copper(II) −cyclen complexes that were similarly used to promote NO release from GSNO.[49] In that example, 25% of available copper was removed after 24 h of immersion in PBS at a GSNO concentration of only 10 μM.[49]

The MOF Cu-BTTri has previously been blended within chitosan membranes at 10 wt % loading, where a similar ability to rapidly induce the decomposition of GSNO was observed.[13] However, control membranes prepared from chitosan alone independently produced a sevenfold increase in NO production relative to the baseline rate of GSNO decomposition. Because the primary amine groups of chitosan facilitate chelation, this phenomenon was attributed to the presence of trace metal ions bound to the polymer. The absence of this behavior in PVA suggests that the polymer matrix functions as an encapsulating support for Cu-BTTri without exerting a chemical influence on the reaction itself. The maximum NO flux observed from 10 wt % Cu-BTTri/PVA was 0.20 ± 0.02 nmol·cm^{-2}·min^{-1}, falling within the 0.05−0.41 nmol·cm^{-2}·min^{-1} range often associated with natural endothelial NO production (Figure S8).[45,51,52] This range has frequently been used as a benchmark to indicate antithrombotic therapeutic potential.[51] As an additional point of comparison, a quantity of Cu-BTTri powder equivalent to the mass contained in 10 wt % Cu-BTTri/PVA membranes was exposed to 10 μM GSNO under identical conditions (Figure 4). The use of this suspension of Cu-BTTri powder did not result in statistically significant differences in total NO yield (90 ± 1%) or completion time (3.3 ± 0.2 h) relative to 10 wt % Cu-BTTri/PVA membrane (Figure S9). However, comparison of the NO release profiles of 10 wt % Cu-BTTri/PVA and an equivalent mass of Cu-BTTri powder shows significantly slower initial release kinetics in the case where the MOF is incorporated within PVA. As a consequence, 50% of available NO is recovered in only 1.3 ± 0.1 h in the case of Cu-BTTri powder, while the required time for 50% recovery is 2.0 ± 0.3 h for 10 wt % Cu-BTTri/PVA. This difference in kinetics likely arises from diffusion of GSNO into the membrane prior to interaction with Cu-BTTri. Following these initial experiments, the 10 wt % Cu-BTTri/PVA membranes were immersed in Millipore water for 3 days and reused in a second set of identical NO release experiments. The reused membranes resulted in an NO yield of 103 ± 8%, with a 34% increase in the duration of the reaction to 4.7 ± 0.4 h (Figure S10).

Notably, the ability of 10 wt % Cu-BTTri/PVA membrane to promote the decomposition of GSNO in pH 7.4 PBS at 37 °C was maintained when the buffer solution was sparged with O_2 in place of N_2. This result was determined by conducting the reaction for 3.5 h (the average completion time under typical measurement conditions) with constant O_2 sparging. Any remaining GSNO was subsequently decomposed by injection of an aqueous solution of copper(II) chloride, and the resulting NO release was quantified under standard analytical conditions. This test resulted in the recovery of only 53 ± 12% of theoretical NO and provides the first experimental confirmation that the reaction proceeds under aerobic

conditions, albeit at a slower rate. A similar outcome for the *thermal* decomposition of RSNOs was observed by Grossi et al., where bubbling with O_2 was found to noticeably decrease the rate of decomposition.[53] Since the combination of NO and O_2 is known to form products such as nitrous acid (HNO_2) under aqueous conditions, it may be the case that the change in chemical environment is solely responsible for the increased reaction time.[54] Alternatively, O_2 may oxidize a putative copper(I) active site to an inactive copper(II) form, a process that has previously been observed in multiple copper-based MOFs.[55] The potential involvement of copper(I) sites has been invoked in computational studies that specifically investigated the interaction of RSNOs with copper-based MOFs, where reduction of framework copper(II) to copper(I) was included as a crucial step in the mechanistic process.[56,57]

However, it remains unclear if this type of mechanism is operative in the case of Cu-BTTri and whether framework copper actively participates in the observed NO-forming activity of the MOF.

Effect of Varying Cu-BTTri Concentration.

The influence of Cu-BTTri concentration on the performance of the Cu-BTTri/PVA membrane was determined by evaluating the ability of 1 and 5 wt % Cu-BTTri/PVA membranes to induce NO release from GSNO in pH 7.4 PBS at 37 °C. At 5 wt % Cu-BTTri, the total NO recovery of 97 ± 6% was obtained after 5.0 ± 0.7 h, representing a statistically significant 43% increase in reaction time compared to the 10 wt % Cu-BTTri/PVA membranes (Figure 5). At 1 wt % Cu-BTTri, total NO recovery (102 ± 3%) required 13 ± 1 h, corresponding to a 270% increase in completion time (Figure 5). The maximum NO fluxes observed for 5 and 1 wt % Cu-BTTri membranes were 0.16 ± 0.06 and 0.051 ± 0.008 $nmol{\cdot}cm^{-2}{\cdot}min^{-1}$, respectively (Figure S8). These results indicate that lowering the concentration of Cu-BTTri from 10 to 1 wt % predictably results in a significant lengthening of the reaction time but does not affect overall NO recovery. The observed reduction in maximum NO flux and extension in overall reaction time that accompanies the decrease in Cu-BTTri concentration may permit application-specific tuning of Cu-BTTri/polymer blends based on the amount of incorporated MOF. This behavior is directly attributable to the reduced availability of active species at lower MOF concentration as a similar relationship was observed by Major et al. in the case of copper nanoparticles blended into a hydrophilic polyurethane at various concentrations.[25]

Effect of Polymer Water Permeability.

The water permeability and uptake of Cu-BTTri/polymer blends influence the rate at which dissolved RSNOs interact with the MOF. To investigate the water uptake properties of 10 wt % Cu-BTTri/PVA membrane, dry samples were weighed and subsequently immersed in water for 24 h.

The 10 wt % Cu-BTTri/PVA membrane exhibited a swelling ratio of 203 ± 3% (Table S1). For comparison, additional 10 wt % Cu-BTTri films were prepared using Tecoflex SG-80A, a comparatively hydrophobic polyurethane often used for medical device fabrication. The swelling ratio of 10 wt % Cu-BTTri/Tecoflex SG-80A films was determined gravimetrically to be 2.0 ± 0.3%, indicating an approximate 100-fold difference in water uptake relative to

hydrophilic PVA-based membranes. The NO release profiles for GSNO in the presence of 10 wt % Cu-BTTri/Tecoflex SG-80A films were subsequently acquired in pH 7.4 PBS at 37 °C over a duration corresponding to the completion time of experiments performed using 10 wt % Cu-BTTri/PVA membranes. Over this period, only 1.5 ± 0.4% of theoretical NO was recovered, compared to 97 ± 6% for the 10% Cu-BTTri/PVA membranes (Figure 6). These results support prior observations that hydrophobic polymers with decreased water permeability/uptake inhibit the NO-forming interaction of RSNOs with Cu-BTTri. In general, it is clear that only hydrophilic polymers such as PVA permit sufficiently rapid transport of GSNO to exploit the NO-forming capability of the MOF. The broader implication of this outcome is that medical devices fabricated from or coated with Cu-BTTri/polymer formulations are likely obligated to use hydrophilic polymers that facilitate diffusion of water and endogenous RSNOs into the polymer matrix. This observation is in agreement with a literature example using copper nanoparticles blended into polyurethane to produce NO from RSNOs.[25] In this case, contact between RSNOs and the active copper species was dependent upon the hydrophilicity of the polymer since the reaction was hypothesized to occur within the polymer itself and must therefore be preceded by RSNO transport into the material.

Effect of Lower Temperature.

Additionally, the impact of lower temperature on the ability of 10 wt % Cu-BTTri/PVA membranes to promote NO release from GSNO was evaluated in pH 7.4 PBS. This assessment is of particular importance for topical blood-contacting applications (e.g., wound dressings) since skin temperature can be anticipated to range from near ambient temperature to a normal physiological value of approximately 37 °C.[58] For 10 wt % Cu-BTTri/PVA membranes, lowering the temperature of the reaction from 37 to 20 °C resulted in an 84% decrease in NO recovery over 3.5 h (the duration of the reaction at 37 °C) from 97 ± 6% (essentially quantitative) to 16 ± 1% (Figure 7a, b, Figure S11). When a suspension of Cu-BTTri powder was used, a similar, statistically significant decrease of 77% was observed (90 ± 1 to 21 ± 1% NO recovery) (Figure 7c, d).

These results indicate that the reaction of GSNO with Cu-BTTri is accelerated at physiological temperature. To further elucidate the role of temperature on the interaction of GSNO with Cu-BTTri, a 10 wt % Cu-BTTri/PVA membrane was used to promote NO release from GSNO at 37 °C and then subsequently reused at 20 °C in an otherwise identical experiment. The rate of NO release remained largely consistent with previous experiments conducted at 20 °C, suggesting that elevation to 37 °C in the presence of GSNO does not activate the MOF in an irreversible manner.

Effect of Varying pH Conditions.

It has been previously observed that the stability of the *S*-nitrosothiol functional group is influenced by pH.[59] However, the influence of pH on the homogeneous copper-catalyzed decomposition of RSNOs or the ability of copper-based MOFs such as Cu-BTTri to promote NO release from RSNOs has not been clearly established. For this reason, the ability of 10 wt % Cu-BTTri/ PVA membranes to decompose GSNO was evaluated in pH 6.0 and 8.0 PBS at 37 °C, supplementing existing data collected at pH 7.4. To avoid the possibility that

alternative buffer systems might independently influence the behavior of the reaction, the pH 7.4 PBS used for other experiments was adjusted to the appropriate pH. At pH values of 6.0 and 8.0, the respective NO yields were 96 ± 1% and 103 ± 4% (Figure 8). Compared to the mean reaction time of 3.5 ± 0.4 h for 10 wt % Cu-BTTri/ PVA at pH 7.4, depression to pH 6.0 resulted in a statistically significant 22% decrease to 2.7 ± 0.2 h (Figure 8). Elevation of the pH to 8.0 increased the required reaction time by 17% to 4.1 ± 0.2 h (Figure 8a, b). To explore the influence of pH specifically on Cu-BTTri, GSNO was exposed to an aqueous suspension of MOF powder in pH 6.0 and 8.0 PBS at 37 °C (Figure 8c, d). At pH 6.0, the total NO recovery was 92 ± 1% after 2.1 ± 0.1 h, corresponding to a statistically significant 36% decrease in reaction time (Figure 8c, d). At pH 8.0, a total of 91 3% of theoretical NO was recovered after 3.2 ± 0.3 h at pH 8.0 (Figure 8c, d). Lower pH appears to consistently promote acceleration of the NO-forming reaction with Cu-BTTri, regardless of whether Cu-BTTri is evaluated as a suspension or incorporated within a PVA matrix. In contrast, slight elevation in pH from 7.4 to 8.0 increases the duration of the reaction with 10 wt % Cu-BTTri/PVA membrane but has no significant impact on the performance of the suspended MOF powder (Figure 8c, d, Figure S12). In the case of 10 wt % Cu-BTTri/PVA membranes, the 17% increase in reaction time at pH 8.0 may be attributable to impaired GSNO diffusion through the PVA matrix. This hypothesis is supported by the observation that no difference in the overall reaction time between pH 7.4 and 8.0 is observed for suspended Cu-BTTri powder. At elevated pH, it may be predicted from the pK_a values of glutathione that an increasing fraction of acidic groups may exist in the negatively charged carboxylate form. This phenomenon may reduce the propensity of GSNO to diffuse through the PVA matrix. At pH 6.0, both the 10 wt % Cu-BTTri/PVA membranes and the suspension of Cu-BTTri powder exhibit accelerated reactions with GSNO (Figure S13). The magnitude of this acceleration is slightly greater in the case of the Cu-BTTri powder suspension, which may indicate a diffusion-related delay as shown in Figure 8.

As a comparative study, aqueous copper(II) chloride was used to decompose 10 μM GSNO in PBS at 37 °C. As anticipated from the use of a homogeneous catalyst, the reaction between solution-phase copper ions and GSNO was significantly faster (<30 min completion time) than the rates observed for Cu-BTTri. In addition, the behavior of the reaction as the pH was reduced from 7.4 to 6.0 was reversed. Unlike the acceleration observed in the case of Cu-BTTri (whether in the form of a suspended powder or blended PVA membrane), the time required for copper(II) chloride to decompose GSNO in PBS at 37 °C was significantly *lengthened* by 74% as the pH was decreased from 7.4 to 6.0. No statistically significant difference manifested at a slight pH elevation to 8.0 in accordance with the performance of suspended Cu-BTTri. These observations with copper(II) chloride and GSNO may support the prevailing understanding of both RSNO stability and the mechanism for homogeneous copper-catalyzed decomposition of RSNOs. The rapid and complete NO-forming decomposition of RSNOs is typically effected by the addition of catalytic quantities of copper(II) salts.[31,60,61] Although copper(II) itself was initially implicated as the active species in this reaction, McAninly et al. proposed a mechanism based on the reduction of copper(II) to copper(I) by thiolate anions.[61] The crucial role of copper(I) in the catalytic decomposition of RSNOs was later supported by the ability of neocuproine (a copper(I)-specific chelator) to arrest the reaction.[62]

The rate-determining, thiol-mediated reduction of copper(II) may result from trace thiol remaining in RSNO samples or the regeneration of thiol from RSNOs in aqueous media.[63] It may therefore be reasoned, a priori, that a decrease in pH (and corresponding decrease in the ratio of thiolate to thiol) could potentially impair the rate at which the active copper(I) species is generated, increasing the completion time of the NO-forming reaction. This hypothesis aligns with the NO-generating performance of copper(II) chloride as a function of pH. For example, it has been theorized that the decomposition of dissolved RSNOs is arrested under acidic conditions due to reduced thiolate concentration, which inhibits reduction of copper(II) to copper(I).[64] In contrast, the significant *acceleration* of the reaction between Cu-BTTri and GSNO as pH is decreased may imply that an alternative process is operative, which is sufficiently distinct in mechanism to produce an inversion of pH sensitivity. While the distinction in behavior between Cu-BTTri and solution-phase copper ions may arise from the comparative role of thiol in each reaction, it must be noted that the ability of copper ions to form sparingly soluble copper phosphate species may influence the kinetics of the latter reaction in PBS. Following the reactions with 10 wt % Cu-BTTri/PVA membrane, the copper concentrations of the pH 6.0 and pH 7.4 buffer solutions were found to correspond to 0.31 ± 0.03 and 0.34 ± 0.08% of theoretical copper in the membranes, respectively. In the case of suspended Cu-BTTri powder, these values were 0.32 ± 0.01 (pH 6.0) and 0.20 ± 0.03% (pH 7.4). The absence of a statistically significant difference between the apparent level of copper leaching as a function of the pH decline from 7.4 to 6.0 indicates that pH-related decomposition of the MOF (and potential copper ion leaching) is not a clear factor in the acceleration of the reaction.

Effect of Thiol Blocking with N-Ethylmalemide.

The ability of thiol to affect the structural characteristics of copper-based MOFs has been previously described. In the specific case of the carboxylate-derived MOF Cu-BTC, exposure to high concentrations of thiophenol or 1,3-propanedithiol in acetonitrile at 70 °C results in the decomposition of the MOF to copper(0) nanoparticles.[65] Ke et al. showed that Cu-BTC could be postsynthetically modified by exposure to dithioglycol (1,2-ethanedithiol), which resulted in grafting of the thiol to coordinatively unsaturated copper centers.[66] As previously discussed, it has also been suggested that the NO-forming behavior of Cu-BTC proceeds through the reduction of copper centers, a process that may be induced by thiol.[56,57] To explore the potential role of thiol/thiolate in the Cu-BTTri-promoted decomposition of GSNO, NO release experiments were performed using GSNO that had been incubated with a 10-fold excess of *N*-ethylmaleimide (NEM). This maleimide-based alkylating reagent reacts with thiol groups to form an effectively irreversible thioether linkage, and it is commonly used to modify cysteine residues in proteins.[67] In the case of 10 wt % Cu-BTTri/PVA membranes, an 84% decrease in NO yield (over 3.5 h) was observed using GSNO that had been treated with NEM (from 97 ± 6% to 16 ± 1%) (Figure 9a, b, Figure S14). A smaller 39% decrease (90 ± 1% to 55 ± 2%) was observed when a suspension of Cu-BTTri powder was evaluated under identical conditions (Figure 9c, d, Figure S14). While it was previously hypothesized that the function of Cu-BTTri may have little dependency on reduction by *thiolate* (as supported by acceleration of the reaction under acidic conditions), the reduction in NO production observed after GSNO was treated with NEM may indicate that residual thiol does have an influence on the activity of the MOF.

However, the fact that this effect is most pronounced when diffusion into the PVA matrix must occur prior to the reaction may support alternative hypotheses. The presence of a 10-fold excess of NEM may simply inhibit the ability of GSNO to reach active sites within the MOF, whether through modification of the primary amine to form a bulkier derivative or through competitive interactions with the MOF itself.[68]

The direct exposure of Cu-BTTri powder to neat ethanethiol results in regeneration of the triazole ligand and loss of the diffraction pattern associated with the MOF. Exposure to 3 mM glutathione (the thiol precursor to GSNO) in pH 7.4 PBS for 24 h at ambient temperature resulted in the release of 16 ± 1% of theoretical copper into solution. These findings suggest that *high* concentrations of thiol are capable of degrading Cu-BTTri. However, previous observations that physiological (10 μM) and elevated (10 mM) concentrations of RSNO do not produce substantial copper leaching or loss of MOF structure suggest that this type of degradation is unlikely to contribute to the NO-forming activity of Cu-BTTri. As further support for this hypothesis, the addition of NEM does not significantly reduce the trace amount of copper recovered subsequent to the reaction, with 0.28 ± 0.03% of theoretical copper determined in the buffer solution by ICP-AES. This outcome suggests that, at physiological RSNO concentrations, the role of thiol/thiolate (if any) is unlikely to include the formation of labile copper species that are subsequently released into solution.

4. SUMMARY AND CONCLUSIONS

Membranes were prepared from hydrophilic PVA and the triazolate-bridged MOF Cu-BTTri and subsequently evaluated for their ability to induce NO release from GSNO, a biomolecule identified in blood. In pH 7.4 PBS at 37 °C, it was demonstrated that 1, 5, and 10 wt % Cu-BTTri/PVA membranes were able to effectively induce NO production from GSNO at levels that may be associated with antithrombotic effects. In the case of 10 wt % Cu-BTTri/ PVA membrane, a 36-fold increase in the rate of NO production was observed relative to the thermal decomposition of GSNO over an identical duration. While incorporation of 10 wt % Cu-BTTri within PVA results in delayed NO production kinetics relative to a suspension of MOF particles, the overall completion time of the reaction was statistically unchanged. The maximum NO flux ranged from 0.051 ± 0.008 (1 wt % Cu-BTTri) to 0.20 ± 0.02 nmol·cm^{-2}·min^{-1} (10 wt % Cu-BTTri) compared to an estimated endothelial flux of 0.05−0.4 nmol·cm^{-2}·min^{-1}. This performance suggests that Cu-BTTri/PVA membranes may be suitable for the production of a physiologically relevant NO flux from endogenous sources. The level of NO production and the time required to consume available GSNO are directly related to the weight percentage of Cu-BTTri blended into the membrane, which indicates the potential for controllable NO generation. This NO-generating activity is apparently unaccompanied by detectable degradation of the MOF itself (pXRD), and only trace quantities of copper (<1% of theoretical) are present in the buffer following the reaction. The ability of Cu-BTTri/PVA materials to initiate NO release from GSNO relies upon the substantial water uptake of the PVA membrane and hypothesized transport of GSNO to the active MOF species. Without this level of water uptake, as in the case of 10 wt % Cu-BTTri films prepared from hydrophobic Tecoflex SG-80A, no short-term increase in NO production is observed. The NO-forming reaction between GSNO and

Cu-BTTri exhibits temperature dependence and dramatically decreases in rate at 20 °C, with an NO yield of only 16 ± 1% over 3.5 h, compared to 97 ± 6% at 37 °C. This finding leads to the conclusion that therapeutically useful activity is most probable at or near physiological temperature, as in the case of flowing blood. This result may also suggest that a significant thermodynamic barrier exists for the rate-determining step of the reaction, in contrast to the rapid and complete reaction of GSNO with homogeneous copper ion catalysts at ambient temperature. The reaction is also pH-dependent, with a substantial increase in the rate of NO production observed as pH declines from 7.4 to 6.0, contrasting with the opposite behavior in aqueous copper(II) chloride. This effect is present regardless of whether the MOF is incorporated within a PVA matrix and is not accompanied by an increase in the quantity of trace copper in the buffer solution. The lack of soluble copper ion formation indicates that pH-related decomposition of either the MOF itself or secondary active species is not a clear factor in the acceleration of the reaction under mildly acidic conditions. When a 10-fold excess of the thiol-blocking reagent NEM is present during the reaction (pH 7.4 PBS, 37 °C), NO generation is reduced by 84% in the case of 10 wt % Cu-BTTri/PVA membrane. A significantly less dramatic 39% decrease in the rate of the reaction is observed for a suspension of Cu-BTTri powder when exposed to NEM-treated GSNO. These findings reveal that trace thiol may influence the reaction, potentially through the reduction of copper sites as described elsewhere for Cu-BTC. The observed properties of Cu-BTTri/PVA blends indicate that the materials are capable of generating NO from the endogenous compound GSNO, and that therapeutically useful levels can be produced at physiological temperature and pH. These findings support the notion that Cu-BTTri/PVA has the potential to produce NO directly from blood, a phenomenon that would be advantageous given the large number of blood-contacting applications for PVA. Taken together, these studies have identified the characteristics (e.g., hydrophilicity) that Cu-BTTri/polymer blends must exhibit for optimized therapeutic use and also provide a greater understanding of factors that influence Cu-BTTri reactivity in an effort to further investigate the role of the MOF in NO generation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors thank Jim Self-and Debbie Weddle in the CSU Soil, Water and Plant Testing Lab for ICP-AES analysis.

Funding

M.M.R acknowledges the National Science Foundation Division of Biomaterials (DMR-1352201), the National Institutes of Health (1R21EB016838−021), and Colorado State University for funding.

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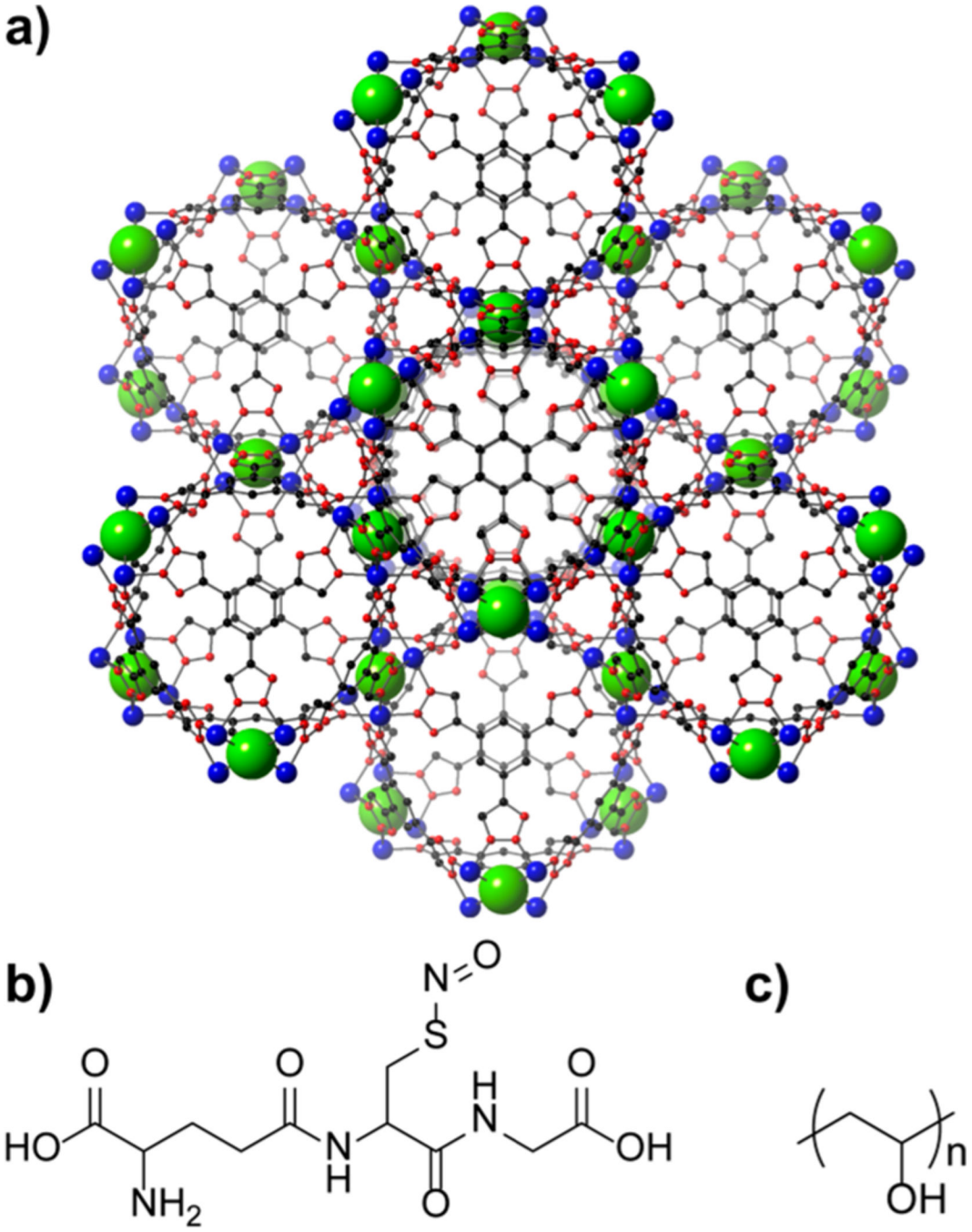

Figure 1.

(a) Structure of Cu-BTTri. Carbon (black), nitrogen (red), chlorine (green), and copper (blue) atoms are depicted with hydrogen atoms omitted for clarity. (b) Structure of S-nitrosoglutathione (GSNO). (c) Repeating unit of poly(vinyl alcohol) (PVA).

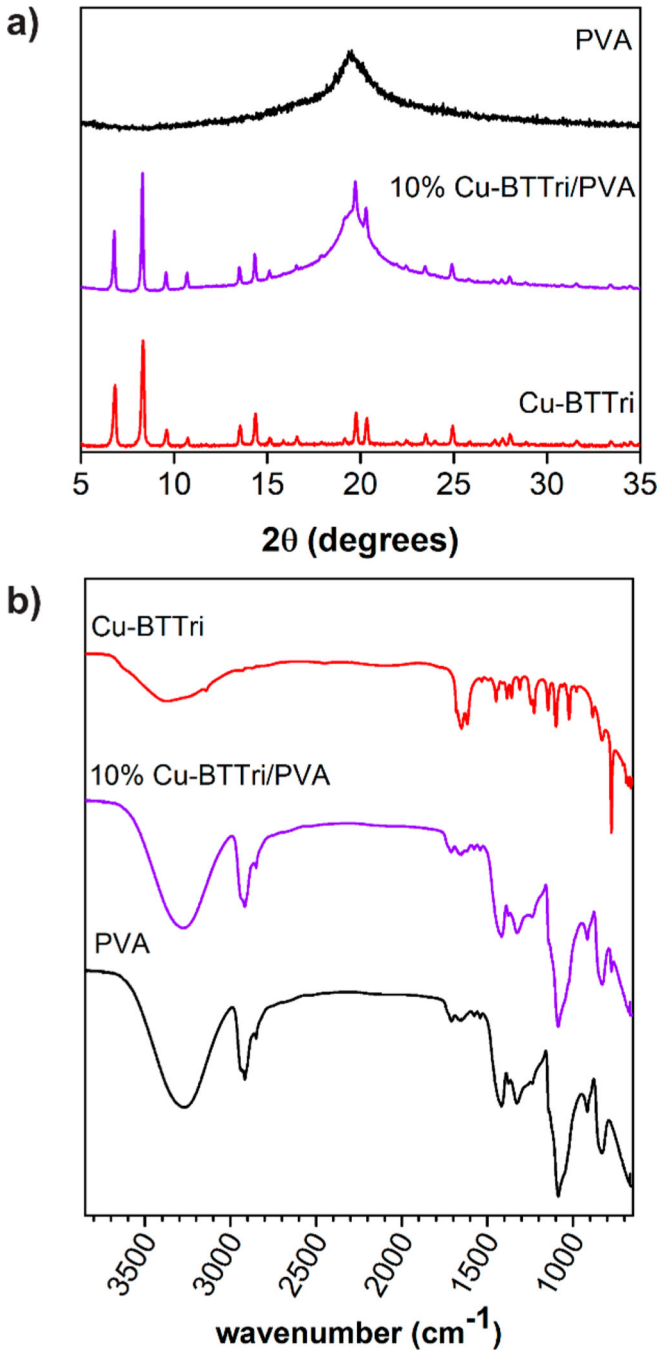

Figure 2.

(a) Powder X-ray diffraction patterns of Cu-BTTri, cross-linked PVA control membrane, and 10 wt % Cu-BTTri/PVA membrane. The diffraction pattern of Cu-BTTri/PVA membrane displays peaks attributable to both PVA and Cu-BTTri, demonstrating retention of the MOF structure following incorporation within the polymer. (b) ATR-FTIR spectra of Cu-BTTri, cross-linked PVA control membrane, and 10 wt % Cu-BTTri/PVA membrane. Infrared absorption bands associated with Cu-BTTri are present at 1616 (C C stretching) and 775 cm $^{-1}$ (C−H out-of-plane bending).

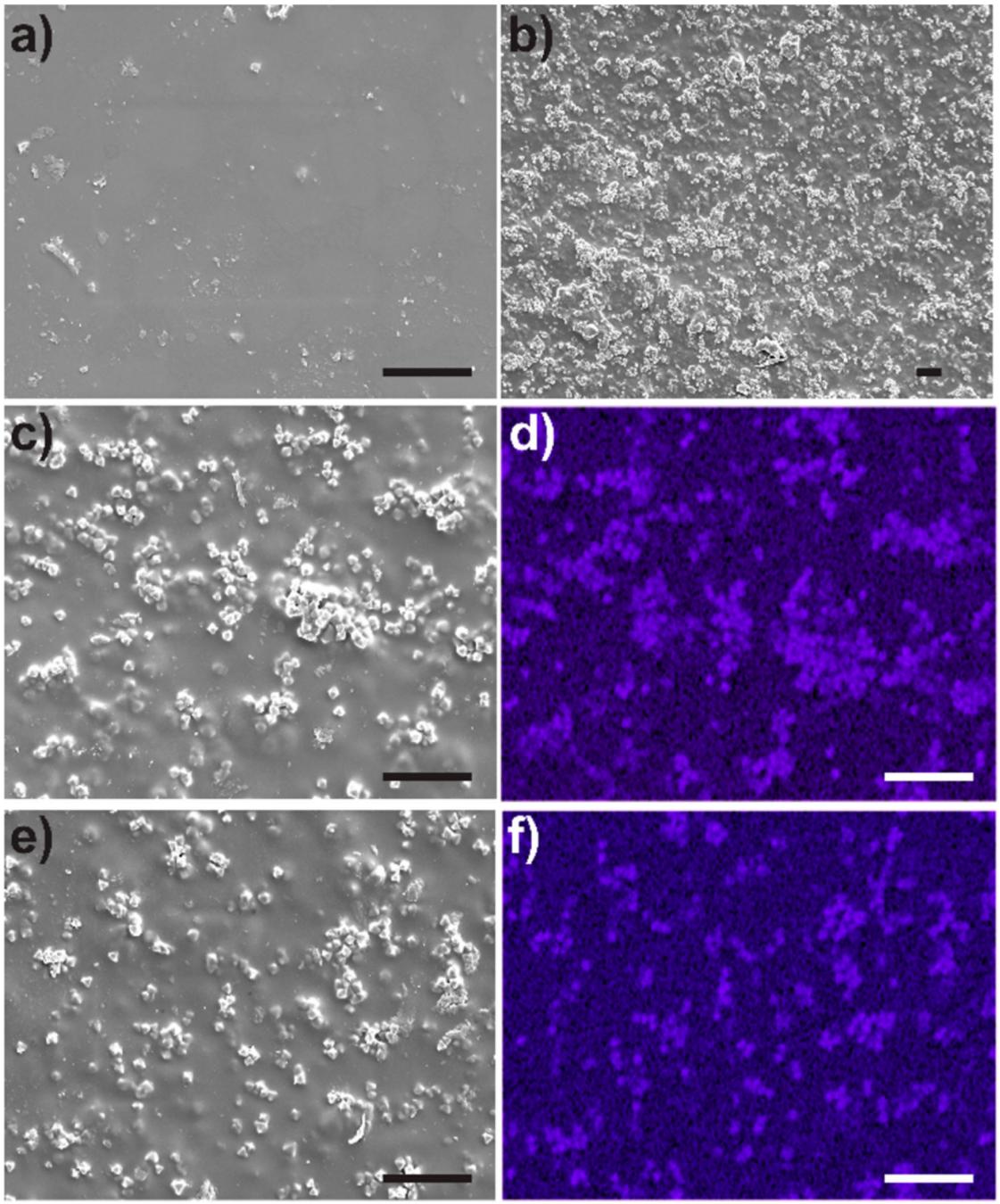

Figure 3.

Scanning electron microscopy images of (a) PVA control at 2000× magnification and (b) 10 wt % Cu-BTTri membrane (air side) at 500× magnification. (c) The 10 wt % Cu-BTTri membrane (air side) at 2000× magnification with (d) EDX copper overlay. (e) The 10 wt % Cu-BTTri membrane (PTFE side) at 2000× magnification with (f) EDX copper overlay. Scale bars (lower right corner) correspond to 10 μm.

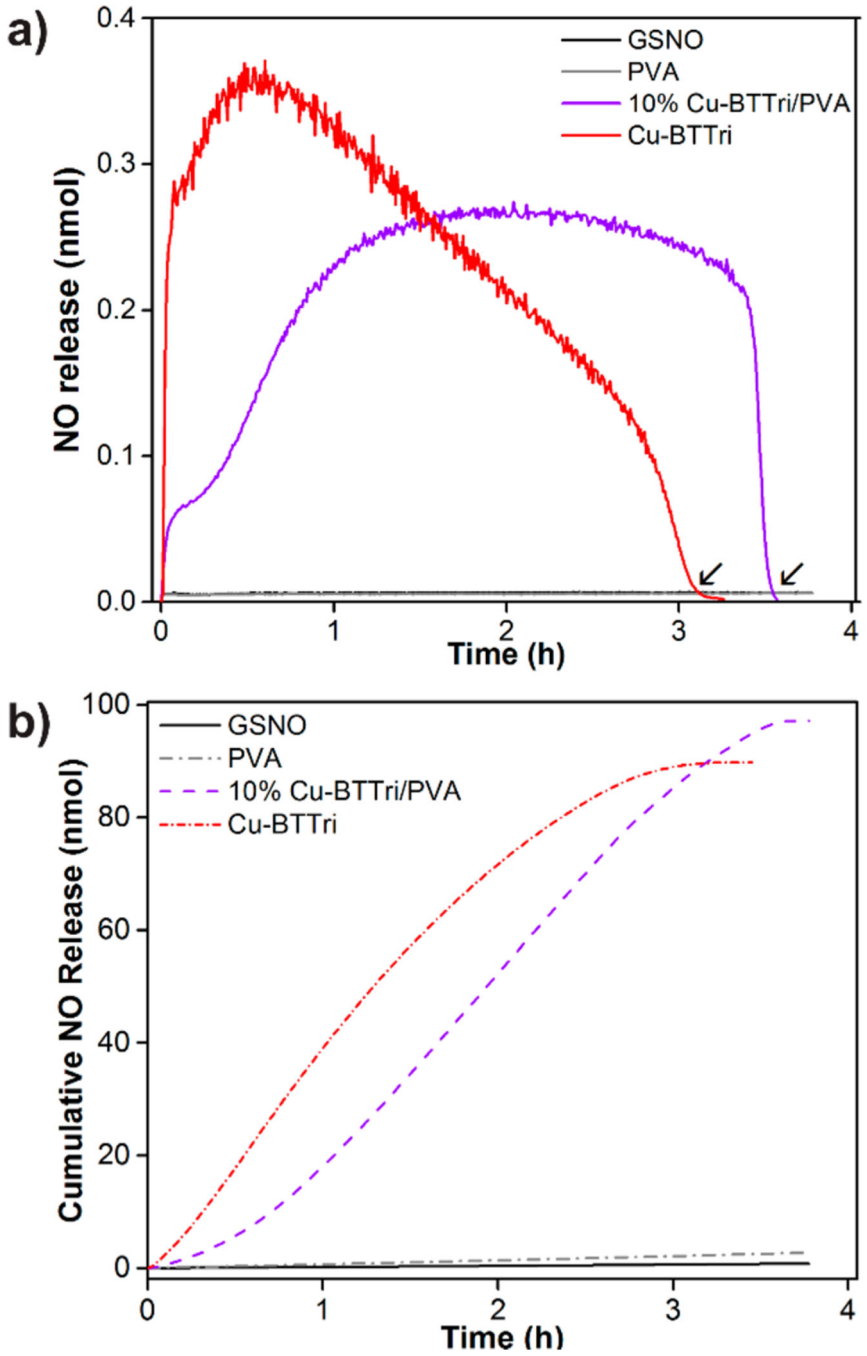

Figure 4.

(a) Representative NO release profiles depicting enhanced NO generation in the presence of Cu-BTTri and 10 wt % Cu-BTTri/ PVA membrane, compared with PVA control membrane and the thermal decomposition of GSNO. Consumption of available GSNO is accompanied by a rapid decrease in detected NO release, as shown by arrows. (b) Cumulative NO release plots for thermal GSNO decomposition, GSNO in the presence of PVA control membranes, and GSNO in the presence of 10% Cu-BTTri/PVA membranes. Experimental conditions: pH 7.4 PBS at 37 °C, with a 10 μM initial GSNO concentration (n ≥ 3).

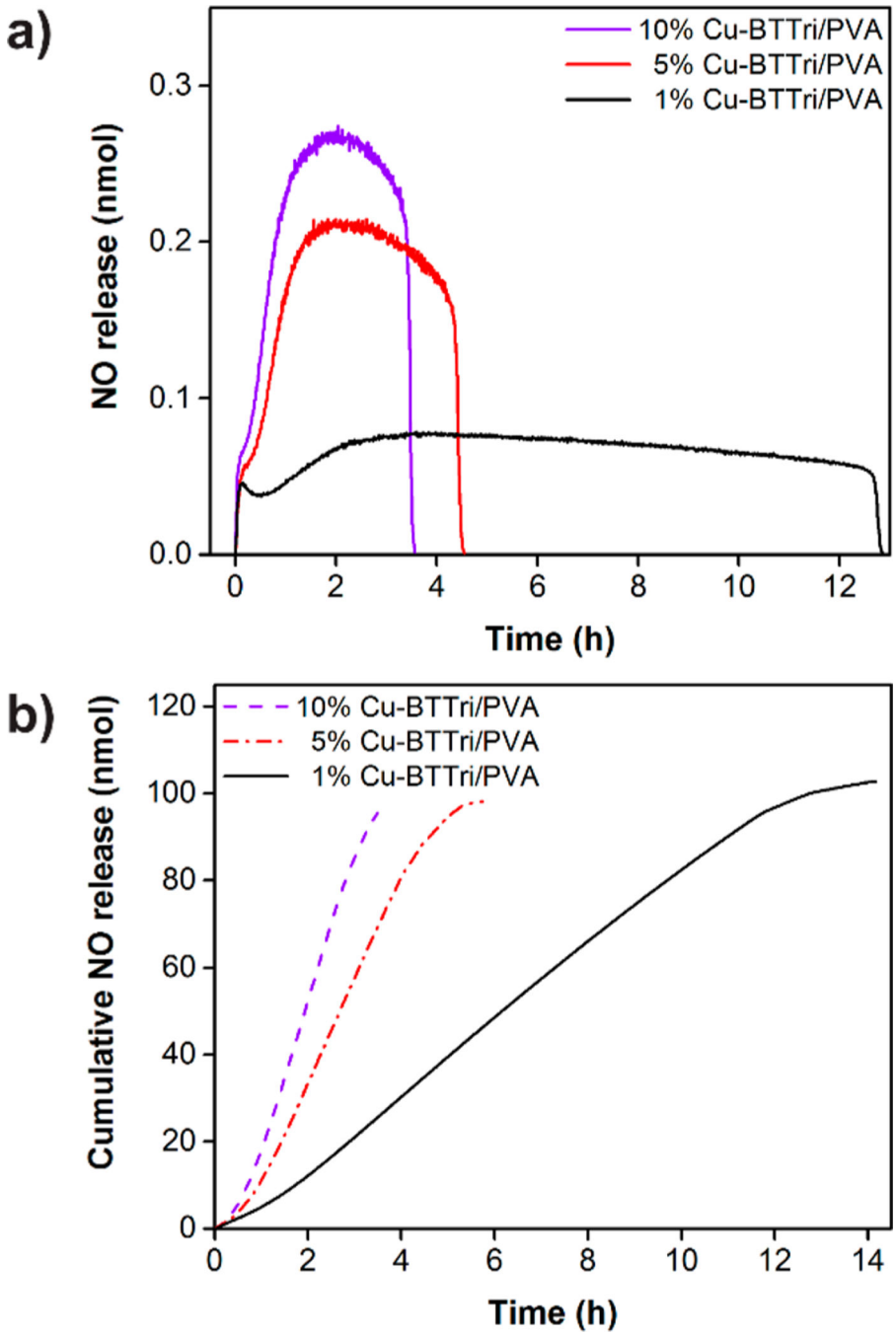

Figure 5.

(a) Representative NO release profiles depicting enhanced NO generation in the presence of the Cu-BTTri/PVA membranes at concentrations of 1, 5, and 10 wt % Cu-BTTri. (b) Cumulative NO release plots for 1, 5, and 10 wt % Cu-BTTri/PVA membranes. Experimental conditions: pH 7.4 PBS at 37 °C, with a 10 μM initial GSNO concentration ($n \geq 3$).

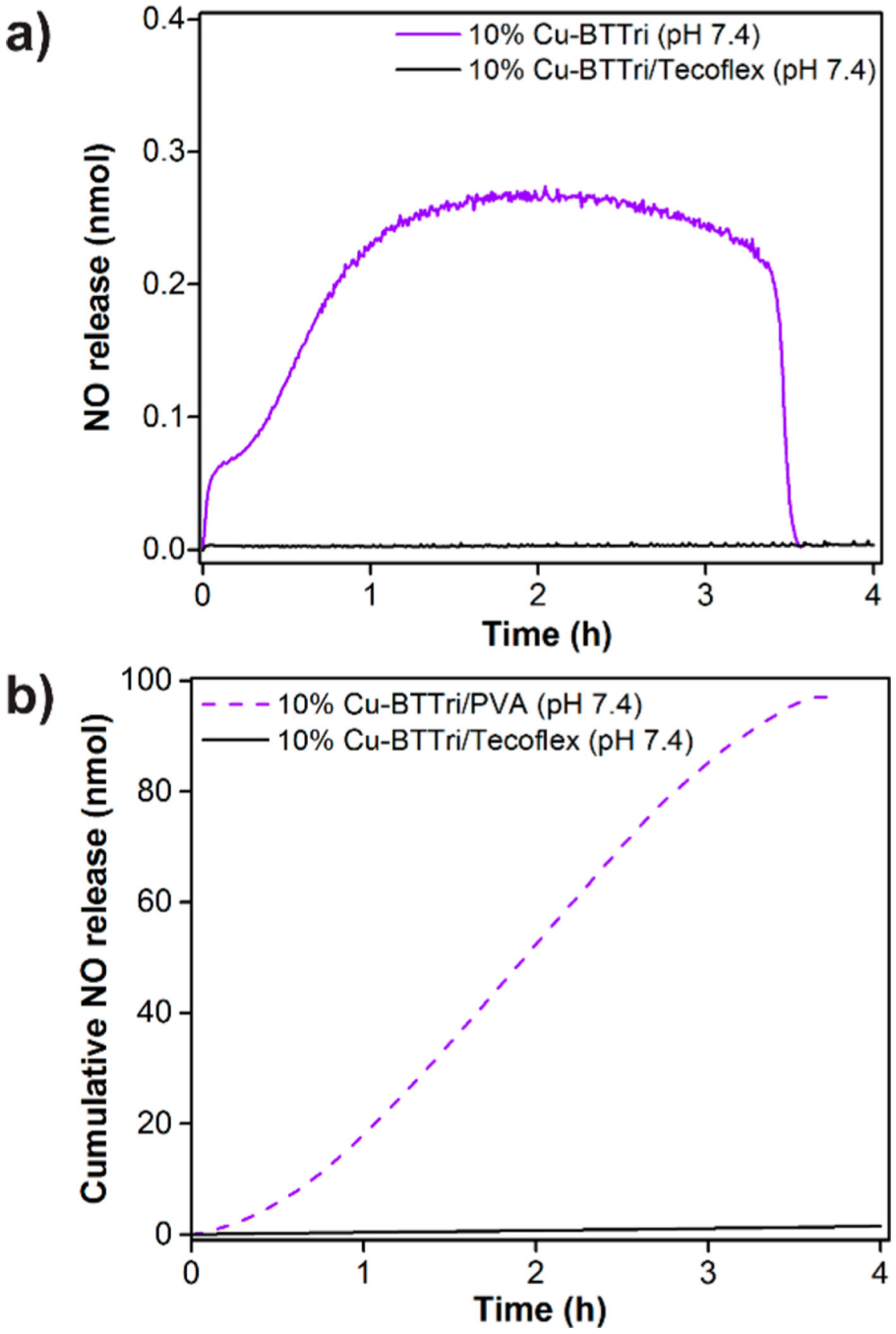

Figure 6.

(a) Representative NO release profiles depicting enhanced NO generation in the presence of 10 wt % Cu-BTTri/PVA and the absence of this effect in the case of 10 wt % Cu-BTTri/Tecoflex SG-80A. This outcome is primarily attributable to differences in water uptake between hydrophilic PVA and hydrophobic Tecoflex SG-80A. Cumulative NO release plots for 10 wt % Cu-BTTri/PVA membrane and 10 wt % Cu-BTTri/Tecoflex SG-80A films. Experimental conditions: pH 7.4 PBS at 37 °C, with a 10 μM initial GSNO concentration ($n \geq 3$).

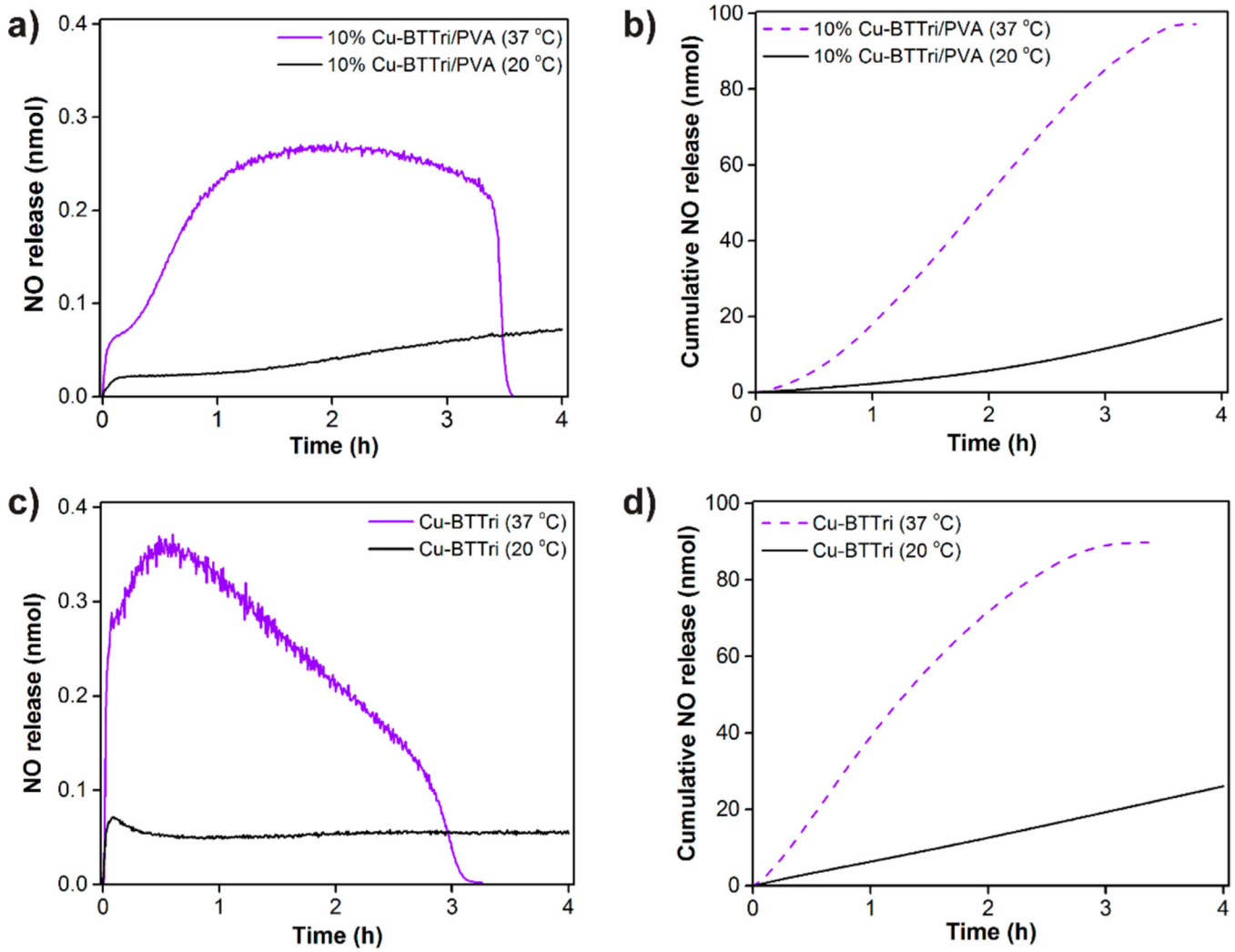

Figure 7.

(a) Representative NO release profiles and (b) cumulative NO release of 10 wt % Cu-BTTri/PVA membranes at 37 and 20 °C. (c) Representative NO release profiles and (d) cumulative NO release plots for suspended Cu-BTTri powder at 37 and 20 °C. Experimental conditions: pH 7.4 PBS, with a 10 μM initial GSNO concentration (n ≥ 3).

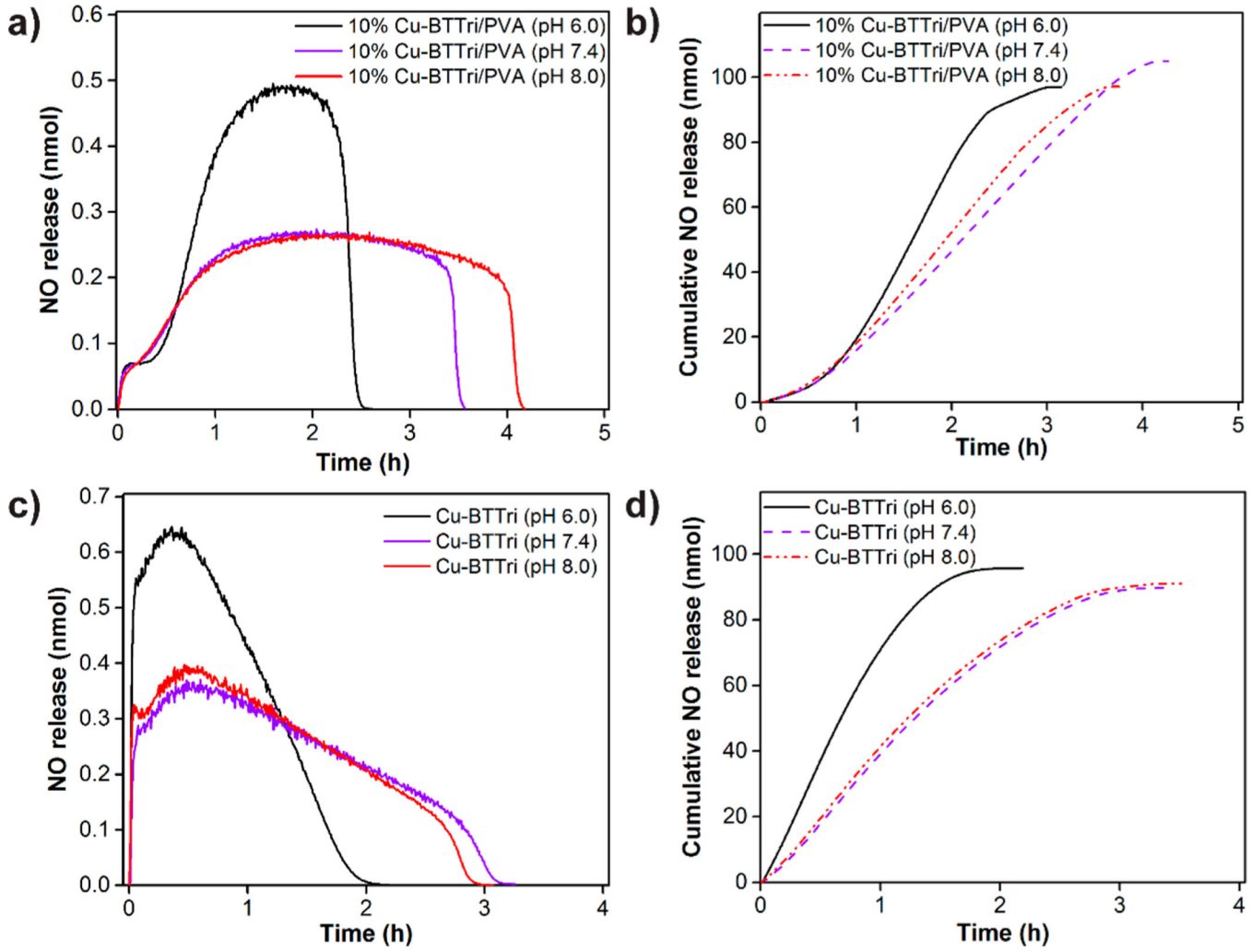

Figure 8.

(a) Representative NO release profiles and (b) cumulative NO release for 10 wt % Cu-BTTri/PVA membranes at pH 6.0, 7.4, and 8.0. (c) Representative NO release profiles and (d) cumulative NO release profiles for suspended Cu-BTTri powder at pH 6.0, 7.4, and 8.0. Experimental conditions: PBS at 37 °C, with a 10 μM initial GSNO concentration ($n \geq 3$).

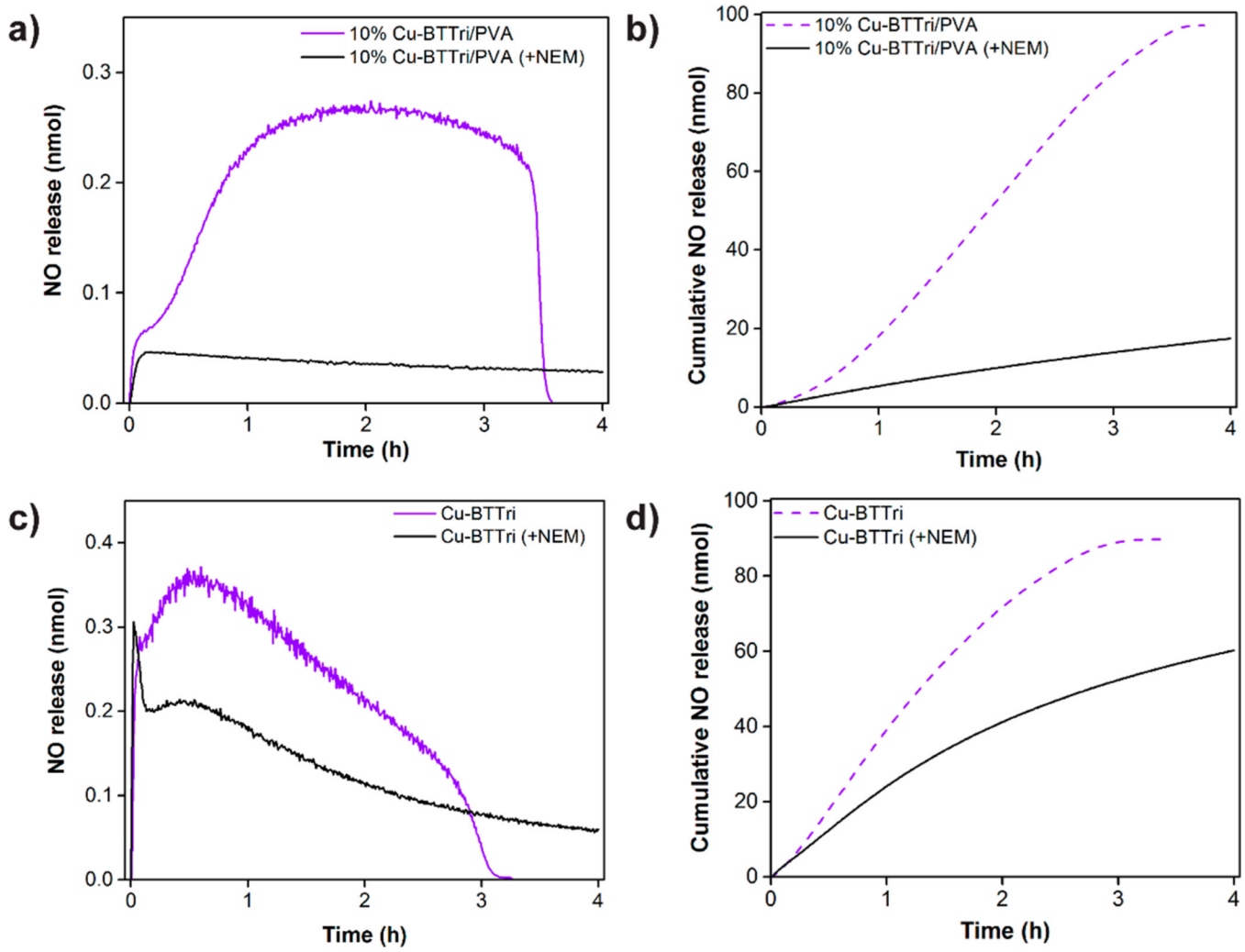

Figure 9.

(a) Representative NO release profiles and (b) cumulative NO release of 10 wt % Cu-BTTri/PVA membranes with and without NEM. (c) Representative NO release profiles and (d) cumulative NO release profiles for Cu-BTTri particles with and without NEM. Experimental conditions: pH 7.4 PBS at 37 °C, with a 10 μM initial GSNO and 100 μM NEM concentration ($n \geq 3$).

Table 1.

Tabulated Nitric Oxide Release Data

reaction conditions[a]	NO yield (%)[c]	completion time (h)[d]
GSNO (pH 7.4, 37 °C)[b]	2.7 ± 0.3	n/a
GSNO + PVA (pH 7.4, 37 °C)[b]	2.5 ± 0.1	n/a
GSNO + 10 wt % Cu-BTTri/Tecoflex SG-80A (pH 7.4, 37 °C)	1.5 ± 0.4	n/a
GSNO + 10 wt % Cu-BTTri/PVA (pH 7.4, 37 °C)	97 ± 6	3.5 ± 0.4
GSNO + 10 wt % Cu-BTTri/PVA (pH 6.0, 37 °C)	96 ± 1	2.7 ± 0.2
GSNO + 10 wt % Cu-BTTri/PVA (pH 8.0, 37 °C)	103 ± 4	4.1 ± 0.2
GSNO + 10 wt % Cu-BTTri/PVA (pH 7.4, 20 °C)	16 ± 1	n/a
GSNO + 1 wt % Cu-BTTri/PVA (pH 7.4, 37 °C)	102 ± 3	13 ± 1
GSNO + 5 wt % Cu-BTTri/PVA (pH 7.4, 37 °C)	97 ± 6	5.0 ± 0.7
GSNO + 10 wt % Cu-BTTri/PVA (reuse, pH 7.4, 37 °C)	103 ± 8	4.7 ± 0.4
GSNO + NEM + 10 wt % Cu-BTTri/PVA (pH 7.4, 37 °C)	16 ± 1	n/a
GSNO + Cu-BTTri (pH 7.4, 37 °C)[b]	90 ± 1	3.3 ± 0.2
GSNO + Cu-BTTri (pH 6.0, 37 °C)[b]	92 ± 1	2.1 ± 0.1
GSNO + Cu-BTTri (pH 8.0, 37 °C)[b]	91 ± 3	3.2 ± 0.3
GSNO + Cu-BTTri (pH 7.4, 20 °C)[b]	21 ± 1	n/a
GSNO + NEM + Cu-BTTri (pH 7.4, 37 °C)[b]	55 ± 2	n/a

[a] Reactions consist of the NO-forming decomposition of 10 μM *S*-nitrosoglutathione (GSNO) at varying temperature, concentration, and pH, or in the presence of *N*-ethylmaleimide (NEM).

[b] Cu-BTTri powder added in a quantity corresponding to the amount present in experiments conducted with 10 wt % Cu-BTTri/PVA.

[c] NO yield refers to measured NO/theoretical GSNO × 100.

[d] Completion time refers to the mean time required for NO measurements to reach baseline levels, which is inferred to represent depletion of available GSNO. Experiments with incomplete NO recovery were performed for fixed intervals corresponding to the mean duration of the parent experiment. All values reported as mean ± standard deviation