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Spatiotemporal defined release of nitric oxide

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Nitric oxide's β-quinol clathrate rapidly and quantitatively releases nitric oxide upon dissolution to give well defined spatiotemporal gradients.

Although nitric oxide donors based on diazeniumdiolates,[1] aromatic nitrite esters,[2] nitrosyl thiols,[3] and metallonitrosyls,[4] are now well established, it is surprisingly difficult to devise donors which release nitric oxide in a biomimetic manner with well defined spatiotemporal control. An important biological example of spatiotemporally defined NO release is in the thin endothelial cell lining of the lumen of arterioles. Here a NO concentration gradient results from its diffusion into surrounding tissues and by the kinetics of its reactions. For example, dioxygen and oxyhemoglobin in arteriole red blood cells rapidly react with nitric oxide by ter- and bimolecular rate laws respectively. Considerable attention has focused on these gradients, with their location, depth, and profile being of particular theoretical importance.[5] Empirical testing of these theories remains difficult, as most available nitric oxide donors create a uniform concentration or "fog" which does not resemble the vasculature gradients. Although the localization, and subsequent release, of NO from polymeric and/or surface bound donors can in principle surmount this problem, their variable NO fluxes create shifting or variable gradients which are difficult to predict or measure. While these donors may ultimately be useful for clinical applications, they are difficult to employ for *in vitro* experiments of cell/tissue response to nitric oxide. An ideal donor for *in vitro* microscopy is a point source of NO, with tunable loading, from which NO can be spontaneously released in a controlled manner. With these goals in mind, we report herein the synthesis and characterization of NO entrapped clathrates which provide an archetype for this type of spatiotemporal donor.

Pioneering studies into the β-quinol NO inclusion clathrate describe highly reactive materials which crystallize with 50% of the sites filled under 40 atmospheres of NO.[6] Characterization of these materials is hampered by their severe sensitivity towards autocatalytic release of nitric oxide and formation of benzoquinone and quinhydrone.[7] For example, merely packing finely powdered samples into a Gouy tube for magnetic susceptibility measurements irreversibly led to rapid decomposition. At the outset of our studies it seemed to us that these and related problems are associated with the transiently high levels of nitric oxide, possibly released from sites of poor crystallinity, which would lead to dinitrogen trioxide. We hypothesized that clathrate stability would be enhanced if the labile NO was thoroughly removed immediately after its preparation, eqn. (1), during its isolation, and before storage.

$$HOC_6H_4OH\ (\text{solution}) + NO \rightarrow \{HOC_6H_4OH\}[NO]_x \quad (1)$$

This proved to be the case in that when the crystals of the hydroquinone NO clathrate, **1**, are filtered onto a large filtration bed, so that the crystals are well dispersed over a large area, we find there is negligible spontaneous decomposition during this initial step. Moreover, when these crystals are immediately dried under high vacuum, most of the nitric oxide is retained in the clathrate and the resulting product can be stored for months in the dark at 4 °C, with little appreciable sign of decomposition. In a typical preparation of **1**, a 40 mL deoxygenated saturated solution of hydroquinone in ethanol at 60 °C in a Fisher–Porter pressure bottle was pressurized to 39 psi with KOH scrubbed commercial nitric oxide. After *ca.* two hours, crystallization was complete and the excess nitric oxide was purged after the bottle cooled to room temperature. The solution was then quickly filtered on a large 20 cm filter bed and the filter and the still moist crystals were transferred to a vacuum oven for rigorous drying over several hours at room temperature. The resulting white crystals, of **1** (1–3 g), decompose slowly in air and often lead to purple quinhydrone-containing phases only after prolonged storage in the open.

We have characterized the resulting clathrates by a number of spectroscopic†and crystallographic‡means. The extent of NO loading has been determined by crystallography, Griess assay,[8] and chemiluminescence measurements which demonstrate that NO loading correlates with NO partial pressures. For example, *ca.* 20% of the sites are filled with NO when the clathrate is prepared at 39 psi, but when a higher pressure such as 475 psi is used in a stainless steel bomb and lines, *ca.* 50% of the cavities are filled with NO. The low temperature structure of 50% loaded clathrate has NO that is disordered within the cage by N/O superposition. Independent refinement of the nitrogen and oxygen site occupancy factors is in accord with the analytical results indicating that all of the NO is located in these sites and available for release.

As was determined by single crystal X-ray diffraction, Fig. 1,‡ the included NO is present in the form of a monomer. The inclusion compound gives a characteristic weak axial signal in the X-band EPR in accord with some of Van Vleck's predictions for this phase although there was no observable ^{14}N hyperfine interaction with a 1 G modulated field.[9] Unfortunately even with the highest NO loading the magnetic susceptibility is still dominated by the diamagnetism of the host lattice and our SQUID measurements are similar to those obtained by Gouy methods.[6,10] The IR spectrum for **1** has a weak band at 2224 cm^{-1} due to the uptake of nitrous oxide

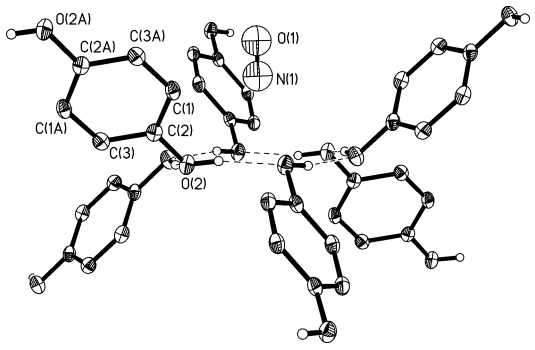

Fig. 1 X-Ray crystal structure of β-quinol clathrate with included NO, **1**, N(1)–O(1) 0.96(1) Å. Note only one of the two superimposed disordered NOs is shown in the bottom half of the cavity. Carbon bound hydrogens have been omitted for clarity.

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impurities in the commercial source, and there is only a weak band at 1855 cm^{-1} which might be attributable to ν(NO). This intensity pattern is similar to that seen for CO clathrate which has a weak ν(CO) at below room temperature and which disappears into the baseline above 20 °C.[11]

Individual crystals of **1** are readily handled and rapidly dissolve in water. For example crystals comparable to that used in the structure determination dissolve in 2 seconds in pH = 7.4 buffers. As described above, this leads to the rapid release of NO from the point of dissolution which would be similar to the NO gradient released from a fish egg shortly after deposition.[12] A consequence, of course, of using **1** for the spatiotemporally defined release of NO is that there is simultaneous release of hydroquinone. While this particular host is anticipated to complicate many biological systems, the concept demonstrated here can be applied to the wide range of materials which are known to form NO inclusion compounds.[13] A clear goal in this research is to prepare NO inclusion compounds with non-redox-active, non-toxic and/or photodegradable hosts.

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Notes and references

† Characteristic data: IR: 22 °C in KBr/cm^{-1}: 3264s, 3030s, 1855m, 1518s, 1476s, 1355s, 1210s, 1096s, 1008m, 826s, 758s, 608s and 516s; mp 171.7 °C; EPR 22 °C, X-band powder spectrum $g_{\perp} = 2.002$, $g_{\parallel} = 2.001$.

‡ NO clathrate, **1**, crystallizes in the rhombohedral space group $R\bar{3}$; common to the β-quinol clathrate phases. Key crystallographic parameters: $a = b = 16.540(6)$ Å, $c = 5.432(3)$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$, $V = 1287.0(9)$ $Å^3$, $\rho_c = 1.337$ Mg m^{-3}, crystal size = $0.62 \times 0.42 \times 0.34$ mm^3, 487 independent data with $I > 2\sigma(I)$, $S_{gof} = 1.097$, $R1 = 3.68\%$, $wR2 = 8.92\%$. Powder diffraction at 30 K gave an identical unit cell. Further details are available on CCDC 232891. See http://www.rsc.org/suppdata/cc/b4/b404728a/ for crystallographic data in .cif format.

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